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Research Paper

Thioredoxin interacting protein and its association with clinical outcome in gastro-oesophageal adenocarcinoma

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ABSTRACT

The overall prognosis for operable gastro-oesophageal adenocarcinoma remains poor and therefore neoadjuvant chemotherapy has become the standard of care, in addition to radical surgery. Certain anticancer agents (e.g. anthracyclines and cisplatin) generate damaging reactive oxygen species as by-products of their mechanism of action. Drug effectiveness can therefore depend upon the presence of cellular redox buffering systems that are often deregulated in cancer. The expression of the redox protein, thioredoxin interacting protein, was assessed in gastro-oesophageal adenocarcinomas. Thioredoxin interacting protein expression was assessed using conventional immunohistochemistry on a tissue microarray of 140 adenocarcinoma patients treated by primary surgery alone and 88 operable cases treated with neoadjuvant chemotherapy. In the primary surgery cases, high thioredoxin interacting protein expression associated with a lack of lymph node involvement ($p=0.005$), no perineural invasion ($p=0.030$) and well/moderate tumour differentiation ($p=0.033$). In the neoadjuvant tumours, high thioredoxin interacting protein expression was an independent marker for improved disease specific survival ($p=0.002$) especially in cases with anthracycline-based regimes ($p=0.008$). This study highlights the potential of thioredoxin interacting protein as a biomarker for response in neoadjuvant treated gastro-oesophageal adenocarcinoma and may represent a useful therapeutic target due to its association with tumour progression.

Introduction

The overall prognosis for patients with gastro-oesphageal adenocarcinomas remains poor and although radical surgery plays a critical role in the management of operable disease, additional therapy is required to improve patient outcomes. The United Kingdom Medical Research Council Adjuvant Gastric Infusional Chemotherapy (MAGIC) trial demonstrated a 25% reduction in the risk of death and a significant improvement in 5 year survival in patients given neoadjuvant chemotherapy compared to those treated with surgery alone [1]. Similar survival benefits have also been demonstrated in the MRC OEO2 trial of surgical resection with or without neoadjuvant chemotherapy (consisting of two cycles of cisplatin and 5-FU chemotherapy) in oesophageal cancer [2]. A recent meta-analysis from 14 trials has also provided further evidence in favour of neoadjuvant chemotherapy on survival in gastric cancer patients [3]. However, delaying surgery in patients that do not respond to neoadjuvant therapy may have a negative influence on clinical outcome. In addition the role of adjuvant chemotherapy in patients who do not respond to neoadjuvant chemotherapy is unknown.

The generation of free radicals has been associated with the cytotoxicity of conventional chemotherapy agents commonly used in the treatment of gastro-oesophageal cancers i.e. cisplatin and more recently epirubicin. Platinum has been a major advance in improving patient outcome with a response rate in gastro-oesophageal tumours of about 40% [1,4]. Although recognised as causing DNA crosslinking, platinum agents also generate free radicals when mixed in a cell-free system with DNA [5] and interaction with radical scavengers and antioxidants such as thioredoxin, decrease their cytotoxic effects [6,7]. Anthracycline based drugs also generate reactive oxygen species (ROS) when their quinone undergoes

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reduction to a semiquinone free radical, in the presence of oxygen. After dismutation, resultant hydrogen peroxide can be converted into the highly damaging hydroxyl radical. The semiquinone radical can also intercalate and damage DNA [8,9].

Multiple mechanisms maintain the redox state of cells and can protect them from oxidative stress. In cancer, these systems are often deregulated/overexpressed to compensate for the increased oxidative stress caused by the accelerated proliferation, altered metabolic activity and persistent growth promoting signalling of tumours [10,11]. One of the key systems involved is the thioredoxin system, exerting its main function as an antioxidant via thioredoxins interaction with its downstream peroxiredoxins [12,13]. Thioredoxin interacting protein (TxNIP), also known as vitamin D3 upregulated protein-1 (VDUP-1), is best known as a competitive inhibitor for thioredoxin and is down regulated in a number of cancers [14,15]. It has been previously demonstrated, via RT-PCR, that Thioredoxin interacting protein gene expression is significantly lower in colorectal and gastric cancers than in adjacent normal tissues [16].

Thioredoxin interacting proteins importance in anthracycline based chemotherapy response has recently been demonstrated, via immunohistochemistry, in locally advanced primary breast cancer [17]. It was found to be an independent prognostic factor for distant metastasis free survival and overall survival. Therefore, the current study was conducted to examine the prognostic and predictive value of Thioredoxin interacting protein expression in gastro-oesophageal adenocarcinoma patients treated with reactive oxygen species generating chemotherapy agents, including a significant proportion with anthracycline based regimes, and as a comparison, in cases with primary surgery alone.

