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Determining the Effects of CD151 and β_1 on Tumor Cell Adhesion and Migration

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Determining the Effects of CD151 and β_1 on Tumor Cell Adhesion and Migration

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THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in Chemical Engineering in the
College of Engineering at the University of Kentucky

By
Rachel Essex
Lexington, Kentucky
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ABSTRACT OF THESIS

DETERMING THE EFFECTS OF CD151 AND β_1 ON TUMOR CELL ADHESION

Previous studies have shown that the upregulation of CD151 and β_1 is associated with poor prognosis in many cancers such as breast cancer. Studies have provided evidence that these proteins are associated with the adhesion and migration of tumor cells. In this study, a microfluidic flow chamber was utilized to determine how CD151 and β_1 affected the firm and initial adhesion of metastatic breast cancer cells to a planar endothelial monolayer under shear stress. This system mimicked the adhesion of metastatic breast cancer cells to the endothelial cells of the circulatory system. CD151 and β_1 increased the firm adhesion of metastatic breast cancer cells onto an endothelial monolayer when subjected to high shear stresses. CD151 and β_1 increased the initial adhesion of metastatic breast cancer cells onto an endothelial monolayer. A transwell assay was utilized to determine how CD151 and β_1 affected random migration through different matrixes and random transendothelial migration. CD151 and β_1 decreased the random migration of metastatic breast cancer cells through matrices. Additionally, background information is provided related to the metastatic cascade, how it can be modeled with microfluidics, and how CD151 and β_1 have been known to effect cancer and metastasis.

KEYWORKDS: metastasis, microfluidics, tumor cell adhesion, transwell assay, MDA-MB-231 metastatic breast cancer cells, HUVEC endothelial cells

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Chapter 1: Introduction

Despite the fact that Richard Nixon declared the war on cancer in 1971, cancer remains to be one of the leading causes of death. In particular, cancer metastasis remains poorly understood[1]. One important aspect of understanding the metastatic cascade is to identify key surface proteins that are involved in the process. In the past decades, CD151 and β_1 shown to be upregulated in many cancer types such as breast, lung, and prostate and have been correlated with poor prognosis[2, 3]. However, how these proteins affect metastasis remains poorly understood[4-7]

This is due to the use of animal models and traditional models that do not mimic the physiological conditions in humans. In recent years, microfluidic models have been utilized to mimic the conditions of the human body. These models implement human cells while maintaining shear that physiologically representative unlike traditional models[8].

In the studies described here, a microfluidic flow chamber produced by Ibidi was utilized to study the adhesion of breast cancer cells to an endothelial monolayer. To study random migration, a Boyden chamber, more commonly known as a transwell assay, was utilized. The specific objects here were:

1) To determine if CD151 and β_1 decrease the firm adhesion of metastatic breast cancer cells onto an endothelial monolayer
2) To determine if CD151 and β_1 decrease the initial adhesion of metastatic breast cancer cells to an endothelial monolayer.

3) To determine how CD151 and β_1 affect the random migration of metastatic breast cancer cells through either gelatin or basement membrane extract both when a endothelial monolayer was present or absent.

Chapter 2: Background

2-1 The Metastatic Cascade

While cancer is the second leading cause of death in the United States, only ten percent of those deaths are caused by the primary tumor[9, 10]. The remaining ninety percent is caused by secondary metastases which are difficult to treat and detect [10, 11]. These secondary tumors are formed after a tumor cell has undergone the metastatic cascade which can be viewed in Figure 2-1. The first step in the metastatic cascade is the process of angiogenesis which is the vascularization of a tumor which is triggered by the upregulation of angiogenic factors and the down regulation of inhibitors of vessel growth[4, 12]. The tumor can be avascular for many years at which time it does not grow beyond a millimeter in diameter nor do metastases occur. However, once vascularization occurs, the tumor can grow quickly in size because it now has necessary nutrients and oxygen, and the tumor cells increase their ability to metastasize because of the close proximity to the vascular[13-15]. Evidence of angiogensis' role in metastasis can be found in studies that demonstrate that the occurrence of metastases in distant sites is correlated with the count of microvessels. In these studies, 49 patients with invasive breast carcinoma had specimen removed, and it was found that with each 10-microvessel increase in the 200 x field, there was a 1.59-fold increase in the chance of metastasis occurring. Once the vessel count exceeded 100 in the 200 x field, 100 percent of patients had secondary metastases[16]. It is also hypothesized that the release of degenerative enzymes during angiogenesis aids the escape of cancer cells from the primary tumor[15, 16].

The next step in the metastatic cascade is the tumor cell undergoing the epithelial to mesenchymal transition (EMT) in which the tumor cell will transition from an epithelial cell to a mesenchymal type cell[4]. There are three instances in which a cell can undergo the EMT. The first is during implantation, embryogenesis, and the development of organs in a fetus. The second is associated with tissue regeneration and organ fibrosis. The final instance is associated with cancer progression and metastasis. It is believed that EMT-inducing signals such as HGF, EGF, and TGF-β are responsible for the induction and activation of a series of transcription factors that control the EMT program[17]. The loss of E-cadherin in particular is vital to the process and leads to dramatic changes in the cells including a loss of intercellular adhesion ability which allows for detachment from the primary tumor, and the morphological change to a motile phenotype. The expression of matrix metalloproteinase during this transition aid in the digestion of laminin and collagen IV which are key components in the basement membrane. Changes to the matrix will then trigger a positive feedback loop which enhances a cell's ability to proliferate and invade the matrix[4].

After a tumor cell undergoes the EMT and detaches from the primary tumor, it can migrate to the vasculature where it can enter through the process of intravasation. After the cell intravasates, the tumor cell can circulate throughout the body. How a secondary site is located can be explained by two hypotheses. The first is the seed and soil hypothesis which was introduced by Paget in 1889. This hypothesis compares the tumor cell to a seed and the secondary site to the soil. He proposed that tumor cells preferred microenvironments in which they were most compatible in. He analyzed over 900 autopsy records of cancer patients to justify this hypothesis. Forty years after the

introduction of the seed and soil hypothesis, Ewing proposed the mechanical hypothesis which states that the secondary site is selected when a tumor cell reaches a site in the vascular that diameter is smaller than the diameter of the cell. This process of getting lodged in the vasculature is physical occlusion. In the 1970s, research was performed showing that secondary sites are selected by a combination of these two events[4, 18]. In the studies described here, physical occlusion was not taken into account. Rather, the steps of interest are those that take place when the secondary site is determined by the seed and soil hypothesis.

If the tumor cell survives the physical stress of the circulatory system, it will come into contact with the vessel wall at which point it will start rolling on the wall. This will occur in vessels with a diameter larger than the diameter of the tumor cell. The tumor cell can interact with the endothelial cells that compose the lining of the vasculature, and the tumor cell can adhere to an endothelial cell. This cell's ability to adhere will depend on a variety of factors including the resident time which is depended on the shear force exercised on the cell and the adhesive forces on the ligand-receptor pairs between the cells. It is also depended on the frequency of the cell colliding with the membrane-bound receptors and the endothelial ligands[4].

The next step in the metastatic cascade is extravasation which is the rate limiting step[19]. In this step, the cancer cell migrates through the endothelial lining of the vessel and penetrates the supporting extracellular matrix. It is widely believed that the CXC-chemokine ligand 12 (CXCL12) is associated with extravasation. This chemokine is secreted by stromal cells in distant organs and attract cells with the CXCR4 and CXCR7

receptors[20]. However, the complete mechanism of extravasation remains relatively not understood.

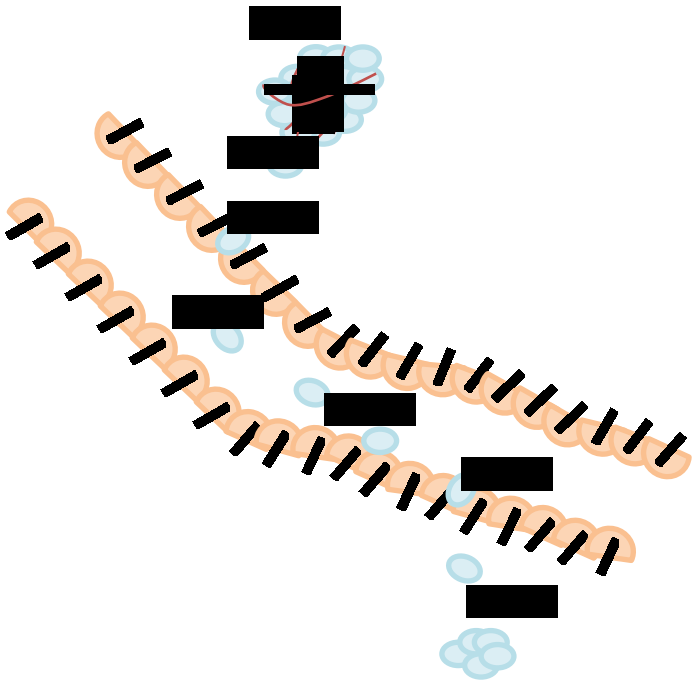

Figure 2-1: The steps of the metastatic cascade. A.) First, angiogenesis, or the vascularization of the tumor must occur. B) Angiogensis is followed by EMT and detachment from primary tumor. C.) After detachment, the tumor cell can then invade the circulatory system by a process called intravasation. D.)The tumor cell will then circulate in vasculature. E.) Once contact has been made, the tumor cell can then adhere to vessel wall. F.) After the tumor cell has adhered, it will then migrate through the endothelial monolayer and penetrate the supporting matrix through a process called extravasation. G.) After exiting the vasculature, a tumor cell can form a secondary tumor.

2-2 Microfluidic Models

2-2.1 Pitfalls of Traditional Models

The main reason that the metastatic cascade is still poorly understood is because of the models that have been utilized to study it. Traditional *in vivo* models such as rat and mouse models have poor imaging capabilities which makes it impossible to determine if tumor cell adhesion or tumor cell extravasation is the step that is being mainly effected by the protein or chemoattractant of interest because it is impossible to visualize the arrest of the tumor cell on the endothelial lining[20, 21]. An alternative to the tradition rat and mouse model is using a zebrafish whose endothelium are transparent. However, it is still impossible to preform tightly controlled parametric studies. It is also impossible to directly relate the effects to humans because the endothelial cells composing the vessels are not human cells, and therefore, the surface expression of proteins is not identical to that seen in humans. This leads to different tumor-endothelial cell interactions than those in humans[8, 20].

The most common *in vitro* studies are static well plate studies. For adhesion studies, endothelial cells would be seeded into the wells and allowed to grow to confluence. Then, the endothelial cells are stimulated to express adhesion molecules before cancer cells are allowed to settle on the endothelial cells. After a given settling time, the cells are then subjected to shear through a series of washes[22, 23]. The problems with this assay is that the shear from the washes would not be physiologically representative, constant, known, nor consistent[23, 24]. It is also impossible to view the cells undergoing detachment[23, 25]. Finally, only firm adhesion can be examined[25].

To adapt a well plate in order to study migration and extravasation, transwell inserts, also known as a Boyden chamber, are added. These are inserts that have a membrane on the bottom. Endothelial cells can be grown directly on the membrane or the membrane can be coated with a matrix such as Basement Membrane Extract onto which endothelial cells can be seeded. Cancer cells are then placed in the transwell, and a chemoattractant in the bottom of the chamber. The number of cancer cells that migrate through the membrane to the bottom of the well can then be counted[20, 26]. However, it is impossible to tightly-regulate the local micro-environment nor can studies be performed under flow[27, 28]. Finally, the Boyden chamber only allows extravasation to be visualized in a plane parallel to the endothelial monolayer making it impossible to visualize the cell to cell interactions[27, 29]. While these studies help understand trends in extravasation, these limitations must be kept in mind when they are being utilized.

