The Transcriptomic and Genomic Analysis of Lamin A/C Expression in the Colon and in Colorectal Cancer

RAHMAN-CASANS, SYED,FIDA,UR

The Transcriptomic and Genomic Analysis of Lamin A/C Expression in the Colon and in Colorectal Cancer

by

Syed Fida-ur Rahman-Casañs

A thesis submitted to University of Durham in fulfilment of the requirements for the degree of Doctor of Philosophy (Ph.D)

University of Durham

School of Biological and Biomedical Sciences

August 2011

Contents

ACKNOWLEDGEMENTS

This project was kindly funded by a grant from the Research and Development department at the James Cook University Hospital, South Tees Acute NHS trust, Middlesbrough.

I am indebted to my supervisors Professor Robert G. Wilson, Professor of Surgery, University of Durham and Medical Director and Chief of Surgery / Consultant Colorectal Surgeon, James Cook University Hospital, Middlesbrough and Professor Christopher J. Hutchison, Chair of Animal Cell Biology, School of Biological and Biomedical Sciences, University of Durham and Principal of St Hild & St Bede College, University of Durham, for their unwavering support, help and kindness throughout my PhD, particularly during the writing up period.

I would also like to acknowledge and thank all patients who volunteered to participate in this project and to all staff on the surgical wards and in theatres at the James Cook University Hospital in Middlesbrough, for their cooperation and support.

I would also like to extend my heartfelt gratitude and thanks to many researchers and technical staff working at the School of Biological and Biomedical Sciences, University of Durham and the department of Pathology, James Cook University Hospital in Middlesbrough. Dr Stefan Przyborski for his invaluable advice and help at various stages of my PhD; Dr Richard Jones, Pathologist at the James Cook University Hospital, Middlesbrough, for personally supervising tissue sampling from colorectal cancer specimens; Mrs Pamela Ritchie for her superb support and technical help at all stages of my work; Dr Ewa Markiewicz for her support and the innumerable discussions we had on various aspects of my work; Dr Fahad Alzoghaibi for his expert advise on RNA work; Dr Nkemcho Ojeh, Dr Georgia Salpingdou, Dr Vanja Pekowicz, Dr Stepan Fenyke and many others for all their help and kind support shown to me during my PhD at the University of Durham.

I would especially like to acknowledge and thank Dr Naomi D. Willis for her kind support, help, advice and above all her patience in guiding me through various stages of my PhD. Finally and most importantly, I am indebted to my very good friend and colleague, Dr Thomas R. Cox, for having extended me his expert advice and help at all stages of my PhD and at the same time, his friendship. It has been a privilege to have worked with such a distinguished team of scientists and researchers, in a centre of excellence at the forefront of cutting edge biological research.

On a personal note, words cannot express my tremendous gratitude towards my wife, son and daughter for their exceptional patience, support and understanding during my PhD. I would also like to show my immense gratitude towards my mother, late father and sisters, who motivated me and supported me at every step of the way and would be very proud of this achievement.

I would like to dedicate this thesis to the loving memory of my late father.

DECLARATION

I hereby declare that this thesis is a result of my own work and all experiments described herein were carried out at the School of Biological Sciences, University of Durham under the supervision of Prof. C.J. Hutchison and Prof R.G. Wilson. Work other than my own is clearly referenced to the appropriate author or their publication. No material contained herein has previously been submitted for a degree at this or any other university. The copyright of this thesis rests solely with the author. No quotation from it should be published in any format, including electronic/digital or the internet, without the author's prior consent. All information derived from this thesis must be acknowledged correctly and in full.

Syed Fida-ur Rahman-Casans

ABSTRACT

Lamins A and C, also known as A-type lamins, are type V nuclear intermediate filament proteins which form an interlacing meshwork of filaments subjacent to the inner nuclear membrane termed the nuclear lamina. A-type lamins have been implicated in DNA replication, gene transcription regulation, apoptosis, regulation of growth promoters and nuclear migration. Traditionally, expression of A-type lamins has been associated with differentiated cells. As such, mutations in A-type lamins have been associated with a diverse range of genetic diseases, including premature ageing syndromes and with increased proliferation, especially in tumours.

In colorectal cancer, expression of A-type lamins, have been shown to impart an adverse prognosis. In order to understand the underlying biological processes responsible for this adverse outcome in patients with colorectal cancer, I sought to clarify the expression profile of A-type lamins and their binding partners in normal colonic/rectal mucosa, prior to investigating the expression of A-type lamins in colorectal cancers. I used fresh tissue specimens obtained from patients with colorectal cancer for my experiments. A unique finding was the expression of lamin A in the putative stem cell niche of colonic / rectal mucosal crypts.

Further studies in the form of a microarray analysis, revealed a very complex picture of up regulation involving various signalling cascades in the cancer samples expressing A-type lamins. There was no evidence to suggest a direct involvement of A-type lamins influencing the Wnt signalling cascade, however, direct involvement of other signalling cascades, such as the IGF signalling cascade, Shh signalling cascade and TGF-β signalling cascades were noted. These signalling cascades were known to influence the Wnt signalling cascades and hence could play a crucial role in the pathogenesis of colorectal cancers expressing A-type lamins.

In addition to these important signalling cascades, other key genes involved in apoptosis, growth promoters, cell adhesion, stem cell regulation, oncogenes and tumour suppression, were noted to have a unique expression profile in the cancer

sample expressing A-type lamins, not observed in the cancer sample lacking A-type lamin expression. These observations were suggestive of A-type lamins having a diverse range of actions via, as yet, undefined pathways. It would appear that A-type lamins were imparting a more motile, less adherent phenotype with stem cell like features in colorectal cancers expressing A-type lamins. This could explain the observed poor prognosis of patients with colorectal cancers expressing A-type lamins.

Creatine kinase brain (CKB), was also identified as an additional, potential, prognostic indicator in the Duke's B group of patients with colorectal cancer expressing A-type lamins. This potential marker, in conjunction with A-type lamin expression could be used to identify a sub group of Duke's B patients at high risk. Whether adjuvant therapy in this group would help improve their long term survival is unknown since no study has been done to assess this.

ABBREVIATIONS

+ve	positive
5-FU	5-Fluorouracil
A	Adenine nucleotide
A	Absorbance
aa	Amino acid
Ab	Antibody
ACF	Aberrant crypt foci
ACVRII	Activin Receptor Type II
ACVRIIB	Activin Receptor Type IIB
AD	Autosomal Dominant
AD-EDMD	Autosomal Dominant – Emery Dreifuss Muscular Dystrophy
AJCC	American Joint Committee on Cancer
ALY	ALY Binding site
AMV-RT	Avian Myeloblastosis Virus – Reverse Transcriptase
ANN	Approximate Nearest Neighbour
APC	Adenomatous Polyposis Coli
APS	Ammonium persulphate
AR	Autosomal recessive
AREG	Amphiregulin
BAF	Barrier to autointegration factor
BAX	*Bcl2* homolog
BCC	Basal cell carcinoma (of skin)
Bcl-2	B-Cell lymphoma/Leukaemia-2
BMP	Bone Morphogenic Protein
BMP4	Bone Morphogenic Protein 4

BMPR1A	Bone Morphogenic Protein Receptor 1A
BMPR1B	Bone Morphogenic Protein Receptor 1B
BMPRII	Bone Morphogenic Protein Receptor Type II
bp	Base pair
BRB	Blot rinse buffer
BRR	Bannayan-Riley-Ruvalcaba
BSA	Bovine serum albumin
C	Cytosine nucleotide
C-	Carboxy terminal
C.elegans	Caenorhabditus elegans
χ^2	chi-square test
$CaCl_2$	Calcium Chloride
CCND1	Cyclin D1 gene
CD44	CD44 antigen
CDH1	Cadherin 1, type 1, E-cadherin (epithelial)
cDNA	complementary DNA
cds	Coding sequence
CEA	Carcinoembryonic antigen
CI	Confidence Interval
CIN	Chromosome Instability
CKB	Creatine Kinase Brain
CKI	Casein Kinase I
CKII	Casein Kinase II
CMT1	Charcot-Marie-Tooth Disease Type 1
CMT2	Charcot-Marie-Tooth Disease Type 2
c-MYC	v-myc avian myecytomatosis viral oncogene homolog
c-NES	C-terminal Nuclear Export Signal

CNS	Central Nervous System
CO_2	Carbon Dioxide
CRC	Colorectal Cancer
CRDGF	Colo Rectum Derived Growth Factor
CREAM	Colorectal Epidemiology and Mutation
CRUK	Cancer Research UK
CS	Cowden Syndrome
CT	Computed Tomography
Da	Daltons
DAB	3,3'-diaminobenzidine tetrahydrochloride
DCC	Deleted in Colon Carcinoma
DCM1A	Dilated Cardiomyopathy type 1A
DEPC	Diethyl Pyrocarbonate
DNA	DeoxyriboNucleic Acid
DNase	DeoxyriboNuclease
dNTP	Deoxynuceotide triphosphate
DoH	Department of Health
Dsh	Dishevelled
DTT	Dithiothreitol
DUSP5	Dual Specificity Phosphatase 5
ECACC	European Collection of Cell Cultures
ECL	Enhanced Chemiluminescence
EDMD	Emery-Dreifuss muscular Dystrophy
EDTA	Ethylenediamine Tetraacetic Acid
EGF	Epidermal Growth Factor
EGFP	Enhanced Green Fluorescent Protein
EGFR	Epidermal Growth Factor Receptor

EGTA	Ethylene glycol-bs[β-aminothyl ether]-N,N,N'N;-tetraacetic acid
EMD	Emerin Gene
EPIC	European Prospective Investigation into Cancer and Nutrition
ER	Endoplasmic Reticulum
ERBB	avian erythroblastosis oncogene B
ERK	Ras/extracellular signal-regulated kinase
EtBr	Ethidium Bromide
f	Female
FAK	Focal Adhesion Kinase
FAP	Familial Adenomatous Polyposis
FBS	Foetal Bovine Serum
Fc	Fold Change
FOBt	Faecal Occult Blood testing
FOSL1	Fos-like Antigen 1
FPLD	Familial Partial Lipodystrophy
FTI	Farnesyl Transferase Inhibitor
Fz	Frizzled
FzR	Frizzled Receptor
G	Guanine Nucleotide
GB	Great Britain
Gcl	Germ-cell-less
GCOS	Gene Chip Operating System
GCT	Gene Class Testing
gDNA	genomic DNA
GFP	Green Fluorescent Protein
GI tract	Gastrointestinal Tract

GO	Gene Ontology
Gro	Groucho
GSK3β	Glycogen Synthase Kinase 3β
H_2O_2	Hydrogen Peroxide
HCA	Heterocyclic Aromatic Amines
HCl	Hydrochloric Acid
HG U133	Human Genome U133 genechip (Affymetrix)
HGPS	Hutchinson-Gilford Progeria Syndrome
hMLH	human mutL homology
hMSH	human mutS homology
HNPCC	Hereditary Non-polyposis Colorectal Cancer
HR	Hazard Ratio
HRAS-VNTR	Harvey ras-1 Variable number tandem repeat polymorphism
HRP	Horse-radish Peroxidase
ICD	International Classification of Diseases
IF	Intermediate Filament
IgG	Immunoglobulin G
IHC	Immunohistochemistry
INM	Inner Nuclear Membrane
JPS	Juvenile Polyposis Syndrome
Kb	kilobase
KCl	Potassium Chloride
kDa	Kilo Dalton
KEGG	Kyoto Encyclopaedia of Genes and Genomes
Ki-ras	Kirsten Ras
KNN	K-Nearest Neighbour
L-15	Leibovitz growth media

LAP	Lamina Associated Protein
LBR	Lamin B Receptor
Lef	Lymphoid enhancing factor
LEM	LAP2, Emerin, MAN1
LGMD1B	Limb Girgle Muscular Dystrophy type 1B
LMNA	Lamin A/C gene
LMNB1	Lamin B1 gene
LMNB2	Lamin B2 gene
LOH	Loss of Heterozygosity
Lrp	Low Density Lipoprotein Receptor related protein
LSM	Laser Scanning Microscope
m	Male
M	Molar
mA	milliAmps
mAb	Monoclonal Antibody
MAD	Mandibuloacral Dysplasia
MAPK	Mitogen-Activated Protein Kinase
MDT	Multidisciplinary team
mg	milligram
$MgCl_2$	Magnesium Chloride
MGED	Microarray and Gene Expression Data
MGMT	O^6-methylguanine-DNA methyltransferase
$MgSO_4$	Magnesium Sulphate
MIAME	Minimum Information About a Microarray Experiment
ml	millilitre
mM	milliMolar
MMP7	matrix metalloproteinase-7 (matrilysin)

MMR	MisMatch Repair
MOK2	Kruppel/TFIIIA-related zinc finger proteins
MRI	Magnetic Resonance Imaging
mRNA	messenger RNA
MSI	Microsatellite Instability
MTHFR	Methylene-Tetra-Hydro-Folate-Reductase
MUTYH	mutY homolog (E-coli)
Mw	Molecular Weight
N-	Amino terminal
N.I.C.E	National Institute of Clinical Excellence
NaCl	Sodium Chloride
NaOAc	Sodium Acetate
NaOH	Sodium Hydroxide
NCR	National Cancer Registry
NE	Nuclear Envelope
ng	Nanogram
NHL	Non-Hodgkin's Lymphoma
NHS	National Health Service
NL	Nuclear lamina
NLCS	Netherlands Cohort Study on Diet and Cancer
NLS	Nuclear Localisation Signal
nm	nanometre
NMSC	Non-melanoma Skin cancer
n-NES	N-terminal Nuclear Export Signal
NPC	Nuclear Pore Complex
NSAID	Non-Steroidal Anti-inflammatory Drug
NAUNCE	Nucleus and ActiN Connecting Element

OD	Optical Density
OMIM	Online Mendelian Inheritance in Man
ONM	Outer Nuclear Membrane
p	Page
p	Probability
$p21^{CIP1/WAF1}$	Cyclin-dependant kinase inhibitor 1A
PAH	Polycyclic Aromatic Hydrocarbons
PALGA	Pathologisch Anatomisch Landelijk Geautomatiseerd Archief
PBS	Phosphate Buffered Saline
PCA	Principal Component Analysis
PCNA	Proliferating Cell Nuclear Antigen
PCR	Polymerase Chain Reaction
PJS	Peutz-Jeghers Syndrome
PLAUR	Plasminogen Activator, Urokinase Receptor
PLS3	T-Plastin
pmol	picomole
PPARD	peroxisome proliferator activator receptor delta
pRB	Retinoblastoma Protein
PSA	Prostate Specific Antigen
PTEN	Phosphate and Tensin Homolog
PUFA	Polyunsaturated Fatty Acids
RD	Restrictive Dermopathy
RECQL2	DNA helicase-like / Werner syndrome ATP-dependent helicase
rhBMP	Recombinant Human BMP
RNA	RiboNucleic Acid
ROBO1	Roundabout, axon guidance receptor, homolog 1 (Drosophila)

rpm	Revolutions per minute
RT	Room Temperature
RT-PCR	Reverse Transcriptase – Polymerase Chain Reaction
SCC	Squamous Cell Carcinoma
SD	Standard Deviation
SDS	Sodium Dioecyl Sulphate
SDS-PAGE	Sodium Dioecyl Sulphate – Polyacrylamide Gel Electrophoresis
Shh	Sonic hedgehog
SLR	Signal Log Ratio
SMAD4	Mothers Against Decapentaplegic (Drosophila) Homolog 4
SOC	Super Optimal Broth [Catabolite Repression]
SOM	Self Organising Maps
SPSS	Statistical Package for the Social Sciences
SREBP-1	sterol regulatory element binding protein 1
STA	Emerin gene
STK11	Serine/Threonine Kinase 11
SUN	Sad1 / UNC-84 homology
SVD	Singular Value Decomposition
SVM	Support Vector Machines
T	Thymine nucleotide
t	Time
TAE	Tris-Acetate-EDTA
Taq	*Thermus aquaticus*
TBE	Tris-Borate-EDTA
TBS	Tris Buffered Saline
Tcf	T-cell Factor

TE	Tris / EDTA
TEMED	N,N,N'N'-tetramethylethylendiamine
TGFα	Transforming Growth Factor α
TGFβ	Transforming Growth Factor β
TGFβIIR	Transforming Growth Factor β Receptor II
Tm	Melt Temperature
TNM	Tumour Node Metastsis
TP53	Tumour Protein 53
Ub	Ubiquitin
UC	Ulcerative Colitis
UK	United Kingdom
US	United States
USA	United States of America
UV	Ultraviolet
V	Volts
v/v	volume / volume
-ve	negative
v-EGF	vascular endothelial growth factor
vs.	versus
w/v	weight / volume
Wg	Wingless
WHO	World Health Organisation
WISP3	WNT-1 inducible signalling pathway protein 3
XL	X-linked
yr	year
β-TrCP	β-Transducin repeat containing protein
δH2O	Distilled Water

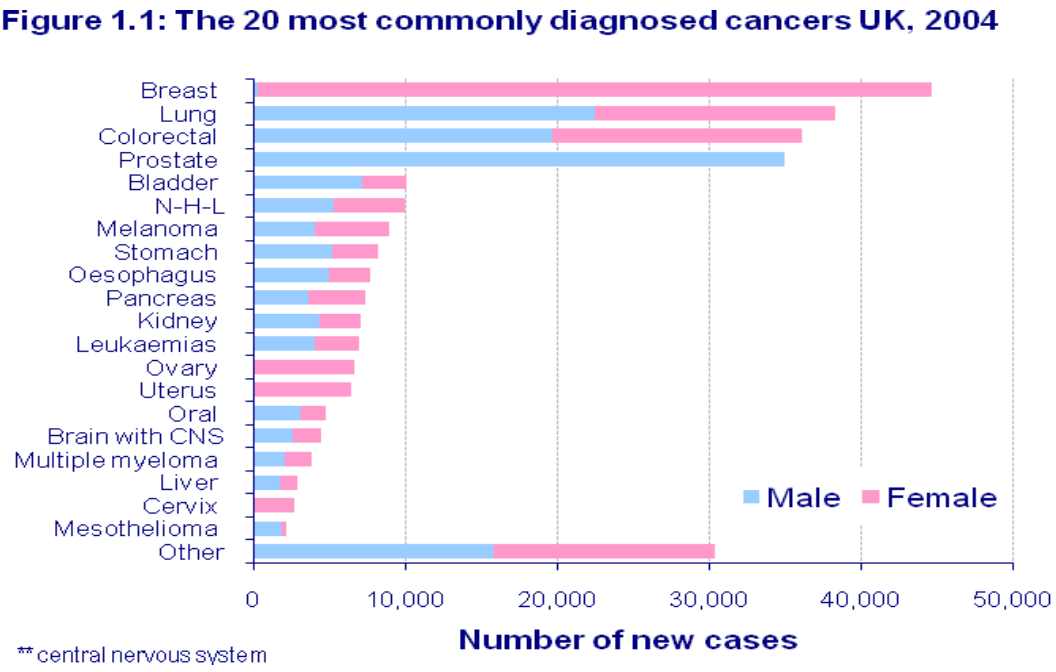
ddH2O	Ultrapure Water
µg	microgram
µl	microlitre
µm	micrometre
µmol	micromole

CHAPTER 1

INTRODUCTION

1.1 Cancer of the colon and rectum Epidemiology

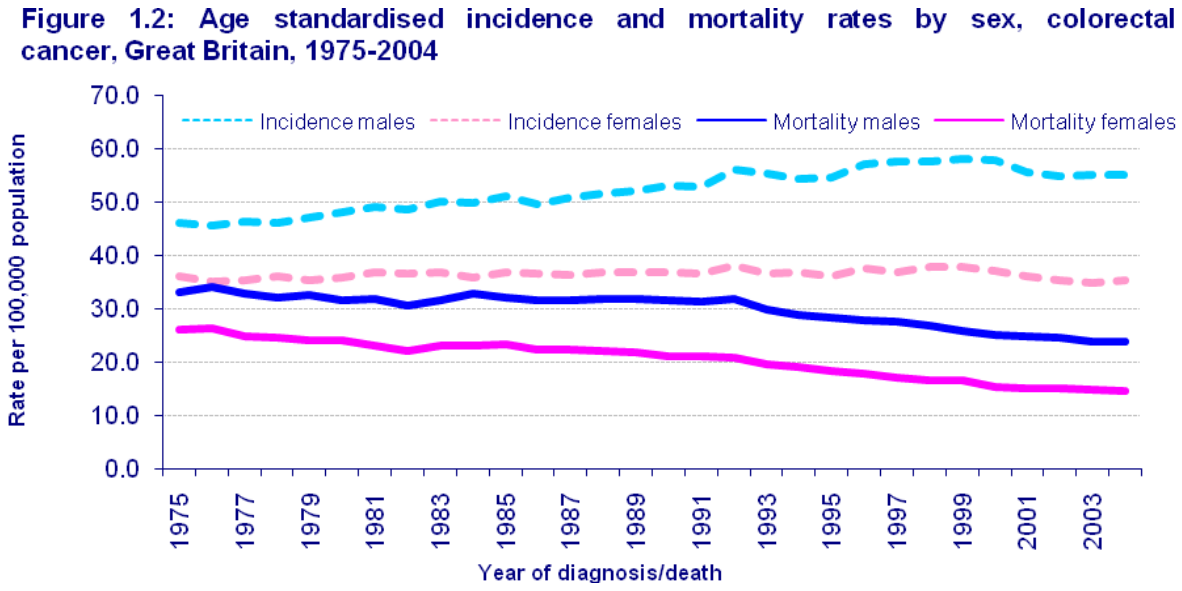
Around the world there were 11 million cases of cancer diagnosed in the year 2002 (cancer research 2008). In the UK there are around 285,000 new cases of cancer diagnosed each year. Breast, lung, bowel and prostate cancers account for over half of all these cases (Figure 1.1). Colorectal cancer is the third most common cancer to affect the population in the UK. In 2004, there were 36,209 cases of bowel cancers registered in the UK, two thirds of these affected the colon and the rest were in the rectum. The male to female ratio stands at 1.2 : 1.0. From 1979 up to 1999, there has been on average a 1% rise per annum in the incidence of male bowel cancer. Thereafter recently there has been a slight decline. During the same period, the incidence of female bowel cancer has remained pretty much the same. There has been a gradual decline in the mortality associated with colorectal cancer (Figure 1.2). However, nearly 16,000 people died in the year 2006 from this cancer. Colorectal cancer is the second most common cause of death after lung cancer in the UK (Figure 1.3). Despite a gradual increase in incidence, the mortality related to this cancer has declined. The five year survival rate for men with colon cancer has increased from 22% (23% for women) in the 1970s to 47% (48% for women) in the late 1990s. A similar trend is seen with rectal cancer over the same time period (cancer research UK 2008).



Source: ***http://info.cancerresearchuk.org/cancerstats/types/bowel***

Figure 1.1

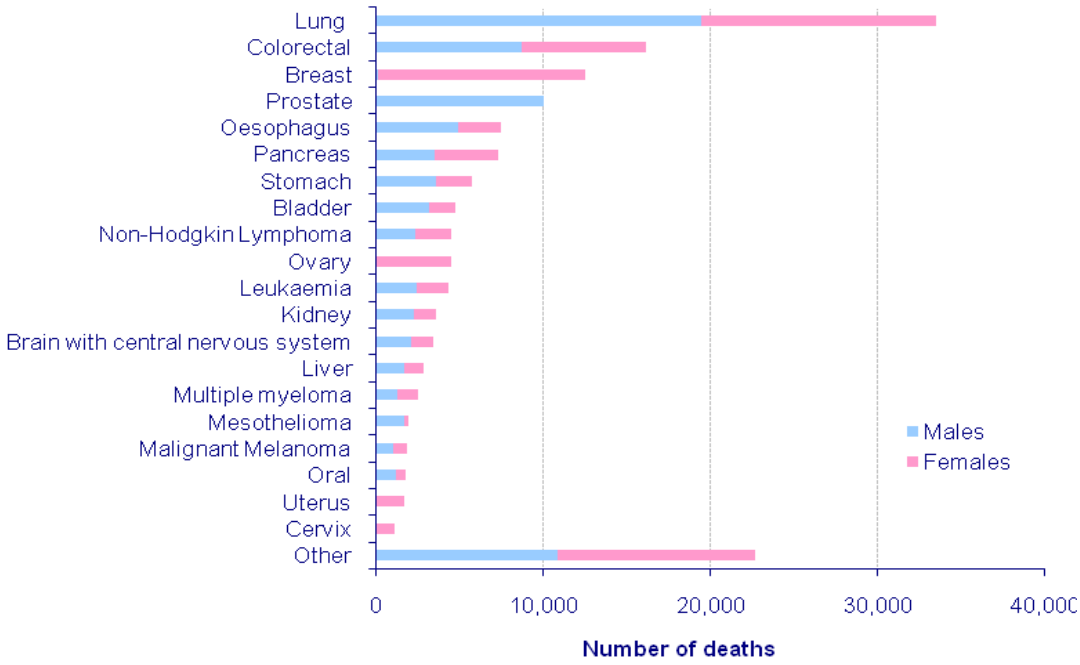
The 20 most commonly diagnosed cancers in the UK



Source: ***http://info.cancerresearchuk.org/cancerstats/types/bowel***

Figure 1.2

Age standardized incidence and mortality rates by sex in the UK.



*Source: **http://info.cancerresearchuk.org/cancerstats/types/bowel***

Figure 1.3
Mortality rates associated with the twenty most common cancers in the UK

1.1.1 Staging of Colon and Rectal Cancer

An important determinant of survival is stage of the disease. Various staging systems are in place. The earliest of these is Dukes' staging system (Dukes, 1932), which takes into account the spread of cancer through various layers of the bowel wall. Dukes' originally proposed three stages, A, B and C. Stage A cancer is limited to the mucosa; stage B cancer indicates spread to the muscular layer and stage C cancer indicates spread to at least one lymph node. Stage C cancer was later modified to include stage C1, which indicates involvement of localised lymph nodes close to the bowel and stage C2, which indicates spread to the highest lymph node in the sample of tissue removed (Gabriel *et al*., 1935). Stage D was added on later by Turnbull (Turnbull et al., 1967) to indicate distant spread of disease (Figure 1.4).

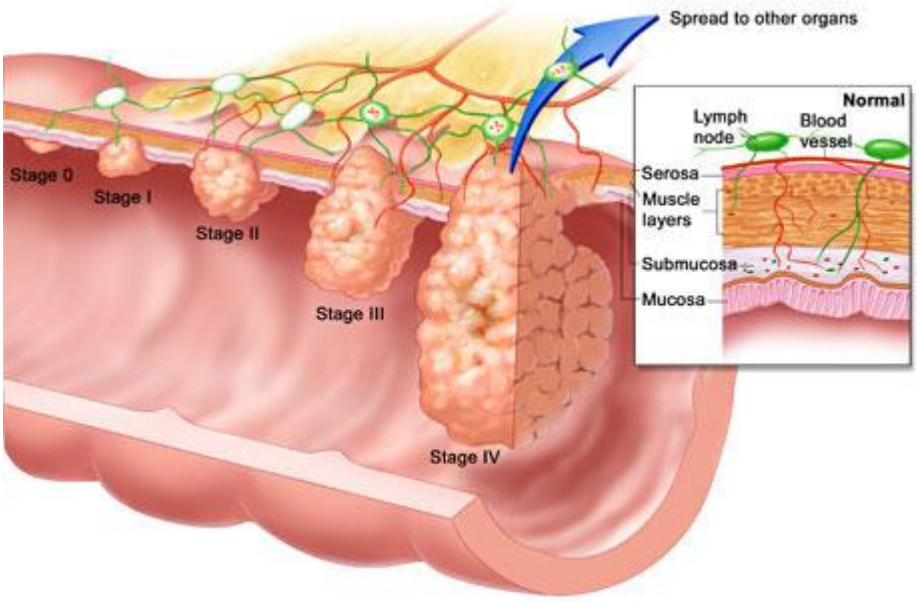


Figure 1.4

Diagrammatic representation of various tumour stages in colorectal cancer. A window shows greater detail of the various layers seen in the colonic / rectal wall.

Source: www.annistononcology.com

Stage 0 corresponds to Dukes' stage A

Stage I and II corresponds to Dukes' stage B

Stage III corresponds to Dukes' stage C

Stage IV corresponds to Dukes' stage D

This modified Dukes' staging system is still widely used throughout the UK. In addition, there is the TNM staging system (AJCC, 1997) where T stands for tumour, N stands for nodes and M stands for metastasis. TNM comprises of stage 0, 1, 2, 3 and 4 depending on the extent of tumour spread, node deposits and metastasis (Figure 1.5). Early stage disease has a much better outcome in terms of five year survival figures as compared to late stage disease. The correlation between various stages and approximate five year survival levels has been demonstrated in tables 1.1, 1.2 and 1.3. Early stage bowel cancer i.e. Dukes' A (equivalent to Stage 1 disease in the TNM system) has an 83% survival rate at 5 years whereas Dukes' D stage has a 3% survival rate at five years. Obviously, the

earlier bowel cancer is diagnosed and treated, the better it is for improving survival.

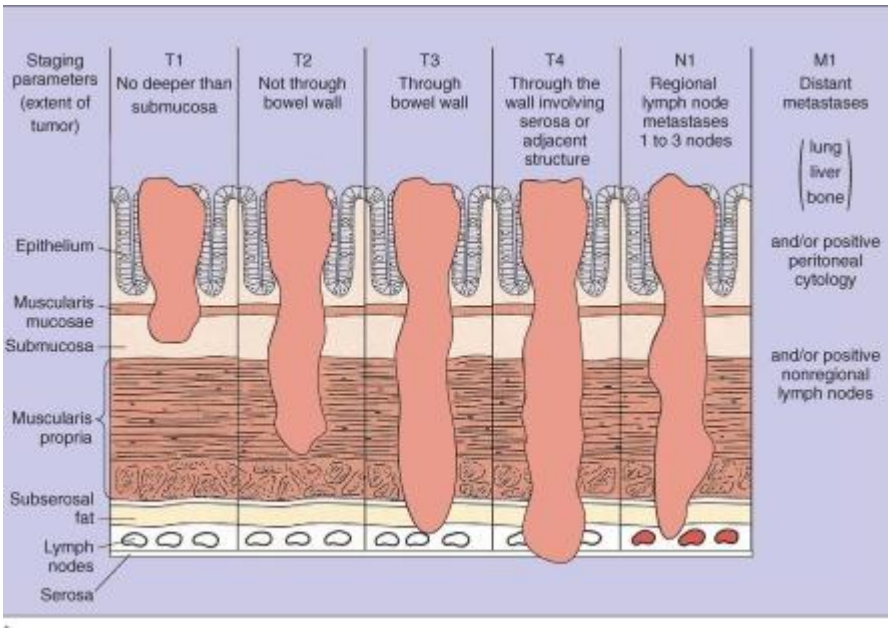


Figure 1.5

Diagrammatic representation of the TNM classification system

Source: www.annistononcology.com

Tumour Node Metastasis (TNM) Definitions agreed by the American Joint Committee on Cancer (AJCC)

Primary Tumour

TX: Primary tumor cannot be assessed
TO: No evidence of primary tumor
Tis: Carcinoma *in situ* (intraepithelial or intramucosal carcinoma)
T1: Tumor invades the submucosa
T2: Tumor invades the muscularis propria
T3: Tumor invades through the muscularis propria into the subserosa or into the nonperitonealized pericolic or perirectal tissues
T4: Tumor directly invades other organs or structures (T4a) or perforates the visceral peritoneum (T4b)

Regional Lymph Nodes

NX: Regional lymph nodes cannot be assessed
NO: No regional lymph node metastasis
N1: Metastasis in 1 to 3 lymph nodes
N2: Metastasis in 4 or more lymph nodes

Distant Metastasis (M)

MX: Presence of distant metastasis cannot be assessed
M0: No distant metastasis
M1: Distant metastasis

Stage	TNM Stage Groupings	Modified Astler-Coller Stage	Dukes Stage - Modified
Stage 0	Tis No Mo	Stage A	Stage A
Stage I	T1 No Mo	N/A	Stage B
	T2 No Mo	Stage B1	Stage B
Stage II	T3 No Mo	Stage B2	Stage B
	T4 No Mo	Stage B3	Stage B
Stage III	Any T N1 Mo	Stage C1(T2); C2(T3); C3(T4)	Stage C 1
	Any T N2 Mo	Stage C1(T2); C2(T3); C3(T4)	Stage C 2
Stage IV	Any T Any N M1	Stage D	Stage D

Table 1.1
Comparison of various colorectal cancer staging protocols in clinical use

TNM Stage	5 Year Survival (%)
Stage 0, I (Tis, T1; No; Mo)	>90
Stage I (T2; No; Mo)	80 – 85
Stage II (T3; T4; No; Mo)	70 – 75
Stage III (T2; N1 – 3; Mo)	70 – 75
Stage III (T3; N 1 – 3; Mo)	50 – 65
Stage III (T4; N 1 – 3; Mo)	25 – 45
Stage IV (M1)	<3

Table 1.2
Correlations between TNM stage and survival in colorectal carcinoma

Dukes Stage	Approximate Frequency at Diagnosis	Approximate Five Year Survival
A	11%	83%
B	35%	64%
C	26%	38%
D	29%	03%

Table 1.3
Correlation between Dukes' stages of colorectal cancer and survival figures

Source: Cancer research UK

http://info.cancerresearchuk.org/cancerstats/types/bowel/survival

1.1.2 Detection and Screening for Bowel cancer

Symptoms related to early bowel cancer can be fairly innocuous and hence by the time significant symptoms do develop, this cancer can be fairly advanced. Various symptoms are attributable to bowel cancer. A change in bowel habit over the last few weeks or months is significant, especially a history of alternating loose stools with episodes of constipation. Passage of dark red blood, either mixed with stools or on its own is also significant. Passage of bright red, rectal blood in the absence of other anal disease such as haemorrhoids, anal fissures, etc, is also a significant symptom. Tenesmus or a feeling of incomplete evacuation of faeces after a bowel motion is highly significant and could signify a low rectal cancer. Various signs are attributable to bowel cancer but anaemia proves to be a fairly common feature of this cancer and can be detected early on. A whole host of investigations are usually carried out if bowel cancer is suspected. Investigations such as examination of the bowel with an endoscope (sigmoidoscopy / colonoscopy) are usually invasive and carry a small but finite risk of damaging the bowel. There are other non invasive modalities to investigate bowel cancer such as computerised tomography (CT), magnetic resonance imaging (MRI) and ultrasound scans, but none of them are capable of yielding a histological diagnosis of bowel cancer and hence they are used mostly for staging and follow up of bowel cancer patients. Some newer, non invasive, tests are available in a few centres such as CT colonography, but their routine use has significant financial constraints (Regge et al., 2009a; Regge et al., 2009b; Robertson et al., 2005).

The early detection and treatment of bowel cancer leads to a reduction in mortality and morbidity. To this end various screening tools have been evaluated. The use of faecal occult blood (FOB) testing as a screening tool has been investigated in randomised control trials. The Minnesota Colon Cancer Control Study assessed the efficacy of annual FOB against FOB testing every two years and a control group (Mandel et al., 1993). They showed a 33 percent decline in mortality associated with annual screening. In addition they also showed an increase in Dukes stage D cancers amongst the control group. In Nottingham, UK, a randomized control trial was undertaken between 1981 and 1991, to assess fecal occult blood testing as a screening tool for colorectal cancer (Hardcastle et al.,

1996). They demonstrated a 15 % reduction in mortality from colorectal cancer in the screened group. The authors' recommendations from the Nottingham study were for the establishment of a national screening program. The UK Department of Health subsequently instituted a UK Colorectal Cancer Screening Pilot study in 2000. The results from this pilot study were in keeping with improved survival and an overall cost benefit. This led to the development of a national bowel screening program, instituted in 2006 using FOB testing.

1.1.3 Treatment of Bowel Cancer

This is dependant on a multitude of factors. Stage of the disease and factors relating to the patients general health are crucial in deciding appropriate management. The decision to offer treatment for bowel cancer is the remit of "Multi Disciplinary Teams" which take into consideration various factors before deciding on various treatment options. Surgery is the mainstay of treatment for bowel cancer. In addition, adjuvant therapy in the form of chemotherapy and in cases of rectal cancer, chemo / radio therapy, are also offered to patients as these have been shown to improve survival. These adjuvant therapies may be given pre or post operatively. In advanced cases, surgery may not be possible and palliative chemo / radio therapy is all that can be offered. According to national guidelines (Association of Coloproctologists of Great Britain and Ireland – ACPGBI), the treatment of Dukes stage A cancers is limited to surgery alone. In cases of Dukes C and D, this is a combination of Surgery and chemo / radio therapy. It is in cases of Dukes B cancers in which there is great debate about which patient will benefit from adjuvant therapy (IMPACT B2, 1999). The decision to offer patients with Dukes B cancers adjuvant therapy is open to debate as there has been no trial which conclusively shows an increase in survival in patients with Dukes' B tumours who receive chemo / radiotherapy. The decision to offer such patients adjuvant therapy is usually based on local hospital policies and the decision of the MDT panel. Various prognostic factors have been studied but as yet there is no conclusive factor which could sway the decision in favour of or against giving patients with Dukes' B tumours adjuvant therapy (1999).

1.1.4 Prognostic Factors in Bowel Cancer

Prior to surgery, all patients have a clinical stage (cTNM) to their disease assigned. This stage is then confirmed and modified, if need be, with a definitive pathological stage (pTNM) of the disease based on histological findings. During histological assessment, other features of the tumour and associated host response are also looked at. It is these additional features which are taken into consideration when assigning a prognosis (related to survival) and determining predictive (related to likelihood of response to adjuvant therapy) values. Established histological features with a significant outcome on prognosis are:

1) Pathological stage of cancer
    a. TNM
    b. Dukes
2) Histological grade of cancer
    a. Well differentiated
    b. Moderately differentiated
    c. Poorly differentiated
3) Tumour type
    a. Adenocarcinoma
    b. Medullary carcinoma
    c. Mucinous (colloid) adenocarcinoma
    d. Signet-ring cell carcinoma
4) Vascular invasion

5) Resection margins
    a. Proximal
    b. Distal
    c. Circumferential resection margins

In addition to these well documented and established prognostic / predictive factors, there are other factors which have been reported in the literature but are

awaiting validation prior to being introduced into routine patient care. These factors are mostly independent of stage. The following is a list of some important prognostic / predictive factors (Cawkwell et al., 1999; Compton, 2003; Parc et al., 2004; Petersen et al., 2002):

1. **Perineural invasion (PNI)**

   Perineural invasion is defined as invasion into Auerbach's plexus (Fujita et al., 2007). The significance of this observation is still controversial, however, several reports have suggested that PNI adversely influences prognosis in patients with colorectal cancer (Burdy et al., 2001; Di Fabio et al., 2004; Huh et al., ; Ueno et al., 2001). PNI is also indicative of an infiltrating tumour border configuration, which is an adverse prognostic factor in its own right, as revealed by other studies (Compton, 2003).

2. **Tumour perforation**

   Transmural involvement of the colonic wall with free perforation of the tumour into the abdominal cavity is a very serious event and is classified as a T4b tumour by the AJCC, Version 6 (Greene and Sobin, 2002). This has been shown to adversely affect outcome and is a poor prognostic indicator (Compton, 2003; Fleming et al., 1997).

3. **Host immune response**

   The presence of lymphocytes in tumour and/or peri-tumoural tissue is indicative of an immunogenic response by the host. Certain studies consider this to a favorable prognostic factor (Compton et al., 2000a; Compton et al., 2000b). However, other studies have failed to reveal any association between host immunogenic response and prognosis. Proctor and Horgan et al (Horgan and McMillan, ; Proctor et al.) carried out an extensive study looking at inflammatory markers in patients with various cancers, including colorectal cancers. Their findings suggest an adverse outcome in patients in whom there is evidence of a strong host immune response.

4. **Total number of lymph nodes examined**

Assessment of regional lymph nodes forms part of the staging process used for colorectal cancer assessment and prognosis. This process is carried out using conventional Immunohistological techniques on lymph nodes identified macroscopically. It has been shown that many nodal metastasis occur in lymph nodes less than 5 mm in diameter and hence a thorough search for lymph nodes is essential (Herrera-Ornelas et al., 1987). The exact number of lymph nodes needed for accurate assessment is still uncertain, however, studies have shown that a minimum of 12 – 15 lymph nodes need to be examined to predict accurate regional lymph node negativity (Scott and Grace, 1989; Tepper et al., 2001). It is generally accepted that a minimum of 12 regional lymph nodes need to be harvested and examined for accurate staging.

1.1.5 Molecular Markers

In the last decade, significant research has been carried out in the field of colorectal cancer at a molecular and genetic level. During this time, many important molecular markers have been identified. The true prognostic value of these markers has been difficult to assess as there is a paucity of trials and other relevant clinical data. However, there are a few important molecular and genetic markers which could, in the near future, be used as prognostic markers in routine patient care. The following is a list of the more important of these markers with brief comments about them:

a. Microsatellite instability (MSI)

   About 5 – 7% of colorectal cancers are part of an inherited condition called Hereditary Non Polyposis Colorectal Cancer (HNPCC). This condition arises as a result of a defect in DNA mismatch repair (MMR) pathways secondary to mutation(s) in the genes controlling these pathways, usually *hMSH2* and *hMLH1* genes. Tumours with mutations in MMR genes exhibit a particular phenotype termed ‘microsatellite instability’ (MSI). Repeated DNA sequences, termed

microsatellites, can be replicated incorrectly. If not corrected by the MMR enzymes, this will result in abnormal lengthening or shortening of the DNA region. Characteristically, 29% of HNPCC tumours assessed show MSI termed as MSI-H (MSI-high). Patients with this have been shown to have a good prognosis but a higher than normal chance of developing another colorectal cancer in a short space of time. There is also some evidence to suggest that these tumours are not sensitive to routinely used chemotherapeutic agents for treating colorectal cancer. Testing for MSI is time consuming and expensive as it requires DNA extraction with subsequent Polymerase Chain Reaction. An indirect way to assess MSI in tumours has been proposed and tested to very good effect (Parc et al., 2004). This involves immunohistochemical analysis using antibodies against *hMSH2* and *hMLH1* genes. There are limitations to this alternate method of identifying patients with MSI such as, missing patients with MSI due to defects in genes for MMR other than the ones being tested for. Despite its limitations, patients with stage II colorectal cancer and MSI-H, have a very good prognosis and do not need adjuvant chemotherapy.

b. *DCC* / 18q LOH (Deleted in Colon Cancer / Chromosome 18q Loss of Heterozygosity)

   DCC is a putative tumour suppressor gene located on chromosome 18q21. Loss of Heterozygosity at this locus results in a poor prognosis with shortened disease free survival time. Patients with stage II colorectal cancer and exhibiting 18q LOH, may benefit from adjuvant chemotherapy, however appropriate trials are awaited.

c. CEA (Carcinoembryonic antigen)

   CEA was first identified in 1965 by Gold and Freedman, when they identified an antigen in fetal colons and colon adenocarcinoma, but absent from healthy adult colon. Hence its name carcino and embryonic antigen. CEA is a glycoprotein involved in cell adhesion

and belongs to the immunoglobulin superfamily. Its main use in colorectal cancer is for detection of recurrence after treatment of the primary tumour. It is also very useful in detection of metastatic spread of colorectal cancer to the liver (Berman et al., 2000; Pietra et al., 1998)

d. *c-myc*

Myc gene was first discovered in Burkitt's lymphoma patients. This gene was similar to myelocytomatosis viral oncogene (*v-Myc*) and hence it was called *c-Myc*. The protein product of this gene is a transcription factor capable of activating many genes (Gearhart et al., 2007). It can also act as a transcriptional repressor and has a direct role in DNA replication (Dominguez-Sola et al., 2007). Various mitogenic signals, like Wnt, Shh and EGF, are capable of activating *Myc* and result in numerous biological functions, such as cell proliferation, cell growth, apoptosis, differentiation and stem cell self renewal (Kanazawa et al., 2003; Taira et al., 1999). *c-Myc* has also been identified as a target in the APC pathway of colorectal cancer (He et al., 1998)

e. *K-ras*

KRAS is a protein encoded by the *K-ras* gene, located on chromosome 12 (McGrath et al., 1983; Popescu et al., 1985). KRAS protein is a GTPase and takes part in many signal transduction pathways. Mutated *K-ras* genes are potent oncogenes that play a significant role in many cancers, including colorectal cancer (Burmer and Loeb, 1989; Kranenburg, 2005). A K-ras mutation in colorectal cancer is also predictive of response to panitumumab and cetuximab therapy in colorectal cancer (Lievre et al., 2006).

1.2 Development and Structure of the Colon and Rectum

1.2.1 Embryological Development

The primitive gut is divided into three parts, foregut, midgut and hindgut. The adult colon and rectum develop from the embryonic hindgut and part of the midgut. In the embryo, the midgut extends from the intestinal portal and is, initially, in wide communication with the yolk sac. This communication subsequently narrows down and the mid gut becomes more tubular and elongated. It eventually develops into the remaining duodenum (the proximal part being derived from the foregut), jejunum, ileum, caecum, ascending colon and most of the transverse colon. The embryonic hindgut extends from the splenic flexure area of the colon up to the cloacal membrane (a more distal or caudal area where the ectoderm and endoderm are in direct opposition to each other).

The primitive embryonic gut is composed of cells derived from three layers:

a) The splanchnopleuric coelomic epithelium forms the outer visceral or serosal layer of the gut.

b) The endodermal epithelium forms the epithelium of the mucosa, the lining cells of adjoining ducts and the secretory cells of associated glands.

c) The splanchnopleuric mesenchyme gives rise to all intervening structures between the above two layers. These are outer connective tissue layers, muscularis externa and submucosal connective tissue layer (muscularis mucosae and lamina propria of the mucosa). In addition, blood vessels and lymphatics are also derived from this layer.

Innervation of the gut is via migrating neural crest cells which form the enteric plexuses. Lymphoid tissue gets incorporated into the gut by two processes; by assimilation of cells within the epithelial layer of the mucosa and by colonization of the submucosa by individual germinal centers of lymphoid follicles (Gray's Anatomy, 1995)

1.2.2 Anatomical Layers

The colon commences at the caecum and includes the ascending, transverse, descending and sigmoid colon. The rectum continues after the sigmoid colon to end at the anal canal. The architecture of the colon and rectum is fairly uniform throughout. The rectum tends to have a thicker and more uniform muscularis externa whereas in the colon, the outer longitudinal part of muscularis externa is condensed into three discreet muscle strips, called “taenia coli” that run the full length of the colon and are responsible for giving it a haustrated appearance. Broadly speaking, the colon and rectum are composed of three layers; mucosa, muscularis externa and serosa (Figure 1.6).

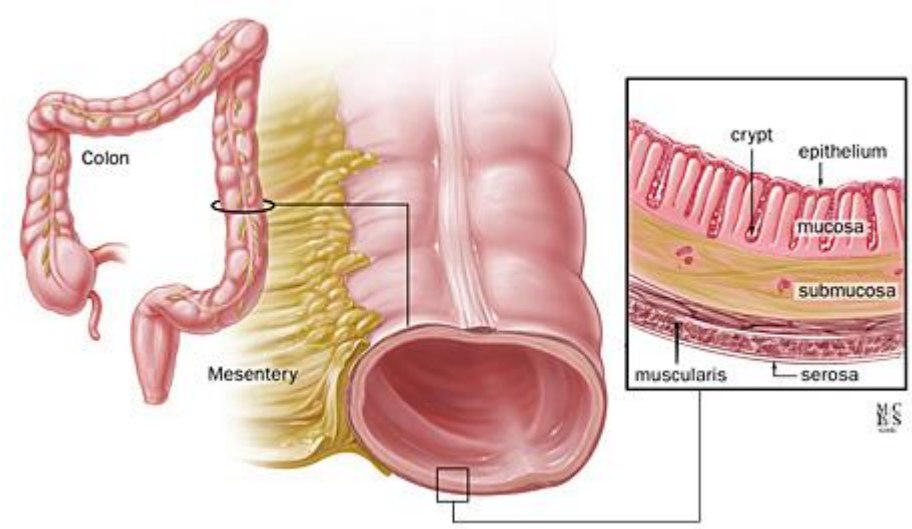


Figure 1.6
Tissue layers of the colon with cut-away detailing various layers of the colon
Source: www.hopkins-gi.org

1.2.2.1 Mucosa

This forms the innermost layer of the colon and rectum. This, one cell thick epithelial layer is composed of columnar cells, mucous secreting cells (goblet cells) and microfold cells, at its luminal surface. The luminal surface is devoid of

any villi in contrast to the small intestine. Numerous pit-like intestinal crypts are present throughout the colon and rectum. These intestinal crypts are the functional units of the colon and rectum. They contain entero-endocrine and stem cells (at their bases) in addition to the other cell types mentioned.

Lamina propria is made of connective tissue and gives support to the overlying epithelium. Solitary lymphoid follicles are also found in this layer.

Muscularis mucosa forms a layer of smooth muscle below the mucosa. Occasional slips of muscle are also noted running in between individual glands.

The *sub mucosa* underlies the mucosa and consists of loose connective tissue within which are found blood vessels, nerves and lymphatics.

1.2.2.2 Muscularis externa

This forms the muscular coat of the colon and rectum. It is composed of two layers, an outer longitudinal layer and an inner circular layer. The outer longitudinal layer is concentrated into three discrete bands of muscle in the colon but completely invests the rectum. In the colon these longitudinal bands of muscle are called the taeniae coli.

1.2.2.3 Serosa

This layer is also known as the visceral peritoneum. It covers the colon and rectum to a variable extent. The transverse colon and sigmoid are completely invested in this layer while the ascending and descending colon and the upper rectum are only partially covered by this layer.

1.2.3 Physiological Function

The colon and rectum act primarily as a reservoir for undigested food particles prior to their evacuation from the anus. Inside this large reservoir, numerous bacteria are living in symbiosis with the human host. Bacterial action on the food residue helps in fermentation with subsequent digestion of food and release of

nutrients, especially minerals. In addition, fermentation of dietary fiber has a potential protective role against colorectal carcinogenesis. The exact underlying mechanisms are not clear though, however, fermentation of fiber leads to the formation of short chain fatty acids (SCFA). Butyrate, a SCFA, is one such by product of the fermentation process and has been shown to be protective against colorectal cancer in some studies (Lupton, 2004). The exact biological mechanism(s) responsible for this protective role of butyrate in colorectal cancer are unknown at present; however, recent studies have shown butyrate influencing the methylation of promoter sequences in the Mis Match Repair (MMR) genes (Dronamraju et al., 2008).

The colon and rectum are also well equipped to absorb water back into the circulation. This process allows the waste products to become more solid and compacted. Most of the residue from the small bowel is liquid but by the time it leaves the human body, it is more or less well formed.

In addition, the large bowel produces copious amounts of mucus which help in lubricating the luminal surface of the mucosal layer and also mucus at the bases of intestinal crypts prevents noxious substances from coming into contact with sensitive cells at these locations. This has very important beneficial effects as harmful or carcinogenic products are prevented from causing damage at the sensitive basal regions of these intestinal glands. The columnar cells lining the mucosa have microvilli at their apical (luminal) surfaces which contain secretory granule containing immunoglobulins of the IgA variety (Guyton 1991).

1.2.4 The Colon Crypt as a Functional Unit and its Genetic Regulation

The mucosal lining of the colon and rectum is characterized by a million pit-like invaginations termed intestinal glands (Figure 1.7 and 1.8). These intestinal glands form the main functional units of the colon and rectum. Colorectal cancer is thought to arise from these intestinal glands, after they undergo mutation. Each crypt or gland contains roughly 2000 cells which are renewed, on average, every

82 hours. The height of each crypt has been determined to be about 82 cells in height but this value is dependant on location in the colon or rectum and has been claimed as 40 in other studies. The total number of cells forming the circumference of each crypt has been estimated to be in the region of 41 cells (Potten et al., 1992; Twombly, 2002).

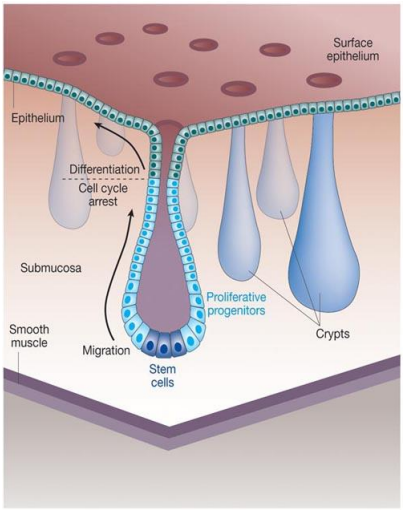


Figure 1.7

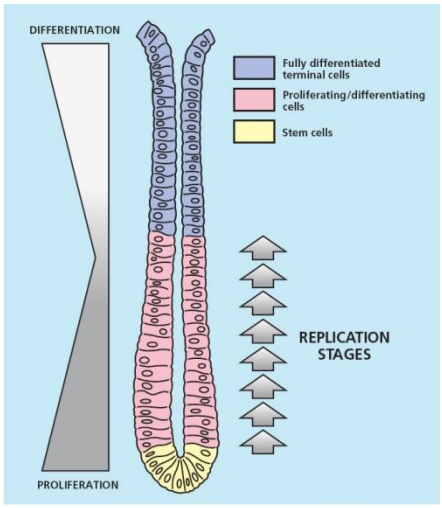


Figure 1.8

Diagrammatic representation of crypts in the colon / rectum illustrating various zones of the crypt in profile, including the stem cell niche area, the proliferative compartment and the differentiated zones, respectively. Also illustrated is the direction of cell migration.

Adapted from (Twombly, 2002)

The crypt can be divided into three zones; stem cell niche area, proliferative zone and differentiated zone. At the base of each crypt is the stem cell niche ranging from 4 to 12 cells. Studies using radiolabelling techniques and other putative stem cell markers have shown cells in the basal region of the crypt with properties in keeping with stem cells (Potten, 1998; Radtke et al., 2006). Cells above this stem cell niche area undergo rapid proliferation and move along the crypt axis towards the lumen. Maximum proliferative activity is noted in the lower third of the crypt (above the stem cell niche area) and gradually diminishes as these rapidly proliferating cells undergo differentiation towards the middle half of the crypt. Cells

above the proliferating zone are differentiated and move towards the lumen of the large bowel (Figure 1.8). At the luminal surface, these differentiated cells spread out like a sheet. Differentiated cells at the luminal surface undergo apoptosis and are shed into the lumen of the large bowel.

Homeostatic control of all three compartments in the crypt is highly complex and involves multiple signaling pathways. These pathways are also implicated in the embryological development of the gut (de Santa Barbara et al., 2003). A few of the more important and better studied pathways are: WNT, Hedgehog, SOX, Notch and Bone Morphogenetic Protein (BMP), signaling pathways. The WNT pathway is considered highly significant in maintaining homeostasis in the crypt (Bienz and Clevers, 2000; Pinto et al., 2003) and is discussed in greater detail below (Figure 1.9).

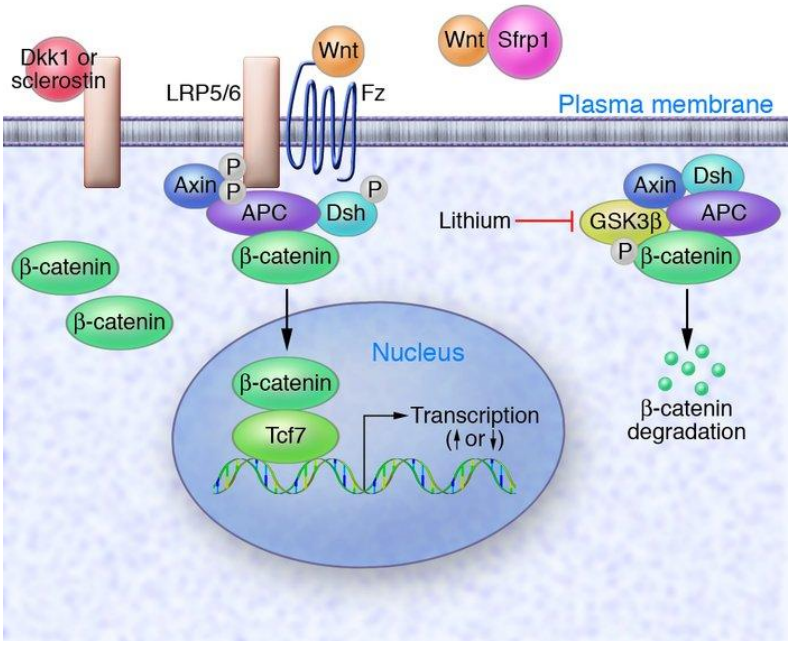


Figure 1.9

A view of the canonical Wnt signalling pathway.

Wnts bind a receptor complex consisting of LRP5 or LRP6 and one of ten Fz proteins. This prevents phosphorylation of β-catenin by GSK3β and other kinases and its subsequent degradation. Stabilized β-catenin accumulates and translocates to the nucleus, where it interacts with Tcf7 and related transcription factors (Lef1, Tcf7L1, Tcf7L2) to regulate gene expression. Outside the cell, molecules that sequester either LRP5 (e.g., Dkk1 and sclerostin) or the Wnt ligand (e.g., Sfrp) negatively control the canonical Wnt signalling pathway.

Wnt genes encode for extracellular signaling molecules called Wnt factors. These are secreted glycoproteins responsible for numerous developmental processes including development and homeostasis in the gut. The source of these secreted Wnt factors is not clear but they are thought to originate at the base of intestinal crypts or underlying mesenchymal tissue (Batlle et al., 2002). Although *Wnt* genes are all included in one family, two distinct signaling pathways can be defined: the Wnt / *β*-catenin / Tcf 4 signalling pathway (also called as the canonical Wnt signaling pathway) and the Wnt / Ca^{2+} signaling pathway. Wnt signals act on cells by signal transduction pathways which ultimately act on gene expression programmes (Peifer, 2002). In the absence of Wnt stimulation, β-catenin is phosphorylated by two

serine / threonine kinases: casein kinase I (CKI) phosphoryates β-catenin at Ser-45, this in turn allows glycogen synthase kinase 3 β (GSK 3 β) to phosphorylate the remaining regulatory sites at Trr-41, Ser-37 and Ser-33 (Polakis, 1997). Phosphorylation of β-catenin takes place in a multiprotein complex made up of APC (Adenomatous Polyposis Coli), Axin (a scaffold protein) and Diversin (the ankyrin repeat protein which helps in recruiting CKI). Phosphorylation of β-catenin results in ubiquitination by the β-TrCP–E3-ligase complex and subsequent degradation by the proteosome (Figure1.9). Stimulation of the canonical Wnt / β-catenin pathway occurs when Wnt glycoproteins bind to the frizzled (Fz) seven-span transmembrane receptors together with the low-density lipoprotein receptor-related proteins 5 and 6 (LRP5/6) single-span transmembrane co-receptors, members of the low-density lipoprotein receptor related family (Pinto and Clevers, 2005b). Intracellularly, degradation of β-catenin is prevented by inhibition of phosphorylation, resulting in its stabilization. In the presence of Wnt factors, Dishevelled (Dsh) prevents degradation of β-catenin by recruiting GBP/Frat-1, which displaces GSK3β from Axin. This prevents Axin from performing its role as a scaffold protein and leads to stabilization of β-catenin in the cytosol from where it translocates to the nucleus (Figure 1.9). Translocation of β-catenin into the nucleus is a poorly understood process. However, in the nucleus, β-catenin

interacts with transcription factors of the T-cell factor / lymphoid enhancing factor (TCF/LEF) family, namely Tcf-4, and forms complexes. This in turn results in transactivation of transcription genes like *c-MYC* and *cyclin D1,* with subsequent loss of its repression on the cell cycle inhibitor $p21^{CIP1/WAF1}$and allows cells to enter S phase from G1. Thus nuclear accumulation of β-catenin is seen as a hallmark of active Wnt signaling. In the absence of Wnt signaling, β-catenin is exported from the nucleus by APC and subsequently degraded (Bienz, 2002). Nuclear accumulation of β-catenin decreases as one moves up along the crypt. This forms a crucial switch between proliferation and differentiation along the crypt axis (van de Wetering et al., 2002).

Any mutational event which inactivates APC or activates β-catenin has the effect of rendering the canonical Wnt signaling pathway permanently on and can lead to tumourigenesis in the colon. It has been shown that mutations in the *APC* gene are noted in about 85% of all colorectal cancers (Kinzler and Vogelstein, 1996). It has also been noted that in aberrant crypt foci (ACF), which are the earliest, benign precursors of colorectal cancer, there is nuclear accumulation of β-catenin (van de Wetering et al., 2002). It is thus widely believed that activation of β-catenin / TCF-4 is the earliest event responsible for initiation of colorectal cancer by allowing unrestricted proliferation of crypt cells.

1.3 Colorectal Carcinogenesis – A Multistep Phenomenon

The initiation, progression and spread of colorectal cancer has been studied extensively and proven to involve multiple stages. Each stage of colorectal carcinogenesis has a unique molecular / genetic signature accompanied by well defined histological features and properties. The adenoma – carcinoma sequence (Fearon and Vogelstein, 1990) sets out a framework of various molecular / genetic changes involved in colorectal carcinogenesis (Figure 1.10). Colorectal carcinogenesis can be divided into four distinct stages:

Aberrant crypt foci → Adenoma formation (Early, Intermediate and Late) → Carcinoma → Metastasis.

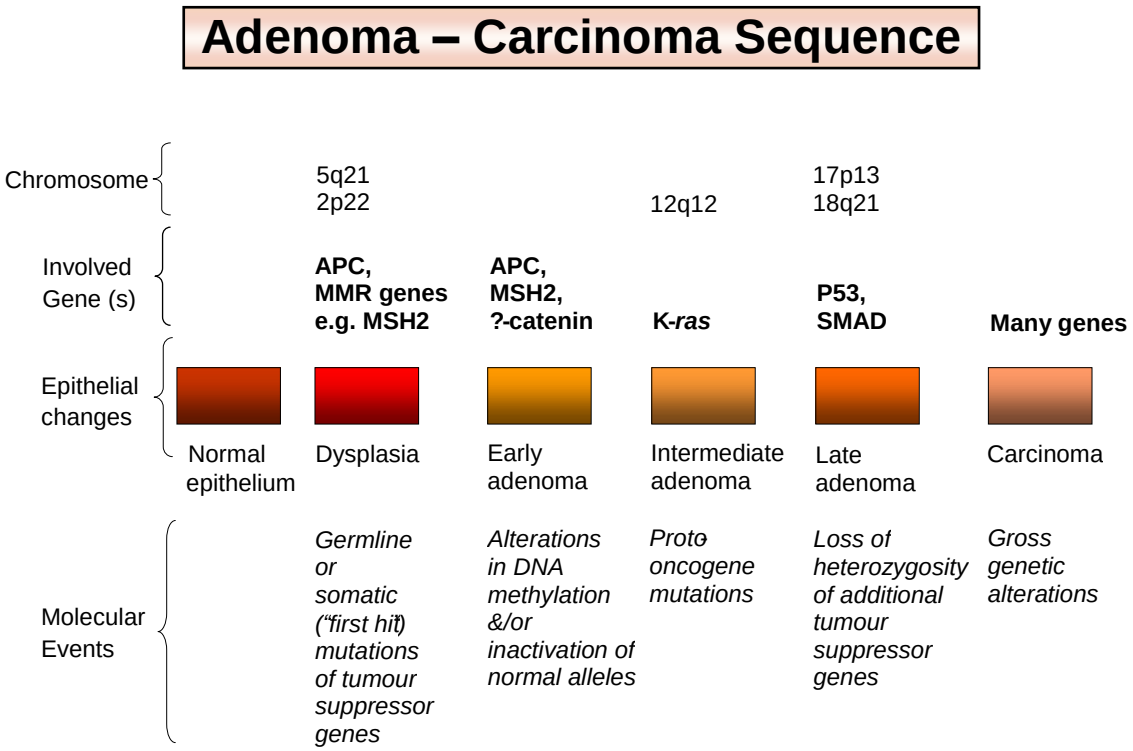


Figure 1.10
Adenoma – Carcinoma Sequence in Colorectal Carcinogenesis.

1.3.1 Aberrant Crypt Foci (ACF)

Aberrant crypt foci (ACF) are generally considered to be the early precursors towards cancer of the colon and rectum in terms of histological, immunological, biochemical, genetic and epigenetic changes seen in these lesions. ACF is the term used to describe abnormally large crypts, either single or multiple, with a thicker epithelial lining and a slit like or elliptical opening. These ACF were first described by Bird *et.al* (1987) in the colons of female mice treated with carcinogens. A year later it was put forward as a potential pre-neoplastic lesion in the colon of mice (McLellan and Bird, 1988). From there onwards further studies have shown that ACF, or at least a subset of ACF, might be the earliest precursors of colorectal cancer. An increase in the Immunohistochemical expression of carcinoembryonic antigen and β-catenin has been noted in ACF (Cheng and Lai, 2003; Pretlow et al., 1994; van de Wetering et al., 2002). Mutations in *APC*, K-*ras*

and P53 are well documented in colorectal carcinogenesis. Similar mutations have also been observed in ACF (Smith et al., 1994). It has been observed that not all ACF are pre destined to become cancerous. The histopathological classification as recognized by the WHO, divides ACF into two main groups, hyperplastic ACF and dysplastic ACF. In hyperplastic ACF, the crypts are elongated with occasional branching and a serrated opening but no layering of epithelium is seen. The nuclei are also enlarged and elongated but there is no nuclear atypia. Mucin depletion is not seen. Cells with positive expression for proliferation markers are usually located at the basal to mid regions of the crypts. On the other hand, ACF with dysplasia show different properties. In addition to elongated and enlarged crypts, there is depolarization of nuclei, depletion of mucin and layering of cells. Expression of proliferative markers were also noted to be higher up in the crypt (Cheng and Lai, 2003).

1.3.2 Early Adenomas – Role of *APC* (5q21)

Adenomata are recognized as pre neoplastic lesions in the colon and rectum. For descriptive purposes these adenomata are also referred to as polyps (which essentially is a descriptive term describing any elevation in the intestinal mucosa). However, not all polyps have the potential towards malignant transformation, e.g. hyperplastic polyps, inflammatory polyps and metaplastic polyps. Macroscopically, Adenomatous polyps are divided into three categories: tubular, tubulo-villous and villous, with increasing malignant potential from the tubular variety towards the villous type. The *APC* gene is located on chromosome 5q21 and encodes a 2,843-amino acid protein. B-catenin is its major downstream target. Mutations in the *APC* gene are usually truncating mutations primarily in the first half of the gene (Miyoshi et al., 1992). In early adenomas, the *APC* gene is either mutated or there is loss of 5q (*APC* gene). This gene is an essential component of the Wnt signaling pathway as discussed earlier. Loss of *APC* results in nuclear accumulation of β-catenin and a drive towards hyperproliferation. This hyperproliferation is noted in the upper third of the colon crypts in contrast to normal crypts where the proliferative

compartment is usually located in the lower two thirds. This results in an abnormal accumulation of cells at the mucosal surface leading to an early adenoma.

1.3.3 Intermediate Adenomas – Proto-oncogene mutations

Intermediate stage adenomas are characterized by mutations in proto-oncogenes. K-*ras* is a proto-oncogene and belongs to the *ras* gene family. They form an integral part of the intracellular signal transduction mechanism. Under normal conditions, K-*ras* is GTP bound and its activity is controlled by a GTPase which converts GTP to GDP, thus inactivating K-*ras.* Activating K-*ras* mutations result in decreased GTPase activity leading to a permanent state of K-*ras* activation with a consequent growth advantage conferred upon daughter cells (Navaratnam et al., 1999). These types of mutations are observed in 35% - 42% of colorectal cancers (Worthley et al., 2007) and are responsible for the further progression of an early adenoma towards an intermediate stage adenoma.

1.3.4 Late Adenomas – Mutations in tumour suppressor genes

The *p53* gene, a tumour suppressor gene, is mutated in 70% of colorectal cancers. This gene encodes for a protein that acts as an important transcription factor that binds to specific sequences of DNA and regulates expression of cell-cycle retarding genes. These include Bax and the BH3 only proteins puma and noxa, which in turn inactivate bcl-2 and bcl-xl, resulting in release of cytochrome c from mitochondria (Fridman and Lowe, 2003). This is important in cases of DNA damage as it allows DNA repair mechanisms time to correct defects / damaged DNA. Should the damage to DNA prove irreparable. *p53* induces pro apoptotic genes that trigger the apoptotic pathway (Figure 1.11). Mutations in *DCC, SMAD2* and *SMAD4* are observed in more advanced adenomas. *SMAD2* and *SMAD4* are involved in TGF-β signaling pathways and hence are involved in regulating growth

as well as apoptosis. All three genes are located at 18q21.1. Allelic loss at this site is observed in a majority of colorectal cancers. *DCC* is also involved in apoptosis. Presumably, mutations in these genes results in the accumulation of genetic defects in cells that would have triggered apoptosis under normal conditions. This in turn could result in malignant transformation of these affected cells. Mutations in *p53* (17p13), usually through allelic loss, is a late feature observed in advanced adenomas and colorectal cancers.

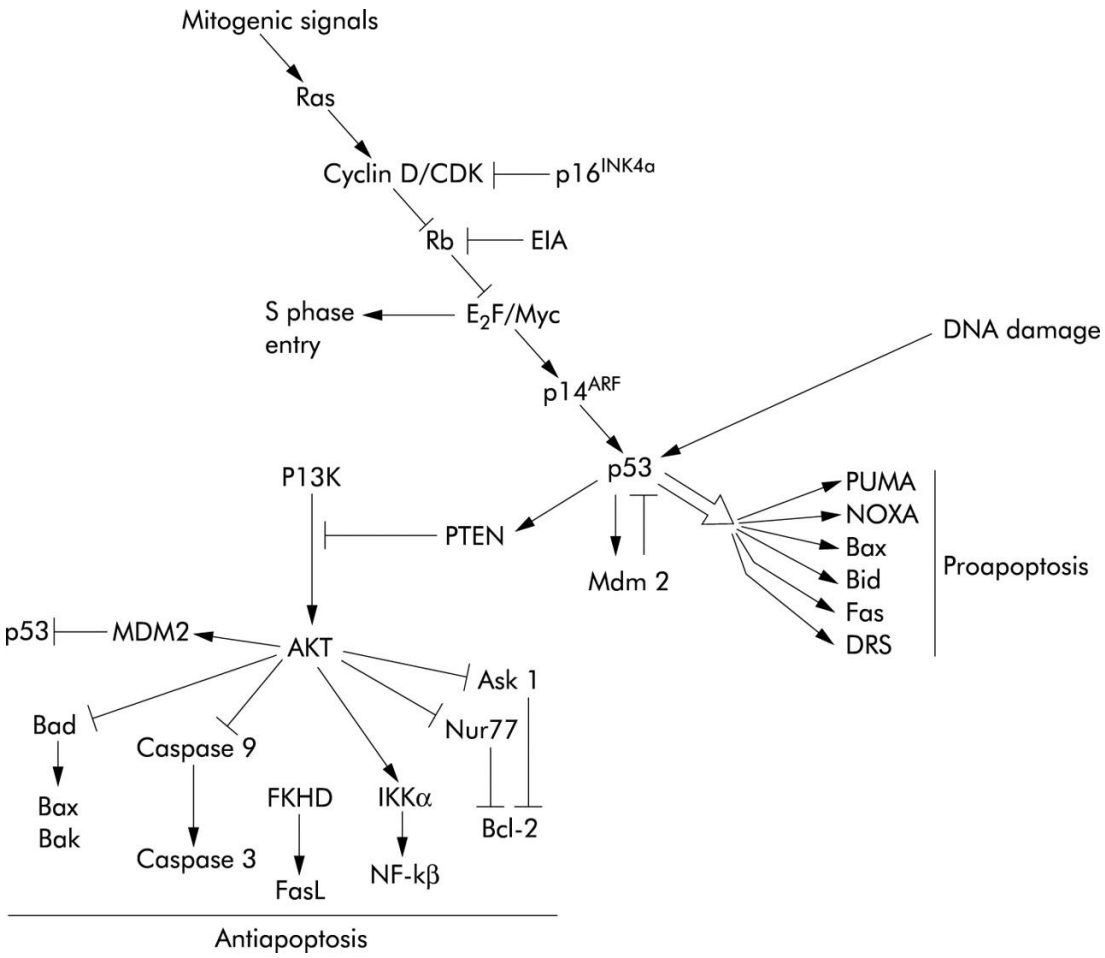


Figure 1.11

Diagram depicting the downstream effects of mitogenic signals in intermediate and late stage colorectal adenomas

Mitogenic signals (including APC mutations) cause activation of Ras. This inhibits the retinoblastoma (Rb) protein allowing E2F and Myc to promote cell cycle progression. p16INK4a is a tumour suppressor whose mutation permits cyclin D dependent kinase (CDK) to inhibit Rb. High levels of E2F or Myc activates p14ARF and thereby p53 which has multiple outputs. A series of proapoptotic bcl-2 family members are stimulated and via PTEN the antiapoptotic actions of AKT are inhibited.

Adapted from AJM Watson 2004

1.4 Sporadic and Inherited Bowel Cancer types

1.4.1 Sporadic Cancer

80% - 85% of colorectal cancers are sporadic where age is the most important determinant of risk (Figure 1.12). The remaining small percentage of CRC incidence is accounted for by patients whose risk is higher than the general population. These patients include those with dominantly inherited CRC syndromes, e.g. familial adenomatous polyposis (FAP), hereditary non-polyposis colorectal cancer (HNPCC). Other patient groups at risk include those with a personal history of CRC, ulcerative colitis (UC) and colonic Crohn's, primary sclerosing cholangitis (PSC) or a liver transplant (LT), and those with familial CRC defined as "the presence of one first degree relative who is affected by CRC aged <45 years or two affected first degree relatives".

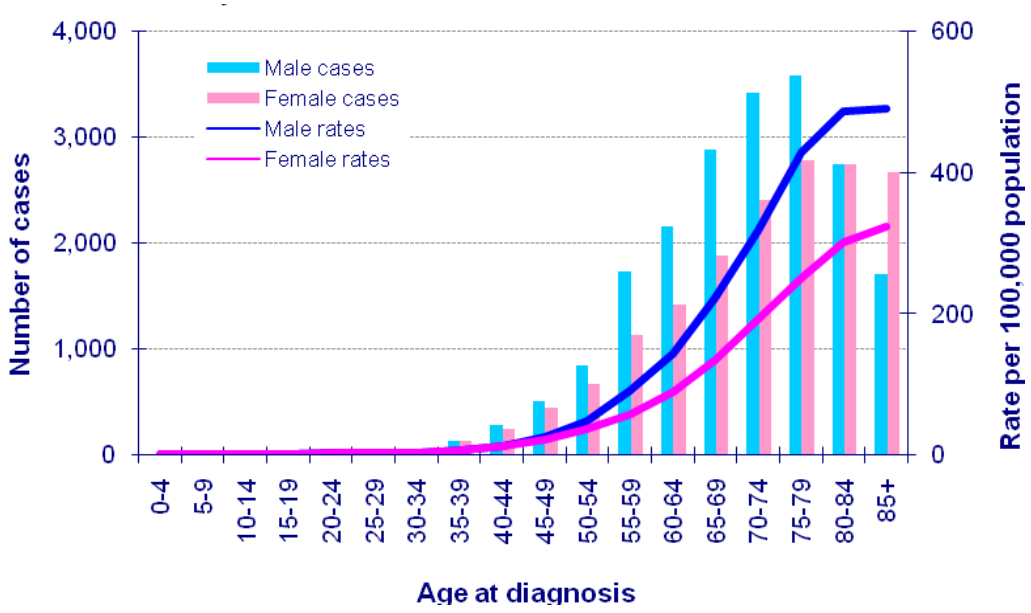


Figure 1.12

Numbers of new cases and age-specific incidence rates, by sex of bowel cancer.

Source: ***http://info.cancerresearchuk.org/cancerstats/types/bowel/incidence/***

The adenoma – carcinoma sequence as proposed by Fearon and Vogelstein (Fearon and Vogelstein, 1990) is based on the accumulation of genetic events which confer a growth advantage to a clonal population of cells with a resultant shift from adenomas to invasive carcinomas. In this model a number of key regulatory oncogenes and tumour suppressor genes acquire mutations which results in either activation or loss of function of theses key genes, as discussed earlier. This in turn drives normal colonic epithelium towards cancer. In the early stages of sporadic colorectal cancer development, somatic mutations are required in both *APC* alleles. For both *APC* alleles to undergo mutation a longer period of time, with appropriate exposure to various risk factors, including carcinogenic stimuli, is required and hence sporadic colorectal cancer is usually a disease affecting an older population group.

1.4.2 Inherited bowel cancer syndromes

1.4.2.1 Familial Adenomatosis Polyposis (FAP)

Around 1% of colorectal cancers are due to FAP. This is an autosomal dominant condition in which one *APC* allele is inherited with a germline mutation. The prevalence of this condition is estimated to be 1 in 8000. Affected individuals are heterozygous for the mutated gene and have a 50% chance of passing it on to their offspring. Males and females are equally affected. These mutations are mostly between codons 169 and 1393 (Spirio et al., 1993). In cases with this condition the incidence of colorectal cancer is disproportionately high. Prior to adenomas developing, the other, normal *APC* allele needs to undergo mutation. Clinically in these patients, their colons are studded with multiple polyps (Muller et al., 2000). These cases usually develop colorectal cancer early in adult life and have a very poor prognosis unless a prophylactic colectomy is undertaken. They need regular surveillance and appropriate surgical intervention when required. In addition to colonic polyps, these patients may also manifest with extra-colonic features such as polyps affecting the upper gastrointestinal tract, osteomas, epidermoid cysts, desmoids formation, congenital hypertrophy of retinal pigment

and other malignant changes such as small bowel cancer, thyroid tumours, hepatoblastomas and brain tumours. Patients with these extra-colonic features are also classified under Gardner's syndrome.

1.4.2.2 Hereditary Non-Polyposis Colorectal Cancer (HNPCC)

HNPCC is an autosomal dominant condition and, unlike FAP, most patients develop a solitary colorectal tumour. HNPCC accounts for about 3% to 5% of all colorectal cancer cases and is also known as Lynch syndrome. HNPCC is caused by inactivating mutations of one of several DNA mismatch repair genes. Patients with HNPCC have an estimated 80% lifetime risk of developing colorectal cancer (Vasen et al., 1996) at an average age of 44years as compared to 64 years for sporadic cancers. These patients are also prone to having synchronous and metachronus colorectal cancers as well as other extra-colonic malignancies such as endometrial, stomach, small bowel, renal, ureteric, ovarian, brain and biliary tree cancers. HNPCC may be diagnosed clinically using the modified Amsterdam criteria:

Modified Amsterdam Criteria:

- There should be at least three relatives with an HNPCC-associated cancer (colorectal cancer, endometrial, small bowel, ureter or renal pelvis malignancy).
- One affected relative should be a first-degree relative of the other two.
- At least two successive generations should be affected.
- At least one malignancy should be diagnosed before age 50 years.
- FAP should be excluded in the colorectal cancer case(s).
- Tumours should be verified by pathological examination.

1.4.2.3 Other Colon Cancer Syndromes

Peutz-Jeghers syndrome (PJS) is an autosomal dominant condition which is associated with multiple gastrointestinal polyps, both hamartomatous and

adenomatous. The risk of developing cancer between the ages of 15 to 64 is 93%. The highest cumulative risk in these patients is for breast, colon, pancreatic, stomach and ovarian cancer.

Juvenile-Polyposis is an autosomal dominant condition associated with hamartomatous polyps throughout the gastrointestinal tract. The underlying defect in this condition, in 15% to 20% cases, is due to a germline mutation in the *MADH4* gene, also known as *SMAD4/DPC4*, on chromosome 18q21(Howe et al., 1998). In 25% to 45% of cases, mutations in the gene-encoding bone morphogenic receptor 1A, residing on chromosome band 10q22, are also noted (Howe et al., 2001).

1.5 The Mammalian Nucleus

In the mammalian cell, the nucleus forms the largest intracellular organelle. Separating the nucleus from the cytoplasm is the nuclear membrane or envelope. The main function of this membrane is to separate the delicate nuclear transcriptional apparatus and genome from the cytoplasm. This envelope is studded with nuclear pore complexes (NPCs) which traverse the full thickness of the nuclear membrane and allows selective movement of various molecules across it (Franke et al., 1981; Gerace and Burke, 1988). The nucleus contains DNA, RNA and proteins and is responsible for transcription and replication of DNA. DNA is packaged into chromatin, which is arranged into loops of 30 -100 kbp, with anchorage of defined DNA sequences to the nuclear matrix (Vogelstein et al., 1980). These DNA sequences are known as matrix or scaffold attachment regions (MARs or SARs respectively).

1.5.1 The Nuclear Envelope and its Dynamic Function

The nuclear envelope consists of two layers enclosing a perinuclear space. Both layers are made of a bilipid layer and are studded with Nuclear Pore Complexes

(NPCs). The outer nuclear membrane (ONM) also incorporates ribosomes on its cytoplasmic surface and is continuous with rough and smooth endoplasmic reticulum (ER). It is functionally similar to the ER (Gerace and Burke, 1988). The inner nuclear membrane (INM), on the other hand, is devoid of ribosomes and is lined on its inner or nucleoplasmic surface by the nuclear lamina, composed of filamentous units known as lamins (Gerace and Blobel, 1980). Nuclear envelope transmembrane proteins (NETs) form another subset of nuclear envelope proteins. Both lamins and NETs are known to have many binding partners (Bengtsson and Wilson, 2004; Zastrow et al., 2004) (Figure 1.13).

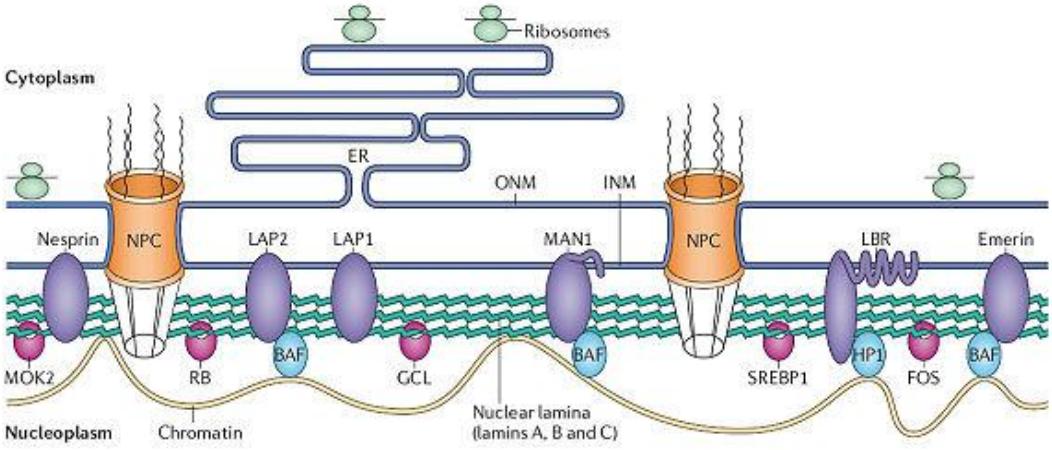


Figure 1.13

Schematic view of the nuclear envelope showing Nuclear Pore Complexes (NPC), organization of the nuclear lamina and their associated proteins (in purple): Lamina associated polypeptides 1 and 2 (LAP1 and 2), Lamin B receptor (LBR), Emerin, Nesprin and MAN1; and transcription fators (in pink): Retinoblastoma transcriptional regulator (RB), Germ Cell Less (GCL), Sterol response element and binding protein 1 (SREBP1), MOK2 and FOS. Barrier to Autointegration factor (BAF) is a chromatin associated protein that also binds to lamins. *Adapted from Coutinhoe et.al 2009*

The importance of these associated binding proteins is significant and, in the form of tissue related complexes, they may be responsible for the observed phenotype in various Laminopathies. To explain nuclear envelope (NE) disease pathology, three mechanisms are suggested – altered gene expression, as regulated from the nuclear periphery; mechanical disruption of lamina-cytoskeletal interactions leading to mechanical instability and disruption of the cell cycle/stem cell

maintenance (Dorner et al., 2007; Malik et al., 2010; Schirmer et al., 2003). Because gene regulation and cytoskeleton connections have both been implicated, this could easily mean that NE proteins could reside either in the inner or outer nuclear membrane. NE proteins have been identified in both inner and outer nuclear membranes. Integral membrane proteins of the ONM are shared by the ER whilst those of the INM consist of lamina-associated polypeptides (LAPs) and lamin B receptors (LBRs) (Foisner and Gerace, 1993; Georgatos et al., 1994; Gerace and Foisner, 1994).