Materials and methods

Clinical samples

The study is reported according to REMARK criteria [18]. Tissue was obtained from patients treated at Nottingham University Hospitals Trust between 2001 and 2008. The cohort consisted of two sets of patients, 140 adenocarcinoma primary surgery cases that had not been exposed to neoadjuvant treatment and 88 operable adenocarcinoma cases that had at least one cycle of neoadjuvant chemotherapy [19]. During the study period, patients in the neoadjuvant arm were treated with either neoadjuvant ECF [Epirubicin (50 mg/m^2), Cisplatin (60 mg/m^2) and continuous infusional 5-FU (200 mg/m^2 per day)] or ECX [Epirubicin (50 mg/m^2), Cisplatin (60 mg/m^2) and capecetabine (625 mg/m^2 p.o. b.d continuously)] chemotherapy, up to three cycles prior to surgery, or CF [cisplatin (80 mg/m^2) and infusional 5-FU (1000 mg/m^2 daily for 4 days)] chemotherapy up to two cycles prior to surgery. The remaining three patients had either Carbo/F or CX. The tumours analysed in the neoadjuvant arm were from surgical samples post neoadjuvant treatment.

Median follow up was 28.4 and 27.3 months and median time to recurrence was 9.1 and 10.2 months for the neoadjuvant and primary surgery cohorts respectively. Disease specific survival was calculated from the date of diagnosis until 26th November 2010 when any remaining survivors were censored. Tumour regression grade (TRG) was defined as per Mandard's criteria [20]. Tables 1 and 2 show the full clinicopathological characteristics of the patient cohort. The conduct of this study was approved by the Ethics Committee of Nottingham University Hospitals.

Tissue microarray

TMAs were constructed as described previously [21]. In short, area-specialised histopathologists identified and marked representative areas on haematoxylin and eosin stained slides from formalin-fixed paraffin-embedded tumour blocks, for tissue microarray construction. Triplicate tissue cores (0.6 mm) were taken from the marked areas and arrayed into separate recipient paraffin block using a manual tissue-arrayer (Beecher Instruments, Silver Spring, MD, USA).

Table 1
Clinical characteristics of patients.

	Neoadjuvant chemotherapy N (%)	Surgery N (%)
Total number of patients	88	140
Median Age (years)	63	74
Sex		
Male	73 (83)	104 (74)
Female	15 (17)	36 (26)
Site of tumour		
Gastric	20 (23)	127 (91)
GOJ	41 (46)	13 (9)
Lower third of oesophagus	27 (31)	–
Surgery		
Total gastrectomy	22 (25)	69 (49)
Partial gastrectomy	5 (6)	52 (37)
Oesophagectomy/ oesophagogastrectomy	61 (69)	19 (14)
Disease Specific Survival		
Alive	30 (34)	42 (30)
Died due to cancer	48 (55)	52 (37)
Other	10 (11)	46 (33)
Recurrence		
None	39 (44)	86 (61)
Recurrence	49 (56)	53 (38)
Unknown	–	1 (1)

Table 2
Pathological criteria of patients showing association with Thioredoxin interacting protein expression $^{*}=p<0.05$ $^{**}=p<0.01$.

	Neoadjuvant Chemotherapy ($N=88$)		Surgery ($N=140$)	
	N (%)	p Value	N (%)	p Value
T stage				
T0,1,2	26 (30)	0.365	62 (44)	0.113
T3,4	61 (69)		78 (56)	
Tx	1 (1)		–	
N stage				
N0	22 (25)	**0.046***	33 (24)	**0.005****
≥N1	66 (75)		107 (76)	
M stage				
M0/Mx	83 (94)	N/A	138 (99)	N/A
M1	5 (6)		2 (1)	
Overall stage				
1,2	26 (30)	0.484	61 (44)	0.162
3,4	62 (70)		79 (56)	
Tumour regression grade response after chemotherapy				
1,2,3	39 (44)	0.300	34 (24)	0.970
4,5	49 (56)		105 (75)	
Unknown	–		1 (1)	
Vascular invasion				
No	40 (46)	0.404	44 (31)	0.338
Yes	48 (54)		96 (69)	
Perineural invasion				
No	75 (85)	0.469	75 (54)	**0.030***
Yes	13 (15)		65 (46)	
Differentiation of tumour				
well/moderate	42 (48)	0.738	62 (44)	**0.033***
poor	46 (52)		78 (56)	

Immunohistochemistry

Immunohistochemistry was performed as previously described [22] with the following modifications. Microwave antigen retrieval in pre-warmed 0.01 mol/L sodium citrate buffer (pH6) was conducted for 10 min at 450 W. The primary antibody was diluted in blocking solution, and incubated for 60 min at room temperature (Thioredoxin interacting protein 1:3000 (MBL International Corporation, Woburn, USA)). Blocking, secondary and ABC reagents were supplied in kit form from Vector Labs (Universal PK-6200 or Goat PK-6105, DAB SK-4100 (Vector Laboratories, Burlingame, USA)) followed by 3,3'-diaminobenzidine (DAB) substrate (K3468 (Dako, Glostrup, Denmark)). Primary antibody was omitted for negative controls. Gastric tissue and historically used breast composite blocks composed of six stage I breast carcinomas, including grade I, II and III tumours were used for initial antibody optimisations and positive controls. Antibody specificity was assessed by Western blotting on gastric and breast cancer cell lysates prior to commencing the study.