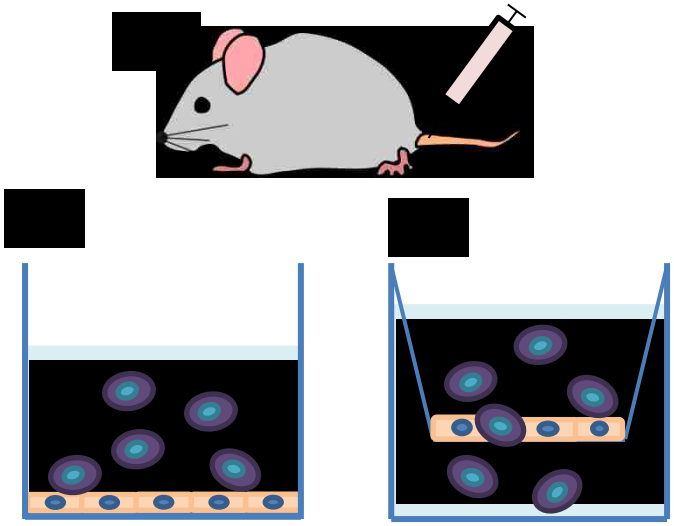

Figure 2-2: Common models to study metastasis. A.) Tail vein injections into mouse and rat models. B.) Static well plate studies in which cancer cells are allowed to adhere to the monolayer. C.) Transwell assays in which cancer cells are allowed to migrate through the endothelial monolayer into a bottom chamber.

2-2.2 Benefits of Microfluidic Models

In recent years, microfluidic models have been utilized to address many of these concerns. The assay that inspired the design of the earliest microfluidic models was the parallel plate flow chamber which was introduced in 1987[30]. This system, which can be viewed in Figure 2-3(a), was novel because it could be connected to a pump which would provide a stable and known shear making much more representative of the physiological conditions than traditional static studies. It consisted of a permanox slide which endothelial cells were grown on, a silastic gasket which separated the slide from the base, and two polycarbonate bases. One of the bases had an entry and an exit that connected to a flow system.

In 2000, Moss et al. preformed work utilizing a parallel plate flow chamber to study the initial adhesion of breast cancer cells to an endothelial monolayer. Flow studies were performed in which cancer cells were flowed into the chamber and their ability to adhere to the monolayer was examined. Studies were performed in which the monolayer was stimulated with TNF-α which were compared to studies without stimulation. The two shear stresses chosen for studies was 0.25 and 1.0 dyne/cm^2. The maximum amount of adhesion occurred at 0.25 dyne/cm^2 with a stimulated monolayer, and no adhesion was viewed at 1.0 dyne/cm^2 without stimulation[31].

Moss et al. continued their work on adhesion by examining initial attachment versus firm attachment. For these studies, a metastatic and nonmetastatic breast cancer cell line were compared. For the initial adhesion studies, it was found that only at high shear stresses is do metastatic cells adhere more to the endothelial monolayer. For studies in which firm attachment was examined, there was no difference in detachment from the monolayer

except at 5 dyne/cm^2 where the metastatic cells were more adherent, and at 10 dyne/cm^2 where the nonmetastatic cancer cells were more adherent[25].

However, with the invention of microfabrication technology such as photolithography, micro-scale flow chambers were able to be produced. These micro-scale, called microfluidic, flow chambers were better able to reproduce physiological conditions, and they required less resources due to their small size[32].

Examples of commercially produced microfluidic flow chambers are those produced by Ibidi. One of special interest is the Ibidi μ-Slide VI$^{0.4}$ which can be viewed in Figure 2-3(b). Each slide contains six channels for flow experiments. The ability to perform multiple experiments on a single slide makes it more resource friendly than the parallel plate flow chamber in which only one study could be performed per permanox slide. Each slide is sterile and treated with a cell culture coating which increases the consistency of the experiments. As can be seen in the comparison in Figure 2-3, unlike the parallel plate flow chamber, the Ibidi slide is also a single piece so the height of the chamber will be constant and there is no possibility of leakage.

A.

B.

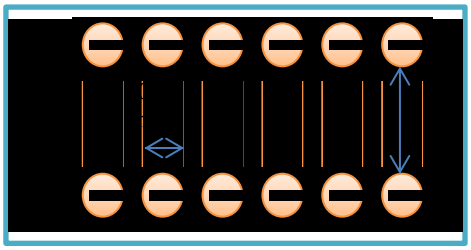

Figure 2-3: Comparison of a parallel plate flow chamber (A.) and the Ibidi µ-slide VI$^{0.4}$ (B.).

2-2.3 Determining Shear Stress in a Microfluidic Flow Chamber

Shear force is defined by de Nevers as the force which tends to make one surface slide parallel to an adjacent force. To determine the shear stress on the surface, the force is divided by the area that is in contact as shown in Equation 1. While solids can resist these shear forces, fluids cannot. Once subject to a shear force, a fluid will move and keep moving as long as the force is applied.

$$\tau = \frac{F}{A} \tag{1}$$

For Newtonian fluids, the shear stress can be described by Equation 2 which is Newton's law of viscosity. This law states that the shear stress, τ, is linearly proportional to the velocity gradient which is represented by $\frac{\partial u}{\partial y}$. The slope of the line is the dynamic viscosity μ. Fluids that do not follow this behavior are non-Newtonian fluids[33].

$$\tau = \mu \frac{\partial u}{\partial y} \tag{2}$$

The shear stress through a vessel that is inelastic, cylindrical, and straight can be computed by the Haagen-Posseuille equation in which Q is the volumetric flowrate and d is the diameter of the vessel. The Haagen-Posseuille equation is Equation 3[34].

$$\tau = 32\mu \frac{Q}{\pi d^3} \tag{3}$$

The channel in the Ibidi μ-slide is rectangular rather than cylindrical. Therefore, a hydrolic radius must be used. The hydraulic radius is allows a noncircular channel to be approximated as a cylindrical channel. The equation for the hydraulic radius is shown in Equation 4[33].

$$HR = {(area\ perpendiucular\ to\ the\ flow)}\Big/{(wetted\ perimeter)} \quad (4)$$

Ibidi provided the equation used to calculate shear stress through the channel of the Ibidi μ-slide which is shown in Equation 5. The constant includes the hydraulic radius. The dynamic viscosity, μ, must be in the units of $\frac{dyne \cdot s}{cm^2}$ and the flow rate, Q, must in in the units of mL/min. The resulting shear stress is in the units of dyne/cm^2.

$$\tau = 176.1\mu Q \quad (5)$$

2-3 CD151 and Cancer

CD151 is a member of the tetraspanin family of proteins. These proteins are named for the fact that they contain four transmembrane domains. In addition to these transmembrane domains, their structure contains a short extracellular and an intracellular loop and a large extracellular loop. This large extracellular loop contains three α-helices and a variable region which contains protein-protein interaction sites. This protein can be viewed in Figure 2-4. Tetraspanins regulate a variety of cell functions including morphology, motility, and invasion[2]. In humans, CD151 is expressed by a variety of cells including nearly all epithelial, endothelial, and fibroblastic cells[35]. Its importance can be demonstrated that if a mutation of the gene for CD151 occurs, the result is a variety of disorders including a skin blistering disease, kidney disruption, and sensorineural deafness[36].

CD151 was the first tetraspanin that was associated with cancer, and since this discovery, it has been found to participate in almost all stages of cancer progression and the formation of metastases[37, 38]. Its upregulation has been associated with many cancer types including breast, lung, endometrial, and prostate[5-7, 39, 40]. This upregulation is correlated with a poor prognosis[38]. One potential reason that CD151 could play a major role in progression of cancer is because it associates tightly with the integrins $\alpha_3\beta_1$, $\alpha_6\beta_1$, $\alpha_6\beta_4$, and $\alpha_7\beta_1$ which interact with laminin, a key component of the extracellular matrix. This interaction also allows CD151 to modulate integrin-dependent functions of the cell such as migration, signaling, and adhesion strengthening all of which play roles in the progression of cancer[2].

Yue et al. performed *in vivo* assays with BDX rats , a variation of the albino rat, in which the rats were injected with $1x10^6$ tumor cells. While when both control ASML, a highly metastatic mouse cancer cell line, and ASML-CD151 kd cells were injected thousands of metastasis formed, there was a delayed metastatic growth of ASML-CD151 kd cells compared to the controls in the lymph nodes. This indicates that CD151 is not required for the formation of metastases but increase the tumor cell's efficiency to reach the lymph nodes since the CD151 kd cell line reached the lymph nodes but at slower rate. In studies in which the adhesion to different matrices was examined, the ASML-CD151 kd cell line did not differ in matrix protein adhesion compared to the control ASML cell line. The ASML-CD151 kd cell line also did not differ in migration through a transwell membrane[41].

Studies performed by Yang et al. demonstrated that CD151 affects the ability of a mammary cell to invade and its motility in both nonmetastatic MCF-10A and MDA-MB-231 cell lines[5]. Mobilization of cells into a gap was examined by scratching a gap in a confluent cell monolayer and observing how the cancer cells invaded the gap. For both cell lines the absence of CD151 reduced the mobilization by nearly 50%. CD151's effect of invasion through a transwell insert was also examined. There was a 78% reduction in invasion for the MDA-MB-231 CD151 kd cell line. There was also a significant reduction in invasion for the J110 (a malignant mouse breast cancer) CD151 kd cell line. Showing that when CD151 is absent, there is an overall reduction in invasion for both cell lines. This study also indicated that CD151 kd MDA-MB-231 cells had a decreased ability to spread on laminin-1 when the α6 integrin was blocked and the cells were not stimulated with EGF. CD151 ablation was not shown to effect spreading on fibronectin.

EGF, phorbol 12-myristate 13-acetate (PMA), nor could insulin-like growth factor I overcome CD151 ablation in invasion or spreading. *In vivo* studies performed on nude mice, in which control MDA-MB-231 cells where injected and the cells expressed either shRNA (small hairpin RNA) or CD151 shRNA, Tumors expressing the control were detected at 8 to 9 weeks while the CD151 ablated cells did not appear until 11 to 12 weeks. Tumor growth was also delayed when tumor cells were injected into mammary fat pads. There was no morphologic differences between the tumors formed[5].

Takeda et al. examined how CD151 affected the first stage of metastasis which is angiogenesis[42]. Studies in which an aoritic ring was embedded into Matrigel showed that aortic rings from CD151-null mice had impaired microvascular sprouting by 35% after 4 days. Similarly, matrigel plugs that were implanted into CD151-null mice showed diminished vascularization after 7 days. When CD151-null mice were injected with Lewis lung carcinoma, LLC, cells, smaller solid tumors formed compared to the wild type mice. There was also a significant decrease in microvessel density. When a corneal micropocket assay was performed, the corneal vessels from CD151-null mice showed minimal bFGF-stimulated sprouting.

In vitro studies showed that there was no significant difference between mouse lung endothelial cells, MLECs, ability to proliferate or adhere when derived from CD151-null or wild type mice. However, several cell functions were altered. The CD151-null cells had decreased spreading on Matrigel by 50 % and to a lesser extent on fibronectin and gelatin. The transwell migration of CD151-null MLECs toward bFGF (basic fibroblastic growth factor) was reduced when the wells were coated with Matrigel or gelatin, but there was not a reduction when fibronetin was the coating. Invasion through Matrigel was

also diminished by 50 % for the CD151-null MLECs. The CD151-null MLECs also had a diminished ability to grow cellular cables, and CD151-null MLEC spheroids had significantly shorter capliary0like sprouts than the wild type. These results allowed Takeda et al. to conclude that CD151 plays a critical role during angiogenesis[42].