The perinuclear space is an aqueous environment with functions similar to the rough ER. This space also harbours the luminal domains of the integral membrane proteins from the ONM and INM, with the possibility of them communicating with each other. The NPCs are complex structures with relative molecular masses of over 100 mDa (Reichelt et al., 1990) and are composed of many proteins collectively known as nucleoporins (Davis and Blobel, 1986). These NPCs are linked by pore-connecting fibrils and by the nuclear lamina lying close to the nucleoplasmic surface of the INM. These NPCs allow the exchange of molecules into and out of the nucleus via either passive diffusion or active transport.

1.5.2 The Cell Cycle

The cell cycle is characterized by four sequential phases: G_1, S, G_2 and M phases. G_1, S and G_2 are collectively termed *interphase.* The phase of mitosis or M phase is responsible for the creation of two genetically identical daughter cells. During mitosis, there is a dynamic sequence of events which can be subdivided into prophase, prometaphase, metaphase, anaphase, teleophase and cytokinesis (Figure 1.14 and 1.5).

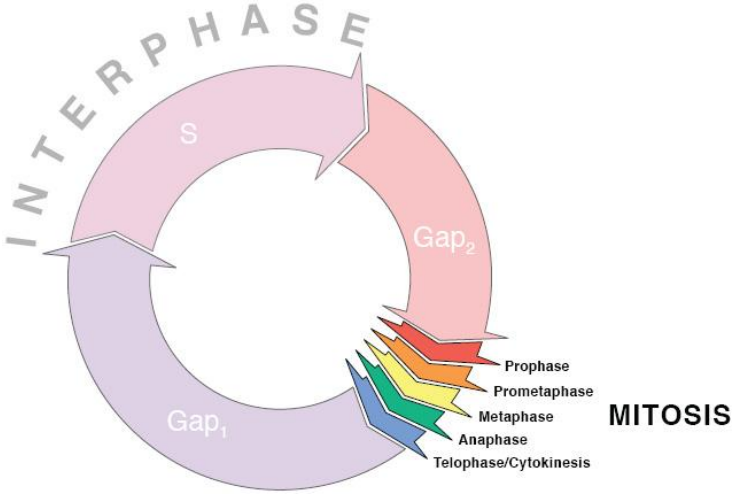


Figure 1.14
The cell cycle

Diagram to illustrate the sequential phases involved in the cell cycle. Phases Gap1, S and Gap2 are collectively known as interphase. The phase of Mitosis is further subdivided into Prophase, Prometaphase, Metaphase, Anaphase and Telophase / Cytokinesis.

Adapted from: http://publications.nigms.nih.gov/insidethecell

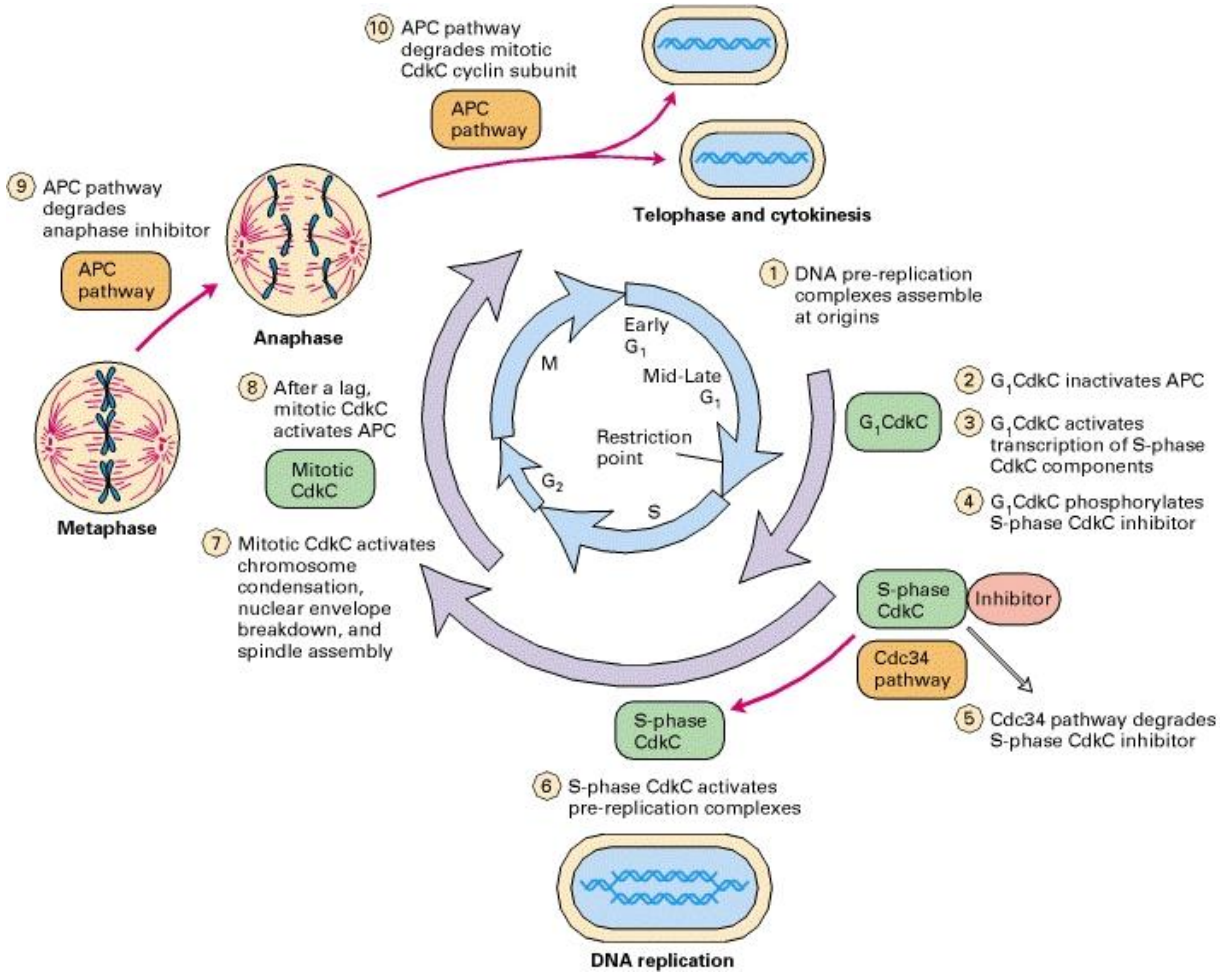


Figure 1.15

Current model for regulation of the eukaryotic cell cycle

Passage through the cycle is controlled by G_1, S-phase, and mitotic cyclin-dependent kinase complexes (CdkCs) highlighted in green. These are composed of a regulatory cyclin subunit and a catalytic cyclin-dependent kinase subunit. Protein complexes (orange) in the Cdc34 pathway and APC pathway polyubiquitinate specific substrates including the S-phase inhibitor, anaphase inhibitor, and mitotic cyclins, marking these substrates for degradation by proteasomes. These pathways thus drive the cycle in one direction because of the irreversibility of protein degradation. Proteolysis of anaphase inhibitors inactivates the protein complexes that connect sister chromatids at metaphase (not shown), thereby initiating anaphase.

Adapted from Molecular Cell Biology; 4th Edn

At prometaphase there is an abrupt breakdown of the nuclear envelope, which includes the nuclear membranes, nuclear lamina and nuclear pore complexes. Reassembly of various components of the nuclear envelope, takes place in a very well coordinated manner towards late anaphase / telophase. It is during prophase that dynein, a minus-end directed microtubule associated motor protein, translocates to the mitotic spindle. This spindle in turn is cross-linked to invaginated areas of the nuclear membrane via its centrosomes. This results in physical disruption of the lamina and nuclear membrane by metaphase (Salina et al., 2002). The nuclear lamina plays an important role in the breakdown and reassembly of the nuclear envelope. This role is dependant upon the phosphorylation status of nuclear lamina (Ottaviano and Gerace, 1985). Upon phosphorylation, the nuclear lamina depolymerises and is dispersed throughout the cytoplasm as soluble dimers (Gerace and Blobel, 1980). This process of nuclear lamina phosphorylation is regulated by M-phase promoting factor (MPF) (Murray and Kirschner, 1989) which is a $p34^{cdc2}$ kinase / cyclin B complex activated early in mitosis (Dessev et al., 1991). Studies have shown that both lamin A and B types become soluble upon depolymerisation (Beaudouin et al., 2002; Daigle et al., 2001)

During telophase each set of daughter chromosomes arrive at the poles of the spindle and decondense. At this stage the nuclear envelope reforms around each set of chromosomes and effectively marks the end of mitosis. Cytokinesis then occurs and each daughter cell then enters into the G_1 phase of the cell cycle. Reformation of the nuclear envelope is accompanied by dephosphorylation of individual lamin components with their subsequent polymerization and recruitment to the inner nuclear membrane, where they form a filamentous meshwork. B-type lamins are thought to be the first nuclear lamins to get incorporated into this polymeric meshwork at the inner nuclear membrane (Moir et al., 2000a). Farnesylation of the cysteine residue in the C-terminal isoprenylation signal, the CAAX motif, and subsequent endoproteolytic cleavage of the AAX tripeptide and carboxymethylation of the terminal cysteine residue, help in allowing anchorage to the inner nuclear membrane. Interaction between integral proteins of the inner nuclear membrane and nuclear lamins is also facilitated (Farnsworth et al., 1989; Firmbach-Kraft and Stick, 1993). The nuclear membrane A-kinase anchoring

protein, AKAP149, targets chromatin-bound protein phosphatase 1 (PP1) to the NE upon nuclear reformation in vitro, this correlates with assembly of B-type lamins into the nuclear lamina (Steen and Collas, 2001).

The exact time at which B-type lamins start accumulating at the inner nuclear membrane is not certain however it is thought to be towards the end of late anaphase / telophase (Daigle et al., 2001). Broers *et al* (1999) have shown that A-type lamins do not migrate to the nuclear periphery until the end of Cytokinesis. Soluble A-type lamins have also been noted in the nucleoplasm of cells for a few hours into G_1 phase of the cell cycle (Moir et al., 2000b). Absence of A-type lamins does not appear to have a significant bearing on formation of the nuclear envelope as evidenced by their absence during the earliest stages of nuclear envelope assembly. However, A-type lamins are very important for the proper functioning of the nucleus. This is obvious in diseases wherein there is a mutation of either the *LMNA* gene or in the lamin A/C protein. In essence then, B-type lamins form a meshwork of fibers adjacent to the inner nuclear membrane upon which, subsequently, A-type lamins get incorporated (Moir et al., 2000a).

1.5.2 The Nuclear Lamina

Intermediate filament proteins are composed of 10 – 13 nm diameter filaments found in the cytoplasm and nucleus. These intermediate filament proteins form cytoskeletal fibers in metazoan cells. In the nucleus, they form a filamentous meshwork lining the inner nuclear membrane and are responsible for providing anchorage sites for chromosomes and nuclear pores. Lamins belong to the intermediate filament protein family group and are principal components of the nuclear lamina. The nuclear lamina is composed of type V intermediate filament proteins (Fisher et al., 1986; McKeon et al., 1986) (Figure 1.16).

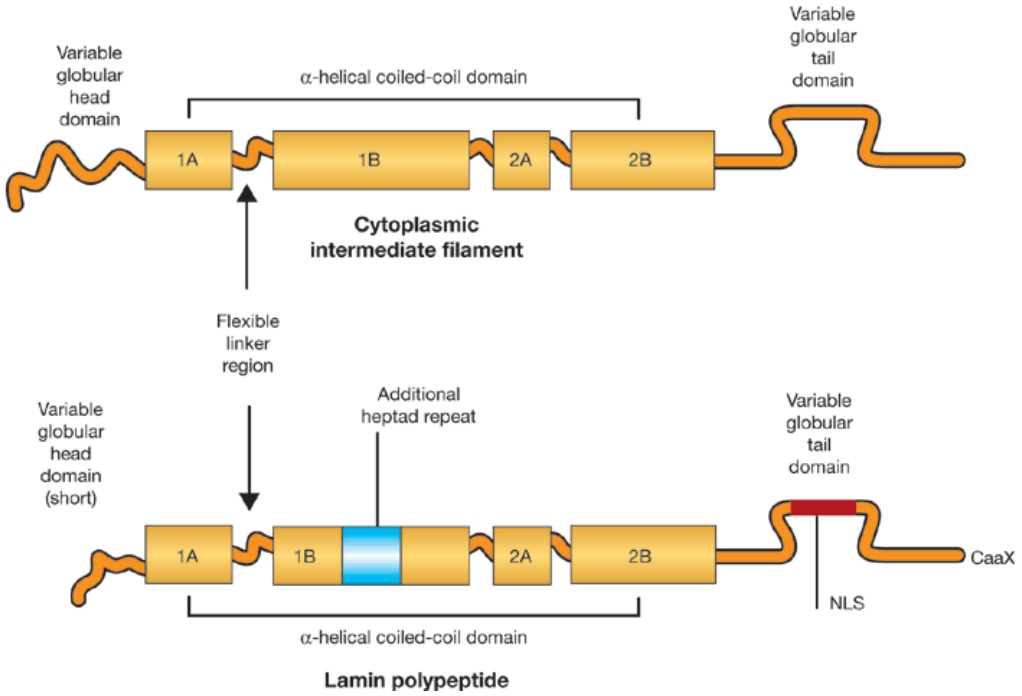


Figure 1.16

Diagrammatic illustration of a cytoplasmic intermediate filament and lamin polypeptide.

Source: Adapted from Hutchison *et al* 2004

Individual polypeptides of intermediate filaments consist of elongated molecules, 48 nm in length, with an extended central α-helical domain. These monomers form a parallel coiled coil with other monomers. These dimers then pair with other dimers in an anti parallel fashion to form staggered tetramers. This represents the soluble subunit of intermediate filaments. These tetramers then stack up in a lateral fashion to form the filament. Each filament is composed of eight parallel protofilaments. This forms a rope like structure with a cross section revealing 32 individual α-helical coils and measuring 10 – 13 nm in diameter. This arrangement allows the filaments to bend easily but makes it very difficult for them to break (Alberts, 2002). The secondary structure of intermediate filaments is composed of a central coiled – coil α-helical rod domain. A non helical amino-terminal forms the head domain while, a non helical, carboxy terminal forms the tail domain. The rod domain is divided into four helical regions, 1A, 1B, 2A and 2B. These are characterized by heptad repeats with three non-helical linker segments separating

them (Fuchs and Weber, 1994). Nuclear lamins, in contrast to cytoplasmic intermediate filaments, contain a 42 amino acid (six heptad repeat) insertion into coil 1B (Erber et al., 1998). Most lamins, except lamin C, have a C-terminal CAAX motif. This is a target for isoprenylation and carboxymethylation This is of importance in helping lamins to localize at the inner nuclear membrane (Nigg, 1992). Also, lamins have a nuclear localization sequence (NLS) in their tail domain (Loewinger and McKeon, 1988) which helps in directing lamins to the nucleus where they line the nucleoplasmic surface of the inner nuclear membrane and form a two dimensional lattice

1.5.2.1 Lamin structure and filament assembly

Up to seven lamin subtypes have been identified in mammals. These can be divided into two subgroups, A and B-type lamins, based on various characteristics such as ultrastructural properties, biochemical properties and mitotic fate (Gerace and Blobel, 1980; Gerace and Burke, 1988; Gerace et al., 1984). A-type lamins are expressed in most differentiated cell types whereas B-type lamins are essential for cell viability and are expressed in virtually all cells. The *LMNA* gene (Figure 1.17), located on chromosome 1q21.1 – 21.3 (Lin and Worman, 1993) encodes for lamins A, AΔ10 and C, which are all alternatively spliced variants of that gene (Fisher et al., 1986; Furukawa et al., 1994; Machiels et al., 1996; McKeon et al., 1986). B-type lamins consist of lamins B1, B2 and B3. The gene encoding laminB1 is *LMNB1,* located on chromosome 5q23.3 – 31.1 (Lin and Worman, 1995) lamins B2 andB3 are alternatively spliced variants of the *LMNB2* gene, located on chromosome 19p13.3 (Biamonti et al., 1992). Lamins C2 and B3 have only been identified in male germ cells (Furukawa and Hotta, 1993).

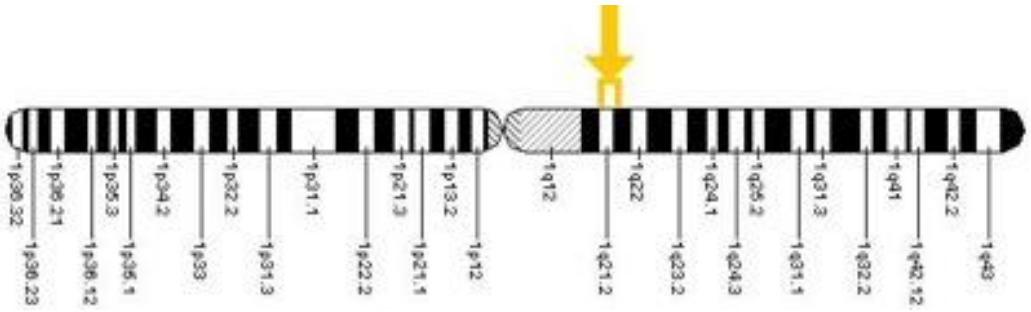


Figure 1.17

Diagrammatic representation of chromosome 1. The LMNA gene is found at location 1q21.2, as marked by the yellow arrow.

Adapted from www.progeriafamilycircle.com

Gene	**LMNA**
Gene Location	**1q22**
Number of Exons	**12**
Alternative splice variants	**Isoform A; Isoform C and isoform ADelta10**
Protein name	**Pre lamin A/C which is cleaved into Lamin A/C**
Protein sequence length	**664 AA**
Protein mass (Da)	**74,139**

Table 1.4

Table summarizing various properties of the LMNA gene and its isoforms

The *LMNA* gene (Table 1.4) contains 12 exons. In exon 10 is located codon 566, which acts as an alternative splice site for prelamin A and lamin C. this gives rise to the 3' – most amino acid common to both lamin A and C mRNAs (Lin and Worman, 1993). Codons 567 to 572 are lamin C specific. The amino acid encoded for by Codon 572 marks the termination of lamin C. In the case of prelamin A, the remaining nucleotides of exons 11 and 12 adjoin the 3' – end of codon 566, thus making these exons lamin A specific. Lamin AΔ10 lacks all 30 amino acids (90 nucleotides) encoded by exon 10 (Machiels et al., 1996), however, it retains the

lamin A specific tail domain. Hence, lamin A subtypes only differ in the sequence of their tail domains (Figure 1.18).

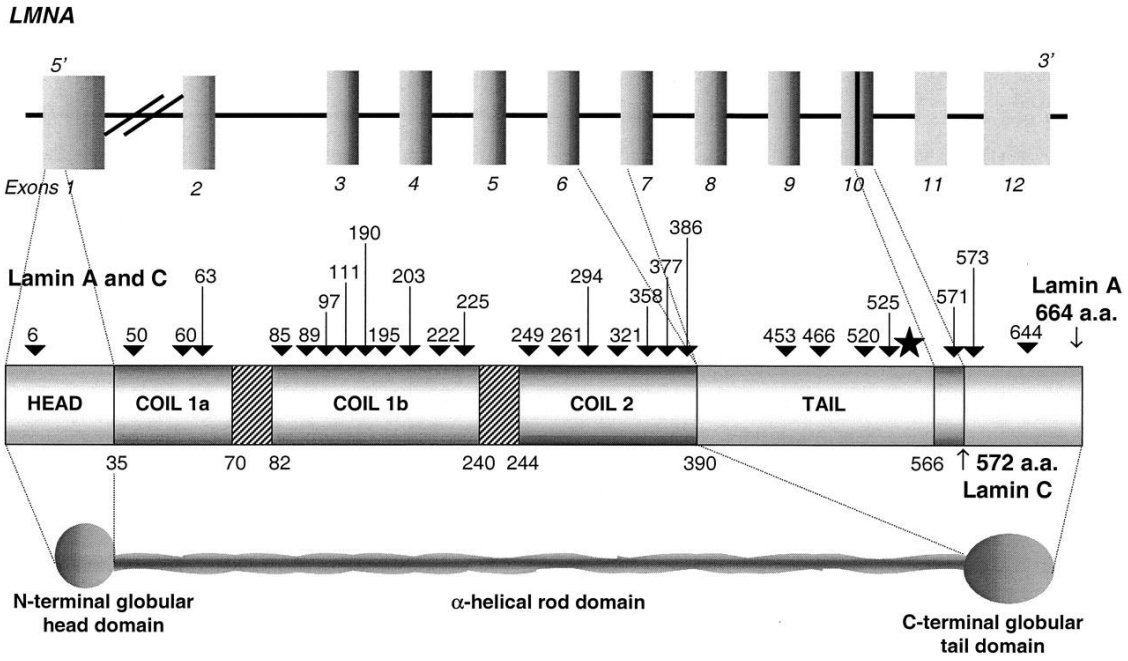


Figure 1.18

Structure of lamin A/C gene *LMNA* and of the lamin A/C protein

Source: Adapted from Taylor, M. R. G. et al. J Am Coll Cardiol 2003

Nuclear lamina assembly is a sequential process in which, initially, parallel, un-staggered dimers form (Heitlinger et al., 1992). These then associate head to tail forming tetramers via highly conserved domains and non-helical amino-terminal head domains (Heitlinger et al., 1992). In contrast to intermediate filaments, lamin tetramers elongate longitudinally to form 2 nm wide protofilaments prior to associating laterally. Lateral growth of these protofilaments are believed to represent half staggered, anti parallel associations of lamin head to tail protofilaments (Goldberg et al., 1999). This gives rise to the observed two dimensional lattice arrangement of 10 nm wide filaments with an average crossover spacing of 52 nm (Aebi et al., 1986).

Hutchison and co workers proposed a model of lamin A and C integration into the nuclear lamina (Hutchison et al., 2001). In their model lamin A and C get incorporated into the nuclear lamina after B-type lamins associate with the inner

nuclear membrane. As lamin C does not possess an isoprenylation signal, it was proposed that its incorporation into the nuclear lamina occurs on the back of lamin A. A-type lamins form anti parallel, half staggered tetramers which associate with B-type lamin tetramers already lying subjacent to the inner nuclear membrane. Vaughan *et al* (Vaughan et al., 2001) have also reported the importance of lamin A in localisation of emerin and lamin C to the nuclear envelope.

1.5.2.2 Lamins and chromatin organization

Nuclear lamins play an important role in DNA replication (Jenkins et al., 1993; Jenkins et al., 1995; Newport et al., 1990). Prior to having any role in DNA replication, nuclear lamins need to interact with the inner nuclear membrane and bind chromatin. Goldberg *et al*. (1995) provided the first direct evidence for the role of nuclear lamins in DNA replication. By the addition of purified lamin B3 to depleted Xenopus nuclear assembly systems, they were able to reinitiate DNA replication. B-type lamins also play an important role in the organisation of interphase chromatin. This is born out by the observation of lamin B1's specific interaction with peripheral matrix attachment regions (MARs) of DNA (Luderus, 1992 #1563). Binding of chromatin by A-type lamins has also been demonstrated (Glass, 1993 #537;Glass, 1990 #1564;Shoeman, 1990 #1565;Taniura, 1995 #1101). By the application of various biochemical studies it has been shown that different regions of the lamin A tail domain interact with chromatin. Hoger *et al*. (1991) discovered the region between amino acid residues 598-641 of lamin A, as being an area of potential chromatin interaction. Similarly, Taniura *et al*. (1995) identifies the region between amino acid residues 394-430 of lamin A as chromatin binding sites.

At present, the expression of A-type lamins is only associated with differentiated cell types. This fact, coupled with the observation that A-type lamins have a greater affinity for binding chromatin as compared to B-type lamins (Hoger et al., 1991; Yuan et al., 1991), has led to the suggestion that chromatin organisation is partly regulated by A-type lamins. Lending further support to this suggestion is the observation that A-type lamins play an important role in the early events of cell

differentiation (Collard et al., 1992; Lourim and Lin, 1992) and in conjunction with lamin C, binds with the growth suppressor $p110^{RB}$ (Mancini et al., 1994; Ozaki et al., 1994).

The association of heterochromatin with lamin A inside the nucleus takes the form of distinct foci (Bridger et al., 1993), thus giving rise to the proposition that lamin A polymerises preferentially on heterochromatin. As A-type lamins relocate to the nuclear envelope, this complex of heterochromatin makes close contact with the nuclear envelope, thereby allowing it to anchor to the nuclear periphery. It is interesting to note that in some cells undergoing rapid proliferation in healthy tissue, A-type lamins are not expressed. Differential expression of A-type lamins is also noted in some cancers (Broers and Ramaekers, 1994; Willis et al., 2008; Worman and Courvalin, 2002).

1.5.2.3 Lamins and Transcription regulation

The mechanisms by which lamins influence transcription regulation are diverse. This is a reflection of the different ways in which lamins interact with transcriptional processes. Lamins do not always bind to transcriptional factors or their regulatory complexes, but act through indirect association with transcription binding partners. The nuclear envelope is known to harbour a large number of transcription factors, mostly repressors. Retinoblastoma protein (Rb) is an important transcription factor and is known to actively repress transcription factor E2F-dependant gene transcription by actively binding to E2F and recruiting histone deacetylases. In its active form, Rb is hypophosphorylated and forms complexes with lamin A/C and lamina associated polypeptide 2 alpha (LAP2α) at the nuclear periphery, during G1 phase of the cell cycle (Hutchison and Worman, 2004; Mancini et al., 1994; Markiewicz et al., 2002; Ozaki et al., 1994). This tethering of Rb at the nuclear periphery is crucial for its normal function and any mutation that disrupts this tethering, results in aberrant function, including development of cancer (Hinds et al., 1992). There is direct evidence now showing the importance of this interaction between A-type lamins and Rb in preventing the degradation of Rb protein by the

proteosome (Johnson et al., 2004), thereby allowing it to function normally in its regulation of cell growth and division (Figure 1.19).

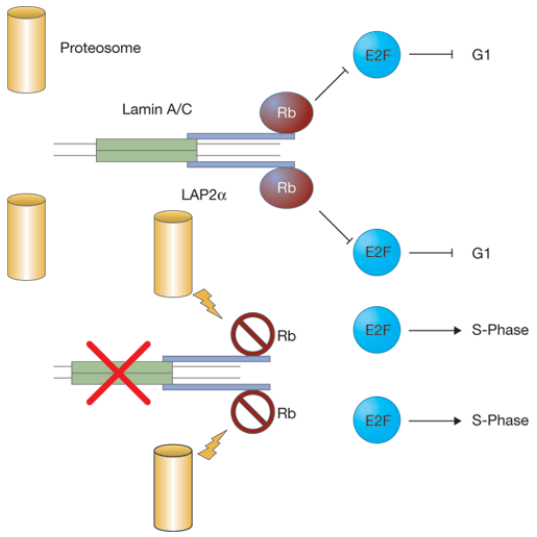


Figure 1.19

Role of lamin A/C and LAP2α in prevention of Rb degradation by the proteosome.

Lamin-A–LAP2α complexes are required for Rb function. A complex of lamin A/C (green) and LAP2α (blue) tethers Rb (red) and thereby prevents its destruction by the proteosome (yellow). In the presence of lamin A/C and LAP2α and the absence of Rb phosphorylation, E2F activity is inhibited and cells remain in G1. When lamin-A/C–LAP2α function is disrupted, Rb is targeted for destruction by the proteosome, thereby de-repressing E2F and allowing cell-cycle progression.

Source: Adapted from Hutchison *et al*. 2004

At the inner nuclear membrane are also located the LEM domain proteins (LAP2 or lamina associated protein 2, Emerin and MAN1) which are the main lamin interacting proteins. These LEM domain proteins share a common 43 amino acid sequence in their N terminus (Cohen et al., 2001), which bind to DNA-binding protein BAF (Barrier to Autointegration Factor) (Furukawa, 1999; Lin et al., 2000). BAF is a highly conserved, small protein, with diffuse nuclear localisation in interphase cells, which localises to chromosomes during mitosis. There are

frequent but transient interactions between BAF and LEM domain proteins. The intermolecular bridging properties of BAF cause compaction of DNA resulting in a higher order of chromosomal structure (Zheng et al., 2000). Hence, various interactions between LEM domain proteins, lamins and BAF can also affect chromatin structure and lead to differences in functional outcomes. Octamer-binding transcription factor (Oct-1) co-localises with B-type lamins at the nuclear envelope and actively represses the ageing associated collagenase gene (Imai et al., 1997). In ageing cells, there is translocation of Oct-1 from the nuclear envelope with loss of transcriptional silencing and increased collagenase activity. Collagenase, an extracellular matrix degrading enzyme involved in tissue repair and remodelling, has been implicated in many tissue degenerative diseases.

LAP2β (Lamina Associated Polypeptide 2 Beta) has also been shown to interact with chromatin via BAF and its LEM domain (Furukawa, 1999). LAP2β also interacts with the transcription factor GCL (Germ-Cell-Less) protein, which localises at the inner nuclear membrane, and is bound to E2F/DP heterodimer. Together they form a repressor complex at the nuclear envelope against the transcriptional activity of E2F/DP3, which is normally under strict control by the Rb protein in G1 phase of the cell cycle (Nili et al., 2001).

Emerin, an integral membrane protein, is known to bind both lamin A/C and BAF (Lee et al., 2001). This interaction results in a functional link to chromatin. Localisation of emerin to the Nuclear envelope is highly dependant on lamin A polymers (Vaughan et al., 2001), the deficiency of which results in emerin being translocated to the cytoplasm. The C-terminal domain of emerin is also known to bind with GCL. This interaction with GCL is disrupted when BAF interacts with the N-terminal LEM domain of emerin (Holaska et al., 2003).

1.5.2.4 Lamins and Apoptosis

Control on the number of cells in a complex, multicellular organism, such as humans, is a very intricate process that requires not only control on the rate of cell division but also on the rate of cell death – programmed cell destruction or

apoptosis. Cells undergoing apoptosis show characteristic features such as, shrinkage and condensation of the cell with collapse of the cytoskeleton followed by nuclear envelope disassembly and fragmentation of DNA. This is then followed by active membrane blebbing and a controlled breakage of the cell into membrane enclosed vesicles, called apoptotic bodies. Of interest is the observation that these apoptotic bodies express surface makers that act as signals for rapid phagocytosis by macrophages or neighbouring cells. The whole process of apoptosis is known to be energy dependant and irreversible once initiated. Central to the whole process of apoptosis is a family of proteases that harbour a cysteine at their active site and cleave target proteins at specific aspartic acids (Alberts, 2002), hence the term caspases is used to describe them. Caspases are synthesised inside the cell, as pro-caspases, which are inactive and require activation by cleavage at their aspartic acid site, usually by other caspases. Nuclear lamins are early targets for caspases. PKCδ, activated by caspases 3, is responsible for hyperphosphorylation of lamins. This allows caspases access to lamins prior to their cleavage (Cross et al., 2000). Caspase 6 cleaves lamins at their α-helical rod domain at position 230 (Lazebnik et al., 1995; Rao et al., 1996), which is an aspartic acid residue. The rod domain in lamins happens to be the site for dimerisation and linking chromatin, hence, cleavage at this site represents an extremely effective mechanism for breaking down lamina and the subsequent release of chromatin. There are differences in the way A-type lamins are broken down as compared to B-type lamins. In case of B-type lamins, they are completely cleaved with the cleaved products closely associating to the Nuclear Envelope. A-type lamins, on the other hand, undergo partial cleavage and are more diffusely dispersed throughout the cytoplasm.

1.5.3 Laminopathies

Laminopathies is the term used to describe a group of genetically inherited disorders with tissue specific phenotypes as a result of mutations in the *LMNA* gene. They are subdivided on the basis of which tissue is affected and also encompass a group of pre-mature ageing syndromes.

1.5.3.1 Laminopathies affecting striated muscle

1.5.3.1.1 Limb girdle muscular dystrophy 1B (LGMD-1B)

This condition is inherited in an autosomal dominant fashion. The underlying defect is a mutation in the *LMNA* gene, linked to chromosome 1q21.1 – q21.3. The results of this mutation manifest themselves as proximal myopathy, affecting the lower limb muscles, during the persons late teen years. This slowly progresses towards the upper limb muscles by the time they are between 30 and 40 yrs of age. Premature death in these people is usually due to defects in their cardiological system.

1.5.3.1.2 Emery – Dreifuss muscular dystrophy (EDMD)

EDMD is an inherited condition characterized by a triad of clinical features: Contractures of tendons, especially at the elbow, Achilles tendon and posterior cervical muscles; cardiomyopathy, heart defects, particularly conduction defects in the heart; and weakness with wasting of skeletal muscles, especially humeral and peroneal muscles. EDMD can be inherited in three different ways: As autosomal dominant or autosomal recessive or X-linked. Both autosomal dominant and X-linked have similar clinical features as described earlier (Dreifuss and Hogan, 1961; Emery and Dreifuss, 1966). The autosomal forms of EDMD are as a result of mutations in the head, rod and tail domains of the *LMNA* gene, on chromosome 1q211 – q21.3 (Raffaele Di Barletta et al., 2000). X-linked EDMD manifests itself in the second or third decade of life as a result of a mutation at the locus mapped to chromosome region Xq28. This is the locus for the *EMD* gene (also called the *STA* gene) which encodes for a inner nuclear membrane protein, emerin (Bione et al., 1994). Emerin interacts with lamin A at the nuclear envelope and provides anchorage to lamin A (Cartegni et al., 1997). Mutations in emerin are widely distributed, however, the majority of these mutations are noted to be in the lamin A binding domain area (Lee et al., 2001). In normal tissue, emerin localizes to similar areas of the nuclear envelope as do A-type lamins. However, in persons with X-linked EDMD, there is a marked absence of emerin from skeletal and cardiac muscle (Nagano et al., 1996)

1.5.3.1.3 Dilated Cardiomyopathy – 1A (CMD-1A)

This condition is inherited in an autosomal dominant fashion. It is characterized by the onset of cardiac conduction defects in the third or fourth decade of life leading to heart failure as a primary cause of mortality. The underlying mutation is in the *LMNA* gene, predominantly in the coiled coil regions 1A and 1B of the rod domain (Brodsky et al., 2000; Fatkin et al., 1999). The phenotypic appearances of this mutation reveal varied skeletal muscular dystrophies.

1.5.3.2 Laminopathies affecting bone, nerve and adipose tissue

1.5.3.2.1 Familial Partial Lipodystrophy (FPLD) - Dunnigan type – laminopathy affecting adipose tissue

FPLD of the Dunnigan type is an autosomal dominant condition due to heterozygous missense mutations in the *LMNA* gene on 1q21 – q23. The locus of this mutation has been isolated to codon 482, in exon 8, which codes for the proximal C-terminal domain of lamin A/C. Clinical features manifest with the onset of puberty and include loss of fat from the limb and trunk, accumulation of fat on the back, groin, neck and face and a predisposition to insulin resistance with resultant complications manifesting as high blood pressure, glucose intolerance, dyslipidaemia, coronary heart disease and liver steatosis (Dunnigan et al., 1974; Vantyghem et al., 2004; Vigouroux et al., 2001).

1.5.3.2.2 Mandibuloacral dysplasia (MAD) – Laminopathy affecting bone

This condition is inherited in an autosomal recessive manner and thus its typical clinical features are only evident in homozygous individuals. These features include growth retardation in the post natal period, hypoplastic mandible with dental overcrowding, other cranio-facial malformations, progeria-like skeletal abnormalities, atrophic skin with mottled cutaneous pigmentation, hair loss and either type A or B lipodystrophy, accompanied by insulin resistance (Simha and Garg, 2002; Young et al., 1971). The underlying homozygous mutation responsible for MAD, R527H has been identified in the tail domain of lamin A/C in cases suffering from type A lipodystrophy. This mutation affects the normal

distribution and localization of lamin A/C at the nuclear envelope (Novelli et al., 2002).

1.5.3.2.3 Charcot-Marie-Tooth type 2B1 (CMT2B1) – Laminopathy affecting neural tissue

This autosomal recessive disease affects motor and sensory nerves, usually in the second decade of life. It can be divided in two groups: CMT type 1 is the demyelinating form of the disease while CMT type 2 affects axons predominantly and can be subdivided into CMT 2 subtype 1 and CMT 2 subtype 2. Persons with CMT 2 suffer from decreased nerve conduction velocity resulting in weakness and wasting of distal lower limb muscles and, in some cases, also proximal muscles. In families with the CMT2B1 disorder, the genetic defect has been isolated to chromosome 1q21, an area that harbours the *LMNA* gene (Bouhouche et al., 1999). Subsequently a homozygous mutation was discovered in the rod domain of lamin A/C in persons with CMT2B1. The genetic mutation giving rise to CMT2B2 has been identified on chromosome 19q13.3 (Leal et al., 2001).

1.5.3.3 Laminopathies and premature ageing syndromes

1.5.3.3.1 Hutchinson – Guildford Progeria Syndrome (HGPS)

HGPS, a premature ageing syndrome, is a rare condition. Since its discovery in 1886, more than 100 cases have been reported worldwide. Its mode of inheritance is more likely to be autosomal dominant, however, autosomal recessive cases are also known. The underlying mutation in this condition involves the *LMNA* gene. Persons suffering from this condition appear to age ten times faster as compared to normal individuals. Clinical features of HGPS include, severe restriction of growth in the post-natal period, microganthia, hypoplasia of the mid face region, alopecia, wrinkled skin secondary to loss of subcutaneous fat, generalized osteodysplasia, hypogonadism, premature atherosclerosis and poor muscle development. The average life expectancy in persons with HGPS is 12 – 15 years with congestive cardiac failure, secondary to advanced atherosclerosis, being a common cause of death. Interestingly, persons with HGPS do not show other

features associated with the ageing process i.e. susceptibility to tumour formation, diabetes, cataract formation, cerebral degeneration leading to cognitive impairment, etc and hence, HGPS is thought to reproduce only partially the ageing process (Martin and Oshima, 2000). De Sandre-Giovannoli *et al* (2003) have reported a single base substitution from C to T in the *LMNA* gene, affecting codon 608 within exon 11, in a subset of persons suffering with HGPS. This does not result in an amino acid change; however, it partially activates a cryptic splice site with deletion of 50 amino acids at the C-terminal end of lamin A. This results in loss of an endoproteolytic cleavage site as well as loss of a cell-cycle dependant phosphorylation site (Ser625). Both these sites play an important role in the post-translational modification of pre-lamin A into lamin A. Presence of this incompletely processed form of prelamin A at the nuclear envelope acts as a dominant mutant (Eriksson et al., 2003). Studies on fibroblasts obtained from persons with HGPS having a G608G mutation, have revealed defects in nuclear structure, for example, thickening of the nuclear lamina, nuclear lobulations and loss of peripheral heterochromatin (Goldman et al., 2004).

1.5.3.3.2 A-typical Werner Syndrome

Werner syndrome is another premature ageing syndrome characterized by atherosclerosis, diabetes, premature graying of hair, subcutaneous calcifications, aged facial appearances, early-onset cataracts, scleroderma like skin changes and early death (McKusick, 1963). Werner syndrome is inherited as an autosomal recessive condition and is due to homozygous mutations in the DNA helicase-like gene *RECQL2*, a 3' – 5' RecQ DNA helicase – exonuclease, responsible for unwinding and cleaving nucleotides from DNA termini (Wang et al., 2000). Atypical Werner syndrome has been identified in a subgroup of patients and represents a more severe form. Persons with atypical Werner syndrome were found to have normal *RECQL2* but were heterozygous for mutations in *LMNA* (Chen et al., 2003). Chen *et al* (2003) noted changes in nuclear morphology and mislocalised lamins in fibroblasts of a person with atypical Werner syndrome due to mutation L140R in the *LMNA* gene.

1.5.3.4 Laminopathies and Fetal Disease

1.5.3.4.1 Restrictive Dermopathy (Fetal Akinesia Deformation Sequence-FADS)

Restrictive dermopathy was first described by Witt *et al* in 1986 (Witt et al., 1986). This is a fatal condition in which tautness of the skin causes fetal akinesia or hypokinesia deformation sequence (FADS). Other manifestations of the condition include a thin, tightly adherent, translucent skin with prominent vessels, generalized joint contractures, typical facial changes (small mouth, pinched nose and microganthia), enlarged fontanels, respiratory insufficiency due to pulmonary hypoplasia, clavicular dysplasia and an enlarged placenta with a short umbilical cord. These abnormalities usually appear after 22 – 24 weeks gestation and are associated with polyhydramnios and reduced fetal movement. Premature delivery usually takes place at around 31 weeks gestation. Live born children usually die within the first week of life (Lenz and Meschede, 1993; Mau et al., 1997; Verloes et al., 1992). These phenotypic features are similar to those observed in Hutchinson-Gilford syndrome, a progeria due to mutations in *LMNA* (De Sandre-Giovannoli, 2003 #1783;Eriksson, 2003 #51). Work done by Navarro *et al* in patients with restrictive dermopathy (Navarro et al., 2005; Navarro et al., 2004) identified truncating splice mutations in *LMNA* exon 11, leading to the production of truncated Lamin A precursors. They also identified a single heterozygous insertion mutation in *ZMPSTE24* exon 9 in another group of patients with restrictive dermopathy not exhibiting mutations in *LMNA*. Mature Lamin A isoforms are processed through a series of post-translational modifications performed on the pre-lamin A precursor. The enzyme responsible for these post-translational modifications is a zinc metalloproteinase ZMPSTE24, for which lamin A is the only known substrate in mammals (Corrigan et al., 2005; Sinensky et al., 1994). Mutations in this enzyme and *LMNA* result in loss of mature lamin A and an increase in the amount of unprocessed, full length lamin A precursors. These defects are associated with phenotypes that show features similar to those observed in Hutchinson-Gilford progeria syndrome (Mounkes and Stewart, 2004; Navarro et al., 2005; Novelli and D'Apice, 2003).

1.5.4 Lamins and Cancer

The differential expression of lamins in cancers of epithelial, mesenchymal and lymphoid tissue has been studied by various researchers and include acute lymhoblastic leukemia, non-Hodgkin's lymphoma (Stadelmann et al., 1990), human small cell lung cancer, non-small cell lung cancer (Broers et al., 1993), (Kaufmann et al., 1991), metastatic leiomyosarcoma, rhabdomyosarcoma, chondrosarcoma (Cance et al., 1992) and basal cell cancer of the skin (Tilli et al., 2003; Venables et al., 2001). There have been varied reports of lamin A/C expression in some cancers. For example, in colorectal cancer, Moss *et al*. (1999) describes a decreased level of lamin A/C expression in colonic adenomas and adenocarcinomas as compared to normal tissue. On the other hand, a study by Canc *et al*. (1992) showed a heterogeneous expression of lamin A/C in two colon adenocarcinomas. These varied results hint towards a greater role of lamins in cancers than was previously believed.

1.6 Stem cells

The human body is made up of many different tissues, many of which are subject to rapid proliferation with a continuous turnover of cells. The human large intestine is a prime example. In this organ, the lifespan of a mature, well differentiated cell can be measured in days. Despite this rapid turnover of cells, the large intestine maintains its unique architecture and mass over time through a tightly regulated process of renovation. Under physiological conditions, this process is sustained by a small minority of cells with extraordinary features, known as stem cells. Stem cells can be defined by three main properties:

1. **Differentiation** – the ability to give rise to a heterogeneous progeny of cells, which progressively diversify and specialize according to hierarchical processes, constantly replenishing the tissue of short lived, mature elements.

2. **Self renewal** – the ability to form new stem cells with an identical and intact potential for proliferation, expansion and differentiation, thereby maintaining the stem cell pool.
3. **Homeostatic control** – the ability to modulate and balance differentiation and self-renewal according to environmental stimuli and genetic constraints.

Individual stem cells are large, primitive cells that do not express markers of lineage differentiation, and are consequently difficult to define and characterize morphologically. Stem cells are typically located in a specialized mesenchymal 'niche' and maintain their capacity for limitless self-replication throughout the lifetime of their host, and can also divide to produce daughter cells, committed to the formation of every adult cell lineage within their tissue of origin (Brittan and Wright, 2004). The study of stem cells has its roots dating back to nearly 50 years or so and is probably best exemplified by the hematopoietic family of blood and immune cells. All the functional cells found in blood and lymph arise from a single common parent cell known as the hematopoietic stem cell (HSC), which resides in the bone marrow. The HSC pool of cells represents less than 0.01% of cells in the bone marrow. Yet, each of these rare cells gives rise to a larger, intermediately differentiated population of progenitor cells. These in turn divide and differentiate further through several stages into mature cells responsible for specific tasks. By the time a cell reaches its final functional stage, it has lost all ability to proliferate or to alter its destiny and is said to be terminally differentiated. Fundamentally, stem cells provide the foundations of every organ in the body, with their ability for self renewal by symmetrical division and their capacity to generate the entire adult cell component within their indigenous tissue via asymmetrical cell division. Stem cells regulate the rate of cell production in a tissue to maintain homeostasis, and can up-regulate cell turnover concurrent to increasing regenerative demand dictated by damage or disease.

A deeper understanding of the role stem cells play in homeostasis of the large intestine has also brought to light the significant link between stem cells and their possible role in colorectal carcinogenesis. At present our understanding about stem cells in the colon / rectum is limited since there are no definitive stem cell markers available that can positively identify these stem cells. However, two putative stem cell markers have been proposed: **Musashi-1** (Msi-1), a neural RNA

binding protein required for asymmetric division of sensory neural precursor cells, has been proposed by Potten et.al (Potten et al., 2003) as a marker of stem and early lineage progenitor cells in murine small intestine. The presence of Msi-1 in the human colon crypt cells was also reported by Nishimura et.al (Nishimura et al., 2003). The second stem cell marker proposed recently by Barker et.al (Barker et al., 2007) is the gene, **Lgr5** (leucine-rich-repeat-containing G-protein-coupled receptor 5). Using lineage-tracing experiments, they were able to show that Lgr-5 positive crypt base columnar cells were able to generate all epithelial lineages over a 60 day period. This was in keeping with observed stem cell characteristics.

In the adult human colon / rectum, stem cells are known to occupy the basal regions of the crypts (Nishimura et al., 2003; Potten, 1998; Potten et al., 2003) and vary in numbers but approximately 4 – 6 stem cells exist in this region. Factors involved in the maintenance of the stem cell niche are incompletely understood, however, two models have been proposed: the **Deterministic or Immortal model** claims that a small number of stem cells reside in a niche, each generating exactly one stem cell and one transit-amplifying cell at each asymmetrical division. Each stem cell is immortal in this model. Studies done by Potten et.al (Potten et al., 1992; Potten et al., 2002) have lent support to this model. They have shown that stem cells protect their DNA from replication errors by selective retention of the template DNA strand. On the other hand, in the **Stochastic** or **Niche model**, many stem cells exist in a niche, with each stem cell division producing either, two stem cells, or one stem cell and a daughter cell or no stem cells and only two daughter cells. Studies done by Yatabe et.al (Yatabe et al., 2001) and Kim et.al (Kim et al., 2005) have advocated support for the Stochastic model.

Various signaling pathways have been implicated in regulation of the crypt – villous axis and stem cell niche. These pathways include the Wnt pathway, the Bone Morphogenetic Protein pathway, the Cdx1 and Cdx2 pathway, the Forkhead Fkh6 signaling pathway, the Hedgehog genes(Shh and Ihh) signaling pathway, the Homeodomain Nkx2-3 pathway and the platelet-derived Growth factor-A (PDGF-A) / PDGFR-α pathway (Androutsellis-Theotokis et al., 2006; Berman et al., 2003; Chen et al., 2004; Gregorieff and Clevers, 2005; Hardwick et al., 2004; Katoh, 2007a; Katoh, 2007b; Katoh, 2007c; Katoh and Katoh, 2006b; Lees et al., 2005; Logan and Nusse, 2004; Pinto and Clevers, 2005a; Pinto and Clevers, 2005b; Reya and Clevers, 2005; van den Brink, 2007; van den Brink and Hardwick, 2006).

The most important pathway involved in homeostasis and colorectal carcinogenesis, has been the Wnt signaling pathway. It is interesting to note that up to date there has been no conclusive direct evidence linking the Wnt signaling pathway to stem cells, the reason(s) for this has been the paucity of definitive stem cell markers in the colon / rectum. All the evidence available is only supportive of a role for these signaling pathways in regulating stem cell function in the colon / rectum.

Finally, the role of stem cells in the pathogenesis of colorectal cancer has been open to debate and speculation. Aberrations in the Wnt signaling cascade, especially mutations in the APC gene, have been shown to lead to tumour formation in the colon (Gregorieff and Clevers, 2005; Logan and Nusse, 2004; Pinto and Clevers, 2005b). Also, there appears to be increasing evidence to suggest that human cancers can be considered a stem cell disease (Dalerba et al., 2007a; Jordan et al., 2006; Reya et al., 2001). According to this 'Cancer Stem Cell' (CSC) theory, tumours are not to be viewed as simple monoclonal expansions of transformed cells, but rather as complex tissues where growth is driven by small pool of pathologically transformed CSCs that, on the one hand, have acquired tumour-related features such as uncontrolled growth with metastatic potential, and, on the other hand, are able to self renew and differentiate into a phenotypically heterogeneous progeny. In support of this hypothesis, three key experimental observations (Al-Hajj et al., 2003; Bonnet and Dick, 1997; Singh et al., 2004) have been made: Firstly, only a minority of cancer cells within each tumour are capable of tumourigenic potential when transplanted into immune-deficient mice: Secondly, tumourigenic cancer cells have a distinct profile of surface markers and can be differentially and reproducibly isolated from non-tumourigenic ones by flow cytometry and thirdly, tumours grown from tumourigenic cells contain mixed populations of both tumourigenic and non-tumourigenic cancer cells, thereby re-creating the full phenotypic heterogeneity of the parent tumour (Dalerba et al., 2007b). At present, CSCs have been identified from several solid tumours such as head and neck cancers (Prince et al., 2007), brain tumours (Galli et al., 2004; Singh et al., 2004), breast cancer (Al-Hajj et al., 2003) and colorectal cancer (O'Brien et al., 2007; Ricci-Vitiani et al., 2007). Colorectal CSCs (Co-CSCs) are identified by expression of $CD133^{+}$ (Ricci-Vitiani et al., 2007) in these cells.

However, additional markers have been described such as CD44 and EpCAM (epithelial cell adhesion molecule) (Dalerba et al., 2007b). The implications of Co-CSCs in colorectal cancer are proving to have a profound impact on our current understanding of this disease process. The CSC model introduces an additional conceptual frame for the interpretation of intra-tumour heterogeneity observed in colorectal cancer. In addition, the CSC model might also help explain unexpected observations in colorectal cancer biology, such as the presence of heterogeneous patterns and non-constitutive mechanisms of telomerase activation within both primary and metastatic colorectal cancers (Dalerba et al., 2005). The observation that colorectal cancer is sustained by a minority subpopulation of CSCs could assist in the development of more effective prognostic indicators and treatment regimens specifically targeting these CSCs.

1.7 Thesis aims

A-type lamins are implicated in various tissue specific disorders such as autosomal inherited EDMD, MAD, etc. Variable expression of A-type lamins has also been observed in a broad range of malignancies affecting epithelial, mesenchymal and lymphoid tissues. Recently, a retrospective study looking at the variable expression of A-type lamins in colorectal cancers has shown an adverse prognosis in patients with colorectal cancers expressing this protein. The underlying biological processes responsible for this adverse outcome in patients with colorectal cancers expressing A-type lamins are still unknown. A-type lamins have been implicated in various signaling pathways and are also known to play an important role in other functions involving the cell, for example, cell to cell adhesion, nuclear chromatin organization, mitosis, cytoskeletal reorganization, cell growth regulatory pathways, amongst other functions.

To try and answer some of the observed features associated with variable A-type lamin expression in colorectal cancers, it is important to characterize the normal

expression profile of A-type lamins and their binding partners in mucosa of the colon / rectum initially followed by a study looking at the expression profile of A-type lamins in colorectal tumours. In addition, the expression profile of β-catenin in the colonic crypts also merits review since this pathway is known to play a key role in colorectal carcinogenesis. To this end, a prospective study of patients with colorectal cancers undergoing definitive treatment is planned. Further studies using microarray based analysis will also be undertaken on tumour samples obtained from patients in the study group.

In colorectal cancer, appropriate treatment of the Duke's B stage group has always posed a dilemma since the benefit of surgery alone or surgery in combination with adjuvant therapy (chemotherapy and / or radiotherapy) is still unproven. A recent retrospective study has shown A-type lamins to be an independent prognostic indicator in colorectal cancer; my work is aimed at trying to identify a cause for this observation by utilizing microarray studies, in which I will compare a Duke's B tumour, expressing A-type lamins, with other stages of colorectal cancers. I would also like to identify any signaling pathways involved in tumours expressing A-type lamins. An attempt will also be made to try and answer the age old question of whether colorectal cancer is primarily a stem cell problem or not. Finally, I would like to identify any additional prognostic indicators that may complement the expression of A-type lamins in colorectal cancer.

CHAPTER 2

MATERIALS AND METHODS

2.1 General Materials and Reagents / Chemicals

All plastic ware / glassware were supplied by Greiner Bio-One Ltd, Gloucestershire, UK. General reagents and chemicals used were supplied by Sigma-Aldrich Company Ltd (Dorset, UK), Melford Laboratories Ltd (Suffolk, UK) and BDH Laboratory Supplies (VWR International Ltd, Leicestershire, UK).

2.2 Patient Selection and Tissue Sampling

2.2.1 Patient selection

After obtaining appropriate Local Research and Ethics Committee approval, patients undergoing elective colorectal cancer surgery at the James Cook University Hospital in Middlesbrough, UK, were identified and consented for tissue sample collection, prospectively. A total of forty two patients were thus identified and appropriate tissue samples were taken for further analysis and research.

2.2.2 Tissue samples for immunohistochemical analysis

Surgically resected specimens of the colon / rectum were opened up longitudinally and thoroughly cleaned with sterile saline. This, in effect, created a rectangular shaped specimen with the tumour located centrally and flanked on either side by macroscopically normal looking colonic / rectal mucosa. Full thickness samples of normal colonic mucosa, tumour and marginal areas between tumour and normal colonic mucosa were obtained by sharp dissection. The same process was repeated for all patients undergoing surgical resection of their tumours. These

samples then underwent an automated process during which they were processed and fixed in 10 % formalin before being embedded in paraffin blocks, at the James Cook University Hospital following standard pathology guidelines. The orientation of samples was crucial to experimental work and so tissue samples were specifically orientated in such a manner as to obtain a full thickness profile of colonic tissue and tumour sample. On an average, six samples were taken from each patient, four samples of normal colonic mucosa from each of the four corners of the resected specimen and one sample each from the central tumour area and the marginal area respectively.

2.2.3 Tissue samples for RNA analysis

Tissue samples of normal colonic mucosa and central tumour region were sampled by sharp dissection. After weighing the samples, an appropriate amount of Trizol® reagent was added to the sample prior to manual homogenisation as per guidelines issued by invitrogen®(Invitrogen™, Paisley, UK). Homogenised samples were aliquoted into 1 ml volumes and stored at minus 80º C in RNAse free eppendorfs. Additionally, a sample of normal colonic mucosa was kept in Dispase II® solution overnight. This assisted in cleaving the basement membrane prior to dissecting the mucosa from the underlying Muscularis. The mucosa was then weighed and an appropriate amount of Trizol® reagent was used for homogenisation, as per issued guidelines. These samples were then stored in a similar fashion.

2.2.4 Tissue samples for cryosectioning

Fresh tissue samples of normal colonic mucosa were also obtained for cryosectioning. Each sample was snap frozen in liquid nitrogen and then stored in a minus 80ºC freezer.

2.3 Immunohistochemistry

2.3.1 Colonic tissue samples

All tissue samples used were sectioned at 5 micrometers thickness. These samples were then transferred to superfrost® slides and incubated at 37ºC overnight to improve adhesion.

2.3.2 Antigen retrieval

Tissue sections were de-paraffinized by immersion in xylene for five minutes, repeated twice. The re-hydration process was undertaken using decreasing concentrations of ethanol: 100% ethanol – 5 minutes, 100% ethanol – 5 minutes, 96% ethanol – 5 minutes, 70% ethanol – 5 minutes before being transferred to distilled water for 3 minutes. Antigen retrieval was carried out using a modified method described by Barker *et al.* (1999). Tissue sections were immersed in a 3% H_2O_2 in methanol solution, for 10 minutes at room temperature, to suppress all endogenous peroxidase activity. This was followed by incubation at 90ºC for 15 minutes of tissue sections in a 0.01 M citrate buffer (pH 6.0) solution, using a water bath. Thereafter, tissue sections were allowed to cool in PBS for at least 10 minutes. Slides containing the tissue sections were then loaded onto Thermo shandon coverplates™ (Thermo Shandon Ltd. UK) and placed into Shandon Sequenza® staining racks (Thermo Fisher Scientific Inc. USA). All experimental work continued with the slides loaded onto the slide holders.

2.3.3 Peroxidase staining reaction

To block non-specific binding, tissue sections were incubated with 2% goat serum – 2% bovine serum albumin in PBS for 20 minutes. After 3 washes with PBS,

tissue sections were incubated with primary antibody, diluted to the appropriate concentration in 0.1% BSA in PBS. 140 µl of antibody solution was applied to each slide and incubated overnight at 4ºC in a dark chamber. Various concentrations and other details about the primary antibody used can be seen in Table 2.1. All slides were then washed 3 times with PBS. Biotinylated IgG was used as secondary antibody at a concentration of 1:400 in 0.1% PBS. 140 µl of this solution was used per slide and left for 45 minutes at RT. Slides were then washed 3 times with PBS. 30 minutes prior to use, StreptABComplex / HRP (Dako Cytomation)

Table 2.1

Various antibodies and corresponding secondary antibodies used for Immunohistochemistry (IHC)

1332 A 2	Lamin A	Mouse monoclonal	Hozak *et al*. (1995)	IHC	1:50	Biotinylated DαM HRP	1:400
Jol 2	Lamin A/C	Mouse monoclonal	Dyer *et al.* (1997)	IHC	1:100	Biotinylated DαM HRP	1:400
RalC	Lamin C	Rabbit polyclonal	Venables *et al.* (2001)	IHC	1:10	Biotinylated DαR HRP	1:400
Emerin	Emerin	Mouse monoclonal	Novocastra®	IHC	1:50	Biotinylated DαM HRP	1:400
PCNA	PCNA	Mouse monoclonal	Immuno-concepts®	IHC	1:100	Biotinylated DαM HRP	1:400
Lap2α	Lap2α	Rabbit polyclonal	ImmuQuest®	IHC	1:10	Biotinylated DαR HRP	1:400
β-Catenin	β-Catenin	Mouse monoclonal	BD Biosciences®	IHC	1:100	Biotinylated DαM HRP	1:400
Anti Active β-Catenin	Active β-Catenin	Mouse monoclonal	Upstate® Cell Signalling Solutions	IHC	1:10	Biotinylated DαM HRP	1:400

was prepared (50 µl Streptavidin and 50 µl biotinylated peroxidase in 5 ml of 0.1% BSA in PBS) and 140 µl of this solution was used per slide and allowed to incubate for 30 minutes at RT. Slides were washed 3 times in PBS. Activated DAB chromogen (Dako Cytomation) was used for immunological detection. Upon adequate visualisation, further reaction was stopped by plunging the slide into dH_2O and the time taken for the reaction was noted. Negative control studies were also conducted using 0.1% BSA in PBS instead of the primary antibody.

2.3.4 Counterstaining

Tissue sections were counterstained with either neat Ehrlich's haematoxylin (Raymond A Lamb Ltd, Eastbourne, East Sussex, UK) for 3 minutes and then rinsed in tap water for 1 minute. Using 1% acid-alcohol for 1 minute, the stain was differentiated and then further matured in 0.04% aqueous ammonia for 30 seconds. Or with neat Mayer's Haemalum for five seconds, before being rinsed in distilled water and fixing the stain with alkaline alcohol (3 ml ammonia solution in 100 ml of 70% ethanol). After washing in dH_2O for 1 minute, tissue sections were de-hydrated by immersing in: 70% ethanol – 1minute, 96% ethanol – 1 minute, 96% ethanol – 1 minute, 100% ethanol – 1 minute, xylene – 3 minutes and xylene – 3 minutes. Cover slips were then applied to the slides using Entellan® (Merck kGaA, Darmstadt, Germany) and left overnight to dry.

2.3.5 Image analysis

A Nikon Diaphot 300 inverted microscope (Nikon Corporation, Tokyo, Japan) fitted with Plan 4x / 0.13, 10x / 0.25, 20x / 0.4 and 40x / 1.3 lenses, was used for image analysis. Images were captured digitally by a Nikon DXM 1200 digital camera using Nikon Act – 1, version 2.2 software. Further image processing was carried out in Adobe® Photoshop® 7.0 (Adobe Systems Inc. San Jose, CA, USA).

2.4 Semi – quantitative RT PCR

2.4.1 Primer design

Specific primers were designed using Primer 3 web interface (http://frodo.wi.mit.edu/primer3/primer3_code.html), developed by the Whitehead Institute, Cambridge, MA and the Howard Hughes Medical Institute, Chevy Chase, MD. This software allows the design of primers from a given DNA sequence based on parameters such as primer length, product size, melt temperature (Tm) and GC %. Primers between 18 to 27 bases long were designed with a minimum Tm of 57°C and a maximum Tm of 63°C. GC content was kept between 45 – 70 %. A maximum Tm difference of 4°C between sense and antisense primers was also ensured. Examination of secondary structures in primer pairs, incorporating hairpin loops, cross-dimers, self dimers, palindromes, runs and repeats which could alter their function or generate aberrant products, was assessed using Net Primer online software (PREMIER Biosoft International, CA / http://www.premierbiosoft.com/netprimer/index.html).Table 2.2 shows a list of primer sequences specific for the coding sequences (cds) of human genes investigated:

2.4.2 RNA Extraction from Colorectal Cancer Tissue Samples

2.4.2.1 RNA extraction

After obtaining appropriate samples, as described in section 2.1.3, RNA isolation was carried out using TRIzol® reagent (Invitrogen™, Paisley, UK), as per their protocol sheet (Technical Manual #15596-026). 1ml of TRIzol® reagent was added to every 100mg of tissue sample. All work surfaces and materials used were RNAse free. This work was carried out in a laminar flow hood.

Gene	Accession Number	Primer Direction	Start (5'– 3')	Deoxyoligonucleotide sequence	Product Size (bp)
Lamin A/C	NM_005572	Sense Antisense	976 1235	GATGCGCTGCAGGAACTGCG CGGGCCAGTGAGTCCTCCAG	259
Lamin A	NM_170707	Sense Antisense	1878 2112	GGTGCGCTCAGTGACTGTGG CGTGACACTGGAGGCAGAAGAG	234
Lamin C	NM_005572	Sense Antisense	1716 1968	GAAGGCTGGGCAGGTGGTGA TCAGCGGCGGCTACCACTCA	252
T Plastin	NM_005032	Sense Antisense	2692 2998	GCATCTTCCCTCTCATACCC GCAAACAGCTTGACAAAGCA	307
E Cadherin	NM_004360	Sense Antisense	4301 4715	CCAAGTGCCTGCTTTTGATG CACAATTATCAGCACCCACAC	415
β Actin	NM_001101	Sense Antisense	257 1090	GGCACCACACCTTCTACAATGAGC CGTCATACTCCTGCTTGCTGATCC	834
Creatine Kinase Brain	NM_001823	Sense Antisense	92 768	CAACAGCCACAACGCACT TGACCCACACCAGGAAGG	677

Table 2.2
Primers for semi-quantitative RT-PCR

2.4.2.2 Homogenisation

Homogenisation of these samples was carried out in a glass Teflon homogeniser. After incubation at RT for 5 minutes, all insoluble material was removed by

centrifugation at 12,000 x g for 10 minutes at 4ºC and transferring the supernatant to 1.5ml RNAse free eppendorfs (1ml of supernatant was added to each 1.5ml RNAse free eppendorfs). At this point, samples were snap frozen in liquid nitrogen and stored at minus 80ºC.

2.4.2.3 Phase separation

Frozen samples were allowed to reach RT before adding 0.2ml of Chloroform for every 1ml of TRIzol® (Invitrogen™, Paisley, UK) reagent used originally. After vigorously shaking the samples to ensure adequate mixing and no obvious phase separation, 15 minutes of centrifugation at 12,000 x g and 4ºC was carried out. This resulted in a colourless, clear aqueous upper phase with a well defined whitish interphase separating it from a pinkish lower phase. The upper, clear phase was very carefully transferred to fresh 1.5ml RNAse free eppendorfs to continue with RNA precipitation.

2.4.2.4 RNA Precipitation

To this clear aqueous phase, 0.5ml of isopropanol was added for every 1 ml of TRIzol® (Invitrogen™, Paisley, UK) used initially. After incubation at RT for 10 minutes, the sample was centrifuged for 10 minutes at 12,000 x g and 4ºC. RNA was precipitated in the form of a small pellet at the base of the tube.

2.4.2.5 RNA Wash

After removal of the supernatant, each pellet was washed with 1ml of 75% RNAse free ethanol. After mixing and vortexing the sample, it was centrifuged at 7500 x g for 5 minutes at 4ºC. After removal of excess ethanol, the RNA sample was allowed to air dry.

2.4.2.6 Redissolving the RNA

RNA was dissolved in 25µl of RNAse free water. This was heated up to 55ºC for 10 minutes and mixed thoroughly by pipetting.

2.4.3 RNA quantification and quality assessment

2.4.3.1 Quantification of RNA

RNA quantification was carried out using a Beckman DU-600 ----- analyser. 2 µl of RNA sample was mixed with 98µl of DEPC treated H_2O. This sample was then loaded onto a 100µl cuvette and analysed. The concentration of RNA in the sample was calculated using the formula:

A_{260} reading x 40µg/ml x Dilution factor

Where:

1) A_{260} is the absorbance of the solution at 260nm
2) 40 µg/ml is a fixed conversion factor relating absorbance to concentration for RNA
3) Dilution Factor was 2 in this case

Knowing the concentration of RNA in the sample, it was easy to calculate the total volume of RNA per sample.

2.4.3.2 Quality assessment of RNA using a 1.2% Formaldehyde denatured gel

A 1.2% Formaldehyde denatured gel was prepared by dissolving 600 mg of Agarose to 40 ml of DEPC treated H_2O, using a microwave oven. The sample was allowed to cool down to RT before adding 5 ml of 10 x MOPS and 5 ml of 37% Formaldehyde. A 50 ml tray, with an appropriate comb, was used to allow the gel

to set in. 1 x MOPS was used as the running buffer solution. All RNA samples were then prepared for gel electrophoresis using RNA sample loading buffer (Sigma®, Sigma-Aldrich Inc, Saint Louise, Missouri, USA), in accordance with the manufacturers instructions. Samples were separated alongside RNA size markers (Sigma®, Sigma-Aldrich Inc, Saint Louise, Missouri, USA) ranging from 0.2 – 10 KB. All gels were prepared in a similar fashion and were run at 80mV for one hour. Bands were visualised using a Gel Doc ™ 2000 UV transilluminator and Quantity One ™, version 4.0.3 software (Bio-Rad). Purity was determined by measuring the ratio of absorbance in DEPC-treated δH_2O at 260nm and 280nm (A_{260}/A_{280}) in a GeneQuest CE2301 Analyser (Cecil Instruments Ltd. Cambridge, UK).

2.4.4 Confirmation of RNA integrity

The human gene β-actin was used to exclude the possibility that geneomic DNA (gDNA) may have been co-isolated with total RNA. Primers to an 834bp fragment of β-actin (see table 2.1) were used to amplify any contaminating gDNA in 0.5µl reaction volume comprising 1 x Reddymix™ PCR Master Mix (ABgene, Epsom, Surrey, UK) [25 units/ml Thermoprine Plus DNA Polymerase (Taq), 75mM Tris-HCl (pH 8.8 at 25°C), 20mM $(NH_4)_2SO_4$, 2.5mM $MgCl_2$, 0.01% Tween®20 (v/v) and 200 µM each dNTPs], 0.4 pmol/µl β-actin sense primer and 0.4 pmol/µl β-actin anti-sense primer. Cycling conditions were as described in section 2.4.6.

The total volume of PCR product (25µl) was electrophoresed on a 1.2% Formaldehyde denatured gel. RNA samples showing evidence of gDNA contamination were treated with 0.075 units/µl RQ1 RNase-free DNase (Promega) in a 1x reaction buffer [40mM Tris-HCl (pH 8.0), 10mM $MgSO_4$, 1mM $CaCl_2$] for 15 minutes at room temperature. The reaction was terminated by incubating the samples with 1µl DNase stop solution (20mM EGTA, pH 8.0) for 10 minutes at 65°C. RNA was then precipitated with 1/10 of the total reaction volume of DEPC-treated 3M Sodium Acetate (NaOAc), pH 5.2 and 3 volumes of 100% RNase free ethanol (-20°C), vortexed for 5 seconds and incubated overnight at -20°C. To recover RNa, samples were centrifuged at 12,000 x g for 10 minutes at 4°C, the supernatant removed and pellets washed with 1ml 70% RNase free alcohol (in

DEPC-treated δH_2O). Centrifugation was repeated 12,000 x g for 5 minutes at 4°C, the supernatant aspirated and residual ethanol allowed to evaporate for 5-10 minutes in a laminar flow hood. The resulting pellet was re-suspended in 20µl RNase free water and the quality, purity (A_{260}/A_{280}) and concentration (A_{280}) of the RNA was re-evaluated as described in section 2.4.3.

2.4.5 RNA storage

All excess RNA samples were immediately snap frozen in liquid nitrogen and stored at minus 80ºC.

2.4.6 Reverse Transcription – Polymerase Chain Reaction (RT-PCR)

Reverse transcription and amplification of target genes was carried out in 2 stages using Promega's Reverse Transcription system (A3500) and PCR Master Mix (M7502). For all samples, RT-PCR was completed in triplicate.

First, a cDNA library was synthesised in a 40µl reaction using Promega's Reverse Transcription System in exact accordance with their technical bulletin TB099 (2006). Two –ve controls substituting RNa and the AMV-RT for δH_2O were used in each run. All resulting cDNA libraries were aliquoted and stored at -20°C.

B-actin was amplified from all cDNA libraries and their accompanying AMV-RT negative controls, plus negative controls where no RNA was added to show equal amounts of cDNA had been reverse transcribed and that reverse transcription and PCR reactions were working successfully.

PCR fragments for the chosen target genes detailed in table 2.1, were amplified from all Reverse Transcriptase reactions using a 25µl reaction containing 1 x Promega PCR Master Mix [25 units/ml Taq DNA polymerase in buffer (pH 8.5), 200µM each dNTPs and 1.5 mM $MgCl_2$], 0.4 pmol/µl sense primer, 0.4 pmol/µl anti-sense primer, 2µl cDNA library and Nuclease-Free water. Optimisation of

PCR profiles was carried out in an Eppendorf Mastercycler™ Gradient Thermal Cycler (Eppendorf UK Ltd, Cambridge, UK) using the following conditions; 94°C for 2 minutes, 26 cycles of [94°C for 45 seconds, Tm°C ± 5°C for 1 minute, 72°C for 1 minute] and finally 72°C for 5 minutes.

2.4.7 Analysis

All amplified products were run on a 1.2% Formaldehyde denatured gel, at 60V for 60 minutes. Product size was compared to a 100bp DNA ladder (Promega Cat #G2101) and bands were visualised using a GelDoc™ 2000 UV transilluminator and Quantity One™ software versin 4.0.3 (Bio-Rad). Differences in expression levels were quantified using densitometry and calculated as a ratio against β-actin expression.

2.5 Microarray Analysis

Genome wide microarray analysis was carried out on selected, human colorectal tumour samples using the Human Genome U133 Plus 2.0 high-density oligonucleotide Affymetrix GeneChip array (Affymetrix, Santa Clara, CA, USA). Normalisation of expression ratios was carried out in accordance with the associated Affymetrix guidelines for the HG U133 GeneChip and a targeted gene list was generated by means of a series of relevance thresholds for detection *p*-value significance, expression change *p*-value significance and Signal Log Ratio (SLR). Analysis of the targeted gene list and biological interpretation of results was carried out using the online NetAffx Analysis Centre from Affymetrix found at (http://www.affymetrix.com/analysis/index.affx). Genes of interest were subjected to cluster analysis by Gene Ontology including; Biological Processes, Molecular Function and Cellular Component. For a detailed description of the online NetAffx tools, see http://www.affymetrix.com/support/index.affx.

CHAPTER 3

EXPRESSION PROFILE OF LAMINS A/C AND THEIR ASSOCIATED BINDING PARTNERS IN HUMAN COLONIC CRYPTS

3.1 Introduction

3.1.1 Histological characteristics of normal colonic and rectal epithelium

As mentioned previously (chapter 1), the mucosal lining of the colon and rectum is a highly complex structure which proliferates rapidly and is under tight control of various homeostatic mechanisms. This ensures a state of physiological equilibrium, essential for the proper functioning of this organ. As described in Gray's anatomy (38th edition), the basic functional units, underpinning the entire colon and rectum, are flask shaped invaginations of mucosa called crypts. Each crypt has a fairly uniform structure and arrangement of cells. However, there is slight variation in the exact number of cells forming any given crypt in the colon and rectum in the same individual or between individuals. Irrespective of this observation, each crypt strictly adheres to a common histological layout of cells which in turn allows the colon and rectum to function properly. Only six types of cells are responsible for giving rise to the millions of cells forming the mucosal lining of the colon and rectum. These include:

Columnar cells (vacuolar absorptive cells) – The most numerous of the epithelial cell types and responsible for ionic exchange / regulation and water resorption. They all bear apical microvilli. Many of these cells also contain secretory granules in their apical cytoplasm containing largely mucins but also antibodies of the IgA type. They are characterised by typical junctional complexes around their apices which limits diffusion from the lumen into the intestine wall.

Mucous (goblet) cells – These cells consist of elongated basal nuclei and have an apical region containing many membrane-bound Mucin granules. Their secretions are important in lubricating the intestinal wall and also in mechanical and chemical protection. Their apical surfaces bear a few short microvilli.

Microfold cells – They are located at sites of lymphoid follicles in the intestinal wall. They have long, widely spaced microvilli between which are numerous endocytic vesicles. These cells are thought to act as sampling sites for antigens in the lumen of the intestine which are then transferred to the underlying lymphoid tissue for further processing and production of appropriate antibodies.

Entero-endocrine cells – These cells are situated mostly at the bases of intestinal glands and are part of the gastro-entero-pancreatic (GEP) endocrine system. They consist of scattered, often solitary, hormone producing cells. Their bases rest on the basal lamina and they produce secretory granules of varying sizes and shapes which diffuse across the basal lamina and are absorbed into the blood stream. Their actions are usually endocrinal.

Brush cells – These are infrequent cell types of unknown function. They have an apical bundle of long, straight microvilli, giving them a characteristic appearance.

Stem cells – These cells reside at the basal regions of crypts and are responsible for giving rise to all cell types. They have no obvious microscopic features that can distinguish them from other cell types in the basal regions of the crypts. They undergo periodic mitosis to give rise to a stream of cells which migrate upwards to populate the entire crypt and are shed off at the boundaries between crypts (Brittan and Wright, 2004; Potten, 1998).

Located between each crypt is the supporting stroma or mesenchyme. Located in the mesenchyme are cells whose functions are poorly understood but appear to secrete important factors which play a significant role in crypt homeostasis for example Wnt factors. Also located in the mesenchyme are scattered lymphocytes. Supporting the mucosa is the muscularis mucosae below which resides the submucosa containing the neurovascular plexus to the intestine. The muscularis externa consists of two layers of smooth muscle, an inner circular and outer longitudinal layer, responsible for peristaltic activity. Surrounding the muscularis

externa is the serosa, a layer of adventitial tissue which is absent in the lower two thirds of the rectum.

To date, many studies have been undertaken to elucidate and characterise various compartments in the crypt, crypt dynamics, stem cell niche areas in the basal region of the crypt and many other crypt characteristics; however there is a paucity of data as regards expression profiles for lamin A/C and its binding partners in the intestinal crypts. Keeping this in mind, my aims are to, initially, characterise the expression profile of A-type lamins and their binding partners in the mucosa of the colon and rectum and, subsequently, to investigate and clarify the role, if any, of A-type lamins in colorectal cancer.

3.1.2 Expression of lamin A/C in different cancerous cells and tissues

The role of lamins in cancer has been studied in a variety of different cancers such as basal skin cell cancer (Venables et al., 2001), acute lymhoblastic leukaemia, non-Hodgkin's lymphoma (Stadelmann et al., 1990), human small cell lung cancer and non-small lung cell cancer (Broers et al., 1993), colorectal cancer (Cance et al., 1992; Moss et al., 1999) and metastatic leiomyosarcoma, rhabdomyosarcoma and chondrosarcoma (Cance et al., 1992).

In some cancers studied, there are conflicting reports about the expression of lamins, for example, in colorectal cancer, Moss et al., (1999) describes a decrease in nuclear staining for A-type lamins and lamin B1 in colon adenomas and adenocarcinomas when compared to normal colonic mucosa, whereas Cance *et al*., (1992) has previously reported heterogeneous expression of A-type lamins in two colon adenocarcinomas. Interestingly, Venables *et al*., (2001) demonstrates a down regulation of lamin A in basal skin cell cancers exhibiting a high proliferative index and a loss of lamin C in slower growing tumours.

In my studies, expression of total lamin A/C, as well as lamin A and C individually, in normal colonic / rectal mucosa was undertaken. Samples were oriented to obtain full thickness profiles of colonic / rectal crypts. All patients participating in

this study underwent expression profiling for lamin A/C, lamin A and lamin C, in a similar fashion. When assessing for lamin A/C expression in colorectal cancers, I took into consideration previous conflicting reports about lamin A/C expression patterns in different patient groups with colorectal cancer. In my studies, I used, commercially available, specific monoclonal / polyclonal antibodies against lamin A, lamin C and lamin A/C. Appropriate negative control studies were done to exclude any bias in the observed results. Similarly, Western blot analysis confirmed specificity of antibody used against lamin A/C (Figure 3.1)

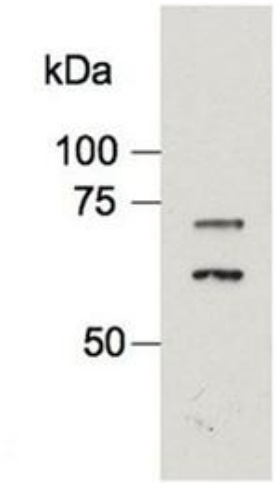


Figure 3.1

Western blot (Human) analysis of whole cell lysates from HeLa cells using Jol 2 (Antibody against lamin A/C). Bands observed at 73 kDa and 64 kDa, corresponding to lamin A and C, respectively.

Courtesy of abcam® laboratories

Quantification of antibody expression in the tissue samples selected using immunohistochemical methods, was undertaken by me. This involved a structured, microscopic analysis of the entire cancer tissue section at 20x magnification. Each 20x field visible was designated 1power field. The tissue section was then divided into rows, corresponding to 1 power field (20x), along the *x* and *y* axis. Each row was assessed for antibody expression in a left → right fashion. For every power field, expression of antibody was categorised into three groups:

Present – If more than 90% of the visualised area was expressing antibody.

Absent – If less than 10% of the visualised area was expressing antibody.

Depleted – If less than 90%, but more than 10%, of the visible area was expressing antibody.

All power fields for each cancer tissue sample were then collated into a single figure and a final assessment regarding expression of antibody was made, based on the same cut off values as used for each individual power field. Analysis of all tissue samples was done by me.

This method of quantification has well known drawbacks. Inter observer error can be seen and reproducibility can be problematic. Similarly, quantification is very subjective. Alternate methods that are more objective, such as quantification studies using an image analyser are difficult to use for this type of analyses due to the patchy areas of antibody expression noted in some cancer tissue samples. The method of antibody expression quantification used by me has previously been used in a separate study (Cox 2007) with very low inter observer error and god reproducibility, hence I opted to use a similar method for my study.

3.2 RESULTS

3.2.1 Optimization of specimen samples for immunohistochemistry

All samples were obtained fresh from appropriately consenting patients undergoing bowel cancer surgery. Orientation of the specimen sample was very important. All samples were meticulously oriented after appropriate fixation and then embedded in liquid paraffin. Half of all specimen samples were oriented flat

with the mucosal surface uppermost, the remaining half were oriented at 90° in paraffin to give full length profiles of colonic crypts. All embedded specimen samples were sectioned as mentioned previously and H&E immunostaining done to assess orientation of the sample prior to undertaking immunostaining using definitive antibodies. All primary antibodies were carefully assessed using gradually increasing dilutions of primary antibody, to assess for optimum expression in the samples. Where possible, monoclonal antibodies were used in preference.

3.2.2 Normal colonic crypts in human adults (Figures 3.2 and 3.3)

The basic functional unit of the large intestine is the crypt. Each crypt is made up of various cell types, as mentioned earlier. The connective tissue surrounding these glands is called the lamina propria. The connective tissue together with the cells forms the mucosa. Separating the mucosa from the sub mucosa is the muscularis mucosae. This is made up of a thin layer of smooth muscle cells. Beneath this layer is the submucosa. In the submucosa are located numerous capillaries, lymphatics, neuro-vascular structures, lymphoid aggregates and scattered lymphocytes. The muscularis externa surrounds the submucosa and is composed of two layers, an outer longitudinal and inner circular layer. Covering the muscularis externa to a

Figure 3.2

Cross section of the colon stained with H & E (10x).

This micrograph shows the three main layers making up the colon. The mucosal layer is adjacent to the GI tract lumen. Colonic crypts are formed by flask shaped invaginations into the mucosal layer. Separating the mucosal layer from the sub-mucosal layer is the muscularis mucosae. The submucosal layer carries in it capillaries, lymphatics and nerves. The outer most layer of the colon is formed by the muscularis externa, composed of the inner circular and outer longitudinal layers of muscle, respectively.