Assessment of expression

Assessment of expression of protein staining was conducted independently by the specialist gastro-intestinal histopathologist

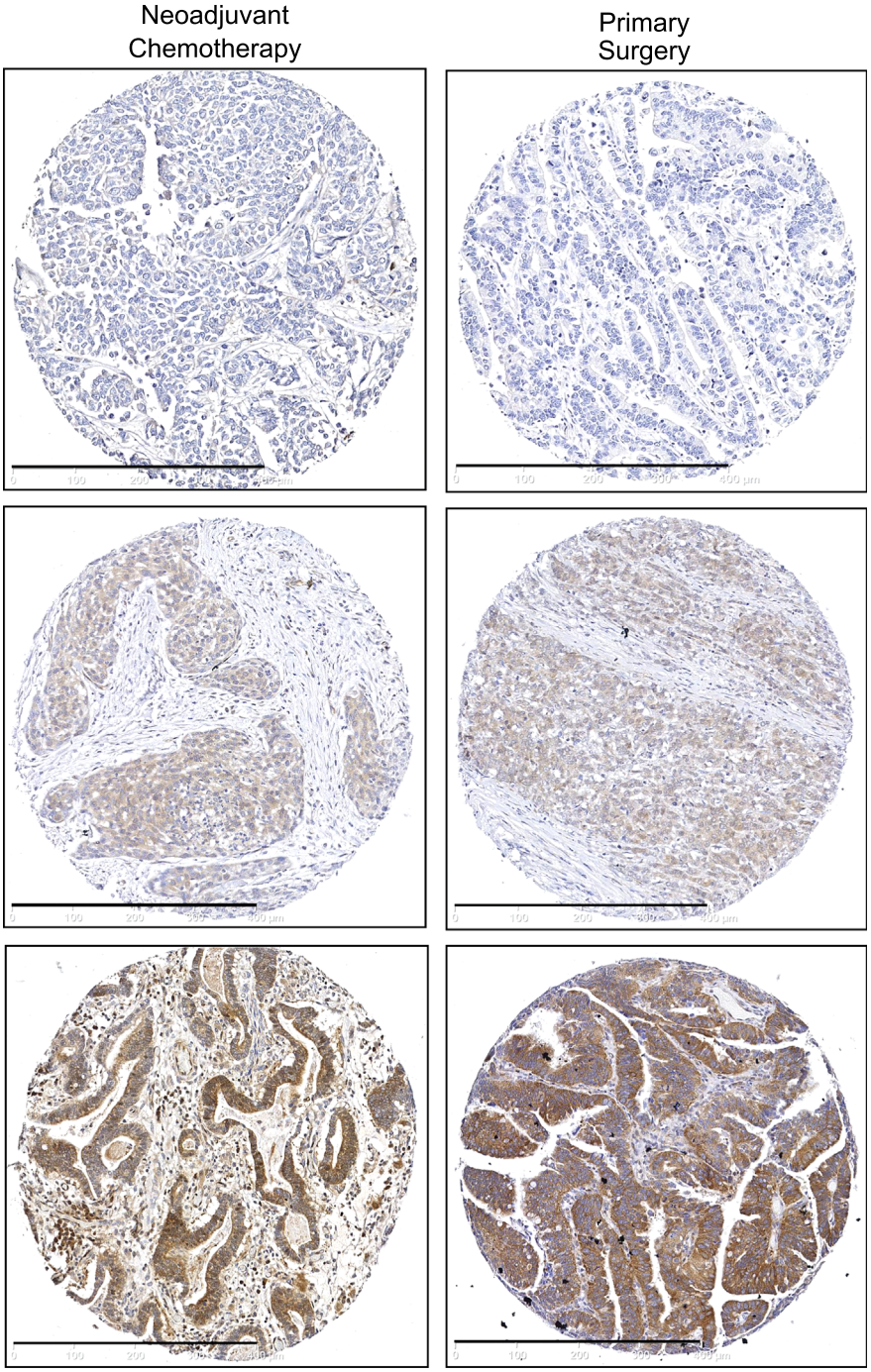

Fig. 1. Photomicrographs of immunohistochemical Thioredoxin interacting protein expression patterns at 10× magnification in tumours after receiving neoadjuvant chemotherapy (left panels) or from primary surgery alone (right panels). Scale bars denote 500 μm.