Takeda et al. continued their work on CD151 and metastasis by examining how metastasis occurred in CD15- null mice[43]. The murine cancer cell lines, B16F10, a melanoma cell line, and LLC were used for these experiments. For *in vivo* experiments, B16F10 cells were injected into the tail veins, and there were fewer metastatic foci in the lungs of the CD151-null mice compared to the wild type after two weeks. The experiments were repeated with LLC cells, and the results were the same. The CD151-null mice also had fewer metastases in the kidneys, peritoneum, and the ovaries. Size distributions were not different between the two genotypes. Further studies were performed where the number of metastases was counted at 6, 24, 48, and 72 hours. Fewer B16F10 cells were recovered from the CD151-null mice at all points. Blood was analyzed to determine if there was a rapid clearance in the CD151-null mice, and the percentage of B16F10 cells was not diminished in the blood of the CD151-null mice at 48 or 72 hours. CD151-null mice did not differ in leukocyte engagement or did not show an increased tendency to undergo cell death.

Takeda et al. also did a series of adhesion and transwell assays using MLECs derived from CD151-null mice or wild type mice. The B16F10 cells adhered significantly less to the monolayers of MLECs derived from the CD151-null mice both with and without the addition of TNF-α. The MLECS were grown on a matrix before being removed, and the cancer's cell's ability to adhere to this matrix was examined. B16F10 cells adhered

significantly less to the ECM derived from the CD151-null mice that suggests that the CD151-null endothelial cells create a deficiency in the ECM. Transwell studies showed that transendothelial migration of B26F10 cells through a monolayer of CD151-null endothelial cells was diminished. When migration of B26F10 cells through a monolayer of keratinocytes, there was no significant difference between the wild type and the CD151-null mice indicating that the effects of CD151 ablation differs between cell lines[43].

Zijilstra et al. examined how the anti-CD151 antibody, mAB 1A5, affected intravasation and metastasis[38]. To determine if CD151 effected spontaneous metastasis, a chick embryo spontaneous metastasis assay was utilized. These animals contained either HEp3 or HT1080 tumors, and were injected with mAB 1A5 or the control antibody mAb 29-7. The anti-CD151 antibody could inhibit spontaneous metastasis. Depsite HT1080 and HEp3 having different CD151 expression, the mAB 1A5 inhibited spontaneous metastasis in both. Experiments using SCID mice had similar results. This indicated that CD151 inhabitation occurred independently of tumor expression.

Next, the migration of HEp3 cells through a matrix was examined. The mAB 1A5 antibody inhibited migration through laminin. However, the antibody also reduced the level of migration through collagen and uncoated filters. It was suggested that the antibody enhanced the matrix interactions. To examine if CD151 effected extravasation and the subsequent formation of secondary tumors, the CAM model was used. HEp3 cells in the presence and absence of mAb 1A5 adhered to the vasculature. Those not treated with the mAB 1A5 antibody disseminated throughout the CAM stroma. However, those treated with mAb 1A5 proliferated but failed to disseminate which suggested to

Zijilstra et al. that the antibody was preventing migration. *In vivo* studies in which HEp3 cells were injected i.v. in the presence of the control or the anti-CD151 antibody showed that mAb 1A5 did not inhibit tumor cell arrest or growth as there were tumor cells found in the LCAM and the lung after four days for both treatments. This indicates that the inhibition of migration plays a greater role in migration from the primary site than in the migration to the secondary site. When HEp3 or HT1080cells were injected into the CAM model, the animals that had been treated with mAB 1A5 had tumors with a defined boarder and little to no invasion of the surrounding stroma compared to the control animals that had an irregular invasive front on the tumor-stromal interface. When HEp3 tumors were implanted into the CAM, it was found that mAB 1A5 inhibited migration velocity, but there was residual movement and the cells' ability to change direction was unaffected. This inhibition was shown to be caused by the rear of the tumor cell not being able to detach. However, when CD151 was silenced with shRNA silencing, neither the migration nor metastasis was limited significantly. Further experiments using the CAM model showed that CD151 inhibition prevented intravasation which inhibits the subsequent metastasis[38].

Deng et al. examined the effects of CD151 on transendothelial migration and migration through Matrigel[44]. The tumor cell line MCF-10A was the cancer cell line and Human Umbilical Vein Endothelial cells were chosen as the endothelial cell line. When no chemoattractant was present, there was no statistical difference in transendothelial migration between the knock down cell line and the control. The same was true when TGF-β was utilized as a chemoattractant. However, when stimulated with EGF, the CD151 knock down cell lines had a reduced migration by 50%. When this study was

repeated to examine the effects of migration through Matrigel, CD151 knock down cells migrated less when stimulated with EGF[45].

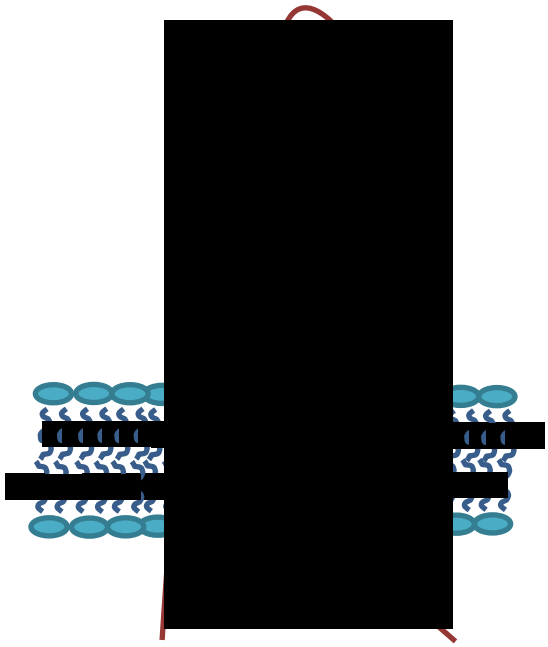

Figure 2-4: The tetraspanin CD151. The name tetraspanin is derived from the four extracellular regions. There are also three loops- a short intera and extracellular loops and one large extracellular loop.

2-4 β_1 and Cancer

β_1 is a member of the integrin family which are heterodimeric cell surface receptors. Each integrin consists of an α and a β subunit that are non-covalently bound[46, 47]. These subunits are transmembrane glycoproteins with large globular amino-terminal extracellular domains that bind to form an ellipsoidal head as can be viewed in Figure 2-5[47]. There are eight different β subunits and 18 different α subunits which interact to form at least 22 different integrins with 10 of these containing β_1[46, 48]. The primary function of β_1 is to bind to laminin, but it can also interact with many of the other components of the extracellular matrix such as type IV and V collagen and fibronectin[47, 49]. This suggests that β_1 potentially plays a large role in extravasation since a key step in extravasation is the tumor cell penetrating the extracellular matrix[4].

There have been examples in literature of β_1 effecting metastasis. One study performed by Fijita et al. examined the effects of β_1 on the invasion and metastasis of colorectal cancer. A total of 51 tissue samples of tumors and normal mucosa were acquired during surgeries and analyzed using an immunoblot assay. Of these samples, the pre-β-unit was increased in 15 samples, and β_1 was increased in two of the samples. Fifteen of these cases with altered expression had lymph node metastases while only 12 of the samples with unaltered expression had lymph node metastases suggesting that β_1 expression plays a key role in the metastasis of colorectal cancer to the lymph nodes[50].

A second study was performed by Elliott et al. which utilized a murine model to determine how the Anti-β_1 integrin IgG affected pulmonary macrometastases. One of two spontaneous intraductal mammary adenocarcinoma cell lines was injected into the mammary fat pad of the animals. The first cell line was SP1 and the second was its

metastatic sister line SP1-3M. Either the Anti-β_1 antibody IgG or the control non-immune IgG was injected every two days. The Anti-β_1 antibody suppressed the growth of overt macrometastases in the SP1 cell line with a significant reduction in the mean diameter of the nodules compared to the control group. However, metastatic tumors still formed suggesting that the β_1 antibody impeded the formation of metastases after extravasation occurred[51].

Cannistra et al. examined how β_1 affected ovarian cancer. Flow cytometry showed that the β_1 chain was widely expressed in the four ovarian cancer cell lines, CAOV-3, SKOV-3, OVCAR-3, and SW626, that they were examining. When tumor samples were harvested from nine patients, all samples expressed the β_1 chain. Adhesion studies were performed in which the cancer cells' ability to adhere to different matrixes was tested. All cell lines had significant binding to collagen type I. The CAOV-3 and the SKOV-3 cells bound significantly to laminin and fibronectin. The OVCAR- and SW626 cells bound to laminin and fibronectin to a lesser extent. To determine who β_1 affected these cells ability to adhere to the matrix, the 4B4 anti-β_1 antibody was utilized. Binding of CAOV-3 cells to collagen was inhibited by the antibody. Binding to laminin was prevented by the anti-β_1 antibody, but not by antibodies for $\alpha 2$ or $\alpha 6$ which indicates that laminin binding is facilitated by β_1. No inhibitory effect was observed when the cells bound to fibronectin. To determine if β_1 was responsible for the binding of cancer cells to the peritoneal mesothelium, adhesion of CAOV-3 cells to confluent monolayers of mesothelial cells was examined when both the anti-β_1 antibody was present or absent. The binding was not inhibited by the presence of the antibody suggesting that more than the β_1 integrin effects the metastasis of ovarian cancer[52].

Canninstra continued studying the effects of $\beta_1$on ovarian cancer with Strobel. In these studies, the ovarian cancer cell lines 36M2, CAOV-3, and SKOV-3 were utilized. All of these cell lines widely expressed the β_1 integrin. For these studies, the 4B4 and the MAB13 anti-β_1 antibodies were utilized. As in the previous study, there was a minimal inhibitory effect of the 4B4 antibody on the binding of cancer cell lines to confluent monolayers of peritoneal mesothelial cells even at high concentrations of the 4B4 antibody. However, when the MAB13 antibody was utilized, the binding was inhibited significantly in all cell lines. This suggests that the inhibitory effect is dependent on type of antibody used. The studies in which CAOV-3 ability to bind to fibronectin were repeated with both of the antibodies. The MAB13 antibody inhibited the binding of the CAOV-3 to a greater degree than the 4B4 antibody. When the cells were exposed to the RAT16 α_5-integrin antibody, the binding of 36M2 and CAOV-3 to the mesothelium was inhibited suggesting that the $\alpha_5\beta_1$ integrin is responsible for the binding of ovarian cancer to the mesothelium[53].

Michelle Chen presented a poster in which the role of β_1 in extravasation was examined. Here, when β_1 was silenced in MDA-MB-231 cells, extravasation was reduced. It was determined to be partially caused by a reduced adhesion rate to the monolayer which was confirmed with flow studies on a planar monolayer[54].

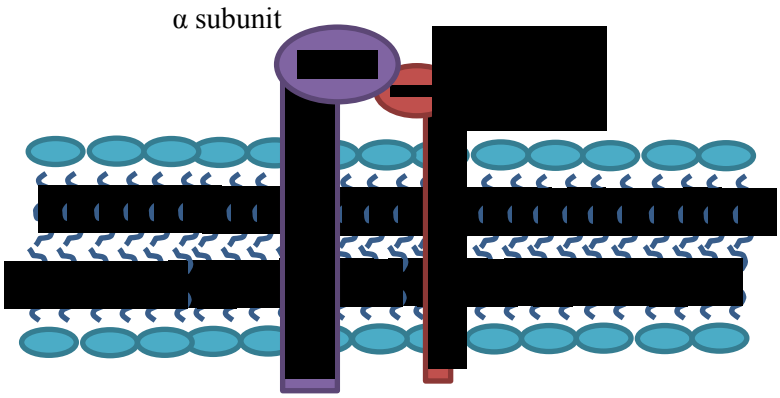

Figure 2-5: A integrin containing the β1 subunit. The α unit and the β1 are transmembrane proteins that bind noncovalently to form an ellipsoidal head.