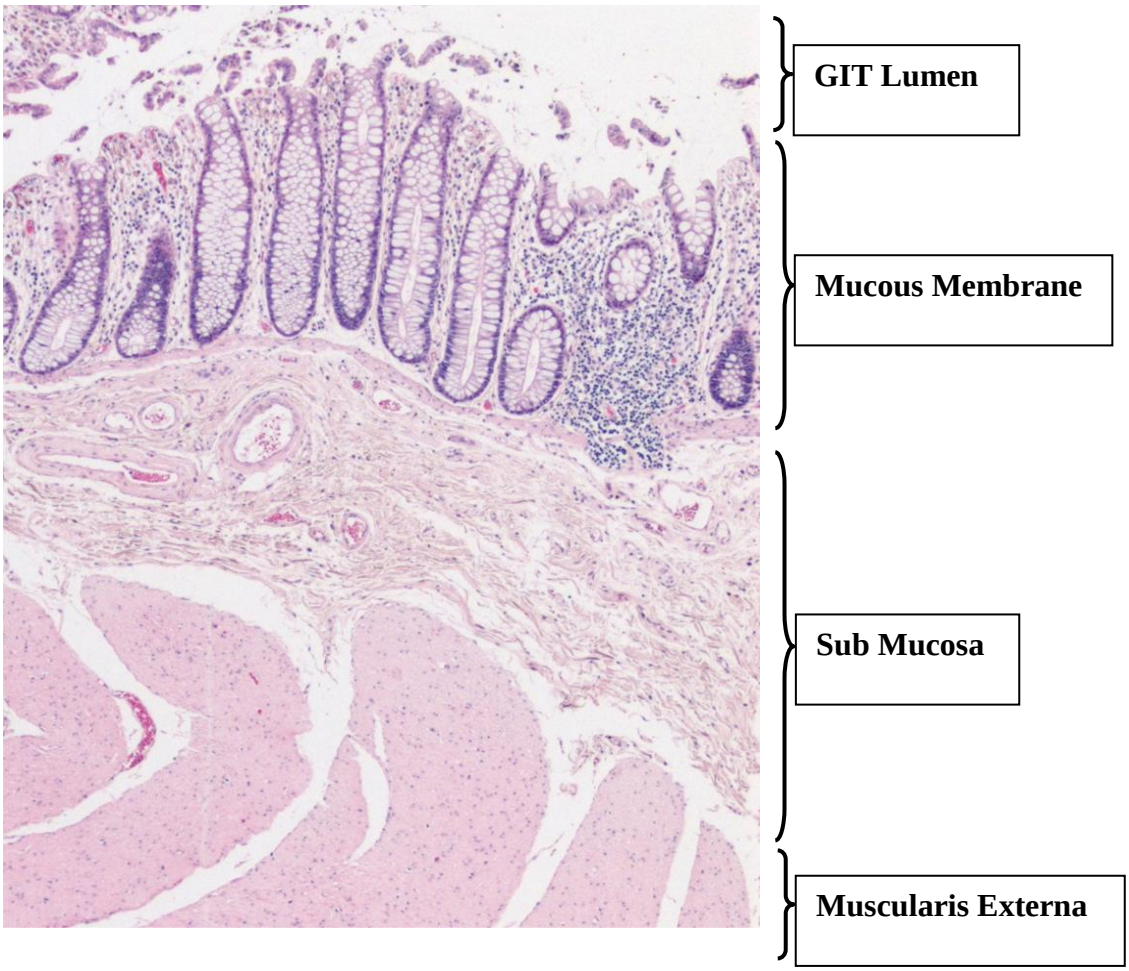


Figure 3.2

Cross section of the colon stained with H & E (10x).

Figure 3.3

Normal crypt from mucosa of the human colon

H & E (20x)

Scale bar represents 100µm.

LP – Lamina Propria **SM** – Sub Mucosa **MM** – Muscularis Mucosae

This micrograph shows in greater detail the full crypt profile. Cells lining these crypts are columnar in shape with basal nuclei. Mucous secreting, goblet cells (indicated by cells with large vacuoles in them) make up a large proportion of cells making up the crypt. The muscularis mucosae and lamina propria are well represented in this section.

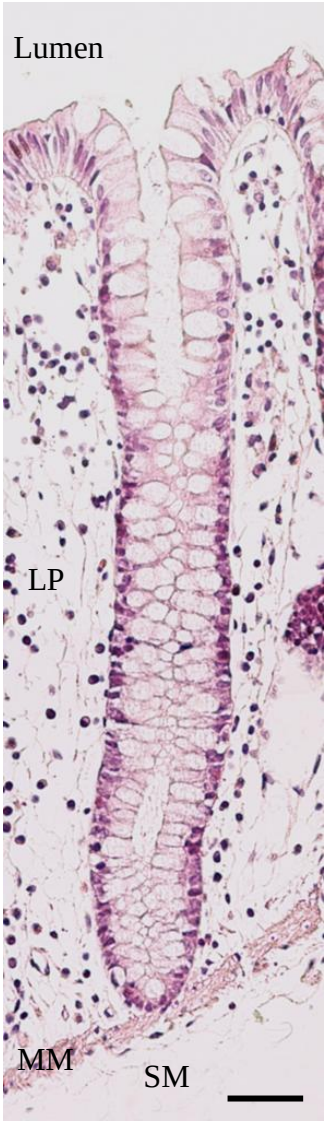


Figure 3.3

Crypt from mucosa of the human colon stained using H & E. (20x)

Scale bar represents 100µm.

variable extent is a layer of visceral peritoneum called the serosa (Figure 3.2 and Figure 3.3).

Significant variation was noted in the total number of cells making up a crypt. On average, the height of a crypt was 80 cells high. The average diameter of a crypt was approximately 200 microns based on serial sections, but this value was highly subjective due to variation based on multiple factors, including tissue processing, tissue orientation, angle at which the specimen was sliced, etc. The mucosal (or luminal) surface of the colon and rectum appeared cribriform, in keeping with multiple luminal openings of intestinal crypts.

3.2.3 Expression of lamin A/C in the adult human colon crypt (Figures 3.4 and 3.5)

The aim of this study was to characterise the expression of lamin A/C subtypes in the human colon crypt. Formalin fixed and paraffin embedded sections of normal adult human colon were stained with the appropriate antibody using standard immunohistochemical methods. To assess the expression of lamin A/C a monoclonal antibody Jol2 (anti lamin A/C) was used. This antibody reacts with the common domain of the lamin A/C tail, aa 464 – 572 and identifies both lamins A and C (Dyer et al., 1997).

Lamin A/C expression was observed in three distinct zones along the crypt axis (Figures 3.4). In the basal region, this was limited to cells lying adjacent to the Muscularis mucosae (figure 3.5 C). On average, the maximum number of cells expressing lamin A/C in this region was eight. Lamin A/C expression was mostly limited to the nucleus, in a heterogeneous distribution. Very little cytoplasmic staining was noted in these cells.

An abrupt change in lamin A/C expression patterns was observed in cells lying above the basal cells. In these cells there was a sudden loss of lamin A/C expression (figure 3.5 A). On average, thirty five to forty five cells above the basal cells had little or no lamin A/C expression. The number of cells

Figure 3.4

Expression of A-type lamins in normal colonic epithelium

(10x) Scale bar represents 200µm.

Arrows point towards the basal regions of the crypts

Arrowheads point towards the top of the crypts.

This photo micrograph shows the expression profile of A-type lamins in the crypts of mucosa from the colon. A-type lamin expression in the crypt is noted in two distinct zones:

Firstly, in the basal zone or putative stem cell niche area of the crypts

Secondly, in cells making up the upper one third of the crypt, this corresponds to the differentiated zone.

A-type lamin expression is also noted in cells making up the muscularis mucosae and lamina propria. At all sites, the expression of A-type lamins is at the nuclear envelope.

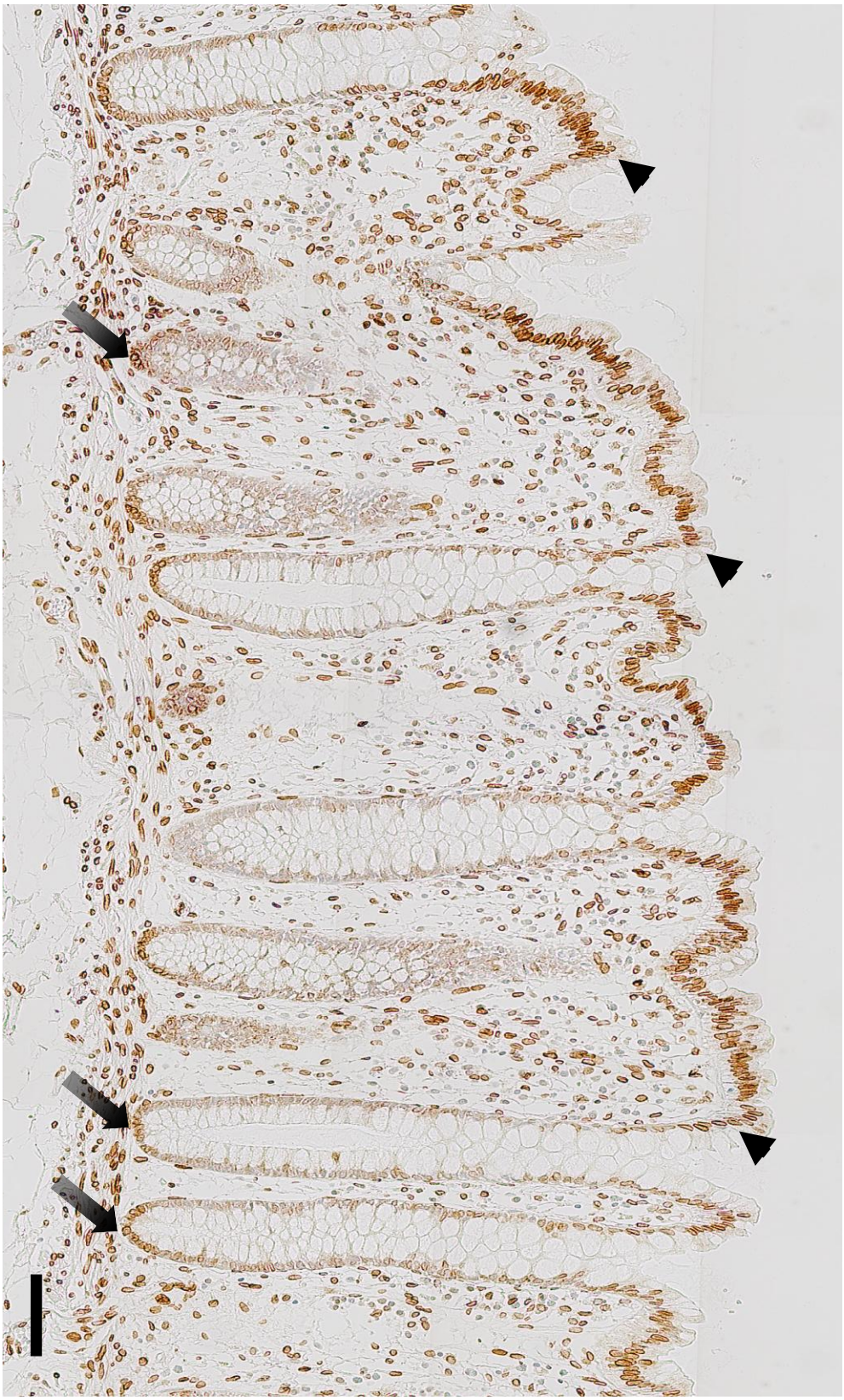


Figure 3.4

Expression of A-type lamins in normal colonic epithelium (10x)

Figure 3.5

Expression profile of lamin A/C in an isolated intestinal crypt from the colon

Figure A

Expression profile of lamin A/C in an isolated intestinal crypt from the colon (20x) Scale bar represents 150µm

Figure B

Expression profile of A-type lamins in cells lining the upper third of the crypt (indicated by arrowheads), corresponding to the differentiated zone

(40x) Scale bar set at 50µm

Figure C

Expression of A-type lamins in cells forming the basal regions of the crypt (indicated by arrows) and corresponding to the putative stem cell niche area.

(40x) Scale bar set at 50µm

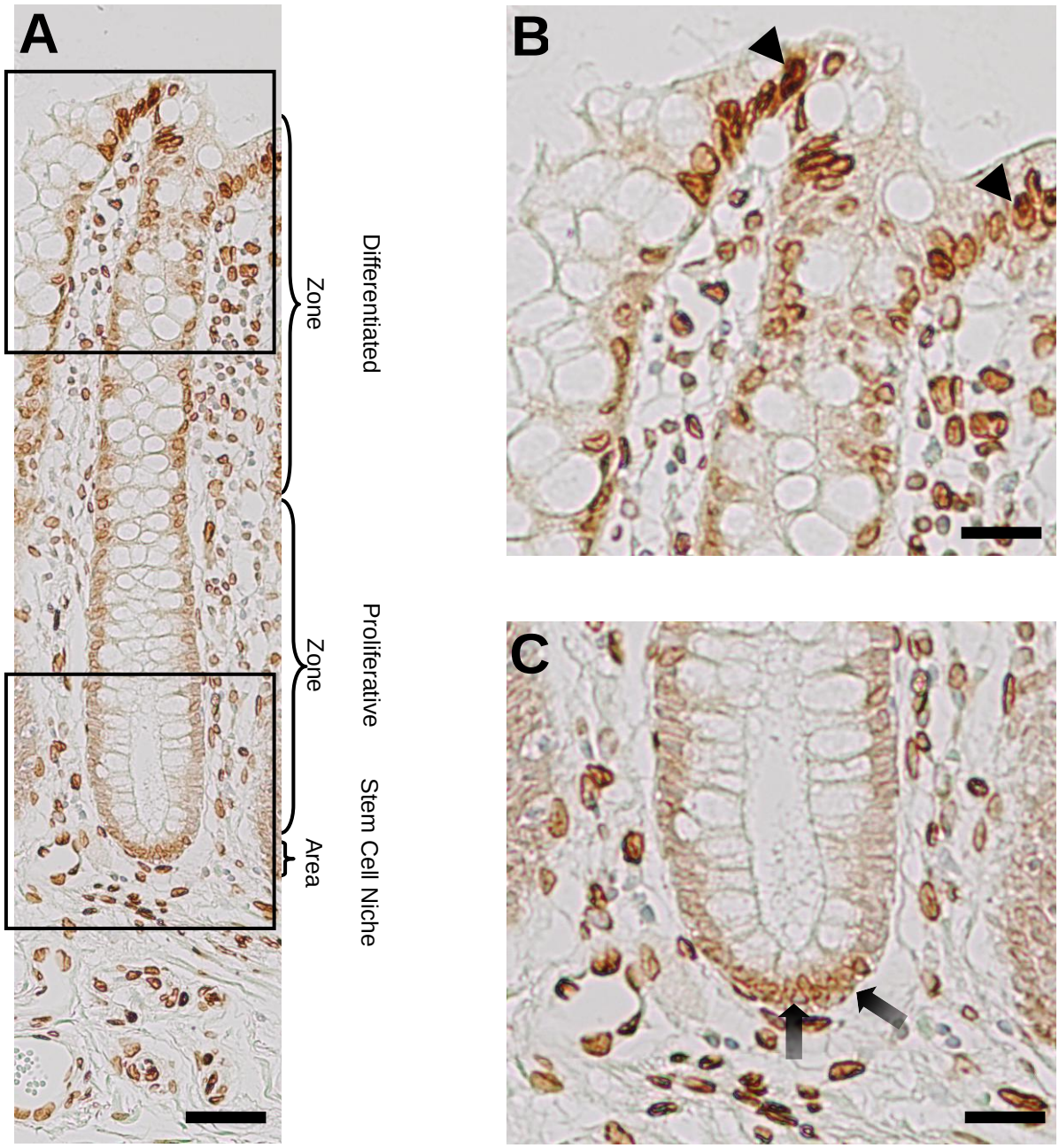


Figure 3.5 (A, B and C)

Expression profile of lamin A/C in an isolated intestinal crypt from the colon

forming this zone was variable between different crypts from the same section as well as between sections taken from different samples, but on average, it comprised two thirds of the crypt axis. This corresponded with the known proliferative zone of the intestinal crypts.

A fairly rapid return of lamin A/C expression in the cells populating the upper one third of the crypt was noted. In these cells lamin A/C expression was predominantly in the nucleus, in a heterogeneous distribution (figure 3.5 B). Lamin A/C expression gradually increased along the crypt axis towards the luminal surface and was most prominent at the luminal surface. This upper third of the crypt was known to be the site for differentiation of cells that moved up from the proliferative lower two thirds of the crypt and is known as the differentiated zone of the crypt.

Of interest was the observation that lamin A/C expression in the mesenchymal tissue surrounding the crypts revealed a uniform expression of lamin A/C in the nuclei of these cells. This expression pattern was observed in all sections from different samples analysed. There did not appear to be any gradient to this staining pattern when compared to the crypts (figure 3.5 A). The morphology of these nuclei expressing lamin A/C in the mesenchymal tissue was very similar to that observed in the nuclei of cells located in the upper most areas of the crypt. These mesenchymal cells exhibited slightly larger nuclei which appeared elongated in some areas. There was variable expression of lamin A/C noted in cells of the submucosa.

3.2.4Expression of lamin A in the adult human colon crypt (Figure 3.6)

Lamin A expression in the colon crypts (figure3.6 A and B) mirrored the expression pattern seen by lamin A/C in crypts. Expression of lamin A was noted in three distinct areas of the crypt and also in the mesenchymal tissue and basement membrane regions. At the basal regions, on an average, eight cells exhibited expression of lamin A. This was predominantly in the nuclei, in

Figure 3.6

Expression profile of lamin A in the intestinal crypts of the colon and rectum.

Figure A

Expression profile of lamin A in cells lining the upper third of the crypt (indicated by arrowheads), corresponding to the differentiated zone.

Scale bar is set at 100µm. (40x)

Figure B

Expression profile of lamin A in cells at the base of the colonic crypt (arrows), corresponding to the stem cell niche area.

Scale bar is set at 100µm. (40x)

In both figures, expression of lamin A is at the nuclear area predominantly. A heterogeneous intranuclear and intra cytoplasmic expression is noted. Cells of the muscularis mucosae and lamina propria also exhibit nuclear area expression of lamin A.

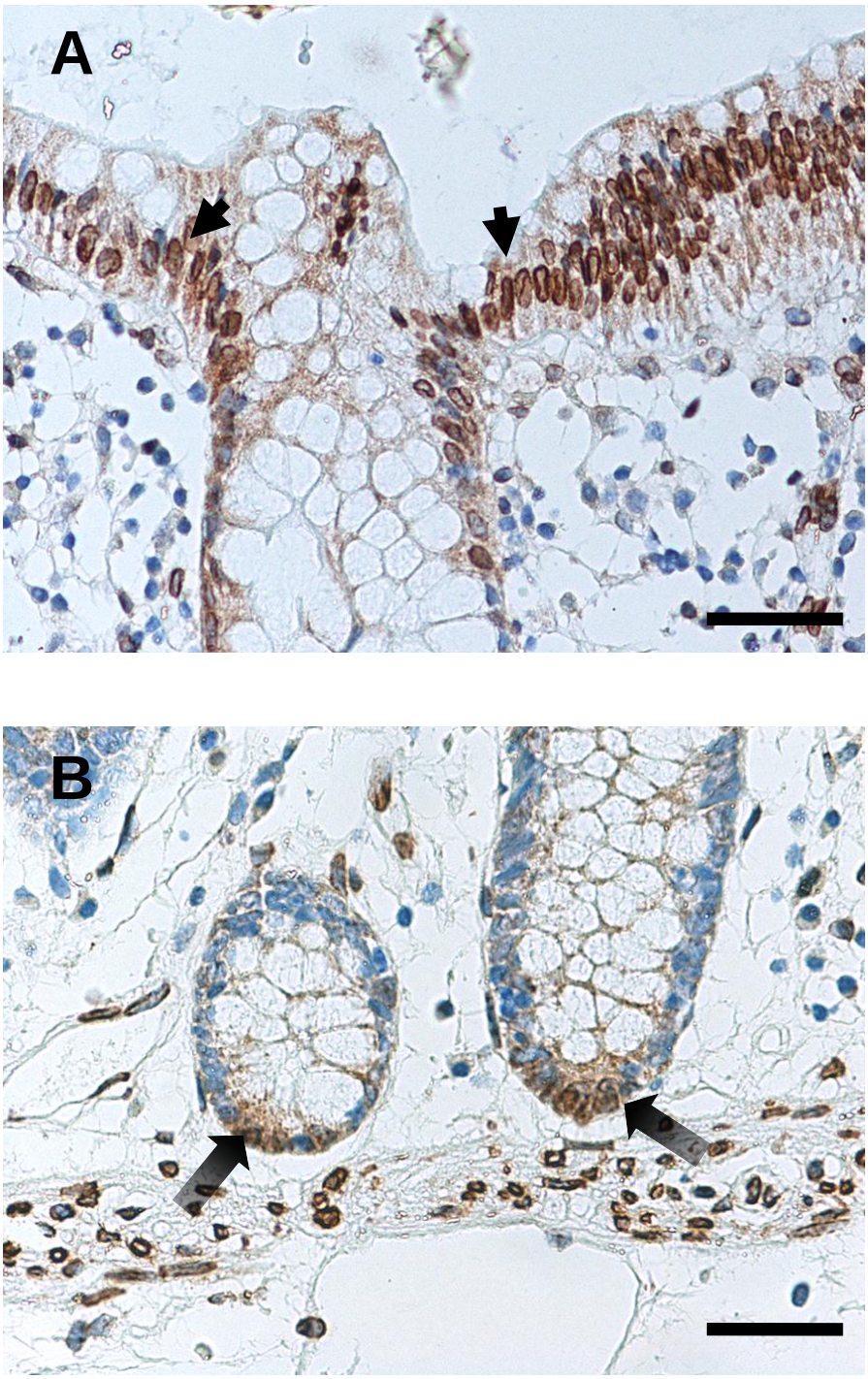


Figure 3.6 (A and B)

Expression profile of lamin A in the intestinal crypts of the colon and rectum

(40x)

a heterogeneous distribution (figure 3.6 B). This region of the crypt is proposed to harbour the putative stem cell niche (Potten, 1998).

As noted with lamin A/C expression, there was an abrupt loss of lamin A expression in cells adjacent to the basal cells expressing lamin A, in the crypt. Two thirds of the way up along the crypt axis, a gradual return of lamin A expression was noted, which became progressively stronger. Expression of Lamin A was highly marked in cells lining the upper one third of the crypt and the mucosal lining of the colonic lumen (figure 3.6 A). At these sites, lamin A was predominantly seen in the nuclei, in a heterogeneous distribution. Small amounts of lamin A were also noted in the cytoplasm of these cells.

The stromal tissue, especially cells making up the basement membrane and muscularis mucosae, exhibited lamin A expression in the nuclei in a heterogeneous distribution. This expression profile of lamin A appeared similar to that seen with lamin A/C expression in the crypt and surrounding mesenchymal tissue.

3.2.5Lamin C expression in the adult human colon crypts (Figure 3.7)

The expression profile of lamin C in the crypt was different to that of lamin A. In the basal regions, at the putative stem cell niche area, there was no obvious expression of lamin C at either the nuclear envelope or in the nucleoplasm (figure 3.7 B). As the crypt axis was traced upwards and into the proliferative compartment, again there was no discernable trace of lamin C expression in the cells or their nuclei.

Approximately two thirds of the way up the crypt axis, there was a progressive increase in lamin C expression, being maximal in the cells lining the mucosal surface. In this area of the crypt, lamin C expression was predominantly at the nuclear area in a heterogeneous distribution pattern (figure 3.7 A).

Figure 3.7

Expression profile of lamin C in the intestinal crypts of the colon and rectum

Figure A

Expression profile of lamin C in cells lining the upper third of the crypt (indicated by arrowheads) and corresponding to the differentiated zone

Scale bar is set at 100µm. (40x)

Figure B

No visible expression of lamin C in cells lining the basal region of the colonic crypt (indicated by arrow). However, expression of lamin C is seen in cells forming the muscularis mucosae

BM indicates Basement Membrane

Scale bar set at 100µm. (40x)

Lamin C expression is noted only in cells making up the differentiated compartment of the crypt and the muscularis mucosae. There was complete absence of lamin C from cells at the base of the crypt or putative stem cell niche area. Expression of lamin C in cells making up the muscularis mucosae was used as a positive internal control test. In cells expressing lamin C, its location was predominantly at the nuclear area.

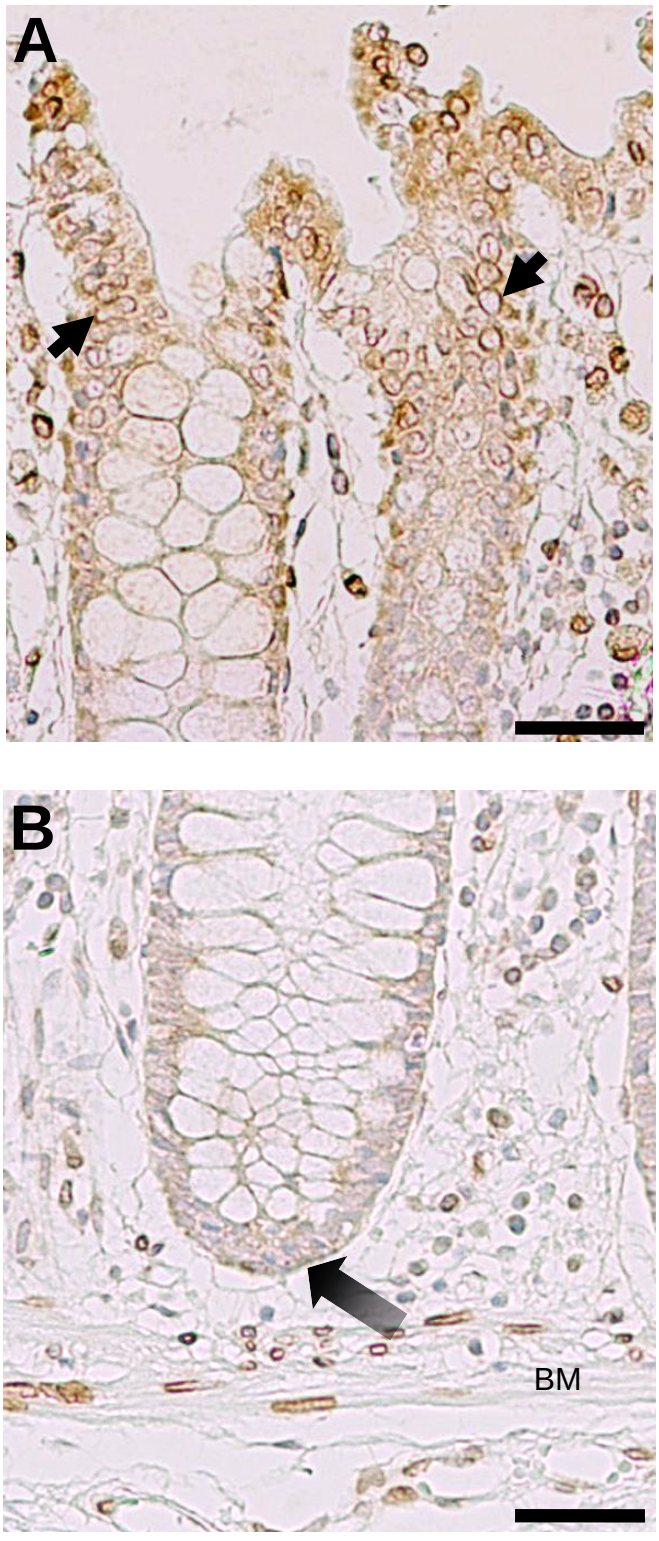


Figure 3.7 (A and B)

Expression profile of lamin C in the intestinal crypts of the colon and rectum.

Expression of Lamin C was noted in cells of the stromal tissue and smooth muscle surrounding the crypts. This was mostly at the nuclei in a heterogeneous distribution. The distribution of lamin C expression in this area was similar to that seen with lamin A/C expression.

3.2.6 Lamina associated polypeptide 2 alpha (Lap 2 alpha) expression in the adult human colon crypts (Figure 3.8)

Expression of Lap 2 alpha was observed along the whole length of the colonic crypt. However, its expression varied at different locations. At the base of the crypt, Lap 2 alpha expression was predominantly at the nuclei of the cells but cytoplasmic staining was also noted (figure 3.8 B). In this location, a heterogeneous distribution of Lap 2 alpha in the cytoplasm was noted. The total number of cells expressing this distribution pattern was difficult to quantify in the basal region. This was mostly due to the continuing expression of Lap 2 alpha in cells of the proliferative compartment. However, interestingly the expression of Lap 2 alpha showed a tendency towards cytoplasmic expression more than nuclear in cells of the proliferative compartment. There was also a decrease in total expression of Lap 2 alpha in cells of the proliferative compartment. This decrease in expression formed a gradient in the lower two thirds of the crypt axis with a gradual return of Lap 2 alpha expression towards the upper one third of the crypt, in the differentiated compartment. Another observation about the distribution of Lap 2 alpha in the differentiated compartment was the granular distribution of Lap2 alpha in the cytoplasm of these cells, especially in cells forming the mucosal lining of the intestine. In these cells, nuclear expression of Lap 2alpha was seen in a heterogeneous distribution (figure 3.8 A).

Lap 2 alpha expression in the stromal tissue surrounding the crypts was very patchy and infrequent. However, the majority of mesenchymal cells that did express Lap 2 alpha were located towards the apical regions of the crypt although occasional cells close to the muscularis mucosae also exhibited expression of LAP

Figure 3.8

Expression profile of LAP2α in the intestinal crypts of the colon and rectum.

Fig A

Expression and distribution of Lap 2α in cells lining the upper half of the colonic crypt. Both nuclear and cytoplasmic expression was noted (short arrows).

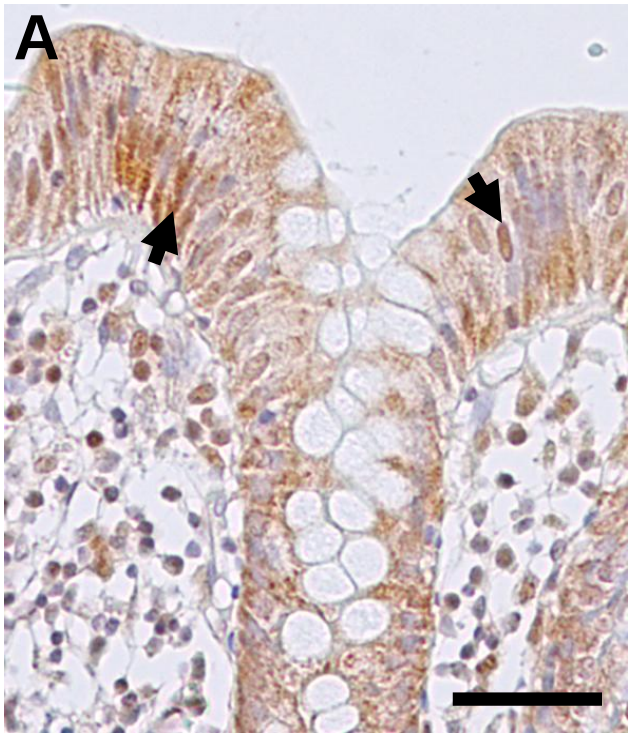
Scale bar set at 150µm. (40x)

Fig B

Expression and distribution of Lap 2α in cells lining the lower half of the colonic crypts (long arrows).

Scale bar set at 150µm. (40x)

The expression of LAP2α was noted in all cells making up the crypt. Due to its granular distribution in the cytoplasm and nucleoplasm as well, it was difficult to assess and ascertain any definitive pattern of expression. However, there appeared to be a generalised increased expression in the nuclear area of cells at the base of the crypt with a loss of nuclear area expression in cells making up the proliferative compartment.



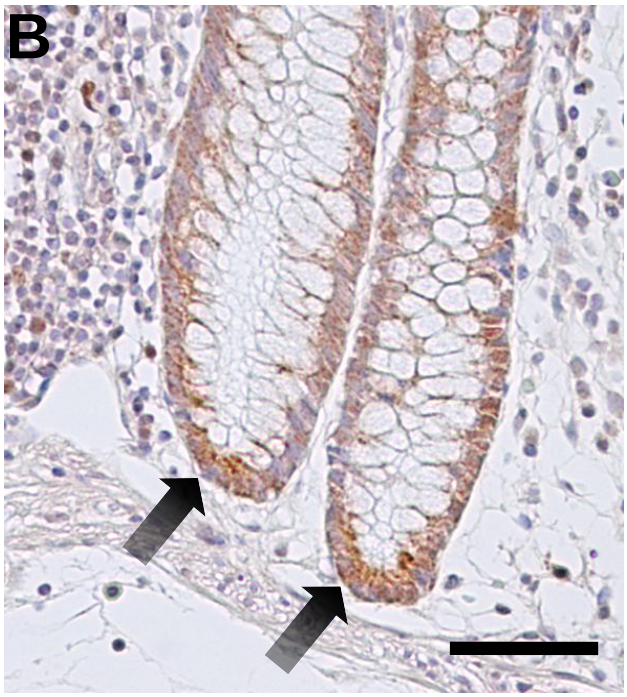


Figure 3.8 (A and B)

Expression profile of LAP2α in the intestinal crypts of the colon and rectum.

2 alpha. In these mesenchymal cells, expression of Lap 2 alpha was nuclear with a heterogeneous distribution. The muscularis mucosa stood out in its absolute lack of Lap 2 alpha expression (figure 3.8 B).

3.2.7 Emerin expression along the adult human colon crypt (Figure 3.9)

The expression of Emerin along the crypt axis revealed a distribution pattern similar to that seen with Lap 2 alpha expression; however there appeared to be some notable exceptions. In the basal areas of the crypt, corresponding to the putative stem cell niche area, an up regulation of Emerin expression was noted. It was concentrated mostly in the nucleus (figure 3.9 B). However, there was also significant expression of emerin in the cytoplasm of these cells, in a heterogeneous distribution. Emerin expression was reduced in the region adjacent to the basal area, in the proliferative zone. This decreased level of emerin expression could be traced upwards to the junction of the upper third and lower two thirds of the crypt region, corresponding to the proliferative compartment of the crypt. Here only a small degree of expression was noted, mostly in the cytoplasm. In the upper third of the crypt, there was significant expression of Emerin, mostly in the cytoplasm of cells, but also at the nucleus. The expression of Emerin in the cytoplasm appeared heterogeneous (figure 3.9 A).

The expression of Emerin in the stromal tissues revealed a pattern different to that seen with Lap 2 alpha expression. The mesenchymal cells of the lamina propria revealed a predominantly cytoplasmic expression of Emerin with no obvious nuclear expression. Also, there did not appear to be any specific gradient of Emerin expression in this area. However, cells in the muscularis mucosae and submucosa expressed Emerin in their nuclei, in a heterogeneous distribution. This was in contrast to Lap 2 alpha expression in the muscularis mucosae, where no discernable expression was seen in these areas (figure 3.9 A & B).

Figure 3.9

Expression profile of Emerin in the intestinal crypts of the colon and rectum

Figure A

Expression of emerin in cells lining the upper half of the colon crypt (short arrows).

Scale bar set at 150 µm. (40x)

Figure B

Expression of emerin in cells forming the basal regions of the colonic crypts (long arrow). Emerin also stains the nuclear envelope of cells in the surrounding stromal tissue.

Scale bar set at 150 µm. (40x)

The expression of emerin is noted in most cells making up the crypt. However, there appears to be an up regulation in its expression in cells located at the base of the crypt, in the putative stem cell niche area (as indicated by the long arrow). In the proliferative compartment of the crypt, a change in expression of emerin was noted with cytoplasmic expression predominating although some nuclear envelope expression was also noted. In the differentiated compartment of the crypt, both heterogeneous cytoplasmic (black short arrow) and nuclear envelope expression (blue short arrow) were also noted.

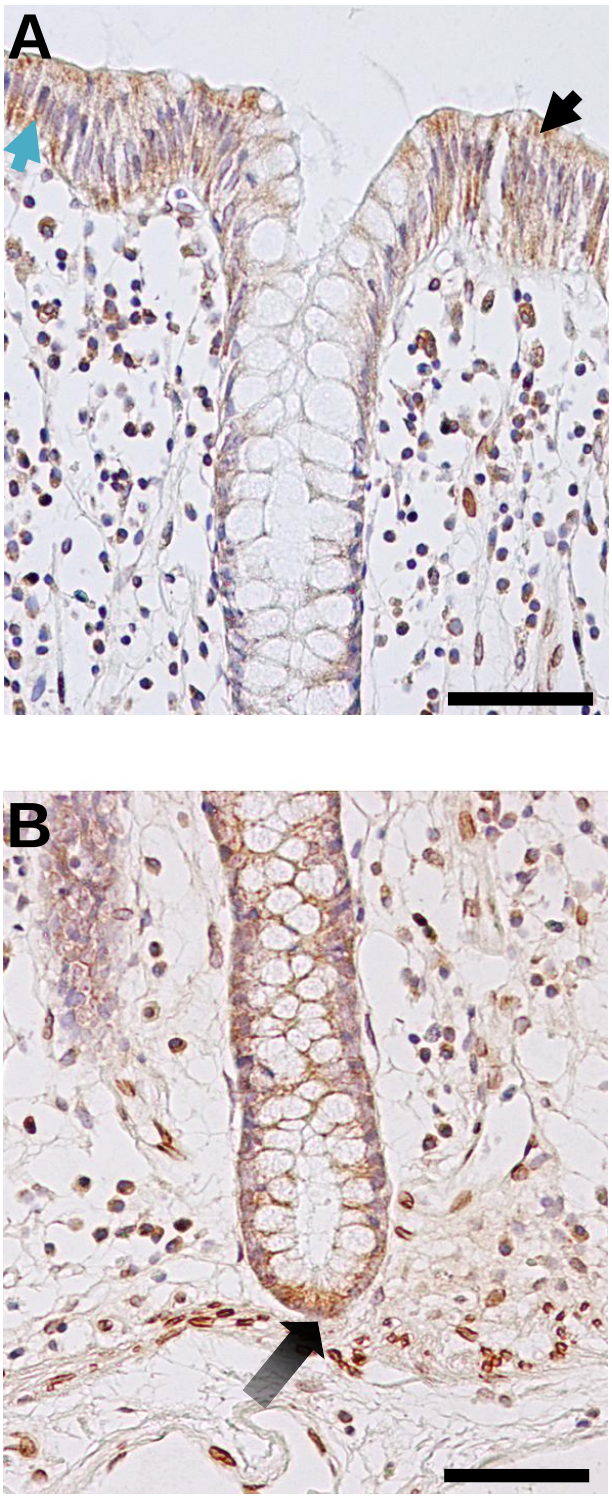


Figure 3.9 (A and B)

Expression profile of Emerin in the intestinal crypts of the colon and rectum.

3.2.8Beta catenin expression in adult human colon crypts

The expression profile of total beta catenin and active beta catenin in normal colonic and rectal tissue samples was assessed in all patients. The colonic samples were from various areas of the colon, including ascending, transverse, descending and sigmoid parts of the colon and did not show any obvious differences in expression profiles based on location.

3.2.8.1 Total beta catenin expression in adult human colon crypts (Figure 3.10)

Total beta catenin was expressed throughout the crypt but a change in expression amongst individual cells was also noted as cells moved up along the crypt axis, towards the lumen of the intestine. In the basal regions of these crypts, total beta catenin was highly expressed in a few cells, corresponding to the putative stem cell niche area. Total beta catenin was expressed predominantly at the nuclear area of these cells. Cytoplasmic and intra-nuclear expression of total beta catenin was also observed in these basal cells. There did not appear to be any significant expression at the cellular junctions in these basal cells (figure 3.10 B).

Above these basal cells in the crypt, total beta catenin expression appeared to diminish somewhat but was still recognisable at the nuclear envelope and cytoplasm. Intra-nuclear expression of total beta catenin was difficult to assess but appeared to diminish to some extent. This pattern of expression in the proliferative compartment of the crypt continued up to the upper third of the crypt, where there was a further change in the expression profile of total beta catenin.

In the upper third of the crypt, total beta catenin expression appeared predominantly cytoplasmic with a shift towards cellular junctions (figure 3.10 A).

Figure 3.10

Expression of total β-catenin in the intestinal crypts of the colon and rectum

Figure A

Expression and distribution profile of total β catenin in cells lining the upper half of the colonic crypt (short arrows).

Scale bars set at 150µm. (40x)

Figure B

Expression and distribution profile of β catenin in cells at the base of the colon crypts (large arrows).

Scale bars set at 150µm. (40x)

The expression of total β-catenin was observed throughout all cells making up the crypt. It was predominantly observed at the nuclear envelope with intranuclear expression also being noted in cells residing at the base of the crypts, at the putative stem cell niche area. In the proliferative compartment, nuclear envelope expression of total β-catenin was also observed. However, in the differentiated compartment, total β-catenin expression was predominantly in the cytoplasm with increased levels of expression being noted at the intercellular junctions.

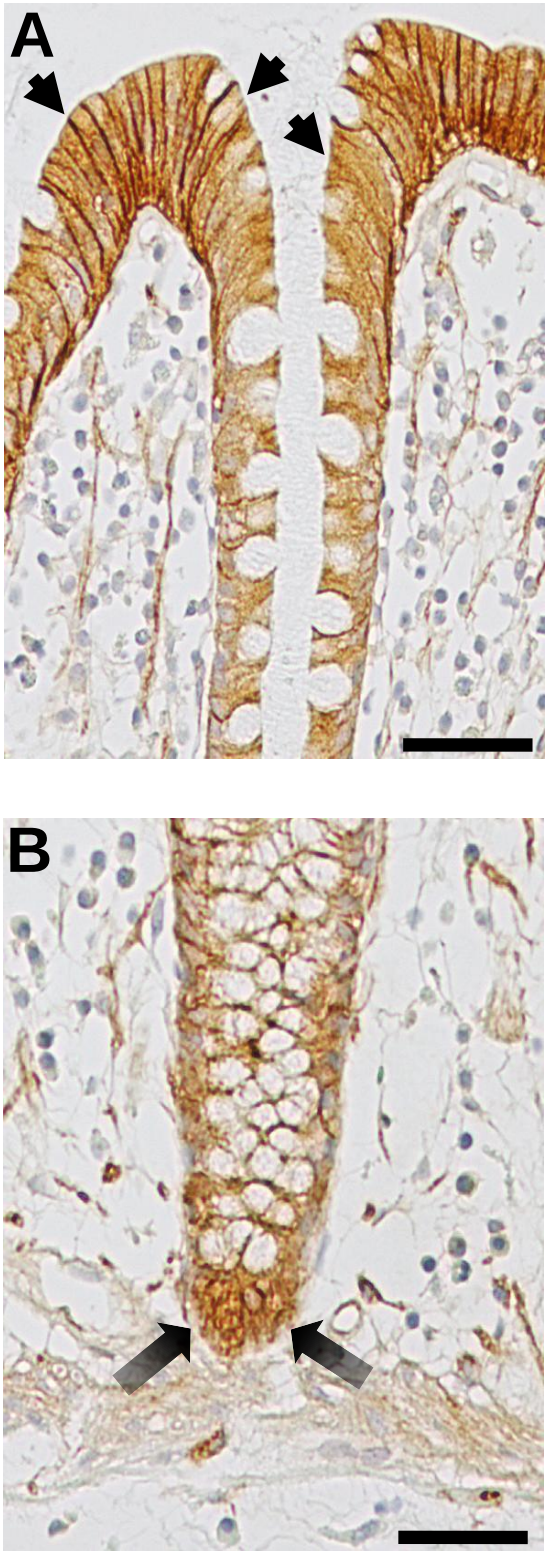


Figure 3.10 (A and B)

Expression of total β-catenin in the intestinal crypts of the colon and rectum

This was more marked in cells at the luminal surface. Some degree of patchy nuclear expression could be discerned though.

The surrounding mesenchymal tissue around each crypt revealed a distinct paucity of total beta catenin expression. Similarly, the muscularis mucosa did not exhibit any distinct expression of total beta catenin.

3.2.8.2 Active beta catenin expression in adult human colon crypts (figure 3.11)

Active beta catenin had a limited expression profile in the crypt and required high resolution imaging to accurately identify its location(s) in the cell. In the basal regions of the crypts (figure 3.11 B), it was seen at the nuclear periphery predominantly. At this site, very little, if any, cytoplasmic expression of active beta catenin was noted.

Above this basal zone, into the proliferative compartment, the expression of active beta catenin further decreased. Due to very low expression levels, it was difficult to ascertain accurately the exact distribution of beta catenin in the proliferative compartment. At this level active beta catenin was mostly concentrated at the nuclear area (figure 3.11 B) in a patchy manner. No obvious cytoplasmic expression of active beta catenin was observed in cells of the proliferative compartment.

About two thirds of the way up the crypt axis, active beta catenin expression was noted at the nuclear area of cells and in the cytoplasm as well. The expression of beta catenin became more prominent towards the luminal surface of the crypt. Interestingly, active beta catenin accumulated at the nuclear envelope in a rather patchy manner, with relatively normal looking portions of nuclear membrane in between. Expression of active beta catenin in the cytoplasm appeared granular and increased expression was noted at intercellular junctions (figure 3.11 A).

Figure 3.11

Expression of active β-catenin in the intestinal crypts of the colon and rectum

Figure A

Expression and distribution profile of active β catenin in cells lining the upper half of the colonic crypt. Active β catenin is expressed predominantly in the cell cytoplasm in a fairly uniform fashion. However, at the intercellular boundaries, this expression is more noticeable (short arrows). Scale bar set at 150µm. (40x)

Figure B

Expression and distribution profile of active β-catenin in the lower half of the intestinal crypts of the colon and rectum (short arrows).

Scale bars set at 150µm. (40x)

The expression of active β-catenin in the lower half of the crypts was difficult to asses due to very low expression levels. However, patchy expression at the nuclear periphery was noted. In the differentiated zone, active β-catenin was predominantly expressed in the cytoplasm of cells with a concentration of active β-catenin at the intercellular junctions being noted.

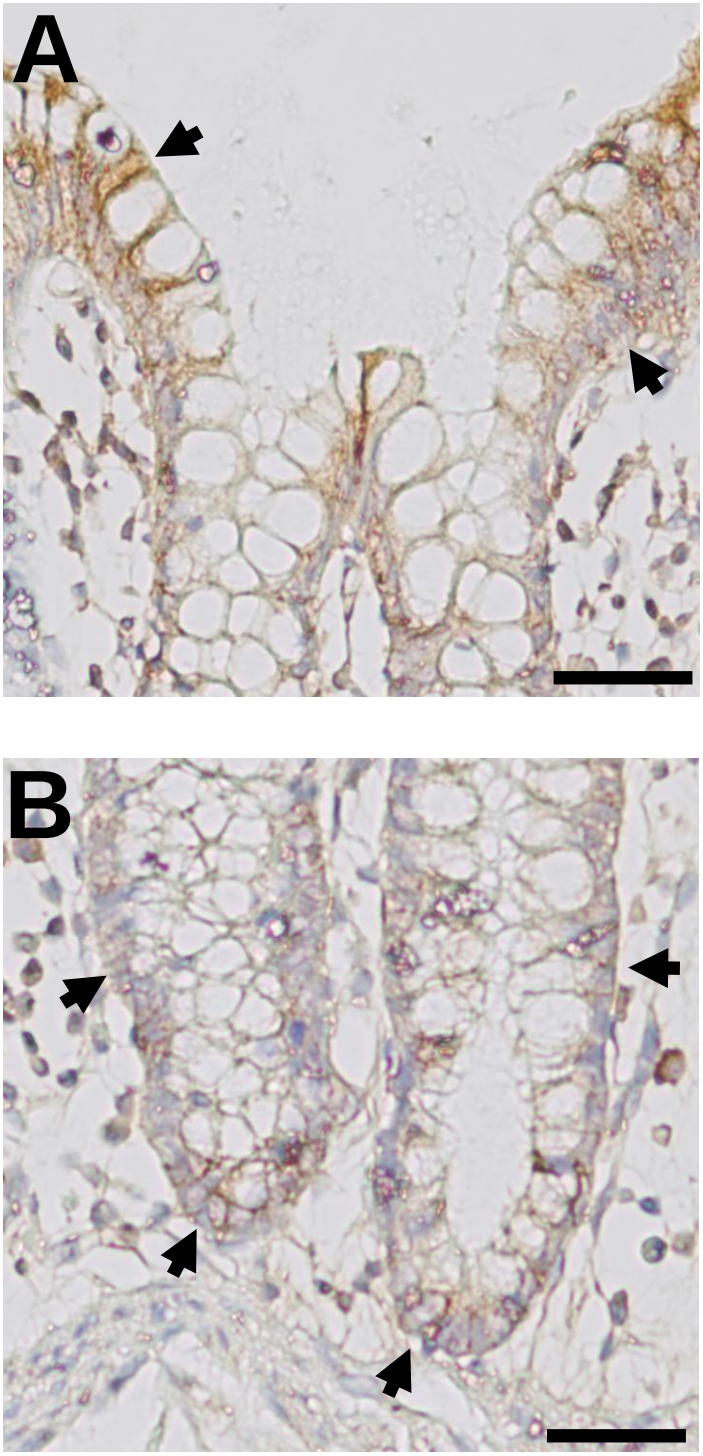


Figure 3.11 (A and B)

Expression of active β-catenin in the intestinal crypts of the colon and rectum

Figure 3.12 – 3.14

Comparative profiles of various nuclear proteins

The mesenchymal tissue surrounding each crypt exhibited occasional cells showing expression of active beta catenin. In cells that did express active beta catenin, it was predominantly located at the nuclear area with no obvious cytoplasmic expression being noted.

Figure 3.12 (A to F) compared, side by side, the expression profiles of Lamin A/C (Figure 3.12 A and B), Lamin A (Figure 3.12 C and D) and Lamin C (Figure 3.12 E and F) in normal colonic crypts.

Figure 3.13 (A to D) compared, side by side, the expression profile of Lamin A/C (Figure 3.13 A and B) and total beta catenin (Figure 3.13 C and D) in normal colonic crypts.

Figure 3.14 (A to D) compared, side by side, the expression profile of LAP 2α (Figure 3.14 A and B) and Emerin (Figure 3.14 C and D) in normal colonic crypts.

Figure 3.12

A comparative analysis between lamin A/C, lamin A and lamin C in the intestinal crypts of the colon and rectum

Figures A & B

The expression and distribution profile of A-type lamins in the cells lining the colonic crypt. Scale bar set at 100 µm. (40x)

Figures C & D

The expression and distribution profile of lamin A in cells lining the colonic crypt. Scale bar set at 150 µm. (40x)

Figures E & F

The expression and distribution profile of lamin C in cells lining the colonic crypt. Scale bar set at 150 µm. (40x)

Heavy arrows point towards the putative stem cell niche area in micrographs B, D and F respectively.

Arrow heads in micrographs B, D and F point out the presumed proliferative compartment area.

Arrow heads in micrographs A, C and E point out the luminal cells or the presumed differentiated compartment area.

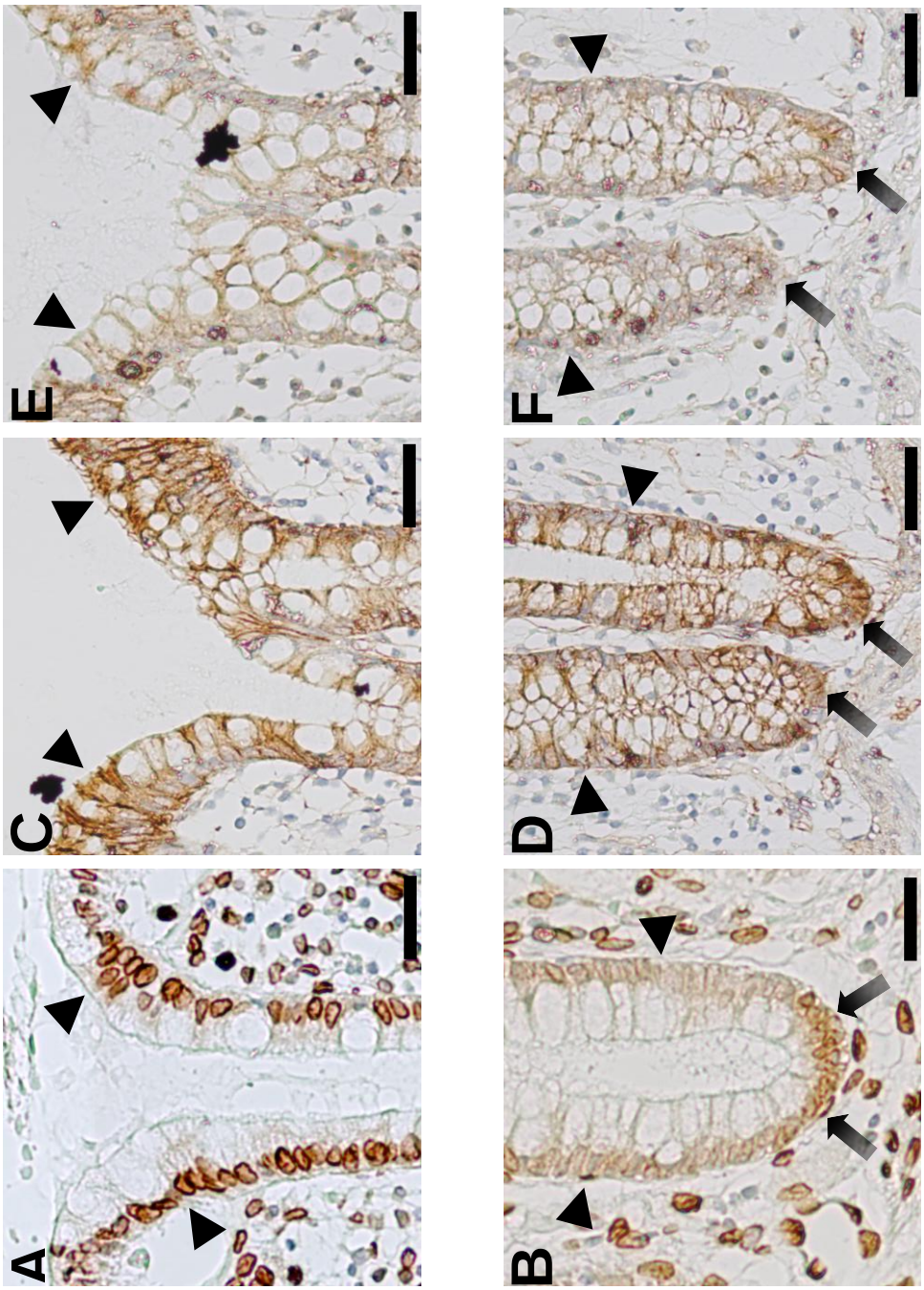


Figure 3.12 (A to F)

A comparative analysis between lamin A/C, lamin A and lamin C in the intestinal crypts of the colon and rectum.

Figure 3.13

Comparative analyses between Lamin A/C and total β-catenin in the intestinal crypts of the colon and rectum

Figures A & B

Lamin A/C expression in the crypt. Scale bar set at 100 µm. (40x)

Figures C & D

Total β-catenin expression in the crypt. Scale bar set at 100 µm. (40x)

Long arrows in micrographs B & D point towards the putative stem cell niche area.

Short arrows in micrographs B & D point towards the proliferative compartment.

Short arrows in micrographs A & C point towards the luminal cells or differentiated compartment.

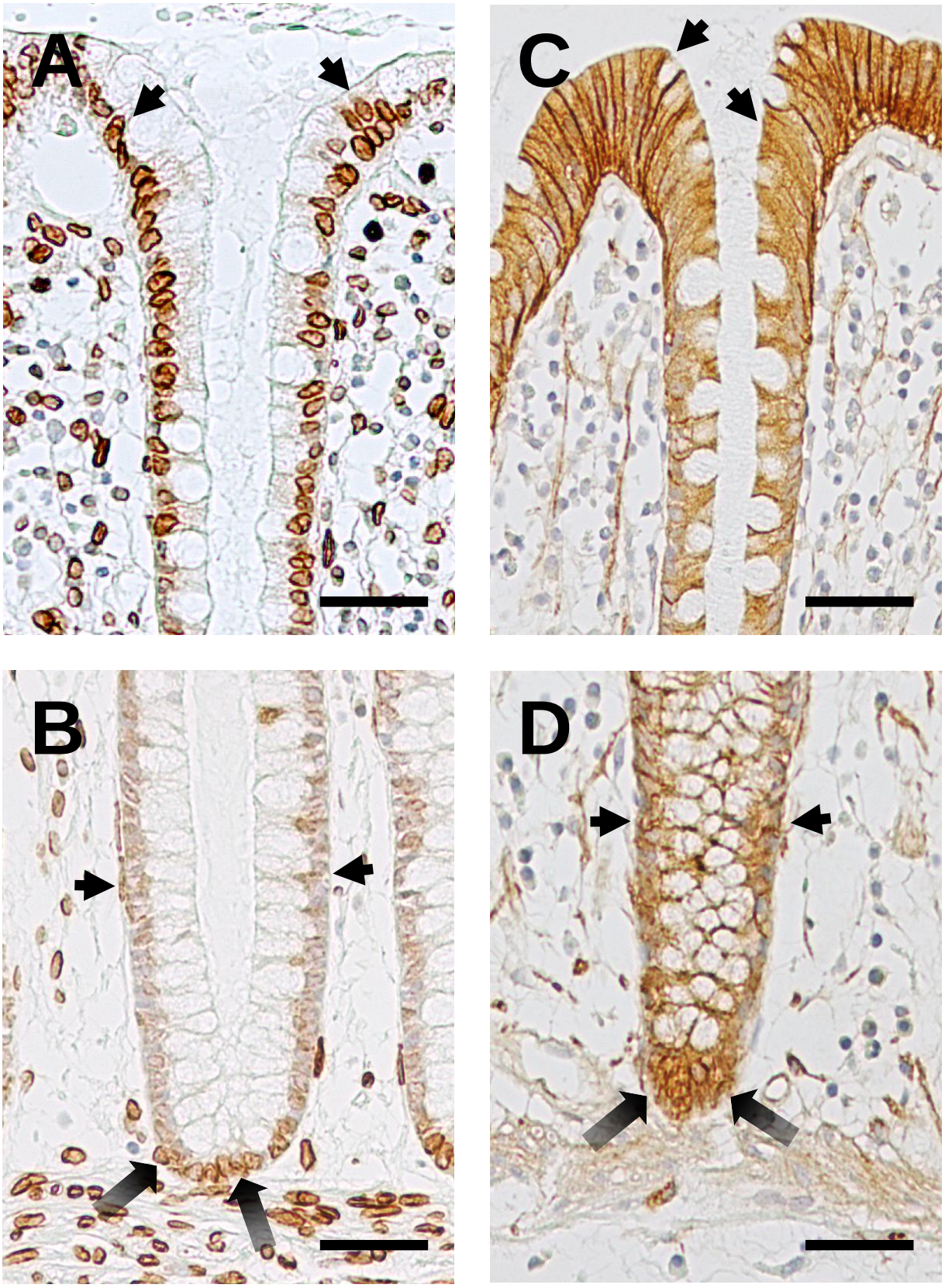


Figure 3.13 (A, B, C and D)

Comparative analyses between Lamin A/C and total β-catenin in the intestinal crypts of the colon and rectum

Figure 3.14

Comparative analyses between expression of lap 2 α and Emerin, in the intestinal crypts of the colon and rectum

Figures A & B

Expression of Lap 2 α in the crypt. Scale bars set at 150 µm. (40x)

Figures C & D

Expression profile of Emerin in the crypt.

Scale bar set at 150 µm. (40x)

Large arrows in micrographs B & D point to crypt base, site of the putative stem cell niche.

Small arrows in micrographs A & C point to the luminal surface of the crypts where differentiated cells reside.

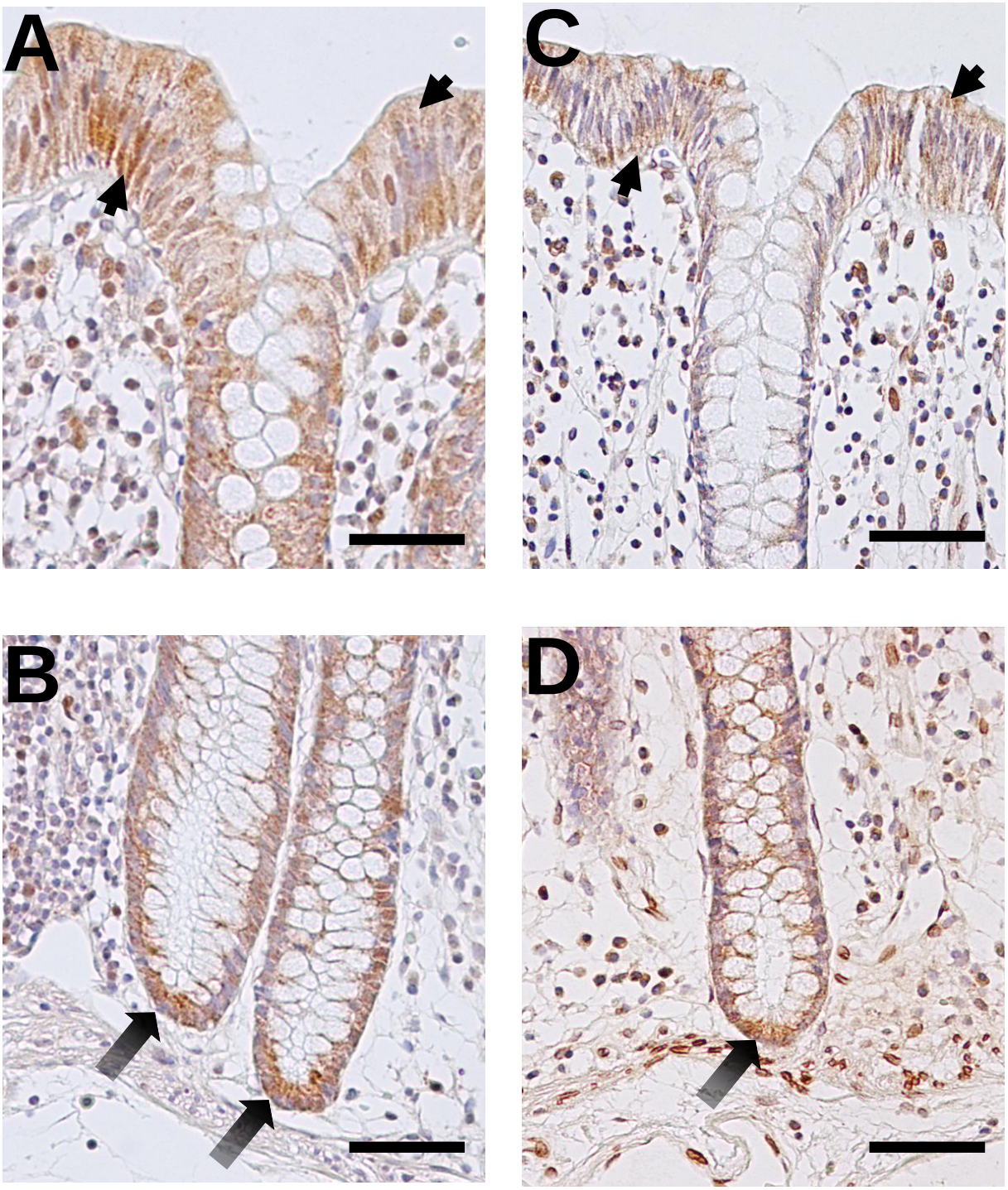


Figure 3.14 (A, B, C and D)

Comparative analyses between expression of lap 2 α and Emerin in the intestinal crypts of the colon and rectum

3.2.9 The stem cell niche in the adult human colon

At the basal regions of the intestinal crypts, in the colon and rectum, is the putative stem cell niche area, above which the proliferative compartment is located (Brittan and Wright, 2004; Potten, 1998). Stem cells are known to divide at a slower but more constant rate and give rise to daughter cells that rapidly divide and move up along the crypt axis, under the influence of various factors and signalling cascades, including Wnt / β-catenin signalling. These rapidly proliferative cells occupy the lower two thirds of the crypt, above the basal or putative stem cell niche region. Lamin A/C is a well known marker for differentiation (Hutchison, 2004), corroborated by its strong expression in the differentiated compartment of the crypt. Its presence in the putative stem cell niche area of the intestinal crypts was confirmed by repeated experiments. To further clarify this observation, antibodies against proliferating cells such as Ki-67 and anti PCNA (Proliferating Cell Nuclear Antigen) were used to study the expression profile of proliferating cells in the crypt. Results obtained with using anti PCNA antibody were more consistent and reproducible and hence I opted to use this antibody in preference to Ki-67. PCNA or Proliferating Cell Nuclear Antigen, also known as cyclin, is an auxiliary protein of DNA polymerase δ that is essential for DNA replication during S-phase (Prives and Gottifredi, 2008) where it is present in the nucleoplasm of continually dividing cells.

Serial sections of normal colonic and rectal mucosa were carefully prepared. After checking for appropriate orientation using H&E staining, serial sections were assessed for lamin A/C expression (figure 3.15 A) alternating with sections assessed for PCNA expression (figure 3.15 B). Repeated experiments confirmed the observation that all cells expressing PCNA did not express lamin A/C and vice versa. Basal cells in the putative stem cell niche area showing expression of lamin A/C did not reveal any PCNA expression, indirectly confirming these cells not to be proliferating cells (figure 3.15 A & B).

Figure 3.15

Serial sections of crypts showing expression of lamin A/C and PCNA in the intestinal crypts of the colon and rectum

Figure A

Expression and distribution of lamin a/c in colonic crypts. Scale bar at 150 µm. (20x)

Figure B

Expression and distribution of PCNA in colonic crypts. Scale bar set at 150 µm. (20x)

There is a reciprocal loss of lamin a/c expression in areas expressing high levels of PCNA and vice versa (compare arrows and arrow heads in both figures).

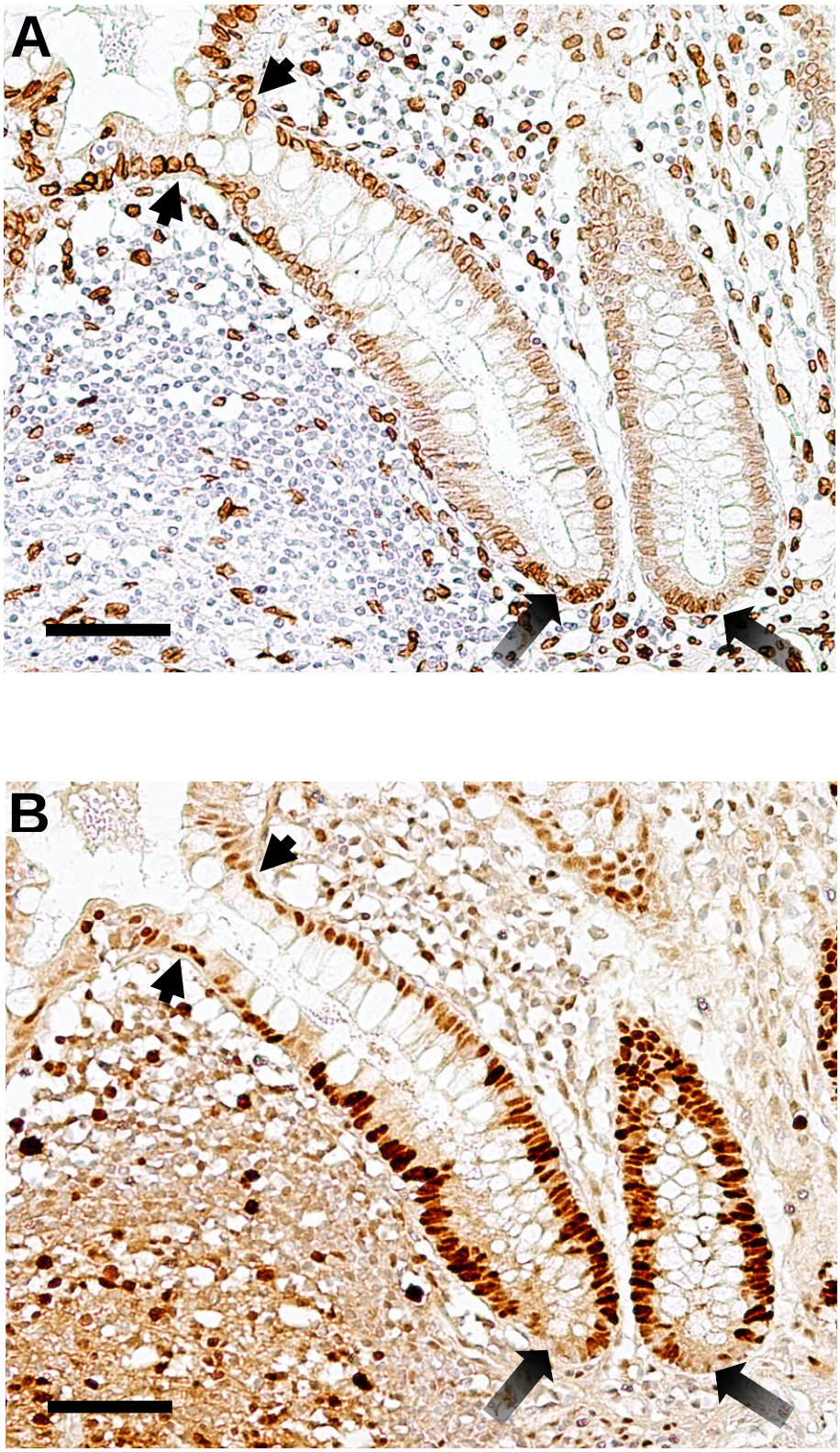


Figure 3.15 (A and B)

Serial sections of crypts showing expression of lamin A/C and PCNA in the intestinal crypts of the colon and rectum

3.2.10 Lamin A/C expression in colorectal cancers (Figure 3.16)

To assess the expression of lamin A/C in colorectal cancers, multiple sections were taken from tumour specimens of all stages i.e. Dukes A through to Dukes C and assessed using Jol2, an antibody directed against lamin A/C. Intriguingly, differential expression of lamin A/C was noted in various samples. In some cases there was a distinct lack of lamin A/C expression in the epithelial component of the cancerous tissue while in other cases a distinct expression of lamin A/C in the epithelial component of the cancerous tissue was noted (Figure 3.16). In all cases, the lamina propria or mesenchymal tissue expressed lamin A/C. This expression of lamin A/C in the lamina propria proved useful as an internal control to assess the effectiveness of lamin A/C antibody used. In the cancer tissue expressing lamin A/C, this expression was noted to be in the nuclear area predominantly in a heterogeneous distribution, but with some increased expression also being noted at the nuclear periphery. The surrounding mesenchymal tissue showed expression of lamin A/C at the nuclear area predominantly in a heterogeneous distribution. This differential expression of lamin A/C in colorectal cancer samples was noted across the whole range of Dukes A to C cancers.

Figure 3.16 (A and B)

Expression of Lamin A/C in colorectal cancer

Figure A

Dukes B colorectal cancer exhibiting expression of lamin A/C in the epithelial layer (arrow). (40x)

Figure B

Dukes B colorectal cancer exhibiting a distinct lack of lamin A/C expression in the epithelial layer. (40x)

Lamina propria (LP), exhibiting expression of lamin A/C in both samples and acting as an internal control.

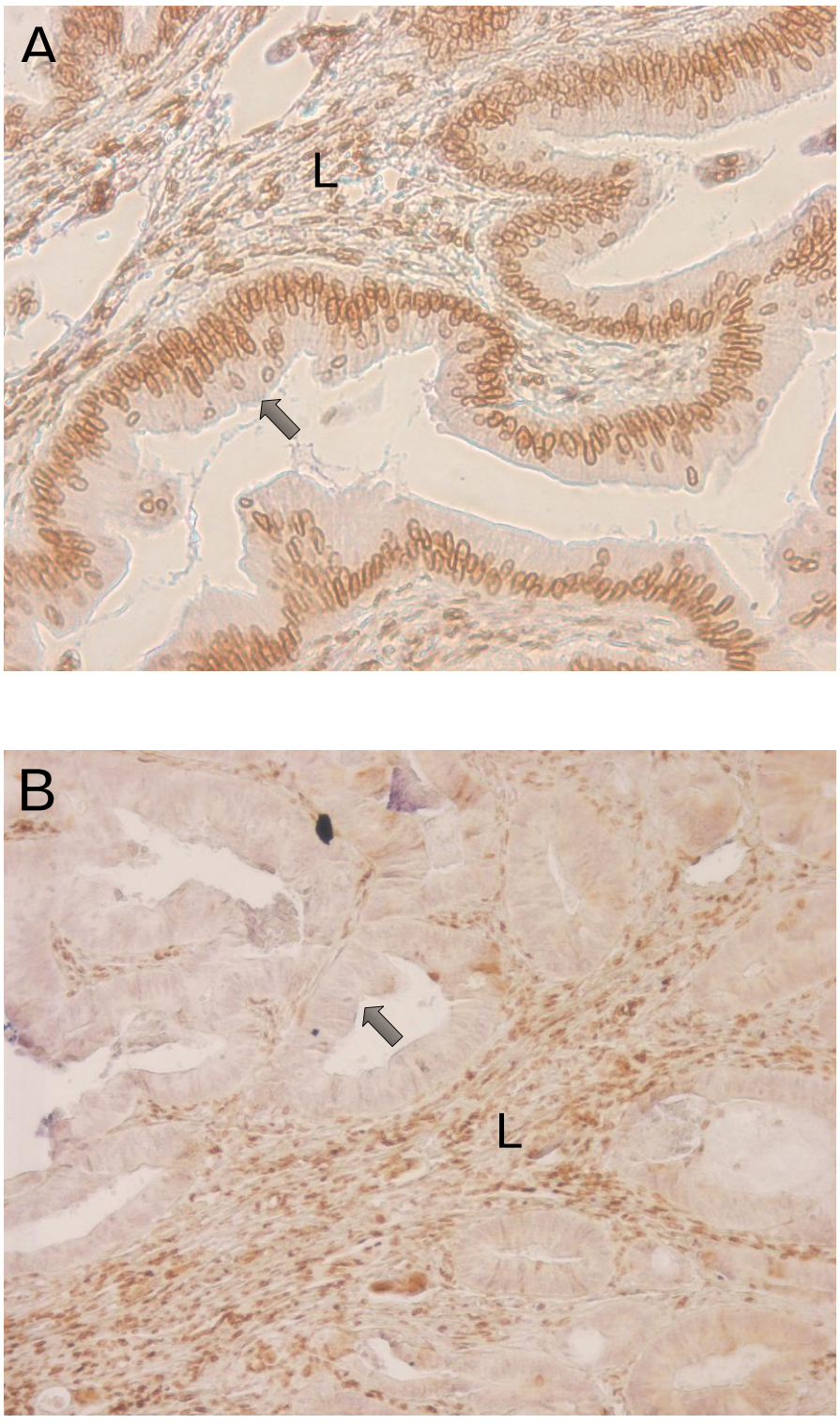


Figure 3.16 (A and B)

Expression of lamin A/C in a Duke's B colorectal cancer from two patients
Figure A – Nuclear envelope expression of lamin A/C is indicated by arrows
Figure B – No nuclear envelope expression of lamin A/C is observed in cells as indicated by the arrow. L – Lamina Propria acts as an internal +ve control.

3.4 DISCUSSION

3.4.1 Normal crypt Architecture

In the large intestine of humans, the smallest functional unit is the intestinal crypt. In anatomical terms the crypt is a tubular or flask shaped structure lined by a single layer of columnar cells. At the base of a crypt, these cells are in close proximity to the muscularis mucosae, a smooth muscle layer. The space in between crypts and muscularis mucosae, the lamina propria, is occupied by mesenchymal cells, of indeterminate function, and lymphocytes that occasionally aggregated to form lymphoid follicles. The actual height of any crypt, in terms of number of cells is very difficult to quantify due to the tremendous variation in crypt structure with regards to cell numbers (Potten, 1998). The other observation noted is the apparent partial division of some crypts into bifid crypts with a common opening onto the luminal aspect. This observation may be in keeping with the reported observation of crypt fission noted by others previously (Brittan and Wright, 2004).

3.4.2 Lamin A/C Expression

There are various studies looking at the expression profiles of lamin A/C in normal colonic mucosa and comparing it with cancers arising from the same tissue. However, there are no substantial studies available that look at the expression profile of Lamin A/C and its binding partners in colonic crypts. By using antibodies specific to lamin A/C, it is observed that the expression profile of lamin A/C in the crypt is restricted to fairly well demarcated zones. Specifically, in the crypt there appear to be two areas in particular that express lamin A/C strongly. In the upper third of the crypt, there is obvious expression of lamin A/C at the nuclear envelope along with heterogeneous intra-nuclear expression. There is an obvious gradient of lamin A/C expression noted as well, with the highest levels of expression at the luminal surface of the crypt, in an area where maximal differentiation is to be

expected. Lamin A/C is a well documented marker for cell differentiation (Lehner et al., 1987; Lourim and Lin, 1992; Rober et al., 1989; Venables et al., 2001). In cells of the upper third of the crypt areas, expression of lamin A/C is accompanied by a loss / substantial decrease in proliferating cell markers, PCNA, which lends further evidence towards these cells being differentiated.

In addition, there is a change in the expression of total β-catenin noted in cells making up the upper third of the crypt. Total β-catenin appears to translocate towards the cytoplasm and intercellular junctions with a decrease in its expression at the nuclear envelope. It is already known that β-catenin together with APC proteins forms part of the canonical Wnt signalling pathway and is responsible for driving cells into a proliferative state (Sancho et al., 2004). This signalling cascade, as discussed earlier in chapter 1, exerts its maximum influence in the lower two thirds of the crypt axis. The total number of cells making up the proliferative compartment or indeed the differentiated compartment is variable, however, on average, the junction of upper one third and lower two thirds marks this transition area. The mesenchymal cells in the lamina propria around each crypt exhibit uniform expression of lamin A/C at the nuclear envelope. The significance of this finding is yet to be determined.

The next step was to identify if lamin A or C, or indeed both, were responsible for expression of lamin A/C noted in the crypt. By using specific antibodies against lamin A and C, the complete lack of lamin C expression in the basal region or putative stem cell niche area, is immediately evident. The other discernable difference between the expression profiles of lamin A and C, in cells making up the differentiated compartment, is the relatively late appearance of lamin C in these cells. In addition, there also appears to be an overall decrease in the relative amount of lamin C expression in the mesenchymal cells surrounding the crypts. Again, the significance of this observation is unclear at present. From previous studies by Pugh *et al* (Pugh et al., 1997), it has been shown that lamin A and C get incorporated into the nuclear lamina via different pathways. In case of lamin A, it gets incorporated directly into the nuclear lamina while lamin C first forms intra nuclear foci before being incorporated into the nuclear lamina. This results in an apparent delay observed in the incorporation of lamin C into the nuclear lamina. This observation seems difficult to explain given that lamin A and C both arise

from the same *LMNA* gene by differential splicing (Lin and Worman, 1993) and also, lamin A differs from lamin C by the presence of a 98 C-terminal amino acid residue (Fisher et al., 1986). However, significantly, lamin C lacks the Caax box motif which then induces lamin C to form intranuclear aggregates (Schmidt and Krohne, 1995).

3.4.3 Lamin A/C expression in relation to its binding partners

The nuclear lamina form the structural framework of a nucleus, which in turn determines the size, shape and mechanical stability of the nucleus (Moir et al., 2000). The major components of lamins are the nuclear lamina, which are type V intermediate filament proteins, and lamina associated polypeptides (LAPs), which are part of the integral membrane proteins of the inner nuclear membrane. Three lamina associated polypeptides have been characterised in detail in mammalian cells: LAP 1, the LAP 2 family and a protein called the lamin B receptor (LBR) (Gerace and Foisner, 1994). LBR and LAP2 preferentially interact with lamin B while LAP1 interacts with both lamin A and B types (Foisner and Gerace, 1993). LAP2 is a family of six alternatively spliced proteins. Four of these are type II membrane proteins: LAP2 β, -δ, -γ and –ε (Berger et al., 1996) and bind lamin B. LAP2α is different, both structurally and functionally. It shares only its N-terminus with the other isoforms and has a distinct C-terminus. It also lacks an apparent transmembrane binding domain (Harris et al., 1995). LAP2α is distributed throughout the nucleus rather than the nuclear envelope (Dechat et al., 1998; Dechat et al., 2000a; Vlcek et al., 1999) and is a specific binding partner of nucleoplasmic A-type lamins (Dechat et al., 2000a).

Emerin is a inner nuclear membrane protein that binds both lamins A and B (Clements et al., 2000; Lee et al., 2001; Sakaki et al., 2001) and is retained at the nuclear envelope by lamin A (Sullivan et al., 1999; Vaughan et al., 2001). It contains two regions with sequence similarity to LAP2. Emerin along with all LAP2 isoforms and MAN1, shares a common structural motif called the LEM-domain, which comprises an ~ 40 amino acid long structural motif (Cai et al., 2001; Laguri

et al., 2001). This domain mediates binding to barrier-to-autointegration factor (BAF), an essential and highly conserved DNA-binding protein. BAF is known to bind double stranded DNA without sequence specificity (Zheng et al., 2000).

In studying the expression profile of LAP2α in the crypt axis, cells known to express lamin A/C, at the basal regions, also exhibit an increased expression of LAP2α, in the region of the nucleus. In contrast, cells in the proliferative compartment exhibit a lack of nuclear expression of LAP2α, however, abundant cytoplasmic expression is evident and at times this makes it difficult to accurately identify LAP2α expression in the nuclear region. This observation indicates that LAP2α translocates to the cytoplasm in cells under-going rapid division as seen in cells of the proliferative compartment of the crypt. From previous studies (Dechat et al., 2004), it has been shown that LAP2α translocates to the cytoplasm during metaphase and returns to the nucleus at the exit of metaphase. In cells forming the differentiated compartment of the crypt, the presence of LAP2α is mostly inside the nucleus. This observation is in keeping with differentiated cells that are not dividing as LAP2α is uniformly distributed through the nuclear interior during interphase.

Emerin expression is linked closely to lamin A expression (Vaughan et al., 2001). In the intestinal crypts, cells at the basal region or the putative intestinal stem cell niche area show expression patterns similar to those of lamin A. However, in the proliferative compartment, cytoplasmic expression of emerin is noted and this is unusual. It is known that emerin disperses into the cytoplasm during metaphase, in the form of membrane bound vesicles also in the absence of lamin A/C (Dabauvalle et al., 1999; Vaughan et al., 2001). Cells in the proliferating compartment are undergoing rapid proliferation, whether this accounts for the cytoplasmic expression of Emerin or not, is not clear at present. In the differentiated compartment, expression of Emerin in the nuclear region is to be expected as this area also expresses lamin A/C; however, Emerin expression is also noted in the cytoplasm. At this stage the significance of this observation is not clear.

3.4.4 Putative stem cell niche and lamin A/C expression

At the base of the crypts, close to the basement membrane, expression of lamin A/C at the nuclear envelope is noted in a handful of cells ~ 8cells. This is unusual since the basal region of the crypt is an area presumed to harbour the stem cell niche (Barker and Clevers, 2007; Potten, 1998). A logical explanation for this observation is difficult to come by as up to date lamin A/C has only been associated with differentiated cell types. Also, there is an abrupt loss of lamin A/C expression noted in cells higher up the crypt, in the presumed proliferative compartment. Why should a limited number of cells at the basal regions of the crypt express this protein? The answer to this question is not clear. One conclusion could be that stem cells in the stem cell niche area are expressing lamin A/C (Willis et al., 2008). If so, what role, if any, does lamin A/C have in adult stem cell function? At present the answer to this remains unclear and one can only hypothesise. However, there has been increasing speculation about the role of lamin A/C in regulation of adult stem cell function to account for the multitude of tissues and organ systems involved in laminopathies (Gotzmann and Foisner, 2006).

3.4.5 Wnt / β-catenin signaling in colonic crypts

The role of Wnt signalling in gut homeostasis was established, unknowingly at the time, more than 15 years ago when the tumour suppressor gene Adenomatous Polyposis Coli (APC) was found mutated in a large number of hereditary and sporadic cases of colorectal cancers (Groden et al., 1991; Kinzler et al., 1991a; Kinzler et al., 1991b; Nagase and Nakamura, 1993; Nakamura et al., 1991). Since then, much work has been carried out in elucidating the role of Wnt signalling in gut homeostasis and colorectal cancer development. At present, the Wnt signalling cascade has been shown to play a central role in regulating the crypt villous axis, at different stages of gut development and cancer (Gregorieff and Clevers, 2005; Pinto and Clevers, 2005b; van de Wetering et al., 2002). In man,

19 Wnt genes have been identified, which encode for cysteine-rich glycoproteins. These Wnts activate coresponding cells by interacting with a seven-span transmembrane protein called Frizzled (Fz) and a single-span transmembrane protein LRP (Bhanot et al., 1996; Pinson et al., 2000; Tamai et al., 2000; Wehrli et al., 2000). This represents the initial step in the so-called Canonical Wnt signalling pathway, which leads to the formation of nuclear Tcf/β-catenin complexes. B-catenin has been identified as the key component of the Wnt canonical pathway. In the absence of Wnts, the scaffolding proteins APC and Axin/Axin2 sequester β-catenin, allowing casein kinase I (CKI) to phosphorylate the N-terminus of β-catenin at Ser S45, a residue often mutated in cancers (Amit et al., 2002; Liu et al., 2002). Subsequently, glycogen synthase kinase 3β (GSK3β) is recruited to phosphorylate additional serine and threonine residues from the N-terminal to S45. Phosphorylated β-catenin is then recognised by the F-box containing protein β-TrCP, which mediates ubiquitination and proteasomal degradation of β-catenin (Hart et al., 1999; Kitagawa et al., 1999; Winston et al., 1999). Together these proteins make up the β-catenin destruction complex.