(IS) and the lead scientist (CW) blinded to the study end points. Staining intensity was divided into four grades, none (0), weak (1) moderate (2) and strong (3). Semiquantitative H scores were calculated by multiplying the percentage of positive tumour cells by the staining intensity, giving a range between 0 and 300. The median H score for the three triplicate cores for each case was then calculated and used in the final assessments. To stratify the cohort of patients into low- vs. high-expressing tumours, the median of the two study populations was calculated and applied (Neoadjuvant 77, Primary surgery 55).

Statistical analysis

SPSS version 15.0 statistical software package was used with patients categorised into two groups, low and high (or negative and positive), depending on whether their staining intensities fell above or below the cut-off values. The Mann Whitney U test was used to examine the Thioredoxin interacting protein expression levels between the primary and neoadjuvant groups. The association between protein expression and clinicopathological criteria was assessed by univariate analysis using a Pearson χ^2 test. Survival analysis comparing protein expression levels with recurrence and overall survival were conducted using Kaplan-Meier curves and the statistical significance evaluated using the log rank test. Cox proportional hazards model was used to test the statistical independence and significance of Thioredoxin interacting protein versus available clinical parameters. Agreement between the two independent observers was assessed using intraclass correlations (0.850).

Results

Thioredoxin interacting protein expression and pathological associations

Fig. 1 demonstrates the diffuse, granular and sometimes heterogeneous cytoplasmic immunostaining for Thioredoxin interacting protein in the tumour samples. In certain tumour cases, there was also heterogeneous staining between the triplicate cores. This had previously been observed in a selection of whole mount sections for this marker but triplicate cores allow for this potential heterogeneity to be represented. From Fig. 2 it can be seen that the levels of Thioredoxin interacting protein expression significantly differed between the treatment approaches when comparing the continuous data from each cohort using the Mann Whitney *U* test ($p=0.007$). There was a higher median of expression in the tumours that had received neoadjuvant chemotherapy (77) than the untreated primary surgery cases (55).

Thioredoxin interacting protein associations in tumours receiving primary surgery only

Table 2 shows the associations between Thioredoxin interacting protein expression and pathological features in the cases that had primary surgery. A significant association was seen with high Thioredoxin interacting protein expression and a lack of lymph node involvement (N stage: X^2 8.027, df=1, $p=0.005$), no perineural invasion (X^2 4.705, df=1, $p=0.030$) and well/moderate tumour differentiation (X^2 4.521, df=1, $p=0.033$).

In the primary surgery cases, no significance was observed between Thioredoxin interacting protein expression and disease specific survival ($p=0.507$) or risk of recurrence ($p=0.722$).

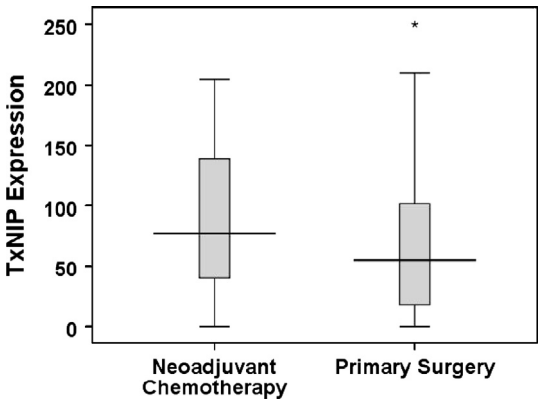

Fig. 2. Stem and Leaf plot demonstrating the differences in expression of Thioredoxin interacting protein (TxNIP) between primary surgery and those that received neoadjuvant chemotherapy. A significantly higher expression was noted in the tumours that had received neoadjuvant chemotherapy compared to the primary surgery cases ($p=0.007$).

Thioredoxin interacting protein associations in tumours receiving neoadjuvant chemotherapy prior to surgery

In the neoadjuvant cohort, Thioredoxin interacting protein only showed association with lymph node involvement (N stage: X^2 3.972, df=1, $p=0.046$), again high expression with a lack of involvement but less significant than in the primary surgery cases (Table 2). No other significant associations were found with pathological features or with risk of recurrence ($p=0.169$).

Fig. 3a shows that a high expression of Thioredoxin interacting protein significantly associates with improved disease specific survival ($p=0.016$) in those patients treated with neoadjuvant chemotherapy. This was analysed against other parameters that were found to be significant under Cox univariate analysis (T stage, N stage, vascular invasion), in a multivariate analysis approach, and Thioredoxin interacting protein was found to be independently associated with outcome ($p=0.002$) (Table 3).