2-5 Mechanisms of Tumor Cell Adhesion and Migration

Cell migration is allowed through a combination of adhesion, protrusion, and contraction. Adhesion forces are present at both the leading edge and the rear of the cell. There is an optimum amount of adhesive forces that allows for the fastest migration. This was demonstrated in a study by Gupton and Waterman-Storer in which epithelial cells were seeded on varying concentration of fibronectin. On low concentrations of fibronectin, the cells had few but dynamic adhesions. On high concentrations of fibroneticin, the cells had many but less dynamic adhesions. On medium concentrations, there were moderate adhesions with well-organized dynamic patterns of actin flow which gave rise to the highest migration rates. This trend can be viewed in Figure 2-6[55].

Adhesion at the leading edge of the cell provides traction for the cell as protrusions form and also initiates signals that are responsible for the dynamics of migrations[56]. This is caused by the polarization of the cell due to external signals and results in the extension of a protrusion from the leading edge which is thought be caused by vesicle trafficking of adhesive molecules to the leading edge[56, 57]. The adhesive forces in the cell must be balanced appropriately. If the adhesive forces at the leading edge of the cell are too weak, the cell cannot pull forward. If the adhesive forces at the rear edge of the cell are too strong, the cell cannot detach from the substrate and move forward[58].

Contractile forces caused by myosin motors work to help break adhesive interactions by the application of physical stress. Studies using fibroblasts suggest that during this process of the rear release, the majority of integrins are left on the substrate suggesting that these integrins play a major role in the attachment and detachment of the rear of the cell. Integrins can also be removed from the rear end of the cell by endocytosis. Once

endocytosis has occurred, the integrins can either accumulate in the cell or are transported to the cell front. This process is called integrin trafficking[57].

Evidence has suggested that there are multiple pathways for β_1 endocytosis. One such pathway is regulated by the AP-2 clathrin adaptor. This pathway involves the binding of AP-2 to the cytoplasmic tail of CD151 which associates with the integrin β_1. The clathrin mediated pathway also includes the cargo-specific endocytic adaptor Numb which binds directly to the β integrin sub-units and interacts with components of the clathrin endocytic mechanism. There are also pathways which are not mediated by clarthrin. Examples of these pathways include the internalization of $\alpha_2\beta_1$ mediated by PKC and caveolae and the internalization of $\alpha L\beta_1$ through a cholesterol-sensitive pathway. These pathways are modeled in Figure 2-7[59].

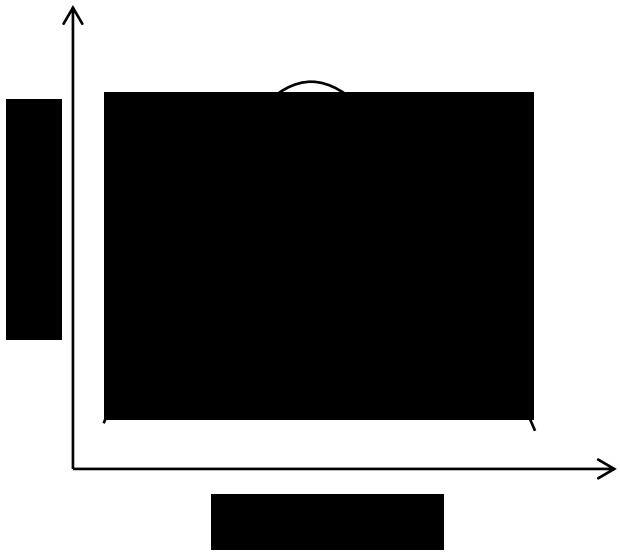

Figure 2-6: Trend in how adhesion strength to the substrate effects random migration. There is an optimum adhesion strength in which the migration is the fastest.

Figure 2-7: The different pathways in which integrin can be endocytosed during integrin trafficking.

2-6 Interaction of CD151 and β_1

As stated previously, CD151 tightly associates with the laminin binding integrins. To support this, one of the laminin binding integrins, α3β1, has not been found present in any tissue or cell in the absence of CD151 association [60, 61]. CD151 forms a stable complex with this integrin and modulates the ligand-binding affinity of the integrin as well as shifting the conformational equilibrium position [60, 62, 63]. This complex can be seen in Figure 2-8. Studies have shown that CD151 and $\alpha_3\beta_1$ will concentrate together at the leading edge of a migratory cell and at the rear of the cell immediately before retraction. This suggests that together, they establish front-rear polarity during migration [64]. It has also been demonstrated that this CD151-α3β1 complex is stable even when treated with the surfactant Triton X-100[63].

Nishiuchi et al. performed studies to determine how the associate of $\alpha_3\beta_1$ with CD151 effects ligand-binding. Studies in which there was CD151-free integrin $\alpha_3\beta_1$ and CD151-bound integrin $\alpha_3\beta_1$ in phosphatidylcholine liposomes were allowed to bind to laminin-10/11 surfaces. Less CD151-free $\alpha_3\beta_1$ liposomes bound to the laminin than CD151-bound $\alpha_3\beta_1$ lipsomes showing that CD151 stabilizes this integrin and increases its potential to bind to laminin-10/11. To determine how the $\alpha_3\beta_1$ association with CD151 affected the adhesion of cells, the mAB 8C3 antibody was utilized. This antibody induces dissociation of CD151 from the $\alpha_3\beta_1$ integrin. When A549 lung carcinoma cells were pretreated with this antibody, there was less adherence to laminin-10/11 than in untreated cells. Knocking down the gene for CD151 also decreased adhesion to laminin-10/11. All of these suggests that CD151 association with $\alpha_3\beta_1$ increases laminin binding[63]. Studies have also

indicated that the association of CD151 with α3β1 is a factor in the binding to laminin-5 during tumor cell-stromal cell interactions [65].

Integrins, especially β_1, interact with CD151 in tetraspanin webs. Tetraspanin webs are described as assemblies of multimolecular signaling complexes at the cell surface. As the name suggests, the primary molecules are tetraspanins. However, many other molecules are included in this web including integrins, membrane receptors, and other signaling molecules. This web creates a tetraspanin enriched microdomin, TEM. Devbhandari et al. examined 57 molecules that are found within a tetraspanin web centered on CD151 and found that β_1 had the highest Mascot score and showed that it was the second most abundant of the tetraspanin partners. In their studies, HCCLM3 cells were utilized because they had the highest expression of CD151 protein. Using immunofluorescence, they determined that CD151 and β_1 co-localized on the plasma membrane. When CD151 was silenced, the internalization of β_1 was impaired which impeded migration. When β_1 was silenced in cells with high CD151 expression, the motility and invasiveness was impaired. However, in cells with low CD151 expression, there was no effect on motility and invasiveness. It should be noted that the cells with low CD151 expression, the motility and invasiveness had been low before gene silencing[66].

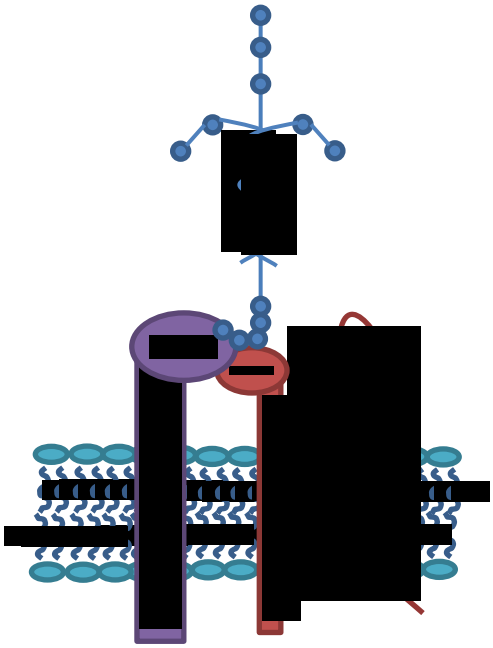

Figure 2-8: Association of $\alpha_3\beta_1$ with CD151. This association enhances binding of the integrin to laminin.

Chapter 3: Experimental Materials

3-1 Flow System

μ-Slide VI$^{0.4}$ ibidiTreat: #1.5 polymeter coverslip, tissue culture treated, sterilized (Cat. # 80606) was obtained from Ibidi, USA (Madison, WI). Male lock stopcocks (Cat. # SI-30600-04), 1/16 inch hose barb male luer (Cat. # EW-45505-00), and Tygon Lab Tubing, Non-DEHP, 1/8x3/16 in (Cat. # EW-07407-75) were obtained from ColeParmer (Vernon Hills, IL). Standard Pump 22 Infusion/Withdraw with standard syringe holder (Cat #. 552226) was obtained from Harvard Apparatus. BD medical 20 mL syringes with leur-lok tip (Cat #. BD302830) were obtained from VWR (Radnor, PA).

3-2 Microscope and Camera Assembly for Adhesion Studies

Axiovert 35 inverted microscope, AxioCam MRm (Cat. # 426509-9902-000), and AxioVision Softer AV 4.9.1 64 bit (Cat. # 4101330906000000) were obtained from Zeiss (Oberkochen, Germany). X-cite series 120Q was obtained from Exceliates Technologies (Miamisburg, OH).

3-3 Treatment of HUVEC Cells

3-3.1 HUVEC Culture

HUVEC cells at passage 1 (Cat. # C2519A), EGM-2 SingleQuot Kit Suppl&Growth Factor (Cat. # CC-4176), EBM Basal Medium Phenol Red Free (Cat. # CC-3129) were obtained from Lonza (Basel, Switzerland).

3-3.2 HUVEC Subculture

HEPES-Buffered Saline Solution (Cat. # CC-5024), Trypsin Ethylenediaminetetraacid Acid, Trypsin/EDTA, in a balanced salt solution (Cat. # CC-5012), and Trypsin

Neutralizing Solution, or TNS, (Cat. # CC-5002) were obtained from Lonza (Basel, Switzerland).

3-4 Treatment of MDA-MB-231 Cells

3-4.1 MDA-MB-231 Culture

All MDA-MB-231 cell lines were provided by Dr. Xiuvwei Yang (Dept. Pharmacology & Nutritional Sciences, University of Kentucky). Dulbecco's Modified Eagle Medium, or DMEM, with high glucose, no glutamine, no phenol red (Cat # 31053-028) was obtained from ThermoFisher Scientific (Waltham, MA). This medium was supplemented with 50,000 U of Penicillin Streptomycin, or Pen Strep, (Cat. #: 15140122) and 1mL of Fungizone Antimycotic (Cat.# 15290-018) to prevent contamination from occurring. These were obtained from ThermoFisher Scientific (Waltham, MA). The medium contained 2 mM L-glutamine (Cat. # 21051024) and 1 mM sodium pyruvate (Cat. # 11360-070) which were obtained from ThermoFisher Scientific (Waltham, MA). The medium was supplemented to a final concentration of 20% with Premium Grade Fetal Bovine Serum, or FBS, (Cat. # 97068-085) which was obtained from VWR (Radnor, PA).

3-4.2 MDA-MB-231 Subculture

Trypsin-EDTA (0.25%/0.53 mM) in Hanks Balanced Salt Solution (Cat. # 30-2101) was obtained from American Type Cell Culture.

3-5 Adhesion Studies

Recombinant Human Tumor Necrosis Factor-alpha, or TNF-α, (Cat. # 210-TA-005) was obtaind from R&D Systems. Dextran from Leuconostoc mesenteroides (Cat. # D4876) was obtained from Sigma Aldrich (St. Louis, MO).

3-6 Staining Cells for Transwell Assay

1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindocarbocyanine Perchlorate, or DiI stain, (Cat. # D-282) and 3,3'-Dioctadecyloxacarbocyanine Perchlorate, or DiO, (Cat #. D-275) was obtained from ThermoFisher Scientific (Waltham, MA).

3-7 Transwell Assay

Corning HTS Transwell-24 well permeable supports (Cat. # CLS3396) and Gelatin from porcine skin (Cat. #G1890) were obtained from Sigma Aldrich (St. Louis, MO). PathClear Cultrex Basement Membrane Extract, or BME, (Cat. # 95036-780) was obtained from VWR (Radnor, PA).