Continued destruction of β-catenin is prevented following Wnt binding to Fz/LRP. Release of β-catenin from the destruction complex allows β-catenin to translocate into the nucleus. Here it associates with the Tcf family of transcription factors (Tcf1, Lef, Tcf3 and Tcf4) (Waterman, 2004). Tcfs function by targeting β-catenin to specific DNA elements found in promoters and enhancers of target genes. In turn, β-catenin recruites a number of nuclear factors responsible for transactivating Tcf target genes. In the absence of β-catenin, Tcfs associate with general transcription repressors such as Groucho (Cavallo et al., 1998).

The downstream target genes for Tcf / β-catenin have been identified. The more important ones include:

c-MYC, a bHLH transcription factor responsible for proliferation (He et al., 1998; van de Wetering et al., 2002);

Cyclin D1, a cell cycle regulator (Hulit et al., 2004);

Id2, an inhibitor of bHLH transcription factors (Russell et al., 2004);

ITF-2, a bHLH transcription factor (Kolligs et al., 2002);

Tcfl, involved in Wnt signalling (Roose et al., 1999);

PPARδ, ligand activated transcription factors (Gupta et al., 2004; Harman et al., 2004; Reed et al., 2004);

COX-2 or cyclooxygenase 2, involved in the prostaglandin pathway (Araki et al., 2003; Zhu et al., 2004);

HDAC2, histone deactylase (Zhu et al., 2004);

FGF18 and **FGF20**, growth factors (Chamorro, 2005 #1861;Shimokawa, 2003 #1862);

BAMBI (BMP and activin membrane bound inhibitor), over expression of which blocks TGFβ mediated growth inhibition (Sekiya et al., 2004);

The role of Wnt signalling in the intestine has been shown to be of key significance in many areas, including maintenance of the stem cell niche, the proliferative compartment and in the switch between proliferation and differentiation along the crypt axis. The presence of nuclear β-catenin is associated with an active Wnt signalling system. My observations, about the expression of β-catenin in the colonic crypts, are in keeping with those from other authors. Interestingly, a higher nuclear expression level of β-catenin is noted in cells forming the basal areas of the colonic crypt, in the putative stem cell niche area. This is significant since previous studies by Korinek et.al (Korinek et al., 1998) have shown the importance of Tcf4 (a down stream target of β-catenin) in maintaining the stem cell niche in the intestine. Similarly, in the proliferative compartment of the crypt, I have observed nuclear β-catenin, which indirectly alludes to an active Wnt signalling system in this area. Pinto et.al (Pinto et al., 2003) have shown the importance of Wnt ligands in driving the proliferative compartment of the crypts. In their studies, Pinto et.al (Pinto et al., 2003) observe disrupted intestinal homeostasis upon inhibition of the Wnt ligands using Dickkopf1 (Dkk1), a known Wnt inhibitor. They conclude that inhibition of β-catenin results in inhibition of c-MYC expression, and subsequent up regulation of $p21^{CIP1/WAF1}$, leading to cell cycle arrest. Loss of nuclear β-catenin expression in cells making up the differentiated compartment of

the crypt corresponds with loss of the Wnt signalling system. This is reflected by expression of lamin A/C in these cells, indicating differentiation. This key event whereby Wnt signals no longer affects cells and there is a switch over from proliferation to differentiation has always been an area of speculation. Studies by Markiewicz *et al* (Markiewicz et al., 2006) have highlighted the role of Emerin and lamin A/C in regulating β-catenin activity. From my observations, there is no sudden change in expression noted between the proliferative and differentiated compartments, but rather a gradual change taking place over 3 or 4 cells. Work done by Batlle et.al (Batlle et al., 2002) has shown that a Wnt signalling gradient controls cell positioning along the crypt axis through regulation of EphB2 and EphB3 gene expression.

Regulation of the Wnt signalling system itself has been an area of speculation. Different views are held on this with two non-mutually exclusive mechanisms that could explain how the stimulatory effects of the Wnt cascade are turned off in the intestine. In one scenario, it is proposed that Wnt signals could gradually and passively dissipate as progenitor cells moved up along the crypt. In the second scenario, it is believed that Wnt signals are actively counteracted by other signalling pathways. In support of the second scenario, various studies have implicated the TGFβ, BMP and Hedgehog signalling pathways as being negative regulators of the Wnt signalling pathway (Haramis et al., 2004; Hardwick et al., 2004; Howe et al., 2001; Sancho et al., 2004; van den Brink, 2007; van den Brink et al., 2004). Further studies will hopefully elucidate the underlying mechanisms of Wnt control in the intestine.

3.4.6 Lamin A/C expression in colorectal cancer

Lamin A/C is a well known marker for differentiation. Well differentiated colorectal cancers have a better prognosis as compared to poorly differentiated ones. The reasons accounting for a better prognosis in well differentiated cancers are many and include features such as a better response to chemotherapy and being less prone to metastasis as compared to poorly differentiated cancers, although this is dependant heavily on stage of the disease. To this effect it is assumed that

colorectal cancers of a higher grade and stage are more likely to express less lamin A/C and vice versa. It is surprising to note that there is a mix of lamin A/C expressing and non-expressing colorectal tumours for every stage of colorectal cancer. Using standard histological and pathological methods to assess these tumours for grade, stage, lympho-vascular invasion, perineural invasion, etc, it is noted that at any stage of this cancer i.e. Dukes A or B or C, there are tumours exhibiting either clear evidence of lamin A/C expression or no expression at all or impaired expression of lamin A/C. The significance of this finding is further explored in a separate study (Willis et al., 2008), who discovered lamin A/C to be an adverse prognostic indicator for patients with colorectal cancer. Lamin A/C proves to be an independent factor in their study on colorectal cancer cell lines and patients with colorectal cancer. To try and identify the underlying reason for this, a few hypotheses are put forward:

Firstly, colorectal cancers expressing lamin A/C have a more motile phenotype, based on in vitro cell mobility assays. This could explain why colorectal cancers expressing lamin A/C could, in theory, be more motile and metastasise early and thereby impart a poorer prognosis.

Secondly, colorectal cancers expressing lamin A/C may be more stem cell like, based on the observations that lamin A/C is expressed in the putative stem cell niche area. The implications for this observation are highly significant as colorectal cancers expressing lamin A/C may in fact be cancer stem cells with properties of stem cells and thus be immortal and capable of self regeneration indefinitely.

No definitive answer to explain this variation in lamin A/C expression in colorectal cancers can be found. There may be other explanations, in addition to the ones described earlier, to account for these observations and further work is needed to elucidate a definitive answer.

Chapter 4

Microarray Analysis of Various Stages Seen In Colorectal Cancer

4.1 Introduction

Developmentally, the human colon and rectum are derived from all three germ layers. Endoderm forms the epithelial lining of the lumen, mesoderm forms the smooth muscle and sub mucosal layers and ectoderm forms the enteric nervous system. Together, these germ layers give rise to a highly specialised organ with a very high cell turnover rate from the epithelial lining of the lumen. The histological structure of the colon, especially the epithelial lining of the lumen, lends itself admirably to a hierarchical system in which stem cells play a major role. The stem cell niche occupies the basal regions of the crypt villus axis followed by a proliferative compartment occupying the lower two thirds of the crypt villus axis and finally a differentiated zone in the upper one third that merges into the inter cryptal table. Control and regulation of the crypt villus axis is very complex indeed and is a site prone to undergoing neoplastic transformation. Multiple signalling cascades, alongside local and regional factors play a crucial role in maintaining a fine balance between optimum function and development of neoplasia.

4.1.1 Stem cell signaling pathways in the human and their significance in maintaining homeostasis, especially in the colon.

4.1.1.1 Canonical Wnt signaling

The canonical Wnt signalling pathway is considered to be the most significant regulator of normal crypt homeostasis and colorectal cancer development (Pinto and Clevers, 2005b; van de Wetering et al., 2002). A detailed discussion on Wnt signalling can be found in chapter one of this thesis. In the absence of a Wnt

signal, members of the T-cell factor / lymphoid enhancing factor (TCF/LEF) family of transcription factors are bound to the transcriptional repressor Groucho (Cavallo et al., 1998). Active beta catenin directly associates with TCF/LEF factors and overcomes this repression imposed by Groucho, thereby transactivating the transcription of downstream targets (van de Wetering et al., 1997). TCF-4 is a member of the TCF/LEF family and is highly expressed in cells forming the epithelial layer of the colon. It forms functionally active complexes with beta catenin (Korinek et al., 1997) which in turn are the principal governors of cell dynamics at the crypt villus junction, preserving a stem cell and proliferating progenitor population in the lower crypt region. This is essential for ensuring an orderly turnover of differentiated cells at the luminal surface (van de Wetering et al., 2002). By inhibiting the β-catenin/TCF-4 complex with inducible expression of dominant negative TCF-4 (dnTCF-4), Van de Wetering *et al.* (2002) were able to demonstrate: Firstly – a concomitant G1 arrest. Secondly – a down-regulation of intestinal markers of proliferation such as *c-MYC, EPHB2, c-MYB, BMP4, ENC1, CD44* and *CLDN1,* known to be expressed in the proliferative compartment of normal crypts, and thirdly – an up-regulation of differentiation markers such as *FABP1* (fatty acid binding protein 1), *CA2* (carbonic anhydrase II) and importantly $p21^{CIP1/WAF1}$. *c-MYC*, a well known oncogene which is responsible for pushing cells from G0 to S-phase of the cell cycle, appears to be one of the most important down-stream targets of the β-catenin/TCF-4 complex (He et al., 1998; Oster et al., 2002). Expression of *c-MYC* at endogenous levels in dn TCF-4 cells caused re-entry into the cell cycle with a concomitant reduction in $p21^{CIP1/WAF1}$ expression. In essence then, β-catenin/TCF-4 complexes appear to maintain a progenitor / stem cell phenotype in intestinal crypts through c-MYC mediated repression of the cell cycle inhibitor $p21^{CIP1/WAF1}$. Wnt signals are absent in the differentiated compartment of the crypt, this results in β-catenin being exported from the nucleus by APC and sequestered for removal by the degradation complex. There is activation of c-MYC in this zone; therefore $p21^{CIP1/WAF1}$ expression is induced which facilitates cell cycle arrest and differentiation (van de Wetering et al., 2002).

Previous studies have shown accumulation of β-catenin in the nuclei of crypt progenitor cells and aberrant crypt foci (ACF), benign precursors of colorectal cancer (van de Wetering et al., 2002), indicating re-initiation of Wnt signalling in

colon tumour cells. Up to 85% of colorectal cancers are sporadic, the result of mutations in the *APC* gene (Kinzler and Vogelstein, 1996) which constitutively activates β-catenin/TCF signalling (Korinek et al., 1997; Morin et al., 1997). Activation of the β-catenin/TCF complex may therefore be the dominant switch responsible in the malignant transformation of colon epithelial cells by imposing a proliferative phenotype at an early stage. This would imply that colorectal cancer is a process of dedifferentiation and increased proliferation as well (van de Wetering et al., 2002).

4.1.1.2 Hedgehog Signaling

The Hedgehog (Hh) signalling pathway was originally described in the development of *Drosophila melanogaster* as a segment polarity gene required for embryonic patterning (Nusslein-Volhard and Wieschaus, 1980). Hh signalling plays a key role in various processes including, embryogenesis, adult tissue repair during chronic inflammation, adult tissue homeostasis and carcinogenesis (Hooper and Scott, 2005; Lum and Beachy, 2004; Pasca di Magliano and Hebrok, 2003). The genes involved in Drosophila are *Hedgehog (Hh), Smoothened (Smo), Patched (Ptc), Hedgehog – interacting protein (HIP1), Costal – 2 (Cos-2), Fused (Fu), Suppressor of Fused [Su(Fu)] and cubitus interruptus (Ci)*. Components of this pathway are highly conserved between species. In vertebrates there are three homologues of Hh (Echelard et al., 1993): *Sonic hedgehog (Shh)* named after a popular Sega computer character*, desert hedgehog (Dhh)* and *Indian hedgehog (Ihh)*. Their biological properties are remarkably similar, differing only in their potency (*Shh>Ihh>Dhh*). They demonstrate different, but frequently overlapping expression profiles (Pathi et al., 2001). There are two homologues for patched, *patched – 1 (Ptc-1)* and *patched – 2 (Ptc-2)*. Both exhibit similar affinity for Hh. *Ptc-1* is expressed on target cells and is up regulated by Hh signalling (Carpenter et al., 1998). There are three homologues for *Ci, Gli-1, Gli-2* and *Gli-3*. These play a major role in Hh signalling (Lees et al., 2005) (Fig 4.1).

Hh itself is a 45-kilodalton precursor protein that undergoes autoproteolysis (Lum, 2004 #1611;Lee, 1994 #1612). This yields a ~ 19 kDa, active, NH_2-terminal signalling domain (N-Hh), to which a cholesterol molecule is added (N-Hhp), and a ~26 kDa, COOH-terminal fragment, responsible for catalysing the cleavage of the precursor Hh protein and also acting as a cholesterol transferase (Bumcrot et al., 1995; Porter et al., 1996). Addition of cholesterol to the N-terminal domain renders it hydrophobic. This allows it to bind with cell membranes and mediate its local actions (Fig 4.1).

In the absence of Hh protein, Ptc inhibits signalling by Smo. Ptc is a transcriptional target for Hh signalling and its expression is up-regulated by Hh activity. Whilst simultaneously initiating signalling it also acts in a negative-feedback loop to restrict the range of Hedgehog signalling in a tissue by sequestering Hh protein. HIP-1 is another cell surface protein that binds to and sequesters Hh (Chuang et al., 2003; Chuang and McMahon, 1999). HIP-1 is up-regulated by activation of the Hh signalling pathway and serves to down-regulate Hh activity.

The central role of Wnt signalling in the intestinal epithelium has been discussed earlier (section 4.1.1.1). Ihh signalling is activated in the differentiated zone of the intestinal epithelium where it induces differentiation (van den Brink, 2004 #1617). Hh signalling acts to restrict expression of wnt targets to the base of the crypt in vivo (van den Brink et al., 2004). In the absence of Hh signalling, full length Gli 1 is phosphorylated by protein kinase A (PKA), casein kinase I (CKI) and glycogen synthase kinase 3β (GSK 3β). The resultant truncated form of Gli 1 is incapable of activating transcription of Hh target genes. However, binding of Hh to its receptor patched (Ptc) results in activation of the Hh signalling receptor smoothened (Smo). Smo in conjunction with Cos 2 and fused kinase (Fu) inhibits the proteolytic processing of Gli 1 leading to its stabilisation and expression of Hh target genes. In vitro studies by Akiyoshi *et.al* (2006) confirm the inhibitory effect of Hh signalling on Wnt signalling. Their studies have implicated Gli 1 as being the main mediator of this inhibition by Hh signalling by interacting with β-catenin at a level below β-catenin phosphorylation (Akiyoshi et al., 2006). At this stage it is not clear whether this antagonism of Wnt is mediated directly by Gli 1 protein or is the result of up or down regulation of a transcriptional target(s) of Hh signalling (van den Brink and Hardwick, 2006).

The inhibitory effect of Hh signalling on Wnt signalling may be indicative of the anti- oncogenic effects of Hh signalling (van den Brink and Hardwick, 2006), a similar view point is also suggested by Berman and colleagues (Berman et al., 2003). Interestingly, Ihh expression is lost very early in the process of colorectal carcinogenesis. Loss or mutations affecting the *APC* gene results in uncontrolled activity of the Wnt signalling pathway, with accumulation of clonal precursor cells (Gregorieff and Clevers, 2005). The vast majority of colorectal cancers are sporadic, approximately 90% harbour *APC* mutations or other mutations that constitutively activate the Wnt signalling pathway (Gregorieff and Clevers, 2005). Loss of Ihh is noted in cases with FAP and in the flat lesions that precede polyp formation in the colon, indicating that loss of Ihh expression may be associated with the earliest stages of colorectal carcinogenesis, probably a direct consequence of overactive wnt stimulation (van den Brink, 2007). There are still many questions unanswered as regards the role of Hedgehog signalling in colonic epithelium and its role in carcinogenesis. Further work needs to be done to clarify its exact role.

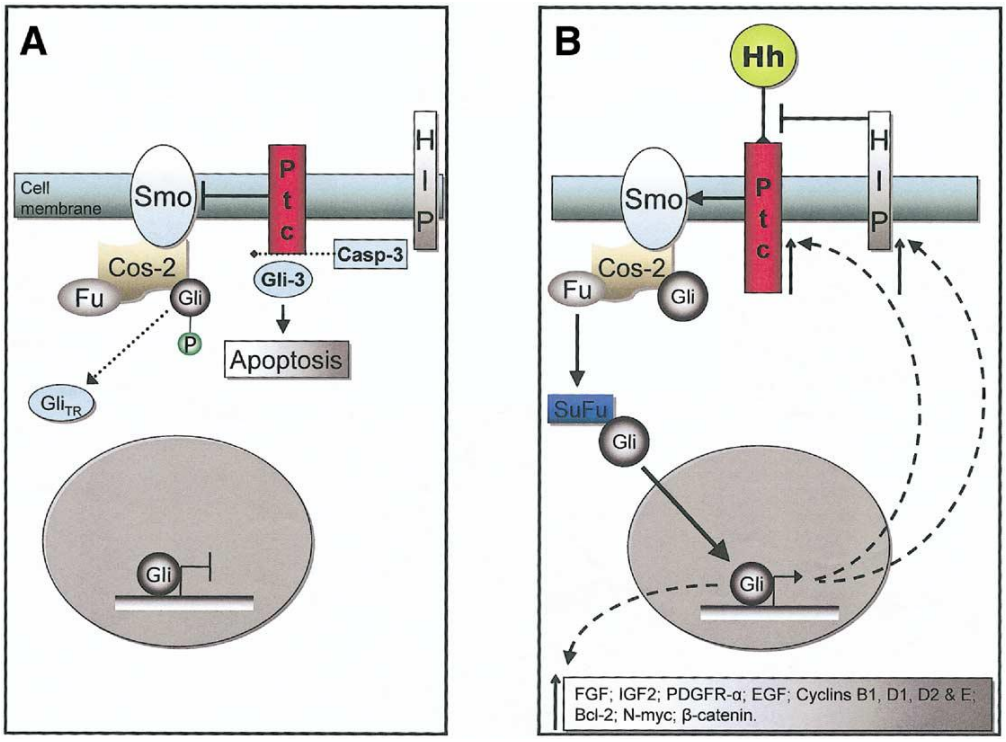


Figure 4.1

Hh signalling in mammalian cells

In the absence of Hh protein (Somech et al.), the 12-transmembrane domain receptor Ptc exerts an inhibitory effect on Smo, a 7-pass transmembrane protein with homology to G-protein–coupled receptors. Smo, in complex with Cos-2, prevents nuclear availability of the full Gli product. This occurs by a combination of microtubule binding of the complex and proteolysis to a truncated Gli (GliTR). Furthermore, when Ptc is unoccupied by Hh, it is suggested that caspase-3 (casp-3) cleavage of its intra-cellular portion exposes a receptor region that transduces an apoptotic signal via Gli-3. In the presence of Hh ligand-binding (*B*), the inhibitory action of Ptc on Smo is released. The full Gli product is now stabilized and transferred to the nucleus. This process is likely mediated in part by conformational change in the Cos-2/Gli/Fu complex and also by interaction of Gli with a phosphorylated Su (Fu). Once in the nucleus, the full Gli product binds to and up-regulates transcriptional targets, including Ptc and another Hh-binding protein, HIP. In this manner, excess Hh is sequestered, and control is exerted on the pathway. *Adapted from (Lees et al., 2005)*

4.1.1.3 Notch signaling

Notch is a transmembrane receptor that coordinates a signalling system known as the Notch pathway. *Notch* itself was identified more than a hundred years ago by a mutant fly with ‘notches’ in its wings (Morgan 1917), which indicated its requirement for wing outgrowth. To date, Notch signalling is considered one of the key pathways constituting the stem cell network and is responsible for self renewal of stem cells, cell fate determination of progenitor cells and terminal differentiation of proliferating cells (Androutsellis-Theotokis et al., 2006; Artavanis-Tsakonas et al., 1999; Lai, 2004). Core components of the Notch signalling pathway consist of a Delta-type ligand, a Notch-type receptor and a transcription factor of the CBF1/Su(H)/LAG1(CSL) family. In mammals, Delta-like 1 (DLL1), Delta-like 2 (DLL2), Delta-like 3 (DLL3), Jagged 1 (JAG1) and Jagged 2 (JAG2) with DSL domain constitute typical Notch ligands, whereas F3/Contactin, DNER and NB-3

without a DSL domain constitute atypical Notch ligands. Notch1, Notch2, Notch3 and Notch4 constitute Notch receptors (Katoh, 2007a; Lai, 2004).

Notch receptors are cleaved at the S1 site by a Furin like convertase to generate a mature heterodimeric receptor. Upon ligand binding to this receptor, further cleavage takes place at the S2 and S3 sites by metalloprotease tumour necrosis factor-α converting enzyme and by γ-secratase complex, respectively. This gives rise to Notch intra cellular domain (NCID) (Katoh, 2007a). Notch signals are transduced to either the canonical pathway or the non-canonical pathway, depending on the expression profile of Notch ligands, Notch receptors and Notch signalling modifiers.

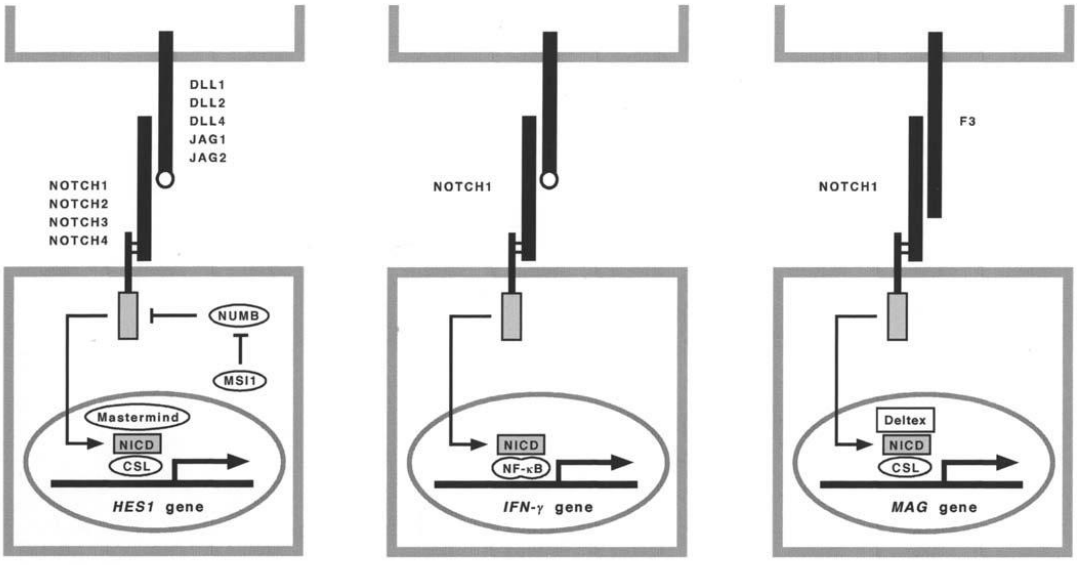
Canonical Notch signalling. Upon binding of appropriate ligand to its Notch receptor, NICD is released and subsequently translocates to the nucleus where it associates with CSL (RBPSUH) transcription factor (Androutsellis-Theotokis et al., 2006). This complex of CSL-NICD is then activated by the Mastermind family of co-activators (MAML1, MAML2 and MAML3) which results in transcriptional activation of *HEY1, HEY2, HEYL, HES1, HES5* and *HES7* genes, which encode for bHLH/orange domain transcriptional repressors (Iso et al., 2003; Katoh, 2004; Li and Harris, 2005). The net effect of Notch signalling to the CSL-NICD-Mastermind complex is the repression of transcription and inhibition of differentiation in stem cells and transit amplifying cells.

Non-canonical Notch signalling. NICD is also known to interact with p-50 or c-Rel in the nucleus. This enhances NF-$_{\kappa}$B activity (Shin et al., 2006) and forms a complex with NICD which results in transcriptional activation of NF-$_{\kappa}$B target genes such as IFN-$_{\gamma}$. Binding of atypical Notch ligands on their own to Notch receptors results in a CSL-NCID-Deltex complex (Cui et al., 2004; Eiraku et al., 2002; Hu et al., 2003). Deltex affects the binding of CSL to DNA which results in transcription of unique target genes, such as *MAG. MAG* is a tissue specific transcription factor responsible for induction of terminal differentiation. Thus atypical Notch signalling to the CSL-NCID-Deltex complex results in differentiation of progenitor cells (Katoh, 2007a) (Fig 4.2).

Notch signalling plays a key role in the stem cell signalling pathways (Wnt, Hedgehog, FGF and BMP) in regulating the balance of proliferation, self renewal

and differentiation among stem cells and progenitor cells in their respective niches (Katoh, 2005a; Katoh, 2005b; Katoh, 2006a; Katoh and Katoh, 2005; Katoh and Katoh, 2006a; Radtke et al., 2006; van den Brink et al., 2004). Notch signalling interacts with other stem cell signalling pathways. For example, , the gene *JAG1* is considered an evolutionary conserved target in the canonical Wnt signalling pathway (Katoh, 2006b). *JAG1* expressed on progenitor cells activates the canonical Notch signalling pathway in adjacent progenitor or stem cells. This activation acts synergistically with the Wnt signalling pathway in maintaining homeostasis in stem and progenitor cells.

In the colon, *JAG1*, *JAG2* and *Notch1* are expressed in the lower half of colonic crypts (Sander and Powell, 2004). Stem cells reside at the basal regions of these crypts. Activation of canonical Notch signalling in this area of the crypt leads to Hes1 up-regulation and down-regulation of Atoh1/Hath1/Math1 (Leow et al., 2004). The net result is inhibition of differentiation and maintenance of stem and progenitor cells. In colorectal cancer, Notch signalling is considered to be oncogenic. It has been observed that *NOTCH1*, *NOTCH2* and *NOTCH3* are up-regulated in colon cancer while *ATOH1* expression is down-regulated (Leow et al., 2004). Notch signalling inhibits terminal differentiation of goblet cells and is considered oncogenic (Katoh, 2007a).



Canonical Notch Pathway

CSL – NICD – Mastermind cascade

Non – canonical Notch Pathway

NF-κB – NICD cascade

Non – canonical Notch Pathway

CSL – NICD – Deltex cascade

Figure 4.2

Notch signalling pathway

Notch signals are transduced to the canonical pathway (CSL-NICD-Mastermind signalling cascade) or the non-canonical pathway (NF-κB-NICD and CSL-NICD-Deltex signalling cascades) based on the expression profile of Notch ligands, Notch receptors, and Notch signalling modifiers. (Left), Binding of DLL1, DLL3, DLL4, JAG1 and JAG2 with DSL domain (open circle) to Notch receptor leads to the release of Notch intracellular domain (NICD) for transcriptional activation of Notch target genes, such as *HES1*, through the CSL-NICD-Mastermind complex (Middle), Notch signalling activation also leads to transcriptional activation of NF-κB target genes, such as *IFN-Á* through direct association between NICD and NF-κB.(Right), Binding of DNER, F3/Contactin and NB-3 without DSL domain to Notch receptor leads to transcriptional activation of differentiation-associated genes, such as *MAG*, through the CSL-NICD-Deltex complex.

Adapted from Katoh 2007

4.1.1.4 BMP Signaling

Bon morphogenetic proteins (BMPs) are members of the transforming growth factor-β superfamily (TGFβ) which are known to regulate cell differentiation, proliferation and apoptosis. They are capable of inducing ectopic bone formation from mesenchymal tissue (Urist et al., 1983). Approximately twenty BMPs have been identified up to date. BMP 1 does not actually belong to the TGF-β family of proteins; it is a Metalloproteinase. BMP ligands bind to serine-threonine kinase receptors, type IA (BMPRIA), type IB (BMPRIB) and type II (BMPRII) for transducing their signals intracellularly (Gilboa et al., 2000; Koenig et al., 1994; ten Dijke et al., 1994). BMP ligands bind to either preformed complexes or initially to BMPRI receptors which then recruits BMPRII (Kirsch et al., 2000). Binding of ligand results in phosphorylation of BMPRI by BMPRII, which in turn phosphorylates intracellular SMAD1, 5 or 8 at their COOH terminus (Kretzschmar et al., 1997). Phosphorylated SMADs then associate with SMAD4 to form a multimeric complex which translocates to the nucleus and activates transcription through interaction with DNA binding proteins or through direct DNA binding to SMAD binding elements (SBE). The net effect is regulation of gene expression of

various genes associated with cell differentiation, cell proliferation and apoptosis (Balemans and Van Hul, 2002).e

The interaction between BMP and Wnt signalling has been studied by Nishanian *et al.* (2004). Using BMP-treated embryonal carcinoma cells, Nishanian *et al.* examined the gene expression profile of these cells and discovered genes implicated in Wnt signalling. Although most of the identified genes (*Axin2, Cerberus/DAN-related inhibitor of Wnts and BMPs, Wnt5a, Wnt5b* and *Dapper*) were antagonists of Wnt signalling, a few genes, such as *Lef1* and *Wnt2b*, were positive regulators of Wnt signalling. Previous reports have shown that *Lef1* is induced by BMP4 in other cell types (Kratochwil et al., 1996). Further tests by Nishanian ***et al.'s*** team to assess whether BMP4 could regulate Wnt signalling in this embryonal carcinoma cells revealed that BMP did in fact activate a Wnt reporter gene by four fold after 72 hours (Nishanian et al., 2004).

In the human colon, it has been shown that BMP2, BMPRIA, BMPRIB, BMPRII, pSMAD1 and SMAD4 are expressed in mature colonocytes at the luminal surface of the colon (Hardwick et al., 2004). Hardwick *et al*. (2004) have also shown that BMP2 inhibits normal growth of colonic epithelial cells by inhibiting proliferation and promoting differentiation and apoptosis. In colon cancer, inactivation of the TGF-β signalling pathway appears to play an important role as inactivation of TGF-β signalling occurs in ~80% of colon cancers (Grady et al., 1998). *ACVR2*, a TGF-β superfamily receptor, undergoes mutation in the majority of colon cancers exhibiting microsatellite instability (Hempen et al., 2003; Jung et al., 2004).

4.1.1.5 FGF Signaling

Fibroblast growth factors (FGFs) belong to a large family of polypeptide growth factors. A variety of multicellular organisms, both vertebrates and invertebrates, harbour these FGFs. FGFs are involved in diverse cellular processes including cell growth, wound healing, differentiation, cell migration, chemotaxis, apoptosis and cell survival (Bottcher and Niehrs, 2005; Powers et al., 2000). FGF signalling pathways interact with other signalling pathways such as Wnt Signalling and NFAT

signalling pathways (Katoh, 2007c). FGFs have been implicated in the development and progression of tumours, for example colorectal cancer (Chamorro et al., 2005).

The family of FGFs in humans consists of 22 members that share certain common features. Members of the FGF family have a high affinity for heparin and heparin-like glycosaminoglycans (HLGAGs) of the extra cellular matrix (ECM) as well as a high sequence homology within a central core domain of 120 amino acids (Ornitz and Itoh, 2001). This central core folds into twelve anti-parallel β-strands and is flanked by more variable amino- and carboxy-terminal regions (Ago et al., 1991; Zhang et al., 1991). FGFs have been arranged into sub-groups depending on their biological properties, structure and expression profiles. FGFs appear diffusible and act in a dose dependant manner (Christen and Slack, 1999; Kengaku and Okamoto, 1995).

FGF receptors (FGFRs) belong to a sub-family of cell surface receptor tyrosine kinases (RTKs), encoded by four closely related genes, *Fgfr1-4* (Johnson and Williams, 1993). These genes code for single spanning transmembrane proteins with an intracellular domain harbouring tyrosine kinase activity and an extracellular domain containing the ligand binding domain. Many different isoforms of FGFR exist, due to alternative splicing of *Fgfr* transcripts (Johnson and Williams, 1993), each isoform displaying distinct FGF-binding specificities (Ornitz et al., 1996). Binding of FGFs to FGFRs leads to receptor dimerisation and triggers tyrosine kinase activation with autophosphorylation of the intracellular domain (McKeehan et al., 1998). Tyrosine autophosphorylation not only serves to control protein tyrosine kinase activity of the receptor but also to recruit and assemble various signalling complexes (Schlessinger, 2000). Further FGF signal transduction can proceed via three pathways of which the RAS / MAPK pathway is most commonly used by FGFs and involves the lipid-anchored docking protein FRS2 that binds to FGFR1 (Kouhara et al., 1997). After activating FGFR1, tyrosine phosphorylated FRS2 acts as a site for the assembly of a multi-protein complex which activates and controls the Ras-MAPK and phosphatidylinositol 3 (PI3)-kinase / Akt signalling cascades. This cascade then leads to phosphorylation of target transcription factors such as *c-myc*, members of the Ets family of transcription factors and AP1. The other two pathways used for signal transduction by FGFs are the

Phospholipase C γ (PLCγ) / Ca^{2+} pathway and the Phosphatidylinositol 3 (PI3)-kinase / Akt pathway, as mentioned earlier.

FGF signalling often takes place across epithelial – mesenchymal boundaries and plays a major role in developmental processes such as mesoderm formation, gastrulation movements, neural induction, AP patterning and endoderm formation. FGF has also been implicated in various pathological conditions such as breast cancer (Dickson et al., 2000), urological cancers (Cronauer et al., 2003), haematological disorders (Moroni et al., 2002) and colorectal cancer (Chamorro, 2005 #1685).

The various signalling pathways discussed are all linked to the stem cell signalling pathways. In addition, there are multiple other pathways, all equally important in maintaining homeostasis in the human body. It is obvious that most of these pathways are intricately linked to one another and dysfunction or mutations in one pathway could lead to a knock-on effect in other pathways. The result is destabilisation of finely tuned homeostatic mechanisms governing and controlling cells, especially stem cells, with resultant pathological manifestations, especially cancer, developing in the affected tissues. Much work still needs to be done to elucidate further interactions with the hope of developing targeted therapeutic interventions to reverse or halt various pathological conditions that result from dysfunction in these pathways.

4.1.2 Sequence of genetic alterations leading to colorectal carcinogenesis

The classic or traditional pathway leading to colorectal cancer was first described by Vogelstein *et al*. in 1988. They described key molecular events that take place in progressive pathological stages, from adenoma to cancer in the colon, and were able to generate the adenoma-carcinoma model (Fearon and Vogelstein, 1990) that has withstood the test of time. This traditional pathway, also known as chromosomal instability pathway (CIN) or the suppressor pathway, accounts for nearly 70% - 85% of observed colorectal cancers (Grady, 2004). Well defined

mutations account for the traditional pathway, such as mutations in *APC* or loss of 5q (APC gene), mutation of K-*ras*, loss of 18q (with loss of *SMAD2*, *SMAD4* and *DCC*) and deletion of 17p, which harbours the tumour suppressor gene *p53* (Grady, 2004). It is important to note that very few colorectal cancers posses the full complement of these molecular events. It is probable that some colorectal cancers bypass certain steps of the traditional pathway by other genetic events (Smith et al., 2002) but manage to achieve the same biological consequences, i.e. colorectal cancer. One such pathway is the microsatellite instability (MSI) pathway or the mutator pathway. Approximately 20% of colorectal cancers exhibit this mutator phenotype. In the MSI pathway, there is dysfunction of the mismatch repair (MMR) system, responsible for correcting replication errors in short repeat sequences (microsatellites). Several microsatellites are present in genes implicated in colorectal carcinogenesis such as, *Bax*, *Caspase 5*, *MSH3*, *MSH6*, *β-catenin*, *APC*, *TGFβRII*, *E2F4* and *IGF-II* (Bresalier, 2002). To aid research and clinicopathological practice, the MSI pathway is sub-divided into MSI-High (MSI-H), MSI-Low (MSI-L) and Microsatellite stable (MSS), based on a standardised panel of microsatellites (Boland et al., 1998). MSI-H tumours occur in two settings: Firstly, as part of an inherited syndrome called Lynch syndrome or Hereditary Non Polyposis Colorectal Cancer (HNPCC), in which there is a germline mutation in the MMR genes *hMLH1* and *hMSH2*. Secondly, as sporadic cases of colorectal cancer due to epigenetic silencing of the *hMLH1* gene (Deng et al., 1999). Another pathway, termed the “methylator pathway” has also been described (Worthley et al., 2007). DNA methylation is an epigenetic means of regulating gene transcription. This occurs at cytosine bases when cytosine and guanine occur in a dinucleotide pair i.e. CpG. In carcinogenesis, CpG island methylation results in epigenetic silencing of gene transcription, which is the equivalent of acquiring an inactivating mutation. Hence, methylation of tumour suppressor genes could have adverse effects by suppressing their normal functions. Type C genes (*MLH1*, *p16*) are associated with CpG methylation in colorectal cancers whereas Type A genes (MINT6, MINT24, MINT32, ER) are predominantly methylated in normal colonic mucosa and only occasionally in cancer (Rashid and Issa, 2004; Toyota et al., 1999). Despite all the available research into colorectal cancer, there still remain many unanswered questions regarding colorectal carcinogenesis. Further research, especially in epigenetic mechanisms driving DNA methylation and the

CIN pathway, is needed. Also, further research into the molecular events underpinning normal colonic mucosa could reveal new insights into other pathways leading to loss of homeostasis in the colonic mucosa.

4.1.3 Expression of lamin A/C in cancerous cells and tissue

The role of lamins in cancer has been studied in a variety of different cancers such as basal skin cell cancer (Venables et al., 2001) acute lymhoblastic leukaemia, non-Hodgkin's lymphoma (Stadelmann et al., 1990), human small cell lung cancer and non-small lung cell cancer (Broers et al., 1993), colorectal cancer (Cance et al., 1992; Moss et al., 1999) and metastatic leiomyosarcoma, rhabdomyosarcoma and chondrosarcoma (Cance et al., 1992).

In some cancers studied, there are conflicting reports about the expression of lamins, for example, in colorectal cancer, Moss et al., (1999) describes a decrease in nuclear staining for A-type lamins and lamin B1in colon adenomas and adenocarcinomas when compared to normal colonic mucosa, whereas Cance *et al*., (1992) previously reports heterogeneous expression of A-type lamins in two colon adenocarcinomas. Interestingly, Venables *et al*., (2001) demonstrates a down regulation of lamin A in basal cell skin cancers exhibiting a high proliferative index and a loss of lamin C in slower growing tumours. When assessing for lamin A/C expression in colorectal cancers, we have to take into consideration previous conflicting reports about lamin A/C expression patterns in different patient groups with this cancer.

4.1.4 Microarray analysis

The term 'microarray' was first used by Schena *et al*. in 1995 (Schena, 1995 #1704). In its original context microarrays were used to describe methods for sequencing and gene expression monitoring in the field of biology. Since then,

there has been a substantial amount of work done in this field with a huge selection of microarray based technologies now available commercially. Microarray based experiments are finding more and more uses in every field of biological research. Initially, researchers designed microarrays by hand or using simple spotting robots to deposit cDNA clone inserts onto a glass slide. Each clone was usually hundreds of base pairs long. Nowadays, commercial oligonucleotide – based microarrays are available. These are less prone to variations, are relatively affordable and offer greater feature densities (Ahmed, 2006). Interpretation of data from microarray based experiments has always been an area fraught with contentious issues. In the early days of microarray technology, researchers often published articles using fold change as the single parameter to indicate a change in gene expression (Carninci et al., 2005; Rihn et al., 2000). This approach often leads to unacceptably high false positive values being observed, especially in cases where transcripts have a high natural variation leading to high fold change values. Over time, the main emphasis has now moved towards developing tools that will enable researchers to use a standard method of analysing microarray results. To this end, in 2001, the Minimum Information About a Microarray Experiment (MIAME) was introduced by the Microarray and Gene Expression Data (MGED) Society (Brazma et al., 2001). These standards are now being used frequently by researchers involved in microarray studies.

4.1.4.1 Microarray data analysis

The main processes involved in data analysis of microarray experiments are, the extraction of spot signal intensities, filtering of data, normalisation of data and assessment of differential expression. Various commercial enterprises such as Affymetrix, offer this level of data analysis as part of their gene chip array software. The resultant lists of data generated then needs further analysis using specialised software. In its simplest form, up and down regulation of genes is relatively straight forward to assess. Further analysis is done using various methods, including cluster analysis. Cluster analysis looks for changes in expression of genes closely related to each other. A large amount of data is generated from a microarray

experiment. Clustering enables subdivision of all this gene expression data into smaller groups based on similarity. There are various methods of performing a cluster analysis. Unsupervised clustering is used for exploratory analysis while supervised clustering is often used to create a diagnostic tool based on previously established gene expression signatures (Meyer and Ginsburg, 2002; Wiese et al., 2007). Data mining of microarray analysis usually gives rise to a long list of differentially expressed genes or clusters of genes. Further analysis of these genes is then needed and there are various methods available to achieve this. From a biological point of view, identification of metabolic pathways is an important part of gene expression profiling, which allows for a mechanistic understanding of the disease under study. Various biological annotations are available for this purpose, such as Gene Ontology (GO) terms, Swissport key words and the Koyoto Encyclopaedia of Genes and Genomes (KEGG) pathways (Ashburner et al., 2000; Kanehisa et al., 2004). KEGG offers the most comprehensive graphical representation of known biochemical pathways (Ogata et al., 1999). The relationship of genes to other biological pathways and processes is another important area to assess during microarray analysis. To this effect, the Gene Ontology (GO) Consortium has developed a vocabulary to describe genes and gene product attributes for biological processes, molecular functions and cellular components(Ashburner et al., 2000). There are commercially available software packages that allow for comprehensive visualisation of gene expression levels in different pathways, such as GeneSpring (Agilent Technologies, USA), alongside other freely available software packages such as GenMAPP (Dahlquist et al., 2002) and Pathway Assist. A number of online access tools are also available which can calculate over-representation statistics for all GO terms with respect to a given data set; so called gene class testing (GCT) (Allison et al., 2006), for example, EASE(Dennis et al., 2003), MAPPfinder(Doniger et al., 2003) and until recently the NetAffx analysis centre by Affymetrix.

4.1.4.2 Validation of Array data

With current microarray technology and data analysis tools, the chances of errors and/or artefacts occurring in a microarray experiment are very small, but nevertheless there is always a possibility for this to occur. It is in this regards that validation of microarray data is always important. Once a number of target genes have been identified in a microarray experiment, it is always prudent to confirm RNA expression levels by an alternate method. The most commonly employed method is Quantitative RT-PCR (Rajeevan et al., 2001a; Rajeevan et al., 2001b) due to its speed and relative cost effectiveness. Alternatively, Northern blot analysis has also been shown to be as effective at validating microarray data (Taniguchi et al., 2001).

In my studies, I opted to use a microarray based method to further assess the implications of variable lamin A/C expression in colorectal tumours exhibiting similar clinical properties and of a similar histological grade. Variable expression of lamin A/C in colorectal cancer in different patients with a similar stage of disease has been noted previously and has been shown to have a direct bearing on prognosis in these patients, despite standard treatment for their colorectal cancer (Willis et al., 2008). Colorectal carcinogenesis is a very complex process involving many signalling pathways and altered gene expression in a number of key genes. The vast amount of data generated from these microarray experiments has to be interpreted with caution and hence, I have analysed this data by two methods:

a) A simple analysis to identify genes with significant up or down regulation. b) A cluster analysis (unsupervised) to identify groups of genes involved in key cell signalling pathways and to assess their differential expression.

4.2 Results

4.2.1 Collection of tissue samples for immunohistochemical analysis of lamin A/C expression

A pilot study to assess the expression of lamin A/C in colorectal cancers at various stages of disease progression was undertaken. This was a prospective pilot study between the period of January 2005 and August 2006. After due approval by the ethics committee at the James Cook University hospital in Middlesbrough and appropriate consent by the patient, a total of 42 patients with colorectal cancer and 1 patient with a tubulo-villous adenoma (Table 1), were enrolled for this pilot study. Under supervision by a consultant histopathologist, appropriate tumour and normal mucosal samples were taken from the resected colon / rectum specimen from all patients. One set of tumour samples was snap frozen in liquid nitrogen and another set of samples placed in formalin solution and subsequent fixation prior to being processed and embedded in paraffin. Corresponding normal colonic mucosa samples were also processed in a similar fashion. To reduce the chances of RNA degradation, the whole process of specimen collection and sampling was done within 25 minutes of removal of tumour from the patient. Similarly, all specimens were washed in 0.9% Sodium Chloride solution to remove any contaminating faecal residue. Paraffin embedded and formalin fixed samples were then sectioned at 4µm thickness prior to being assessed for lamin A/C expression using standard immunohistochemical methods, as described in chapter 2. Mouse Monoclonal antibodies against lamin A/C were used throughout for immunohistochemical analyses. Snap frozen tissue samples were weighed and placed in an appropriate volume of Trizol® reagent (using 1ml of reagent for every 100mg of tissue) and homogenised as per manufacturer's protocol, using a glass Teflon homogeniser. Subsequent steps in total RNA extraction were as outlined in chapter 2, Materials and Methods.

4.2.2 Lamin A/C expression profile at various stages of colorectal cancer as observed in different regions of the colon and rectum

A total of 43 patients participated in this study. Of these patients, 42 had a histological diagnosis of colorectal cancer while 1 patient had a tubulo-villous adenoma. The distribution of colorectal cancer according to Dukes' stage was as follows: Dukes' A – 5 patients (11.91%); Dukes' B – 22 patients (52.38%); Dukes' C1 – 13 patients (30.95%); Dukes' C2 – 2 patients (4.76%). In each Duke's stage, the presence, absence or depletion of nuclear lamin A/C expression was also assessed. Amongst the Dukes' A group of patients, 2 patient samples had strong lamin A/C expression, 2 patient samples did not exhibit any lamin A/C expression and 1 patient sample had weak nuclear lamin A/C expression. In the Dukes' B group of patients, 9 patient samples had strong nuclear expression of lamin A/C, 7 patient samples had no nuclear expression of lamin A/C and 6 patient samples had weak nuclear expression of lamin A/C. In the Dukes' C1 group of patients, 4 patient samples had strong nuclear expression of lamin A/C, 3 patient samples had no nuclear expression of lamin A/C and 6 patient samples had weak nuclear expression of lamin A/C. In the Dukes' C2 group only two patients were identified, 1 patient sample had strong nuclear expression of lamin A/C and 1 patient sample had weak nuclear expression of lamin A/C in their colorectal cancer respectively (Table 4.2 A). The incidence of colorectal cancer affecting various areas in the colon and rectum was studied and an attempt to correlate this with Dukes' staging and lamin A/C expression was made using a Chi square cross tabulation analysis (Table 4.2 B). This did not reveal any significant relation ($p = 0.85$).

A total of 9 patients with ascending colon cancer were identified: 6 patients had Dukes' B stage tumours; 1 patient with Dukes' A stage tumour and 2 patients with Dukes' C1stage tumours. No lamin A/C expression was noted in tumours from 8 patients and only 1 patient with a Duke's C1 cancer had weak nuclear expression of lamin A/C.

A total of 3 patients with cancer at the hepatic flexure were identified: 2 patients had a Dukes' C1 tumour and 1 patient had a Dukes' B tumour. Strong nuclear expression of Lamin A/C was noted in all patients with cancer at the hepatic

flexure. None of the patients participating in this pilot study had cancer of the transverse colon.

A total of 2 patients were noted to have cancer at the splenic flexure: 1 patient had a Dukes' A tumour, showing no expression of nuclear lamin A/C, and the other patient with a Dukes' B tumour, had strong nuclear lamin A/C expression.

A total of 12 patients were noted to have cancer in the sigmoid colon: 6 patients had a Dukes' B stage tumour, of which two tumours revealed high expression levels of nuclear lamin A/C and four tumours expressed low levels of nuclear lamin A/C; 4 patients had a Dukes' C1 stage tumour, of which two tumours had no detectable nuclear expression of lamin A/C while two other tumours exhibited low nuclear lamin A/C expression; 2 patients had a Dukes' C2 stage tumours at this site, of which one tumour expressed strong nuclear lamin A/C and the other tumour expressed only low levels of nuclear lamin A/C.

At the recto-sigmoid junction area, 4 patients are noted to have cancer: 2 patients have Duke's stage A cancer with one tumour expressing strong nuclear lamin A/C levels while the other tumour has low expression levels of nuclear lamin A/C; 1 patient has a Duke's stage B tumour, expressing high levels of nuclear lamin A/C; 1 patient has a Duke's stage C1 tumour also expressing high nuclear lamin A/C levels.

In the rectum, 12 patients were noted to have cancer: 1 patient had a Dukes' stage A tumour, exhibiting strong nuclear expression of lamin A/C; 7 patients had a Dukes' B stage tumour, in which 4 tumours expressed strong nuclear lamin A/C, 2 tumours had no detectable expression of nuclear lamin A/C and 1 tumour expressed low levels of nuclear lamin A/C expression; 4 patients had a Dukes' stage C1 tumour, in which, 1 tumour expressed strong nuclear lamin A/C levels and 3 tumours expressed low levels of nuclear lamin A/C expression (Table 4.3).

4.2.3 Extraction of total RNA and its qualitative and quantitative assessment

Initial attempts at extraction of total RNA were not adequate due to extensive contamination resulting in degradation of total RNA. After various attempts at

optimisation, it was found that use of a decontaminated flow hood, snap freezing tissue samples, smaller sample size to aid in homogenisation and cleaning of the initial tissue sample with sterile 0.9% sodium Chloride solution, greatly aided in the extraction of total RNA of sufficient purity to allow further experimental work to proceed.

Using a Beckman DU – 600 Analyser, the concentration of total RNA in each sample was determined. Samples with an A260/A280 ratio of 1.7 and above were then further assessed on an Agarose Formaldehyde Gel with 50µl wells. An 80mV current was used in all tests. Using an appropriate RNA marker, 28S (4712 bp) and 18S (1950 bp) ribosomal bands were analysed in each sample. Four tumour samples exhibiting the best quality RNA were then selected for microarray studies (Figure 4.2). The tumour samples selected were as follows:

1) Patient sample number 29 →Tubulo-villous adenoma – lamin negative tumour.

2) Patient sample number 33 → Duke's C1 – lamin negative tumour.

3) Patient sample number 37 → Duke's B – lamin positive tumour.

4) Patient sample number 38 → Duke's B – lamin negative tumour.

The quality of RNA extracted from the remaining tumour samples was not deemed of sufficient purity to enable an accurate microarray analysis to be carried out. Similarly, the quality of RNA extracted from normal colonic samples was of a poor quality and would interfere with interpretation of the microarray results. The decision regarding quality of extracted RNA was taken by Dr P Heiko, an independent observer, highly experienced in RNA / microarray work at the Centre for Life in Newcastle upon Tyne. The four samples selected contained the best quality RNA and were selected for microarray analysis.

RNA Concentration Ratios in Tissue Samples

$A_{260/280}$ ratios of samples used for microarray analysis

Patient Sample No	Absorbance at 260.0 nm	Absorbance at 280.0 nm	Background absorbance at 320.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm
29	2.1953	1.1976	0.0167	1.8449	0.5420
33	1.1576	0.6535	0.0078	1.7807	0.5616
37	0.4249	0.2409	0.0073	1.7874	0.5595
38	0.4621	0.2587	0.0121	1.8246	0.5481

Interestingly, by substituting DEPC treated dH_2O for TRIS during analyses, there was a substantial difference in the A260 nm / A280 nm ratio. For example, in sample 29 the original ratio noted using DEPC treated dH2O was 1.8449 whereas this ratio was 2.128 if TRIS were used instead. This effect was well known and the difference in the A260/A280 ratio was related to a decrease in absorbance at 280 nm when the ionic strength and/or pH of the solution increased (Wilfinger, 1997 #1706). The concentration of RNA in each case was determined using the following formula:

X (A260 nm reading) x 40 µg/ml x Dilution factor (50) = Y µg/ml (RNA concentration)

The concentration values of RNA in each sample were as follows:

Sample 29 → 2.1953 x 40 x 50 = 4390.6 µg/ml OR 4.39 µg/µl

Sample 33 → 1.1576 x 40 x 50 = 2315.2 µg/ml OR 2.32 µg/µl

Sample 37 → 0.4249 x 40 x 50 = 849.8 µg/ml OR 0.85 µg/µl

Sample 38 → 0.4621 x 40 x 50 = 924.2 µg/ml OR 0.92 µg/µl

4.2.4 Microarray analysis using The Human Genome U133A Plus 2.0 Array® by Affymetrix®

A qualitative Genome-wide DNA microarray pair-wise screening was carried out on four separate tissue samples, corresponding to advancing stages of colorectal neoplasia from the adenoma stage up to Dukes' C stage. In addition, a comparative analysis of a Dukes' B tumour expressing lamin A/C with a Dukes' B tumour not expressing lamin A/C was also undertaken. This study was carried out using the Human Genome U133A Plus 2.0 Array® Genechip by Affymetrix® (Affymetrix, Santa Clara, CA, USA). This array offered a comprehensive analysis of genome-wide expression on a single array. Analyses of the relative expression levels of more than 47,000 transcripts and variants', including more than 38,500 well characterised genes and UniGenes (Figure 4.3) was done. Further detailed analysis of the results, including extraction of spot signal intensity, filtering and normalisation of data was carried out in accordance with the associated Affymetrix® guidelines for the GH-U133A using the GeneChip® Operating Software by Affymetrix®. A preliminary gene list was generated by means of a series of relevance thresholds for signal detection level, signal detection *p*-value significance, expression change in *p*-value significance, and Signal Log Ratio (SLR), for each analysis. After generating appropriate data lists, these were then imported into Microsoft Excel® for further analysis, including fold change analysis. Genes of interest were identified and a difference in their expression profile between different tissue samples was analysed.

4.2.4.1 Comparative gene analysis of a Tubulo-Villous (TV) adenoma of the colon with Dukes' B stage and Dukes' C stage colorectal tumours (Table 4.4)

Assessment of the microarray data from the TV adenoma tissue sample revealed no signal detection in 31163 transcripts, positive signal detection in 22664 transcripts, and only a moderate signal detected in the remaining 848 transcripts. Similarly, analysis of the microarray data from the lamin A/C negative Dukes' B

stage colorectal tumour sample revealed no signal detection in 28598 transcripts, positive signal detection in 25235 transcripts and only a moderate signal in the remaining 842 transcripts. Analysis of the array data from the lamin A/C positive Dukes' B stage colorectal tumour sample revealed no signal detection in 27656 transcripts, positive signal detection in 26250 transcripts and only a moderate signal in 769 transcripts. Finally, analysis of the microarray data from the Duke's C stage colorectal tumour sample showed no detectable signal from 28907 transcripts, positive signal detection in 24976 transcripts and only a moderate signal in 792 transcripts. In all four tissue microarray experiments, transcripts exhibiting a positive signal had a detection *p*-value of $p < 0.05$ (Table 4.4).

The next stage of this analysis focused on simple fold changes observed in these four different tissue samples. For comparative purposes a baseline set of data was needed against which to compare and calculate the relevant fold changes. To this end data obtained from the tissue sample consisting of a tubulo-villous adenoma was used as a comparative base line against which microarray data from the other three tissue samples was compared. Since I was not performing a cluster analysis, it was safe to assign a cut off value for assessing the fold change. Arbitrarily, a fold change value of 2 was assigned and appropriate comparative expression analysis of the microarray data done.

4.2.4.1.1 A comparative microarray analysis of changes in functional genes between a Dukes' B adenocarcinoma (nuclear lamin A/C negative) tumour sample and a Tubulo-Villous adenoma (nuclear lamin A/C negative) tumour sample (Table 4.5)

Comparative expression analysis between both tissue samples revealed a total of 527 transcripts exhibiting a fold change of 2 or more in the Dukes' B sample devoid of lamin A/C expression. 190 transcripts were noted to be down-regulated while 337 transcripts were up-regulated. This indicated a significant increase of 63.94% in the number of up-regulated transcripts in the Dukes' B tissue sample. Given the large number of transcripts exhibiting a fold change of 2 or more, an

initial assessment was made only of the 20 most up and down regulated transcripts. Refer to table 5 for a full list and description of these transcripts.

The highest fold change observed amongst the up-regulated transcripts was +388.02 (p=0.00002). This highly up-regulated transcript was encoding for a gene called insulin-like growth factor 2 (Somatomedin A or *IGF2*). The, maximum fold change noted amongst the down-regulated transcripts was -59.71, corresponding to the gene encoding for Gremlin 1(*GREM1*).

4.2.4.1.2 A comparative microarray analysis of changes in functional genes between a Dukes' C adenocarcinoma (nuclear lamin A/C negative) tumour sample and a Tubulo-Villous adenoma (nuclear lamin A/C negative) tumour sample (Table 4.6)

Comparative expression analysis between both tissue samples revealed a total of 1080 transcripts exhibiting a fold change of 2 or more in the Dukes' C sample. Of these transcripts, 216 were down-regulated while 864 transcripts were up-regulated. This indicated a highly significant increase of up to 80% in the number of transcripts that were up-regulated in the Dukes' C tissue sample. Assessment of 1080 transcripts individually would prove to be prohibitive and very time consuming, instead an assessment of 20 transcripts that were highly up-regulated and a further 20 transcripts that were highly down regulated was undertaken. Refer to Table 4.6 for a full list and description of these transcripts.

The maximum fold change observed amongst the up-regulated transcripts was +78.79 (*p*=0.00002), corresponding to the gene encoding for homosapiens carbonic anhydrase 1(*CA1*). Similarly the maximum fold change observed amongst the down-regulated transcripts was -84.45 (*p*=0.99998), corresponding to the gene encoding for homo sapiens regulator of G protein signalling (*RGS13*).

4.2.4.1.3 A comparative microarray analysis of changes in functional genes between a Dukes' C adenocarcinoma (nuclear lamin A/C negative) tumour sample and a Dukes' B adenocarcinoma (nuclear lamin A/C negative) tumour sample (Table 4.7).

Comparative expression analysis between both tissue samples revealed a total of 2591 transcripts exhibiting a fold change of 2 or more in the Dukes' C tissue sample. Of these transcripts, 572 were down-regulated while 2019 transcripts were up-regulated. This indicated a highly significant increase of up to 77.92% in the number of transcripts that were up-regulated amongst the Dukes' C tissue sample. Given the large volume of transcripts identified, further assessment of these individually would prove to be very time consuming, instead an assessment of 20 transcripts that were highly up-regulated and a further 20 transcripts that were highly down regulated was undertaken. Refer to table 4.7 for a full list and description of these transcripts.

The maximum fold change observed in the up-regulated transcripts was +97.01. This corresponded to the gene encoding for homo-sapiens carbonic anhydrase 1(*CA1*). The maximum fold change observed in the down-regulated transcripts was -128.00 and corresponded to the gene encoding for insulin like growth factor II (Somatomedin A or *IGF2*).

4.2.4.1.4 A comparative microarray analysis of changes in functional genes between a Dukes' B adenocarcinoma (nuclear lamin A/C positive) tumour sample and a Dukes' B adenocarcinoma (nuclear lamin A/C negative) tumour sample (Table 4.8)

A comparative microarray analysis of two tissue samples belonging to the same stage and grade of colorectal cancer i.e. Dukes' B stage and moderately well differentiated cancer, was undertaken. The only difference between both tissue samples was the complete lack of nuclear lamin A/C expression in one sample and a strong nuclear expression of lamin A/C in the other. Data obtained after

analysis of the tissue sample lacking nuclear lamin A/C expression was used as a baseline against which a comparative expression analysis was done using data obtained from analysis of the tissue sample expressing nuclear lamin A/C.

Initial comparative analyses revealed a very large number of transcripts amounting to more than 50,000 that were identified as showing a fold change. This data was narrowed down by adding a filter in the form of a fold change cut off at +/- 2. This substantially narrowed down the analysed data to a total of 4487 transcripts. Of these transcripts, 557 were noted to be up-regulated while 3930 transcripts were down-regulated, in the Dukes' B, lamin A/C expressing tumour sample. These results indicated a very significant overall down-regulation of genes (87.58%) in the tumour sample expressing nuclear lamin A/C. A detailed analysis of each and every transcript was beyond the scope of this chapter, instead, by limiting the transcripts to just 20 each from the most up-regulated and down-regulated transcripts, a list of appropriate genes was created and analysed further. Refer to Table 4.8 for a detailed list of these transcripts.

The maximum fold change observed in the down-regulated transcripts was -97.01. This corresponded to the gene *SPP1* (Secreted Phosphoprotein 1) encoding for human nephropontin (also known as Osteopontin or Bone Sialoprotein or Early T Lymphocyte activation 1). The maximum fold change observed in the up-regulated transcripts was +84.45 and corresponded to the genes encoding for human creatine kinase brain *(CKB*) and 3-hydroxy-3-methylglutaryl-Coenzyme A synthase *(HMGCS2),* respectively.

4.2.4.2 Comparative microarray analysis of functional gene groups involved in intestinal homeostasis and carcinogenesis

A targeted analysis of the microarray data was undertaken. This involved compiling a list of relevant genes and arranging them into functional groups associated with intestinal epithelial morphogenesis, maintenance of the proliferative and differentiated compartments of mature crypts, adult stem cells,

tumour suppressor genes, oncogenes and other key genes affected in colorectal neoplasia. A number of nucleoskeletal and cytoskeletal genes were also assessed. This list of genes was compiled in collaboration with key researchers in the laboratory, after a thorough literature search. A total of 164 genes that made up 16 functional groups, were used in the analysis. Fold change values were not assigned any specific cut off level as the main focus here was to assess these genes as a functional group and by assigning fold change cut off values, many genes that exhibited no fold change, would be omitted. Expression batch queries were carried out on all genes identified, using data mining tools available freely from the Affymetrix web site. This expression batch analysis focused on the following areas:

- Gene ontology biological process classifications
- Gene ontology molecular function classifications
- Gene ontology cellular component classifications
- Ortholog targets
- GenMAPP pathway details
- Entrez Annotations and grades
- SwissProt and InterPro descriptions
- OMIM, Ensembl, UniGene and RefSeq entries
- Chromosomal locations
- Gene bank descriptions
- Affymetrix unique Id codes

Emphasis was focused on two key areas of interest: Wnt signalling and stem cells. Twenty two (22) genes were included in the Wnt signalling group with the omission of *c-myc* and *APC*, as both these genes were recognised oncogenes and hence they were classified accordingly to their relevant functional group. Thirty (30) genes were identified and classified under the stem cell differentiation group. The

remaining genes were allocated to their representative groups. Microarray data was then analysed focusing on the areas specified above and relevant information was organised appropriately using Microsoft Excel® (tables 4.9 – 4.14).

4.2.4.2.1 A comparative microarray analysis of changes in functional gene groups between a Dukes' B adenocarcinoma (nuclear lamin A/C negative) tumour sample and a Tubulo-villous adenoma (nuclear lamin A/C negative) tumour sample (Table 4.9)

The signal log ratio (SLR) profiles of various genes expressed in the tubulo-villous adenoma sample were used as a reference level from which fold change (FC) values were calculated for genes expressed in the Dukes' B (nuclear lamin A/C negative) tumour sample. A higher than 9 FC value was assigned to identify key genes of interest. The following genes were identified as being significantly up regulated in the Dukes' B (nuclear lamin A/C negative) tumour sample:

1) *IGF2* – FC value of +388.02 → Insulin like growth factor 2 (Somatomedin A).
2) *FN* – FC value of +64 → Fibronectin.
3) *FN1* – FC value of +18.38 → Cellular fibronectin.
4) *TCF1* – FC value +11.31 → Transcription factor 1, hepatic
5) *SCF* – FC value of +10.56 → Stem cell factor.
6) *MACF1* – FC value of +9.85 → Microtubule-actin crosslinking factor 1.

Similarly, the following genes were identified as being significantly down regulated:

1) *MAPT* – FC value of -18.38 → Microtubule-associated protein tau.
2) *PICK3CG* – FC value of -10.56 → Phosphoinositide-3-kinase, catalytic, gamma polypeptide.
3) *PRG3* – FC value of -10.56 → p53 responsive gene.

Key genes had been clustered into various functional groups and their expression profiles had been assessed. This had revealed certain interesting findings:

- A general down regulation of key genes involved in regulating apoptosis.
- A subtle down regulation noted in key genes involved with protein translation, processing, transport and degradation.
- A down regulation of key genes involved with Tumour suppression.
- Amongst the genes studied in the Wnt functional group, there was a mixed picture but overall an increase in the expression profile of genes had been noted.
- In the stem cell functional group, no conclusive evidence was available to decide between either an overall up or down regulation of gene expression.
- In the remaining functional groups, no significant overall up or down regulation in gene expression was noted.

4.2.4.2.2 A comparative microarray analysis of changes in functional gene groups between a Tubulo-villous adenoma (nuclear lamin A/C negative) tumour sample and a Dukes' B adenocarcinoma (nuclear lamin A/C positive) tumour sample (Table 4.10)

The signal log ratio (SLR) profiles of various genes expressed in the Dukes' B (nuclear lamin A/C positive) tumour sample were used as a reference level from which fold change (FC) values were calculated for genes expressed in the tubulo-villous adenoma sample. A higher than 9 FC value had been assigned to identify key genes of interest. The following genes were identified as being significantly up regulated in the tubulo-villous (nuclear lamin A/C negative) adenoma sample:

1) *FN* – FC value of +103.97 → Fibronectin.
2) *FN1* – FC value of +32 → Cellular fibronectin

3) *SPP1* – FC value of +24.25 → Secreted Phosphoprotein 1(Osteopontin).

4) *EPHB1* – FC value of +19.7 → Epherin receptor B1.

5) *CSPG2* – FC value of +13 → Chondroitin sulphate proteoglycan 2 (versican).

6) *COL1A2* – FC value of +10.56 → Collagen type 1, alpha 2.

7) *MACF1* – FC value of +9.85 → Microtubule-actin crosslinking factor 1.

Similarly, the following genes were identified as being significantly down regulated:

1) *FABP1* – FC value of -1097.5 → Fatty acid binding protein 1, hepatic.

2) *CKB* – FC value of -84.45 → Creatine kinase brain.

3) *BMP4* – FC value of -45.25 → Bone morphogenetic protein 4.

4) *ASCL2* – FC value of -32 → Achaete-scute complex-like 2.

5) *PLP* – FC value of -24.25 → Proteolipid protein.

6) *TDGF1* – FC value of -18.38 → Teratocarcinoma derived growth factor 1.

7) *CCNA1* – FC value of -17.15 → Cyclin A1.

8) *EGF* – FC value of -13.93 → Epidermal growth factor.

9) *MLH1* – FC value of -13.93 → MutL homolog , colon cancer, non-polyposis type 2.

10) *SLC16A1* – FC value of -13.93 → Solute carrier family 16, member 1.

11) *PRG3* – FC value of -12.13 → p53 responsive gene.

12) *PLS3* – FC value of -9.19 → Plastin 3 (T isoform)

Key genes had been clustered into various functional groups and their expression profiles had been assessed. These revealed:

- Down regulation of key genes involved in regulating apoptosis.

- Down regulation of all key genes studied, apart from one gene, involved in cell cycle progression, proliferation and growth.
- Down regulation of key genes involved in DNA replication and repair.
- Down regulation of key oncogenes
- Down regulation of key genes involved in protein translation, processing, transport and degradation.
- An overall down regulation of key genes involved in stem cell differentiation.
- Down regulation of key tumour suppressor genes studied apart from one gene i.e *EPHB1*.
- The remaining functional groups studied had not revealed any significant differences.

4.2.4.2.3 A comparative microarray analysis of changes in functional gene groups between a Dukes' C adenocarcinoma (nuclear lamin A/C negative) tumour sample and a Tubulo-villous adenoma (nuclear lamin A/C negative) tumour sample (Table 4.11)

The signal log ratio (SLR) profiles of various genes expressed in the tubulo-villous adenoma sample were used as a reference level from which fold change (FC) values were calculated for genes expressed in the Dukes' C (nuclear lamin A/C negative) tumour sample. A higher than 9 FC value had been assigned to identify key genes of interest. The following genes were identified as being significantly up regulated:

1) *FN* – FC value of +294.07 → Fibronectin.
2) *FN1* – FC value of +84.45 → Cellular fibronectin.
3) *TF* – FC value of +9.85 → Transferrin.
4) *ITGB1* – Fc value of +9.19 → Integrin beta 1.

Similarly, the following genes were identified as being significantly down regulated:

1) *EGF* – FC value of -24.25 → Epidermal growth factor.

Key genes had been clustered into various functional groups and their expression profiles had been assessed. These revealed:

- Down regulation of key genes involved in regulating apoptosis.
- Up regulation of key genes involved in gene transcription.
- The remaining functional groups studied had not revealed any significant differences.

4.2.4.2.4 A comparative microarray analysis of changes in functional gene groups between a Dukes' B adenocarcinoma (nuclear lamin A/C positive) tumour sample and a Dukes' B adenocarcinoma (nuclear lamin A/C negative) tumour sample (Table 4.12)

The signal log ratio (SLR) profiles of various genes expressed in the Dukes' B (nuclear lamin A/C negative) tumour sample were used as a reference level from which fold change (FC) values were calculated for genes expressed in the Dukes' B (nuclear lamin A/C positive) tumour sample. A higher than 9 FC value had been assigned to identify key genes of interest. The following genes were identified as being significantly up regulated:

1) *IGF2* – FC value of +1024 → Insulin-like growth factor 2 (Somatomedin A).
2) *FABP1* – FC value of +776.05 → Fatty acid binding protein 1, liver.
3) *CKB* – FC value of +84.45 → Creatine kinase brain.
4) *BMP4* – FC value of +21.11 → Bone morphogenetic protein 4.
5) *NEFL* – FC value of +9.19 → Neurofilament light polypeptide.
6) *PLS3* – FC value of +9.19 → Plastin 3 (T isoform)

Similarly, the following genes were identified as being significantly down regulated:

1) *SPP1* – FC value of -97.01 → Secreted phosphoprotein 1 (Osteopontin).
2) *PTGS2* – FC value of -19.7 → Prostaglandin-endoperoxide synthase 2 (cyclooxygenase)
3) *CD14* – FC value of -18.38 → CD14 antigen.
4) *MAPT* – FC value of -16 → Microtubule associated protein tau.
5) *LCP1* – FC value of -9.19 → Lymphocyte cytosolic protein 1 (L-Plastin).
6) *CDH1* – FC value of -1.32 → Cadherin 1, type1, E-cadherin (epithelial)

Key genes had been clustered into various functional groups and their expression profiles had been assessed. These revealed:

- An overall down regulation of key genes studied involved in regulation of the cytoskeleton and nucleoskeleton.
- Slight down regulation of key genes studied involved in regulation of gene expression / transcription.
- Down regulation of key genes studied involved in signal transduction.
- Down regulation of key genes studied involved in tumour suppression.
- The remaining functional groups studied had not revealed any significant differences.

4.2.4.2.5 A comparative microarray analysis of changes in functional gene groups between a Dukes' B adenocarcinoma (nuclear lamin A/C positive) tumour sample and a Dukes' C adenocarcinoma (nuclear lamin A/C negative) tumour sample (Table 4.13)

The signal log ratio (SLR) profiles of various genes expressed in the Dukes' C (nuclear lamin A/C negative) tumour sample were used as a reference level from

which fold change (FC) values were calculated for genes expressed in the Dukes' B (nuclear lamin A/C positive) tumour sample. A higher than 9 FC value had been assigned to identify key genes of interest. The following genes were identified as being significantly up regulated:

1) *FABP1* – FC value of +724.08 → Fatty acid binding protein 1, liver.
2) *MMP3* – FC value of +107 → Matrix metalloproteinase 3 (Stromelysin 1, gelatinase).
3) *PLP* – FC value of +51.98 → Proteolipid protein.
4) *CKB* – FC value of +29.86 → Creatine kinase brain.
5) *PRG3* – FC value of +29.86 → p53-responsive gene.
6) *ASCL2* – FC value of +22.63 → Achaete-scute complex-like 2.
7) *TDGF1* – FC value of +12.13 → Teratocarcinoma-derived growth factor 1.
8) *FGF5* – FC value of +11.31 → Fibrobalst growth factor 5.
9) *PLS3* – FC value of +9.66 → Plastin 3 (T isoform)

Similarly, the following genes were identified as being significantly down regulated:

1) *SPP1* – FC value of -48.5 → secreted phosphoprotein 1 (osteopontin).
2) *PTGS2* – FC value of -45.25 → prostaglandin-endoperoxide synthase 2 (cyclooxygenase).
3) *CD14* – FC value of -11.31 → CD 14 antigen.
4) *SOD 2* – FC value of -9.19 → superoxide dismutase 2, mitochondrial.

Key genes had been clustered into various functional groups and their expression profiles had been assessed. These revealed:

- An overall increase in gene expression related to protein translation, processing, transport and degradation

- A comparative increase in the expression of genes associated with stem cell differentiation
- The remaining functional groups studied had not revealed any significant differences, apart from the Wnt group, where a gene was significantly up regulated i.e *FABP1*.

4.2.4.2.6 A comparative microarray analysis of changes in functional gene groups between a Dukes' C adenocarcinoma (nuclear lamin A/C negative) tumour sample and a Dukes' B adenocarcinoma (nuclear lamin A/C negative) tumour sample (Table 4.14)

The signal log ratio (SLR) profiles of various genes expressed in the Dukes' B (nuclear lamin A/C negative) tumour sample were used as a reference level from which fold change (FC) values were calculated for genes expressed in the Dukes' C (nuclear lamin A/C negative) tumour sample. A higher than 9 FC value had been assigned to identify key genes of interest. The following genes were identified as being significantly up regulated:

1) *MAPT* – FC value of +21.11 → Microtubule-associated protein tau.
2) *BGN* – FC value of +10.56 → Biglycan.
3) *FGF5* – FC value of +9.85 → Fibroblast growth factor 5.
4) *PLP* – FC value of +9.85 → Proteolipid protein.

Similarly, the following genes were identified as being significantly down regulated:

1) *IGF2* – FC value of -724.08 → Insulin-like growth factor 2 (somatomedin A).
2) *EGF* – FC value of -9.85 → Epidermal growth factor.

Key genes had been clustered into various functional groups and their expression profiles had been assessed. These revealed:

- An overall increase in the expression of key, selected genes involved in extracellular matrix: components, processing, cellular attachment, proteases and protease inhibitors.
- Up-regulation of key selected genes involved in signal transduction.
- An overall increase in the expression of key, selected genes involved in stem cell differentiation.
- Up regulation of key, selected genes involved in tumour suppression.
- The remaining functional groups displayed equivocal expression levels.

All results of up and down regulated genes in the functional groups had been closely studied and a detailed literature search carried out. Interest was focused on *CKB* (creatine kinase brain), T-Plastin and E-Cadherin. The reason for this interest was due to previous studies showing a significant role these genes played in colorectal cancer. Further confirmation of the presence of the aforementioned genes and lamin A/C status in each experimental sample, was done by RT-PCR.

4.2.5 RT-PCR studies to confirm lamin A/C status and gene expression in various tumour samples

4.2.5.1 RT-PCR to assess expression of lamin A and C in a tubulo-villous adenoma sample (Figure 4.4)

Using immunohistochemical techniques, no discernable nuclear expression of lamin A/C was noted in the tubulo-villous adenoma sample. However, to confirm this, semi quantitative RT – PCR was carried out on equal concentrations of total RNA (verified by a β-actin RT – PCR – Figure 4.4). No obvious expression of lamin A/C was observed. This observation was in keeping with the microarray results.

4.2.5.2 RT-PCR to assess expression of lamin A and C in a Dukes' C tumour sample (Figure 4.5)

Immunohistochemical analyses of the Dukes' C tumour sample did not show any expression of lamin A/C. To confirm this observation a semi quantitative RT-PCR was carried out on equal concentrations of total RNA (verified by a β-actin RT-PCR, figure 4.5). The RT-PCR did not show any evidence of lamin A/C expression in this tumour sample. Similarly, the microarray data also suggested no significant change of lamin A/C expression to be found in this specific tumour sample.

4.2.5.3 RT-PCR to assess expression of lamin A and C in a Dukes' B tumour sample known to express lamin A/C and its comparison with a similar Dukes' B tumour sample deficient in lamin A/C expression (Figure 4.6)

Immunohistochemical analyses of two Dukes' B tumour samples revealed strong nuclear envelope expression of lamin A/C in one tumour sample and complete absence of lamin A/C expression in the other tumour sample. To confirm these observations a semi quantitative RT-PCR was carried out on equal concentrations of total RNA from both tumour samples (verified by a β-actin RT-PCR, figure 4.6). The results of the RT-PCR supported the aforementioned observations of strong lamin A/C expression in one tumour sample and no expression of lamin A/C in the other tumour sample, respectively. The microarray data also confirmed these observations.

4.2.5.4 RT-PCR to assess the expression of creatine kinase brain (CKB) in a tubulo-villous adenoma (devoid of nuclear lamin A/C expression), a Dukes' B tumour sample (expressing strong nuclear lamin A/C), a Dukes' B tumour sample (devoid of nuclear lamin A/C) and a Dukes' C tumour sample (devoid of nuclear lamin A/C expression) (Figure 4.7)

Comparative microarray analysis of all tumour samples revealed a significant up regulation of creatine kinase brain (CKB) in the Dukes' B tumour sample expressing lamin A/C. To confirm this observation, a semi quantitative RT-PCR was carried on equal concentrations of total RNA from all tumour samples (verified by a β-actin RT-PCR, figure 4.8) as illustrated in figure 4.7. The results supported the microarray observation of increased expression of CKB in the Dukes' B tumour sample expressing lamin A/C.

4.2.5.5 RT-PCR to assess expression of T-Plastin and E-cadherin between two Dukes' B tumour samples, one expressing nuclear lamin A/C and the other devoid of nuclear lamin A/C expression (Figure 4.9)

A comparative microarray analysis between two Duke's B tumour samples, one expressing strong nuclear lamin A/C and the other devoid of nuclear lamin A/C expression, revealed an up regulation in the levels of T-plastin with a concomitant low expression of E-cadherin in the Dukes' B tumour sample expressing lamin A/C. On the other hand, significantly decreased expression levels of T-plastin with increased expression levels of E-cadherin were noted in the Dukes' B tumour sample exhibiting no lamin A/C expression. These observations were confirmed by

a semi quantitative RT-PCR using equal volumes of total RNA (verified by a β-actin RT-PCR, figure 4.9).

4.3 Tables and Figures

Table 4.1

Table 4.1 is a list of all patients with colorectal cancer participating in this study. A total of 43 patients participated in this study. Tumour site, Dukes' staging and lamin A/C expression profiles (positive, depleted or negative), in each case are listed.

Table 4.1

Table of all patients participating in the study, showing site of tumour, Dukes' stage and lamin A/C profile

Number	Site	Dukes Stage	Lamin A/C expression (Nuclear)
1	Rectum	A	Positive
2	Sigmoid	B	Lamin depleted
3	Recto-sigmoid junction	A	Lamin depleted
4	Ascending colon	C1	Lamin depleted
5	Sigmoid	B	Lamin depleted
6	Ascending colon	B	Negative
7	Ascending colon	B	Lamin depleted
8	Rectum	B	Negative
9	Rectum	C1	Positive
10	Hepatic Flexure	C1	Positive
11	Recto-sigmoid junction	B	Positive
12	Sigmoid	C1	Negative
13	Rectum	B	Lamin depleted
14	Hepatic Flexure	C1	Positive
15	Hepatic Flexure	B	Positive
16	Ascending colon	B	Negative
17	Rectum	B	Positive
18	Recto-sigmoid junction	C1	Positive
19	Ascending colon	C1	Negative
20	Rectum	B	Positive
21	Sigmoid	B	Lamin depleted
22	Sigmoid	C2	Positive
23	Rectum	C1	Lamin depleted
24	Sigmoid	C1	Lamin depleted
25	Sigmoid	B	Positive
26	Sigmoid	C2	Lamin depleted
27	Ascending colon	B	Negative
28	Rectum	C1	Lamin depleted
29	Rectum	TV - Adenoma	Negative
30	Sigmoid	C1	Lamin depleted
31	Sigmoid	B	Positive
32	Recto-sigmoid junction	A	Positive
33	Rectum	C1	Lamin depleted
34	Rectum	B	Positive
35	Sigmoid	C1	Negative
36	Rectum	B	Negative
37	Splenic flexure	B	Positive
38	Sigmoid	B	Lamin depleted
39	Ascending colon	A	Negative
40	Splenic flexure	A	Negative
41	Ascending colon	B	Negative
42	Ascending colon	B	Negative
43	Rectum	B	Positive

Table 4.2 (A and B)

Table 4.2 illustrates the relationship between various Dukes' stages and lamin A/C expression in tumour samples taken from all patients participating in this study. Due to low numbers, it is difficult to establish any statistical significance.

Table 4.2 A

Distribution of various Duke's stages and associated expression profiles of lamin A/C at each stage.

Duke's Stage	% Frequency	Lamin +ve	Lamin -ve	Lamin Depleted
A	11.91% (05 patients)	2 (40%)	2 (40%)	1 (20%)
B	52.38% (22 patients)	9 (40.91%)	7 (31.82%)	6 (27.27%)
C1	30.95% (13 patients)	4 (30.77%)	3 (23.08%)	6 (46.15%)
C2	04.76% (02 patients)	1 (50%)	0 (0%)	1 (50%)

Table 4.2 B

Chi square cross tabulation between Duke's stage and lamin A/C expression.

Observed Values	Lamin +ve	Lamin –ve	Lamin Depleted	Total
Duke's A	2	2	1	5
Duke's B	9	7	6	22
Duke's C1	4	3	6	13
Duke's C2	1	0	1	2
Total	16	12	14	42
Expected values	**Lamin +ve**	**Lamin –ve**	**Lamin Depleted**	**Total**
Duke's A	1.9	1.4	1.7	5
Duke's B	8.4	6.3	7.3	22
Duke's C1	5.0	3.7	4.3	13
Duke's C2	0.8	0.6	0.7	2
Total	16	12	14	42
			***p* =**	**0.85**

Simple chi-square cross-tabulation of Duke's stage against lamin status shows no significant correlation (p=0.85) although the size of the population (n=42) significantly limits the power of such statistical analysis.

Table 4.3

A comparative analysis of colorectal cancer according to Dukes' stage, lamin A/C expression and anatomical location

Site	Duke's A	Duke's B	Duke's C1	Duke's C2	Total
Ascending colon *Lamin A/C +ve* *Lamin A/C –ve* *Lamin A/C depleted*	**1** *0* *1* *0*	**6** *0* *6* *0*	**2** *0* *1* *1*	**0** *0* *0* *0*	**9 (21.42%)** *0* *8* *1*
Hepatic flexure *Lamin A/C +ve* *Lamin A/C –ve* *Lamin A/C depleted*	**0** *0* *0* *0*	**1** *1* *0* *0*	**2** *2* *0* *0*	**0** *0* *0* *0*	**3 (7.14%)** *3* *0* *0*
Transverse colon	**0**	**0**	**0**	**0**	**0**
Splenic flexure *Lamin A/C +ve* *Lamin A/C –ve* *Lamin A/C depleted*	**1** *0* *1* *0*	**1** *1* *0* *0*	**0** *0* *0* *0*	**0** *0* *0* *0*	**2 (4.76%)** *1* *1* *0*
Descending colon	**0**	**0**	**0**	**0**	**0**
Sigmoid colon *Lamin A/C +ve* *Lamin A/C –ve* *Lamin A/C depleted*	**0** *0* *0* *0*	**6** *2* *0* *4*	**4** *0* *2* *2*	**2** *1* *0* *1*	**12 (28.57%)** *3* *2* *7*
Recto-sigmoid junction *Lamin A/C +ve* *Lamin A/C –ve* *Lamin A/C depleted*	**2** *1* *0* *1*	**1** *1* *0* *0*	**1** *1* *0* *0*	**0** *0* *0* *0*	**4 (9.52%)** *3* *0* *1*
Rectum *Lamin A/C +ve* *Lamin A/C –ve* *Lamin A/C depleted*	**1** *1* *0* *0*	**7** *4* *2* *1*	**4** *1* *0* *3*	**0** *0* *0* *0*	**12 (28.57%)** *6* *2* *4*
Total	**5**	**22**	**13**	**2**	***42***

Table 4.4

List of total transcripts analysed by microarray with their corresponding signal detection values, from each tissue sample analysed

Sample	Total No of Transcripts	+ve Signal detection	-ve Signal detection	Moderate Signal detection	Detection *p*-value
TV Adenoma	54,675	22,664	31,163	848	≤0.05
Duke's B lamin A/C –ve	54,675	25,235	28,598	842	≤0.05
Duke' B Lamin A/C +ve	54,675	26,250	27,656	769	≤0.05
Duke's C Lamin A/C –ve	54,675	24,976	28,907	792	≤0.05

Table 4.5

A comparative microarray analysis of changes in functional genes between a Dukes' B adenocarcinoma (nuclear lamin A/C negative) tumour sample and a Tubulo-Villous adenoma (nuclear lamin A/C negative) tumour sample

Using microarray data obtained from the tubulo-villous (nuclear lamin A/C negative) adenoma sample as a baseline, a comparative microarray expression analysis was made to assess the twenty most up (red) and down (green) regulated genes in the Dukes' B (nuclear lamin A/C negative) adenocarcinoma sample. Fold change values have been shown as well as a brief description of the involved genes in the Dukes' B (nuclear lamin A/C negative) adenocarcinoma sample.