In this cohort, some received chemotherapy containing anthracyclines (ECX/ECF) and some non-anthracycline based (CF/CX/CarboF) treatment. Therefore we examined whether there was a difference in the disease specific survival between these two groups. A high expression of Thioredoxin interacting protein in the anthracycline based treatment group still associated with improved disease specific survival ($p=0.022$) whereas in those treated only with cisplatin and 5-FU, no association was seen ($p=0.561$) (Fig. 3b and c).

Discussion

The results presented in the current study have demonstrated that a high expression level of Thioredoxin interacting protein is an independent determinant of good disease specific survival in gastro-oesophageal patients that have received neoadjuvant chemotherapy (p=0.002). The predictive power of Thioredoxin interacting protein appears more relevant to those neoadjuvant cases that received treatment containing anthracyclines compared to those in the CF treatment group (5-FU/cisplatin) but further validation studies would be required to confirm this. Woolston et al., have previously shown the potentially predictive/prognostic role of redox protein expression in tumours taken pre and post neoadjuvant anthracycline based chemotherapy in locally advanced primary breast cancer patients [17]. High Thioredoxin interacting protein was an independent prognostic factor for an improved disease free- and overall survival as well as a significant increase in expression between paired pre- and post-chemotherapy tumour samples.

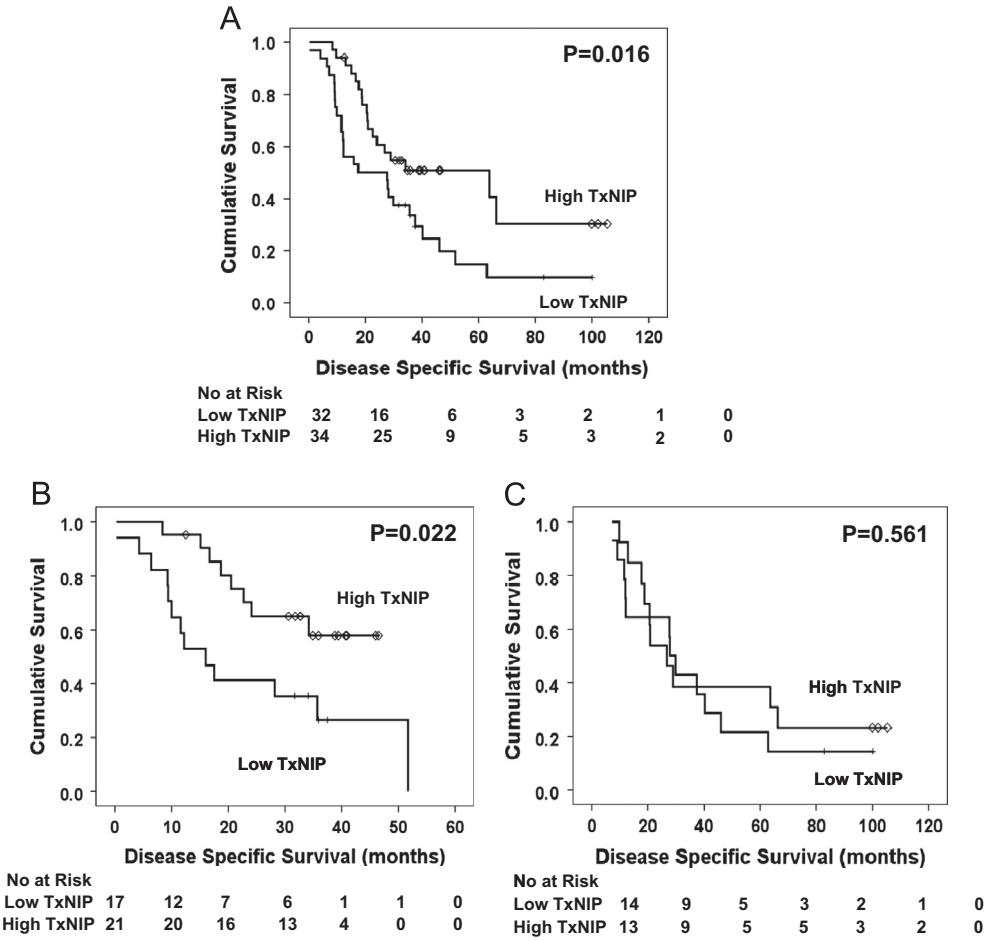

Fig. 3. Kaplan Meier survival curves. (A) High expression of Thioredoxin interacting protein (TxNIP) significantly associated with a better disease specific survival ($p=0.016$). This was more apparent in the cases that had (B) anthracyclines included in their neoadjuvant treatment regime ($p=0.022$) compared to those (C) without ($p=0.561$).