Chapter 4: Experimental Methods

4-1 Cell Culture

HUVECs were utilized at passages five through eight. The HUVECs were cultured at 37 °C and 5 % CO_2 in EGM-2 which was changed every other day at minimum until the cells were confluent.

Three different variations of the breast carcinoma cell line MDA-MB-231 were utilized. The control cell line had been transfected with the green florescent protein (GFP) tag to negate any effect the GFP tag had on adhesion in the kd cell lines. Cell lines of MDA-MB-231 with the genes for CD151 and β_1 were used as the test cell lines. The gene for either CD151 or β_1 was knocked down using shRNA gene silencing which degrades the mRNA. This process was performed by Dr. Yang. A GFP tag was used to indicate if the gene had been successfully knocked down in an individual cell. All cancer cells were cultured at 37 °C and 5 % in DMEM which was changed every other day a minimum until cells were confluent.

4-2 Adhesion Studies

4-2.1 Preparation of Endothelial Monolayer

Once the HUVECs reached confluency, the HUVECs were washed with HEPES and trypsinized. The cell suspension was then neutralized with TNS, spun down, and the supernatant removed before being resuspended and counted using a cellometer. Cells were then diluted with EGM-2 to reach the desired concentration, and 30 μL of cell suspension was seeded into the channels of an Ibidi μ-slide. The seeding density varied depending on when studies were going to be performed. The HUVECs were allowed to settle for thirty minutes before the reservoirs of the channels were filled with 60 μL of

EMG-2. HUVECs were cultured in the Ibidi μ-slide at 37 °C and 5% CO_2 until the cells were confluent at which time, the monolayer was stimulated with 20 ng/mL of TNF-α. This stimulated important adhesion molecules such as ICAM-1 and PECAM-1. The cells were incubated with TNF-α for 4 hours before experiments were conducted.

4-2.2 Preparation of Breast Cancer Cells

A confluent flask of cancer cells were trypsinized, spun down, and the supernatant was removed. The cells were resuspended and counted using a cellometer. The cell suspension was then diluted to the desired concentration, 5.0×10^5 cells/mL for attachment experiments and 1.25×10^6 cells/mL for the detachment experiments. The cells were resuspended in a solution of dextran in EGM-2. The dextran solution had a concentration of 7.5 g per 100 mL.

4-2.3 Experimental Set Up

One syringe was filled with the cell suspension and a second syringe was filled with the solution of dextran in EGM-2. For attachment experiments the cell suspension syringe was connected to the syringe pump while the syringe containing the dextran solution was connected to the line of tubing through a valve. The tubing before the valve was filled with the dextran solution while the tubing after the valve leading to the pump was filled with the cell suspension. This was to minimize the number of cancer cells introduced into the chamber before flow. This set up was reversed for detachment experiments in which the tubing before the valve was filled with the cell suspension while the tubing after the pump was filled with the dextran solution. The tubing was connected to one of the reservoirs of the Ibidi flow chamber and a second piece of tubing was connected to the corresponding outlet reservoir. This second piece of tubing led to a waste reservoir. This

setup can be viewed in Figure 4-1. The flow chamber was placed on top of the stage of an inverted microscope. The monolayer could be viewed on the desktop computer screen through use of a camera and the corresponding software. The flow chamber was taped to the stage to ensure that the same location could be viewed for the entire length of the trial as shown in Figure 4-2.

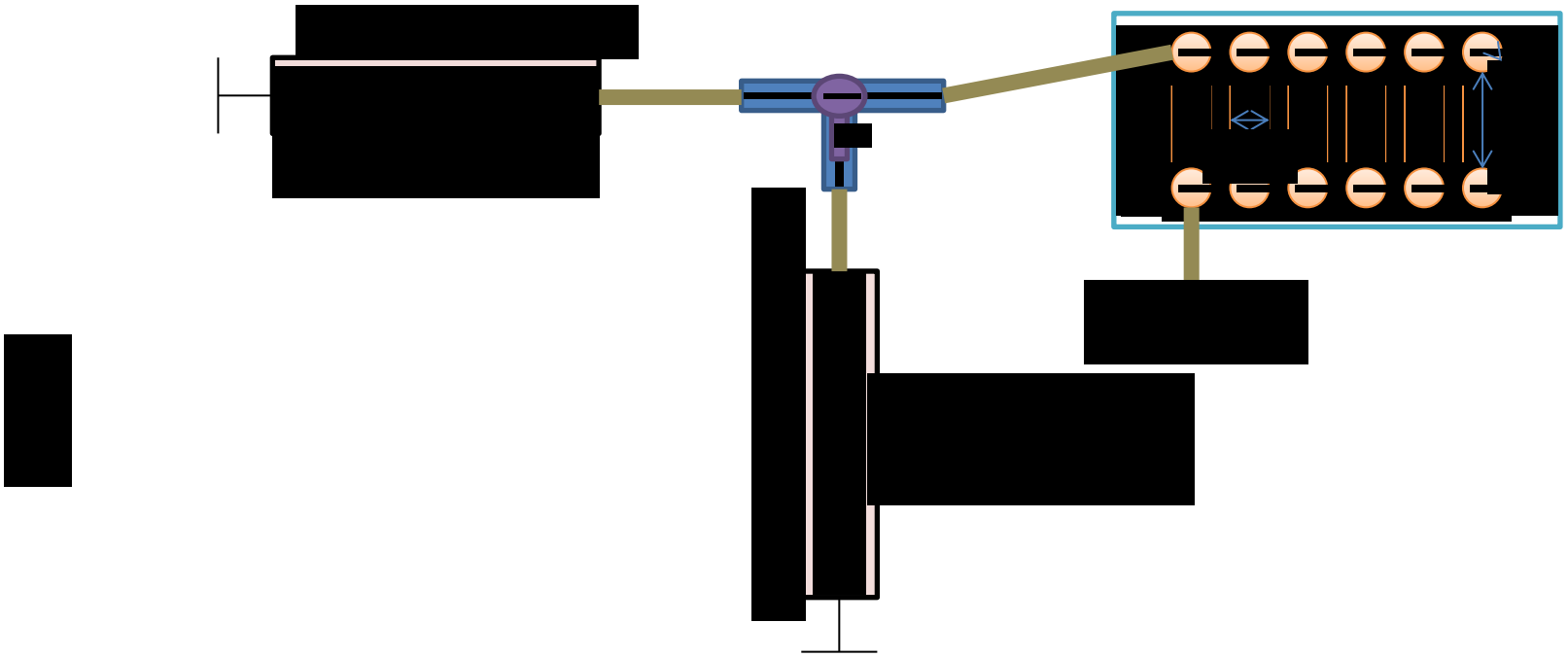

Figure 4-1: Syringe set up for adhesion studies.

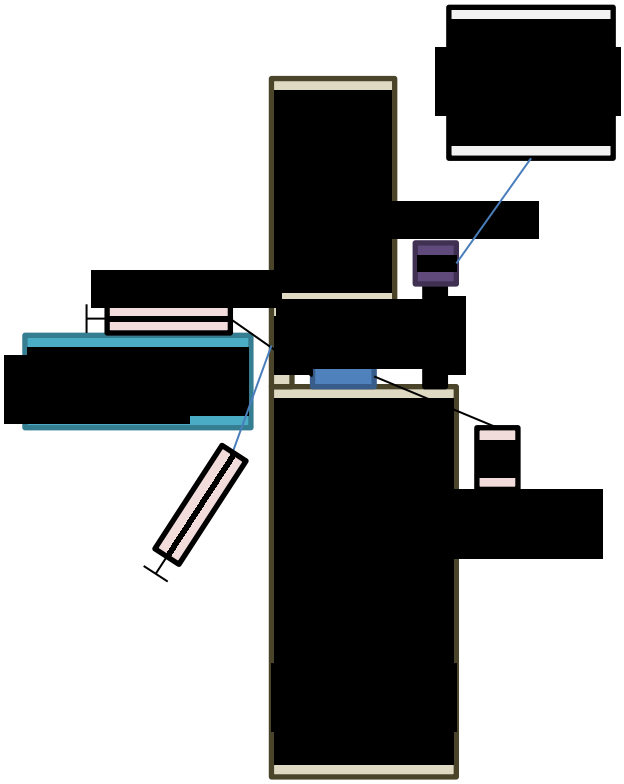

Figure 4-2: Experimental setup for all flow studies.

4-2.4 Detachment Experiments

To determine how the cancer cells firmly adhere to an endothelial monolayer, detachment studies were carried out in the Ibidi flow chamber. A suspension of cancer cells at a concentration of 1.25 x 106 cells/mL were introduced into the chamber through the secondary syringe. These cancer cells were allowed to settle on the endothelial monolayer for 30 min. After the cells were allowed to settle, initial bright field and florescent images were taken. The monolayer was then perfused for 1 minute at a flow rate of 0.01 mL/min to remove any nonadherent cancer cells, loose endothelial cells, or cell debris. Bright field and florescent images were taken again after this wash. The monolayer was then perfused at the flow rate required to produce the required shear of either 2 or 8 dyne/cm^2 [25]. Final bright field and florescent images were taken. The wash and the 10 minute trial were recorded using the time lapse option on the Axiovision software. This consisted of the software automatically taking images very second.

4-2.5 Attachment Experiments

To determine how the cancer cells initially adhere to an endothelial monolayer, attachment studies were carried out in the Ibidi flow chamber. The front end of the line of tubing was filled with a dextran solution in EGM-2 through use of a secondary syringe and the primary syringe contained the cell suspension at a density of 5 x 105 cells/mL. Initial bright field and florescent images of the monolayer were taken. The suspension was perfused into the chamber at a shear stress of 0.25 dyne/cm^2 [25, 31]. Experiments were conducted for a total of 40 minutes. The trial was recorded using the time lapse option on the Axiovision software at a frame rate of one frame per second.

4-3 Random Migration Studies

4-3.1 Matrix Coatings

Six of the twelve transwell membranes were coated with 0.5% gelatin to ensure that cells would adhere to the membrane. Enough gelatin to coat the membrane was added to the transwell before being placed in a 37 °C incubator for 2 hours before being allowed to dry at room temperature overnight[67]. The other six transwells were coated with BME, which unlike gelatin, contains laminin, the protein to which both CD151 and β1 bind to. After being thawed on ice, the 20 μL aliquot of BME was diluted with ice-cold sterile deionized water to 0.125 mg/mL. 150 μL was injected into the transwell before 50 μL was removed. This step ensured a uniform layer. The transwells were allowed to dry overnight[68].

4-3.2 Preparation of Endothelial Monolayer

Once the HUVECs reached confluency, the HUVECs were washed with HEPES and trypsinized. The cell suspension was then neutralized with TNS, spun down, and the supernatant removed before being resuspended and counted using a cellometer. The cell suspension was diluted with PBS to a concentration of 1×10^6 cells/mL, and a working solution of DiI was added to 1 mL of the cell suspension. This cell suspension was incubated for 20 minutes at 37 °C before being wash three times was EGM-2. Using a cellometer, the cell suspension was measured, and the suspension was diluted to a concentration of 5×10^5 cells/mL using EGM-2. 100 μL of cell suspension was added to tranwell inserts that had been precoated with the matrix. The transwells were placed in an incubator at 37 °C and 5% CO_2 overnight. In the morning, the HUVECs were examined using a Nikon microscope.

4-3.3 Seeding of Cancer Cells into Transwells

After checking the HUVECs in the transwells, a confluent flask of cancer cells were trypsinized, spun down, and the supernatant was removed. The cells were resuspended and counted using a cellometer. The cell suspension was then diluted to the desired concentration of 1.0×10^6 cells/mL. For the knocked down cell lines, this concentration was based off of the GFP transfection rate. For the control cells, 1 mL of suspension was added to a working solution of diO and the cells were responded in PBS. This cell suspension was incubated for 20 minutes at 37 °C before being wash three times in EGM-2. Using a cellometer, the cell suspension was measured, and the suspension was diluted to a concentration of 1×10^6 cells/mL using EGM-2. For all cell lines, the media had been removed from the transwell, and 100 μL of cancer cell suspension was added to each transwell. The transwells were placed in an incubator at 37 °C and 5% CO_2 for 24 hours.