Table 4.5				
Identification of Up and Down Regulated Transcripts in a Duke's B (Lamin A/C negative) tumour when compared to a Tubulo-Villous adenoma				
Number	**Gene**	**Product**	**Fold Change**	**Up or Down regulated**
1	*GREM 1*	Gremlin 1, cysteine knot superfamily, homolog (Xenopus laevis)	59.71	Down
2	*PSPH*	Phosphoserine phosphatase	32	Down
3	*SPON1*	Spondin 1, (f-spondin) extracellular matrix protein	19.7	Down
4	*SI*	Sucrase isomaltase	18.38	Down
5	*KLK10*	Kallikrein 10	16	Down
6	*LYZ*	Lysozyme precursor	13.93	Down
7	*ATDC*	Ataxia-telangiectasia group D-associatedprotein	12.13	Down
8	*FKBP1B*	FK506-binding protein 1B (12.6 kD)	12.13	Down
9	*RARRES1*	Retinoic acid receptor responder (tazarotene induced) 1	11.31	Down
10	*MT1F*	Metallothionein 1F (functional)	10.56	Down
11	*MT1G*	Metallothionein 1G	9.85	Down
12	*LOC56901*	NADH:ubiquinone oxidoreductase MLRQ subunit homolog	9.85	Down
13	*KLK11*	Kallikrein 11	9.19	Down
14	*RARRES1*	Retinoic acid receptor responder (tazaroteneinduced) 1	9.19	Down
15	*SCYB11*	Interferon stimulated T-cell alphachemoattractant precursor	9.19	Down
16	*MT1X*	Metallothionein 1X	8.57	Down
17	*MT1H*	Metallothionein 1H	8	Down
18	*L6*	Human tumor antigen (L6)	8	Down
19	*MT1E*	Metallothionein 1E (functional)	8	Down
20	*HLA-DRB3*	Major histocompatibility complex, class II, DR beta 3	8	Down

Table 4.5				
Identification of Up and Down Regulated Transcripts in a Duke's B (Lamin A/C negative) tumour when compared to a Tubulo-Villous adenoma				
Number	**Gene**	**Product**	**Fold Change**	**Up or Down regulated**
21	*RPS11*	Ribosomal protein S11	6.06	Up
22	*PDZ-GEF1*	PDZ domain containing guanine nucleotide exchange factor(GEF)1	6.5	Up
23	*MEGT1*	Megakaryocyte-enhanced gene transcript 1protein	6.96	Up
24	*GZMB*	Granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1)	6.96	Up
25	*ARGBP2*	ArgAbl-interacting protein ArgBP2	6.96	Up
26	*ACE2*	Angiotensin I converting enzyme(peptidyl-dipeptidase A) 2	8.57	Up
27	*SRPUL*	Sushi-repeat protein	9.19	Up
28	*SAA2*	Serum amyloid A2	10.56	Up
29	*LACS5*	Long-chain acyl-CoA synthetase 5	10.56	Up
30	*NR1I3*	Nuclear receptor subfamily 1, group I, member 3	12.13	Up
31	*CKMT2*	Sarcomeric mitochondrial creatine kinase precursor	12.13	Up
32	*SLC26A2*	Solute carrier family 26 (sulfate transporter), member 2	13	Up
33	*PAP*	Pancreatitis-associated protein	16	Up
34	*SYN47*	Homer, neuronal immediate early gene, 1B	22.63	Up
35	*SNCAIP*	Synuclein, alpha interacting protein (synphilin)	48.5	Up
36	*SERPINB3*	Serine proteinase inhibitor, cladeB (Ovalbumin), member 3	59.71	Up
37	*FN1*	Fibronectin precursor	64	Up
38	*UGT2B17*	UDP Glycosyltransferase 2 family, polypeptide B 17	90.51	Up
39	*APOA2*	Apolipoprotein A-II	103.97	Up
40	*IGF2*	Insulin-like growth factor 2 (somatomedin A)	388.02	Up

Table 4.6

A comparative microarray analysis of changes in functional genes between a Dukes' C adenocarcinoma (nuclear lamin A/C negative) tumour sample and a Tubulo-Villous adenoma (nuclear lamin A/C negative) tumour sample

Using microarray data obtained from the tubulo-villous (nuclear lamin A/C negative) adenoma sample as a baseline, a comparative microarray expression analysis was made to assess the twenty most up (red) and down (green) regulated genes in the Dukes' C (nuclear lamin A/C negative) adenocarcinoma sample. Fold change values have been shown as well as a brief description of the involved genes in the Dukes' C (nuclear lamin A/C negative) adenocarcinoma sample.

Table 4.6				
Identification of Up and Down Regulated Transcripts in a Duke's C (Lamin A/C negative) tumour when compared to a Tubulo-Villous adenoma				
Number	**Gene**	**Product**	**Fold Change**	**Up or Down regulated**
1	RGS13	regulator of G protein signaling	84.45	Down
2	cPLA2	phosphatidylcholine 2-acylhydrolase	21.11	Down
3	DF	adipsin complement factor D precursor	19.7	Down
4	SLC21A8	solute carrier family 21 (organic aniontransporter), member 8	19.7	Down
5	H174	putative alpha chemokine	17.15	Down
6	SERPINB5	serine (or cysteine) proteinase inhibitor, cladeB (ovalbumin), member 5	13	Down
7	L6	Human tumor antigen (L6)	13	Down
8	MSLN	megakaryocyte potentiating factor precursor	12.13	Down
9	CA9	carbonic anhydrase IX	12.13	Down
10	KIAA1199	KIAA1199 protein	12.13	Down
11	ECM1	extracellular matrix protein 1	10.56	Down
12	CRIP1	cysteine-rich protein 1 (intestinal)	9.85	Down
13	SCYB11	interferon stimulated T-cell alphachemoattractant precursor	9.85	Down
14	TACSTD2	Human gastrointestinal tumor-associated antigen GA733-1 protein gene	9.19	Down
15	APG-1	heat shock protein (hsp110 family)	8.57	Down
16	UBD	diubiquitin	8.57	Down
17	IGFBP2	insulin-like growth factor binding protein 2(36kD)	7.46	Down
18	S100A4	S100 calcium-binding protein A4	7.46	Down
19	RARRES1	retinoic acid receptor responder (tazaroteneinduced) 1	7.46	Down
20	PHLDA1	pleckstrin homology-like domain, family A,member 1	7.46	Down

Table 4.6				
Identification of Up and Down Regulated Transcripts in a Duke's C (Lamin A/C negative) tumour when compared to a Tubulo-Villous adenoma				
Number	**Gene**	**Product**	**Fold Change**	**Up or Down regulated**
21	COL5A2	collagen, type V, alpha 2	10.56	Up
22	MMP2	Homo sapiens matrix metalloproteinase 2 (gelatinase A, 72kD gelatinase, 72kD type IV collagenase)	11.31	Up
24	RAB7L1	RAB7, member RAS oncogene family-like 1	11.31	Up
25	NID	nidogen (enactin)	12.13	Up
26	PDZ-GEF1	PDZ domain containing guanine nucleotide exchange factor(GEF)1	12.13	Up
27	CSPG2	chondroitin sulfate proteoglycan 2 (versican)	13	Up
28	PROS1	protein S (alpha)	13.93	Up
29	NPD009	NPD009	13.93	Up
30	LOC64167	aminopeptidase	13.93	Up
31	COL1A2	collagen, type I, alpha 2	18.38	Up
32	IGFBP5	Human insulin-like growth factor binding protein 5 (IGFBP5)	18.38	Up
33	CSPG2	chondroitin sulfate proteoglycan 2 (versican)	21.11	Up
34	PPBP	pro-platelet basic protein	29.86	Up
35	COL1A2	collagen, type I, alpha 2	32	Up
36	GUCA1B	guanylate cyclase activator 1B (retina)	36.76	Up
37	CLCA4	calcium activated chloride channel 4	39.4	Up
38	SLC26A2	solute carrier family 26 (sulfate transporter), member 2	42.22	Up
39	CHAK2	channel-kinase 2	45.25	Up
40	CA1	carbonic anhydrase I	78.79	Up

Table 4.7

A comparative microarray analysis of changes in functional genes between a Dukes' C adenocarcinoma (nuclear lamin A/C negative) tumour sample and a Dukes' B (nuclear lamin A/C negative) tumour sample

Using microarray data obtained from the Dukes' B (nuclear lamin A/C negative) adenocarcinoma sample as a baseline, a comparative microarray expression analysis was made to assess the twenty most up (red) and down (green) regulated genes in the Dukes' C (nuclear lamin A/C negative) adenocarcinoma sample. Fold change values have been shown as well as a brief description of the involved genes in the Dukes' C (nuclear lamin A/C negative) adenocarcinoma sample.

Table 4.7				
Identification of Up and Down Regulated Transcripts in a Duke's C (Lamin A/C negative) tumour when compared to a Duke's B (Lamin A/C negative) stage tumour				
Number	**Gene**	**Product**	**Fold Change**	**Up or Down regulated**
1	IGF2	insulin-like growth factor 2 (somatomedin A)	128	Down
2	CaCC1	calcium-activated chloride channel protein 1	18.38	Down
3	PNLIPRP2	pancreatic lipase-related protein 2	16	Down
4	UBD	diubiquitin	14.93	Down
5	MMP10	matrix metalloproteinase 10 preproprotein	13	Down
6	PAP	pancreatitis-associated protein	13	Down
7	KIAA1199	KIAA1199 protein	13	Down
8	RPS4Y	ribosomal protein S4, Y-linked	12.13	Down
9	HOXA9	Human class I homeoprotein	12.13	Down
10	EIF5A	eukaryotic translation initiation factor 5A	11.31	Down
11	CES1	acyl coenzyme A:cholesterol acyltransferase	11.31	Down
12	ID4	inhibitor of DNA binding 4, dominant negative helix-loop-helix protein	9.85	Down
13	APOA2	apolipoprotein A-II precursor	8.57	Down
14	SERPINA1	serine (or cysteine) proteinase inhibitor, cladeA (alpha-1 antiproteinase, antitrypsin), member 1	8.57	Down
15	KCNS3	potassium voltage-gated channel,delayed-rectifier, subfamily S, member 3	8	Down
16	PAH	phenylalanine hydroxylase	8	Down
17	OR216	olfactory receptor, family 2, subfamily I, member 6	8	Down
18	IGFBP2	insulin-like growth factor binding protein 2	6.96	Down
19	EGR3	early growth response 3	6.5	Down
20	FDXR	ferredoxin reductase isoform 2 precursor	6.5	Down

Table 4.7				
Identification of Up and Down Regulated Transcripts in a Duke's C (Lamin A/C negative) tumour when compared to a Duke's B (Lamin A/C negative) stage tumour				
Number	**Gene**	**Product**	**Fold Change**	**Up or Down regulated**
21	LOC63928	hepatocellular carcinoma antigen gene 520	6.96	Up
22	PCK1	phosphoenolpyruvate carboxykinase 1 (soluble)	7.46	Up
23	ADH2	class I alcohol dehydrogenase (ADH2) beta-1 subunit	7.46	Up
24	LOC64167	aminopeptidase	7.46	Up
25	CHAK2	channel-kinase 2	7.46	Up
26	COL1A2	collagen, type I, alpha 2	7.46	Up
27	KIAA1775	MT-protocadherin	8	Up
28	ROBO1	roundabout (axon guidance receptor, Drosophila) homolog 1	8	Up
29	SLC4A4	solute carrier family 4, sodium bicarbonatecotransporter, member 4	8.57	Up
30	GUCA1B	guanylate cyclase activator 1B (retina)	9.85	Up
31	CLCA4	calcium activated chloride channel 4	10.56	Up
32	KIAA0193	KIAA0193 gene product	11.31	Up
33	PIPOX	sarcosine oxidase	12.13	Up
34	PSPHL	L-3-phosphoserine phosphatase homolog	17.15	Up
35	SI	sucrase-isomaltase	18.38	Up
36	MUC12	transmembrane mucin 12	22.63	Up
37	PPBP	pro-platelet basic protein	29.86	Up
38	EREG	epiregulin precursor	34.3	Up
39	CA4	carbonic anhydrase IV precursor	51.98	Up
40	CA1	carbonic anhydrase I	97.01	Up

Table 4.8

A comparative microarray analysis of changes in functional genes between a Dukes' B (nuclear lamin A/C positive) tumour sample and a Dukes' B adenocarcinoma (nuclear lamin A/C negative) tumour sample

Using microarray data obtained from the Dukes' B (nuclear lamin A/C negative) adenocarcinoma sample as a baseline, a comparative microarray expression analysis was made to assess the twenty most up (red) and down (green) regulated genes in the Dukes' B (nuclear lamin A/C positive) adenocarcinoma sample. Fold change values have been shown as well as a brief description of the involved genes in the Dukes' B (nuclear lamin A/C positive) adenocarcinoma sample.

Table 4.8

Identification of up and down regulated transcripts in a Duke's B (lamin A/C positive) tumour when compared to a Duke's B (lamin A/C negative) Tumour

Number	Gene	Product	Fold Change	Up or Down regulated
1	HMGCS2	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2(mitochondrial)	84.45	Up
2	CKB	creatine kinase, brain	84.45	Up
3	DUOX2	dual oxidase-like domains 2	68.59	Up
4	SRPUL	sushi-repeat protein	59.71	Up
5	SLC3A1	amino acid transport protein	55.72	Up
6	KRT20	cytokeratin 20	51.98	Up
7	AZGP1	zinc-alpha2-glycoprotein precursor	48.5	Up
8	ACE2	angiotensin I converting enzyme (peptidyl-dipeptidase A) 2	42.22	Up
9	PCANAP6	prostate cancer associated protein 6	39.4	Up
10	IGFBP2	insulin-like growth factor binding protein 2	39.4	Up
11	SLC26A2	solute carrier family 26 (sulfate transporter), member 2	39.4	Up
12	PCCA	Propionyl-Coenzyme A carboxylase, alphapolypeptide	32	Up
13	SLU7	step II splicing factor SLU7	32	Up
14	CCND2	cyclin D2	29.86	Up
15	VA3	vav 3 oncogene	25.99	Up
16	LR8	LR8 protein	25.99	Up
17	UGT1A9	UDP glycosyltransferase 1 family, polypeptide A9	24.25	Up
18	ARSE	arylsulfatase E	22.63	Up
19	NOX1	NADPH oxidase 1 isoform long	22.63	Up
20	MEP1A	meprin A, alpha (PABA peptide hydrolase)	21.11	Up

Table 4.8				
Identification of up and down regulated transcripts in a Duke's B lamin A/C positive tumour when compared to a Duke's B lamin A/C negative Tumour				
Number	**Gene**	**Product**	**Fold Change**	**Up or Down regulated**
21	CD14	CD14 antigen precursor	9.19	Down
22	CLECSF6	C-type (calcium dependent,carbohydrate-recognition domain) lectin, superfamilymember 6	9.85	Down
24	LCP2	lymphocyte cytosolic protein 2	21.11	Down
25	RARRES1	retinoic acid receptor responder (tazarotene induced) 1	22.63	Down
26	CD163	M130 antigen cytoplasmic variant 1	11.31	Down
27	BCL2A1	BCL2-related protein A1	11.31	Down
28	PRG1	proteoglycan 1, secretory granule	12.13	Down
29	CD69	early activation antigen CD69	13.93	Down
30	TNFAIP6	tumor necrosis factor, alpha-induced protein 6	27.86	Down
31	HSAPOMUCIN	apomucin	16	Down
32	PTGS2	cyclooxygenase 2b	18.38	Down
33	TREM1	triggering receptor expressed on myeloid cells1	18.38	Down
34	SCYB5	small inducible cytokine subfamily B, member 5	22.63	Down
35	MUC5B	mucin 5, subtype B, tracheobronchial	45.25	Down
36	IL8	interleukin 8 C-terminal variant	22.63	Down
37	FPR2	formyl peptide receptor	45.25	Down
38	PIWIL1	piwi (Drosophila)-like 1	48.5	Down
39	VNN1	vanin 1	51.98	Down
40	nephropontin	nephropontin	97.01	Down

Table 4.9

A comparative microarray analysis of changes in functional gene groups between a Dukes' B (nuclear lamin A/C negative) tumour sample and a Tubulo-villous adenoma (nuclear lamin A/C negative) tumour sample

Using microarray data obtained from the tubulo-villous (nuclear lamin A/C negative) adenoma sample as a baseline, a comparative microarray expression analysis was made to assess changes in sixteen functional gene groups, containing 164 genes in total, in the Dukes' B (nuclear lamin A/C negative) adenocarcinoma sample. Affymetrix Id numbers, unique Gene codes, fold change (FC) values, using an arbitrary cut off level set at +/- 9, and a brief description of the genes were also included amongst other criteria, as mentioned in section 4.2.4.3. Genes of interest were appropriately highlighted to indicate either an up (highlighted in red) or down (highlighted in green) regulation. Some genes not exhibiting a significant FC values were also highlighted (in blue) as this information was deemed important in analysis of the functional gene group as a whole.

Table 4.9					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's B (Iam -ve) vs TV Adenoma (Iam -ve)		Gene Description
			Fold Change		
			UP	Down	
Apoptosis and apoptotic inhibitors					
AMID / PRG3	220811_at	NM_006093.2		10.56	p53-responsive gene
BAK1	203728_at	NM_001188.1	*1*		BCL2-antagonist/killer 1
CASP3	202763_at	NM_004346.1		1.52	caspase 3, apoptosis-related cysteine protease
CAST	207467_x_at	NM_001750.2		1.23	calpastatin
FASLG	210865_at	NM_000639.1		1.52	Fas ligand (TNF superfamily, member 6)
NCKAP1 / NAP1	207738_s_at	NM_013436.1		1.41	NCK-associated protein 1
TNFRSF10A / TRAILR1	1552648_a_at	NM_003844.2		1.23	tumour necrosis factor receptor superfamily, member 10a
Cell adhesion					
CTNNA1	200764_s_at	NM_001903.1		1.23	catenin (cadherin-associated protein), alpha 1
Cell cycle progression / cell proliferation and growth					
CCNA1	205899_at	NM_003914.1		8	cyclin A1
CCNB1	228729_at	N90191	1.15		cyclin B1
CCNB2	202705_at	NM_004701.2	1.15		cyclin B2
CCND1	208711_s_at	BC000076.1		1.07	cyclin D1
CCND3	201700_at	NM_001760.1		1.23	cyclin D3
CDC2	203214_x_at	NM_001786.1		1.32	cell division cycle 2, G1 to S and G2 to M / cdk1
CDKN1B	209112_at	NM_004064.1		1.15	cyclin-dependant kinase inhibitor 1B
CHK1	205393_s_at	NM_001274.1		1.87	checkpoint kinase Chk1
CHK2	210416_s_at	NM_007194.1	1.62		checkpoint kinase Chk2
CSPG2	204620_s_at	NM_004385.1	3.03		chondroitin sulfate proteoglycan 2 (versican)

Table 4.9					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's B (lam -ve) vs TV Adenoma (lam -ve)		Gene Description
			Fold Change		
			UP	Down	
EGF	206254_at	NM_001963.2		3.48	epidermal growth factor
FGF5	208378_x_at	NM_004464.1		1.32	fibroblast growth factor 5
GPC3	209220_at	NM_004484.2	8		glypican 3
IGF2	202410_x_at	NM_000612.2	388.02		insulin-like growth factor 2 (somatomedin A)
TGFA	205015_s_at	NM_003236.1		1	transforming growth factor, alpha
TGFB1 / BIGH3	203084_at	NM_000660.1	4.92		transforming growth factor, beta-induced
VEGF	210512_s_at	NM_003376.1	1.15		vascular endothelial growth factor
Cytoskeleton and nucleoskeleton					
ACTG1	201550_x_at	NM_001614.2	1		actin, gamma 1
KRT1	205900_at	NM_006121.1	3.25		keratin 1
KRT18	201596_x_at	NM_000224.1		1.07	keratin 18
KRT8	209008_x_at	NM_002273.1		1.23	keratin 8
LCP1	208885_at	NM_002298.2		1.07	lymphocyte cytosolic protein 1 (L-plastin)
LMNA (lamin C)	203411_s_at	NM_005572.1		2.3	lamin C
LMNB1	203276_at	NM_005573.1	2		lamin B1
MACF1	1553407_at	NM_033044.1	9.85		microtubule-actin crosslinking factor1
NEBL	203962_s_at	NM_006393.1		1.32	nebulette
NEFH	204412_s_at	NM_021076.1		2.46	neurofilament, heavy polypeptide 200 kDa
PLS3	201215_at	NM_005032.2	1.07		plastin 3 (T isoform)
CDH1	201131_s_at	NM_004360.1		1.32	cadherin 1, type 1, E-cadherin (epithelial)
PPL	203407_at	NM_002705.1		2	periplakin

Table 4.9					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's B (lam -ve) vs TV Adenoma (lam -ve)		Gene Description
			Fold Change		
			UP	Down	
TMPO(A)	203432_at	NM_003276.1	1.41		thymopoietin alpha / lamina-associated polypeptide 2 alpha
TUBB2	208977_x_at	NM_006088.1		1.62	tubulin, beta 2
TUBG	201714_at	NM_001070.1	1.07		tubulin, gamma 1
TUBG2	203894_at	NM_016437.1	2.14		tubulin, gamma 2
VCL	200930_s_at	NM_014000.1	1.32		vinculin
VIL2	208621_s_at	NM_003379.2		2.64	villin 2 (ezrin)
VIM	201426_s_at	NM_003380.1		1.15	vimentin
DNA replication and repair					
ATM	208442_s_at	NM_000051.1	1.62		ataxia telangectasia mutated
MLH1	202520_s_at	NM_000249.1		1.32	mutL homolog 1, colon cancer, non-polyposis type 2
MSH2	209421_at	NM_000251.1		1.52	mutS homolog 2, colon cancer, non-polyposis type 1
NBS1	202907_s_at	NM_002485.2	1.32		Nijmegen breakage syndrome 1 (nibrin)
PRKDC	210543_s_at	U34994.3	1.32		DNA-dependant protein kinase catalytic subunit
RPA1	201528_at	NM_002945.1	1		replication protein A1
XRCC4	205071_x_at	AB017445.1		1.07	DNA-repair protein XRCC4
XRCC5	208642_s_at	NM_021141.2		1.07	Ku autoantigen
Extracellular matrix: components, processing, cellular attachment, proteases and protease inhibitors					
BGN	201262_s_at	NM_001711.1		6.5	biglycan
COLIA2	202404_s_at	NM_000089.1	5.28		collagen type I, alpha 2
FN1 / FN	212464_s_at	X02761.1	64		fibronectin, alt splice
HPSE	219403_s_at	NM_006665.1		2	heparanase

Table 4.9					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's B (lam -ve) vs TV Adenoma (lam -ve)		Gene Description
			Fold Change		
			UP	Down	
ITGA1	214660_at	X68742.1	2		integrin, alpha 1
ITGA2	205032_at	NM_002203.2		1.23	integrin, alpha 2
ITGA3	229257_at	AI625045	1.87		integrin, alpha 3
ITGAV	202351_at	NM_002210.1		2.46	integrin, alpha V (vitronectin receptor)
ITGB1	216190_x_at	AA215854	2.14		integrin, beta 1
LAMA4	202202_s_at	NM_002290.2	2.14		laminin alpha 4
LAMB1	211651_s_at	M20206.1	1		laminin beta 1
MMP14	202827_s_at	NM_004995.2	2.83		matrix metalloproteinase 14 (membrane inserted)
MMP3	205828_at	NM_002422.2	2.83		matrix metalloproteinase 3 (stromelysin 1, progelatinase)
SDC4	202071_at	NM_002999.1		1.41	syndecan 4 (amphiglycan, ryudocan)
SPP1	209875_s_at	M83248.1		3.25	secreted phosphoprotein 1 (osteopontin)
TIMP1	201666_at	NM_003254.1	2.64		tissue inhibitor of metalloproteinase 1
Oncogenes					
ABL1	202123_s_at	NM_005157.2	1.07		v-abl Abelson murine leukemia viral oncogene homolg 1
HRAS	212983_at	NM_005343.1	1.15		v-Ha-ras Harvey rat sarcoma viral oncogene homolog
JUN	201465_s_at	NM_002228.2		2.46	v-jun sarcoma virus 17 oncogene homolog
KRAS	204009_s_at	NM_004985.1		1.23	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
MOS	221367_at	NM_005372.1	1.15		v-mos Moloney murine sarcoma viral oncogene homolog
MYC	202431_s_at	NM_002467.1		1.52	v-myc myelocytomatosis viral oncogene homolog
MYCN	209757_s_at	NM_005378.1	4.29		N-myc proto-oncogene protein
RAF1	201244_s_at	NM_002880.1	1.15		v-raf-1 murine leukemia viral oncogene homolog 1

Table 4.9					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's B (lam -ve) vs TV Adenoma (lam -ve)		Gene Description
			Fold Change		
			UP	Down	
SRC	221281_at	NM_005417.1		1.32	v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog
Protein translation, processing, transport and degradation					
AIM1	206513_at	NM_004833.1		1.07	absent in melanoma 1
EIF3S	221912_s_at	AL049795	1.62		eukaryocytic translation initiation factor 3, subunit 2 beta
HSP70-1	219212_at	NM_016299.1		1.41	heat shock 70kD protein 1
HSPA8 / HSC70	221891_x_at	AA704004		1.32	constitutive heat shock protein 70
HSPB2	205824_at	NM_001541.1		1.52	heat shock 27kDa protein 2
NUP153	202097_at	NM_005124.1		1.32	nucleporin 153kDa
RPL21	200012_x_at	NM_000982.1		1.15	ribosomal protein L21
RPL31	200963_x_at	NM_000993.1		1.15	ribosomal protein L31
Regulation of gene expression (transcription)					
GTF3A	201338_x_at	NM_002097.1		1.52	general transcription factor IIIA
RARA	203750_s_at	NM_000964.1	1.87		retinoic acid receptor, alpha
RXRA	202449_s_at	NM_002957.2		1.62	retinoid X receptor, alpha
TCF1	216930_at	X71347.1	11.31		transcription factor 1, hepatic
TCF4	203753_at	NM_003199.1		1.07	transcription factor 4
ZFP91	206059_at	NM_003430.1	1.15		zinc finger protein 91 homolog (mouse)
Signal transduction					
CCR7	206337_at	NM_001838.1	1.41		chemokine (C-C motif) receptor 7
CD14	201743_at	NM_000591.1		1.74	CD14 antigen
FCGR2B	210889_s_at	M31933.1	1		IGFR2 / Fc fragment of IgG, low affinity IIb, receptor

Table 4.9					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's B (lam -ve) vs TV Adenoma (lam -ve)		Gene Description
			Fold Change		
			UP	Down	
GNAL	206356_s_at	NM_002071.1		6.96	G-s-alpha / guanine nucleotide binding protein (G protein)
MAPK1	208351_s_at	NM_002745.1		1.52	mitogen-activated protein kinase 1
PIK3CG	206370_at	NM_002649.1		10.56	phosphoinositide-3-kinase, catalytic, gamma polypeptide
PTK2B / PKB	203111_s_at	U33284.1	1		PTK2B protein tyrosine kinase 2 beta
RAC1	208640_at	NM_006908.2		1.52	rho family, small GTP binding protein Rac1
RGS2	202388_at	NM_002923.1		1.41	regulator of G-protein signaling 2 (G0/G1 switch regulatory protein 8
TIAM1	206409_at	NM_003253.1		1.41	T-cell lymphoma invasion and metastasis 1
Stem cell differentitaion					
ASTN	209693_at	AF116574.1		1.23	Astrotactin
BMP4	211518_s_at	D30751.1		1.41	bone morphogenetic protein 4
BMP6	206176_at	NM_001718.2	3.25		bone morphogenetic protein 6
FN1	216442_x_at	AK026737.1	18.38		cellular fibronectin
FUT1	206109_at	NM_000148.1	2		fucosyltransferase 1
FUT2	208505_s_at	NM_000511.1	1.32		fucosyltransferase 2 (secretor status included)
GAP43	204471_at	NM_002045.1		1.07	growth associated protein 43
GJA1	201667_at	NM_000165.2		1.23	gap juction protein, alpha 1 (connexin 43)
LAMB1	201505_at	NM_002291.1		1.23	laminin, beta 1
MAPT / TAU	203929_s_at	NM_016835.1		18.38	microtubule-associated protein tau
MASH1 / ASCL1	209985_s_at	NM_004316.1	2.64		achaete-scute homolog 1
MASH2 / ASCL2	207607_at	NM_005170.1		3.73	achaete-scute complex-like 2

Table 4.9					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's B (lam -ve) vs TV Adenoma (lam -ve)		Gene Description
			Fold Change		
			UP	Down	
MYOD1	206657_s_at	NM_002478.2		1.07	myogenic factor 3
NEFL	221801_x_at	NM_006158.1	3.03		neurofilament, light polypeptide 68kDa
NEUROD2	1552953_a_at	NM_006160.2	1.74		neurogenic differentiation 2
NEUROD1	206282_at	NM_002500.1		1.07	neurogenic differentiation 3
NEUROD4	221318_at	NM_021191.1		4.59	neurogenic differentiation 4
NHLH2	214497_s_at	NM_005599.1		1.23	nescient helix loop helix 2
NOTCH2	202445_s_at	NM_024408.1		1.62	Notch homolog 2 (Drosophila)
NSE	201313_at	NM_001975.1	1.52		neuron-specific enolase
PAX6	205646_s_at	NM_000280.1	1		paired box gene 6 (aniridia, keratitis)
PLP	210198_s_at	BC002665.1		3.73	proteolipid protein
REST	204535_s_at	NM_005612.1		1.32	RE1-silencing transcription factor
SOX1	208533_at	NM_005986.1	2.14		SRY (sex determining region)-box 1
SOX17	219568_x_at	NM_018419.1		1.32	SRY (sex determining region)-box 17
SOX2	214178_s_at	AI356682	1.41		SRY (sex determining region)-box 2
SYP	213200_at	U93305	1.32		synaptophysin
TDGF1	206286_s_at	NM_003212.1		1.74	teratocarcinoma-derived growth factor 1
TF	203400_s_at	NM_001063.1	1.23		transferrin
VTN	204534_at	NM_000638.1	4.92		vitronectin
Stress response					
SOD1	200642_at	NM_000454.1		1.23	superoxide dismutase 1, soluble
SOD2	216841_s_at	X15132.1	1.23		superoxide dismutase 2, mitochondrial

Table 4.9					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's B (lam -ve) vs TV Adenoma (lam -ve)		Gene Description
			Fold Change		
			UP	Down	
Transporters, carriers					
ATP2A3	207522_s_at	NM_005173.1		1.32	ATPase, Ca++ transporting, ubiquitous
SLC16A1	202234_s_at	NM_003051.1		1.32	solute carrier family 16 (monocarboxylic acid transporters), member 1
SLC2A1 / GLUT1	201250_s_at	NM_006516.1		2.64	solute carrier family 2 (facilitated glucose transporter), member 1
Tumour suppressor genes					
APC	203527_s_at	NM_000038.1		1.23	adenomatosis polyposis coli
DCC	206939_at	NM_005215.1		2.83	deleted in colorectal carcinoma
RB1	203132_at	NM_000321.1		1.87	retinoblastoma 1
TP53	201746_at	NM_000546.2		1.15	tumour protein p53 (Li-Fraumeni syndrome)
Wnt signalling					
BIRC5	210334_x_at	AB028869.1	1.15		apoptosis inhibitor 4 - survivin
CDKN1A	202284_s_at	NM_000389.1	2.3		cyclin-dependant kinase inhibitor 1A (p21, Cip1)
CEACAM1 / BGP1	206576_s_at	NM_001712.1	1.62		carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)
CHAF1A	203976_s_at	NM_005483.1	1.41		chromatin assembly factor 1, subunit A (p150)
CLDN1	218182_s_at	NM_021101.1		1.32	claudin 1
CLDN4	201428_at	NM_001305.1		3.03	claudin 4
DLEU1	205677_s_at	NM_005887.1		2	deleted in lymphocyte leukemia, 1
ENC1	201341_at	NM_003633.1	1.52		ectodermal-neural cortex (with BTB-like domain)
EPHB1	230425_at	AI674183	4.59		EPH receptor B1
EPHB2	209588_at	AL530874	1.15		EPH receptor B2
ETS2	201328_at	NM_005239.1		1.32	v-ets erythroblastosis virus E26 oncogene homolog 2

Table 4.9					
Gene Symbol	**Affymetrix Id No**	**NM GENE**	**Duke's B (lam -ve) vs TV Adenoma (lam -ve)**		**Gene Description**
			Fold Change		
			UP	**Down**	
FABP1	205892_s_at	NM_001443.1	1.32		fatty acid binding protein 1, liver
GPX2	202831_at	NM_002083.1	1.23		glutathione peroxidase 2 (gastrointestinal)
JPO1 / LOC442172	224428_s_at	AY029179.1		1.23	c-MYC target JPO1
MCM3	201555_at	NM_002388.2	1.23		MCM3 minichromosome maintenance deficient 3
MUC2	204673_at	NM_002457.1		2.14	mucin 2, intestinal/tracheal
MYB	204798_at	NM_005375.1	1.32		Myb proto-oncogene protein (C-myb)
PRKCD	202545_at	NM_006254.1	1		protein kinase C, delta
RBBP4	210371_s_at	NM_005610.1		2	retinoblastoma binding protein 4
SCF / KITLG	216974_at	S80491.1	10.56		stem cell factor / kit ligand precursor / Mast cell growth factor (MGF)
TCOF1	202385_s_at	NM_000356.1	1.32		treacher Collins-Franceschetti syndrome 1
UNG	202330_s_at	NM_003362.1		1.32	uracil-DNA glycosylase
Miscellaneous					
PTGS2 / COX2	204748_at	NM_000963.1		1.23	prostaglandin-endoperoxide synthase 2 (cyclooxygenase)
CKB	200884_at	NM_001823.1	1		Creatine kinase brain
Gene used as a positive control test					
ß-Actin	200801_x_at	NM_001101.2		1.07	Beta actin

Table 4.10

A comparative microarray analysis of changes in functional gene groups between a Dukes' B (nuclear lamin A/C positive) tumour sample and a Tubulo-villous adenoma (nuclear lamin A/C negative) tumour sample

Using microarray data obtained from the tubulo-villous (nuclear lamin A/C negative) adenoma sample as a baseline, a comparative microarray expression analysis was made to assess changes in sixteen functional gene groups, containing 164 genes in total, in the Dukes' B (nuclear lamin A/C positive) adenocarcinoma sample. Affymetrix Id numbers, unique Gene codes, fold change (FC) values, using an arbitrary cut off level set at +/- 9, and a brief description of the genes were also included amongst other criteria, as mentioned in section 4.2.4.3. Genes of interest were appropriately highlighted to indicate either an up (highlighted in red) or down (highlighted in green) regulation. Some genes not exhibiting a significant FC values were also highlighted (in blue) as this information was deemed important in analysis of the functional gene group as a whole.

Table 4.10					
Gene Symbol	Affymetrix Id No	NM GENE	TV Adenoma (lam -ve) vs Duke's B (lam +ve)		Gene Description
			Fold change		
			UP	DOWN	
Apoptosis and apoptotic inhibitors					
AMID / PRG3	220811_at	NM_006093.2	12.13		p53-responsive gene
BAK1	203728_at	NM_001188.1	2.14		BCL2-antagonist/killer 1
CASP3	202763_at	NM_004346.1	1.52		caspase 3, apoptosis-related cysteine protease
CAST	207467_x_at	NM_001750.2	2.46		calpastatin
FASLG	210865_at	NM_000639.1	1.52		Fas ligand (TNF superfamily, member 6)
NCKAP1 / NAP1	207738_s_at	NM_013436.1	2.14		NCK-associated protein 1
TNFRSF10A / TRAILR1	1552648_a_at	NM_003844.2	3.25		tumour necrosis factor receptor superfamily, member 10a
Cell adhesion					
CTNNA1	200764_s_at	NM_001903.1	1.74		catenin (cadherin-associated protein), alpha 1
Cell cycle progression / cell proliferation and growth					
CCNA1	205899_at	NM_003914.1	17.15		cyclin A1
CCNB1	228729_at	N90191	1.32		cyclin B1
CCNB2	202705_at	NM_004701.2	1.52		cyclin B2
CCND1	208711_s_at	BC000076.1	1.15		cyclin D1
CCND3	201700_at	NM_001760.1	1.32		cyclin D3
CDC2	203214_x_at	NM_001786.1	1.52		cell division cycle 2, G1 to S and G2 to M / cdk1
CDKN1B	209112_at	NM_004064.1	3.73		cyclin-dependant kinase inhibitor 1B
CHK1	205393_s_at	NM_001274.1	2.3		checkpoint kinase Chk1
CHK2	210416_s_at	NM_007194.1	1.15		checkpoint kinase Chk2
CSPG2	204620_s_at	NM_004385.1		13	chondroitin sulfate proteoglycan 2 (versican)

Table 4.10					
Gene Symbol	Affymetrix Id No	NM GENE	TV Adenoma (lam -ve) vs Duke's B (lam +ve)		Gene Description
			Fold change		
			UP	DOWN	
EGF	206254_at	NM_001963.2	13.93		epidermal growth factor
FGF5	208378_x_at	NM_004464.1	1.32		fibroblast growth factor 5
GPC3	209220_at	NM_004484.2	1.52		glypican 3
IGF2	202410_x_at	NM_000612.2	2.46		insulin-like growth factor 2 (somatomedin A)
TGFA	205015_s_at	NM_003236.1	1.87		transforming growth factor, alpha
TGFB1 / BIGH3	203084_at	NM_000660.1	1.32		transforming growth factor, beta-induced
VEGF	210512_s_at	NM_003376.1	1.74		vascular endothelial growth factor
Cytoskeleton and nucleoskeleton					
ACTG1	201550_x_at	NM_001614.2	1.62		actin, gamma 1
KRT1	205900_at	NM_006121.1	1.87		keratin 1
KRT18	201596_x_at	NM_000224.1	2.64		keratin 18
KRT8	209008_x_at	NM_002273.1	3.73		keratin 8
LCP1	208885_at	NM_002298.2		3.48	lymphocyte cytosolic protein 1 (L-plastin)
LMNA (lamin C)	203411_s_at	NM_005572.1	2.83		lamin C
LMNB1	203276_at	NM_005573.1		1.87	lamin B1
MACF1	1553407_at	NM_033044.1		9.85	microtubule-actin crosslinking factor1
NEBL	203962_s_at	NM_006393.1	1.87		nebulette
NEFH	204412_s_at	NM_021076.1		2.14	neurofilament, heavy polypeptide 200 kDa
PLS3	201215_at	NM_005032.2	9.19		plastin 3 (T isoform)
CDH1	201131_s_at	NM_004360.1		2	cadherin 1, type 1, E-cadherin (epithelial)
PPL	203407_at	NM_002705.1	4.92		periplakin

Table 4.10					
Gene Symbol	Affymetrix Id No	NM GENE	TV Adenoma (lam -ve) vs Duke's B (lam +ve)		Gene Description
			Fold change		
			UP	DOWN	
TMPO(A)	203432_at	NM_003276.1		1.87	thymopoietin alpha / lamina-associated polypeptide 2 alpha
TUBB2	208977_x_at	NM_006088.1	2.46		tubulin, beta 2
TUBG	201714_at	NM_001070.1	1.41		tubulin, gamma 1
TUBG2	203894_at	NM_016437.1		1.15	tubulin, gamma 2
VCL	200930_s_at	NM_014000.1	1.15		vinculin
VIL2	208621_s_at	NM_003379.2	3.03		villin 2 (ezrin)
VIM	201426_s_at	NM_003380.1		2.83	vimentin
DNA replication and repair					
ATM	208442_s_at	NM_000051.1	1.15		ataxia telangectasia mutated
MLH1	202520_s_at	NM_000249.1	13.93		mutL homolog 1, colon cancer, non-polyposis type 2
MSH2	209421_at	NM_000251.1	1.87		mutS homolog 2, colon cancer, non-polyposis type 1
NBS1	202907_s_at	NM_002485.2	1.23		Nijmegen breakage syndrome 1 (nibrin)
PRKDC	210543_s_at	U34994.3		1.07	DNA-dependant protein kinase catalytic subunit
RPA1	201528_at	NM_002945.1	1.52		replication protein A1
XRCC4	205071_x_at	AB017445.1	2.3		DNA-repair protein XRCC4
XRCC5	208642_s_at	NM_021141.2	2.64		Ku autoantigen
Extracellular matrix: components, processing, cellular attachment, proteases and protease inhibitors					
BGN	201262_s_at	NM_001711.1	1.41		biglycan
COLIA2	202404_s_at	NM_000089.1		10.56	collagen type I, alpha 2
FN1 / FN	212464_s_at	X02761.1		103.97	fibronectin, alt splice
HPSE	219403_s_at	NM_006665.1		1.74	heparanase

Table 4.10					
Gene Symbol	Affymetrix Id No	NM GENE	TV Adenoma (lam -ve) vs Duke's B (lam +ve)		Gene Description
			Fold change		
			UP	DOWN	
ITGA1	214660_at	X68742.1		1	integrin, alpha 1
ITGA2	205032_at	NM_002203.2	2.64		integrin, alpha 2
ITGA3	229257_at	AI625045		1.23	integrin, alpha 3
ITGAV	202351_at	NM_002210.1	2.3		integrin, alpha V (vitronectin receptor)
ITGB1	216190_x_at	AA215854		6.96	integrin, beta 1
LAMA4	202202_s_at	NM_002290.2		2.14	laminin alpha 4
LAMB1	211651_s_at	M20206.1	4.29		laminin beta 1
MMP14	202827_s_at	NM_004995.2		2.46	matrix metalloproteinase 14 (membrane inserted)
MMP3	205828_at	NM_002422.2	2.14		matrix metalloproteinase 3 (stromelysin 1, progelatinase)
SDC4	202071_at	NM_002999.1	2.3		syndecan 4 (amphiglycan, ryudocan)
SPP1	209875_s_at	M83248.1		24.25	secreted phosphoprotein 1 (osteopontin)
TIMP1	201666_at	NM_003254.1		2.14	tissue inhibitor of metalloproteinase 1
Oncogenes					
ABL1	202123_s_at	NM_005157.2	1.52		v-abl Abelson murine leukemia viral oncogene homolg 1
HRAS	212983_at	NM_005343.1	1.07		v-Ha-ras Harvey rat sarcoma viral oncogene homolog
JUN	201465_s_at	NM_002228.2	2.64		v-jun sarcoma virus 17 oncogene homolog
KRAS	204009_s_at	NM_004985.1	1.74		v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
MOS	221367_at	NM_005372.1	1.07		v-mos Moloney murine sarcoma viral oncogene homolog
MYC	202431_s_at	NM_002467.1	4.29		v-myc myelocytomatosis viral oncogene homolog
MYCN	209757_s_at	NM_005378.1		1	N-myc proto-oncogene protein
RAF1	201244_s_at	NM_002880.1		1	v-raf-1 murine leukemia viral oncogene homolog 1

Table 4.10					
Gene Symbol	Affymetrix Id No	NM GENE	TV Adenoma (lam -ve) vs Duke's B (lam +ve)		Gene Description
			Fold change		
			UP	DOWN	
SRC	221281_at	NM_005417.1	2		v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog
Protein translation, processing, transport and degradation					
AIM1	206513_at	NM_004833.1	1.15		absent in melanoma 1
EIF3S	221912_s_at	AL049795	1.23		eukaryocytic translation initiation factor 3, subunit 2 beta
HSP70-1	219212_at	NM_016299.1	3.48		heat shock 70kD protein 1
HSPA8 / HSC70	221891_x_at	AA704004	2.3		constitutive heat shock protein 70
HSPB2	205824_at	NM_001541.1	2.83		heat shock 27kDa protein 2
NUP153	202097_at	NM_005124.1	2.3		nucleporin 153kDa
RPL21	200012_x_at	NM_000982.1	2.64		ribosomal protein L21
RPL31	200963_x_at	NM_000993.1	2.46		ribosomal protein L31
Regulation of gne expression (transcription)					
GTF3A	201338_x_at	NM_002097.1	3.73		general transcription factor IIIA
RARA	203750_s_at	NM_000964.1		1.15	retinoic acid receptor, alpha
RXRA	202449_s_at	NM_002957.2	1.32		retinoid X receptor, alpha
TCF1	216930_at	X71347.1		4.29	transcription factor 1, hepatic
TCF4	203753_at	NM_003199.1		1.41	transcription factor 4
ZFP91	206059_at	NM_003430.1	1.41		zinc finger protein 91 homolog (mouse)
Signal transduction					
CCR7	206337_at	NM_001838.1	1.52		chemokine (C-C motif) receptor 7
CD14	201743_at	NM_000591.1		6.06	CD14 antigen
FCGR2B	210889_s_at	M31933.1		3.73	IGFR2 / Fc fragment of IgG, low affinity IIb, receptor

Table 4.10					
Gene Symbol	Affymetrix Id No	NM GENE	TV Adenoma (lam -ve) vs Duke's B (lam +ve)		Gene Description
			Fold change		
			UP	DOWN	
GNAL	206356_s_at	NM_002071.1	8.57		G-s-alpha / guanine nucleotide binding protein (G protein)
MAPK1	208351_s_at	NM_002745.1	1.41		mitogen-activated protein kinase 1
PIK3CG	206370_at	NM_002649.1	13		phosphoinositide-3-kinase, catalytic, gamma polypeptide
PTK2B / PKB	203111_s_at	U33284.1	1.32		PTK2B protein tyrosine kinase 2 beta
RAC1	208640_at	NM_006908.2	2.83		rho family, small GTP binding protein Rac1
RGS2	202388_at	NM_002923.1		3.25	regulator of G-protein signaling 2 (G0/G1 switch regulatory protein 8
TIAM1	206409_at	NM_003253.1	2.14		T-cell lymphoma invasion and metastasis 1
Stem cell differentitaion					
ASTN	209693_at	AF116574.1	4		Astrotactin
BMP4	211518_s_at	D30751.1	45.25		bone morphogenetic protein 4
BMP6	206176_at	NM_001718.2		1.62	bone morphogenetic protein 6
FN1	216442_x_at	AK026737.1		32	cellular fibronectin
FUT1	206109_at	NM_000148.1	1.52		fucosyltransferase 1
FUT2	208505_s_at	NM_000511.1		1	fucosyltransferase 2 (secretor status included)
GAP43	204471_at	NM_002045.1	8		growth associated protein 43
GJA1	201667_at	NM_000165.2	1.32		gap juction protein, alpha 1 (connexin 43)
LAMB1	201505_at	NM_002291.1	5.66		laminin, beta 1
MAPT / TAU	203929_s_at	NM_016835.1	1.87		microtubule-associated protein tau
MASH1 / ASCL1	209985_s_at	NM_004316.1	1.41		achaete-scute homolog 1
MASH2 / ASCL2	207607_at	NM_005170.1	32		achaete-scute complex-like 2

Table 4.10					
Gene Symbol	Affymetrix Id No	NM GENE	TV Adenoma (lam -ve) vs Duke's B (lam +ve)		Gene Description
			Fold change		
			UP	DOWN	
MYOD1	206657_s_at	NM_002478.2	2.14		myogenic factor 3
NEFL	221801_x_at	NM_006158.1	2.46		neurofilament, light polypeptide 68kDa
NEUROD2	1552953_a_at	NM_006160.2	1.52		neurogenic differentiation 2
NEUROD1	206282_at	NM_002500.1	6.96		neurogenic differentiation 3
NEUROD4	221318_at	NM_021191.1	5.66		neurogenic differentiation 4
NHLH2	214497_s_at	NM_005599.1	1.52		nescient helix loop helix 2
NOTCH2	202445_s_at	NM_024408.1	1.62		Notch homolog 2 (Drosophila)
NSE	201313_at	NM_001975.1	2.14		neuron-specific enolase
PAX6	205646_s_at	NM_000280.1	1.32		paired box gene 6 (aniridia, keratitis)
PLP	210198_s_at	BC002665.1	24.25		proteolipid protein
REST	204535_s_at	NM_005612.1	4.29		RE1-silencing transcription factor
SOX1	208533_at	NM_005986.1	1.74		SRY (sex determining region)-box 1
SOX17	219568_x_at	NM_018419.1		2.3	SRY (sex determining region)-box 17
SOX2	214178_s_at	AI356682		1.52	SRY (sex determining region)-box 2
SYP	213200_at	U93305	1.87		synaptophysin
TDGF1	206286_s_at	NM_003212.1	18.38		teratocarcinoma-derived growth factor 1
TF	203400_s_at	NM_001063.1	1.15		transferrin
VTN	204534_at	NM_000638.1		1.32	vitronectin
Stress response					
SOD1	200642_at	NM_000454.1	1.87		superoxide dismutase 1, soluble
SOD2	216841_s_at	X15132.1		2.83	superoxide dismutase 2, mitochondrial

Table 4.10					
Gene Symbol	Affymetrix Id No	NM GENE	TV Adenoma (lam -ve) vs Duke's B (lam +ve)		Gene Description
			Fold change		
			UP	DOWN	
Transporters, carriers					
ATP2A3	207522_s_at	NM_005173.1		1.23	ATPase, Ca++ transporting, ubiquitous
SLC16A1	202234_s_at	NM_003051.1	13.93		solute carrier family 16 (monocarboxylic acid transporters), member 1
SLC2A1 / GLUT1	201250_s_at	NM_006516.1	6.5		solute carrier family 2 (facilitated glucose transporter), member 1
Tumour suppressor genes					
APC	203527_s_at	NM_000038.1	1.52		adenomatosis polyposis coli
DCC	206939_at	NM_005215.1	2.64		deleted in colorectal carcinoma
RB1	203132_at	NM_000321.1	2		retinoblastoma 1
TP53	201746_at	NM_000546.2	2		tumour protein p53 (Li-Fraumeni syndrome)
Wnt signalling					
BIRC5	210334_x_at	AB028869.1	1.32		apoptosis inhibitor 4 - survivin
CDKN1A	202284_s_at	NM_000389.1	2.3		cyclin-dependant kinase inhibitor 1A (p21, Cip1)
CEACAM1 / BGP1	206576_s_at	NM_001712.1	4.92		carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)
CHAF1A	203976_s_at	NM_005483.1		1	chromatin assembly factor 1, subunit A (p150)
CLDN1	218182_s_at	NM_021101.1	1.15		claudin 1
CLDN4	201428_at	NM_001305.1	4.59		claudin 4
DLEU1	205677_s_at	NM_005887.1	2.64		deleted in lymphocyte leukemia, 1
ENC1	201341_at	NM_003633.1	2.83		ectodermal-neural cortex (with BTB-like domain)
EPHB1	230425_at	AI674183		19.7	EPH receptor B1
EPHB2	209588_at	AL530874	2.14		EPH receptor B2
ETS2	201328_at	NM_005239.1	2		v-ets erythroblastosis virus E26 oncogene homolog 2

Table 4.10					
Gene Symbol	Affymetrix Id No	NM GENE	TV Adenoma (Iam -ve) vs Duke's B (Iam +ve)		Gene Description
			Fold change		
			UP	DOWN	
FABP1	205892_s_at	NM_001443.1	1097.5		fatty acid binding protein 1, liver
GPX2	202831_at	NM_002083.1	4.29		glutathione peroxidase 2 (gastrointestinal)
JPO1 / LOC442172	224428_s_at	AY029179.1	3.25		c-MYC target JPO1
MCM3	201555_at	NM_002388.2	1.52		MCM3 minichromosome maintenance deficient 3
MUC2	204673_at	NM_002457.1	1.15		mucin 2, intestinal/tracheal
MYB	204798_at	NM_005375.1	2		Myb proto-oncogene protein (C-myb)
PRKCD	202545_at	NM_006254.1	1.15		protein kinase C, delta
RBBP4	210371_s_at	NM_005610.1	1.52		retinoblastoma binding protein 4
SCF / KITLG	216974_at	S80491.1	2		stem cell factor / kit ligand precursor / Mast cell growth factor (MGF)
TCOF1	202385_s_at	NM_000356.1	1.32		treacher Collins-Franceschetti syndrome 1
UNG	202330_s_at	NM_003362.1	1.87		uracil-DNA glycosylase
Miscellaneous					
PTGS2 / COX2	204748_at	NM_000963.1		8	prostaglandin-endoperoxide synthase 2 (cyclooxygenase)
CKB	200884_at	NM_001823.1	84.45		Creatine kinase brain
Gene used as a positive control test					
β-Actin	200801_x_at	NM_001101.2		1.74	Beta actin

Table 4.11

A comparative microarray analysis of changes in functional gene groups between a Dukes' C (nuclear lamin A/C negative) tumour sample and a Tubulo-villous adenoma (nuclear lamin A/C negative) tumour sample

Using microarray data obtained from the tubulo-villous (nuclear lamin A/C negative) adenoma sample as a baseline, a comparative microarray expression analysis was made to assess changes in sixteen functional gene groups, containing 164 genes in total, in the Dukes' C (nuclear lamin A/C negative) adenocarcinoma sample. Affymetrix Id numbers, unique Gene codes, fold change (FC) values, using an arbitrary cut off level set at +/- 9, and a brief description of the genes were also included amongst other criteria, as mentioned in section 4.2.4.3. Genes of interest were appropriately highlighted to indicate either an up (highlighted in red) or down (highlighted in green) regulation. Some genes not exhibiting a significant FC values were also highlighted (in blue) as this information was deemed important in analysis of the functional gene group as a whole.

Table 4.11					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's C (lam -ve) vs TV Adenoma (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
Apoptosis and apoptotic inhibitors					
AMID / PRG3	220811_at	NM_006093.2		6.96	p53-responsive gene
BAK1	203728_at	NM_001188.1		1.32	BCL2-antagonist/killer 1
CASP3	202763_at	NM_004346.1		1.52	caspase 3, apoptosis-related cysteine protease
CAST	207467_x_at	NM_001750.2		1.15	calpastatin
FASLG	210865_at	NM_000639.1		4	Fas ligand (TNF superfamily, member 6)
NCKAP1 / NAP1	207738_s_at	NM_013436.1		1.52	NCK-associated protein 1
TNFRSF10A / TRAILR1	1552648_a_at	NM_003844.2		1.87	tumour necrosis factor receptor superfamily, member 10a
Cell adhesion					
CTNNA1	200764_s_at	NM_001903.1		1.32	catenin (cadherin-associated protein), alpha 1
Cell cycle progression / cell proliferation and growth					
CCNA1	205899_at	NM_003914.1		4	cyclin A1
CCNB1	228729_at	N90191		1.32	cyclin B1
CCNB2	202705_at	NM_004701.2	1		cyclin B2
CCND1	208711_s_at	BC000076.1		1.23	cyclin D1
CCND3	201700_at	NM_001760.1	1.87		cyclin D3
CDC2	203214_x_at	NM_001786.1		1.52	cell division cycle 2, G1 to S and G2 to M / cdk1
CDKN1B	209112_at	NM_004064.1	1.07		cyclin-dependant kinase inhibitor 1B
CHK1	205393_s_at	NM_001274.1		1.52	checkpoint kinase Chk1
CHK2	210416_s_at	NM_007194.1	1.62		checkpoint kinase Chk2
CSPG2	204620_s_at	NM_004385.1	13		chondroitin sulfate proteoglycan 2 (versican)

Table 4.11					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's C (lam -ve) vs TV Adenoma (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
EGF	206254_at	NM_001963.2		24.25	epidermal growth factor
FGF5	208378_x_at	NM_004464.1	7.46		fibroblast growth factor 5
GPC3	209220_at	NM_004484.2	1.07		glypican 3
IGF2	202410_x_at	NM_000612.2	1		insulin-like growth factor 2 (somatomedin A)
TGFA	205015_s_at	NM_003236.1	1.15		transforming growth factor, alpha
TGFB1 / BIGH3	203084_at	NM_000660.1	4.29		transforming growth factor, beta-induced
VEGF	210512_s_at	NM_003376.1	1		vascular endothelial growth factor
Cytoskeleton and nucleoskeleton					
ACTG1	201550_x_at	NM_001614.2		1.07	actin, gamma 1
KRT1	205900_at	NM_006121.1	1.62		keratin 1
KRT18	201596_x_at	NM_000224.1		2.14	keratin 18
KRT8	209008_x_at	NM_002273.1		2.14	keratin 8
LCP1	208885_at	NM_002298.2		1.23	lymphocyte cytosolic protein 1 (L-plastin)
LMNA (lamin C)	203411_s_at	NM_005572.1		2.3	lamin C
LMNB1	203276_at	NM_005573.1	1.74		lamin B1
MACF1	1553407_at	NM_033044.1	16		microtubule-actin crosslinking factor1
NEBL	203962_s_at	NM_006393.1	1.07		nebulette
NEFH	204412_s_at	NM_021076.1	3.25		neurofilament, heavy polypeptide 200 kDa
PLS3	201215_at	NM_005032.2		1.52	plastin 3 (T isoform)
CDH1	201131_s_at	NM_004360.1	1.15		cadherin 1, type 1, E-cadherin (epithelial)
PPL	203407_at	NM_002705.1		1.15	periplakin

Table 4.11					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's C (lam -ve) vs TV Adenoma (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
TMPO(A)	203432_at	NM_003276.1		1.74	thymopoietin alpha / lamina-associated polypeptide 2 alpha
TUBB2	208977_x_at	NM_006088.1		1.52	tubulin, beta 2
TUBG	201714_at	NM_001070.1		1.23	tubulin, gamma 1
TUBG2	203894_at	NM_016437.1	2.64		tubulin, gamma 2
VCL	200930_s_at	NM_014000.1		1.74	vinculin
VIL2	208621_s_at	NM_003379.2		1.41	villin 2 (ezrin)
VIM	201426_s_at	NM_003380.1	1.62		vimentin
DNA replication and repair					
ATM	208442_s_at	NM_000051.1	2.3		ataxia telangectasia mutated
MLH1	202520_s_at	NM_000249.1		1.23	mutL homolog 1, colon cancer, non-polyposis type 2
MSH2	209421_at	NM_000251.1		1.74	mutS homolog 2, colon cancer, non-polyposis type 1
NBS1	202907_s_at	NM_002485.2		1.07	Nijmegen breakage syndrome 1 (nibrin)
PRKDC	210543_s_at	U34994.3	1.32		DNA-dependant protein kinase catalytic subunit
RPA1	201528_at	NM_002945.1		1.41	replication protein A1
XRCC4	205071_x_at	AB017445.1		1.52	DNA-repair protein XRCC4
XRCC5	208642_s_at	NM_021141.2		1.52	Ku autoantigen
Extracellular matrix: components, processing, cellular attachment, proteases and protease inhibitors					
BGN	201262_s_at	NM_001711.1	1.41		biglycan
COLIA2	202404_s_at	NM_000089.1	32		collagen type I, alpha 2
FN1 / FN	212464_s_at	X02761.1	294.07		fibronectin, alt splice
HPSE	219403_s_at	NM_006665.1	1.15		heparanase

Table 4.11					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's C (lam -ve) vs TV Adenoma (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
ITGA1	214660_at	X68742.1	2		integrin, alpha 1
ITGA2	205032_at	NM_002203.2		1.74	integrin, alpha 2
ITGA3	229257_at	AI625045	1.41		integrin, alpha 3
ITGAV	202351_at	NM_002210.1		2	integrin, alpha V (vitronectin receptor)
ITGB1	216190_x_at	AA215854	9.19		integrin, beta 1
LAMA4	202202_s_at	NM_002290.2	11.31		laminin alpha 4
LAMB1	211651_s_at	M20206.1		1.15	laminin beta 1
MMP14	202827_s_at	NM_004995.2	4		matrix metalloproteinase 14 (membrane inserted)
MMP3	205828_at	NM_002422.2	1.07		matrix metalloproteinase 3 (stromelysin 1, progelatinase)
SDC4	202071_at	NM_002999.1	1.74		syndecan 4 (amphiglycan, ryudocan)
SPP1	209875_s_at	M83248.1		1.23	secreted phosphoprotein 1 (osteopontin)
TIMP1	201666_at	NM_003254.1	1.87		tissue inhibitor of metalloproteinase 1
Oncogenes					
ABL1	202123_s_at	NM_005157.2	1.15		v-abl Abelson murine leukemia viral oncogene homolg 1
HRAS	212983_at	NM_005343.1	1.41		v-Ha-ras Harvey rat sarcoma viral oncogene homolog
JUN	201465_s_at	NM_002228.2		2	v-jun sarcoma virus 17 oncogene homolog
KRAS	204009_s_at	NM_004985.1		1.15	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
MOS	221367_at	NM_005372.1	1.74		v-mos Moloney murine sarcoma viral oncogene homolog
MYC	202431_s_at	NM_002467.1		1.52	v-myc myelocytomatosis viral oncogene homolog
MYCN	209757_s_at	NM_005378.1		1.23	N-myc proto-oncogene protein
RAF1	201244_s_at	NM_002880.1	1.15		v-raf-1 murine leukemia viral oncogene homolog 1

Table 4.11					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's C (lam -ve) vs TV Adenoma (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
SRC	221281_at	NM_005417.1	4		v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog
Protein translation, processing, transport and degradation					
AIM1	206513_at	NM_004833.1	1.32		absent in melanoma 1
EIF3S	221912_s_at	AL049795	2.64		eukaryocytic translation initiation factor 3, subunit 2 beta
HSP70-1	219212_at	NM_016299.1		1.15	heat shock 70kD protein 1
HSPA8 / HSC70	221891_x_at	AA704004	1		constitutive heat shock protein 70
HSPB2	205824_at	NM_001541.1	1.52		heat shock 27kDa protein 2
NUP153	202097_at	NM_005124.1		1.15	nucleporin 153kDa
RPL21	200012_x_at	NM_000982.1	1		ribosomal protein L21
RPL31	200963_x_at	NM_000993.1		1.23	ribosomal protein L31
Regulation of gne expression (transcription)					
GTF3A	201338_x_at	NM_002097.1	1.32		general transcription factor IIIA
RARA	203750_s_at	NM_000964.1	1.23		retinoic acid receptor, alpha
RXRA	202449_s_at	NM_002957.2	1.32		retinoid X receptor, alpha
TCF1	216930_at	X71347.1	5.66		transcription factor 1, hepatic
TCF4	203753_at	NM_003199.1	4.59		transcription factor 4
ZFP91	206059_at	NM_003430.1	2		zinc finger protein 91 homolog (mouse)
Signal transduction					
CCR7	206337_at	NM_001838.1	1.52		chemokine (C-C motif) receptor 7
CD14	201743_at	NM_000591.1		1.07	CD14 antigen
FCGR2B	210889_s_at	M31933.1	1.74		IGFR2 / Fc fragment of IgG, low affinity IIb, receptor

Table 4.11					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's C (lam -ve) vs TV Adenoma (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
GNAL	206356_s_at	NM_002071.1		6.5	G-s-alpha / guanine nucleotide binding protein (G protein)
MAPK1	208351_s_at	NM_002745.1		1.41	mitogen-activated protein kinase 1
PIK3CG	206370_at	NM_002649.1		1.23	phosphoinositide-3-kinase, catalytic, gamma polypeptide
PTK2B / PKB	203111_s_at	U33284.1	1.52		PTK2B protein tyrosine kinase 2 beta
RAC1	208640_at	NM_006908.2	1.07		rho family, small GTP binding protein Rac1
RGS2	202388_at	NM_002923.1		1.23	regulator of G-protein signaling 2 (G0/G1 switch regulatory protein 8
TIAM1	206409_at	NM_003253.1		1.07	T-cell lymphoma invasion and metastasis 1
Stem cell differentitaion					
ASTN	209693_at	AF116574.1		1.62	Astrotactin
BMP4	211518_s_at	D30751.1		4.92	bone morphogenetic protein 4
BMP6	206176_at	NM_001718.2	1.32		bone morphogenetic protein 6
FN1	216442_x_at	AK026737.1	84.45		cellular fibronectin
FUT1	206109_at	NM_000148.1	2.46		fucosyltransferase 1
FUT2	208505_s_at	NM_000511.1	1.32		fucosyltransferase 2 (secretor status included)
GAP43	204471_at	NM_002045.1		6.96	growth associated protein 43
GJA1	201667_at	NM_000165.2	1.41		gap juction protein, alpha 1 (connexin 43)
LAMB1	201505_at	NM_002291.1		1.87	laminin, beta 1
MAPT / TAU	203929_s_at	NM_016835.1	1.15		microtubule-associated protein tau
MASH1 / ASCL1	209985_s_at	NM_004316.1	1.32		achaete-scute homolog 1
MASH2 / ASCL2	207607_at	NM_005170.1		1.62	achaete-scute complex-like 2

Table 4.11					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's C (lam -ve) vs TV Adenoma (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
MYOD1	206657_s_at	NM_002478.2	1.15		myogenic factor 3
NEFL	221801_x_at	NM_006158.1	3.48		neurofilament, light polypeptide 68kDa
NEUROD2	1552953_a_at	NM_006160.2	1.23		neurogenic differentiation 2
NEUROD1	206282_at	NM_002500.1	3.25		neurogenic differentiation 3
NEUROD4	221318_at	NM_021191.1		3.03	neurogenic differentiation 4
NHLH2	214497_s_at	NM_005599.1	1.62		nescient helix loop helix 2
NOTCH2	202445_s_at	NM_024408.1	1		Notch homolog 2 (Drosophila)
NSE	201313_at	NM_001975.1		1.87	neuron-specific enolase
PAX6	205646_s_at	NM_000280.1	1.07		paired box gene 6 (aniridia, keratitis)
PLP	210198_s_at	BC002665.1	3.73		proteolipid protein
REST	204535_s_at	NM_005612.1		3.03	RE1-silencing transcription factor
SOX1	208533_at	NM_005986.1	1.32		SRY (sex determining region)-box 1
SOX17	219568_x_at	NM_018419.1		1.23	SRY (sex determining region)-box 17
SOX2	214178_s_at	AI356682	3.03		SRY (sex determining region)-box 2
SYP	213200_at	U93305		1.15	synaptophysin
TDGF1	206286_s_at	NM_003212.1	1.23		teratocarcinoma-derived growth factor 1
TF	203400_s_at	NM_001063.1	9.85		transferrin
VTN	204534_at	NM_000638.1		1.23	vitronectin
Stress response					
SOD1	200642_at	NM_000454.1		1.07	superoxide dismutase 1, soluble
SOD2	216841_s_at	X15132.1		1.62	superoxide dismutase 2, mitochondrial

Table 4.11					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's C (lam -ve) vs TV Adenoma (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
Transporters, carriers					
ATP2A3	207522_s_at	NM_005173.1	1		ATPase, Ca++ transporting, ubiquitous
SLC16A1	202234_s_at	NM_003051.1	1		solute carrier family 16 (monocarboxylic acid transporters), member 1
SLC2A1 / GLUT1	201250_s_at	NM_006516.1		3.25	solute carrier family 2 (facilitated glucose transporter), member 1
Tumour suppressor genes					
APC	203527_s_at	NM_000038.1		1.07	adenomatosis polyposis coli
DCC	206939_at	NM_005215.1	1.74		deleted in colorectal carcinoma
RB1	203132_at	NM_000321.1		1.07	retinoblastoma 1
TP53	201746_at	NM_000546.2	1.41		tumour protein p53 (Li-Fraumeni syndrome)
Wnt signalling					
BIRC5	210334_x_at	AB028869.1		1.41	apoptosis inhibitor 4 - survivin
CDKN1A	202284_s_at	NM_000389.1		1.52	cyclin-dependant kinase inhibitor 1A (p21, Cip1)
CEACAM1 / BGP1	206576_s_at	NM_001712.1	1.41		carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)
CHAF1A	203976_s_at	NM_005483.1		1.15	chromatin assembly factor 1, subunit A (p150)
CLDN1	218182_s_at	NM_021101.1		4	claudin 1
CLDN4	201428_at	NM_001305.1		1.87	claudin 4
DLEU1	205677_s_at	NM_005887.1		1.87	deleted in lymphocyte leukemia, 1
ENC1	201341_at	NM_003633.1		1.32	ectodermal-neural cortex (with BTB-like domain)
EPHB1	230425_at	AI674183	6.5		EPH receptor B1
EPHB2	209588_at	AL530874		1.62	EPH receptor B2
ETS2	201328_at	NM_005239.1		1.32	v-ets erythroblastosis virus E26 oncogene homolog 2

Table 4.11					
Gene Symbol	**Affymetrix Id No**	**NM GENE**	**Duke's C (lam -ve) vs TV Adenoma (lam -ve)**		**Gene Description**
			Fold change		
			UP	**DOWN**	
FABP1	205892_s_at	NM_001443.1	1.23		fatty acid binding protein 1, liver
GPX2	202831_at	NM_002083.1		1.41	glutathione peroxidase 2 (gastrointestinal)
JPO1 / LOC442172	224428_s_at	AY029179.1	1		c-MYC target JPO1
MCM3	201555_at	NM_002388.2		1.32	MCM3 minichromosome maintenance deficient 3
MUC2	204673_at	NM_002457.1		1.32	mucin 2, intestinal/tracheal
MYB	204798_at	NM_005375.1	1.62		Myb proto-oncogene protein (C-myb)
PRKCD	202545_at	NM_006254.1	1.23		protein kinase C, delta
RBBP4	210371_s_at	NM_005610.1		2	retinoblastoma binding protein 4
SCF / KITLG	216974_at	S80491.1	2.14		stem cell factor / kit ligand precursor / Mast cell growth factor (MGF)
TCOF1	202385_s_at	NM_000356.1	1.87		treacher Collins-Franceschetti syndrome 1
UNG	202330_s_at	NM_003362.1		1.52	uracil-DNA glycosylase
Miscellaneous					
PTGS2 / COX2	204748_at	NM_000963.1		3.03	prostaglandin-endoperoxide synthase 2 (cyclooxygenase)
CKB	200884_at	NM_001823.1		1.87	Creatine kinase brain
Gene used as a positive control test					
β-Actin	200801_x_at	NM_001101.2	1		Beta actin

Table 4.12

A comparative microarray analysis of changes in functional gene groups between a Dukes' B (nuclear lamin A/C positive) tumour sample and a Dukes' B (nuclear lamin A/C negative) tumour sample

Using microarray data obtained from the Dukes' B (nuclear lamin A/C negative) adenocarcinoma sample as a baseline, a comparative microarray expression analysis was made to assess changes in sixteen functional gene groups, containing 164 genes in total, in the Dukes' B (nuclear lamin A/C positive) adenocarcinoma sample. Affymetrix Id numbers, unique Gene codes, fold change (FC) values, using an arbitrary cut off level set at +/- 9, and a brief description of the genes were also included amongst other criteria, as mentioned in section 4.2.4.3. Genes of interest were appropriately highlighted to indicate either an up (highlighted in red) or down (highlighted in green) regulation. Some genes not exhibiting a significant FC values were also highlighted (in blue) as this information was deemed important in analysis of the functional gene group as a whole.

Table 4.12					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's B (lam +ve) vs Duke's B (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
Apoptosis and apoptotic inhibitors					
AMID / PRG3	220811_at	NM_006093.2		1.74	p53-responsive gene
BAK1	203728_at	NM_001188.1	1.32		BCL2-antagonist/killer 1
CASP3	202763_at	NM_004346.1		1.87	caspase 3, apoptosis-related cysteine protease
CAST	207467_x_at	NM_001750.2	1.07		calpastatin
FASLG	210865_at	NM_000639.1		2	Fas ligand (TNF superfamily, member 6)
NCKAP1 / NAP1	207738_s_at	NM_013436.1		1.41	NCK-associated protein 1
TNFRSF10A / TRAILR1	1552648_a_at	NM_003844.2	1.62		tumour necrosis factor receptor superfamily, member 10a
Cell adhesion					
CTNNA1	200764_s_at	NM_001903.1		1.23	catenin (cadherin-associated protein), alpha 1
Cell cycle progression / cell proliferation and growth					
CCNA1	205899_at	NM_003914.1	1		cyclin A1
CCNB1	228729_at	N90191		1.23	cyclin B1
CCNB2	202705_at	NM_004701.2		1.15	cyclin B2
CCND1	208711_s_at	BC000076.1		1.41	cyclin D1
CCND3	201700_at	NM_001760.1		1.41	cyclin D3
CDC2	203214_x_at	NM_001786.1		1.52	cell division cycle 2, G1 to S and G2 to M / cdk1
CDKN1B	209112_at	NM_004064.1	1.52		cyclin-dependant kinase inhibitor 1B
CHK1	205393_s_at	NM_001274.1		1.32	checkpoint kinase Chk1
CHK2	210416_s_at	NM_007194.1	1.07		checkpoint kinase Chk2
CSPG2	204620_s_at	NM_004385.1		6.96	chondroitin sulfate proteoglycan 2 (versican)

Table 4.12					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's B (lam +ve) vs Duke's B (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
EGF	206254_at	NM_001963.2	2.64		epidermal growth factor
FGF5	208378_x_at	NM_004464.1		1.15	fibroblast growth factor 5
GPC3	209220_at	NM_004484.2	4.29		glypican 3
IGF2	202410_x_at	NM_000612.2	1024		insulin-like growth factor 2 (somatomedin A)
TGFA	205015_s_at	NM_003236.1	1.15		transforming growth factor, alpha
TGFB1 / BIGH3	203084_at	NM_000660.1	1.41		transforming growth factor, beta-induced
VEGF	210512_s_at	NM_003376.1	1		vascular endothelial growth factor
Cytoskeleton and nucleoskeleton					
ACTG1	201550_x_at	NM_001614.2		1.15	actin, gamma 1
KRT1	205900_at	NM_006121.1	5.28		keratin 1
KRT18	201596_x_at	NM_000224.1	1.41		keratin 18
KRT8	209008_x_at	NM_002273.1	1.62		keratin 8
LCP1	208885_at	NM_002298.2		9.19	lymphocyte cytosolic protein 1 (L-plastin)
LMNA (lamin C)	203411_s_at	NM_005572.1		1.52	lamin C
LMNB1	203276_at	NM_005573.1		1.87	lamin B1
MACF1	1553407_at	NM_033044.1		1.52	microtubule-actin crosslinking factor1
NEBL	203962_s_at	NM_006393.1		1.23	nebulette
NEFH	204412_s_at	NM_021076.1		8	neurofilament, heavy polypeptide 200 kDa
PLS3	201215_at	NM_005032.2	9.19		plastin 3 (T isoform)
CDH1	201131_s_at	NM_004360.1		1.32	cadherin 1, type 1, E-cadherin (epithelial)
PPL	203407_at	NM_002705.1	1.07		periplakin

Table 4.12					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's B (lam +ve) vs Duke's B (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
TMPO(A)	203432_at	NM_003276.1		1.32	thymopoietin alpha / lamina-associated polypeptide 2 alpha
TUBB2	208977_x_at	NM_006088.1		1.32	tubulin, beta 2
TUBG	201714_at	NM_001070.1		1.15	tubulin, gamma 1
TUBG2	203894_at	NM_016437.1		1.32	tubulin, gamma 2
VCL	200930_s_at	NM_014000.1		1.07	vinculin
VIL2	208621_s_at	NM_003379.2		1.52	villin 2 (ezrin)
VIM	201426_s_at	NM_003380.1		6.06	vimentin
DNA replication and repair					
ATM	208442_s_at	NM_000051.1		1.07	ataxia telangectasia mutated
MLH1	202520_s_at	NM_000249.1	6.06		mutL homolog 1, colon cancer, non-polyposis type 2
MSH2	209421_at	NM_000251.1		1.52	mutS homolog 2, colon cancer, non-polyposis type 1
NBS1	202907_s_at	NM_002485.2		1.15	Nijmegen breakage syndrome 1 (nibrin)
PRKDC	210543_s_at	U34994.3		1.52	DNA-dependant protein kinase catalytic subunit
RPA1	201528_at	NM_002945.1		1.23	replication protein A1
XRCC4	205071_x_at	AB017445.1	1.32		DNA-repair protein XRCC4
XRCC5	208642_s_at	NM_021141.2	1.32		Ku autoantigen
Extracellular matrix: components, processing, cellular attachment, proteases and protease inhibitors					
BGN	201262_s_at	NM_001711.1		6.5	biglycan
COLIA2	202404_s_at	NM_000089.1		4.29	collagen type I, alpha 2
FN1 / FN	212464_s_at	X02761.1		3.03	fibronectin, alt splice
HPSE	219403_s_at	NM_006665.1		6.06	heparanase

Table 4.12					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's B (lam +ve) vs Duke's B (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
ITGA1	214660_at	X68742.1	1.15		integrin, alpha 1
ITGA2	205032_at	NM_002203.2	1.41		integrin, alpha 2
ITGA3	229257_at	AI625045		1.32	integrin, alpha 3
ITGAV	202351_at	NM_002210.1		2	integrin, alpha V (vitronectin receptor)
ITGB1	216190_x_at	AA215854		5.28	integrin, beta 1
LAMA4	202202_s_at	NM_002290.2		1.74	laminin alpha 4
LAMB1	211651_s_at	M20206.1	2.46		laminin beta 1
MMP14	202827_s_at	NM_004995.2		2.3	matrix metalloproteinase 14 (membrane inserted)
MMP3	205828_at	NM_002422.2	2.64		matrix metalloproteinase 3 (stromelysin 1, progelatinase)
SDC4	202071_at	NM_002999.1		1.07	syndecan 4 (amphiglycan, ryudocan)
SPP1	209875_s_at	M83248.1		97.01	secreted phosphoprotein 1 (osteopontin)
TIMP1	201666_at	NM_003254.1	1.32		tissue inhibitor of metalloproteinase 1
Oncogenes					
ABL1	202123_s_at	NM_005157.2		1.15	v-abl Abelson murine leukemia viral oncogene homolg 1
HRAS	212983_at	NM_005343.1		1.15	v-Ha-ras Harvey rat sarcoma viral oncogene homolog
JUN	201465_s_at	NM_002228.2		1.87	v-jun sarcoma virus 17 oncogene homolog
KRAS	204009_s_at	NM_004985.1		1.23	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
MOS	221367_at	NM_005372.1		1.74	v-mos Moloney murine sarcoma viral oncogene homolog
MYC	202431_s_at	NM_002467.1	1.41		v-myc myelocytomatosis viral oncogene homolog
MYCN	209757_s_at	NM_005378.1	2.14		N-myc proto-oncogene protein
RAF1	201244_s_at	NM_002880.1		1.41	v-raf-1 murine leukemia viral oncogene homolog 1

Table 4.12					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's B (lam +ve) vs Duke's B (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
SRC	221281_at	NM_005417.1	1.07		v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog
Protein translation, processing, transport and degradation					
AIM1	206513_at	NM_004833.1		1.52	absent in melanoma 1
EIF3S	221912_s_at	AL049795		1.32	eukaryocytic translation initiation factor 3, subunit 2 beta
HSP70-1	219212_at	NM_016299.1	1.41		heat shock 70kD protein 1
HSPA8 / HSC70	221891_x_at	AA704004	1.07		constitutive heat shock protein 70
HSPB2	205824_at	NM_001541.1		1.23	heat shock 27kDa protein 2
NUP153	202097_at	NM_005124.1	1		nucleporin 153kDa
RPL21	200012_x_at	NM_000982.1	1.23		ribosomal protein L21
RPL31	200963_x_at	NM_000993.1	1.23		ribosomal protein L31
Regulation of gne expression (transcription)					
GTF3A	201338_x_at	NM_002097.1	1.23		general transcription factor IIIA
RARA	203750_s_at	NM_000964.1	1.07		retinoic acid receptor, alpha
RXRA	202449_s_at	NM_002957.2		1.87	retinoid X receptor, alpha
TCF1	216930_at	X71347.1		1.15	transcription factor 1, hepatic
TCF4	203753_at	NM_003199.1		2.46	transcription factor 4
ZFP91	206059_at	NM_003430.1		1.23	zinc finger protein 91 homolog (mouse)
Signal transduction					
CCR7	206337_at	NM_001838.1		1.07	chemokine (C-C motif) receptor 7
CD14	201743_at	NM_000591.1		18.38	CD14 antigen
FCGR2B	210889_s_at	M31933.1		8.57	IGFR2 / Fc fragment of IgG, low affinity IIb, receptor

Table 4.12					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's B (lam +ve) vs Duke's B (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
GNAL	206356_s_at	NM_002071.1		1.15	G-s-alpha / guanine nucleotide binding protein (G protein)
MAPK1	208351_s_at	NM_002745.1		2.3	mitogen-activated protein kinase 1
PIK3CG	206370_at	NM_002649.1		1.52	phosphoinositide-3-kinase, catalytic, gamma polypeptide
PTK2B / PKB	203111_s_at	U33284.1		1.41	PTK2B protein tyrosine kinase 2 beta
RAC1	208640_at	NM_006908.2	1		rho family, small GTP binding protein Rac1
RGS2	202388_at	NM_002923.1		8	regulator of G-protein signaling 2 (G0/G1 switch regulatory protein 8)
TIAM1	206409_at	NM_003253.1		1.23	T-cell lymphoma invasion and metastasis 1
Stem cell differentitaion					
ASTN	209693_at	AF116574.1	2.46		Astrotactin
BMP4	211518_s_at	D30751.1	21.11		bone morphogenetic protein 4
BMP6	206176_at	NM_001718.2		1.32	bone morphogenetic protein 6
FN1	216442_x_at	AK026737.1		3.25	cellular fibronectin
FUT1	206109_at	NM_000148.1	1.32		fucosyltransferase 1
FUT2	208505_s_at	NM_000511.1		1.23	fucosyltransferase 2 (secretor status included)
GAP43	204471_at	NM_002045.1	4		growth associated protein 43
GJA1	201667_at	NM_000165.2		1.74	gap juction protein, alpha 1 (connexin 43)
LAMB1	201505_at	NM_002291.1	2.14		laminin, beta 1
MAPT / TAU	203929_s_at	NM_016835.1		16	microtubule-associated protein tau
MASH1 / ASCL1	209985_s_at	NM_004316.1	2.46		achaete-scute homolog 1
MASH2 / ASCL2	207607_at	NM_005170.1	2		achaete-scute complex-like 2

Table 4.12					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's B (lam +ve) vs Duke's B (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
MYOD1	206657_s_at	NM_002478.2		1.07	myogenic factor 3
NEFL	221801_x_at	NM_006158.1	9.19		neurofilament, light polypeptide 68kDa
NEUROD2	1552953_a_at	NM_006160.2		1.15	neurogenic differentiation 2
NEUROD1	206282_at	NM_002500.1	2.3		neurogenic differentiation 3
NEUROD4	221318_at	NM_021191.1	1.23		neurogenic differentiation 4
NHLH2	214497_s_at	NM_005599.1		1.41	nescient helix loop helix 2
NOTCH2	202445_s_at	NM_024408.1		1.74	Notch homolog 2 (Drosophila)
NSE	201313_at	NM_001975.1	2		neuron-specific enolase
PAX6	205646_s_at	NM_000280.1		1.23	paired box gene 6 (aniridia, keratitis)
PLP	210198_s_at	BC002665.1	2		proteolipid protein
REST	204535_s_at	NM_005612.1	1.41		RE1-silencing transcription factor
SOX1	208533_at	NM_005986.1	1		SRY (sex determining region)-box 1
SOX17	219568_x_at	NM_018419.1		6.06	SRY (sex determining region)-box 17
SOX2	214178_s_at	AI356682		1.41	SRY (sex determining region)-box 2
SYP	213200_at	U93305	1.07		synaptophysin
TDGF1	206286_s_at	NM_003212.1	4.29		teratocarcinoma-derived growth factor 1
TF	203400_s_at	NM_001063.1		1.23	transferrin
VTN	204534_at	NM_000638.1	1.41		vitronectin
Stress response					
SOD1	200642_at	NM_000454.1		1.15	superoxide dismutase 1, soluble
SOD2	216841_s_at	X15132.1		4.59	superoxide dismutase 2, mitochondrial

Table 4.12					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's B (lam +ve) vs Duke's B (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
Transporters, carriers					
ATP2A3	207522_s_at	NM_005173.1		3.03	ATPase, Ca++ transporting, ubiquitous
SLC16A1	202234_s_at	NM_003051.1	5.66		solute carrier family 16 (monocarboxylic acid transporters), member 1
SLC2A1 / GLUT1	201250_s_at	NM_006516.1	1.23		solute carrier family 2 (facilitated glucose transporter), member 1
Tumour suppressor genes					
APC	203527_s_at	NM_000038.1		1.23	adenomatosis polyposis coli
DCC	206939_at	NM_005215.1		2.14	deleted in colorectal carcinoma
RB1	203132_at	NM_000321.1		1.87	retinoblastoma 1
TP53	201746_at	NM_000546.2		1.32	tumour protein p53 (Li-Fraumeni syndrome)
Wnt signalling					
BIRC5	210334_x_at	AB028869.1		1.15	apoptosis inhibitor 4 - survivin
CDKN1A	202284_s_at	NM_000389.1	2.83		cyclin-dependant kinase inhibitor 1A (p21, Cip1)
CEACAM1 / BGP1	206576_s_at	NM_001712.1	4.29		carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)
CHAF1A	203976_s_at	NM_005483.1		1.52	chromatin assembly factor 1, subunit A (p150)
CLDN1	218182_s_at	NM_021101.1		2	claudin 1
CLDN4	201428_at	NM_001305.1		1.07	claudin 4
DLEU1	205677_s_at	NM_005887.1		1.23	deleted in lymphocyte leukemia, 1
ENC1	201341_at	NM_003633.1	2.46		ectodermal-neural cortex (with BTB-like domain)
EPHB1	230425_at	AI674183		6.5	EPH receptor B1
EPHB2	209588_at	AL530874	1.52		EPH receptor B2
ETS2	201328_at	NM_005239.1		1.41	v-ets erythroblastosis virus E26 oncogene homolog 2

Table 4.12					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's B (lam +ve) vs Duke's B (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
FABP1	205892_s_at	NM_001443.1	776.05		fatty acid binding protein 1, liver
GPX2	202831_at	NM_002083.1	2.83		glutathione peroxidase 2 (gastrointestinal)
JPO1 / LOC442172	224428_s_at	AY029179.1	1.52		c-MYC target JPO1
MCM3	201555_at	NM_002388.2	1.07		MCM3 minichromosome maintenance deficient 3
MUC2	204673_at	NM_002457.1		3.48	mucin 2, intestinal/tracheal
MYB	204798_at	NM_005375.1	1.41		Myb proto-oncogene protein (C-myb)
PRKCD	202545_at	NM_006254.1		1.41	protein kinase C, delta
RBBP4	210371_s_at	NM_005610.1		2.3	retinoblastoma binding protein 4
SCF / KITLG	216974_at	S80491.1	6.5		stem cell factor / kit ligand precursor / Mast cell growth factor (MGF)
TCOF1	202385_s_at	NM_000356.1	1.62		treacher Collins-Franceschetti syndrome 1
UNG	202330_s_at	NM_003362.1		1.23	uracil-DNA glycosylase
Miscellaneous					
PTGS2 / COX2	204748_at	NM_000963.1		19.7	prostaglandin-endoperoxide synthase 2 (cyclooxygenase 2)
CKB	200884_at	NM_001823.1	84.45		Creatine kinase brain
Gene used as a positive control test					
β-Actin	200801_x_at	NM_001101.2		1.15	Beta actin

Table 4.13

A comparative microarray analysis of changes in functional gene groups between a Dukes' B (nuclear lamin A/C positive) tumour sample and a Dukes' C (nuclear lamin A/C negative) tumour sample

Using microarray data obtained from the Dukes' C (nuclear lamin A/C negative) adenocarcinoma sample as a baseline, a comparative microarray expression analysis was made to assess changes in sixteen functional gene groups, containing 164 genes in total, in the Dukes' B (nuclear lamin A/C negative) adenocarcinoma sample. Affymetrix Id numbers, unique Gene codes, fold change (FC) values, using an arbitrary cut off level set at +/- 9, and a brief description of the genes were also included amongst other criteria, as mentioned in section 4.2.4.3. Genes of interest were appropriately highlighted to indicate either an up (highlighted in red) or down (highlighted in green) regulation. Some genes not exhibiting a significant FC values were also highlighted (in blue) as this information was deemed important in analysis of the functional gene group as a whole.