Table 3
Cox proportional hazards analysis for disease specific survival in the neoadjuvant treated patients and anthracycline based neoadjuvant treated patients. HR=Hazard ratio, CI=Confidence Interval, *=$p<0.05$.

	Neoadjuvant treated patients		
	Thioredoxin interacting protein		
	Hazard ratio	95% CI	Significance
Protein	0.4	0.2–0.7	0.002*
T stage	3.0	1.1–7.8	0.0.28*
N stage	3.0	1.3–6.7	0.009*
Vascular invasion	2.5	1.3–5.0	0.009*
	Anthracycline based patients		
	Thioredoxin interacting protein		
	Hazard Ratio	95% CI	Significance
Protein	0.3	0.1–0.7	0.008*
T stage	8.4	1.0–66.6	0.045*
N stage	2.2	0.7–6.9	0.159
Vascular invasion	2.5	0.7–8.1	0.136

Lim et al., demonstrated that a low Thioredoxin and high Thioredoxin interacting protein gene expression in 68 stage III gastric cancer cases had a better recurrence free survival. In 85% of gastric cancers that they went on to examine by immunohistochemistry, 85% under expressed Thioredoxin interacting protein compared to normal tissues with a tendency for this under expression to occur in two poor prognostic groups: diffuse histology and high stage [23].

It may well be that it is the differences in tumour site between groups (i.e. 23 vs. 91% gastric) that is influencing the expression differences seen between the neoadjuvant and primary surgery groups; however, 5-FU could also be contributing to the expression differences. 5-FU upregulated Thioredoxin interacting protein levels in a gene expression study [24] and is known to trigger reactive oxygen species generation through as yet undetermined mechanisms [25]. 5-FU also decreased Focal adhesion kinase protein expression and up-regulated Thioredoxin interacting protein expression in 293 kidney cells [26]. The higher overall expression of Thioredoxin interacting protein seen in tumours examined after neoadjuvant therapy compared to primary surgery ($p=0.007$) may be indicative of redox pathways being activated in response to therapy but further work is required in this area, potentially by examining the expression of TxNIP in normal tissue at different sites from which the tumours arise.

The effects of Thioredoxin interacting protein could be via thioredoxin inhibition, allowing the reactive oxygen species generated by the cytotoxic agents to exert their damaging effects. An increased level of reactive oxygen species has been observed in fibroblasts with increased levels of Thioredoxin interacting protein [27] and preventing thioredoxin interacting with PAG or ASK-1 can sensitise cells to oxidative stress [28]. However, in contrast, gene silencing of Thioredoxin interacting protein in melanoma cells reduced reactive oxygen species production [29] suggesting a potential tumour specific effect. In gastric cancer, primary tumours expressing high levels of thioredoxin have been shown to have a

higher proliferative rate and lower rate of spontaneous cell death [30] and knockout of Thioredoxin interacting protein in a mouse model induced Helicobacter pylori related gastric cancer [31]. Forced expression of Thioredoxin interacting protein in stomach cancer and promyelocytic leukaemia cell lines inhibited their proliferation [14]. Thioredoxin interacting protein is also regulated by class II HDACs such as HDAC10. HDAC10 transcriptional down-regulation of Thioredoxin interacting protein leads to altered reactive oxygen species signalling in human gastric cancer cells [32]. It appears, from such reports and from current results, that indirect modulation of Thioredoxin interacting protein by agents such as HDAC inhibitors may represent a promising therapeutic approach.

In the primary surgery cohort of patients, although not significant for survival or recurrence, Thioredoxin interacting protein did show significant association with prognostic features such as lymph node involvement (N stage $p=0.005$), perineural invasion ($p=0.030$) and differentiation status of the tumour ($p=0.033$). M stage cases were not included in the analyses as only 6% of primary surgery cases and 1% of neoadjuvant cases had distant metastases reported. Thioredoxin interacting protein has been demonstrated to suppress tumour growth and metastasis in transplantation models [33] and suppress cell invasiveness and tumour metastases of melanoma B16-F10 in in vivo models possibly via its association with the β domain of von Hippel-Lindau protein [34]. Loss of Thioredoxin interacting protein has been demonstrated to be enough to induce malignancy, forming hepatocellular carcinomas in mice [35]. In the case of differentiation, Thioredoxin interacting protein is thought to play a role in epithelial differentiation in the GI tract [36] with Thioredoxin expression predominantly seen in undifferentiated gastric cancers [37]. Such reports support current findings where high Thioredoxin interacting protein expression associates with better tumour differentiation, lack of lymph node involvement and no perineural invasion.