4-3.4 Imaging of Transwells

The media was removed from the transwells and the wells before the transwells were washed twice with PBS. A fixing solution of 3.5% Formaldehyde was prepared, and 100 μL was placed in the transwell, and 600 μL was placed in the well. After 2 minutes, the formaldehyde solution was removed, and the transwells were washed twice in PBS. Using a cotton swab, the cells were removed from the apical side of the transwell, and the transwell was placed on a coverslip. An inverted Nikon microscope was used to image the transwells. Five representative images were taken-one in the center and one in each corner. To analyze the images of cells, the NIS Basic Software package was used. The object count function was used with the intensity option. For all images, the clean option

was set to x1, and any objects smaller than 5 μm in diameter were assumed to be cell debris.

4-3.5 Statistical Analysis

A Two-Way ANOVA was utilized to determine the statistical significance of the results. For all studies, the first condition was cell line. For the flow studies, the second condition was time while for the transwell assay studies, the second condition was matrix coating with and without HUVECs. The pairs were compared using the Holm-Sidak method for pairwise multiple comparison procedures. The analysis was performed using SimgaPlot.

Chapter 5: Results and Discussion

5-1.Detachment Results

The detachment of the MDA-MB-231 cell lines at 2 dyne/cm^2 and 8 dyne/cm^2 are shown in Figure 5-1 and Figure 5-2. When a shear stress of 2 dyne/cm^2 is applied to the cells, there was no difference in the detachment of the cells for the control and the two knock down cell lines. However, when a shear stress of 8 dyne/cm^2 is applied to the cells, the CD151 kd and the β_1 kd MDA-MB-231 cells detach to a greater degree than the control MDA-MB-231 cells. This indicates that low shear stresses, such as those found in the venous circulation, the cancer cells can compensate for the lack of CD151 and β_1. However, the cells cannot compensate for the loss of either protein at high shear stresses.

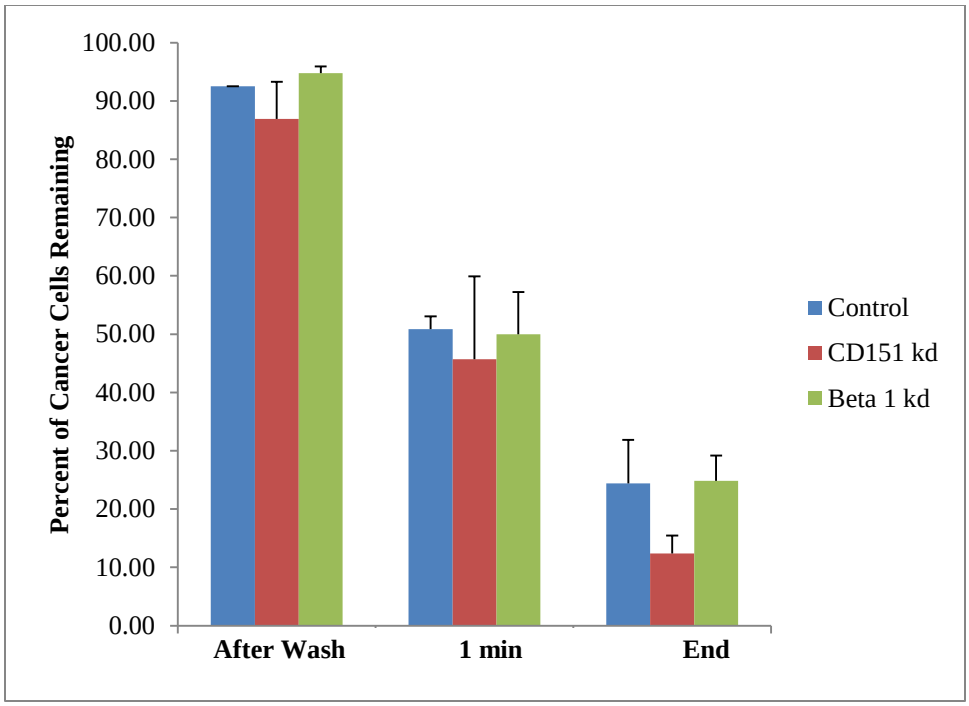

Figure 5-1: Detachment of MDA-MB-231 cells from an endothelial monolayer when the monolayer is subjected to a shear stress of 2 dyne/cm^2. N=6. Error bars represent SE. * indicates p-value < 0.05.

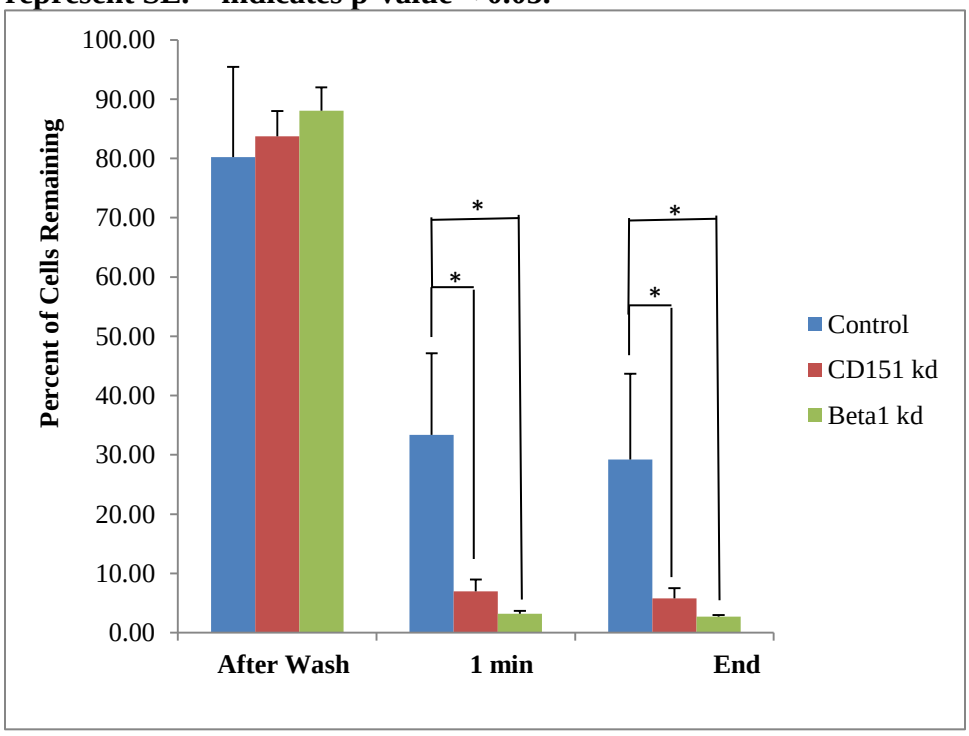

Figure 5-2: Detachment of MDA-MB-231 cells from an endothelial monolayer when the monolayer is subjected to a shear stress of 8 dyne/cm^2. N=6. Error bars represent SE. * indicates p-value < 0.05.

5-2 Attachment Results

The attachment of the different MDA-MB-231 cell lines is shown in Figure 5-3. At a shear stress of 0.25 dyne/cm^2, the control cell line had significantly greater attachment than the two knocked down cell lines. This indicates that CD151 and β_1 effect initial attachment. With attachment studies it is postulated cancer cells do not have a long enough residence time to invoke a response would overcome the loss of CD151 and β_1 the adhesion proteins. It is also possible that since one of the proteins is absent, the two cannot associate to aid in adhesion. These results are the first that have examined how these proteins effect initial adhesion as static studies can only determine the effects on firm adhesion and *in vivo* studies do not have the capabilities to separate the two different types of adhesion into different studies. Chen et al. found that β_1 kd MDA-MB-231 cells were less adhesive to an endothelial monolayer than the control MDA-MB-231 cells which is in agreement to the detachment experiments at 8 dyne/cm^2 and the attachment experiments reported here[54].

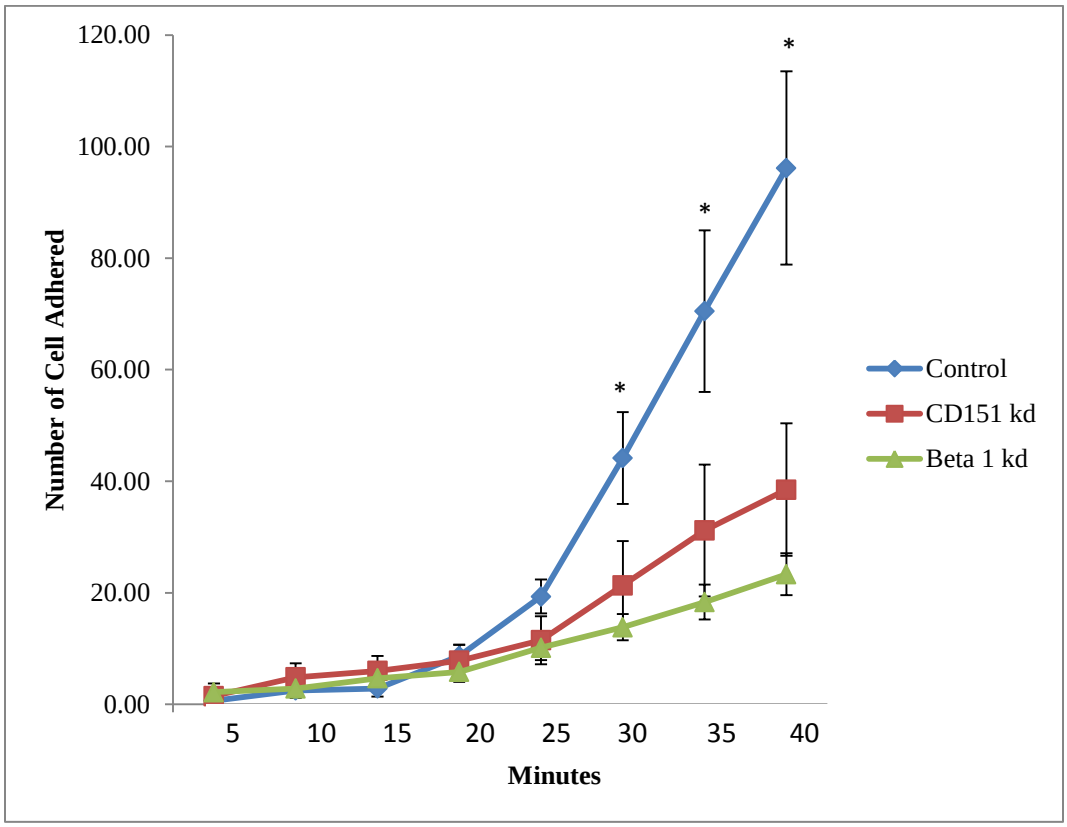

Figure 5-3: Attachment of cancer cells to an endothelial monolayer the cancer cells are introduced at a flowrate that produces a shear stress of 0.25 dyne/cm^2 on the endothelial monolayer. N=6. Error bars represent SE. * indicates p-value < 0.05.

5-3 Random Migration Results

Transwell assays were performed to determine how CD151 and β_1 effected random migration on different surfaces. Four different surface conditions were examined and the results were shown here in Figure 5-4. When cancer cells were seeded directly on gelatin, the control cancer cells migrated less than the CD151 kd and the β_1 kd cancer cell lines. When a confluent monolayer of endothelial cells were introduced on the control cancer cells migrated less than the β_1 kd cells. However, there was no significant difference between the migration of the control cells and the CD151 kd cells. When cancer cells were placed directly onto basement membrane extract, the migration for all cell lines was statistically different with the control cells migrating the least and the CD151 kd cells migrating the most. When an endothelial cell monolayer was introduced on the basement membrane extract, there was no statistical difference in migration flux.