Table 4.13					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's B (lam +ve) vs Duke's C (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
Apoptosis and apoptotic inhibitors					
AMID / PRG3	220811_at	NM_006093.2	29.86		p53-responsive gene
BAK1	203728_at	NM_001188.1	1.07		BCL2-antagonist/killer 1
CASP3	202763_at	NM_004346.1		2.14	caspase 3, apoptosis-related cysteine protease
CAST	207467_x_at	NM_001750.2	1.15		calpastatin
FASLG	210865_at	NM_000639.1		6.06	Fas ligand (TNF superfamily, member 6)
NCKAP1 / NAP1	207738_s_at	NM_013436.1		1.15	NCK-associated protein 1
TNFRSF10A / TRAILR1	1552648_a_at	NM_003844.2		1.23	tumour necrosis factor receptor superfamily, member 10a
Cell adhesion					
CTNNA1	200764_s_at	NM_001903.1		1.32	catenin (cadherin-associated protein), alpha 1
Cell cycle progression / cell proliferation and growth					
CCNA1	205899_at	NM_003914.1	1		cyclin A1
CCNB1	228729_at	N90191		1.87	cyclin B1
CCNB2	202705_at	NM_004701.2		1.32	cyclin B2
CCND1	208711_s_at	BC000076.1		1.87	cyclin D1
CCND3	201700_at	NM_001760.1	1.52		cyclin D3
CDC2	203214_x_at	NM_001786.1		1.74	cell division cycle 2, G1 to S and G2 to M / cdk1
CDKN1B	209112_at	NM_004064.1	2.14		cyclin-dependant kinase inhibitor 1B
CHK1	205393_s_at	NM_001274.1		1.32	checkpoint kinase Chk1
CHK2	210416_s_at	NM_007194.1		1.07	checkpoint kinase Chk2
CSPG2	204620_s_at	NM_004385.1		1.87	chondroitin sulfate proteoglycan 2 (versican)

Table 4.13					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's B (lam +ve) vs Duke's C (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
EGF	206254_at	NM_001963.2		3.03	epidermal growth factor
FGF5	208378_x_at	NM_004464.1	11.31		fibroblast growth factor 5
GPC3	209220_at	NM_004484.2		1.52	glypican 3
IGF2	202410_x_at	NM_000612.2	1.07		insulin-like growth factor 2 (somatomedin A)
TGFA	205015_s_at	NM_003236.1	1.62		transforming growth factor, alpha
TGFB1 / BIGH3	203084_at	NM_000660.1	1.23		transforming growth factor, beta-induced
VEGF	210512_s_at	NM_003376.1		1.07	vascular endothelial growth factor
Cytoskeleton and nucleoskeleton					
ACTG1	201550_x_at	NM_001614.2		1.23	actin, gamma 1
KRT1	205900_at	NM_006121.1	2.3		keratin 1
KRT18	201596_x_at	NM_000224.1		1.41	keratin 18
KRT8	209008_x_at	NM_002273.1		1.15	keratin 8
LCP1	208885_at	NM_002298.2		7.46	lymphocyte cytosolic protein 1 (L-plastin)
LMNA (lamin C)	203411_s_at	NM_005572.1		1.52	lamin C
LMNB1	203276_at	NM_005573.1		2.14	lamin B1
MACF1	1553407_at	NM_033044.1		1.07	microtubule-actin crosslinking factor1
NEBL	203962_s_at	NM_006393.1	1.07		nebulette
NEFH	204412_s_at	NM_021076.1		1.52	neurofilament, heavy polypeptide 200 kDa
PLS3	201215_at	NM_005032.2	9.66	1.74	plastin 3 (T isoform)
CDH1	201131_s_at	NM_004360.1	1.23		cadherin 1, type 1, E-cadherin (epithelial)
PPL	203407_at	NM_002705.1	2		periplakin

Table 4.13					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's B (lam +ve) vs Duke's C (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
TMPO(A)	203432_at	NM_003276.1		1.62	thymopoietin alpha / lamina-associated polypeptide 2 alpha
TUBB2	208977_x_at	NM_006088.1		1.23	tubulin, beta 2
TUBG	201714_at	NM_001070.1		1.74	tubulin, gamma 1
TUBG2	203894_at	NM_016437.1	1.15		tubulin, gamma 2
VCL	200930_s_at	NM_014000.1		2.64	vinculin
VIL2	208621_s_at	NM_003379.2	1.32		villin 2 (ezrin)
VIM	201426_s_at	NM_003380.1		3.73	vimentin
DNA replication and repair					
ATM	208442_s_at	NM_000051.1	1.52		ataxia telangectasia mutated
MLH1	202520_s_at	NM_000249.1	6.06		mutL homolog 1, colon cancer, non-polyposis type 2
MSH2	209421_at	NM_000251.1		1.74	mutS homolog 2, colon cancer, non-polyposis type 1
NBS1	202907_s_at	NM_002485.2		1.52	Nijmegen breakage syndrome 1 (nibrin)
PRKDC	210543_s_at	U34994.3		1.41	DNA-dependant protein kinase catalytic subunit
RPA1	201528_at	NM_002945.1		1.62	replication protein A1
XRCC4	205071_x_at	AB017445.1		1.15	DNA-repair protein XRCC4
XRCC5	208642_s_at	NM_021141.2		1.07	Ku autoantigen
Extracellular matrix: components, processing, cellular attachment, proteases and protease inhibitors					
BGN	201262_s_at	NM_001711.1	1.23		biglycan
COLIA2	202404_s_at	NM_000089.1	1.41		collagen type I, alpha 2
FN1 / FN	212464_s_at	X02761.1	1.41		fibronectin, alt splice
HPSE	219403_s_at	NM_006665.1		2.46	heparanase

Table 4.13					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's B (lam +ve) vs Duke's C (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
ITGA1	214660_at	X68742.1		1.23	integrin, alpha 1
ITGA2	205032_at	NM_002203.2		1.52	integrin, alpha 2
ITGA3	229257_at	AI625045		1.52	integrin, alpha 3
ITGAV	202351_at	NM_002210.1		1.74	integrin, alpha V (vitronectin receptor)
ITGB1	216190_x_at	AA215854		1.52	integrin, beta 1
LAMA4	202202_s_at	NM_002290.2	3.03		laminin alpha 4
LAMB1	211651_s_at	M20206.1	2		laminin beta 1
MMP14	202827_s_at	NM_004995.2		1.52	matrix metalloproteinase 14 (membrane inserted)
MMP3	205828_at	NM_002422.2	107		matrix metalloproteinase 3 (stromelysin 1, progelatinase)
SDC4	202071_at	NM_002999.1	2.14		syndecan 4 (amphiglycan, ryudocan)
SPP1	209875_s_at	M83248.1		48.5	secreted phosphoprotein 1 (osteopontin)
TIMP1	201666_at	NM_003254.1		1.87	tissue inhibitor of metalloproteinase 1
Oncogenes					
ABL1	202123_s_at	NM_005157.2	1		v-abl Abelson murine leukemia viral oncogene homolg 1
HRAS	212983_at	NM_005343.1	1.07		v-Ha-ras Harvey rat sarcoma viral oncogene homolog
JUN	201465_s_at	NM_002228.2		1.23	v-jun sarcoma virus 17 oncogene homolog
KRAS	204009_s_at	NM_004985.1		1.23	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
MOS	221367_at	NM_005372.1	1		v-mos Moloney murine sarcoma viral oncogene homolog
MYC	202431_s_at	NM_002467.1	1.62		v-myc myelocytomatosis viral oncogene homolog
MYCN	209757_s_at	NM_005378.1		2.46	N-myc proto-oncogene protein
RAF1	201244_s_at	NM_002880.1		1.52	v-raf-1 murine leukemia viral oncogene homolog 1

Table 4.13					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's B (lam +ve) vs Duke's C (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
SRC	221281_at	NM_005417.1	7.46		v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog
Protein translation, processing, transport and degradation					
AIM1	206513_at	NM_004833.1	1.32		absent in melanoma 1
EIF3S	221912_s_at	AL049795	1.62		eukaryocytic translation initiation factor 3, subunit 2 beta
HSP70-1	219212_at	NM_016299.1	1.52		heat shock 70kD protein 1
HSPA8 / HSC70	221891_x_at	AA704004	1.15		constitutive heat shock protein 70
HSPB2	205824_at	NM_001541.1	1.32		heat shock 27kDa protein 2
NUP153	202097_at	NM_005124.1	1		nucleporin 153kDa
RPL21	200012_x_at	NM_000982.1	1.41		ribosomal protein L21
RPL31	200963_x_at	NM_000993.1	1.07		ribosomal protein L31
Regulation of gne expression (transcription)					
GTF3A	201338_x_at	NM_002097.1	2.46		general transcription factor IIIA
RARA	203750_s_at	NM_000964.1		2	retinoic acid receptor, alpha
RXRA	202449_s_at	NM_002957.2		1.15	retinoid X receptor, alpha
TCF1	216930_at	X71347.1	1		transcription factor 1, hepatic
TCF4	203753_at	NM_003199.1	1.74		transcription factor 4
ZFP91	206059_at	NM_003430.1	1.62		zinc finger protein 91 homolog (mouse)
Signal transduction					
CCR7	206337_at	NM_001838.1	1.15		chemokine (C-C motif) receptor 7
CD14	201743_at	NM_000591.1		11.31	CD14 antigen
FCGR2B	210889_s_at	M31933.1		5.66	IGFR2 / Fc fragment of IgG, low affinity IIb, receptor

Table 4.13					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's B (lam +ve) vs Duke's C (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
GNAL	206356_s_at	NM_002071.1	2		G-s-alpha / guanine nucleotide binding protein (G protein)
MAPK1	208351_s_at	NM_002745.1		1.87	mitogen-activated protein kinase 1
PIK3CG	206370_at	NM_002649.1	4.29		phosphoinositide-3-kinase, catalytic, gamma polypeptide
PTK2B / PKB	203111_s_at	U33284.1	1.07		PTK2B protein tyrosine kinase 2 beta
RAC1	208640_at	NM_006908.2	1.52		rho family, small GTP binding protein Rac1
RGS2	202388_at	NM_002923.1		6.96	regulator of G-protein signaling 2 (G0/G1 switch regulatory protein 8
TIAM1	206409_at	NM_003253.1	1.15		T-cell lymphoma invasion and metastasis 1
Stem cell differentitaion					
ASTN	209693_at	AF116574.1	1.07		Astrotactin
BMP4	211518_s_at	D30751.1	6.06		bone morphogenetic protein 4
BMP6	206176_at	NM_001718.2		2.3	bone morphogenetic protein 6
FN1	216442_x_at	AK026737.1	1.41		cellular fibronectin
FUT1	206109_at	NM_000148.1	1.52		fucosyltransferase 1
FUT2	208505_s_at	NM_000511.1		1.15	fucosyltransferase 2 (secretor status included)
GAP43	204471_at	NM_002045.1		1.23	growth associated protein 43
GJA1	201667_at	NM_000165.2		1.07	gap juction protein, alpha 1 (connexin 43)
LAMB1	201505_at	NM_002291.1	1.74		laminin, beta 1
MAPT / TAU	203929_s_at	NM_016835.1	1		microtubule-associated protein tau
MASH1 / ASCL1	209985_s_at	NM_004316.1		1.23	achaete-scute homolog 1
MASH2 / ASCL2	207607_at	NM_005170.1	22.63		achaete-scute complex-like 2

Table 4.13					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's B (lam +ve) vs Duke's C (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
MYOD1	206657_s_at	NM_002478.2	1.23		myogenic factor 3
NEFL	221801_x_at	NM_006158.1	6.06		neurofilament, light polypeptide 68kDa
NEUROD2	1552953_a_at	NM_006160.2	1.32		neurogenic differentiation 2
NEUROD1	206282_at	NM_002500.1	16		neurogenic differentiation 3
NEUROD4	221318_at	NM_021191.1	1.62		neurogenic differentiation 4
NHLH2	214497_s_at	NM_005599.1	3.73		nescient helix loop helix 2
NOTCH2	202445_s_at	NM_024408.1		1.41	Notch homolog 2 (Drosophila)
NSE	201313_at	NM_001975.1		1.87	neuron-specific enolase
PAX6	205646_s_at	NM_000280.1	1.15		paired box gene 6 (aniridia, keratitis)
PLP	210198_s_at	BC002665.1	51.98		proteolipid protein
REST	204535_s_at	NM_005612.1		1.62	RE1-silencing transcription factor
SOX1	208533_at	NM_005986.1	1.32		SRY (sex determining region)-box 1
SOX17	219568_x_at	NM_018419.1		5.28	SRY (sex determining region)-box 17
SOX2	214178_s_at	AI356682		1.15	SRY (sex determining region)-box 2
SYP	213200_at	U93305		1.23	synaptophysin
TDGF1	206286_s_at	NM_003212.1	12.13		teratocarcinoma-derived growth factor 1
TF	203400_s_at	NM_001063.1	4		transferrin
VTN	204534_at	NM_000638.1		1.74	vitronectin
Stress response					
SOD1	200642_at	NM_000454.1		1.07	superoxide dismutase 1, soluble
SOD2	216841_s_at	X15132.1		9.19	superoxide dismutase 2, mitochondrial

Table 4.13					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's B (lam +ve) vs Duke's C (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
Transporters, carriers					
ATP2A3	207522_s_at	NM_005173.1		1.87	ATPase, Ca++ transporting, ubiquitous
SLC16A1	202234_s_at	NM_003051.1	8		solute carrier family 16 (monocarboxylic acid transporters), member 1
SLC2A1 / GLUT1	201250_s_at	NM_006516.1	1		solute carrier family 2 (facilitated glucose transporter), member 1
Tumour suppressor genes					
APC	203527_s_at	NM_000038.1		1.32	adenomatosis polyposis coli
DCC	206939_at	NM_005215.1	2.14		deleted in colorectal carcinoma
RB1	203132_at	NM_000321.1	1		retinoblastoma 1
TP53	201746_at	NM_000546.2	1.52		tumour protein p53 (Li-Fraumeni syndrome)
Wnt signalling					
BIRC5	210334_x_at	AB028869.1		1.87	apoptosis inhibitor 4 - survivin
CDKN1A	202284_s_at	NM_000389.1		1.23	cyclin-dependant kinase inhibitor 1A (p21, Cip1)
CEACAM1 / BGP1	206576_s_at	NM_001712.1	3.25		carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)
CHAF1A	203976_s_at	NM_005483.1		2.3	chromatin assembly factor 1, subunit A (p150)
CLDN1	218182_s_at	NM_021101.1		5.66	claudin 1
CLDN4	201428_at	NM_001305.1	1.41		claudin 4
DLEU1	205677_s_at	NM_005887.1		1.23	deleted in lymphocyte leukemia, 1
ENC1	201341_at	NM_003633.1	1.15		ectodermal-neural cortex (with BTB-like domain)
EPHB1	230425_at	AI674183		2	EPH receptor B1
EPHB2	209588_at	AL530874		1.41	EPH receptor B2
ETS2	201328_at	NM_005239.1		1.32	v-ets erythroblastosis virus E26 oncogene homolog 2

Table 4.13					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's B (lam +ve) vs Duke's C (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
FABP1	205892_s_at	NM_001443.1	724.08		fatty acid binding protein 1, liver
GPX2	202831_at	NM_002083.1	1.74		glutathione peroxidase 2 (gastrointestinal)
JPO1 / LOC442172	224428_s_at	AY029179.1	1.74		c-MYC target JPO1
MCM3	201555_at	NM_002388.2		1.62	MCM3 minichromosome maintenance deficient 3
MUC2	204673_at	NM_002457.1		2.3	mucin 2, intestinal/tracheal
MYB	204798_at	NM_005375.1	1.87		Myb proto-oncogene protein (C-myb)
PRKCD	202545_at	NM_006254.1	1		protein kinase C, delta
RBBP4	210371_s_at	NM_005610.1		2.3	retinoblastoma binding protein 4
SCF / KITLG	216974_at	S80491.1	1.41		stem cell factor / kit ligand precursor / Mast cell growth factor (MGF)
TCOF1	202385_s_at	NM_000356.1	1.62		treacher Collins-Franceschetti syndrome 1
UNG	202330_s_at	NM_003362.1		1.52	uracil-DNA glycosylase
Miscellaneous					
PTGS2 / COX2	204748_at	NM_000963.1		45.25	prostaglandin-endoperoxide synthase 2 (cyclooxygenase)
CKB	200884_at	NM_001823.1	29.86		Creatine kinase brain
Gene used as a positive control test					
β-Actin	200801_x_at	NM_001101.2		1.07	Beta Actin

Table 4.14

A comparative microarray analysis of changes in functional gene groups between a Dukes' B (nuclear lamin A/C negative) tumour sample and a Dukes' C (nuclear lamin A/C negative) tumour sample

Using microarray data obtained from the Dukes' C (nuclear lamin A/C negative) adenocarcinoma sample as a baseline, a comparative microarray expression analysis was made to assess changes in sixteen functional gene groups, containing 164 genes in total, in the Dukes' B (nuclear lamin A/C negative) adenocarcinoma sample. Affymetrix Id numbers, unique Gene codes, fold change (FC) values, using an arbitrary cut off level set at +/- 9, and a brief description of the genes were also included amongst other criteria, as mentioned in section 4.2.4.3. Genes of interest were appropriately highlighted to indicate either an up (highlighted in red) or down (highlighted in green) regulation. Some genes not exhibiting a significant FC values were also highlighted (in blue) as this information was deemed important in analysis of the functional gene group as a whole.

Table 4.14					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's C (lam-ve) vs Duke's B (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
Apoptosis and apoptotic inhibitors					
AMID / PRG3	220811_at	NM_006093.2	2.14		p53-responsive gene
BAK1	203728_at	NM_001188.1		1.32	BCL2-antagonist/killer 1
CASP3	202763_at	NM_004346.1		1.15	caspase 3, apoptosis-related cysteine protease
CAST	207467_x_at	NM_001750.2	1.07		calpastatin
FASLG	210865_at	NM_000639.1		3.03	Fas ligand (TNF superfamily, member 6)
NCKAP1 / NAP1	207738_s_at	NM_013436.1	1		NCK-associated protein 1
TNFRSF10A / TRAILR1	1552648_a_at	NM_003844.2		1.32	tumour necrosis factor receptor superfamily, member 10a
Cell adhesion					
CTNNA1	200764_s_at	NM_001903.1		1.07	catenin (cadherin-associated protein), alpha 1
Cell cycle progression / cell proliferation and growth					
CCNA1	205899_at	NM_003914.1	2.14		cyclin A1
CCNB1	228729_at	N90191		1.87	cyclin B1
CCNB2	202705_at	NM_004701.2		1.07	cyclin B2
CCND1	208711_s_at	BC000076.1		1.23	cyclin D1
CCND3	201700_at	NM_001760.1	2.3		cyclin D3
CDC2	203214_x_at	NM_001786.1		1.15	cell division cycle 2, G1 to S and G2 to M / cdk1
CDKN1B	209112_at	NM_004064.1	1.32		cyclin-dependant kinase inhibitor 1B
CHK1	205393_s_at	NM_001274.1	1.23		checkpoint kinase Chk1
CHK2	210416_s_at	NM_007194.1		1.15	checkpoint kinase Chk2
CSPG2	204620_s_at	NM_004385.1	4		chondroitin sulfate proteoglycan 2 (versican)

Table 4.14					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's C (lam-ve) vs Duke's B (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
EGF	206254_at	NM_001963.2		9.85	epidermal growth factor
FGF5	208378_x_at	NM_004464.1	9.85		fibroblast growth factor 5
GPC3	209220_at	NM_004484.2		6.5	glypican 3
IGF2	202410_x_at	NM_000612.2		724.08	insulin-like growth factor 2 (somatomedin A)
TGFA	205015_s_at	NM_003236.1	1.23		transforming growth factor, alpha
TGFB1 / BIGH3	203084_at	NM_000660.1		1.23	transforming growth factor, beta-induced
VEGF	210512_s_at	NM_003376.1		1.07	vascular endothelial growth factor
Cytoskeleton and nucleoskeleton					
ACTG1	201550_x_at	NM_001614.2		1.07	actin, gamma 1
KRT1	205900_at	NM_006121.1		1.23	keratin 1
KRT18	201596_x_at	NM_000224.1		2	keratin 18
KRT8	209008_x_at	NM_002273.1		1.87	keratin 8
LCP1	208885_at	NM_002298.2	1.15		lymphocyte cytosolic protein 1 (L-plastin)
LMNA (lamin C)	203411_s_at	NM_005572.1	1.07		lamin C
LMNB1	203276_at	NM_005573.1		1.15	lamin B1
MACF1	1553407_at	NM_033044.1	1.87		microtubule-actin crosslinking factor1
NEBL	203962_s_at	NM_006393.1	1.32		nebulette
NEFH	204412_s_at	NM_021076.1	4.59		neurofilament, heavy polypeptide 200 kDa
PLS3	201215_at	NM_005032.2		1.62	plastin 3 (T isoform)
CDH1	201131_s_at	NM_004360.1	1.23		cadherin 1, type 1, E-cadherin (epithelial)
PPL	203407_at	NM_002705.1	1.74		periplakin

Table 4.14					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's C (lam-ve) vs Duke's B (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
TMPO(A)	203432_at	NM_003276.1		1.32	thymopoietin alpha / lamina-associated polypeptide 2 alpha
TUBB2	208977_x_at	NM_006088.1	1		tubulin, beta 2
TUBG	201714_at	NM_001070.1		1.32	tubulin, gamma 1
TUBG2	203894_at	NM_016437.1	1.62		tubulin, gamma 2
VCL	200930_s_at	NM_014000.1		2.64	vinculin
VIL2	208621_s_at	NM_003379.2	1.74		villin 2 (ezrin)
VIM	201426_s_at	NM_003380.1	1.62		vimentin
DNA replication and repair					
ATM	208442_s_at	NM_000051.1	1.74		ataxia telangectasia mutated
MLH1	202520_s_at	NM_000249.1	1.07		mutL homolog 1, colon cancer, non-polyposis type 2
MSH2	209421_at	NM_000251.1		1.07	mutS homolog 2, colon cancer, non-polyposis type 1
NBS1	202907_s_at	NM_002485.2		1.32	Nijmegen breakage syndrome 1 (nibrin)
PRKDC	210543_s_at	U34994.3	1.07		DNA-dependant protein kinase catalytic subunit
RPA1	201528_at	NM_002945.1		1.23	replication protein A1
XRCC4	205071_x_at	AB017445.1		1.41	DNA-repair protein XRCC4
XRCC5	208642_s_at	NM_021141.2		1.41	Ku autoantigen
Extracellular matrix: components, processing, cellular attachment, proteases and protease inhibitors					
BGN	201262_s_at	NM_001711.1	10.56		biglycan
COLIA2	202404_s_at	NM_000089.1	6.5		collagen type I, alpha 2
FN1 / FN	212464_s_at	X02761.1	4.59		fibronectin, alt splice
HPSE	219403_s_at	NM_006665.1	2.46		heparanase

Table 4.14					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's C (lam-ve) vs Duke's B (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
ITGA1	214660_at	X68742.1		1.23	integrin, alpha 1
ITGA2	205032_at	NM_002203.2		2	integrin, alpha 2
ITGA3	229257_at	AI625045		1.41	integrin, alpha 3
ITGAV	202351_at	NM_002210.1	1.15		integrin, alpha V (vitronectin receptor)
ITGB1	216190_x_at	AA215854	3.03		integrin, beta 1
LAMA4	202202_s_at	NM_002290.2	4.92		laminin alpha 4
LAMB1	211651_s_at	M20206.1	1		laminin beta 1
MMP14	202827_s_at	NM_004995.2	1.23		matrix metalloproteinase 14 (membrane inserted)
MMP3	205828_at	NM_002422.2		2.46	matrix metalloproteinase 3 (stromelysin 1, progelatinase)
SDC4	202071_at	NM_002999.1	2.3		syndecan 4 (amphiglycan, ryudocan)
SPP1	209875_s_at	M83248.1	1.87		secreted phosphoprotein 1 (osteopontin)
TIMP1	201666_at	NM_003254.1		1.41	tissue inhibitor of metalloproteinase 1
Oncogenes					
ABL1	202123_s_at	NM_005157.2	1.23		v-abl Abelson murine leukemia viral oncogene homolg 1
HRAS	212983_at	NM_005343.1	1.32		v-Ha-ras Harvey rat sarcoma viral oncogene homolog
JUN	201465_s_at	NM_002228.2	1.41		v-jun sarcoma virus 17 oncogene homolog
KRAS	204009_s_at	NM_004985.1	1.07		v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
MOS	221367_at	NM_005372.1	2.14		v-mos Moloney murine sarcoma viral oncogene homolog
MYC	202431_s_at	NM_002467.1	1.07		v-myc myelocytomatosis viral oncogene homolog
MYCN	209757_s_at	NM_005378.1		5.66	N-myc proto-oncogene protein
RAF1	201244_s_at	NM_002880.1	1.07		v-raf-1 murine leukemia viral oncogene homolog 1

Table 4.14					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's C (lam-ve) vs Duke's B (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
SRC	221281_at	NM_005417.1	6.5		v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog
Protein translation, processing, transport and degradation					
AIM1	206513_at	NM_004833.1	2		absent in melanoma 1
EIF3S	221912_s_at	AL049795	1.74		eukaryocytic translation initiation factor 3, subunit 2 beta
HSP70-1	219212_at	NM_016299.1	1.15		heat shock 70kD protein 1
HSPA8 / HSC70	221891_x_at	AA704004	1.15		constitutive heat shock protein 70
HSPB2	205824_at	NM_001541.1	1.74		heat shock 27kDa protein 2
NUP153	202097_at	NM_005124.1	1		nucleporin 153kDa
RPL21	200012_x_at	NM_000982.1	1.15		ribosomal protein L21
RPL31	200963_x_at	NM_000993.1		1.07	ribosomal protein L31
Regulation of gne expression (transcription)					
GTF3A	201338_x_at	NM_002097.1	1.87		general transcription factor IIIA
RARA	203750_s_at	NM_000964.1		2.14	retinoic acid receptor, alpha
RXRA	202449_s_at	NM_002957.2	1.87		retinoid X receptor, alpha
TCF1	216930_at	X71347.1		1.23	transcription factor 1, hepatic
TCF4	203753_at	NM_003199.1	4.59		transcription factor 4
ZFP91	206059_at	NM_003430.1	1.87		zinc finger protein 91 homolog (mouse)
Signal transduction					
CCR7	206337_at	NM_001838.1	1.23		chemokine (C-C motif) receptor 7
CD14	201743_at	NM_000591.1	1.62		CD14 antigen
FCGR2B	210889_s_at	M31933.1	1.62		IGFR2 / Fc fragment of IgG, low affinity IIb, receptor

Table 4.14					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's C (lam-ve) vs Duke's B (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
GNAL	206356_s_at	NM_002071.1	1.62		G-s-alpha / guanine nucleotide binding protein (G protein)
MAPK1	208351_s_at	NM_002745.1		1.07	mitogen-activated protein kinase 1
PIK3CG	206370_at	NM_002649.1	5.28		phosphoinositide-3-kinase, catalytic, gamma polypeptide
PTK2B / PKB	203111_s_at	U33284.1	1.52		PTK2B protein tyrosine kinase 2 beta
RAC1	208640_at	NM_006908.2	1.62		rho family, small GTP binding protein Rac1
RGS2	202388_at	NM_002923.1	1.15		regulator of G-protein signaling 2 (G0/G1 switch regulatory protein 8
TIAM1	206409_at	NM_003253.1	1.07		T-cell lymphoma invasion and metastasis 1
Stem cell differentitaion					
ASTN	209693_at	AF116574.1		1.52	Astrotactin
BMP4	211518_s_at	D30751.1		3.48	bone morphogenetic protein 4
BMP6	206176_at	NM_001718.2		2.14	bone morphogenetic protein 6
FN1	216442_x_at	AK026737.1	4.92		cellular fibronectin
FUT1	206109_at	NM_000148.1	1.07		fucosyltransferase 1
FUT2	208505_s_at	NM_000511.1	1.07		fucosyltransferase 2 (secretor status included)
GAP43	204471_at	NM_002045.1		8.57	growth associated protein 43
GJA1	201667_at	NM_000165.2	1.87		gap juction protein, alpha 1 (connexin 43)
LAMB1	201505_at	NM_002291.1		1.32	laminin, beta 1
MAPT / TAU	203929_s_at	NM_016835.1	21.11		microtubule-associated protein tau
MASH1 / ASCL1	209985_s_at	NM_004316.1		2.83	achaete-scute homolog 1
MASH2 / ASCL2	207607_at	NM_005170.1	5.66		achaete-scute complex-like 2

Table 4.14					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's C (lam-ve) vs Duke's B (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
MYOD1	206657_s_at	NM_002478.2	1.41		myogenic factor 3
NEFL	221801_x_at	NM_006158.1	1		neurofilament, light polypeptide 68kDa
NEUROD2	1552953_a_at	NM_006160.2	1.62		neurogenic differentiation 2
NEUROD1	206282_at	NM_002500.1	3.48		neurogenic differentiation 3
NEUROD4	221318_at	NM_021191.1	1.74		neurogenic differentiation 4
NHLH2	214497_s_at	NM_005599.1	3.03		nescient helix loop helix 2
NOTCH2	202445_s_at	NM_024408.1	1		Notch homolog 2 (Drosophila)
NSE	201313_at	NM_001975.1		3.25	neuron-specific enolase
PAX6	205646_s_at	NM_000280.1	1		paired box gene 6 (aniridia, keratitis)
PLP	210198_s_at	BC002665.1	9.85		proteolipid protein
REST	204535_s_at	NM_005612.1		2	RE1-silencing transcription factor
SOX1	208533_at	NM_005986.1	1.23		SRY (sex determining region)-box 1
SOX17	219568_x_at	NM_018419.1	1.32		SRY (sex determining region)-box 17
SOX2	214178_s_at	AI356682	1.87		SRY (sex determining region)-box 2
SYP	213200_at	U93305		1.74	synaptophysin
TDGF1	206286_s_at	NM_003212.1	2.3		teratocarcinoma-derived growth factor 1
TF	203400_s_at	NM_001063.1	5.28		transferrin
VTN	204534_at	NM_000638.1		4.92	vitronectin
Stress response					
SOD1	200642_at	NM_000454.1	1.15		superoxide dismutase 1, soluble
SOD2	216841_s_at	X15132.1		1.87	superoxide dismutase 2, mitochondrial

Table 4.14					
Gene Symbol	Affymetrix Id No	NM GENE	Duke's C (lam-ve) vs Duke's B (lam -ve)		Gene Description
			Fold change		
			UP	DOWN	
Transporters, carriers					
ATP2A3	207522_s_at	NM_005173.1	1.74		ATPase, Ca++ transporting, ubiquitous
SLC16A1	202234_s_at	NM_003051.1	1.41		solute carrier family 16 (monocarboxylic acid transporters), member 1
SLC2A1 / GLUT1	201250_s_at	NM_006516.1		1.23	solute carrier family 2 (facilitated glucose transporter), member 1
Tumour suppressor genes					
APC	203527_s_at	NM_000038.1	1.07		adenomatosis polyposis coli
DCC	206939_at	NM_005215.1	3.03		deleted in colorectal carcinoma
RB1	203132_at	NM_000321.1	1.74		retinoblastoma 1
TP53	201746_at	NM_000546.2	1.62		tumour protein p53 (Li-Fraumeni syndrome)
Wnt signalling					
BIRC5	210334_x_at	AB028869.1		1.23	apoptosis inhibitor 4 - survivin
CDKN1A	202284_s_at	NM_000389.1		3.48	cyclin-dependant kinase inhibitor 1A (p21, Cip1)
CEACAM1 / BGP1	206576_s_at	NM_001712.1		1.15	carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)
CHAF1A	203976_s_at	NM_005483.1		1.62	chromatin assembly factor 1, subunit A (p150)
CLDN1	218182_s_at	NM_021101.1		2.64	claudin 1
CLDN4	201428_at	NM_001305.1	1.62		claudin 4
DLEU1	205677_s_at	NM_005887.1	1		deleted in lymphocyte leukemia, 1
ENC1	201341_at	NM_003633.1		2.14	ectodermal-neural cortex (with BTB-like domain)
EPHB1	230425_at	AI674183	2.46		EPH receptor B1
EPHB2	209588_at	AL530874		2.14	EPH receptor B2
ETS2	201328_at	NM_005239.1	1.07		v-ets erythroblastosis virus E26 oncogene homolog 2

Table 4.14					
Gene Symbol	**Affymetrix Id No**	**NM GENE**	**Duke's C (lam-ve) vs Duke's B (lam -ve)**		**Gene Description**
			Fold change		
			UP	**DOWN**	
FABP1	205892_s_at	NM_001443.1	1		fatty acid binding protein 1, liver
GPX2	202831_at	NM_002083.1		1.74	glutathione peroxidase 2 (gastrointestinal)
JPO1 / LOC442172	224428_s_at	AY029179.1	1.15		c-MYC target JPO1
MCM3	201555_at	NM_002388.2		1.62	MCM3 minichromosome maintenance deficient 3
MUC2	204673_at	NM_002457.1	1.62		mucin 2, intestinal/tracheal
MYB	204798_at	NM_005375.1	1.32		Myb proto-oncogene protein (C-myb)
PRKCD	202545_at	NM_006254.1	1.52		protein kinase C, delta
RBBP4	210371_s_at	NM_005610.1	1		retinoblastoma binding protein 4
SCF / KITLG	216974_at	S80491.1		4	stem cell factor / kit ligand precursor / Mast cell growth factor (MGF)
TCOF1	202385_s_at	NM_000356.1	1		treacher Collins-Franceschetti syndrome 1
UNG	202330_s_at	NM_003362.1		1.41	uracil-DNA glycosylase
Miscellaneous					
PTGS2 / COX2	204748_at	NM_000963.1		2.3	prostaglandin-endoperoxide synthase 2 (cyclooxygenase)
CKB	200884_at	NM_001823.1		2	Creatine kinase brain
Gene used as a positive control test					
β-Actin	200801_x_at	NM_001101.2	1.07		Beta Actin

Figure 4.2

Quality and purity of total RNA used in microarray experiments.

Total RNA was extracted from four different samples using Trizol® reagent, as per manufacturer's recommendations:

Sample 29 – Tubulo-villous adenoma (nuclear lamin A/C negative)

Sample 33 – Duke's C cancer sample (nuclear lamin A/C negative)

Sample 37 – Duke's B cancer sample (nuclear lamin A/C positive)

Sample 38 – Duke's B cancer sample (nuclear lamin A/C negative)

Quality was verified by gel electrophoresis. 28S and 18S ribosomal bands were visualised in all cases. In lane 1 some RNA degradation was noted, however no obvious DNA contamination was noted. RNA purity was determined by measuring the ratio of absorbance in DEPC treated dH_2O at 260 nm and 280 nm ($A_{260/280}$). An $A_{260/280}$ value greater than1.8 indicates very pure RNA. M indicate RNA size markers in base pairs (bp).

Patient Sample No	Absorbance at 260.0 nm	Absorbance at 280.0 nm	Background absorbance at 320.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm
29	2.1953	1.1976	0.0167	1.8449	0.5420
33	1.1576	0.6535	0.0078	1.7807	0.5616
37	0.4249	0.2409	0.0073	1.7874	0.5595
38	0.4621	0.2587	0.0121	1.8246	0.5481

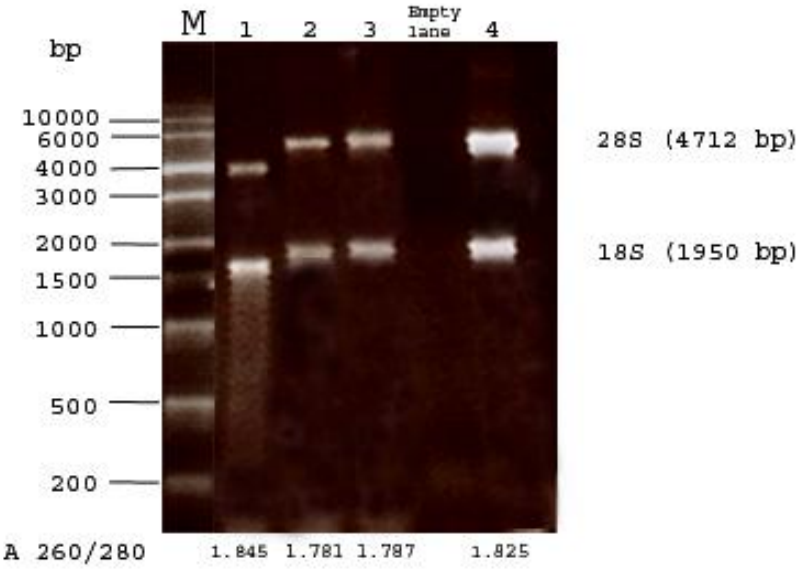


Figure 4.2

Agarose Formaldehyde Gel showing RNA expression levels in tissue sample 29 (lane 1), 33 (lane 2), 37 (lane 3) and 38 (lane 4) respectively. M denotes RNA marker

Figure 4.3

GeneChip HG-U133 Plus 2.0 array® by Affymetrix used in microarray analysis of tissue samples. Each array utilised probe sets with 11µm features to provide multiple, independent measurements for each transcript. The GeneChip array was scanned using a GeneArray® 2500 scanner to generate a GeneChip® Human Genome U133 Plus 2.0 .dat file with appropriate .CEL files exhibiting cell intensities. More than 54000 probe sets were used to analyse the expression level of more than 47000 transcripts and variants, including approximately 38500 well characterised human genes. All four samples subjected for microarray analysis were analysed using this specific array.

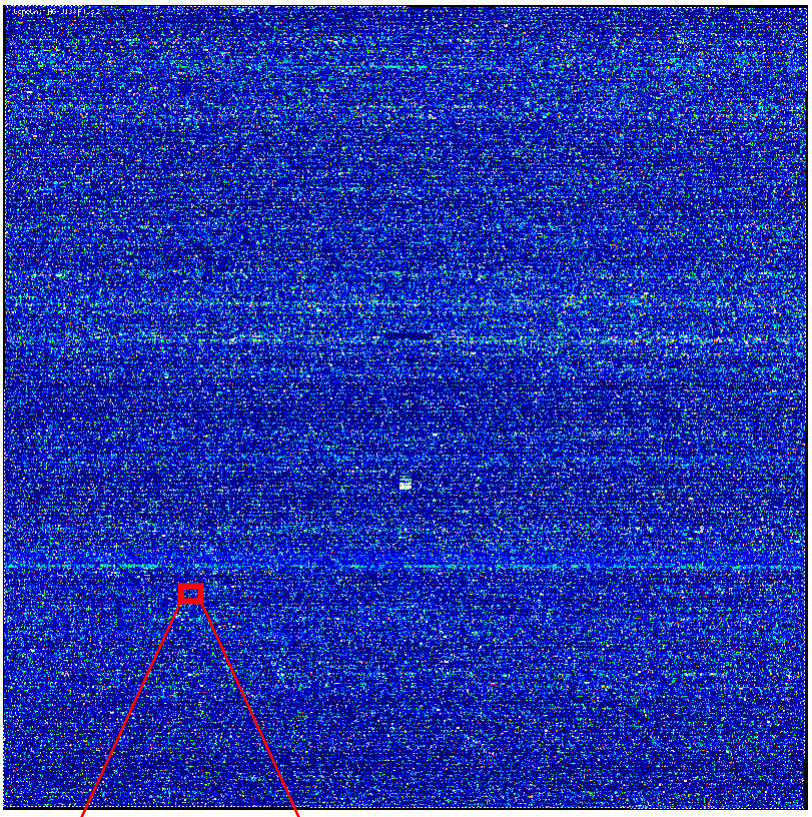
A) GeneChip® Human Genome U133 Plus 2.0 .CEL file illustrating probe sets exhibiting varying expression levels for each transcript. Black squares indicate no discernable expression whereas red squares indicate highly significant expression levels.

B) Expanded view to illustrate individual probe sets.

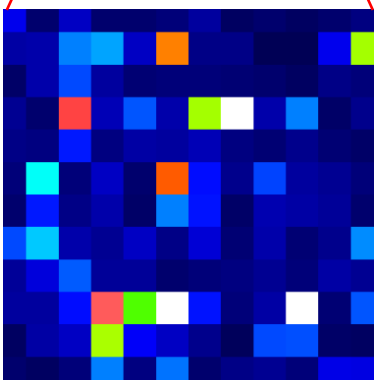
Figure 4.3

GeneChip® Human Genome U133 Plus 2.0 .CEL file

A)



B)



Magnified view of each 11µm probe set in the chip

Figure 4.4

RT-PCR to assess expression of lamin A and C in Tubulo-villous adenoma sample.

Immunohistological analysis revealed no discernable expression of lamin A and C in the tissue sample. This was confirmed on semi-quantitative RT-PCR. Primers were designed to amplify lamin A and C in the sample. Equal loading of starting material was verified by monitoring the transcriptional activity of β-actin, seen as an 834 bp product. Lane M was used a marker lane, lane 1 was used for β-actin, lane 2 was used for lamin A and lane 3 was used for lamin C. Three separate gels were run to examine any variation between each experiment. The results were analysed by densitometric analysis and standard deviation measured.

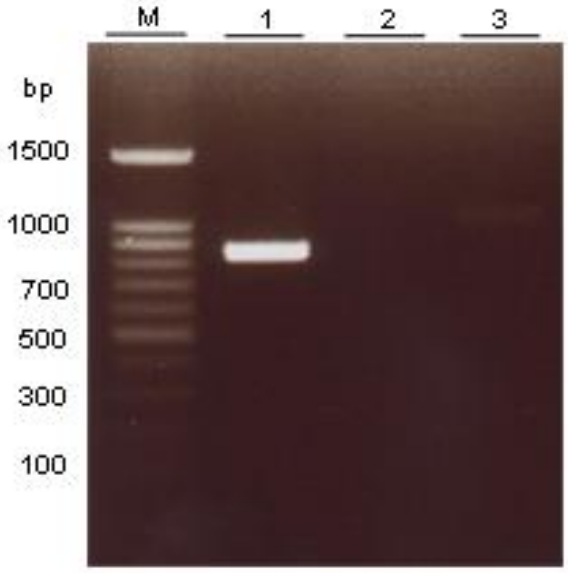


Figura 4.4

RT-PCR showing no expression of lamin A and C in Tubulo-villous adenoma sample. Beta actin is visible as a 834 bp product.

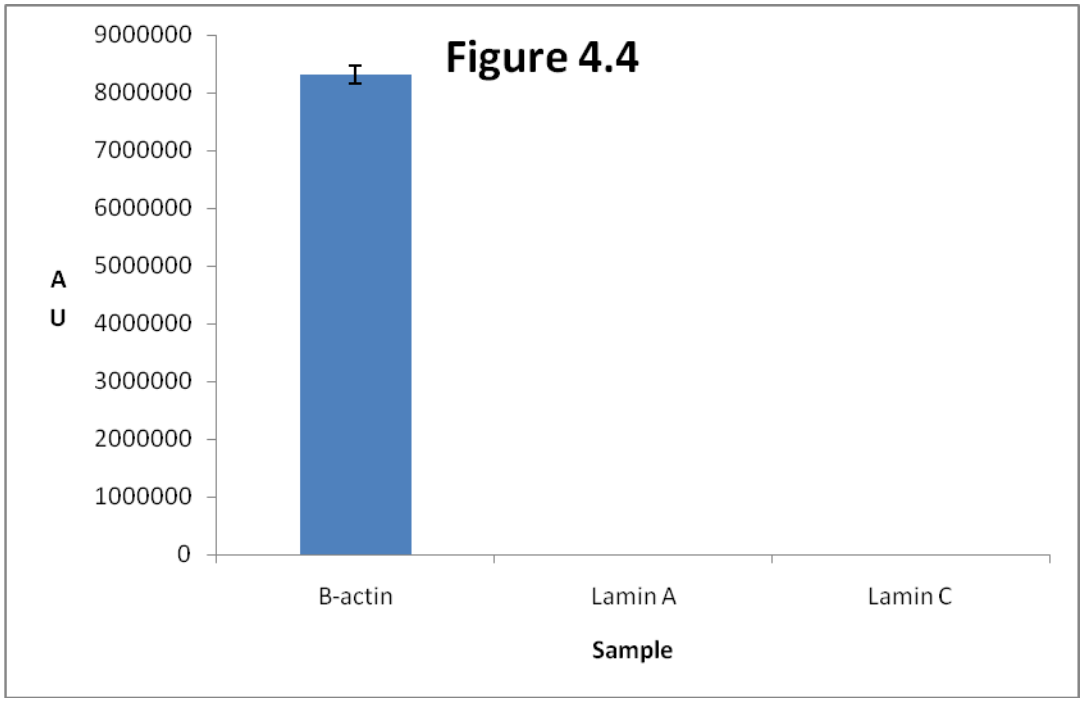


Figure 4.4

Graph showing densitometric analysis of three separate RT-PCR samples assessing the expression of lamin A and C in a tubulo-villous adenoma sample.

Standard Deviation was measured.

AU – Arbitrary Units

Figure 4.5

RT-PCR to assess expression of lamin A and C in a Dukes' C tumour sample.

Immunohistological analysis revealed no discernable expression of lamin A and C in the tissue sample. This was confirmed on semi-quantitative RT-PCR. Primers were designed to amplify lamin A and C in the sample. Equal loading of starting material was verified by monitoring the transcriptional activity of β-actin, seen as an 834 bp product. Lane M was used a marker lane, lane 1 was used for β-actin, lane 2 was used for lamin A and lane 3 was used for lamin C. Three separate gels were run to examine any variation between each experiment. The results were analysed by densitometric analysis and standard deviation measured.

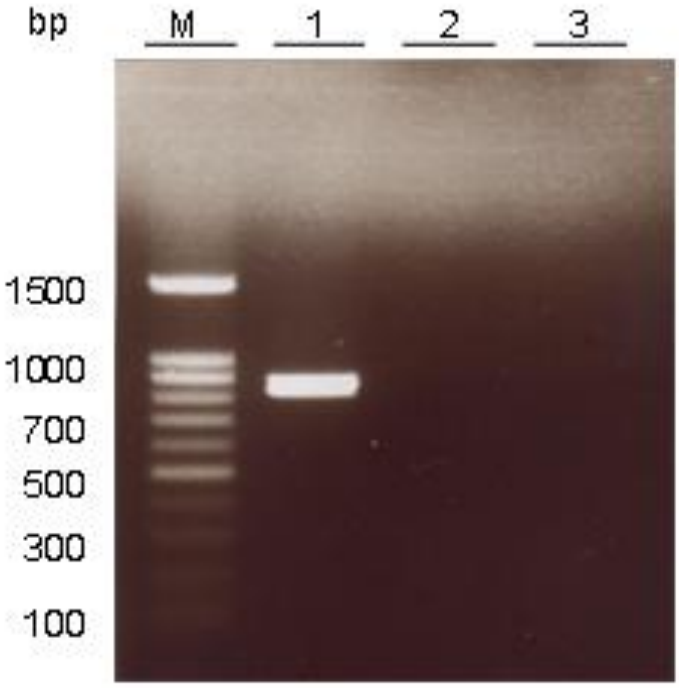


Figure 4.5

RT-PCR showing no expression of of lamin A and C in Duke's C tumour sample. Beta actin is visible as a 834 bp product.

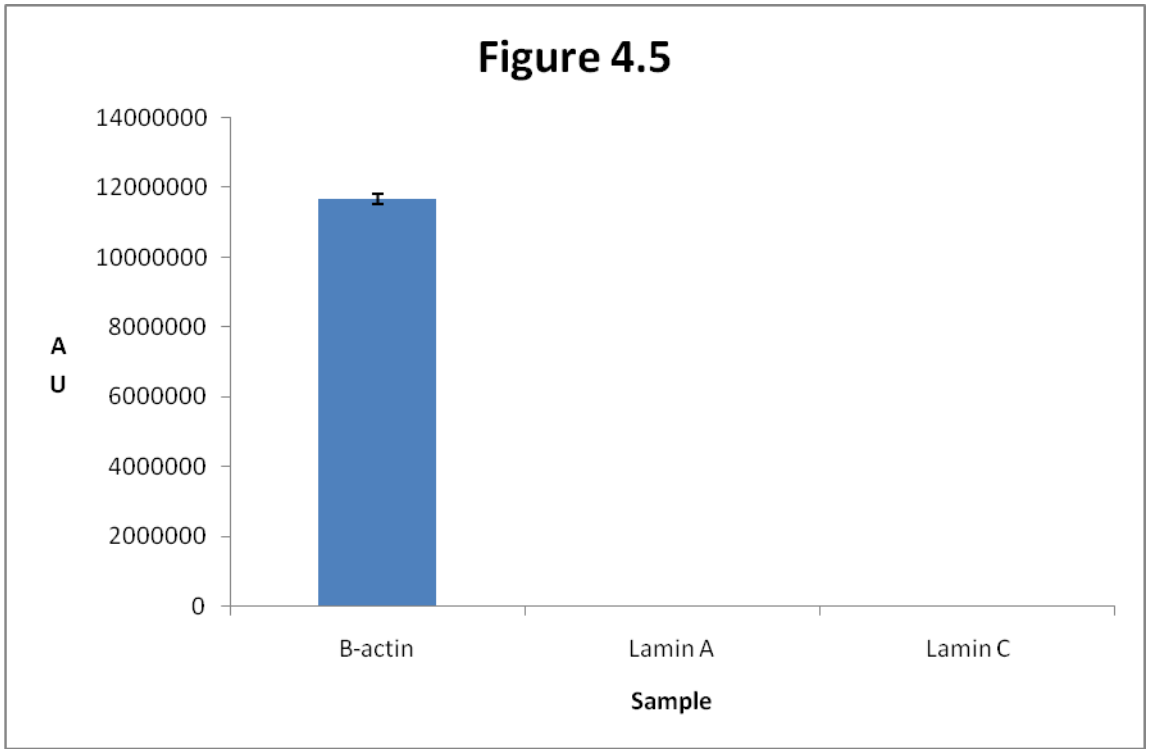


Figure 4.5

Graph showing densitometric analysis of three separate RT-PCR samples assessing the expression of lamin A and C in a Dukes' C tumour sample.

Standard Deviation was measured.

AU – Arbitrary Units

Figure 4.6

RT-PCR to assess expression of lamin A and C in a Dukes' B tumour sample (sample 37) known to express lamin A/C and its comparison with a similar Dukes' B tumour sample (sample 38) deficient in lamin A/C expression.

Immunohistological analysis revealed no discernable expression of lamin A and C in the tissue sample number 38, known to be a Dukes' stage B tumour. This was confirmed on semi-quantitative RT-PCR. Similarly, tissue sample number 37, a Dukes' B stage tumour, was known to express lamin A/C and this was confirmed on semi-quantitative RT-PCR. Primers were designed to amplify lamin A and C in both samples. Equal loading of starting material was verified by monitoring the transcriptional activity of β-actin, seen as an 834 bp product. Lane M was used a marker lane, lanes 1 and 4 were used for β-actin, lanes 2 and 5 were used for lamin A and lanes 3 and 6 were used for lamin C. Lamin A expression was noted by observing a band at 1137 bp while lamin C expression was noted by observing a band at 993 bp. Three separate gels were run to examine any variation between each experiment. The results were analysed by densitometric analysis and standard deviation measured.

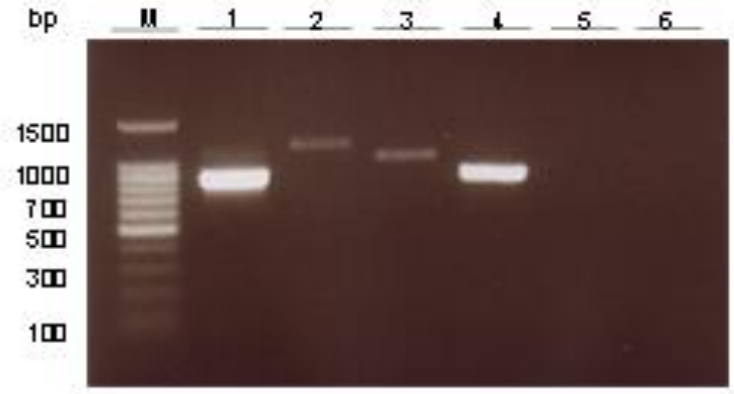


Figure 4.6

RT-PCR showing expression of lamin A and C, in lanes 2 and 3 respectively, in the Duke's B (nuclear lamin A/C positive) tumour sample and no lamin A or C expression being observed in lanes 5 and 6, corresponding to the Dukes' B (nuclear lamin A/C negative) tumour sample. Beta actin is visible as a 834 bp product.

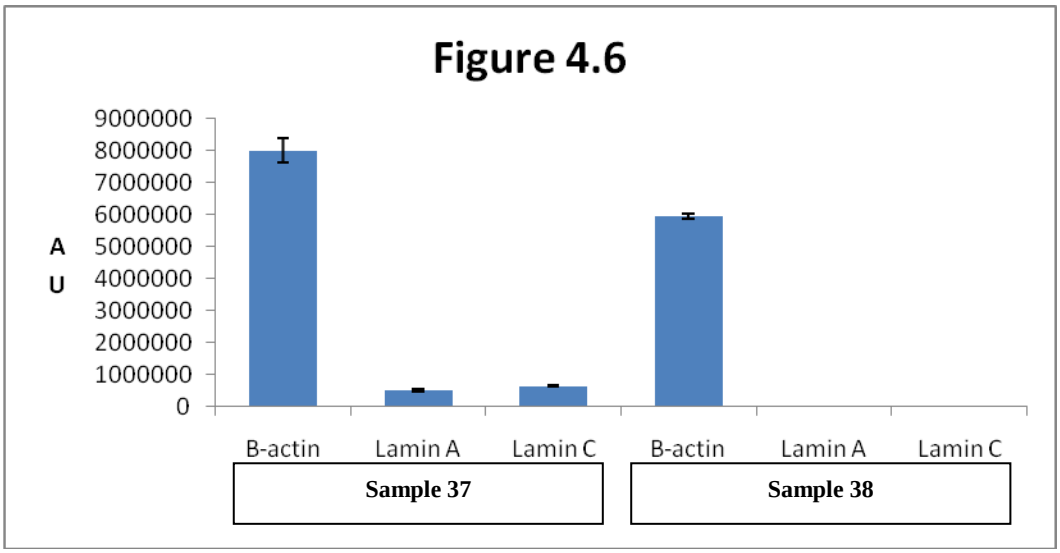


Figure 4.6

Graph showing densitometric analysis of three separate RT-PCR samples assessing the expression of lamin A and C in two Dukes' B tumour samples (sample 37 and 38 respectively).

Standard Deviation was measured.

AU – Arbitrary Units

Figure 4.7

RT-PCR to assess for expression of creatine kinase brain (*CKB*) in a tubulo villous adenoma (lamin A/C negative), Dukes' B (lamin A/C positive), Dukes' B (lamin A/C negative) and Dukes' C tumour samples (lamin A/C negative).

All tumour samples were assessed for the expression of creatine kinase brain. Lane 1 contained a sample from a tubulo villous adenoma; lane 2 contained a sample from a Dukes' B (lamin A/C positive) tumour; lane 3 contained a sample from a Dukes' B (lamin A/C negative) tumour and lane 4 contained a sample from a Dukes' C (lamin A/C negative) tumour sample. Lane M was used a marker lane. Three separate gels were run to examine any variation between each experiment. The results were analysed by densitometric analysis and standard deviation measured.

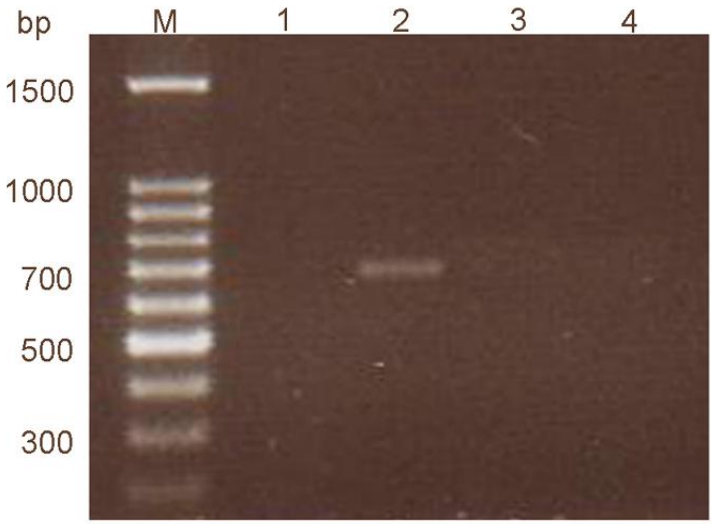


Figure 4.7

RT-PCR demonstrating a 687 bp product in lane 2, in keeping with Creatine Kinase Brain.
Lane 1 - Tubulo-villous adenoma sample.
Lane 2 - Duke's B (lamin A/C positive) sample.
Lane 3 - Duke's B (lamin A/C negative) sample.
Lane 4 - Duke's C (lamin A/C negative) sample.
M indicates a marker lane.

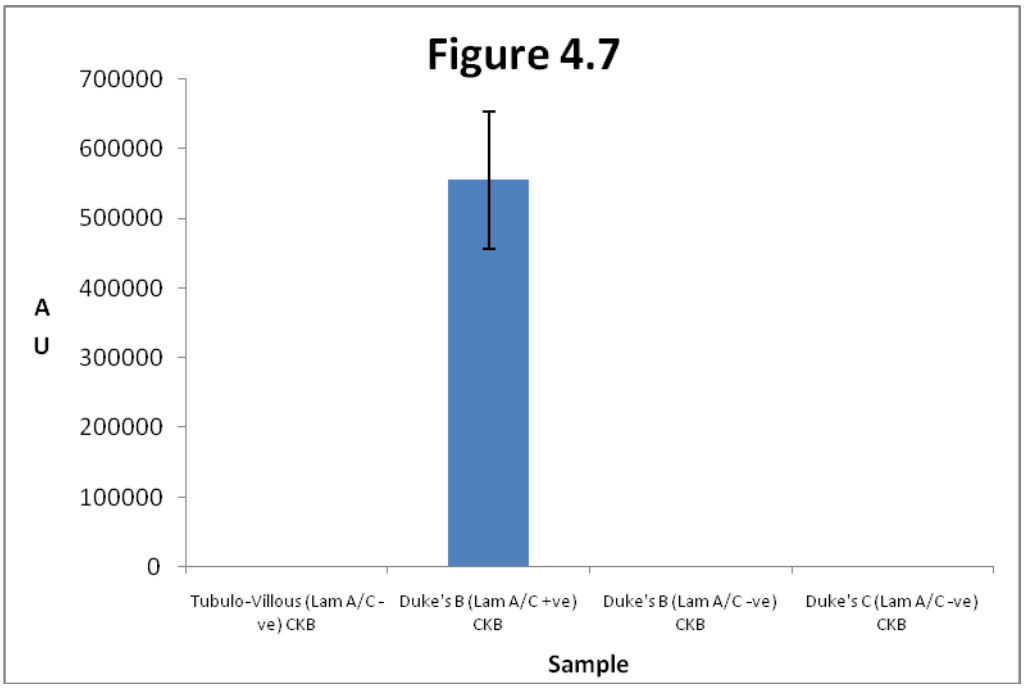


Figure 4.7

Graph showing densitometric analysis of three separate RT-PCR samples assessing the expression of CKB in all tumour samples.

Standard Deviation was measured.

AU – Arbitrary Units

Figure 4.8

RT-PCR showing expression of Beta-actin in all tumour samples used for assessing expression levels of creatine kinase brain.

This figure shows the expression of beta-actin in all tumour samples used for the assessment of creatine kinase brain, as shown in figure 4.6. Beta actin is expressed uniformly in all samples. Lane 1 corresponds to a tubulo-villous (lamin A/C negative) sample; lane 2 corresponds to a Dukes' B (lamin A/C negative) sample; lane 3 corresponds to a Dukes' B (lamin A/C positive) sample and lane 4 corresponds to a Dukes' C (lamin A/C negative) tumour sample.

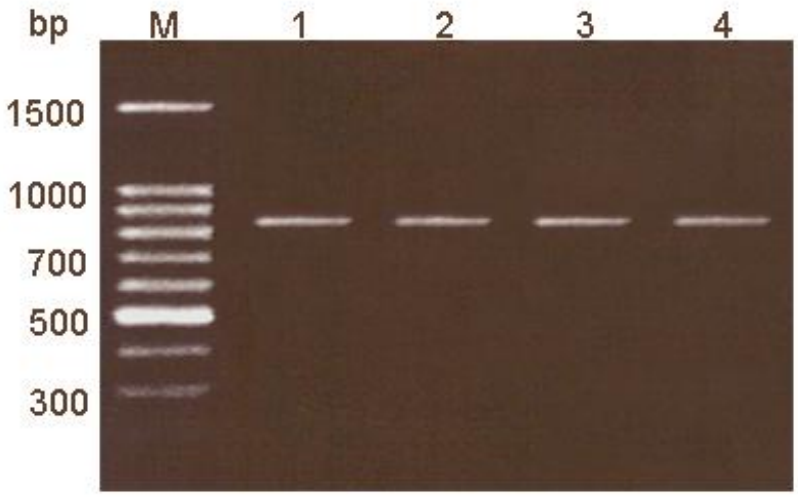


Figure 4.8

RT-PCR showing expression of 834 bp product in all lanes, in keeping with beta-actin.
Lane 1 - Tubulo-villous adenoma (lamin A/C negative) sample
Lane 2 - Duke's B (lamin A/C positive) sample.
Lane 3 - Duke's B (lamin A/C negative) sample.
Lane 4 - Duke's C (lamin A/C negative) sample.
M indicates marker lane.

Figure 4.9

Expression of T-plastin and E-cadherin in two Dukes' B tumour samples, one expressing lamin A/C and the other devoid of any lamin A/C expression.

Semi quantitative RT-PCR to assess for levels of T-plastin and E-cadherin in Dukes' B (lamin A/C) and Dukes' B (lamin A/C negative) tumour samples revealed high expression of T-plastin in the tumour sample expressing lamin A/C (lane 3) whereas low levels were detected in the tumour sample lacking nuclear expression of lamin A/C (lane 4). On the other hand, low levels of E-cadherin were detected in the tumour sample expressing lamin A/C (lane 5) as compared to the tumour sample lacking nuclear expression of lamin A/C (lane 6). M indicates a DNA marker lane. Lane 1 exhibits beta-actin from a Dukes' B (lamin A/C) tumour sample while lane 2 exhibits beta-actin from a Dukes' B (lamin A/C negative) tumour sample.

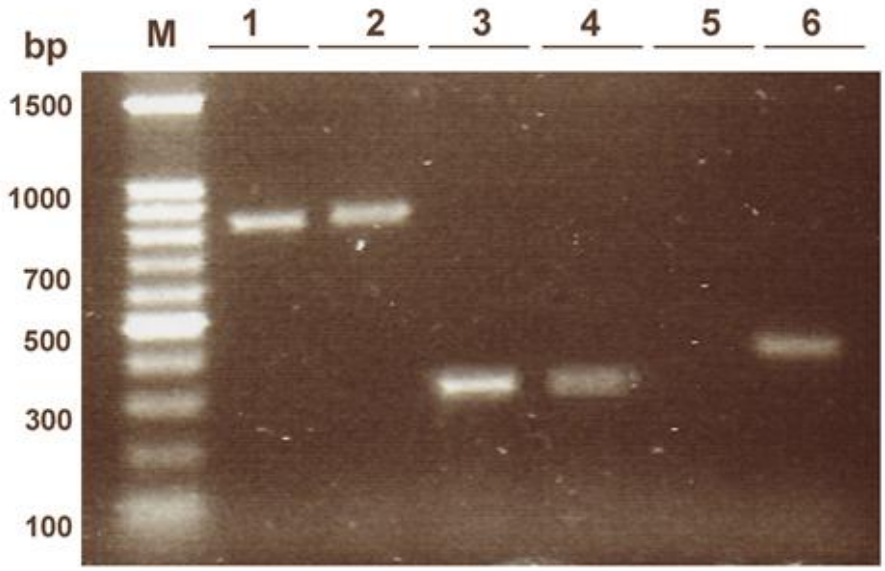


Figure 4.9

RT-PCR demonstrating T-Plastin in lanes 3 and 4. E-Cadherin is observed in lane 6 but is absent in lane 5. M is a marker lane. Lane 1 and 2 show beta-actin. Lanes 1, 3 and 5 correspond to Duke's B (lamin A/C positive) tumour positive samples. Lanes 2, 4 and 6 correspond to Duke's B (lamin A/C negative) tumour samples.

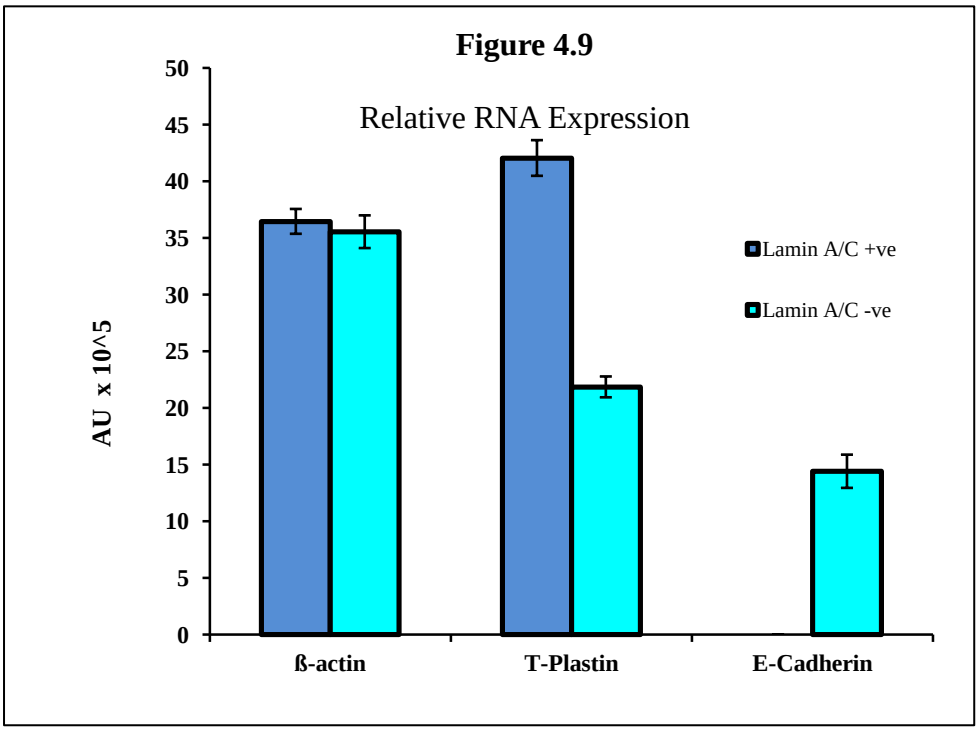


Figure 4.9

Graph illustrating densitometric analysis of three separate RT-PCR samples assessing the expression of T-plastin and E-cadherin in two Dukes' B tumour samples, one expressing lamin A/C and the other devoid of lamin A/C expression, respectively. B-actin was also measured.

Standard error was measured.

AU – Arbitrary units

4.4 Discussion

Work done previously by Cox, Willis (Willis et al., 2008) has shown differential expression of lamin A/C in colorectal cancers of the same stage and grade. Hence, to confirm their findings and explore the underlying mechanism(s) leading to their observations, a prospective study involving 42 patients with bowel cancer, undergoing elective surgery, were recruited (*n=42*) from a local hospital after due approval by the ethics committee. Initial efforts were focused on assessing the expression of lamin A/C using jol2, monoclonal antibodies (Dyer et al., 1997) in cancer tissue samples from these patients. Looking at the cohort as a whole, the incidence of bowel cancer according to Dukes' staging reveals no significant difference in the Dukes' A group when compared with UK national values (cancer research UK 2002). However, in the Dukes' B group the observed incidence of 52% is higher than expected from the UK national value of 35%. Similarly in the Dukes' C group the observed incidence value of 36% is higher than the UK national average of 26%. The main reason for these differences in observation is due to the exclusion of Dukes' D patients from the cohort studied. Dukes' D patients are not deemed suitable candidates for definitive treatment and receive palliative care only, hence their exclusion from the study. To assess whether or not lamin A/C expression influences the stage of colorectal cancer, a simple chi-square cross-tabulation of Dukes' stage against lamin status was carried out. This shows no significant correlation (p=0.85) although the size of the population (n=42) puts significant limits on the power of such a statistical analysis. Comparing the incidence of colorectal cancer according to site involved in the colon and rectum, the observed values are more or less in keeping with those observed nationally in the UK (cancer research UK 2002).

4.4.1 Comparative microarray analysis of Dukes' B and C (nuclear lamin A/C negative) tumour samples with a tubulo-villous adenoma (nuclear lamin A/C negative) sample

The genetic alterations involved in the progression of a colorectal adenoma towards a fully developed colorectal cancer and its metastatic spread, have been elucidated excellently by previous studies and have given rise to the well known adenoma – carcinoma sequence (Fearon and Vogelstein, 1990). However, knowledge about lamin A/C imparting a less favourable outcome in patients with colorectal cancer (Willis et al., 2008) has recently been brought to light. In order to get a clearer picture of the various genetic alterations involved in colorectal carcinogenesis, a microarray experiment has been designed using three different tissue samples, each from a different patient; a tubulo-villous adenoma, a Dukes' B cancer and a Dukes' C cancer, respectively. Intentionally, all samples selected are lacking in nuclear lamin A/C expression in order to achieve minimal bias in the results. Using microarray data obtained from analysis of the tubulo-villous adenoma sample as a base line, comparative arrays are carried out on the other two samples and the results analysed. In the Dukes' B group, the gene encoding for *insulin-like growth factor 2* (*somatomedin A*) exhibits the highest level of up regulation, while the gene encoding for *Gremlin 1* (part of the cysteine knot superfamily) is down regulated the most. In the Dukes' C group, the gene encoding for *carbonic anhydrase 1* is up regulated the most while the gene encoding for *regulator of G protein signalling* is down regulated the most. By using the microarray data from the Dukes' B tumour sample as a base line, a comparative array study of the Dukes' C sample reveals maximal up regulation in the gene encoding for *carbonic anhydrase 1* and maximal down regulation in the gene encoding for *insulin-like growth factor 2* (*Somatomedin A*). From this data it appears that the gene encoding for *insulin-like growth factor 2* (*Somatomedin A*) is uniquely up regulated in the Dukes' B tumour sample only and has no bearing on increasing stage of colorectal cancer, if anything, it is greatly suppressed. Similarly, the gene encoding for *carbonic anhydrase 1* is uniquely up regulated in the Dukes' C tumour sample only.

By clustering key genes into functional groups it is observed that genes involved with apoptosis are universally down regulated in both Dukes' B and Dukes' C tumour samples. This finding is not unusual as loss of these apoptotic inhibitors is usually seen in cancer cells.

Examination of key genes involved in tumour suppression also reveal an overall down regulation in the Dukes' B sample but no significant change is seen in the Dukes' C sample. No specific reason can be found to explain this observation in the Dukes' C group.

A general up regulation of key genes involved in stem cell differentiation is noted in the Dukes' C sample. This observation is difficult to explain as there are no plausible explanations found in the literature. However, various studies have suggested that colorectal cancer may be a consequence of adult stem cell dysfunction. Also, many cancers are known to exhibit certain characteristics that are typical of stem cells, for example, immortality, self renewal, monoclonality, etc. To this end, it appears that colorectal cancers, through some unknown mechanism(s), might be acquiring stem cell like attributes. At what stage does this change occur is open to debate. Some authors are firm proponents of colorectal cancer being a stem cell problem right from the beginning (Preston, 2003), while others believe in the top down morphogenesis of colorectal cancer in which a colorectal cancer invades downwards into the crypt and eventually encroaches upon the stem cell niche and manages to acquire stem cell like properties (Shih et al., 2001), thus priming the stage for possible metastatic spread. From the microarray data it appears that acquisition of stem cell properties may be a late event and is observed in later stages of colorectal cancer, however definitive proof is lacking and further studies are needed to clarify this.

The Wnt signalling pathway in the colon is well known for driving the proliferative compartment of the crypt and aberrations in this pathway, especially mutations in the APC (Adenomatous Polyposis Coli) gene are known to lead towards polyp formation and constitute part of the adenoma carcinoma pathway (Fearon and Vogelstein, 1990). No significant changes in either APC or downstream mediators of Wnt signalling, such as c-MYC, are noted. This result is expected as all three tumour samples will already harbour changes in the APC gene and its associated

pathways i.e. the Wnt signalling pathway and hence no significant change between each sample is expected. An exception here is the observation that epherin receptor B1 (EPHB1) is up regulated in all samples compared. At present no explanation is forthcoming for this observation.

4.4.2 Comparative microarray analysis of a Dukes' B (nuclear lamin A/C positive) tumour sample with a tubulo-villous adenoma (nuclear lamin A/C negative), Dukes' B (nuclear lamin A/C negative) and Dukes' C (nuclear lamin A/C negative) tumour samples

Patients with Dukes' B colorectal cancer pose a unique challenge to clinicians as regards optimum treatment. The reasons for this dilemma lie in the results of various trials that have not shown a conclusive benefit of adjuvant therapy in improving long term survival figures (IMPACT B2 trials - 1999). In this context, assessment of two Dukes' B tumour samples from different patients with identical stage and grades but different expression of nuclear lamin A/C, reveal very interesting features. The following genes are greatly up regulated in the Dukes' B (lamin A/C positive) tumour sample: *Insulin-like growth factor 2 (Somatomedin A)*; *Fatty acid binding protein 1, liver*; *T plastin*; *creatine kinase brain* and *bone morphogenetic protein 4*. On the other hand, key genes that are down regulated in the Dukes' B (lamin A/C positive) tumour sample included: *Secreted Phosphoprotein 1 (Osteopontin), E-cadherin* and *cyclooxygenase* (*COX2*) amongst other down regulated genes.

Comparative analysis between the tubulo-villous adenoma sample and Dukes' B (lamin A/C positive) sample show a general up regulation in key genes involved with apoptotic inhibitors, cell cycle progression / proliferation and growth, oncogenes, stem cell differentiation and Wnt signalling, with the notable exception of epherin receptor B1 which is down regulated. When compared with the other Dukes' B sample (nuclear lamin A/C negative), no clear cut change can be observed in any functional gene group as a whole, although certain genes are significantly up regulated in the Dukes' B (lamin A/C positive) group when

compared with the Dukes' C (lamin A/C negative) sample. These key genes are: Matrix metalloproteinase 3 (progelatinase); fatty acid binding protein 1, liver; bone morphogenetic protein 4; creatine kinase brain; proteolipid protein and T-plastin. On the other hand, key genes that are down regulated in the Dukes' B (lamin A/C positive) tumour sample include: Secreted Phosphoprotein 1 (Osteopontin), E-cadherin and cyclooxygenase (*COX2*) amongst other down regulated genes.

4.4.3 Key genes of interest identified from the microarray experiment and their role in colorectal cancer

A brief description of key genes of interest identified through the microarray studies and their possible role in colorectal cancer is further explored and reveals the following:

Creatine kinase brain (CKB) is an enzyme known to act as a catalyst in the conversion of creatine to phosphocreatine and adenosine diphosphate. Phosphocreatine acts as an energy reservoir and plays an important role in the rapid buffering and regeneration of adenosine triphosphate in tissues, via the phosphocreatine shuttle (Wallimann et al., 1992). In human cells, creatine kinase consists of two subunits (B-Brain; M-Muscle) and three isoforms (BB, MM and MB). The genes encoding for the B subunit are on chromosome 14q32. The increased levels of creatine kinase brain subunit, observed in the Dukes' B, lamin A/C positive tumours is intriguing. Proteomic studies done by Friedman et.al (Friedman et al., 2004) describe low levels of creatine kinase observed in colorectal cancers. Their observations are supported by other studies as well (Joseph et al., 1997). However, Balasubramani et.al (Balasubramani et al., 2006) observes high levels of creatine kinase in the nuclear matrix but low levels in the cytoplasm of cells taken from colorectal cancer tissue samples. No definite explanation for this observation is available, however, it is postulated that due to post-translational modification(s), creatine kinase preferentially translocates to the nuclear matrix with a resultant low level observed in the cytoplasm. In other studies, high creatine kinase levels are observed in cancers of the lung and breast (Arenas et al., 1989; Joseph et al., 1997; Zarghami et al., 1996). Also, serum

levels of creatine kinase are known to be elevated in cancers of the lung, colon, ovary and prostate. Joseph et.al speculates that increased levels of creatine kinase observed in the serum of patients with colorectal and lung cancer are probably due to enhanced enzyme release due to necrosis of tumour cells (Joseph et al., 1997). Creatine kinase would thus seem to be more of a generic marker rather than a specific marker for colorectal cancer (Arenas et al., 1989; Mercer and Talamo, 1985). The key interest in this enzyme from my point of view is the observation that it is closely associated with the nuclear matrix, of which lamins A/C are an integral part. This raises the possibility that lamins A/C can potentially play a part in nuclear energy metabolic pathways, which could be a unique finding if proven true. Also, the prospect of assessing a patient's serum for CKB levels and using this information, in conjunction with nuclear lamin A/C status in the colorectal tumour sample, to impart an accurate prognosis can prove a very useful tool. Obviously, further studies are needed to confirm this.

T-plastin is a member of the actin bundling protein group and includes L-plastin and I-plastin. All three isoforms share between a 70% and 78% homology with each other (Delanote et al., 2005). T-plastin is found in epithelial and mesenchymal tissue. Neoplastic transformation in these tissues also results in synthesis of T-plastin and L-plastin. The actin based cytoskeleton is known to be involved in a multitude of functions such as cell interaction, cytokinesis and cell migration. Work done by Willis et.al (Willis et al., 2008) reveals an increase in T-plastin levels with a concomitant decrease in E-cadherin levels, in SW480 cell lines transfected with lamin A/C. SW480 cell lines are known to be deficient in lamin A/C expression (Willis et al., 2008). Their results show an increase in cell motility and invasiveness associated with increased expression of T-plastin and decreased expression of E-cadherin in SW480 cells transfected with lamin A/C. E-cadherin is a well known regulator of adhesive properties in epithelial cells (Masciari et al., 2007) and is a known downstream target for the BMP signalling cascade. E-cadherin has been implicated in many solid tumours such as breast and pancreatic cancers (von der Hardt et al., 2007; Zeisberg et al., 2005). Significant expression of T-plastin with a decrease in E-cadherin expression in the Dukes' B lamin A/C positive tumour might suggest an adverse prognosis in this

group of cancers as the tumour cells may be less adhesive and more invasive with a higher likelihood of metastatic spread. Further studies will, of course, be required to prove this.