In summary, this study highlights the potential of Thioredoxin interacting protein as a biomarker for response to reactive oxygen species generating chemotherapy agents used in the neoadjuvant setting in gastro-oesophageal adenocarcinoma. It also indicates its role in tumour progression through its associations with pathological criteria.

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References

[1] D. Cunningham, W.H. Allum, S.P. Stenning, et al., Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer, New England Journal of Medicine 355 (2006) 11–20.
[2] Medical Research Council Oesophageal Cancer Working Group, Surgical resection with or without preoperative chemotherapy in oesophageal cancer: a randomised controlled trial, Lancet 359 (2002) 1727–1733.
[3] W. Li, J. Qin, Y.-H. Sun, T.-S. Liu, Neoadjuvant chemotherapy for advanced gastric cancer: a meta-analysis, World Journal of Gastroenterology 16 (2010) 5621–5628.
[4] D. Cunningham, N Starling, S. Rao, et al., Capecita bine and oxaliplatin for advanced esophagogastric cancer, New England Journal of Medicine 358 (2008) 36–46.
[5] H. Masuda, T. Tanaka, U. Takahama, Cisplatin generates superoxide anion by interaction with DNA in a cell-free system, Biochemical and Biophysical Research Communications 203 (1994) 1175–1180.
[6] K. Sugihara, M. Gemba, Modification of cisplatin toxicity by antioxidants, Japanese Journal of Pharmacology 40 (1986) 353–355.
[7] T. Sasada, H. Nakamura, S. Ueda, et al., Secretion of thioredoxin enhances cellular resistance to cis-diamminedichloroplatinum(II), Antioxid Redox Signaling 2 (2000) 695–705.
[8] I. Muller, D. Niethammer, G. Bruchelt, Anthracycline-derived chemotherapeutics in apoptosis and free radical cytotoxicity (review), International Journal of Molecular Medicine 1 (1998) 491–494.
[9] A Ravid, D. Rocker, A. Machlenkin, et al., 1,25-Dihydroxyvitamin D3 enhances the susceptibility of breast cancer cells to doxorubicin-induced oxidative damage, Cancer Research 59 (1999) 862–867.
[10] J.D. Pennington, T.J. Wang, P. Nguyen, et al., Redox-sensitive signalling factors as a novel molecular targets for cancer therapy, Drug Resistance Updates 8 (2005) 322–330.
[11] M. Valko, D. Leibfritz, J. Moncol, M.T.D. Cronin, M. Mazur, J. Telser, Free radicals and antioxidants in normal physiological functions and human disease, International Journal of Biochemistry and Cell Biology 39 (2007) 44–84.
[12] A. Mukherjee, S.G. Martin, The thioredoxin system: a key target in tumour and endothelial cells, British Journal of Radiology 81 (2008) S57–S68.
[13] CM Woolston, S.J. Storr, I.O. Ellis, D.A. Morgan, S.G. Martin, Expression of thioredoxin system and related peroxiredoxin proteins is associated with clinical outcome in radiotherapy treated early stage breast cancer, Radiotherapy Oncology 100 (2011) 308–313.
[14] S.H. Han, J.H. Jeon, H.R. Ju, et al., VDUP1 upregulated by TGF-β1 and 1,25-dihydroxyvitamin D3 inhibits tumour cell growth by blocking cell-cycle progression, Oncogene 22 (2003) 4035–4046.
[15] L.M. Butler, X. Zhou, W.S. Xu, et al., The histone deacetylase inhibitor SAHA arrests cancer cell growth, up-regulates thioredoxin-binding protein-2, and down-regulates thioredoxin, Proceedings of the National Academy of Sciences USA 99 (2002) 11700–11705.
[16] M. Ikarashi, Y. Takahashi, Y. Ishii, T. Nagata, S. Asai, K. Ishikawa, Vitamin D3 up-regulated protein 1 (VDUP1) expression in gastrointestinal cancer and its relation to stage of disease, Anticancer Research 22 (2002) 4045–4048.
[17] C.M. Woolston, L. Zhang, S.J. Storr, et al., The prognostic and predictive power of redox protein expression for anthracycline-based chemotherapy response in locally advanced breast cancer, Modern Pathology (2012), http://dx.doi.org/10.1038/modpathol.2012.60.
[18] L.M. McShane, D.G. Altman, W. Sauerbrei, S.E. Taube, M. Gion, G.M. Clark, Statistics Subcommittee of the NCI-EORTC Working Group on Cancer Diagnostics. Reporting recommendations for tumour marker prognostic studies, Journal of Clinical Oncology 23 (2005) 9067–9072.