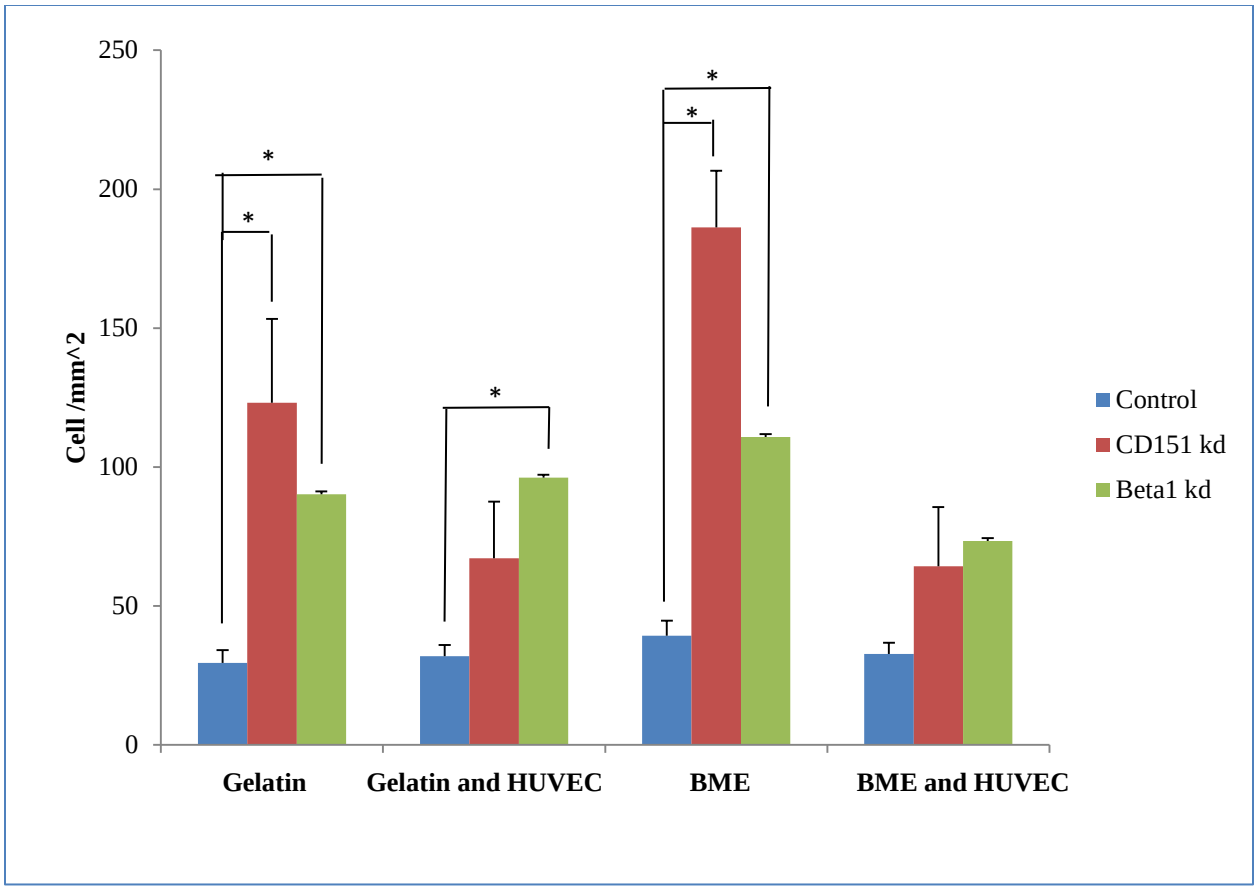

Figure 5-4: Random migration flux of cancer cell through a 0.4 µm pore under various conditions. N=12. Error bars represent SE. * indicates p-value < 0.05.

5-4 Discussion

In the detachment studies, there was no significant difference in the detachment of the kd cell lines from an endothelial monolayer than the control at a low shear stress of 2 dyne/cm^2. However, at a higher shear stress of 8 dyne/cm^2, the kd cells detached significantly more than the control cells. This indicates that CD151 and β_1 are crucial for firm adhesion at high shear stress such as those found in the arterial system but not at low shear stresses. Attachment studies determined that both CD151 and β_1 play a significant role in initial adhesion since the attachment to the endothelial monolayer was significantly impaired when CD151 and β_1 is knocked down. The role of CD151 in adhesion to an endothelial monolayer was confirmed by Takeda et al. who demonstrated that cancer cells adhered less to CD151-null MLECs. These studies were performed in a static well plate so direct comparisons cannot be made only trends can be observed[43]. Chen et a. confirmed that β_1 affected adhesion to a planar HUVEC monolayer under shear flow. It was determined that β_1 kd cells had significantly decreased adhesion[54]. Ad stated previously, CD151 and β_1 tightly associate to form complexes that are stable under even extreme conditions such as exposure to Triton-X. When either protein is absent, this complex cannot be formed which could be the cause in decrease in adhesion.

In the migration studies, both kd cell lines migrated more than the control cells when seeded directly on the matrix. When endothelial cells were seeded on the gelatin matrix before the introduction of cancer cells, only the β_1 migrated more than the control cells, and when endothelial cells were seeded on the basement membrane extract, there was no difference in migration. As described earlier, the cell's ability to migrate depends on the adhesive forces. CD151 and β_1 interact to aid in laminin binding and binding to different

types of collagen. Since basement membrane extract contains both laminin and collagen IV and gelatin is a collagen based compound, the control cells should adhere more to these compounds to a greater extent than the kd cells which would decrease the migration rate of the control cells. When Deng et al. examined migration of MCF-10A cells through an endothelial monolayer when no chemoattractant was present, there was not difference in migration between the control cells and the CD151 kd cells which was in agreement to what was observed in these studies[44]. Figure 5-5 shows how our data fit into and adds to current literature.

In conclusion, CD151 and β_1 significantly affect firm adhesion of MDA-MB-231 cells to an endothelial monolayer at high shear stress but not low shear stresses. CD151 and β_1 affected the initial adhesion of MDA-MB-231 cells to an endothelial monolayer. When CD151 and β_1 was absent, MDA-MB-231 cells migrated to a greater amount through the either a gelatin or basement membrane extract matrix.

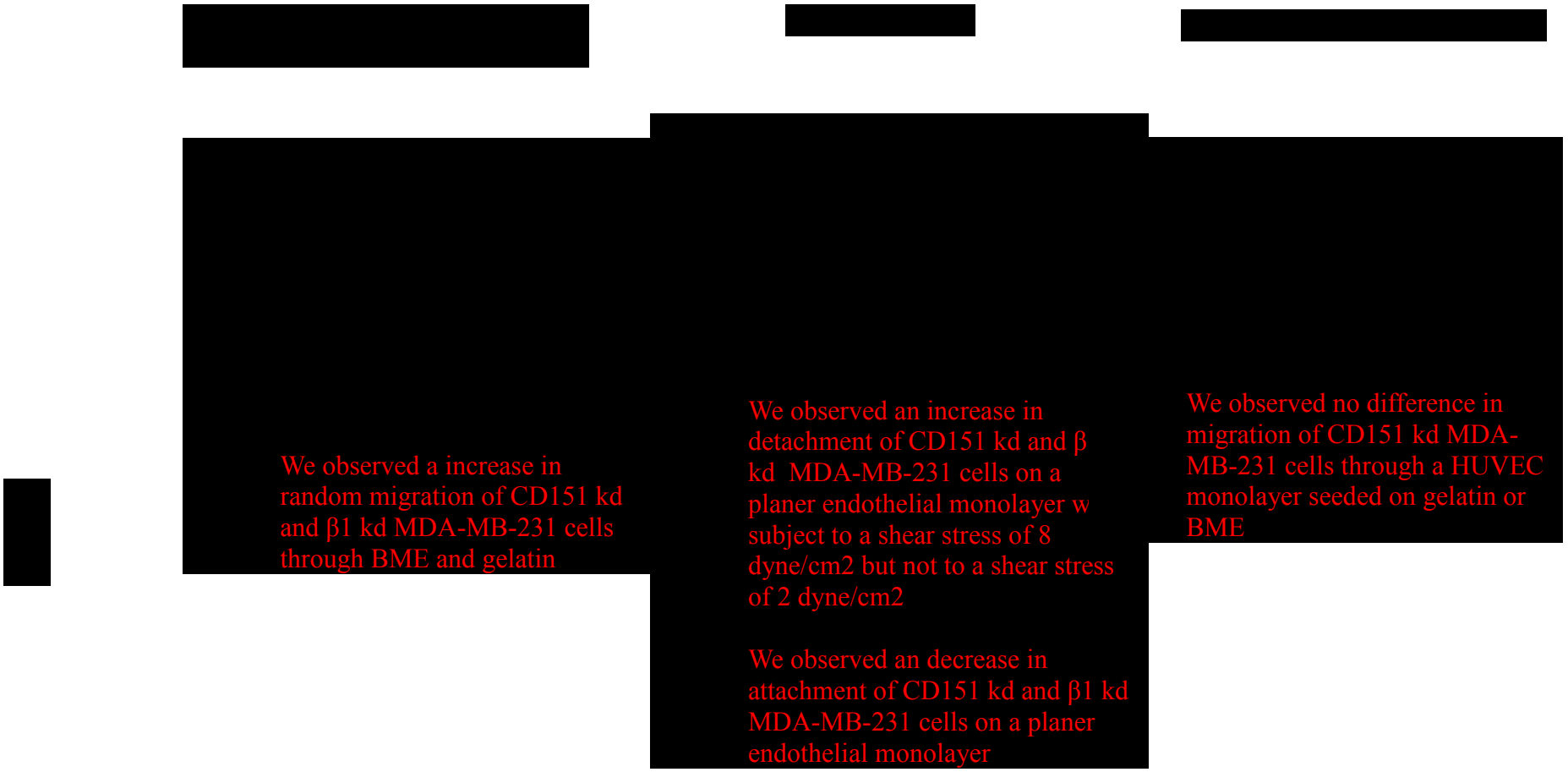

Figure 5-5: Summary of our results written in red and how it fits in with other research in the same area of interest.

5-5 Future Work

5-5.1 Determining the Effects of Shear on Extravasation

The model that was used to study migration here was a static transwell assay. To gain a more comprehensive view on the effects of CD151 and β_1 on tumor cell extravasation, a model that allows for the cells to be under shear stress needs to be utilized. An example of this type of model are microfluidic models such as those proposed by Jeon et al. and Zhang et al[8, 69]. As noted with the detachment studies reported here, cells will detach to different extents under different shear stresses. It is possible that when subjected to shear stress, a different pattern in migration will occur than that in the transwell assay.

5-5.2 Investigating Alternate Cancer Cell Lines

The chosen cell line for these studies was MDA-MB-231. This breast cancer cell line is referred to as a triple negative phenotype because it does not express ERα, PR, and HER2. It has the classification of Caudin-low[70]. This cell line is one of the most commonly used metastatic breast cancer cell lines for *in vitro* studies. It is possible that an underlying characteristic of the MDA-MB-231 cell line is effects how the cancer cells adhere and migrate when CD151 or β_1 is absent. Therefore, these studies should be repeated with alternate cell lines. One option would be to look at a breast cancer cell line that is not triple negative for example MDA-MB-453 which does not express ERα or PR but is positive for HER2. Another would be to examine the effects on a different cancer such as the hepatocellular carcinoma cell line HCCLM3 which has been shown to have very high expression of CD151.

5-5.3 Investigating Alternate Endothelial Cell Lines

HUVECs, as denoted by the name, are an endothelial cell line harvested from the umbilical cord vein. Endothelial cells will take on different morphologies and configuration depending on their location in the circulation meaning that vein, arterial, and microvascular cells all have different morphologies. For example, there are intercellular gaps in arterial and venous segments but not in capillary segments. This indicates that cells would adhere and extravasate different in these sections of the circulatory system[71]. A microvascular endothelial cell line such as Human Dermal Microvascular Endothelial Cells, HMVEC, and an arterial cell line such as Human Pulmonary Artery Endothelial cells, HPAEC, should be utilized in repeated experiments to determine if the effects of CD151 and β_1 on adhesion and migration vary depending on endothelial cell line.