Bone morphogenetic protein 4 (BMP4) is a member of the Transforming Growth Factor – Beta (TGF-β) superfamily with more than 20 different BMP isoforms having been identified in mammals and Drosophila. In addition to regulating bone and cartilage formation, BMPs are also involved in development, morphogenesis, chemotaxis, cell proliferation and apoptosis (Leivonen and Kahari, 2007), in a diverse range of cells and tissues. BMPs exert their biological effects by binding to heteromeric type I / type II receptor complexes, containing serine / threonine kinase domains (Chen et al., 2004). Upon BMP binding, the heteromeric complex between type I and type II receptors initiate intracellular signalling by phosphorylation of Smad1, Smad 5 and Smad 8 (Kretzschmar et al., 1997). These phosphorylated Smads in turn associate with Smad 4 and translocate to the nucleus where they modulate the transcription of target genes involved in cell proliferation, cell differentiation and apoptosis (Balemans and Van Hul, 2002). Three different type I BMP receptors have been identified: activin receptor-like kinase-2 (ALK- 2), BMP type IA receptor (BMPR-IA)/ALK-3 and BMPR-IB/ALK-6 (Koenig et al., 1994; Liu et al., 1995; ten Dijke et al., 1994). Three different BMP type II receptors have been isolated: BMPR-II, activin type II receptor (ActR-II) and ActR-IIB (Liu et al., 1995; Rosenzweig et al., 1995). BMP2 and BMP4 appear to only bind to BMPR-IA and BMPR-II receptors.

Dysregulation of BMP signalling has been linked to various cancers. Involvement of the BMP signalling pathway in colorectal cancer was first detected in patients with juvenile Polyposis syndrome, in whom a BMPR-IA mutation was noted (Howe et al., 2001). Further studies by Hardwick *et.al* (Hardwick et al., 2004) reveal that BMP 2 has an inhibitory effect on normal colonic epithelial growth by promoting differentiation and apoptosis and inhibiting cell proliferation. It is not surprising to find that inactivation of the TGF-β signalling mechanisms is noted in approximately 80% of all colorectal cancers (Grady et al., 1998). Interestingly, the expression of BMP 4 is elevated in early colorectal cancers but then declines in colorectal

cancers that metastasise to lymph nodes or distant organs (Deng et al., 2007). No obvious explanation is found for this observation but the implications are that BMP 4 may help initiate metastasis of colorectal cancer but then has a lesser role in maintaining these metastasis. Over expression of BMP 4 has also been shown to promote a more invasive phenotype in HCT116 colorectal cancer cell lines. The exact mechanisms for this are not clear but it is observed that over expression of BMP 4 in HCT116 colorectal cancer cells induces the expression of urokinase plasminogen activator (uPA) mRNA and secretion of uPA protein, which is an extracellular matrix degrading proteinase. BMP 4 signalling can thus be involved in the regulation of uPA activity which may enhance the malignant potential of cancer cells by increasing their invasive potential. It has also been observed that BMP 4 over expression can protect colon cancer cells from apoptotic death under stressful conditions (Deng et al., 2007). What interactions do lamin A/C expressing colorectal cancer cells have with BMP 4 signalling that confers a poorer prognosis in this sub-group, is open to debate. Several intriguing possibilities are noted, as mentioned above, but no conclusive evidence is available to establish a definite link between BMP 4 signalling and lamin A/C status in colorectal cancer. Further work will be needed to explore these observations.

Osteopontin (OPN) is a phosphoglycoprotein, found in the extra-cellular matrix and secreted by a number of cells. OPN is known to bind with CD44 and integrin receptors (Ding et al., 2002) and is believed to be involved in a number of pathological / physiological processes (Sodek et al., 2000). It has been shown to play a major role in tumourigenesis, tumour invasion and metastasis in lung, breast, prostate and colon cancer (Agrawal et al., 2002; Chambers et al., 1996; Thalmann et al., 1999; Tuck et al., 1997). OPN levels are notably elevated in tumour samples with high metastatic potential (Craig et al., 1990; Oates et al., 1996). In addition OPN has also been shown to play a role in cellular processes such as adhesion, motility, invasion and angiogenesis, in a diverse range of tissues (Hirama et al., 2003; Liaw et al., 1995; Tuck et al., 2003). The increased expression of OPN in a variety of cancers, including bowel cancer, has been associated with a poorer prognosis (Irby et al., 2004; Rohde et al., 2007). It has

been proposed previously that OPN may contribute towards tumour invasion and metastasis via an integrin mediated signalling pathway (Irby et al., 2004).

Rohde et.al (Rohde et al., 2007) has linked OPN over-expression to aberrant Wnt signalling in colorectal cancers and has observed a very strong and independent association of OPN over-expression with increasing tumour stage, especially liver metastasis, and poor survival in patients with colorectal cancer. The molecular mechanisms linking OPN to Wnt signalling are not very clear but the observation that mitogenic DNA fragments are able to induce OPN expression via TCF4/LEF (TCF T-cell factor; Lymphoid enhancer factor) in a breast cancer model, suggest that OPN may be a target of the Wnt signalling pathway. The role of OPN in colorectal cancer has been studied by other groups and there appears to be a common association between increased levels of OPN and advanced tumour stage with a subsequent poorer prognosis.

The reduced expression levels of OPN observed in the Dukes' B (lamin A/C positive) group appear to suggest an early stage tumour since elevated levels of OPN are typically associated with higher stage / metastatic tumours. Whether any other mechanism(s) exists to explain this decrease in OPN levels in the nuclear lamin A/C expressing Dukes' B tumour sample, is unknown. Further work is needed in this direction since OPN appears to be an important marker for colorectal cancer progression.

Insulin-like growth factor 2 (Somatomedin A) and insulin-like growth factor 1, are known to be important regulators of cell differentiation and growth (Chao and D'Amore, 2008) and are structurally similar to insulin. In adult humans, insulin-like growth factor 2 (IGF2) is the predominant insulin-like growth factor (IGF) (LeRoith and Roberts, 2003) and its altered expression is implicated in many pathological conditions (Docquier et al., 2005), including colon cancer (Kaneda and Feinberg, 2005). In adult humans, IGF2 is found to be the major gene expressed amongst the IGFs (LeRoith and Roberts, 2003). The regulation and expression of *Igf2* is very complex. The gene encoding for IGF2 is found to be imprinted, or expressed monoallelically, and active only on the paternally inherited gene (DeChiara et al., 1991). Loss of imprinting, with resultant over expression of *Igf2*, is shown to be a

fairly common observation in many cancers [reviewed in (Feinberg et al., 2006)], including colorectal cancer (Kaneda and Feinberg, 2005). The exact underlying mechanisms involved are still not clear but it has been shown that Sonic Hedgehog (shh) can transcriptionally activate *Igf2* (Ingram et al., 2002). Activation of Gli is the last stage involved in shh signalling cascade, resulting in activation of key target genes. In mouse models, it is shown that *Igf2* is up-regulated in mesenchymal cells transfected with *Gli1*and also points towards a putative binding site for *Gli1* on the *Igf2* promoter (Ingram et al., 2002). Another important association is also noted between IGF2 and its ability to up-regulate VEGF, partly by indirectly up-regulating HIF (Kim et al., 1998). These associations serve only to highlight the importance of IGF2 in tumour development. The striking features observed in my experiments are the observation that IGF2 levels are greatly up-regulated only in the Dukes' B stage but not the Dukes' C stage. This is unusual as one can expect a continually rising level of IGF2 in advancing tumours. The only difference in Dukes' stage B and C tumours is the spread of tumour to surrounding lymph nodes. Upon a literature search I cannot find any studies comparing IGF2 levels to increasing tumour stage in colorectal cancer. If IGF2 levels are genuinely increased only in Dukes' B tumours then the implications of this may have significant bearing on our understanding of colorectal cancer pathogenesis. It might imply a defining moment in the tumours capacity to spread or metastasize to other areas. Obviously, further work is needed to confirm this and to clarify any other possible explanation(s) for this observation.

Fatty acid binding protein 1 (liver) belongs to a group of low molecular weight proteins mostly involved in transport of fatty acids and other lipophilic substances between extracellular and intracellular membranes. Its gene location is mapped out on chromosome 2. These proteins are known to play a key role in the intracellular compartmentalisation of long chain fatty acids, thereby enhancing their cellular uptake and further processing (Storch and Thumser, 2000). Fatty acid binding proteins are known to play an active role in fatty acid mediated signal transduction pathways and regulation of gene expression (Hertzel and Bernlohr, 1998) and consequently their active role in modulating cell division (Sorof, 1994), cell growth and differentiation (Schroeder et al., 2001). Given their diverse role(s)

in various physiological functions and their altered expression in various cancers, it is proposed that fatty acid binding proteins might play a role in tumourigenesis (Jing et al., 2000). The role of fatty acid binding protein 1, found in the liver, is further explored by Lawrie et.al (Lawrie et al., 2004). Their team conclude that fatty acid binding protein 1 is greatly decreased in colorectal cancers, especially at the adenoma stage of tumour development. My own observations from the microarray experiments do not coincide with their observations. However, the level of fatty acid binding protein 1 is greatly up-regulated in the Dukes' B, lamin A/C positive group and this can be interpreted in different ways. Lamin A/C is a known marker for differentiation and is expressed predominantly in the differentiated compartment of the colonic crypts; the same area of expression is also noted for fatty acid binding protein 1. It might be feasible to propose then that the presence of lamin A/C in colorectal tumours imposes a more differentiated phenotype thus preserving the expression of fatty acid binding protein 1 in these tissues. After a literature search, no definite explanation can be found for this observation though and further experimental work would be needed to clarify this.

4.5 Summary

This comparative microarray study has shown clearly the differences existing between two tumours that were classed as Dukes' B with similar grading. Both patients from whom these samples were obtained would have had the same treatment and follow up. The patient expressing lamin A/C in his / her tumour would have had a higher likelihood of mortality as compared with the patient not expressing lamin A/C (Willis et al., 2008). The explanation for this observed increase in mortality associated with lamin A/C expression is difficult to explain, but in vitro work done by Dr Willis et al (2008) reveals a possible mechanism of action to explain the poor outcome associated with lamin A/C expression. Firstly, cells expressing lamin A/C are more motile / less adherent and so can spread with ease. Secondly, cells expressing lamin A/C have potentially a more stem cell like phenotype. My own observations support those made by Dr Willis et.al. Furthermore, I have identified genes and certain pathways that might explain why lamin A/C expressing tumours are more motile and thereby impart an adverse prognosis. Obviously further experimental work is needed to find the missing links

and map out in detail various signalling pathways through which lamin A/C expressing colorectal cancers may act to impart a poorer prognosis and potentially identify therapeutic targets and / or further prognostic markers.

CHAPTER 5

General Discussion

5.1 Background to this project

The role of non-functional A-type lamins and their binding partners in tissue specific diseases is well established and observed in tissue-specific familial disorders such as autosomal inherited Emery-Dreifuss Muscular Dystrophy, Dilated Cardiomyopathy type-1A, Dunnigan type—Familial Partial Lipodystrophy, Mandibulosacral Dysplasia, Hutchinson-Gilford progeria and Charcot-Marie-Tooth type-2B1 (De Sandre-Giovannoli, 2003 #1783;Bonne, 1999 #268;Fatkin, 1999 #1577;Hegele, 2000 #583;Hegele, 2000 #582;Raffaele Di Barletta, 2000 #956;De Sandre-Giovannoli, 2002 #1582;Novelli, 2002 #897). Mutations in the lamin A binding region of Emerin also gives rise to an X-linked form of Emery-Dreifuss Muscular Dystrophy (Bione et al., 1994; Lee et al., 2001). Altered expression and distribution of A-type lamins has also been reported in a growing number of neoplasms affecting epithelial, mesenchymal and lymphoid lineages. These observations highlight the importance of A-type lamins and their binding partners in the regulation of growth pathways and their possible link(s) with tumour progression. In the UK, colorectal cancer is the second most common cause of death after lung cancer. In this thesis an attempt has been made to explore the role of A-type lamins and their binding partners in normal colonic mucosa and at various stages of colorectal cancer.

5.2 Expression of A-type lamins and their binding partners in normal colonic mucosa

5.2.1 Expression of A-Type Lamins in normal colonic / rectal mucosa

The expression and distribution profile of A-type lamins (lamins A and C), and their binding partners LAP2α and Emerin, in the colon and rectum is assessed using standard immunohistochemical techniques, with emphasis being placed on the orientation of tissue samples, in order to maximise complete crypt profile studies. The observed distribution of A-type lamins is, as expected, in the differentiated compartment of the crypt, specifically in the upper third. Here A-type lamins are predominantly expressed at the nuclear periphery with some heterogeneous intranuclear distribution of A-type lamins also being noted. The expression of A-type lamins is noted to follow a gradient with increasing expression towards the luminal surface. This is in keeping with increasing differentiation of these cells as confirmed by decreasing / absence of PCNA, a marker for proliferating cells in these areas. In the lower two thirds of the crypt, except the basal zone, lamin A/C expression is virtually absent with concomitant high expression of PCNA, indicating active proliferation of cells in this region.

The switch from proliferation to differentiation is a complex area with many factors involved in its regulation, many of which are unknown. However, Wnt signalling pathways are known to play a key role in maintaining this proliferative zone. After asymmetric division of stem cells at the base of the crypt, the daughter cells, under the influence of Wnt signals arising from mesenchymal cells, enter into a phase of rapid proliferation. These Wnt factors are known to activate β-catenin and lead to activation of Tcf/Lef transcription factors, namely Tcf-4 in the colon. These in turn result in transactivation of downstream target genes such as c-MYC, CCND1, MMP7, CD44, PLAUR and PPARD (Jo and Chung, 2005). These downstream targets are typically associated with initiating and driving cellular proliferation, for example c-MYC which represses the cell cycle inhibitor p21 $^{CIP1/WAF1}$ and allows cells to leave G1 and enter S phase of the cell cycle. It would thus seem that β-catenin/Tcf-4 driven gene transcription is the major regulator driving the

proliferating progenitor population in the lower two thirds of the crypt and is responsible for maintaining the crypt villus axis and replenishing the continual turnover of differentiated cells at the crypt lumen (van de Wetering et al., 1997; van de Wetering et al., 2002). Since Wnt signalling appears to be switched off higher up the crypt, in the differentiated zone, it appears to play a key role in the proliferation/differentiation switch associated with crypt regeneration. The study of Eph-receptors and epherins in the intestine has shed further light into the actual mechanisms involved in separating proliferating cells from differentiated cells and in cell positioning along the crypt axis. The Eph family of receptors are known to represent the largest known subfamily of receptor tyrosine kinases (Tuzi and Gullick, 1994). Based on their ligand binding specificity, Eph receptors are divided into two groups, EphA receptors, binding A-type epherins, and EphB receptors binding B-type epherins (Frisen et al., 1999). The Eph – epherin interactions are known to be important in development, especially in cell to cell interactions, which frequently results in repulsion forces between cells. The role of Eph-receptors and epherins in determining cell position along the crypt axis is highlighted in experiments done by Batlle et al 2002 (Batlle et al., 2002). These studies reveal the inverse relationship associated with β-catenin/TCF signalling and EphB/Epherin B in cell positioning in the intestine. They also show a reciprocal gradient of epherin B to that of β-catenin/TCF signalling i.e. the concentration of epherin B is maximal at the mucosal surface and decreases further down the crypt. These studies also demonstrate loss of cell positioning along the crypt axis and intermingling of differentiated cells with proliferating cells in the crypts of mice with mutations involving EphB receptors. Work done by Holmberg et.al (Holmberg et al., 2006) has focused on intestinal stem cells and they were able to identify EphB receptors as major regulators of proliferation in the intestinal stem cell niche. In addition Holmberg et.al demonstrated EphB kinase-dependant signalling in the intestine promoting cell-cycle re-entry and extending the proliferative domain in crypts. However, a complete picture as to the exact underlying mechanisms involved in this switch from proliferation to differentiation, is still not clear. The role of A-type lamins in this setting and whether it has any bearing on influencing this switch from proliferation to differentiation is still obscure, and at best, hypothetical. However, with cell cycle arrest, indicating a halt to proliferation and differentiation setting in, it is no surprise to find A-type lamins being expressed in this setting.

There is however an unusual expression of lamin A, but not lamin C, at the base of the crypts in the colon / rectum mucosa, in an area known to harbour the stem cell niche. An explanation for this observation is not available as there are no previous studies done in this area with which I can compare my findings. Can lamin A be expressed in adult stem cells of the colon crypt? From my observations, this appears to be very plausible based on:

- **Firstly,** the anatomical location and cell numbers exhibiting expression of lamin A in the basal regions of the crypts corresponds with studies that identify stem cells in these locations.
- **Secondly,** adult stem cells are not, in the strictest sense, fully undifferentiated cells as is the case with embryonic stem cells, and so one can argue that these cells have taken the first step(s) towards differentiation and in the process of doing so they can potentially exhibit markers of differentiation, such as lamin A.

Also, it is well known that A-type lamins are completely disassembled during cell division and reassembled afterwards in the new daughter cells. In case of the proliferating compartment of the crypts in colonic mucosa, the Wnt signalling pathway has always been assumed to follow a concentration gradient with maximum concentration at the basal regions of the crypts with progressively decreasing expression levels further up the crypt until the concentration of Wnt signals gets so weak that it can no longer exert its proliferative effects on crypt cells. At this stage then, cells are known to undergo cell cycle arrest and terminally differentiate prior to undergoing apoptosis at the luminal surface and being shed. The current explanation for the role of Wnt signalling in preventing differentiation, and subsequently expression of A-type lamins in proliferating cells of crypt, are well known and relate to the transactivation of downstream target genes which result in cellular proliferation by different mechanisms, including inhibition of cell cycle inhibitors, as mentioned earlier. Why then is lamin A being expressed in a handful of cells at the basal regions of the crypts, in the putative stem cell niche area, where the expression of Wnt signals is supposed to be at its maximum? What is protecting these cells from the influence of Wnt signals? Can the presence of lamin A in these cells confer a protective effect from Wnt signals? If so what are the underlying molecular mechanisms for this? Can A-type lamins in conjunction

with Emerin be responsible for this effect by inhibiting the entry of β-catenin into these putative stem cells and thereby blocking the proliferative effects of Wnt signalling? These questions and many more are difficult to answer at this stage. It is felt however that A-type lamins in some way have an important role to play in preserving the '**Stemness**' of these putative stem cells in the basal regions of the intestinal crypts. Future studies into this area will hopefully clarify this picture and probably highlight in greater detail the role of A-type lamins in adult stem cells of the colon.

5.2.2 Expression of Emerin in normal colonic / rectal mucosa

The expression of Emerin in crypts of the colonic / rectal mucosa reveal an expression pattern similar to that of A-type lamins, but with a few notable differences. In the basal regions of the crypts, at the putative stem cell niche area, Emerin is strongly expressed in the nuclear area; however, heterogeneous intra-cytoplasmic expression is also noted which is in contrast to A-type lamin expression in this area. In the proliferative zone of the crypts, Emerin expression is greatly reduced and only small traces of this protein are noted in the cytoplasm. This expression profile changes higher up the crypt axis, in the differentiated zone, where Emerin expression once again becomes evident in the nuclear area in addition to heterogeneous intra-cytoplasmic expression. Significant expression of Emerin in the nuclear area is also observed in cells making up the muscularis mucosae however, expression of Emerin in cells making up the surrounding mesenchyme is predominantly cytoplasmic with only a few cells expressing Emerin in the nucleus. It would appear that Emerin and A-type lamins follow a very similar pattern of expression in crypts located in the colon / rectal mucosa and the main difference(s) in expression between each is related to greater intra-cytoplasmic expression of Emerin in corresponding cells.

Emerin is a type II integral membrane protein of the inner nuclear membrane (INM) belonging to the 'LEM-domain' family of nuclear proteins. The localisation of Emerin at the INM is through its association with A-type lamins, type V

intermediate filament proteins, (Sullivan et al., 1999; Vaughan et al., 2001), which form part of the nuclear lamina (Hutchison, 2002). Emerin on its own is dispensable for cell survival (Harborth et al., 2001) and normal development (Gruenbaum et al., 2002), but has overlapping functions in cell division and chromosome segregation with the INM protein MAN1 (Liu et al., 2003), another LEM-protein. Other family members of LEM-proteins include otefin, Lem-3 and SANE (Cohen et al., 2001; Dechat et al., 2000b; Lin et al., 2000; Raju et al., 2003). The LEM-domains of Emerin and LAP2β are known to bind with Barrier-to-Autointegration Factor (BAF), a highly conserved chromatin protein essential for the viability of dividing cells (Zheng et al., 2000). BAF is known to bind DNA, has structural roles during nuclear assembly and dictates higher order chromatin structure via unknown mechanisms (Segura-Totten et al., 2002; Zheng et al., 2000). BAF also represses gene expression by inhibiting transcription activators (Wang et al., 2002). The central region of Emerin is essential for binding lamin A (Lee et al., 2001; Vaughan et al., 2001) and this coupled with distinct binding regions for BAF and lamins on LAP2β (Foisner and Gerace, 1993; Shumaker et al., 2001), suggest that BAF links chromatin directly to membrane anchored LEM-proteins and indirectly to lamins. In addition, Emerin is also shown to interact with GCL (germ-cell-less) a gene regulatory protein that is known to repress E2F-DP regulated genes (Holaska et al., 2003). The binding site for GCL is mapped to two regions in Emerin that overlaps proposed binding regions for BAF and lamin A. Interestingly, competition assays demonstrate that Emerin forms stable tertiary complexes with either lamin A plus BAF, or lamin A plus GCL. However, Emerin cannot bind simultaneously to both GCL and BAF and BAF's estimated concentration at the NE (nuclear envelope) can be sufficient to inhibit GCL binding (Holaska et al., 2003). Emerin is also known to bind two other proteins implicated in gene expression: *Btf* and *YT521-B* (Wilkinson et al., 2003). *YT521-B* is involved in selecting sites for alternative mRNA splicing and Emerin influences this splice site selection. *Btf* is a known transcription repressor that induces cell death when over-expressed (Kasof et al., 1999). It is speculated that Emerin sequesters *Btf* and, thus, suppresses apoptosis (Tzur et al., 2002). The aforementioned roles of Emerin imply strongly that Emerin plays a significant role directly or indirectly in transcription regulation.

No studies are available to indicate what role Emerin plays in homeostasis of crypts from colonic / rectal mucosa. In this regards, my study is unique. Work done by Markiewicz *et.al* (Markiewicz et al., 2006) in human fibroblasts has shed light on the interaction between Emerin and β-catenin. Studies by Markiewicz et.al (2006) has shown that Emerin interacts with β-catenin through a C-terminal APC-like domain. They show that over-expression of Emerin inhibits β-catenin signalling by preventing its nuclear accumulation. In contrast, expression of a mutant form of Emerin that lacks the β-catenin-binding domain dominantly stimulates β-catenin accumulation in the nucleus and its signalling potential. The underlying mechanism(s) are unclear; however, Emerin appears to act by stimulating the export of β-catenin from the nucleus. They also show that correct localisation of Emerin at the INM is necessary for its influence on nuclear accumulation of β-catenin. These findings are of great significance to my work since I have demonstrated the presence of Emerin in the nuclear area in differentiated cells and its absence in proliferating cells of the crypt. Also, cells at the putative stem cell niche area only express lamin A and, from previous studies, it is known that Emerin binds preferentially to lamin A (Lee et al., 2001; Vaughan et al., 2001). It is plausible that Emerin in conjunction with lamin A and other unknown factors can be responsible for maintaining the stem cell niche in crypts of the colon / rectal mucosa. This can be achieved by increased shuttling of β-catenin out of the nucleus and thereby protecting stem cells from the proliferative effects of β-catenin and hence the Wnt signalling cascade. The prospect of Emerin playing a significant role in stem cell signalling could add another dimension to the functional role(s) of Emerin.

5.2.3 Expression of LAP2α in normal colonic / rectal mucosa

LAP2α is expressed in the majority of cells involved in the crypt. However, the distribution of LAP2α is not uniform at various sites in the crypt. In the basal regions of the crypt, corresponding to the putative stem cell niche area, LAP2α is distributed in a heterogeneous manner in the nucleoplasm and cytoplasm. In the

proliferative compartment of the crypt, the expression of LAP2α is mostly cytoplasmic with a granular appearance. Interestingly, LAP2α exhibits a concentration gradient in this zone with gradually increasing levels of expression in the cytoplasm being observed higher up along the crypt axis. In the differentiated zone of the crypt, the expression of LAP2α is again noted in the nuclear area with maximal concentration levels being observed in cells lining the mucosal surfaces of the crypts. In addition, LAP2α is also expressed in a heterogeneous distribution in the nucleoplasm and cytoplasm of these same cells. A striking difference between the expression of LAP2α and Emerin in the crypts is the complete absence of LAP2α expression in cells making up the muscularis mucosae. Cells making up the mesenchyme surrounding each crypt also exhibit LAP2α expression in the nuclear area with heterogeneous nucleoplasmic and cytoplasmic expression but this expression is in a patchy distribution.

Lamina associated polypeptides (LAPs) are known to form part of the integral membrane family of proteins at the inner nuclear membrane. In mammalian cells, three lamina associated polypeptides have been characterised in detail: LAP 1, LAP 2 and a protein called the lamin B receptor (LBR) (Gerace and Foisner, 1994). LBR and LAP2 are known to preferentially interact with lamin B while LAP1 interacts with both lamin A and B types (Foisner and Gerace, 1993). The LAP2 family members are known to comprise six alternatively spliced proteins; four of these are type II membrane proteins: LAP2 β, -δ, -γ and –ε (Berger et al., 1996) that bind lamin B. LAP2α is different, both structurally and functionally as it shares only its N-terminus with the other isoforms and has a distinct C-terminus. It also lacks an apparent transmembrane binding domain (Harris et al., 1995). The distribution of LAP2α in cells is throughout the nucleus rather than the nuclear envelope (Dechat et al., 1998; Dechat et al., 2000a; Vlcek et al., 1999). LAP2α is a well known specific binding partner of nucleoplasmic A-type lamins (Dechat et al., 2000a). At their N-terminus, LAP2 proteins share a LEM (LAP2, Emerin and MAN1) domain (Lin et al., 2000), which binds to BAF (barrier-to-autointegration-factor), an essential, highly conserved DNA-binding protein in eukaryotes (Segura-Totten and Wilson, 2004). Based on these interactions, LEM domain proteins in the nuclear lamina and the nuclear interior have been implicated in chromatin organisation (Dechat et al., 2004; Segura-Totten and Wilson, 2004). In addition,

LAP2α interacts with chromosomes via its α-specific C-terminal domain in a phosphorylation dependant manner (Dechat et al., 2004; Vlcek et al., 1999). Over-expression of C-terminal fragments of LAP2α are shown to dominantly inhibit assembly of endogenous LAP2α, nuclear membranes and A-type lamins in in-vitro nuclear assembly assays and to cause-cell cycle arrest at interphase, indicating a role for LAP2α in cell cycle progression (Vlcek et al., 2002).

The retinoblastoma protein (Rb) is known to regulate the activity of E2F transcription factors, which in turn are responsible for controlling the expression of cell cycle regulatory genes (Classon et al., 2000; Frolov and Dyson, 2004). In non-cycling cells, Rb is hypophosphorylated and thereby active as a transcriptional repressor by binding to and inhibiting E2F transcription factors. A-type lamins and LAP2α complexes are known to bind with the C-terminal nuclear anchorage domain of Rb and keep it hypophosphorylated, resulting in repression of E2F (Dorner et al., 2006; Mancini et al., 1994; Markiewicz et al., 2002; Ozaki et al., 1994). Rb has been shown to be essential for the differentiation of various tissues, including skeletal muscle and adipocytes (Hansen et al., 2004; Huh et al., 2004) by maintaining a balance between initiation and differentiation in adult stem cells (Classon et al., 2000). Adult stem cells are known not to proliferate; instead, they enter the cell cycle upon specific signals to self propagate and maintain a stable population of adult stem cells in the tissue. At the same time, they are also known to differentiate and regenerate damaged tissue or maintain a basic turnover of differentiated cells (Wagers and Weissman, 2004). Lap2α and A-type lamins are known to be essential co-factors of RB in maintaining the balance between proliferation and differentiation in adult stem cells (Dorner et al., 2006). Given the expression profiles of A-type lamins and LAP2α in the crypts of the colon / rectal mucosa, it is plausible that LAP2α might play a significant role in the stem cell niche and also in differentiation of colonocytes along the crypt axis. In support of this view, proliferating cells in the lower third of the crypts do not exhibit any significant expression of LAP2α in the nucleoplasm of the cells. The exact mechanisms underlying the observed expression profile of LAP2α in the crypts is still not clear and probably multiple signalling pathways are involved; however, the close link between LAP2α and A-type lamin expression in the nuclei of differentiated cells and of the putative stem cells could support the view that, in

addition to other well documented pathways involved in crypt homeostasis, LAP2α in association with A-type lamins might play a significant role in maintaining the crypt profile via the retinoblastoma-E2F pathway.

5.2.4 Expression of β-catenin in normal colonic / rectal mucosa

In the crypts of the colon, the expression profile of total β-catenin exhibits certain interesting features. At the basal region of the crypt, in the putative stem cell niche area, significant expression of total β-catenin is noted in the nuclear area in addition to cytoplasmic expression. In the proliferative compartment of the crypt, total β-catenin expression is observed in the nuclear area and cytoplasm of cells but nucleoplasmic expression has diminished considerably. A further change in expression of total β-catenin is then observed in cells making up the differentiated compartment of the crypt. In these differentiated cells, total β-catenin is predominantly expressed in the cytoplasm with an increased concentration being observed at the inter-cellular junctions.

β-catenin plays a central role in the canonical Wnt signalling pathway, which is considered to be the most significant regulator of normal crypt homeostasis and colorectal cancer development (Pinto and Clevers, 2005b; van de Wetering et al., 2002). In the absence of a Wnt signal, members of the T-cell factor / lymphoid enhancing factor (TCF/LEF) family of transcription factors are bound to the transcriptional repressor Groucho (Cavallo et al., 1998). Active beta catenin is known to directly associate with TCF/LEF factors and overcome this repression imposed by Groucho, thereby transactivating the transcription of downstream targets (van de Wetering et al., 1997). TCF-4 is a member of the TCF/LEF family and is highly expressed in cells forming the epithelial layer of the colon. It forms functionally active complexes with beta catenin (Korinek et al., 1997) which in turn are the principal governors of cell dynamics at the crypt villous junction, preserving a stem cell and proliferating progenitor population in the lower crypt region. This is essential for ensuring an orderly turnover of differentiated cells at the luminal surface (van de Wetering et al., 2002). Inhibition of the β-catenin/TCF-4 complex

(van de Wetering et al., 2002) has been shown to result in the following: Firstly – a concomitant G1 arrest. Secondly – a down-regulation of intestinal markers of proliferation such as *c-MYC, EPHB2, c-MYB, BMP4, ENC1, CD44* and *CLDN1,* known to be expressed in the proliferative compartment of normal crypts, and thirdly – an up-regulation of differentiation markers such as *FABP1* (fatty acid binding protein 1), *CA2* (carbonic anhydrase II) and importantly $p21^{CIP1/WAF1}$. *c-MYC*, a well known oncogene responsible for pushing cells from G0 to S-phase of the cell cycle, appears to be one of the most important down-stream targets of the β-catenin/TCF-4 complex (He et al., 1998; Oster et al., 2002). In essence then, β-catenin/TCF-4 complexes appear to maintain a progenitor / stem cell phenotype in intestinal crypts through c-MYC mediated repression of the cell cycle inhibitor $p21^{CIP1/WAF1}$. Wnt signals are absent in the differentiated compartment of the crypt, resulting in β-catenin being exported from the nucleus by APC and sequestered for removal by the degradation complex. Activation of c-MYC also occurs in this zone resulting in $p21^{CIP1/WAF1}$ being induced which facilitates cell cycle arrest and differentiation (van de Wetering et al., 2002). After a literature search, no direct role for A-type lamins in the Wnt signalling pathway has been observed, up to date. From my observations, the expression profile of Lamin A/C and β-catenin in the proliferation and differentiation regions of the crypt appears to be in keeping with their known functions. However, at the base of the crypt a very interesting picture is emerging. Both A-type lamins and β-catenin are being expressed in the nuclear area with β-catenin also being expressed in the cytoplasm of these cells located in the stem cell niche area. It appears that stem cells are expressing both lamin A/C and total β-catenin and yet are not actively proliferating, as evidenced by the lack of PCNA (a well known marker of proliferation), nor are they known to be fully differentiated. It is difficult to explain this observation; however, it appears that A-type lamins are somehow preventing the effects of β-catenin on the nuclear machinery by limiting its movement across the nuclear envelope and hence the observations mentioned earlier. Also, it is well known that stem cells are able divide asymmetrically and give rise to another stem cell and a daughter cell that then undergoes rapid proliferation prior to terminal differentiation in the crypt. In addition, stem cells are also known to divide symmetrically and give rise to two adult stem cells which can then restore the total numbers of adult stem cells making up the niche. The exact factors and signalling pathways controlling both

types of cell division in adult stem cells are not yet known. At this stage I can only hypothesise about the role(s) of lamin A/C and β-catenin in stem cells, for example; could the presence of lamin A/C in adult stem cells protect them from the effects of β-catenin at a nuclear level and help in maintaining an adult stem cell phenotype at this location? Are there as yet undiscovered pathways that could link lamin A/C with the Wnt signalling pathway directly? The answers to these questions and many more require further work in this direction.

5.3 Expression of A-type lamins in colorectal cancer

The expression profile of A-type lamins in various stages of colorectal neoplasia was studied in a cohort of forty two patients. Tumour samples from all stages of colorectal neoplasia, including polyps, were studied using standard immunohistochemical techniques. Although the sample size is not large enough to make any definite conclusions, certain observations are intriguing:

- Variable expression of A-type lamins is noted in colorectal cancer specimens ranging from very strong nuclear area expression of A-type lamins to virtually no expression in the nucleus.
- This variation is not limited to cancer stage or differentiation status.
- Irrespective of nuclear area expression, the cells making the mesenchyme and lamina propria, exhibit strong expression of A-type lamins in the nucleus.

Given that A-type lamins are well known as markers for differentiation, the natural tendency is to assume that cancers expressing A-type lamins are more differentiated as compared to other cancers of a similar stage not expressing A-type lamins. However, this proves not to be case since well differentiated cancers (defined by using standard histo-pathological criteria) are found to be lacking expression of A-type lamins and vice versa.

The expression of nuclear lamins has previously been studied most extensively in lung cancer (Broers and Ramaekers, 1994; Broers et al., 1993; Kaufmann et al., 1991) and keratinocytic tumours of the skin (Oguchi et al., 2002; Venables et al., 2001), but other studies have also focussed on acute lymphoblastic leukaemia, non-Hodgkin's lymphoma, (Prokocimer et al., 2006; Stadelmann et al., 1990; Wu et al., 2009), gastric cancer (Wu et al., 2009) and colorectal cancer (Cance et al., 1992; Moss et al., 1999; Prokocimer et al., 2006; Willis et al., 2008).

The transcriptional and post-transcriptional regulation of A-type lamins has also been reported previously. Broers et.al (Broers et al., 1993) demonstrate a decrease in lamin A and C mRNA levels accompanied down-regulation of protein expression in two small cell lung cancer (SCLC) cell lines. Work by Machiels et.al (Machiels et al., 1995) demonstrates preferential repression of lamin A protein expression relative to lamin C in a human lung adenocarcinoma cell line and ascribe this imbalance to a reduction in the amount of lamin A transcript. Equally, up-regulation of lamin A/C protein expression can be reflected in mRNA levels. Expression of the mutant v-Ha-ras oncogene in a SCLC cell line induces a 10 fold increase in lamin A/C protein levels which are accompanied by elevation in the corresponding mRNA species (Kaufmann et al., 1991). Post transcriptional mechanisms by which lamin A expression might be abrogated but lamin C unaffected have been described in a study on the premature ageing disease Hutchinson-Gilford progeria syndrome. De novo point mutations in the lamin A-specific tail domain are thought to prevent complete processing of prelamin A, while lamin C is processed as normal (Eriksson et al., 2003).

A decrease in the expression of A-type lamins has previously been reported in colorectal cancer by Moss et.al (Moss et al., 1999), but they do not distinguish between individual A-type lamin polypeptides. The first evidence of a differential relationship between lamins A and C in tumours is described by Venables et.al (Venables et al., 2001). They report a mutually exclusive pattern of lamin A and Ki67 expression in basal cell cancers (BCC) of the skin and correlate loss of lamin A with rapidly proliferating tumours. However, further studies by Willis et.al (2005), using in vitro studies, demonstrate no correlation between lamin A expression and growth rate or proliferation and are therefore not consistent with the observations made by Venables et.al (Venables et al., 2001).

A complex relationship between lamin A/C expression and cellular proliferation / differentiation has emerged. A decrease in A-type lamin levels has been correlated with proliferation in Swiss 3T3 murine fibroblasts (Pugh et al., 1997). Similarly lamin A/C expression has been inversely correlated with proliferation in lung tumours (Coates et al., 1996; Rowlands et al., 1994). However, many groups have reported a coincident appearance of lamin A/C during tissue and cellular differentiation, whereas at least one B-type lamin is always expressed (Lebel et al., 1987; Paulin-Levasseur et al., 1989; Rober et al., 1989; Stewart and Burke, 1987). Further to the work by Venables et.al (Venables et al., 2001) on BCC, Oguchi et.al (Oguchi et al., 2002) have examined a range of keratinocytic tumours of the skin and report the greatest reduction in lamin A/C in poorly differentiated tumours. Tilli et.al (Tilli et al., 2003) also come to the conclusion that A-type lamin expression is strongly associated with well-differentiated tumours, but that it does not follow that these cells possess no proliferative capacity. They observe simultaneous expression of lamin A/C and Ki67 in approximately 50% of the proliferating cells in BCC and Squamous cell cancer (SCC) of the skin.

In general these investigations have reported down regulation of A-type lamins in association with increased proliferation and a strong association of A-type lamins with increasing differentiation of tumours. These studies have provided significant insight into the role(s) A-type lamins might play in the development and progression of cancer. However, there is still controversy about the exact role of A-type lamins in colorectal cancer due to the contradictory findings related to A-type lamin expression in colorectal tumours. Attempts to link changes in expression to patient prognosis or directly to tumour progression result in conflicting results between different groups (Cance et al., 1992; Moss et al., 1999; Willis et al., 2008).

Recently, Willis et.al (Willis et al., 2008), have described an adverse outcome in a group of patients with colorectal cancer expressing lamin A/C. Their study involves 656 patients taking part in the Netherlands Cohort Study on Diet and Cancer (van den Brandt et al., 1990). Willis et.al (Willis et al., 2008). They are able to demonstrate clearly that patients expressing A-type lamins in their colorectal tumour are almost twice as likely to die from colorectal cancer related causes as compared to clinicopathologically identical patients that do not express A-type

lamins in their tumours. Their studies have linked this adverse outcome to increased motility and invasiveness in colorectal cancer cells expressing lamin A/C. They also propose that colorectal cancers exhibiting A-type lamins in their cells might be a reflection of these cells acquiring a more stem cell like phenotype.

5.4 Comparative microarray analysis of various stages involved in colorectal cancer

Previous studies, apart from that carried out by Willis et.al (Willis et al., 2008), have all hinted towards A-type lamins being associated with increasing differentiation in tumours from various regions of the body and thereby implying a better prognosis in these patients exhibiting A-type lamins in their tumours. My own findings are not in keeping with these previous observations. To try and clarify the role of A-type lamins in colorectal cancer, I carried out a comparative microarray analysis comparing various stages of colorectal cancer tumours. To this end the Affymetrix Gene Chip® was used for the microarray experiment. Microarray data obtained in this manner does have its limitations and needs to be interpreted with caution. To obtain meaningful results from a microarray experiment one has to pay particular attention to the experimental design and the statistical methods used for interpreting the results. A good experimental design will minimise variance. Variance can arise from multiple areas, including RNA handling and isolation, chip to chip variability, hybridization conditions, sample to sample differences, etc (Pan et al., 2007). A reduction in variance is achieved by using multiple biological replicates and multiple arrays which can then allow for appropriate calibration, also known as normalisation (Huber et al., 2002). The ratio of these calibrated intensities is also known as the ‘Fold Change’ and is a useful way of quantifying transcript abundance. In replicate microarray studies, the variance for measured spot intensities increases with their mean and hence the need for biological and array replicate studies. Appropriate use of statistical methods in microarray data analysis is another crucial area. To validate all significant genes thus identified by RT-PCR or Northern Blot analysis is prohibitive and impractical and hence some form of multiple testing correction needs to be

applied, typically Bonferroni (very stringent) or Benjamani-Hochberg. This will control the false discovery rate (FDR). In reality, FDR is not widely used and could explain why so many array studies have results that cannot be reproduced (Pan et al., 2007). The lack of biological and array replicates in my experiments are areas of potential contention with the results I have described. Also, the statistical methods used to analyse the data are not stringent enough to allow a very high degree of confidence in the results. Subsequently, there is some minor lack of correlation between the microarray data and the RT-PCR data used to validate the results from the microarray studies. On a plus note, the Affymetrix Genechip® has about a hundred so called 'house keeping' genes that help keep variance down to a minimum and are useful indicators to this effect. Ideally I would have used array data from normal tissue samples as a base line against which various comparative studies would be carried out, unfortunately, the quality of RNA from the normal samples was poor and hence a decision was taken to use only tumour samples for the microarray study. Despite these drawbacks to my experiments, it was felt that useful information could be obtained from the selected tumour samples.

From a clinical point of view, there has always been controversy about managing patients with a Dukes' B adenocarcinoma since there are no clear prognostic indicators to identify which patients are at high risk of an adverse outcome from their cancer and thereby require adjuvant treatment to decrease this risk. The mainstay of treatment for Dukes' A cancers is surgery alone and for Dukes' C patients, either neo-adjuvant or adjuvant treatment in addition to surgery. The identification of a genuine prognostic indicator, especially in Dukes' B adenocarcinomas, will greatly aid in the decision making policy for treatment in these patients. Initially, a comparative analysis between three tumour samples of different stages, and all exhibiting no nuclear lamin A/C expression, was assessed. This was then followed by a comparative microarray analysis of two Dukes' B adenocarcinoma samples, one with strong nuclear expression of lamin A/C and the other with complete absence of nuclear expression of lamin A/C.

5.4.1 Comparative microarray analysis between a tubulo-villous adenoma, a Dukes' B and Dukes' C adenocarcinoma, all lacking nuclear lamin A/C expression.

Three tumour samples were selected based on their complete lack of nuclear lamin A/C expression. These samples are representative of an increasing stage of colorectal neoplasia i.e. from a polyp to a Dukes' C adenocarcinoma. Given the lack of nuclear lamin A/C expression in these samples, it is felt that absence of A-type lamins will not confound the results of the microarray analysis. Two different approaches were undertaken to analyse the data. The first approach looks at the highest and lowest up-regulation of genes while the second approach clusters key genes into functional groups and then assessments are made to look for any unusual activation or suppression overall in the functional group being examined.

By using the array data obtained from microarray analysis of the Tubulo-villous adenoma sample as a base line against which to compare array data obtained from the Dukes' B & C cancer samples, it is observed that the gene encoding for *insulin like growth factor 2 (Somatomedin A)* is maximally up regulated only in the Dukes' B sample while the gene encoding for *carbonic anhydrase 1* is maximally up regulated in the Dukes' C sample only. The genes encoding for *Gremlin 1* (part of the cysteine knot family) and *regulator of G protein signalling* are down regulated significantly in the Dukes' B and C samples respectively. Interestingly, when the array data from the Dukes' B sample is used as a baseline and a comparison made with the Dukes' C sample array data, it shows maximal down regulation in the gene encoding for *insulin like growth factor 2 (Somatomedin A)* and maximal up regulation in the gene encoding for *carbonic anhydrase 1*, in the Dukes' C sample. It is thus immediately apparent that up regulation of the gene encoding for *insulin like growth factor 2 (Somatomedin A)* is uniquely up regulated only in the Dukes' B stage tumour.

By clustering key genes into functional groups and using the array data obtained from the microarray analysis of the tubulo-villous adenoma sample as a base line for comparison, it is observed that:

- Key genes involved in **apoptosis** are down regulated significantly in both Dukes' B and C samples.
- Key genes involved in **tumour suppression** are down regulated only in the Dukes' B sample but not in the Dukes' C sample.
- Key genes involved in **stem cell differentiation** are generally up regulated in the Dukes' C sample only.
- No obvious changes are noted in key genes involved with the **Wnt signalling pathway** or its downstream mediators, such as *c-MYC*.
- Other functional groups studied do not reveal any significant changes as a group, although instances of individual genes in a functional group do reveal significant changes.

At this stage of the analysis from the microarray data obtained from three different stages of colorectal neoplasia, all devoid of nuclear lamin A/C expression, significant changes affecting the transition of colorectal cancer from the Dukes' B stage towards the Dukes' C stage are noted. From a clinical point of view, the main difference between these stages is related to the development of lymphatic spread in the Dukes' C cancers. Up to date, various factors involved in this transition are still not clear and one can only speculate at best. The observation that key genes involved in tumour suppression are down regulated in the Dukes' B cancer probably indicates a defining moment in the cancers ability to develop the potential to metastasise. Once metastasis takes place, the role of specific genes involved in tumour suppression might not be very significant, as illustrated by no significant changes in these genes in the Dukes' C cancer; however, this is purely speculative and no definite evidence to support this viewpoint is found in the literature. The other key change noted is the up regulation of known key genes involved in stem cell differentiation, in the Dukes' C cancer only. No definite explanation can be offered for this observation. However, stem cells are implicated by various authors in the development of colorectal cancer with some groups favouring the development of colorectal cancer due to a primary stem cell dysfunction (Preston et al., 2003) while other groups are of the opinion that colorectal cancers invade the stem cell niche and then take on attributes of stem cells (Shih et al., 2001) prior to attaining metastatic potential. From the array results, it appears that if any stem cell involvement is contributing to colorectal

cancer progression, it will be at a later stage of the cancer. This observation is in keeping with that proposed by Vogelstein et.al (Shih et al., 2001). This could also explain certain overlapping features of cancers with stem cells as regards immortality, self renewal, capacity to develop in different environments, etc. What are the individual factors and signalling cascades responsible for this switch in expression of key functional genes between a Dukes' B and a Dukes' C cancer, are still elusive, however, significantly the Wnt signalling cascade does not appear to contribute in any significant manner to these changes.

5.4.2 Comparative microarray analysis of a Dukes' B tumour sample expressing nuclear lamin A/C with a tubulo-villous adenoma, Dukes' B and Dukes' C tumour samples, devoid of nuclear lamin A/C expression.

Assessment of two Dukes' B tumour samples from different patients with identical stage and grades but different expression of nuclear lamin A/C, reveal very interesting features. The following genes are greatly up regulated in the Dukes' B (lamin A/C positive) tumour sample: *Insulin-like growth factor 2 (Somatomedin A)*; *Fatty acid binding protein 1, liver*; *T plastin*; *creatine kinase brain* and *bone morphogenetic protein 4 (BMP4)*. On the other hand, key genes that are down regulated in the Dukes' B (nuclear lamin A/C positive) tumour sample include: *Secreted Phosphoprotein 1 (Osteopontin)* and *cyclooxygenase* (*COX2*) amongst other down regulated genes. In addition, a microarray comparison using the Dukes' B cancer sample expressing nuclear lamin A/C as a base line and comparing it with a tubulo-villous adenoma and a Dukes' C tumour sample, both devoid of nuclear lamin A/C expression, reveals up regulation in the following additional key genes in the Dukes' B sample expressing nuclear lamin A/C: Matrix metalloproteinase 3 (progelatinase) and proteolipid protein. Microarray analysis to assess for key down regulated genes reveals a similar set of genes as to those already observed when compared with the Dukes' B sample devoid of nuclear lamin A/C expression.

Microarray assessment of key genes making up relevant functional groups reveals up regulation of the following functional gene groups in the Dukes' B sample expressing nuclear lamin A/C when compared to the other tumour samples not expressing nuclear lamin A/C:

- Apoptotic inhibitors
- Cell cycle progression / proliferation and growth
- Oncogenes
- Stem cell differentiation
- Wnt signalling (with the notable exception of epherin receptor B1 which was down regulated)

In the Dukes' B cancer sample expressing lamin A/C, it is interesting to observe an up regulation in key genes involved in Wnt signalling, especially fatty acid binding protein 1, liver (FABP1). Similarly, in the stem cell functional group, an overall increase in the expression of key genes is noted, with the gene encoding for BMP4 (bone morphogenetic protein 4) being highly expressed. Amongst the functional group of genes involved with the cytoskeleton / nucleoskeleton, T-plastin (PLS3) is highly expressed. This is accompanied by a modest down regulation of E-cadherin. Of interest is the observation that CKB (creatine kinase brain) is greatly up regulated.

Given the microarray data analysis, a very complex picture is emerging as regards the role of lamin A/C in colorectal cancer. Many different signalling cascades are being implicated in cancers expressing lamin A/C. Work done by Willis *et.al* (Willis et al., 2008) has shown that cells expressing lamin A/C cause a downstream up regulation of **T-plastin** which in turn leads to a down regulation of **E-cadherin**. T-plastin has previously been shown to be a member of the actin bundling protein group and includes L-plastin and I-plastin. All three isoforms share between a 70% and 78% homology with each other (Delanote et al., 2005). T-plastin expression has been identified in epithelial and mesenchymal tissues. The actin based cytoskeleton is known to be involved in a multitude of functions such as cell interaction, cytokinesis and cell migration. Neoplastic transformation in these tissues is accompanied by an increase in synthesis of T-plastin and L-plastin. Work done by Willis *et.al* (Willis et al., 2008) shows an increase in T-plastin levels

with a concomitant decrease in E-cadherin levels, in SW480 cell lines transfected with lamin A/C. SW480 cell lines are known to be deficient in lamin A/C expression (Willis et al., 2008). Their results show an increase in cell motility and invasiveness associated with increased expression of T-plastin and decreased expression of E-cadherin. E-cadherin is a well known regulator of adhesive properties in epithelial cells (Masciari et al., 2007) and is a known downstream target for the BMP signalling cascade. E-cadherin has been implicated in many solid tumours such as breast and pancreatic cancers (von der Hardt et al., 2007; Zeisberg et al., 2005). Significant expression of T-plastin with a decrease in E-cadherin expression in the Dukes' B sample expressing nuclear lamin A/C may be associated with an adverse prognosis in this group of cancers as the tumour cells could be less adhesive and potentially more invasive with a higher likelihood of metastatic spread.

BMP4 is a known member of the transforming growth factor-β superfamily (TGFβ), members of which are known to play an important role in regulating cell growth, differentiation, proliferation and apoptosis via the TGFβ signalling pathway (BMP signalling pathway). Both up and down regulation of BMPs and their receptors have been reported in association with the progression of a variety of cancers. For example, up regulation of BMPR-IA, BMPR-II and BMP 2 mRNA levels has been reported in pancreatic cancers with 55% of these cancers exhibiting biallelic loss of SMAD4 (Moskaluk et al., 1997; Zhang et al., 1997). A strong expression of BMP 4, 6 and 7 has also been detected in prostatic adenocarcinomas with established metastasis, thereby linking the role of BMPs in the development of skeletal metastasis in prostate cancers (Autzen et al., 1998; Hamdy et al., 1997; Masuda et al., 2003). An up-regulation of BMP 4 and BMP 7 expression has also been reported in several melanoma cell lines and increased expression is noted in primary and metastatic melanoma compared with naevi (Rothhammer et al., 2005). In addition, Nishanian *et.al* (Nishanian et al., 2004) also demonstrate a link between Wnt signalling and BMP signalling. The role of BMP signalling pathway in colorectal cancer was first reported in patients with juvenile Polyposis syndrome (Howe et al., 2001). Further studies by Hardwick *et.al* (Hardwick et al., 2004) reveal that BMP 2 has an inhibitory effect on normal colonic epithelial growth by promoting differentiation and apoptosis and inhibiting cell proliferation. In the

development of colorectal cancer, uncontrolled and abnormal cellular proliferation coupled with aberrant cell death mechanisms, are known to play a very important role, amongst other mechanisms. It is not surprising then to find that inactivation of the TGF-β signalling mechanisms have been noted in approximately 80% of all colorectal cancers (Grady et al., 1998). The implications of these observations are that BMP signalling has a suppressive role in the development of colorectal cancer. However, the expression of BMP 4 has been detected in several colon cancer cell lines and is induced by an *APC* gene mutation(s) and / or activated β-catenin, which occurs very frequently in colorectal tumours (Kim et al., 2002). Up-regulation of BMP 4 in early colorectal cancers as compared to normal colonic mucosa has also been observed in a separate study by Nosho *et.al* (Nosho et al., 2005). Interestingly, the expression of BMP 4 is noted to be elevated in early colorectal cancers but then declines in colorectal cancers that metastasise to lymph nodes or distant organs (Deng et al., 2007). No obvious explanation is found for this observation but the implications are that BMP 4 may help initiate metastasis of colorectal cancer but then plays a lesser role in maintaining these metastasis. Over expression of BMP 4 has also been shown to promote a more invasive phenotype in HCT116 colorectal cancer cell lines. The exact mechanisms for this are not clear but it is noted that over expression of BMP 4 in HCT116 colorectal cancer cells induces the expression of urokinase plasminogen activator (uPA) mRNA and secretion of uPA protein, which is an extracellular matrix degrading proteinase. BMP 4 signalling can thus be involved in the regulation of uPA activity which can enhance the malignant potential of cancer cells by increasing their invasive potential. A further study observes that BMP 4 over expression can protect colon cancer cells from apoptotic death under stressful conditions (Deng et al., 2007). What interaction(s) does lamin A/C expressing colorectal cancer cells have with BMP 4 signalling that confers a poorer prognosis in this sub-group? The answer to this question is not clear at present. Several intriguing possibilities are noted but no conclusive evidence is available to establish a definite link between BMP 4 signalling and lamin A/C status in colorectal cancer. Further work is needed to explore these observations since A-type lamins might be implicated in BMP / TGFβ signalling pathways and if proved, this will be a novel finding, adding a further dimension to the known properties of A-type lamins.

Another gene found to be uniquely up regulated only in the Dukes' B cancer sample expressing lamin A/C, is **creatine kinase brain (CKB)**. CKB is an enzyme known to act as a catalyst in the conversion of creatine to phosphocreatine and adenosine diphosphate, via the phosphocreatine shuttle (Wallimann et al., 1992). Phosphocreatine is to known to act as an energy reservoir and plays an important role in the rapid buffering and regeneration of adenosine triphosphate in tissues. Studies done by Balasubramani *et.al* (Balasubramani et al., 2006) reveal high levels of creatine kinase in the nuclear matrix but low levels in the cytoplasm of cells taken from colorectal cancer tissue samples. There is no definite explanation for their observations; however, they propose post translational modifications as being responsible for the preferential translocation of CKB to the nuclear matrix with resultant low expression levels in the cytoplasm. In other studies involving cancers of the lung and breast (Arenas et al., 1989; Joseph et al., 1997; Zarghami et al., 1996), high levels of CKB expression are also noted in tumour samples. Another observation about CKB, which is very relevant and of significance to my work, is the observation that CKB levels are elevated in the serum of patients with cancer of the colon, lungs, ovaries and prostate. These high levels of CKB expression in the serum are difficult to explain but one theory put forward by Joseph *et.al* (Joseph et al., 1997) is that necrosis of tumour cells results in the high expression levels of serum CKB noted in these patients. Another key interest in this enzyme is the observation that it is closely associated with the nuclear matrix, of which lamins A/C are an integral part, raising the possibility of A-type lamins being involved, in some way, with nuclear energy metabolic pathways. This will add a new facet to the already known functions of lamin A/C in the nucleus and can explain partially why tumour cells expressing lamin A/C have an adverse prognosis. CKB has the potential for being developed as a prognostic tool in colorectal cancer, even though it is not very specific for colorectal cancer, its use as a serum marker and the fact that it is greatly up regulated only in lamin A/C expressing tumours, which are known to have an adverse prognosis, are features making it very attractive for use as a prognostic tool. Obviously, further experiments and trials will be needed to clarify the exact role of CKB as a prognostic tool in conjunction with lamin A/C expression.

Insulin-like growth factors (IGFs) are known to play an important role in cell growth and differentiation in many species (Chao and D'Amore, 2008). IGF1 and IGF2 are two proteins involved in mediating the observed effects of IGFs and are structurally similar to insulin. Both IGF1 and IGF2 are known to mediate their mitogenic signals through type I IGF receptors (IGF1R), present on most cells. Upon binding to IGF1R, the IGFs can activate Ras/Raf/MAPK and PI3-kinase/Akt cascades, and depending on the cell type, stimulate proliferation, differentiation or both (Baserga et al., 2003). PI3-kinase activation can lead to anti-apoptotic signals, and components of this pathway are frequently amplified or mutated in cancers (Hennessy et al., 2005). In humans, IGF2 is the predominant IGF (LeRoith and Roberts, 2003) and its altered expression is observed in many pathological conditions (Docquier et al., 2005), including colon cancer (Kaneda and Feinberg, 2005). The regulation and expression of *Igf2* is very complex since the gene encoding for IGF2 is found to be imprinted, or expressed monoallelically, and active only on the paternally inherited gene (DeChiara et al., 1991). Loss of imprinting, with resultant over expression of *Igf2*, is a fairly common observation in many cancers [reviewed in (Feinberg et al., 2006)], including colorectal cancer (Kaneda and Feinberg, 2005). Interestingly, transcriptional activation of IGF2 by the sonic hedgehog signalling pathway is also demonstrated in other studies (Ingram et al., 2002). Sonic hedgehog (Shh) along with Indian hedgehog (Ihh) and desert hedgehog (Ihh), make up three homologues of the Hedgehog (Hh) signalling pathway, each with remarkably similar biological properties, differing only in their potency (Shh>Ihh>Dhh). Hedgehog (Hh) signalling is known to inhibit Wnt signalling in the differentiated zone of the colonic crypts and thereby allow differentiation of cells to take place (van den Brink, 2007). Hedgehog (Hh) signalling is also known to restrict expression of Wnt targets to the base of the crypt in vivo (van den Brink et al., 2004), at the putative stem cell niche area, and thereby act as an anti-oncogenic factor towards Wnt signalling. The vast majority of colorectal cancers are sporadic, with approximately 90% harbouring *APC* mutations or other mutations that constitutively activate the Wnt signalling pathway (Gregorieff and Clevers, 2005). Interestingly, Ihh expression is lost very early in the process of colorectal carcinogenesis. Also, loss of Ihh has previously been noted in cases with FAP and in the flat lesions that precede polyp formation in the colon, indicating that loss of Ihh expression can be associated with the earliest

stages of colorectal carcinogenesis, probably as a direct consequence of overactive Wnt stimulation (van den Brink, 2007). From my microarray data, it is evident that IGF2 expression levels are low in the earliest stages of colorectal carcinogenesis; these then peak to a maximum in the Dukes' B tumour expressing lamin A/C before dramatically tapering off in the Dukes' C stage of colorectal cancer. It is difficult to explain this finding but it is apparent that significant events are taking place in the Dukes' B tumour involving the expression of lamin A/C and potentially linking A-type lamins to IGFs. Can the cancer at this stage be undergoing changes that will then prime the stage for metastatic spread? Are these changes indicative of lamin A/C imposing a more differentiated phenotype on the cancer? Alternatively, is this the stage at which cancer cells are interacting with stem cells at the basal regions of the crypt and taking on a more stem cell like phenotype prior to attaining the potential for metastatic spread? An obvious explanation for these observations is lacking but a significant role for A-type lamins in controlling cell growth and differentiation via interacting with IGFs is a distinct possibility and merits further studies.

Fatty acid binding protein 1 (liver) belongs to a group of low molecular weight proteins mostly involved in transport of fatty acids and other lipophilic substances between extracellular and intracellular membranes. Fatty acid binding proteins are known to play an active role in fatty acid mediated signal transduction pathways and regulation of gene expression (Hertzel and Bernlohr, 1998) and consequently its active role in modulating cell division (Sorof, 1994), cell growth and differentiation (Schroeder et al., 2001). Due to their diverse physiological roles, and altered expression in various cancers, it is proposed that fatty acid binding proteins can play a role in tumourigenesis (Jing et al., 2000). Studies by Lawrie *et.al* (Lawrie et al., 2004) describe significantly decreased levels of fatty acid binding protein 1 expression in colorectal cancers, especially at the adenoma stage of tumour development. Their findings are in contrast to my observations. The high expression levels of fatty acid binding protein-1 noted in the Dukes' B stage tumour expressing lamins A/C is intriguing. No definitive explanation can be given for this observation. However one possible theory to explain this finding is that A-type lamins are known markers for differentiation as is FABP 1, and hence their expression predominantly in the differentiated compartment of the colonic

crypts is to be expected. It is feasible to propose that the presence of lamin A/C in colorectal tumours imposes a more differentiated phenotype and thereby preserves the expression of fatty acid binding protein 1 in these tissues.

5.5 Summary

In summary then, I profiled the expression patterns of A-type lamins and their associated binding partners, Emerin and LAP2α, in crypts from the colon and rectum. A unique finding is the expression of lamin A in the putative stem cell niche area. The obvious questions here are 'Does lamin A play a role in maintaining the stem cell niche? Can lamin A/C be a marker for stem cells? What are the underlying mechanisms resulting in this expression?' In an attempt to answer these questions and many more, I identified potential mechanisms that can explain why lamin A/C is expressed in the stem cell niche area and also the intricate interactions of A-type lamins and their binding partners with known signalling mechanism at work in the crypts. A definitive link between Wnt signalling and lamin A/C is theoretically possible through Emerin. In addition, the retinoblastoma – E2F pathway is also potentially implicated in maintaining homeostasis in the crypts and can play a key role in the stem cell niche area.

Looking at the role of A-type lamins in colorectal cancers, it is noted that lamin A/C expression in cancer cells imparts a poor prognosis, irrespective of stage or differentiation status. Further experimental work and microarray analysis yield indicative information as to the potential mechanisms responsible for A-type lamins imparting a poor prognosis in colorectal cancers. Of key interest are the observations that tumours expressing lamin A/C have a more motile and less adherent phenotype due to up regulation of T-plastin and down regulation of E-

cadherin. These tumours also exhibit a general up regulation of genes involved in stem cell differentiation, especially in the Dukes' C stage of the tumour; and a down regulation of genes involved in apoptosis and inhibition of tumour suppressor genes.

Concentrating on the Dukes' B stage tumour, I note significant differences between the nuclear lamin A/C expressing tumours and tumours not expressing lamin A/C. In the tumour sample expressing lamin A/C, there appears to be a general up regulation of genes involved with inhibition of apoptosis, cell cycle progression / proliferation and growth, oncogenes, stem cell differentiation and Wnt signalling pathways. These observations also add further insight as to why lamin A/C expressing tumours can potentially be more aggressive.

In studying individual key genes showing significant change, I note with interest the significant role BMP / TGFβ signalling might play in the group of cancers expressing lamin A/C. Similarly, up regulation of IGF2 in the lamin A/C expressing tumour sample hints towards involvement of the hedgehog signalling pathway. The Wnt signalling pathway is also implicated in the lamin A/C expressing tumour sample. Another unique gene up regulated significantly in the lamin A/C expressing tumour is *CKB*. The product of this gene, CKB protein appeared to hold good promise as a prognostic indicator in colorectal cancers in conjunction with lamin A/C.

An answer to the question of "Is colorectal cancer primarily a stem cell problem or not?" is still not clear. However, from my observations, there appear to be key changes taking place mostly in the Dukes' B group of tumours only, this can potentially indicate that colorectal cancers do adopt a more stem cell like phenotype at this stage and not before, thereby favouring a top down morphogenesis of colorectal cancer. It is stressed at this point that additional samples or pooled samples from multiple patients are needed to carry out further confirmatory studies. My results are based on single samples from individual patients. I will conclude this work by proposing that "Lamin A/C expressing tumours appear to be motile, more invasive and less adherent and adopt a stem cell like phenotype which can impart an adverse outcome in this group of patients".

REFERENCES

(1999). Efficacy of adjuvant fluorouracil and folinic acid in B2 colon cancer. International Multicentre Pooled Analysis of B2 Colon Cancer Trials (IMPACT B2) Investigators. J Clin Oncol *17*, 1356-1363.

Aebi, U., Cohn, J., Buhle, L., and Gerace, L. (1986). The nuclear lamina is a meshwork of intermediate-type filaments. Nature *323*, 560-564.

Ago, H., Kitagawa, Y., Fujishima, A., Matsuura, Y., and Katsube, Y. (1991). Crystal structure of basic fibroblast growth factor at 1.6 A resolution. J Biochem *110*, 360-363.

Agrawal, D., Chen, T., Irby, R., Quackenbush, J., Chambers, A. F., Szabo, M., Cantor, A., Coppola, D., and Yeatman, T. J. (2002). Osteopontin identified as lead marker of colon cancer progression, using pooled sample expression profiling. J Natl Cancer Inst *94*, 513-521.

Ahmed, F. E. (2006). Microarray RNA transcriptional profiling: part I. Platforms, experimental design and standardization. Expert Rev Mol Diagn *6*, 535-550.

Akiyoshi, T., Nakamura, M., Koga, K., Nakashima, H., Yao, T., Tsuneyoshi, M., Tanaka, M., and Katano, M. (2006). Gli1, downregulated in colorectal cancers, inhibits proliferation of colon cancer cells involving Wnt signalling activation. Gut *55*, 991-999.

Alberts, B., . Jhonson, A,. Lewis, J,. Raff, M,. Roberts, K,. Walter, P,. ed. (2002). Molecular Biology Of The Cell, Fourth edn (Garland Sciences).

Al-Hajj, M., Wicha, M. S., Benito-Hernandez, A., Morrison, S. J., and Clarke, M. F. (2003). Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A *100*, 3983-3988.

Allison, D. B., Cui, X., Page, G. P., and Sabripour, M. (2006). Microarray data analysis: from disarray to consolidation and consensus. Nat Rev Genet *7*, 55-65.

Amit, S., Hatzubai, A., Birman, Y., Andersen, J. S., Ben-Shushan, E., Mann, M., Ben-Neriah, Y., and Alkalay, I. (2002). Axin-mediated CKI phosphorylation of beta-catenin at Ser 45: a molecular switch for the Wnt pathway. Genes Dev *16*, 1066-1076.

Androutsellis-Theotokis, A., Leker, R. R., Soldner, F., Hoeppner, D. J., Ravin, R., Poser, S. W., Rueger, M. A., Bae, S. K., Kittappa, R., and McKay, R. D. (2006). Notch signalling regulates stem cell numbers in vitro and in vivo. Nature *442*, 823-826.

Araki, Y., Okamura, S., Hussain, S. P., Nagashima, M., He, P., Shiseki, M., Miura, K., and Harris, C. C. (2003). Regulation of cyclooxygenase-2 expression by the Wnt and ras pathways. Cancer Res *63*, 728-734.

Arenas, J., Diaz, A. E., Alcaide, M. J., Santos, I., Martinez, A., and Culebras, J. M. (1989). Serum CK-BB as a tumor marker in patients with carcinoma confirmed histologically. Clin Chim Acta *182*, 183-193.

Artavanis-Tsakonas, S., Rand, M. D., and Lake, R. J. (1999). Notch signaling: cell fate control and signal integration in development. Science *284*, 770-776.

Ashburner, M., Ball, C. A., Blake, J. A., Botstein, D., Butler, H., Cherry, J. M., Davis, A. P., Dolinski, K., Dwight, S. S., Eppig, J. T., *et al.* (2000). Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet *25*, 25-29.

Autzen, P., Robson, C. N., Bjartell, A., Malcolm, A. J., Johnson, M. I., Neal, D. E., and Hamdy, F. C. (1998). Bone morphogenetic protein 6 in skeletal metastases from prostate cancer and other common human malignancies. Br J Cancer *78*, 1219-1223.

Balasubramani, M., Day, B. W., Schoen, R. E., and Getzenberg, R. H. (2006). Altered expression and localization of creatine kinase B, heterogeneous nuclear ribonucleoprotein F, and high mobility group box 1 protein in the nuclear matrix associated with colon cancer. Cancer Res *66*, 763-769.

Balemans, W., and Van Hul, W. (2002). Extracellular regulation of BMP signaling in vertebrates: a cocktail of modulators. Dev Biol *250*, 231-250.

Barker, N., and Clevers, H. (2007). Tracking down the stem cells of the intestine: strategies to identify adult stem cells. Gastroenterology *133*, 1755-1760.

Barker, N., van Es, J. H., Kuipers, J., Kujala, P., van den Born, M., Cozijnsen, M., Haegebarth, A., Korving, J., Begthel, H., Peters, P. J., and Clevers, H. (2007). Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature *449*, 1003-1007.

Baserga, R., Peruzzi, F., and Reiss, K. (2003). The IGF-1 receptor in cancer biology. Int J Cancer *107*, 873-877.

Batlle, E., Henderson, J. T., Beghtel, H., van den Born, M. M., Sancho, E., Huls, G., Meeldijk, J., Robertson, J., van de Wetering, M., Pawson, T., and Clevers, H. (2002). Beta-catenin and TCF mediate cell positioning in the intestinal epithelium by controlling the expression of EphB/ephrinB. Cell *111*, 251-263.

Beaudouin, J., Gerlich, D., Daigle, N., Eils, R., and Ellenberg, J. (2002). Nuclear envelope breakdown proceeds by microtubule-induced tearing of the lamina. Cell *108*, 83-96.

Bengtsson, L., and Wilson, K. L. (2004). Multiple and surprising new functions for emerin, a nuclear membrane protein. Curr Opin Cell Biol *16*, 73-79.

Berger, R., Theodor, L., Shoham, J., Gokkel, E., Brok-Simoni, F., Avraham, K. B., Copeland, N. G., Jenkins, N. A., Rechavi, G., and Simon, A. J. (1996). The characterization and localization of the mouse thymopoietin/lamina-associated polypeptide 2 gene and its alternatively spliced products. Genome Res *6*, 361-370.

Berman, D. M., Karhadkar, S. S., Maitra, A., Montes De Oca, R., Gerstenblith, M. R., Briggs, K., Parker, A. R., Shimada, Y., Eshleman, J. R., Watkins, D. N., and Beachy, P. A. (2003). Widespread requirement for Hedgehog ligand stimulation in growth of digestive tract tumours. Nature *425*, 846-851.

Berman, J. M., Cheung, R. J., and Weinberg, D. S. (2000). Surveillance after colorectal cancer resection. Lancet *355*, 395-399.

Bhanot, P., Brink, M., Samos, C. H., Hsieh, J. C., Wang, Y., Macke, J. P., Andrew, D., Nathans, J., and Nusse, R. (1996). A new member of the frizzled family from Drosophila functions as a Wingless receptor. Nature *382*, 225-230.

Biamonti, G., Giacca, M., Perini, G., Contreas, G., Zentilin, L., Weighardt, F., Guerra, M., Della Valle, G., Saccone, S., Riva, S., and et al. (1992). The gene for a novel human lamin maps at a highly transcribed locus of chromosome 19 which replicates at the onset of S-phase. Mol Cell Biol *12*, 3499-3506.

Bienz, M. (2002). The subcellular destinations of APC proteins. Nat Rev Mol Cell Biol *3*, 328-338.

Bienz, M., and Clevers, H. (2000). Linking colorectal cancer to Wnt signaling. Cell *103*, 311-320.

Bione, S., Maestrini, E., Rivella, S., Mancini, M., Regis, S., Romeo, G., and Toniolo, D. (1994). Identification of a novel X-linked gene responsible for Emery-Dreifuss muscular dystrophy. Nat Genet *8*, 323-327.

Boland, C. R., Thibodeau, S. N., Hamilton, S. R., Sidransky, D., Eshleman, J. R., Burt, R. W., Meltzer, S. J., Rodriguez-Bigas, M. A., Fodde, R., Ranzani, G. N., and Srivastava, S. (1998). A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res *58*, 5248-5257.

Bonnet, D., and Dick, J. E. (1997). Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med *3*, 730-737.

Bottcher, R. T., and Niehrs, C. (2005). Fibroblast growth factor signaling during early vertebrate development. Endocr Rev *26*, 63-77.

Bouhouche, A., Benomar, A., Birouk, N., Mularoni, A., Meggouh, F., Tassin, J., Grid, D., Vandenberghe, A., Yahyaoui, M., Chkili, T., *et al.* (1999). A locus for an axonal form of autosomal recessive Charcot-Marie-Tooth disease maps to chromosome 1q21.2-q21.3. Am J Hum Genet *65*, 722-727.

Brazma, A., Hingamp, P., Quackenbush, J., Sherlock, G., Spellman, P., Stoeckert, C., Aach, J., Ansorge, W., Ball, C. A., Causton, H. C., *et al.* (2001). Minimum information about a microarray experiment (MIAME)-toward standards for microarray data. Nat Genet *29*, 365-371.

Bresalier, R. S. (2002). Malignant neoplasms of the large intestine. In Sleisenger & Fordtran's gastrointestinal and liver disease: pathophysiology, diagnosis, management., F. L. Feldman M, Sleisenger MH, ed. (Philadelphia, Saunders), pp. 2215-2261.

Bridger, J. M., Kill, I. R., O'Farrell, M., and Hutchison, C. J. (1993). Internal lamin structures within G1 nuclei of human dermal fibroblasts. J Cell Sci *104 (Pt 2)*, 297-306.

Brittan, M., and Wright, N. A. (2004). Stem cell in gastrointestinal structure and neoplastic development. Gut *53*, 899-910.

Brodsky, G. L., Muntoni, F., Miocic, S., Sinagra, G., Sewry, C., and Mestroni, L. (2000). Lamin A/C gene mutation associated with dilated cardiomyopathy with variable skeletal muscle involvement. Circulation *101*, 473-476.

Broers, J. L., and Ramaekers, F. C. (1994). Differentiation markers for lung-cancer sub-types. A comparative study of their expression in vivo and in vitro. Int J Cancer Suppl *8*, 134-137.

Broers, J. L., Raymond, Y., Rot, M. K., Kuijpers, H., Wagenaar, S. S., and Ramaekers, F. C. (1993). Nuclear A-type lamins are differentially expressed in human lung cancer subtypes. Am J Pathol *143*, 211-220.

Bumcrot, D. A., Takada, R., and McMahon, A. P. (1995). Proteolytic processing yields two secreted forms of sonic hedgehog. Mol Cell Biol *15*, 2294-2303.

Burdy, G., Panis, Y., Alves, A., Nemeth, J., Lavergne-Slove, A., and Valleur, P. (2001). Identifying patients with T3-T4 node-negative colon cancer at high risk of recurrence. Dis Colon Rectum *44*, 1682-1688.

Burmer, G. C., and Loeb, L. A. (1989). Mutations in the KRAS2 oncogene during progressive stages of human colon carcinoma. Proc Natl Acad Sci U S A *86*, 2403-2407.

Cai, M., Huang, Y., Ghirlando, R., Wilson, K. L., Craigie, R., and Clore, G. M. (2001). Solution structure of the constant region of nuclear envelope protein LAP2 reveals two LEM-domain structures: one binds BAF and the other binds DNA. Embo J *20*, 4399-4407.

Cance, W. G., Chaudhary, N., Worman, H. J., Blobel, G., and Cordon-Cardo, C. (1992). Expression of the nuclear lamins in normal and neoplastic human tissues. J Exp Clin Cancer Res *11*, 233-246.

Carninci, P., Kasukawa, T., Katayama, S., Gough, J., Frith, M. C., Maeda, N., Oyama, R., Ravasi, T., Lenhard, B., Wells, C., *et al.* (2005). The transcriptional landscape of the mammalian genome. Science *309*, 1559-1563.

Carpenter, D., Stone, D. M., Brush, J., Ryan, A., Armanini, M., Frantz, G., Rosenthal, A., and de Sauvage, F. J. (1998). Characterization of two patched receptors for the vertebrate hedgehog protein family. Proc Natl Acad Sci U S A *95*, 13630-13634.

Cartegni, L., di Barletta, M. R., Barresi, R., Squarzoni, S., Sabatelli, P., Maraldi, N., Mora, M., Di Blasi, C., Cornelio, F., Merlini, L., *et al.* (1997). Heart-specific localization of emerin: new insights into Emery-Dreifuss muscular dystrophy. Hum Mol Genet *6*, 2257-2264.

Cavallo, R. A., Cox, R. T., Moline, M. M., Roose, J., Polevoy, G. A., Clevers, H., Peifer, M., and Bejsovec, A. (1998). Drosophila Tcf and Groucho interact to repress Wingless signalling activity. Nature *395*, 604-608.

Cawkwell, L., Gray, S., Murgatroyd, H., Sutherland, F., Haine, L., Longfellow, M., O'Loughlin, S., Cross, D., Kronborg, O., Fenger, C., *et al.* (1999). Choice of management

strategy for colorectal cancer based on a diagnostic immunohistochemical test for defective mismatch repair. Gut *45*, 409-415.

Chambers, A. F., Wilson, S. M., Kerkvliet, N., O'Malley, F. P., Harris, J. F., and Casson, A. G. (1996). Osteopontin expression in lung cancer. Lung Cancer *15*, 311-323.

Chamorro, M. N., Schwartz, D. R., Vonica, A., Brivanlou, A. H., Cho, K. R., and Varmus, H. E. (2005). FGF-20 and DKK1 are transcriptional targets of beta-catenin and FGF-20 is implicated in cancer and development. Embo J *24*, 73-84.

Chao, W., and D'Amore, P. A. (2008). IGF2: epigenetic regulation and role in development and disease. Cytokine Growth Factor Rev *19*, 111-120.

Chen, D., Zhao, M., and Mundy, G. R. (2004). Bone morphogenetic proteins. Growth Factors *22*, 233-241.

Chen, L., Lee, L., Kudlow, B. A., Dos Santos, H. G., Sletvold, O., Shafeghati, Y., Botha, E. G., Garg, A., Hanson, N. B., Martin, G. M., *et al.* (2003). LMNA mutations in atypical Werner's syndrome. Lancet *362*, 440-445.

Cheng, L., and Lai, M. D. (2003). Aberrant crypt foci as microscopic precursors of colorectal cancer. World J Gastroenterol *9*, 2642-2649.

Christen, B., and Slack, J. M. (1999). Spatial response to fibroblast growth factor signalling in Xenopus embryos. Development *126*, 119-125.

Chuang, P. T., Kawcak, T., and McMahon, A. P. (2003). Feedback control of mammalian Hedgehog signaling by the Hedgehog-binding protein, Hip1, modulates Fgf signaling during branching morphogenesis of the lung. Genes Dev *17*, 342-347.

Chuang, P. T., and McMahon, A. P. (1999). Vertebrate Hedgehog signalling modulated by induction of a Hedgehog-binding protein. Nature *397*, 617-621.

Classon, M., Kennedy, B. K., Mulloy, R., and Harlow, E. (2000). Opposing roles of pRB and p107 in adipocyte differentiation. Proc Natl Acad Sci U S A *97*, 10826-10831.

Clements, L., Manilal, S., Love, D. R., and Morris, G. E. (2000). Direct interaction between emerin and lamin A. Biochem Biophys Res Commun *267*, 709-714.

Coates, P. J., Hobbs, R. C., Crocker, J., Rowlands, D. C., Murray, P., Quinlan, R., and Hall, P. A. (1996). Identification of the antigen recognized by the monoclonal antibody BU31 as lamins A and C. J Pathol *178*, 21-29.

Cohen, M., Lee, K. K., Wilson, K. L., and Gruenbaum, Y. (2001). Transcriptional repression, apoptosis, human disease and the functional evolution of the nuclear lamina. Trends Biochem Sci *26*, 41-47.

Collard, J. F., Senecal, J. L., and Raymond, Y. (1992). Redistribution of nuclear lamin A is an early event associated with differentiation of human promyelocytic leukemia HL-60 cells. J Cell Sci *101 (Pt 3)*, 657-670.

Compton, C., Fenoglio-Preiser, C. M., Pettigrew, N., and Fielding, L. P. (2000a). American Joint Committee on Cancer Prognostic Factors Consensus Conference: Colorectal Working Group. Cancer *88*, 1739-1757.

Compton, C. C. (2003). Colorectal carcinoma: diagnostic, prognostic, and molecular features. Mod Pathol *16*, 376-388.

Compton, C. C., Fielding, L. P., Burgart, L. J., Conley, B., Cooper, H. S., Hamilton, S. R., Hammond, M. E., Henson, D. E., Hutter, R. V., Nagle, R. B., *et al.* (2000b). Prognostic factors in colorectal cancer. College of American Pathologists Consensus Statement 1999. Arch Pathol Lab Med *124*, 979-994.

Corrigan, D. P., Kuszczak, D., Rusinol, A. E., Thewke, D. P., Hrycyna, C. A., Michaelis, S., and Sinensky, M. S. (2005). Prelamin A endoproteolytic processing in vitro by recombinant Zmpste24. Biochem J *387*, 129-138.

Craig, A. M., Bowden, G. T., Chambers, A. F., Spearman, M. A., Greenberg, A. H., Wright, J. A., McLeod, M., and Denhardt, D. T. (1990). Secreted phosphoprotein mRNA is induced during multi-stage carcinogenesis in mouse skin and correlates with the metastatic potential of murine fibroblasts. Int J Cancer *46*, 133-137.

Cronauer, M. V., Schulz, W. A., Seifert, H. H., Ackermann, R., and Burchardt, M. (2003). Fibroblast growth factors and their receptors in urological cancers: basic research and clinical implications. Eur Urol *43*, 309-319.

Cross, T., Griffiths, G., Deacon, E., Sallis, R., Gough, M., Watters, D., and Lord, J. M. (2000). PKC-delta is an apoptotic lamin kinase. Oncogene *19*, 2331-2337.

Cui, X. Y., Hu, Q. D., Tekaya, M., Shimoda, Y., Ang, B. T., Nie, D. Y., Sun, L., Hu, W. P., Karsak, M., Duka, T., *et al.* (2004). NB-3/Notch1 pathway via Deltex1 promotes neural progenitor cell differentiation into oligodendrocytes. J Biol Chem *279*, 25858-25865.

Dabauvalle, M. C., Muller, E., Ewald, A., Kress, W., Krohne, G., and Muller, C. R. (1999). Distribution of emerin during the cell cycle. Eur J Cell Biol *78*, 749-756.

Dahlquist, K. D., Salomonis, N., Vranizan, K., Lawlor, S. C., and Conklin, B. R. (2002). GenMAPP, a new tool for viewing and analyzing microarray data on biological pathways. Nat Genet *31*, 19-20.

Daigle, N., Beaudouin, J., Hartnell, L., Imreh, G., Hallberg, E., Lippincott-Schwartz, J., and Ellenberg, J. (2001). Nuclear pore complexes form immobile networks and have a very low turnover in live mammalian cells. J Cell Biol *154*, 71-84.

Dalerba, P., Cho, R. W., and Clarke, M. F. (2007a). Cancer stem cells: models and concepts. Annu Rev Med *58*, 267-284.

Dalerba, P., Dylla, S. J., Park, I. K., Liu, R., Wang, X., Cho, R. W., Hoey, T., Gurney, A., Huang, E. H., Simeone, D. M., *et al.* (2007b). Phenotypic characterization of human colorectal cancer stem cells. Proc Natl Acad Sci U S A *104*, 10158-10163.

Dalerba, P., Guiducci, C., Poliani, P. L., Cifola, I., Parenza, M., Frattini, M., Gallino, G., Carnevali, I., Di Giulio, I., Andreola, S., *et al.* (2005). Reconstitution of human telomerase

reverse transcriptase expression rescues colorectal carcinoma cells from in vitro senescence: evidence against immortality as a constitutive trait of tumor cells. Cancer Res *65*, 2321-2329.

Davis, L. I., and Blobel, G. (1986). Identification and characterization of a nuclear pore complex protein. Cell *45*, 699-709.

de Santa Barbara, P., van den Brink, G. R., and Roberts, D. J. (2003). Development and differentiation of the intestinal epithelium. Cell Mol Life Sci *60*, 1322-1332.

Dechat, T., Gajewski, A., Korbei, B., Gerlich, D., Daigle, N., Haraguchi, T., Furukawa, K., Ellenberg, J., and Foisner, R. (2004). LAP2alpha and BAF transiently localize to telomeres and specific regions on chromatin during nuclear assembly. J Cell Sci *117*, 6117-6128.