[19] S.J. Storr, X. Pu, J. Davis, D. Lobo, et al., Expression of the calpain system is associated with poor clinical outcome in gastro-oesophageal adenocarcinomas, Journal of Gastroenterology (2013), PMID:23329366 [Epub ahead of print].
[20] A.M. Mandard, F. Dalibard, J.C. Mandard, et al., Pathologic assessment of tumour regression after preoperative chemoradiotherapy of esophageal carcinoma. Clinicopathologic correlations, Cancer 73 (1994) 2680–2686.
[21] J. Kononen, L. Bubendorf, A. Kallioniemi, et al., Tissue microarrays for high-throughput molecular profiling of tumour specimens, Nature Medicine 4 (1998) 844–847.
[22] C.M. Woolston, S. Deen, A. Al-Attar, M. Shehata, S.Y. Chan, S.G. Martin, Redox protein expression predicts progression-free and overall survival in ovarian cancer patients treated with platinum-based chemotherapy, Free Radical Biology and Medicine 49 (2010) 1263–1272.
[23] J.Y. Lim, S.O. Yoon, S.W. Hang, J.W. Kim, S.H. Choi, J.Y. Cho, Thioredoxin and thioredoxin-interacting protein as prognostic markers for gastric cancer recurrence, World Journal of Gastroenterology 18 (2012) 5581–5588.
[24] Y. Takahashi, T. Nagata, Y. Ishii, M. Ikarashi, K. Ishikawa, S. Asai, Up-regulation of vitamin D3 up-regulated protein 1 gene in response to 5-fluorouracil in colon carcinoma SW620, Oncology Reports 9 (2002) 75–79.
[25] I.T. Hwang, Y.M. Chung, J.J. Kim, et al., Drug resistance to 5-FU linked to reactive oxygen species modulator 1, Biochemical and Biophysical Research Communications 359 (2007) 304–310.
[26] B Ho, G Huang, VM. Golubovskaya, Focal adhesion kinase regulates expression of thioredoxin-interacting protein (TxNIP) in cancer cells, Anticancer Agents in Medicinal Chemistry (2013), PMID:23387972 [Epub ahead of print].
[27] J. Yoshioka, P.C. Schulze, M. Cupesi, et al., Thioredoxin-interacting protein controls cardiac hypertrophy through regulation of thioredoxin activity, Circulation 109 (2004) 2581–2586.
[28] E. Junn, S.H. Han, J.Y. Im, et al., Vitamin D3 up-regulated protein 1 mediates oxidative stress via suppressing the thioredoxin function, Journal of Immunology 164 (2000) 6287–6295.
[29] H. Song, D. Cho, J.H. Jeon, et al., Vitamin D(3) up-regulating protein 1 (VDUP1) antisense DNA regulates tumorigenicity and melanogenesis of murine melanoma cells via regulating the expression of fas ligand and reactive oxygen species, Immunology Letters 86 (2003) 235–247.
[30] T.M. Grogan, C. Fenoglio-Prieser, R. Zeheb, et al., Thioredoxin, a putative oncogene product, is overexpressed in gastric carcinoma and associated with increased proliferation and increased cell survival, Human Pathology 31 (475) (2000) 481.
[31] H.J. Kwan, Y.S. Won, K.T. Nam, et al., Vitamin D3 upregulated protein 1 deficiency promotes N-methyl-N-nitrosourea and Helicobacter pylori-induced gastric carcinogenesis in mice, Gut 61 (2012) 53–63.
[32] J.H. Lee, E.G. Jeong, M.C. Choi, et al., Inhibition of histone deacetylase 10 induces thioredoxin-interacting protein and causes accumulation of reactive oxygen species in SNU-620 human gastric cancer cells, Molecules and Cells 30 (2010) 107–112.

[33] S.F. Goldberg, M.E. Miele, N. Hatta, et al., Melanoma metastasis suppression by chromosome 6: evidence for a pathway regulated by CRSP3 and TXNIP, Cancer Research 63 (2003) 432–440.
[34] D. Shin, J.H. Jeon, M. Jeong, et al., VDUP1 mediates nuclear export of HIF1α via CRM1-dependent pathway, Biochimica et Biophysica Acta 1783 (2008) 838–848.
[35] S.S. Sheth, J.S. Bodnar, A. Ghazalpour, et al., Hepatocellular carcinoma in Txnip-deficient mice, Oncogene 25 (2006) 3528–3536.
[36] Y Takahashi, Y. Ishii, A. Murata, T. Nagata, S. Asai, Localization of thioredoxin-interacting protein (TxNIP) mRNA in epithelium of human gastrointestinal tract, Journal of Histochemistry and Cytochemistry 51 (2003) 973–976.
[37] N. Noda, A. Ochiai, K. Miyazaki, T. Sugimura, M. Terada, H. Wakasugi, Detection of thioredoxin in gastric cancer: association with histological type, Antioxidants and Redox Signaling 2 (2000) 519–528.