Dechat, T., Gotzmann, J., Stockinger, A., Harris, C. A., Talle, M. A., Siekierka, J. J., and Foisner, R. (1998). Detergent-salt resistance of LAP2alpha in interphase nuclei and phosphorylation-dependent association with chromosomes early in nuclear assembly implies functions in nuclear structure dynamics. Embo J *17*, 4887-4902.

Dechat, T., Korbei, B., Vaughan, O. A., Vlcek, S., Hutchison, C. J., and Foisner, R. (2000a). Lamina-associated polypeptide 2alpha binds intranuclear A-type lamins. J Cell Sci *113 Pt 19*, 3473-3484.

Dechat, T., Vlcek, S., and Foisner, R. (2000b). Review: lamina-associated polypeptide 2 isoforms and related proteins in cell cycle-dependent nuclear structure dynamics. J Struct Biol *129*, 335-345.

DeChiara, T. M., Robertson, E. J., and Efstratiadis, A. (1991). Parental imprinting of the mouse insulin-like growth factor II gene. Cell *64*, 849-859.

Delanote, V., Vandekerckhove, J., and Gettemans, J. (2005). Plastins: versatile modulators of actin organization in (patho)physiological cellular processes. Acta Pharmacol Sin *26*, 769-779.

Deng, G., Chen, A., Hong, J., Chae, H. S., and Kim, Y. S. (1999). Methylation of CpG in a small region of the hMLH1 promoter invariably correlates with the absence of gene expression. Cancer Res *59*, 2029-2033.

Deng, H., Makizumi, R., Ravikumar, T. S., Dong, H., Yang, W., and Yang, W. L. (2007). Bone morphogenetic protein-4 is overexpressed in colonic adenocarcinomas and promotes migration and invasion of HCT116 cells. Exp Cell Res *313*, 1033-1044.

Dennis, G., Jr., Sherman, B. T., Hosack, D. A., Yang, J., Gao, W., Lane, H. C., and Lempicki, R. A. (2003). DAVID: Database for Annotation, Visualization, and Integrated Discovery. Genome Biol *4*, P3.

Dessev, G., Iovcheva-Dessev, C., Bischoff, J. R., Beach, D., and Goldman, R. (1991). A complex containing p34cdc2 and cyclin B phosphorylates the nuclear lamin and disassembles nuclei of clam oocytes in vitro. J Cell Biol *112*, 523-533.

Di Fabio, F., Nascimbeni, R., Villanacci, V., Baronchelli, C., Bianchi, D., Fabbretti, G., Casella, C., and Salerni, B. (2004). Prognostic variables for cancer-related survival in node-negative colorectal carcinomas. Dig Surg *21*, 128-133.

Dickson, C., Spencer-Dene, B., Dillon, C., and Fantl, V. (2000). Tyrosine kinase signalling in breast cancer: fibroblast growth factors and their receptors. Breast Cancer Res *2*, 191-196.

Ding, Q., Stewart, J., Jr., Prince, C. W., Chang, P. L., Trikha, M., Han, X., Grammer, J. R., and Gladson, C. L. (2002). Promotion of malignant astrocytoma cell migration by osteopontin expressed in the normal brain: differences in integrin signaling during cell adhesion to osteopontin versus vitronectin. Cancer Res *62*, 5336-5343.

Docquier, F., Farrar, D., D'Arcy, V., Chernukhin, I., Robinson, A. F., Loukinov, D., Vatolin, S., Pack, S., Mackay, A., Harris, R. A., *et al.* (2005). Heightened expression of CTCF in breast cancer cells is associated with resistance to apoptosis. Cancer Res *65*, 5112-5122.

Dominguez-Sola, D., Ying, C. Y., Grandori, C., Ruggiero, L., Chen, B., Li, M., Galloway, D. A., Gu, W., Gautier, J., and Dalla-Favera, R. (2007). Non-transcriptional control of DNA replication by c-Myc. Nature *448*, 445-451.

Doniger, S. W., Salomonis, N., Dahlquist, K. D., Vranizan, K., Lawlor, S. C., and Conklin, B. R. (2003). MAPPFinder: using Gene Ontology and GenMAPP to create a global gene-expression profile from microarray data. Genome Biol *4*, R7.

Dorner, D., Gotzmann, J., and Foisner, R. (2007). Nucleoplasmic lamins and their interaction partners, LAP2alpha, Rb, and BAF, in transcriptional regulation. Febs J *274*, 1362-1373.

Dorner, D., Vlcek, S., Foeger, N., Gajewski, A., Makolm, C., Gotzmann, J., Hutchison, C. J., and Foisner, R. (2006). Lamina-associated polypeptide 2alpha regulates cell cycle progression and differentiation via the retinoblastoma-E2F pathway. J Cell Biol *173*, 83-93.

Dreifuss, F. E., and Hogan, G. R. (1961). Survival in x-chromosomal muscular dystrophy. Neurology *11*, 734-737.

Dronamraju, S. S., Coxhead, J. M., Kelly, S. B., and Mathers, J. C. (2008). Role of DNA-mismatch repair in anti-neoplastic effects of butyrate. Proc Nutr Soc *67*, E87.

Dukes, C. E. (1932). The classification of cancer of the rectum. Journal of Pathology and Bacteriology *35*, 323-332.

Dunnigan, M. G., Cochrane, M. A., Kelly, A., and Scott, J. W. (1974). Familial lipoatrophic diabetes with dominant transmission. A new syndrome. Q J Med *43*, 33-48.

Dyer, J. A., Kill, I. R., Pugh, G., Quinlan, R. A., Lane, E. B., and Hutchison, C. J. (1997). Cell cycle changes in A-type lamin associations detected in human dermal fibroblasts using monoclonal antibodies. Chromosome Res *5*, 383-394.

Echelard, Y., Epstein, D. J., St-Jacques, B., Shen, L., Mohler, J., McMahon, J. A., and McMahon, A. P. (1993). Sonic hedgehog, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity. Cell *75*, 1417-1430.

Eiraku, M., Hirata, Y., Takeshima, H., Hirano, T., and Kengaku, M. (2002). Delta/notch-like epidermal growth factor (EGF)-related receptor, a novel EGF-like repeat-containing protein targeted to dendrites of developing and adult central nervous system neurons. J Biol Chem *277*, 25400-25407.

Emery, A. E., and Dreifuss, F. E. (1966). Unusual type of benign x-linked muscular dystrophy. J Neurol Neurosurg Psychiatry *29*, 338-342.

Erber, A., Riemer, D., Bovenschulte, M., and Weber, K. (1998). Molecular phylogeny of metazoan intermediate filament proteins. J Mol Evol *47*, 751-762.

Eriksson, M., Brown, W. T., Gordon, L. B., Glynn, M. W., Singer, J., Scott, L., Erdos, M. R., Robbins, C. M., Moses, T. Y., Berglund, P., *et al.* (2003). Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. Nature *423*, 293-298.

Farnsworth, C. C., Wolda, S. L., Gelb, M. H., and Glomset, J. A. (1989). Human lamin B contains a farnesylated cysteine residue. J Biol Chem *264*, 20422-20429.

Fatkin, D., MacRae, C., Sasaki, T., Wolff, M. R., Porcu, M., Frenneaux, M., Atherton, J., Vidaillet, H. J., Jr., Spudich, S., De Girolami, U., *et al.* (1999). Missense mutations in the rod domain of the lamin A/C gene as causes of dilated cardiomyopathy and conduction-system disease. N Engl J Med *341*, 1715-1724.

Fearon, E. R., and Vogelstein, B. (1990). A genetic model for colorectal tumorigenesis. Cell *61*, 759-767.

Feinberg, A. P., Ohlsson, R., and Henikoff, S. (2006). The epigenetic progenitor origin of human cancer. Nat Rev Genet *7*, 21-33.

Firmbach-Kraft, I., and Stick, R. (1993). The role of CaaX-dependent modifications in membrane association of Xenopus nuclear lamin B3 during meiosis and the fate of B3 in transfected mitotic cells. J Cell Biol *123*, 1661-1670.

Fisher, D. Z., Chaudhary, N., and Blobel, G. (1986). cDNA sequencing of nuclear lamins A and C reveals primary and secondary structural homology to intermediate filament proteins. Proc Natl Acad Sci U S A *83*, 6450-6454.

Fleming, I. D., Phillips, J. L., Menck, H. R., Murphy, G. P., and Winchester, D. P. (1997). The National Cancer Data Base report on recent hospital cancer program progress toward complete American Joint Committee on Cancer/TNM staging. Cancer *80*, 2305-2310.

Foisner, R., and Gerace, L. (1993). Integral membrane proteins of the nuclear envelope interact with lamins and chromosomes, and binding is modulated by mitotic phosphorylation. Cell *73*, 1267-1279.

Franke, W. W., Scheer, U., Krohne, G., and Jarasch, E. D. (1981). The nuclear envelope and the architecture of the nuclear periphery. J Cell Biol *91*, 39s-50s.

Fridman, J. S., and Lowe, S. W. (2003). Control of apoptosis by p53. Oncogene *22*, 9030-9040.

Friedman, D. B., Hill, S., Keller, J. W., Merchant, N. B., Levy, S. E., Coffey, R. J., and Caprioli, R. M. (2004). Proteome analysis of human colon cancer by two-dimensional difference gel electrophoresis and mass spectrometry. Proteomics *4*, 793-811.

Frisen, J., Holmberg, J., and Barbacid, M. (1999). Ephrins and their Eph receptors: multitalented directors of embryonic development. Embo J *18*, 5159-5165.

Frolov, M. V., and Dyson, N. J. (2004). Molecular mechanisms of E2F-dependent activation and pRB-mediated repression. J Cell Sci *117*, 2173-2181.

Fuchs, E., and Weber, K. (1994). Intermediate filaments: structure, dynamics, function, and disease. Annu Rev Biochem *63*, 345-382.

Fujita, S., Nakanisi, Y., Taniguchi, H., Yamamoto, S., Akasu, T., Moriya, Y., and Shimoda, T. (2007). Cancer invasion to Auerbach's plexus is an important prognostic factor in patients with pT3-pT4 colorectal cancer. Dis Colon Rectum *50*, 1860-1866.

Furukawa, K. (1999). LAP2 binding protein 1 (L2BP1/BAF) is a candidate mediator of LAP2-chromatin interaction. J Cell Sci *112 (Pt 15)*, 2485-2492.

Furukawa, K., and Hotta, Y. (1993). cDNA cloning of a germ cell specific lamin B3 from mouse spermatocytes and analysis of its function by ectopic expression in somatic cells. Embo J *12*, 97-106.

Furukawa, K., Inagaki, H., and Hotta, Y. (1994). Identification and cloning of an mRNA coding for a germ cell-specific A-type lamin in mice. Exp Cell Res *212*, 426-430.

Galli, R., Binda, E., Orfanelli, U., Cipelletti, B., Gritti, A., De Vitis, S., Fiocco, R., Foroni, C., Dimeco, F., and Vescovi, A. (2004). Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. Cancer Res *64*, 7011-7021.

Gearhart, J., Pashos, E. E., and Prasad, M. K. (2007). Pluripotency redux--advances in stem-cell research. N Engl J Med *357*, 1469-1472.

Georgatos, S. D., Meier, J., and Simos, G. (1994). Lamins and lamin-associated proteins. Curr Opin Cell Biol *6*, 347-353.

Gerace, L., and Blobel, G. (1980). The nuclear envelope lamina is reversibly depolymerized during mitosis. Cell *19*, 277-287.

Gerace, L., and Burke, B. (1988). Functional organization of the nuclear envelope. Annu Rev Cell Biol *4*, 335-374.

Gerace, L., Comeau, C., and Benson, M. (1984). Organization and modulation of nuclear lamina structure. J Cell Sci Suppl *1*, 137-160.

Gerace, L., and Foisner, R. (1994). Integral membrane proteins and dynamic organization of the nuclear envelope. Trends Cell Biol *4*, 127-131.

Gilboa, L., Nohe, A., Geissendorfer, T., Sebald, W., Henis, Y. I., and Knaus, P. (2000). Bone morphogenetic protein receptor complexes on the surface of live cells: a new oligomerization mode for serine/threonine kinase receptors. Mol Biol Cell *11*, 1023-1035.

Goldberg, M., Harel, A., and Gruenbaum, Y. (1999). The nuclear lamina: molecular organization and interaction with chromatin. Crit Rev Eukaryot Gene Expr *9*, 285-293.

Goldman, R. D., Shumaker, D. K., Erdos, M. R., Eriksson, M., Goldman, A. E., Gordon, L. B., Gruenbaum, Y., Khuon, S., Mendez, M., Varga, R., and Collins, F. S. (2004). Accumulation of mutant lamin A causes progressive changes in nuclear architecture in Hutchinson-Gilford progeria syndrome. Proc Natl Acad Sci U S A *101*, 8963-8968.

Gotzmann, J., and Foisner, R. (2006). A-type lamin complexes and regenerative potential: a step towards understanding laminopathic diseases? Histochem Cell Biol *125*, 33-41.

Grady, W. M. (2004). Genomic instability and colon cancer. Cancer Metastasis Rev *23*, 11-27.

Grady, W. M., Rajput, A., Myeroff, L., Liu, D. F., Kwon, K., Willis, J., and Markowitz, S. (1998). Mutation of the type II transforming growth factor-beta receptor is coincident with the transformation of human colon adenomas to malignant carcinomas. Cancer Res *58*, 3101-3104.

Greene, F. L., and Sobin, L. H. (2002). The TNM system: our language for cancer care. J Surg Oncol *80*, 119-120.

Gregorieff, A., and Clevers, H. (2005). Wnt signaling in the intestinal epithelium: from endoderm to cancer. Genes Dev *19*, 877-890.

Groden, J., Thliveris, A., Samowitz, W., Carlson, M., Gelbert, L., Albertsen, H., Joslyn, G., Stevens, J., Spirio, L., Robertson, M., and et al. (1991). Identification and characterization of the familial adenomatous polyposis coli gene. Cell *66*, 589-600.

Gruenbaum, Y., Lee, K. K., Liu, J., Cohen, M., and Wilson, K. L. (2002). The expression, lamin-dependent localization and RNAi depletion phenotype for emerin in C. elegans. J Cell Sci *115*, 923-929.

Gupta, R. A., Wang, D., Katkuri, S., Wang, H., Dey, S. K., and DuBois, R. N. (2004). Activation of nuclear hormone receptor peroxisome proliferator-activated receptor-delta accelerates intestinal adenoma growth. Nat Med *10*, 245-247.

Hamdy, F. C., Autzen, P., Robinson, M. C., Horne, C. H., Neal, D. E., and Robson, C. N. (1997). Immunolocalization and messenger RNA expression of bone morphogenetic protein-6 in human benign and malignant prostatic tissue. Cancer Res *57*, 4427-4431.

Hansen, J. B., Jorgensen, C., Petersen, R. K., Hallenborg, P., De Matteis, R., Boye, H. A., Petrovic, N., Enerback, S., Nedergaard, J., Cinti, S., *et al.* (2004). Retinoblastoma protein functions as a molecular switch determining white versus brown adipocyte differentiation. Proc Natl Acad Sci U S A *101*, 4112-4117.

Haramis, A. P., Begthel, H., van den Born, M., van Es, J., Jonkheer, S., Offerhaus, G. J., and Clevers, H. (2004). De novo crypt formation and juvenile polyposis on BMP inhibition in mouse intestine. Science *303*, 1684-1686.

Harborth, J., Elbashir, S. M., Bechert, K., Tuschl, T., and Weber, K. (2001). Identification of essential genes in cultured mammalian cells using small interfering RNAs. J Cell Sci *114*, 4557-4565.

Hardcastle, J. D., Chamberlain, J. O., Robinson, M. H., Moss, S. M., Amar, S. S., Balfour, T. W., James, P. D., and Mangham, C. M. (1996). Randomised controlled trial of faecal-occult-blood screening for colorectal cancer. Lancet *348*, 1472-1477.

Hardwick, J. C., Van Den Brink, G. R., Bleuming, S. A., Ballester, I., Van Den Brande, J. M., Keller, J. J., Offerhaus, G. J., Van Deventer, S. J., and Peppelenbosch, M. P. (2004). Bone morphogenetic protein 2 is expressed by, and acts upon, mature epithelial cells in the colon. Gastroenterology *126*, 111-121.

Harman, F. S., Nicol, C. J., Marin, H. E., Ward, J. M., Gonzalez, F. J., and Peters, J. M. (2004). Peroxisome proliferator-activated receptor-delta attenuates colon carcinogenesis. Nat Med *10*, 481-483.

Harris, C. A., Andryuk, P. J., Cline, S. W., Mathew, S., Siekierka, J. J., and Goldstein, G. (1995). Structure and mapping of the human thymopoietin (TMPO) gene and relationship of human TMPO beta to rat lamin-associated polypeptide 2. Genomics *28*, 198-205.

Hart, M., Concordet, J. P., Lassot, I., Albert, I., del los Santos, R., Durand, H., Perret, C., Rubinfeld, B., Margottin, F., Benarous, R., and Polakis, P. (1999). The F-box protein beta-TrCP associates with phosphorylated beta-catenin and regulates its activity in the cell. Curr Biol *9*, 207-210.

He, T. C., Sparks, A. B., Rago, C., Hermeking, H., Zawel, L., da Costa, L. T., Morin, P. J., Vogelstein, B., and Kinzler, K. W. (1998). Identification of c-MYC as a target of the APC pathway. Science *281*, 1509-1512.

Heitlinger, E., Peter, M., Lustig, A., Villiger, W., Nigg, E. A., and Aebi, U. (1992). The role of the head and tail domain in lamin structure and assembly: analysis of bacterially expressed chicken lamin A and truncated B2 lamins. J Struct Biol *108*, 74-89.

Hempen, P. M., Zhang, L., Bansal, R. K., Iacobuzio-Donahue, C. A., Murphy, K. M., Maitra, A., Vogelstein, B., Whitehead, R. H., Markowitz, S. D., Willson, J. K., *et al.* (2003). Evidence of selection for clones having genetic inactivation of the activin A type II receptor (ACVR2) gene in gastrointestinal cancers. Cancer Res *63*, 994-999.

Hennessy, B. T., Smith, D. L., Ram, P. T., Lu, Y., and Mills, G. B. (2005). Exploiting the PI3K/AKT pathway for cancer drug discovery. Nat Rev Drug Discov *4*, 988-1004.

Herrera-Ornelas, L., Justiniano, J., Castillo, N., Petrelli, N. J., Stulc, J. P., and Mittelman, A. (1987). Metastases in small lymph nodes from colon cancer. Arch Surg *122*, 1253-1256.

Hertzel, A. V., and Bernlohr, D. A. (1998). Regulation of adipocyte gene expression by polyunsaturated fatty acids. Mol Cell Biochem *188*, 33-39.

Hinds, P. W., Mittnacht, S., Dulic, V., Arnold, A., Reed, S. I., and Weinberg, R. A. (1992). Regulation of retinoblastoma protein functions by ectopic expression of human cyclins. Cell *70*, 993-1006.

Hirama, M., Takahashi, F., Takahashi, K., Akutagawa, S., Shimizu, K., Soma, S., Shimanuki, Y., Nishio, K., and Fukuchi, Y. (2003). Osteopontin overproduced by tumor cells acts as a potent angiogenic factor contributing to tumor growth. Cancer Lett *198*, 107-117.

Hoger, T. H., Krohne, G., and Kleinschmidt, J. A. (1991). Interaction of Xenopus lamins A and LII with chromatin in vitro mediated by a sequence element in the carboxyterminal domain. Exp Cell Res *197*, 280-289.

Holaska, J. M., Lee, K. K., Kowalski, A. K., and Wilson, K. L. (2003). Transcriptional repressor germ cell-less (GCL) and barrier to autointegration factor (BAF) compete for binding to emerin in vitro. J Biol Chem *278*, 6969-6975.

Holmberg, J., Genander, M., Halford, M. M., Anneren, C., Sondell, M., Chumley, M. J., Silvany, R. E., Henkemeyer, M., and Frisen, J. (2006). EphB receptors coordinate migration and proliferation in the intestinal stem cell niche. Cell *125*, 1151-1163.

Hooper, J. E., and Scott, M. P. (2005). Communicating with Hedgehogs. Nat Rev Mol Cell Biol *6*, 306-317.

Horgan, P. G., and McMillan, D. C. (2011). Surgeons and selection of adjuvant therapy for node-negative colonic cancer. Br J Surg *97*, 1459-1460.

Howe, J. R., Bair, J. L., Sayed, M. G., Anderson, M. E., Mitros, F. A., Petersen, G. M., Velculescu, V. E., Traverso, G., and Vogelstein, B. (2001). Germline mutations of the gene encoding bone morphogenetic protein receptor 1A in juvenile polyposis. Nat Genet *28*, 184-187.

Howe, J. R., Roth, S., Ringold, J. C., Summers, R. W., Jarvinen, H. J., Sistonen, P., Tomlinson, I. P., Houlston, R. S., Bevan, S., Mitros, F. A., *et al.* (1998). Mutations in the SMAD4/DPC4 gene in juvenile polyposis. Science *280*, 1086-1088.

Hu, Q. D., Ang, B. T., Karsak, M., Hu, W. P., Cui, X. Y., Duka, T., Takeda, Y., Chia, W., Sankar, N., Ng, Y. K., *et al.* (2003). F3/contactin acts as a functional ligand for Notch during oligodendrocyte maturation. Cell *115*, 163-175.

Huber, W., von Heydebreck, A., Sultmann, H., Poustka, A., and Vingron, M. (2002). Variance stabilization applied to microarray data calibration and to the quantification of differential expression. Bioinformatics *18 Suppl 1*, S96-104.

Huh, J. W., Kim, H. R., and Kim, Y. J. Prognostic Value of Perineural Invasion in Patients with Stage II Colorectal Cancer. Ann Surg Oncol.

Huh, M. S., Parker, M. H., Scime, A., Parks, R., and Rudnicki, M. A. (2004). Rb is required for progression through myogenic differentiation but not maintenance of terminal differentiation. J Cell Biol *166*, 865-876.

Hulit, J., Wang, C., Li, Z., Albanese, C., Rao, M., Di Vizio, D., Shah, S., Byers, S. W., Mahmood, R., Augenlicht, L. H., *et al.* (2004). Cyclin D1 genetic heterozygosity regulates colonic epithelial cell differentiation and tumor number in ApcMin mice. Mol Cell Biol *24*, 7598-7611.

Hutchison, C. J. (2002). Lamins: building blocks or regulators of gene expression? Nat Rev Mol Cell Biol *3*, 848-858.

Hutchison, C. J., Alvarez-Reyes, M., and Vaughan, O. A. (2001). Lamins in disease: why do ubiquitously expressed nuclear envelope proteins give rise to tissue-specific disease phenotypes? J Cell Sci *114*, 9-19.

Hutchison, C. J., and Worman, H. J. (2004). A-type lamins: guardians of the soma? Nat Cell Biol *6*, 1062-1067.

Imai, S., Nishibayashi, S., Takao, K., Tomifuji, M., Fujino, T., Hasegawa, M., and Takano, T. (1997). Dissociation of Oct-1 from the nuclear peripheral structure induces the cellular aging-associated collagenase gene expression. Mol Biol Cell *8*, 2407-2419.

Ingram, W. J., Wicking, C. A., Grimmond, S. M., Forrest, A. R., and Wainwright, B. J. (2002). Novel genes regulated by Sonic Hedgehog in pluripotent mesenchymal cells. Oncogene *21*, 8196-8205.

Irby, R. B., McCarthy, S. M., and Yeatman, T. J. (2004). Osteopontin regulates multiple functions contributing to human colon cancer development and progression. Clin Exp Metastasis *21*, 515-523.

Iso, T., Kedes, L., and Hamamori, Y. (2003). HES and HERP families: multiple effectors of the Notch signaling pathway. J Cell Physiol *194*, 237-255.

Jenkins, H., Holman, T., Lyon, C., Lane, B., Stick, R., and Hutchison, C. (1993). Nuclei that lack a lamina accumulate karyophilic proteins and assemble a nuclear matrix. J Cell Sci *106 (Pt 1)*, 275-285.

Jenkins, H., Whitfield, W. G., Goldberg, M. W., Allen, T. D., and Hutchison, C. J. (1995). Evidence for the direct involvement of lamins in the assembly of a replication competent nucleus. Acta Biochim Pol *42*, 133-143.

Jing, C., Beesley, C., Foster, C. S., Rudland, P. S., Fujii, H., Ono, T., Chen, H., Smith, P. H., and Ke, Y. (2000). Identification of the messenger RNA for human cutaneous fatty acid-binding protein as a metastasis inducer. Cancer Res *60*, 2390-2398.

Jo, W. S., and Chung, D. C. (2005). Genetics of hereditary colorectal cancer. Semin Oncol *32*, 11-23.

Johnson, B. R., Nitta, R. T., Frock, R. L., Mounkes, L., Barbie, D. A., Stewart, C. L., Harlow, E., and Kennedy, B. K. (2004). A-type lamins regulate retinoblastoma protein function by promoting subnuclear localization and preventing proteasomal degradation. Proc Natl Acad Sci U S A *101*, 9677-9682.

Johnson, D. E., and Williams, L. T. (1993). Structural and functional diversity in the FGF receptor multigene family. Adv Cancer Res *60*, 1-41.

Jordan, C. T., Guzman, M. L., and Noble, M. (2006). Cancer stem cells. N Engl J Med *355*, 1253-1261.

Joseph, J., Cardesa, A., and Carreras, J. (1997). Creatine kinase activity and isoenzymes in lung, colon and liver carcinomas. Br J Cancer *76*, 600-605.

Jung, B., Doctolero, R. T., Tajima, A., Nguyen, A. K., Keku, T., Sandler, R. S., and Carethers, J. M. (2004). Loss of activin receptor type 2 protein expression in microsatellite unstable colon cancers. Gastroenterology *126*, 654-659.

Kanazawa, S., Soucek, L., Evan, G., Okamoto, T., and Peterlin, B. M. (2003). c-Myc recruits P-TEFb for transcription, cellular proliferation and apoptosis. Oncogene *22*, 5707-5711.

Kaneda, A., and Feinberg, A. P. (2005). Loss of imprinting of IGF2: a common epigenetic modifier of intestinal tumor risk. Cancer Res *65*, 11236-11240.

Kanehisa, M., Goto, S., Kawashima, S., Okuno, Y., and Hattori, M. (2004). The KEGG resource for deciphering the genome. Nucleic Acids Res *32*, D277-280.

Kasof, G. M., Goyal, L., and White, E. (1999). Btf, a novel death-promoting transcriptional repressor that interacts with Bcl-2-related proteins. Mol Cell Biol *19*, 4390-4404.

Katoh, M. (2004). Identification and characterization of human HES2, HES3, and HES5 genes in silico. Int J Oncol *25*, 529-534.

Katoh, M. (2005a). Comparative genomics on FGF8, FGF17, and FGF18 orthologs. Int J Mol Med *16*, 493-496.

Katoh, M. (2005b). Comparative genomics on FGF20 orthologs. Oncol Rep *14*, 287-290.

Katoh, M. (2006a). Cross-talk of WNT and FGF signaling pathways at GSK3beta to regulate beta-catenin and SNAIL signaling cascades. Cancer Biol Ther *5*, 1059-1064.

Katoh, M. (2006b). Notch ligand, JAG1, is evolutionarily conserved target of canonical WNT signaling pathway in progenitor cells. Int J Mol Med *17*, 681-685.

Katoh, M. (2007a). Notch signaling in gastrointestinal tract (review). Int J Oncol *30*, 247-251.

Katoh, M. (2007b). WNT antagonist, DKK2, is a Notch signaling target in intestinal stem cells: augmentation of a negative regulation system for canonical WNT signaling pathway by the Notch-DKK2 signaling loop in primates. Int J Mol Med *19*, 197-201.

Katoh, M. (2007c). WNT signaling pathway and stem cell signaling network. Clin Cancer Res *13*, 4042-4045.

Katoh, Y., and Katoh, M. (2005). Hedgehog signaling pathway and gastric cancer. Cancer Biol Ther *4*, 1050-1054.

Katoh, Y., and Katoh, M. (2006a). FGF signaling inhibitor, SPRY4, is evolutionarily conserved target of WNT signaling pathway in progenitor cells. Int J Mol Med *17*, 529-532.

Katoh, Y., and Katoh, M. (2006b). WNT antagonist, SFRP1, is Hedgehog signaling target. Int J Mol Med *17*, 171-175.

Kaufmann, S. H., Mabry, M., Jasti, R., and Shaper, J. H. (1991). Differential expression of nuclear envelope lamins A and C in human lung cancer cell lines. Cancer Res *51*, 581-586.

Kengaku, M., and Okamoto, H. (1995). bFGF as a possible morphogen for the anteroposterior axis of the central nervous system in Xenopus. Development *121*, 3121-3130.

Kim, J. S., Crooks, H., Dracheva, T., Nishanian, T. G., Singh, B., Jen, J., and Waldman, T. (2002). Oncogenic beta-catenin is required for bone morphogenetic protein 4 expression in human cancer cells. Cancer Res *62*, 2744-2748.

Kim, J. Y., Siegmund, K. D., Tavare, S., and Shibata, D. (2005). Age-related human small intestine methylation: evidence for stem cell niches. BMC Med *3*, 10.

Kim, K. W., Bae, S. K., Lee, O. H., Bae, M. H., Lee, M. J., and Park, B. C. (1998). Insulin-like growth factor II induced by hypoxia may contribute to angiogenesis of human hepatocellular carcinoma. Cancer Res *58*, 348-351.

Kinzler, K. W., Nilbert, M. C., Su, L. K., Vogelstein, B., Bryan, T. M., Levy, D. B., Smith, K. J., Preisinger, A. C., Hedge, P., McKechnie, D., and et al. (1991a). Identification of FAP locus genes from chromosome 5q21. Science *253*, 661-665.

Kinzler, K. W., Nilbert, M. C., Vogelstein, B., Bryan, T. M., Levy, D. B., Smith, K. J., Preisinger, A. C., Hamilton, S. R., Hedge, P., Markham, A., and et al. (1991b). Identification of a gene located at chromosome 5q21 that is mutated in colorectal cancers. Science *251*, 1366-1370.

Kinzler, K. W., and Vogelstein, B. (1996). Lessons from hereditary colorectal cancer. Cell *87*, 159-170.

Kirsch, T., Sebald, W., and Dreyer, M. K. (2000). Crystal structure of the BMP-2-BRIA ectodomain complex. Nat Struct Biol *7*, 492-496.

Kitagawa, M., Hatakeyama, S., Shirane, M., Matsumoto, M., Ishida, N., Hattori, K., Nakamichi, I., Kikuchi, A., and Nakayama, K. (1999). An F-box protein, FWD1, mediates ubiquitin-dependent proteolysis of beta-catenin. Embo J *18*, 2401-2410.

Koenig, B. B., Cook, J. S., Wolsing, D. H., Ting, J., Tiesman, J. P., Correa, P. E., Olson, C. A., Pecquet, A. L., Ventura, F., Grant, R. A., and et al. (1994). Characterization and cloning of a receptor for BMP-2 and BMP-4 from NIH 3T3 cells. Mol Cell Biol *14*, 5961-5974.

Kolligs, F. T., Nieman, M. T., Winer, I., Hu, G., Van Mater, D., Feng, Y., Smith, I. M., Wu, R., Zhai, Y., Cho, K. R., and Fearon, E. R. (2002). ITF-2, a downstream target of the

Wnt/TCF pathway, is activated in human cancers with beta-catenin defects and promotes neoplastic transformation. Cancer Cell *1*, 145-155.

Korinek, V., Barker, N., Morin, P. J., van Wichen, D., de Weger, R., Kinzler, K. W., Vogelstein, B., and Clevers, H. (1997). Constitutive transcriptional activation by a beta-catenin-Tcf complex in APC-/- colon carcinoma. Science *275*, 1784-1787.

Korinek, V., Barker, N., Willert, K., Molenaar, M., Roose, J., Wagenaar, G., Markman, M., Lamers, W., Destree, O., and Clevers, H. (1998). Two members of the Tcf family implicated in Wnt/beta-catenin signaling during embryogenesis in the mouse. Mol Cell Biol *18*, 1248-1256.

Kouhara, H., Hadari, Y. R., Spivak-Kroizman, T., Schilling, J., Bar-Sagi, D., Lax, I., and Schlessinger, J. (1997). A lipid-anchored Grb2-binding protein that links FGF-receptor activation to the Ras/MAPK signaling pathway. Cell *89*, 693-702.

Kranenburg, O. (2005). The KRAS oncogene: past, present, and future. Biochim Biophys Acta *1756*, 81-82.

Kratochwil, K., Dull, M., Farinas, I., Galceran, J., and Grosschedl, R. (1996). Lef1 expression is activated by BMP-4 and regulates inductive tissue interactions in tooth and hair development. Genes Dev *10*, 1382-1394.

Kretzschmar, M., Liu, F., Hata, A., Doody, J., and Massague, J. (1997). The TGF-beta family mediator Smad1 is phosphorylated directly and activated functionally by the BMP receptor kinase. Genes Dev *11*, 984-995.

Laguri, C., Gilquin, B., Wolff, N., Romi-Lebrun, R., Courchay, K., Callebaut, I., Worman, H. J., and Zinn-Justin, S. (2001). Structural characterization of the LEM motif common to three human inner nuclear membrane proteins. Structure (Camb) *9*, 503-511.

Lai, E. C. (2004). Notch signaling: control of cell communication and cell fate. Development *131*, 965-973.

Lawrie, L. C., Dundas, S. R., Curran, S., and Murray, G. I. (2004). Liver fatty acid binding protein expression in colorectal neoplasia. Br J Cancer *90*, 1955-1960.

Lazebnik, Y. A., Takahashi, A., Moir, R. D., Goldman, R. D., Poirier, G. G., Kaufmann, S. H., and Earnshaw, W. C. (1995). Studies of the lamin proteinase reveal multiple parallel biochemical pathways during apoptotic execution. Proc Natl Acad Sci U S A *92*, 9042-9046.

Leal, A., Morera, B., Del Valle, G., Heuss, D., Kayser, C., Berghoff, M., Villegas, R., Hernandez, E., Mendez, M., Hennies, H. C., *et al.* (2001). A second locus for an axonal form of autosomal recessive Charcot-Marie-Tooth disease maps to chromosome 19q13.3. Am J Hum Genet *68*, 269-274.

Lebel, S., Lampron, C., Royal, A., and Raymond, Y. (1987). Lamins A and C appear during retinoic acid-induced differentiation of mouse embryonal carcinoma cells. J Cell Biol *105*, 1099-1104.

Lee, K. K., Haraguchi, T., Lee, R. S., Koujin, T., Hiraoka, Y., and Wilson, K. L. (2001). Distinct functional domains in emerin bind lamin A and DNA-bridging protein BAF. J Cell Sci *114*, 4567-4573.

Lees, C., Howie, S., Sartor, R. B., and Satsangi, J. (2005). The hedgehog signalling pathway in the gastrointestinal tract: implications for development, homeostasis, and disease. Gastroenterology *129*, 1696-1710.

Lehner, C. F., Stick, R., Eppenberger, H. M., and Nigg, E. A. (1987). Differential expression of nuclear lamin proteins during chicken development. J Cell Biol *105*, 577-587.

Leivonen, S. K., and Kahari, V. M. (2007). Transforming growth factor-beta signaling in cancer invasion and metastasis. Int J Cancer *121*, 2119-2124.

Lenz, W., and Meschede, D. (1993). Historical note on restrictive dermopathy and report of two new cases. Am J Med Genet *47*, 1235-1237.

Leow, C. C., Romero, M. S., Ross, S., Polakis, P., and Gao, W. Q. (2004). Hath1, down-regulated in colon adenocarcinomas, inhibits proliferation and tumorigenesis of colon cancer cells. Cancer Res *64*, 6050-6057.

LeRoith, D., and Roberts, C. T., Jr. (2003). The insulin-like growth factor system and cancer. Cancer Lett *195*, 127-137.

Li, J. L., and Harris, A. L. (2005). Notch signaling from tumor cells: a new mechanism of angiogenesis. Cancer Cell *8*, 1-3.

Liaw, L., Skinner, M. P., Raines, E. W., Ross, R., Cheresh, D. A., Schwartz, S. M., and Giachelli, C. M. (1995). The adhesive and migratory effects of osteopontin are mediated via distinct cell surface integrins. Role of alpha v beta 3 in smooth muscle cell migration to osteopontin in vitro. J Clin Invest *95*, 713-724.

Lievre, A., Bachet, J. B., Le Corre, D., Boige, V., Landi, B., Emile, J. F., Cote, J. F., Tomasic, G., Penna, C., Ducreux, M., *et al.* (2006). KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res *66*, 3992-3995.

Lin, F., Blake, D. L., Callebaut, I., Skerjanc, I. S., Holmer, L., McBurney, M. W., Paulin-Levasseur, M., and Worman, H. J. (2000). MAN1, an inner nuclear membrane protein that shares the LEM domain with lamina-associated polypeptide 2 and emerin. J Biol Chem *275*, 4840-4847.

Lin, F., and Worman, H. J. (1993). Structural organization of the human gene encoding nuclear lamin A and nuclear lamin C. J Biol Chem *268*, 16321-16326.

Lin, F., and Worman, H. J. (1995). Structural organization of the human gene (LMNB1) encoding nuclear lamin B1. Genomics *27*, 230-236.

Liu, C., Li, Y., Semenov, M., Han, C., Baeg, G. H., Tan, Y., Zhang, Z., Lin, X., and He, X. (2002). Control of beta-catenin phosphorylation/degradation by a dual-kinase mechanism. Cell *108*, 837-847.

Liu, F., Ventura, F., Doody, J., and Massague, J. (1995). Human type II receptor for bone morphogenic proteins (BMPs): extension of the two-kinase receptor model to the BMPs. Mol Cell Biol *15*, 3479-3486.

Liu, J., Lee, K. K., Segura-Totten, M., Neufeld, E., Wilson, K. L., and Gruenbaum, Y. (2003). MAN1 and emerin have overlapping function(s) essential for chromosome segregation and cell division in Caenorhabditis elegans. Proc Natl Acad Sci U S A *100*, 4598-4603.

Loewinger, L., and McKeon, F. (1988). Mutations in the nuclear lamin proteins resulting in their aberrant assembly in the cytoplasm. Embo J *7*, 2301-2309.

Logan, C. Y., and Nusse, R. (2004). The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol *20*, 781-810.

Lourim, D., and Lin, J. J. (1992). Expression of wild-type and nuclear localization-deficient human lamin A in chick myogenic cells. J Cell Sci *103 (Pt 3)*, 863-874.

Lum, L., and Beachy, P. A. (2004). The Hedgehog response network: sensors, switches, and routers. Science *304*, 1755-1759.

Lupton, J. R. (2004). Microbial degradation products influence colon cancer risk: the butyrate controversy. J Nutr *134*, 479-482.

Machiels, B. M., Broers, J. L., Raymond, Y., de Ley, L., Kuijpers, H. J., Caberg, N. E., and Ramaekers, F. C. (1995). Abnormal A-type lamin organization in a human lung carcinoma cell line. Eur J Cell Biol *67*, 328-335.

Machiels, B. M., Zorenc, A. H., Endert, J. M., Kuijpers, H. J., van Eys, G. J., Ramaekers, F. C., and Broers, J. L. (1996). An alternative splicing product of the lamin A/C gene lacks exon 10. J Biol Chem *271*, 9249-9253.

Malik, P., Korfali, N., Srsen, V., Lazou, V., Batrakou, D. G., Zuleger, N., Kavanagh, D. M., Wilkie, G. S., Goldberg, M. W., and Schirmer, E. C. (2010). Cell-specific and lamin-dependent targeting of novel transmembrane proteins in the nuclear envelope. Cell Mol Life Sci *67*, 1353-1369.

Mancini, M. A., Shan, B., Nickerson, J. A., Penman, S., and Lee, W. H. (1994). The retinoblastoma gene product is a cell cycle-dependent, nuclear matrix-associated protein. Proc Natl Acad Sci U S A *91*, 418-422.

Mandel, J. S., Bond, J. H., Church, T. R., Snover, D. C., Bradley, G. M., Schuman, L. M., and Ederer, F. (1993). Reducing mortality from colorectal cancer by screening for fecal occult blood. Minnesota Colon Cancer Control Study. N Engl J Med *328*, 1365-1371.

Markiewicz, E., Dechat, T., Foisner, R., Quinlan, R. A., and Hutchison, C. J. (2002). Lamin A/C binding protein LAP2alpha is required for nuclear anchorage of retinoblastoma protein. Mol Biol Cell *13*, 4401-4413.

Markiewicz, E., Tilgner, K., Barker, N., van de Wetering, M., Clevers, H., Dorobek, M., Hausmanowa-Petrusewicz, I., Ramaekers, F. C., Broers, J. L., Blankesteijn, W. M., *et al.*

(2006). The inner nuclear membrane protein emerin regulates beta-catenin activity by restricting its accumulation in the nucleus. Embo J *25*, 3275-3285.

Martin, G. M., and Oshima, J. (2000). Lessons from human progeroid syndromes. Nature *408*, 263-266.

Masciari, S., Larsson, N., Senz, J., Boyd, N., Kaurah, P., Kandel, M. J., Harris, L. N., Pinheiro, H. C., Troussard, A., Miron, P., *et al.* (2007). Germline E-cadherin mutations in familial lobular breast cancer. J Med Genet *44*, 726-731.

Masuda, H., Fukabori, Y., Nakano, K., Takezawa, Y., T, C. S., and Yamanaka, H. (2003). Increased expression of bone morphogenetic protein-7 in bone metastatic prostate cancer. Prostate *54*, 268-274.

Mau, U., Kendziorra, H., Kaiser, P., and Enders, H. (1997). Restrictive dermopathy: report and review. Am J Med Genet *71*, 179-185.

McGrath, J. P., Capon, D. J., Smith, D. H., Chen, E. Y., Seeburg, P. H., Goeddel, D. V., and Levinson, A. D. (1983). Structure and organization of the human Ki-ras proto-oncogene and a related processed pseudogene. Nature *304*, 501-506.

McKeehan, W. L., Wang, F., and Kan, M. (1998). The heparan sulfate-fibroblast growth factor family: diversity of structure and function. Prog Nucleic Acid Res Mol Biol *59*, 135-176.

McKeon, F. D., Kirschner, M. W., and Caput, D. (1986). Homologies in both primary and secondary structure between nuclear envelope and intermediate filament proteins. Nature *319*, 463-468.

McKusick, V. A. (1963). Medical Genetics 1962. J Chronic Dis *16*, 457-634.

McLellan, E. A., and Bird, R. P. (1988). Aberrant crypts: potential preneoplastic lesions in the murine colon. Cancer Res *48*, 6187-6192.

Mercer, D. W., and Talamo, T. S. (1985). Multiple markers of malignancy in sera of patients with colorectal carcinoma: preliminary clinical studies. Clin Chem *31*, 1824-1828.

Meyer, J. M., and Ginsburg, G. S. (2002). The path to personalized medicine. Curr Opin Chem Biol *6*, 434-438.

Miyoshi, Y., Ando, H., Nagase, H., Nishisho, I., Horii, A., Miki, Y., Mori, T., Utsunomiya, J., Baba, S., Petersen, G., and et al. (1992). Germ-line mutations of the APC gene in 53 familial adenomatous polyposis patients. Proc Natl Acad Sci U S A *89*, 4452-4456.

Moir, R. D., Spann, T. P., Lopez-Soler, R. I., Yoon, M., Goldman, A. E., Khuon, S., and Goldman, R. D. (2000). Review: the dynamics of the nuclear lamins during the cell cycle--relationship between structure and function. J Struct Biol *129*, 324-334.

Moir, R. D., Spann, T. P., Lopez-Soler, R. I., Yoon, M., Goldman, A. E., Khuon, S., and Goldman, R. D. (2000a). Review: the dynamics of the nuclear lamins during the cell cycle--relationship between structure and function. J Struct Biol *129*, 324-334.

Moir, R. D., Yoon, M., Khuon, S., and Goldman, R. D. (2000b). Nuclear lamins A and B1: different pathways of assembly during nuclear envelope formation in living cells. J Cell Biol *151*, 1155-1168.

Morin, P. J., Sparks, A. B., Korinek, V., Barker, N., Clevers, H., Vogelstein, B., and Kinzler, K. W. (1997). Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. Science *275*, 1787-1790.

Moroni, E., Dell'Era, P., Rusnati, M., and Presta, M. (2002). Fibroblast growth factors and their receptors in hematopoiesis and hematological tumors. J Hematother Stem Cell Res *11*, 19-32.

Moskaluk, C. A., Hruban, R. H., Schutte, M., Lietman, A. S., Smyrk, T., Fusaro, L., Fusaro, R., Lynch, J., Yeo, C. J., Jackson, C. E., *et al.* (1997). Genomic sequencing of DPC4 in the analysis of familial pancreatic carcinoma. Diagn Mol Pathol *6*, 85-90.

Moss, S. F., Krivosheyev, V., de Souza, A., Chin, K., Gaetz, H. P., Chaudhary, N., Worman, H. J., and Holt, P. R. (1999). Decreased and aberrant nuclear lamin expression in gastrointestinal tract neoplasms. Gut *45*, 723-729.

Mounkes, L. C., and Stewart, C. L. (2004). Aging and nuclear organization: lamins and progeria. Curr Opin Cell Biol *16*, 322-327.

Muller, H. H., Heinimann, K., and Dobbie, Z. (2000). Genetics of hereditary colon cancer - a basis for prevention? Eur J Cancer *36*, 1215-1223.

Murray, A. W., and Kirschner, M. W. (1989). Cyclin synthesis drives the early embryonic cell cycle. Nature *339*, 275-280.

Nagano, A., Koga, R., Ogawa, M., Kurano, Y., Kawada, J., Okada, R., Hayashi, Y. K., Tsukahara, T., and Arahata, K. (1996). Emerin deficiency at the nuclear membrane in patients with Emery-Dreifuss muscular dystrophy. Nat Genet *12*, 254-259.

Nagase, H., and Nakamura, Y. (1993). Mutations of the APC (adenomatous polyposis coli) gene. Hum Mutat *2*, 425-434.

Nakamura, Y., Nishisho, I., Kinzler, K. W., Vogelstein, B., Miyoshi, Y., Miki, Y., Ando, H., Horii, A., and Nagase, H. (1991). Mutations of the adenomatous polyposis coli gene in familial polyposis coli patients and sporadic colorectal tumors. Princess Takamatsu Symp *22*, 285-292.

Navaratnam, R. M., Chowaniec, J., and Winslet, M. C. (1999). The molecular biology of colorectal cancer development and the associated genetic events. Ann R Coll Surg Engl *81*, 312-319.

Navarro, C. L., Cadinanos, J., De Sandre-Giovannoli, A., Bernard, R., Courrier, S., Boccaccio, I., Boyer, A., Kleijer, W. J., Wagner, A., Giuliano, F., *et al.* (2005). Loss of ZMPSTE24 (FACE-1) causes autosomal recessive restrictive dermopathy and accumulation of Lamin A precursors. Hum Mol Genet *14*, 1503-1513.

Navarro, C. L., De Sandre-Giovannoli, A., Bernard, R., Boccaccio, I., Boyer, A., Genevieve, D., Hadj-Rabia, S., Gaudy-Marqueste, C., Smitt, H. S., Vabres, P., *et al.*

(2004). Lamin A and ZMPSTE24 (FACE-1) defects cause nuclear disorganization and identify restrictive dermopathy as a lethal neonatal laminopathy. Hum Mol Genet *13*, 2493-2503.

Newport, J. W., Wilson, K. L., and Dunphy, W. G. (1990). A lamin-independent pathway for nuclear envelope assembly. J Cell Biol *111*, 2247-2259.

Nigg, E. A. (1992). Assembly-disassembly of the nuclear lamina. Curr Opin Cell Biol *4*, 105-109.

Nili, E., Cojocaru, G. S., Kalma, Y., Ginsberg, D., Copeland, N. G., Gilbert, D. J., Jenkins, N. A., Berger, R., Shaklai, S., Amariglio, N., *et al.* (2001). Nuclear membrane protein LAP2beta mediates transcriptional repression alone and together with its binding partner GCL (germ-cell-less). J Cell Sci *114*, 3297-3307.

Nishanian, T. G., Kim, J. S., Foxworth, A., and Waldman, T. (2004). Suppression of tumorigenesis and activation of Wnt signaling by bone morphogenetic protein 4 in human cancer cells. Cancer Biol Ther *3*, 667-675.

Nishimura, S., Wakabayashi, N., Toyoda, K., Kashima, K., and Mitsufuji, S. (2003). Expression of Musashi-1 in human normal colon crypt cells: a possible stem cell marker of human colon epithelium. Dig Dis Sci *48*, 1523-1529.

Nosho, K., Yamamoto, H., Adachi, Y., Endo, T., Hinoda, Y., and Imai, K. (2005). Gene expression profiling of colorectal adenomas and early invasive carcinomas by cDNA array analysis. Br J Cancer *92*, 1193-1200.

Novelli, G., and D'Apice, M. R. (2003). The strange case of the "lumper" lamin A/C gene and human premature ageing. Trends Mol Med *9*, 370-375.

Novelli, G., Muchir, A., Sangiuolo, F., Helbling-Leclerc, A., D'Apice, M. R., Massart, C., Capon, F., Sbraccia, P., Federici, M., Lauro, R., *et al.* (2002). Mandibuloacral dysplasia is caused by a mutation in LMNA-encoding lamin A/C. Am J Hum Genet *71*, 426-431.

Nusslein-Volhard, C., and Wieschaus, E. (1980). Mutations affecting segment number and polarity in Drosophila. Nature *287*, 795-801.

Oates, A. J., Barraclough, R., and Rudland, P. S. (1996). The identification of osteopontin as a metastasis-related gene product in a rodent mammary tumour model. Oncogene *13*, 97-104.

O'Brien, C. A., Pollett, A., Gallinger, S., and Dick, J. E. (2007). A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature *445*, 106-110.

Ogata, H., Goto, S., Sato, K., Fujibuchi, W., Bono, H., and Kanehisa, M. (1999). KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Res *27*, 29-34.

Oguchi, M., Sagara, J., Matsumoto, K., Saida, T., and Taniguchi, S. (2002). Expression of lamins depends on epidermal differentiation and transformation. Br J Dermatol *147*, 853-858.

Ornitz, D. M., and Itoh, N. (2001). Fibroblast growth factors. Genome Biol *2*, REVIEWS3005.

Ornitz, D. M., Xu, J., Colvin, J. S., McEwen, D. G., MacArthur, C. A., Coulier, F., Gao, G., and Goldfarb, M. (1996). Receptor specificity of the fibroblast growth factor family. J Biol Chem *271*, 15292-15297.

Oster, S. K., Ho, C. S., Soucie, E. L., and Penn, L. Z. (2002). The myc oncogene: MarvelouslY Complex. Adv Cancer Res *84*, 81-154.

Ottaviano, Y., and Gerace, L. (1985). Phosphorylation of the nuclear lamins during interphase and mitosis. J Biol Chem *260*, 624-632.

Ozaki, T., Saijo, M., Murakami, K., Enomoto, H., Taya, Y., and Sakiyama, S. (1994). Complex formation between lamin A and the retinoblastoma gene product: identification of the domain on lamin A required for its interaction. Oncogene *9*, 2649-2653.

Pan, J. Z., Eckenhoff, R. G., and Eckenhoff, M. F. (2007). Limitations of microarray studies. Anesth Analg *104*, 1300-1301; author reply 1301-1302.

Parc, Y., Gueroult, S., Mourra, N., Serfaty, L., Flejou, J. F., Tiret, E., and Parc, R. (2004). Prognostic significance of microsatellite instability determined by immunohistochemical staining of MSH2 and MLH1 in sporadic T3N0M0 colon cancer. Gut *53*, 371-375.

Pasca di Magliano, M., and Hebrok, M. (2003). Hedgehog signalling in cancer formation and maintenance. Nat Rev Cancer *3*, 903-911.

Pathi, S., Pagan-Westphal, S., Baker, D. P., Garber, E. A., Rayhorn, P., Bumcrot, D., Tabin, C. J., Blake Pepinsky, R., and Williams, K. P. (2001). Comparative biological responses to human Sonic, Indian, and Desert hedgehog. Mech Dev *106*, 107-117.

Paulin-Levasseur, M., Giese, G., Scherbarth, A., and Traub, P. (1989). Expression of vimentin and nuclear lamins during the in vitro differentiation of human promyelocytic leukemia cells HL-60. Eur J Cell Biol *50*, 453-461.

Peifer, M. (2002). Developmental biology: colon construction. Nature *420*, 274-275, 277.

Petersen, V. C., Baxter, K. J., Love, S. B., and Shepherd, N. A. (2002). Identification of objective pathological prognostic determinants and models of prognosis in Dukes' B colon cancer. Gut *51*, 65-69.

Pietra, N., Sarli, L., Costi, R., Ouchemi, C., Grattarola, M., and Peracchia, A. (1998). Role of follow-up in management of local recurrences of colorectal cancer: a prospective, randomized study. Dis Colon Rectum *41*, 1127-1133.

Pinson, K. I., Brennan, J., Monkley, S., Avery, B. J., and Skarnes, W. C. (2000). An LDL-receptor-related protein mediates Wnt signalling in mice. Nature *407*, 535-538.

Pinto, D., and Clevers, H. (2005a). Wnt control of stem cells and differentiation in the intestinal epithelium. Exp Cell Res *306*, 357-363.

Pinto, D., and Clevers, H. (2005b). Wnt, stem cells and cancer in the intestine. Biol Cell *97*, 185-196.

Pinto, D., Gregorieff, A., Begthel, H., and Clevers, H. (2003). Canonical Wnt signals are essential for homeostasis of the intestinal epithelium. Genes Dev *17*, 1709-1713.

Polakis, P. (1997). The adenomatous polyposis coli (APC) tumor suppressor. Biochim Biophys Acta *1332*, F127-147.

Popescu, N. C., Amsbaugh, S. C., DiPaolo, J. A., Tronick, S. R., Aaronson, S. A., and Swan, D. C. (1985). Chromosomal localization of three human ras genes by in situ molecular hybridization. Somat Cell Mol Genet *11*, 149-155.

Porter, J. A., Ekker, S. C., Park, W. J., von Kessler, D. P., Young, K. E., Chen, C. H., Ma, Y., Woods, A. S., Cotter, R. J., Koonin, E. V., and Beachy, P. A. (1996). Hedgehog patterning activity: role of a lipophilic modification mediated by the carboxy-terminal autoprocessing domain. Cell *86*, 21-34.

Potten, C. S. (1998). Stem cells in gastrointestinal epithelium: numbers, characteristics and death. Philos Trans R Soc Lond B Biol Sci *353*, 821-830.

Potten, C. S., Booth, C., Tudor, G. L., Booth, D., Brady, G., Hurley, P., Ashton, G., Clarke, R., Sakakibara, S., and Okano, H. (2003). Identification of a putative intestinal stem cell and early lineage marker; musashi-1. Differentiation *71*, 28-41.

Potten, C. S., Kellett, M., Roberts, S. A., Rew, D. A., and Wilson, G. D. (1992). Measurement of in vivo proliferation in human colorectal mucosa using bromodeoxyuridine. Gut *33*, 71-78.

Potten, C. S., Owen, G., and Booth, D. (2002). Intestinal stem cells protect their genome by selective segregation of template DNA strands. J Cell Sci *115*, 2381-2388.

Powers, C. J., McLeskey, S. W., and Wellstein, A. (2000). Fibroblast growth factors, their receptors and signaling. Endocr Relat Cancer *7*, 165-197.

Preston, S. L., Wong, W. M., Chan, A. O., Poulsom, R., Jeffery, R., Goodlad, R. A., Mandir, N., Elia, G., Novelli, M., Bodmer, W. F., *et al.* (2003). Bottom-up histogenesis of colorectal adenomas: origin in the monocryptal adenoma and initial expansion by crypt fission. Cancer Res *63*, 3819-3825.

Pretlow, T. P., Roukhadze, E. V., O'Riordan, M. A., Chan, J. C., Amini, S. B., and Stellato, T. A. (1994). Carcinoembryonic antigen in human colonic aberrant crypt foci. Gastroenterology *107*, 1719-1725.

Prince, M. E., Sivanandan, R., Kaczorowski, A., Wolf, G. T., Kaplan, M. J., Dalerba, P., Weissman, I. L., Clarke, M. F., and Ailles, L. E. (2007). Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. Proc Natl Acad Sci U S A *104*, 973-978.

Prives, C., and Gottifredi, V. (2008). The p21 and PCNA partnership: a new twist for an old plot. Cell Cycle *7*, 3840-3846.

Proctor, M. J., Morrison, D. S., Talwar, D., Balmer, S. M., O'Reilly, D. S., Foulis, A. K., Horgan, P. G., and McMillan, D. C. (2011). An inflammation-based prognostic score (mGPS) predicts cancer survival independent of tumour site: a Glasgow Inflammation Outcome Study. Br J Cancer.

Prokocimer, M., Margalit, A., and Gruenbaum, Y. (2006). The nuclear lamina and its proposed roles in tumorigenesis: projection on the hematologic malignancies and future targeted therapy. J Struct Biol *155*, 351-360.

Promega (2002). Wizard SV Gel and PCR Clean-Up System Technical Bulletin #TB308.

Pugh, G. E., Coates, P. J., Lane, E. B., Raymond, Y., and Quinlan, R. A. (1997). Distinct nuclear assembly pathways for lamins A and C lead to their increase during quiescence in Swiss 3T3 cells. J Cell Sci *110 (Pt 19)*, 2483-2493.

Radtke, F., Clevers, H., and Riccio, O. (2006). From gut homeostasis to cancer. Curr Mol Med *6*, 275-289.

Raffaele Di Barletta, M., Ricci, E., Galluzzi, G., Tonali, P., Mora, M., Morandi, L., Romorini, A., Voit, T., Orstavik, K. H., Merlini, L., *et al.* (2000). Different mutations in the LMNA gene cause autosomal dominant and autosomal recessive Emery-Dreifuss muscular dystrophy. Am J Hum Genet *66*, 1407-1412.

Rajeevan, M. S., Ranamukhaarachchi, D. G., Vernon, S. D., and Unger, E. R. (2001a). Use of real-time quantitative PCR to validate the results of cDNA array and differential display PCR technologies. Methods *25*, 443-451.

Rajeevan, M. S., Vernon, S. D., Taysavang, N., and Unger, E. R. (2001b). Validation of array-based gene expression profiles by real-time (kinetic) RT-PCR. J Mol Diagn *3*, 26-31.

Raju, G. P., Dimova, N., Klein, P. S., and Huang, H. C. (2003). SANE, a novel LEM domain protein, regulates bone morphogenetic protein signaling through interaction with Smad1. J Biol Chem *278*, 428-437.

Rao, L., Perez, D., and White, E. (1996). Lamin proteolysis facilitates nuclear events during apoptosis. J Cell Biol *135*, 1441-1455.

Rashid, A., and Issa, J. P. (2004). CpG island methylation in gastroenterologic neoplasia: a maturing field. Gastroenterology *127*, 1578-1588.

Reed, K. R., Sansom, O. J., Hayes, A. J., Gescher, A. J., Winton, D. J., Peters, J. M., and Clarke, A. R. (2004). PPARdelta status and Apc-mediated tumourigenesis in the mouse intestine. Oncogene *23*, 8992-8996.

Regge, D., Hassan, C., Pickhardt, P. J., Laghi, A., Zullo, A., Kim, D. H., Iafrate, F., and Morini, S. (2009a). Impact of computer-aided detection on the cost-effectiveness of CT colonography. Radiology *250*, 488-497.

Regge, D., Laudi, C., Galatola, G., Della Monica, P., Bonelli, L., Angelelli, G., Asnaghi, R., Barbaro, B., Bartolozzi, C., Bielen, D., *et al.* (2009b). Diagnostic accuracy of computed tomographic colonography for the detection of advanced neoplasia in individuals at increased risk of colorectal cancer. Jama *301*, 2453-2461.

Reichelt, R., Holzenburg, A., Buhle, E. L., Jr., Jarnik, M., Engel, A., and Aebi, U. (1990). Correlation between structure and mass distribution of the nuclear pore complex and of distinct pore complex components. J Cell Biol *110*, 883-894.

Reya, T., and Clevers, H. (2005). Wnt signalling in stem cells and cancer. Nature *434*, 843-850.

Reya, T., Morrison, S. J., Clarke, M. F., and Weissman, I. L. (2001). Stem cells, cancer, and cancer stem cells. Nature *414*, 105-111.

Ricci-Vitiani, L., Lombardi, D. G., Pilozzi, E., Biffoni, M., Todaro, M., Peschle, C., and De Maria, R. (2007). Identification and expansion of human colon-cancer-initiating cells. Nature *445*, 111-115.

Rihn, B. H., Mohr, S., McDowell, S. A., Binet, S., Loubinoux, J., Galateau, F., Keith, G., and Leikauf, G. D. (2000). Differential gene expression in mesothelioma. FEBS Lett *480*, 95-100.

Rober, R. A., Weber, K., and Osborn, M. (1989). Differential timing of nuclear lamin A/C expression in the various organs of the mouse embryo and the young animal: a developmental study. Development *105*, 365-378.

Robertson, D. J., Greenberg, E. R., Beach, M., Sandler, R. S., Ahnen, D., Haile, R. W., Burke, C. A., Snover, D. C., Bresalier, R. S., McKeown-Eyssen, G., *et al.* (2005). Colorectal cancer in patients under close colonoscopic surveillance. Gastroenterology *129*, 34-41.

Rohde, F., Rimkus, C., Friederichs, J., Rosenberg, R., Marthen, C., Doll, D., Holzmann, B., Siewert, J. R., and Janssen, K. P. (2007). Expression of osteopontin, a target gene of de-regulated Wnt signaling, predicts survival in colon cancer. Int J Cancer *121*, 1717-1723.

Roose, J., Huls, G., van Beest, M., Moerer, P., van der Horn, K., Goldschmeding, R., Logtenberg, T., and Clevers, H. (1999). Synergy between tumor suppressor APC and the beta-catenin-Tcf4 target Tcf1. Science *285*, 1923-1926.

Rosenzweig, B. L., Imamura, T., Okadome, T., Cox, G. N., Yamashita, H., ten Dijke, P., Heldin, C. H., and Miyazono, K. (1995). Cloning and characterization of a human type II receptor for bone morphogenetic proteins. Proc Natl Acad Sci U S A *92*, 7632-7636.

Rothhammer, T., Poser, I., Soncin, F., Bataille, F., Moser, M., and Bosserhoff, A. K. (2005). Bone morphogenic proteins are overexpressed in malignant melanoma and promote cell invasion and migration. Cancer Res *65*, 448-456.

Rowlands, D. C., Bunce, C. M., Crocker, J., Ayres, J. G., Johnson, G. D., Ling, N., and Brown, G. (1994). Expression of a nuclear envelope protein recognized by the monoclonal antibody BU31 in lung tumours: relationship to Ki-67 antigen expression. J Pathol *173*, 89-96.

Russell, R. G., Lasorella, A., Dettin, L. E., and Iavarone, A. (2004). Id2 drives differentiation and suppresses tumor formation in the intestinal epithelium. Cancer Res *64*, 7220-7225.

Sakaki, M., Koike, H., Takahashi, N., Sasagawa, N., Tomioka, S., Arahata, K., and Ishiura, S. (2001). Interaction between emerin and nuclear lamins. J Biochem (Tokyo) *129*, 321-327.

Salina, D., Bodoor, K., Eckley, D. M., Schroer, T. A., Rattner, J. B., and Burke, B. (2002). Cytoplasmic dynein as a facilitator of nuclear envelope breakdown. Cell *108*, 97-107.

Sancho, E., Batlle, E., and Clevers, H. (2004). Signaling pathways in intestinal development and cancer. Annu Rev Cell Dev Biol *20*, 695-723.

Sander, G. R., and Powell, B. C. (2004). Expression of notch receptors and ligands in the adult gut. J Histochem Cytochem *52*, 509-516.

Schirmer, E. C., Florens, L., Guan, T., Yates, J. R., 3rd, and Gerace, L. (2003). Nuclear membrane proteins with potential disease links found by subtractive proteomics. Science *301*, 1380-1382.

Schlessinger, J. (2000). Cell signaling by receptor tyrosine kinases. Cell *103*, 211-225.

Schmidt, M., and Krohne, G. (1995). In vivo assembly kinetics of fluorescently labeled Xenopus lamin A mutants. Eur J Cell Biol *68*, 345-354.

Schroeder, F., Atshaves, B. P., Starodub, O., Boedeker, A. L., Smith, R. R., 3rd, Roths, J. B., Foxworth, W. B., and Kier, A. B. (2001). Expression of liver fatty acid binding protein alters growth and differentiation of embryonic stem cells. Mol Cell Biochem *219*, 127-138.

Scott, K. W., and Grace, R. H. (1989). Detection of lymph node metastases in colorectal carcinoma before and after fat clearance. Br J Surg *76*, 1165-1167.

Segura-Totten, M., Kowalski, A. K., Craigie, R., and Wilson, K. L. (2002). Barrier-to-autointegration factor: major roles in chromatin decondensation and nuclear assembly. J Cell Biol *158*, 475-485.

Segura-Totten, M., and Wilson, K. L. (2004). BAF: roles in chromatin, nuclear structure and retrovirus integration. Trends Cell Biol *14*, 261-266.

Sekiya, T., Adachi, S., Kohu, K., Yamada, T., Higuchi, O., Furukawa, Y., Nakamura, Y., Nakamura, T., Tashiro, K., Kuhara, S., *et al.* (2004). Identification of BMP and activin membrane-bound inhibitor (BAMBI), an inhibitor of transforming growth factor-beta signaling, as a target of the beta-catenin pathway in colorectal tumor cells. J Biol Chem *279*, 6840-6846.

Shih, I. M., Wang, T. L., Traverso, G., Romans, K., Hamilton, S. R., Ben-Sasson, S., Kinzler, K. W., and Vogelstein, B. (2001). Top-down morphogenesis of colorectal tumors. Proc Natl Acad Sci U S A *98*, 2640-2645.

Shin, H. M., Minter, L. M., Cho, O. H., Gottipati, S., Fauq, A. H., Golde, T. E., Sonenshein, G. E., and Osborne, B. A. (2006). Notch1 augments NF-kappaB activity by facilitating its nuclear retention. Embo J *25*, 129-138.

Shumaker, D. K., Lee, K. K., Tanhehco, Y. C., Craigie, R., and Wilson, K. L. (2001). LAP2 binds to BAF.DNA complexes: requirement for the LEM domain and modulation by variable regions. Embo J *20*, 1754-1764.

Simha, V., and Garg, A. (2002). Body fat distribution and metabolic derangements in patients with familial partial lipodystrophy associated with mandibuloacral dysplasia. J Clin Endocrinol Metab *87*, 776-785.

Sinensky, M., Fantle, K., Trujillo, M., McLain, T., Kupfer, A., and Dalton, M. (1994). The processing pathway of prelamin A. J Cell Sci *107 (Pt 1)*, 61-67.

Singh, S. K., Hawkins, C., Clarke, I. D., Squire, J. A., Bayani, J., Hide, T., Henkelman, R. M., Cusimano, M. D., and Dirks, P. B. (2004). Identification of human brain tumour initiating cells. Nature *432*, 396-401.

Smith, A. J., Stern, H. S., Penner, M., Hay, K., Mitri, A., Bapat, B. V., and Gallinger, S. (1994). Somatic APC and K-ras codon 12 mutations in aberrant crypt foci from human colons. Cancer Res *54*, 5527-5530.

Smith, G., Carey, F. A., Beattie, J., Wilkie, M. J., Lightfoot, T. J., Coxhead, J., Garner, R. C., Steele, R. J., and Wolf, C. R. (2002). Mutations in APC, Kirsten-ras, and p53--alternative genetic pathways to colorectal cancer. Proc Natl Acad Sci U S A *99*, 9433-9438.

Sodek, J., Ganss, B., and McKee, M. D. (2000). Osteopontin. Crit Rev Oral Biol Med *11*, 279-303.

Somech, R., Izraeli, S., and A, J. S. (2004). Histone deacetylase inhibitors--a new tool to treat cancer. Cancer Treat Rev *30*, 461-472.

Sorof, S. (1994). Modulation of mitogenesis by liver fatty acid binding protein. Cancer Metastasis Rev *13*, 317-336.

Spirio, L., Olschwang, S., Groden, J., Robertson, M., Samowitz, W., Joslyn, G., Gelbert, L., Thliveris, A., Carlson, M., Otterud, B., and et al. (1993). Alleles of the APC gene: an attenuated form of familial polyposis. Cell *75*, 951-957.

Stadelmann, B., Khandjian, E., Hirt, A., Luthy, A., Weil, R., and Wagner, H. P. (1990). Repression of nuclear lamin A and C gene expression in human acute lymphoblastic leukemia and non-Hodgkin's lymphoma cells. Leuk Res *14*, 815-821.

Steen, R. L., and Collas, P. (2001). Mistargeting of B-type lamins at the end of mitosis: implications on cell survival and regulation of lamins A/C expression. J Cell Biol *153*, 621-626.

Stewart, C., and Burke, B. (1987). Teratocarcinoma stem cells and early mouse embryos contain only a single major lamin polypeptide closely resembling lamin B. Cell *51*, 383-392.

Storch, J., and Thumser, A. E. (2000). The fatty acid transport function of fatty acid-binding proteins. Biochim Biophys Acta *1486*, 28-44.

Sullivan, T., Escalante-Alcalde, D., Bhatt, H., Anver, M., Bhat, N., Nagashima, K., Stewart, C. L., and Burke, B. (1999). Loss of A-type lamin expression compromises nuclear envelope integrity leading to muscular dystrophy. J Cell Biol *147*, 913-920.

Taira, T., Sawai, M., Ikeda, M., Tamai, K., Iguchi-Ariga, S. M., and Ariga, H. (1999). Cell cycle-dependent switch of up-and down-regulation of human hsp70 gene expression by interaction between c-Myc and CBF/NF-Y. J Biol Chem *274*, 24270-24279.

Tamai, K., Semenov, M., Kato, Y., Spokony, R., Liu, C., Katsuyama, Y., Hess, F., Saint-Jeannet, J. P., and He, X. (2000). LDL-receptor-related proteins in Wnt signal transduction. Nature *407*, 530-535.

Taniguchi, M., Miura, K., Iwao, H., and Yamanaka, S. (2001). Quantitative assessment of DNA microarrays--comparison with Northern blot analyses. Genomics *71*, 34-39.

ten Dijke, P., Yamashita, H., Sampath, T. K., Reddi, A. H., Estevez, M., Riddle, D. L., Ichijo, H., Heldin, C. H., and Miyazono, K. (1994). Identification of type I receptors for osteogenic protein-1 and bone morphogenetic protein-4. J Biol Chem *269*, 16985-16988.

Tepper, J. E., O'Connell, M. J., Niedzwiecki, D., Hollis, D., Compton, C., Benson, A. B., 3rd, Cummings, B., Gunderson, L., Macdonald, J. S., and Mayer, R. J. (2001). Impact of number of nodes retrieved on outcome in patients with rectal cancer. J Clin Oncol *19*, 157-163.

Thalmann, G. N., Sikes, R. A., Devoll, R. E., Kiefer, J. A., Markwalder, R., Klima, I., Farach-Carson, C. M., Studer, U. E., and Chung, L. W. (1999). Osteopontin: possible role in prostate cancer progression. Clin Cancer Res *5*, 2271-2277.

Tilli, C. M., Ramaekers, F. C., Broers, J. L., Hutchison, C. J., and Neumann, H. A. (2003). Lamin expression in normal human skin, actinic keratosis, squamous cell carcinoma and basal cell carcinoma. Br J Dermatol *148*, 102-109.

Toyota, M., Ahuja, N., Ohe-Toyota, M., Herman, J. G., Baylin, S. B., and Issa, J. P. (1999). CpG island methylator phenotype in colorectal cancer. Proc Natl Acad Sci U S A *96*, 8681-8686.

Tuck, A. B., Hota, C., Wilson, S. M., and Chambers, A. F. (2003). Osteopontin-induced migration of human mammary epithelial cells involves activation of EGF receptor and multiple signal transduction pathways. Oncogene *22*, 1198-1205.

Tuck, A. B., O'Malley, F. P., Singhal, H., Tonkin, K. S., Harris, J. F., Bautista, D., and Chambers, A. F. (1997). Osteopontin and p53 expression are associated with tumor progression in a case of synchronous, bilateral, invasive mammary carcinomas. Arch Pathol Lab Med *121*, 578-584.

Turnbull, R. B., Jr., Kyle, K., Watson, F. R., and Spratt, J. (1967). Cancer of the colon: the influence of the no-touch isolation technic on survival rates. Ann Surg *166*, 420-427.

Tuzi, N. L., and Gullick, W. J. (1994). eph, the largest known family of putative growth factor receptors. Br J Cancer *69*, 417-421.

Twombly, R. (2002). Decoding the cryptic origins of colon cancer. Environ Health Perspect *110*, A394-395.

Tzur, Y. B., Hersh, B. M., Horvitz, H. R., and Gruenbaum, Y. (2002). Fate of the nuclear lamina during Caenorhabditis elegans apoptosis. J Struct Biol *137*, 146-153.

Ueno, H., Hase, K., and Mochizuki, H. (2001). Criteria for extramural perineural invasion as a prognostic factor in rectal cancer. Br J Surg *88*, 994-1000.

Urist, M. R., DeLange, R. J., and Finerman, G. A. (1983). Bone cell differentiation and growth factors. Science *220*, 680-686.

van de Wetering, M., Cavallo, R., Dooijes, D., van Beest, M., van Es, J., Loureiro, J., Ypma, A., Hursh, D., Jones, T., Bejsovec, A., *et al.* (1997). Armadillo coactivates transcription driven by the product of the Drosophila segment polarity gene dTCF. Cell *88*, 789-799.

van de Wetering, M., Sancho, E., Verweij, C., de Lau, W., Oving, I., Hurlstone, A., van der Horn, K., Batlle, E., Coudreuse, D., Haramis, A. P., *et al.* (2002). The beta-catenin/TCF-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. Cell *111*, 241-250.

van den Brandt, P. A., Goldbohm, R. A., van 't Veer, P., Volovics, A., Hermus, R. J., and Sturmans, F. (1990). A large-scale prospective cohort study on diet and cancer in The Netherlands. J Clin Epidemiol *43*, 285-295.

van den Brink, G. R. (2007). Hedgehog Signaling in Development and Homeostasis of the Gastrointestinal Tract
10.1152/physrev.00054.2006. Physiol Rev *87*, 1343-1375.

van den Brink, G. R., Bleuming, S. A., Hardwick, J. C., Schepman, B. L., Offerhaus, G. J., Keller, J. J., Nielsen, C., Gaffield, W., van Deventer, S. J., Roberts, D. J., and Peppelenbosch, M. P. (2004). Indian Hedgehog is an antagonist of Wnt signaling in colonic epithelial cell differentiation. Nat Genet *36*, 277-282.

van den Brink, G. R., and Hardwick, J. C. (2006). Hedgehog Wnteraction in colorectal cancer. Gut *55*, 912-914.

Vantyghem, M. C., Pigny, P., Maurage, C. A., Rouaix-Emery, N., Stojkovic, T., Cuisset, J. M., Millaire, A., Lascols, O., Vermersch, P., Wemeau, J. L., *et al.* (2004). Patients with familial partial lipodystrophy of the Dunnigan type due to a LMNA R482W mutation show muscular and cardiac abnormalities. J Clin Endocrinol Metab *89*, 5337-5346.

Vasen, H. F., Wijnen, J. T., Menko, F. H., Kleibeuker, J. H., Taal, B. G., Griffioen, G., Nagengast, F. M., Meijers-Heijboer, E. H., Bertario, L., Varesco, L., *et al.* (1996). Cancer risk in families with hereditary nonpolyposis colorectal cancer diagnosed by mutation analysis. Gastroenterology *110*, 1020-1027.

Vaughan, A., Alvarez-Reyes, M., Bridger, J. M., Broers, J. L., Ramaekers, F. C., Wehnert, M., Morris, G. E., Whitfield, W. G. F., and Hutchison, C. J. (2001). Both emerin and lamin C depend on lamin A for localization at the nuclear envelope. J Cell Sci *114*, 2577-2590.

Venables, R. S., McLean, S., Luny, D., Moteleb, E., Morley, S., Quinlan, R. A., Lane, E. B., and Hutchison, C. J. (2001). Expression of individual lamins in basal cell carcinomas of the skin. Br J Cancer *84*, 512-519.

Verloes, A., Mulliez, N., Gonzales, M., Laloux, F., Hermanns-Le, T., Pierard, G. E., and Koulischer, L. (1992). Restrictive dermopathy, a lethal form of arthrogryposis multiplex with skin and bone dysplasias: three new cases and review of the literature. Am J Med Genet *43*, 539-547.

Vigouroux, C., Auclair, M., Dubosclard, E., Pouchelet, M., Capeau, J., Courvalin, J. C., and Buendia, B. (2001). Nuclear envelope disorganization in fibroblasts from lipodystrophic patients with heterozygous R482Q/W mutations in the lamin A/C gene. J Cell Sci *114*, 4459-4468.

Vlcek, S., Just, H., Dechat, T., and Foisner, R. (1999). Functional diversity of LAP2alpha and LAP2beta in postmitotic chromosome association is caused by an alpha-specific nuclear targeting domain. Embo J *18*, 6370-6384.

Vlcek, S., Korbei, B., and Foisner, R. (2002). Distinct functions of the unique C terminus of LAP2alpha in cell proliferation and nuclear assembly. J Biol Chem *277*, 18898-18907.

Vogelstein, B., Pardoll, D. M., and Coffey, D. S. (1980). Supercoiled loops and eucaryotic DNA replicaton. Cell *22*, 79-85.

von der Hardt, S., Bakkers, J., Inbal, A., Carvalho, L., Solnica-Krezel, L., Heisenberg, C. P., and Hammerschmidt, M. (2007). The Bmp gradient of the zebrafish gastrula guides migrating lateral cells by regulating cell-cell adhesion. Curr Biol *17*, 475-487.

Wagers, A. J., and Weissman, I. L. (2004). Plasticity of adult stem cells. Cell *116*, 639-648.

Wallimann, T., Wyss, M., Brdiczka, D., Nicolay, K., and Eppenberger, H. M. (1992). Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the 'phosphocreatine circuit' for cellular energy homeostasis. Biochem J *281 (Pt 1)*, 21-40.

Wang, L., Ogburn, C. E., Ware, C. B., Ladiges, W. C., Youssoufian, H., Martin, G. M., and Oshima, J. (2000). Cellular Werner phenotypes in mice expressing a putative dominant-negative human WRN gene. Genetics *154*, 357-362.

Wang, X., Xu, S., Rivolta, C., Li, L. Y., Peng, G. H., Swain, P. K., Sung, C. H., Swaroop, A., Berson, E. L., Dryja, T. P., and Chen, S. (2002). Barrier to autointegration factor interacts with the cone-rod homeobox and represses its transactivation function. J Biol Chem *277*, 43288-43300.

Waterman, M. L. (2004). Lymphoid enhancer factor/T cell factor expression in colorectal cancer. Cancer Metastasis Rev *23*, 41-52.

Wehrli, M., Dougan, S. T., Caldwell, K., O'Keefe, L., Schwartz, S., Vaizel-Ohayon, D., Schejter, E., Tomlinson, A., and DiNardo, S. (2000). arrow encodes an LDL-receptor-related protein essential for Wingless signalling. Nature *407*, 527-530.

Wiese, A. H., Auer, J., Lassmann, S., Nahrig, J., Rosenberg, R., Hofler, H., Ruger, R., and Werner, M. (2007). Identification of gene signatures for invasive colorectal tumor cells. Cancer Detect Prev *31*, 282-295.

Wilkinson, F. L., Holaska, J. M., Zhang, Z., Sharma, A., Manilal, S., Holt, I., Stamm, S., Wilson, K. L., and Morris, G. E. (2003). Emerin interacts in vitro with the splicing-associated factor, YT521-B. Eur J Biochem *270*, 2459-2466.

Willis, N. D., Cox, T. R., Rahman-Casans, S. F., Smits, K., Przyborski, S. A., van den Brandt, P., van Engeland, M., Weijenberg, M., Wilson, R. G., de Bruine, A., and Hutchison, C. J. (2008). Lamin A/C is a risk biomarker in colorectal cancer. PLoS ONE *3*, e2988.

Winston, J. T., Strack, P., Beer-Romero, P., Chu, C. Y., Elledge, S. J., and Harper, J. W. (1999). The SCFbeta-TRCP-ubiquitin ligase complex associates specifically with phosphorylated destruction motifs in IkappaBalpha and beta-catenin and stimulates IkappaBalpha ubiquitination in vitro. Genes Dev *13*, 270-283.

Witt, D. R., Hayden, M. R., Holbrook, K. A., Dale, B. A., Baldwin, V. J., and Taylor, G. P. (1986). Restrictive dermopathy: a newly recognized autosomal recessive skin dysplasia. Am J Med Genet *24*, 631-648.

Worman, H. J., and Courvalin, J. C. (2002). The nuclear lamina and inherited disease. Trends Cell Biol *12*, 591-598.

Worthley, D. L., Whitehall, V. L., Spring, K. J., and Leggett, B. A. (2007). Colorectal carcinogenesis: road maps to cancer. World J Gastroenterol *13*, 3784-3791.

Wu, Z., Wu, L., Weng, D., Xu, D., Geng, J., and Zhao, F. (2009). Reduced expression of lamin A/C correlates with poor histological differentiation and prognosis in primary gastric carcinoma. J Exp Clin Cancer Res *28*, 8.

Yatabe, Y., Tavare, S., and Shibata, D. (2001). Investigating stem cells in human colon by using methylation patterns. Proc Natl Acad Sci U S A *98*, 10839-10844.

Young, L. W., Radebaugh, J. F., Rubin, P., Sensenbrenner, J. A., Fiorelli, G., and McKusick, V. A. (1971). New syndrome manifested by mandibular hypoplasia, acroosteolysis, stiff joints and cutaneous atrophy (mandibuloacral dysplasia) in two unrelated boys. Birth Defects Orig Artic Ser *7*, 291-297.

Yuan, J., Simos, G., Blobel, G., and Georgatos, S. D. (1991). Binding of lamin A to polynucleosomes. J Biol Chem *266*, 9211-9215.

Zarghami, N., Giai, M., Yu, H., Roagna, R., Ponzone, R., Katsaros, D., Sismondi, P., and Diamandis, E. P. (1996). Creatine kinase BB isoenzyme levels in tumour cytosols and survival of breast cancer patients. Br J Cancer *73*, 386-390.

Zastrow, M. S., Vlcek, S., and Wilson, K. L. (2004). Proteins that bind A-type lamins: integrating isolated clues. J Cell Sci *117*, 979-987.

Zeisberg, M., Shah, A. A., and Kalluri, R. (2005). Bone morphogenic protein-7 induces mesenchymal to epithelial transition in adult renal fibroblasts and facilitates regeneration of injured kidney. J Biol Chem *280*, 8094-8100.

Zhang, J. D., Cousens, L. S., Barr, P. J., and Sprang, S. R. (1991). Three-dimensional structure of human basic fibroblast growth factor, a structural homolog of interleukin 1 beta. Proc Natl Acad Sci U S A *88*, 3446-3450.

Zhang, L., Zhou, W., Velculescu, V. E., Kern, S. E., Hruban, R. H., Hamilton, S. R., Vogelstein, B., and Kinzler, K. W. (1997). Gene expression profiles in normal and cancer cells. Science *276*, 1268-1272.

Zheng, R., Ghirlando, R., Lee, M. S., Mizuuchi, K., Krause, M., and Craigie, R. (2000). Barrier-to-autointegration factor (BAF) bridges DNA in a discrete, higher-order nucleoprotein complex. Proc Natl Acad Sci U S A *97*, 8997-9002.

Zhu, P., Martin, E., Mengwasser, J., Schlag, P., Janssen, K. P., and Gottlicher, M. (2004). Induction of HDAC2 expression upon loss of APC in colorectal tumorigenesis. Cancer Cell *5*, 455-463.