5-5.4 Investigating the Effects of Smooth Muscle Cells

In the migration studies described here, it was determined how the matrix and how a monolayer of endothelial cells affected migration. However, the endothelial cells of the veins and arteries are surrounded by smooth muscle cells which would add another layer of resistance and could greatly affect how cells extravasate. Therefore, transwell studies need to be repeated using a smooth muscle cell line such as Primary Aortic Smooth Muscle cells.

5-5.5 Determining the Threshold of Detachment

At 2 dyne/cm^2 there was no significance in detachment. However, at a shear stress of 8 dyne/cm^2, there the knocked down cells detached significantly more than the control cells. These shear stresses had been chosen as representative shear stresses of the venous

and arterial circulation. The shear stress in the venous circulation can range up to 4 dyne/cm^2, and the arterial circulation can range as low as 4 dyne/cm^2. Due to a gap of 6 dyne/cm^2, it is unknown at what threshold the shear stress is high enough to cause significant detachment. Ideally, the next set of experiments should be conducted at 4 dyne/cm^2 since this is the overlapping shear stress for the venous and arterial circulation.

5-5.6 Determining the Effects of Cell Cycle on Metastasis

In many of the transwell studies performed here, there were clusters of multiple cells. This indicates that either the cells had migrated through as a cluster, clustered after migration, or had divided and multiplied after migrating. As discussed earlier, since the imaging is performed in the plane parallel to migration, it is impossible to determine which is occurring using transwell assays alone. However, to give further insight on if the cells are multiplying, one of two modifications can be made to these studies. The first is to decrease the amount of time that the cells are allowed to migrate. For example, one common shorter endpoint for transwell assays is four hours. This would give the cells less time to undergo the cell cycle. The second option would be to synchronize the cells by culturing them in serum free media for 24 hours prior to seeding in the transwells. This would ensure that all cells are on the same phase of the cell cycle at the beginning of the experiments. Previous studies have shown that nearly all cells that adhere to a vessel wall are in the G1 phase while the cells that migrate across the vessel wall are in the G1/S transition indicating that cell cycle does play a role in these processes[72].

5-5.7 Alternative Transwell Assays

Depending on how the transwell assay is preform, these studies can answer many different questions. In the studies that have been described here, we determined how CD151 and β_1 effected random migration. However, to determine how these proteins effect directed migration, studies need to be performed in which serum free media is placed inside of the insert and media containing FBS is placed inside of the well. This concentration difference will act to polarize the cells and enhance migration. Alternative migration studies cold also be performed in which the HUVECs are seeded on the opposite side of the membrane. This would mimic the configuration of intravasation rather than extravasation.

5-5.8 Determining the Effects of shRNA Gene Silencing vs Antibodies

There are two ways to negate the effects of a protein in a process. The first is through gene silencing. As mentioned earlier, shRNA was used to knock down the genes for CD151 and β_1 in the studies that were described there. There is also the method of siRNA (small interfacing RNA) to knock down genes. These methods both work by degrading the target mRNA (messenger RNA). The second method to negate the effects of a protein is through an antibody. An antibody will physically bind to the protein of interest and physically prevent the protein from interacting with its environment. These methods will affect the cell and its adhesion differently. For example, the loss of a protein could cause structural differences in the cell while the antibody could also block other proteins that are not the targeted protein. Doing comparison studies would determine if CD151 and β_1 were the cause of decreased adhesion or if it was due to an unknown side effect of the gene silencing.

List of Abbreviations

ATCC	American Type Cell Culture
BME	Basement Membrane Extract
CAM	Chick Chorioallantoic Membrane
CD151	Cluster of Differentiation 151
CXCL12	Chemokine (C-X-C motif) ligand 12
CXCR4	chemokine receptor type 4
CXCR7	chemokine receptor type 7
DMEM	Dulbecco's Modified Eagle Medium
DiI	1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindocarbocyanine Perchlorate
DiO	3,3'-Dioctadecyloxacarbocyanine Perchlorate
EBM	Endothelial Growth Basal Medium
EDTA	Trypsin Ethylenediaminetetraacid Acid
EGF	Epidermal Growth Factor
EGM-2	Endothelial Growth Medium 2
EMT	Epithelial to Mesenchymal Transition
FBS	Fetal Bovine Serum
GFP	Green Florescent Protein
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HGF	Hepatocyte Growth Factor
HUVEC	Human Umbicial Vein Endothelial Cell
ICAM-1	Intercellular Adhesion Molecule
IgG	Immunoglubulin G
LLC	Lewis lung carcinoma
MLEC	Mouse lung endothelial cells
mRNA	Messanger RNA
PBS	Phosphate-buffered saline
PECAM-1	Platelet endothelial cell adhesion molecule
PMA	phorbol 12-myristate 13-acetate

shRNA	small/short hairpin RNA
siRNA	small interfacing RNA
TGF-β	Transforming Growth Factor
TNF-α	Tumor Necrosis Factor-α
TNS	Trypsin Neutralizing Solution

References

1. Jemal, A., E. Ward, and M. Thun, *Declining death rates reflect progress against cancer.* PLoS One, 2010. **5**(3): p. e9584.
2. Hemler, M.E., *Tetraspanin functions and associated microdomains.* Nat Rev Mol Cell Biol, 2005. **6**(10): p. 801-811.
3. Lebrun, J.-J., *The Dual Role of TGF in Human Cancer: From Tumor Suppression to Cancer Metastasis.* ISRN Molecular Biology, 2012. **2012**: p. 28.
4. Wirtz, D., K. Konstantopoulos, and P.C. Searson, *The physics of cancer: the role of physical interactions and mechanical forces in metastasis.* Nat Rev Cancer, 2011. **11**(7): p. 512-522.
5. Yang, X.H., et al., *CD151 Accelerates Breast Cancer by Regulating α6 Integrin Function, Signaling, and Molecular Organization.* Cancer Research, 2008. **68**(9): p. 3204-3213.
6. Tokuhara, T., et al., *Clinical Significance of CD151 Gene Expression in Non-Small Cell Lung Cancer.* Clinical Cancer Research, 2001. **7**(12): p. 4109-4114.
7. Ang, J., et al., *CD151 Protein Expression Predicts the Clinical Outcome of Low-Grade Primary Prostate Cancer Better than Histologic Grading: A New Prognostic Indicator?* Cancer Epidemiology Biomarkers & Prevention, 2004. **13**(11): p. 1717-1721.
8. Jeon, J.S., et al., *In Vitro Model of Tumor Cell Extravasation.* PLoS ONE, 2013. **8**(2): p. e56910.
9. Siegel, R., D. Naishadham, and A. Jemal, *Cancer statistics, 2013.* CA: A Cancer Journal for Clinicians, 2013. **63**(1): p. 11-30.
10. Weigelt, B., J.L. Peterse, and L.J. van't Veer, *Breast cancer metastasis: markers and models.* Nat Rev Cancer, 2005. **5**(8): p. 591-602.
11. Weber, G.F., *Why does cancer therapy lack effective anti-metastasis drugs?* Cancer Letters, 2013. **328**(2): p. 207-211.
12. Nishida, N., et al., *Angiogenesis in Cancer.* Vascular Health and Risk Management, 2006. **2**(3): p. 213-219.
13. Folkman, J., *Tumor Angiogenesis: A Possible Control Point in Tumor Growth.* Annals of Internal Medicine, 1975. **82**(1): p. 96-100.
14. Carmeliet, P. and R.K. Jain, *Angiogenesis in cancer and other diseases.* Nature, 2000. **407**(6801): p. 249-257.
15. Deryugina, E.I. and J.P. Quigley, *Tumor angiogenesis: MMP-mediated induction of intravasation- and metastasis-sustaining neovasculature.* Matrix Biology, 2015. **44**–**46**: p. 94-112.
16. Weidner, N., et al., *Tumor Angiogenesis and Metastasis — Correlation in Invasive Breast Carcinoma.* New England Journal of Medicine, 1991. **324**(1): p. 1-8.
17. Kalluri, R. and R.A. Weinberg, *The basics of epithelial-mesenchymal transition.* The Journal of Clinical Investigation, 2009. **119**(6): p. 1420-1428.
18. Hart, I.R., *'Seed and soil' revisited: mechanisms of site-specific metastasis.* Cancer Metastasis Rev, 1982. **1**(1): p. 5-16.
19. Koop, S., et al., *Independence of metastatic ability and extravasation: metastatic ras-transformed and control fibroblasts extravasate equally well.* Proceedings of

the National Academy of Sciences of the United States of America, 1996. **93**(20): p. 11080-11084.
20. Reymond, N., B.B. d'Agua, and A.J. Ridley, *Crossing the endothelial barrier during metastasis.* Nat Rev Cancer, 2013. **13**(12): p. 858-870.
21. Chen, M.B., et al., *Mechanisms of tumor cell extravasation in an in vitro microvascular network platform.* Integr Biol (Camb), 2013. **5**(10): p. 1262-71.
22. Dustin, M.L. and T.A. Springer, *Lymphocyte function-associated antigen-1 (LFA-1) interaction with intercellular adhesion molecule-1 (ICAM-1) is one of at least three mechanisms for lymphocyte adhesion to cultured endothelial cells.* The Journal of Cell Biology, 1988. **107**(1): p. 321-331.
23. Butler, L.M., H.M. McGettrick, and G.B. Nash, *Static and Dynamic Assays of Cell Adhesion Relevant to the Vasculature*, in *Angiogenesis Protocols*, C. Murray and S. Martin, Editors. 2009, Humana Press. p. 211-228.
24. Prabhakarpandian, B., et al., *Microfluidic devices for modeling cell-cell and particle-cell interactions in the microvasculature.* Microvasc Res, 2011. **82**(3): p. 210-20.
25. Moss, M.A. and K.W. Anderson, *Adhesion of Cancer Cells to Endothelial Monolayers: A Study of Initial Attachment Versus Firm Adhesion.* The Journal of Adhesion, 2000. **74**(1-4): p. 19-40.
26. Chung, S., et al., *Cell migration into scaffolds under co-culture conditions in a microfluidic platform.* Lab Chip, 2009. **9**(2): p. 269-75.
27. Riahi, R., et al., *A microfluidic model for organ-specific extravasation of circulating tumor cells.* Biomicrofluidics, 2014. **8**(2): p. 024103.
28. Reese, B.E., et al. *Microfluidic device for studying tumor cell extravasation in cancer metastasis*. in *Biomedical Sciences and Engineering Conference (BSEC), 2010*. 2010.
29. Heyder, C., et al., *Realtime visualization of tumor cell/endothelial cell interactions during transmigration across the endothelial barrier.* Journal of Cancer Research and Clinical Oncology, 2002. **128**(10): p. 533-538.
30. Lawrence, M.B., L.V. McIntire, and S.G. Eskin, *Effect of flow on polymorphonuclear leukocyte/endothelial cell adhesion.* Blood, 1987. **70**(5): p. 1284-90.
31. Moss, M.A., S. Zimmer, and K.W. Anderson, *Role of metastatic potential in the adhesion of human breast cancer cells to endothelial monolayers.* Anticancer Res, 2000. **20**(3a): p. 1425-33.
32. Sia, S.K. and G.M. Whitesides, *Microfluidic devices fabricated in Poly(dimethylsiloxane) for biological studies.* ELECTROPHORESIS, 2003. **24**(21): p. 3563-3576.
33. De Nevers, N., *Fluid Mechanics for Chemical Engineers*. 2004: McGraw-Hill Higher Education.
34. Papaioannou, T.G. and C. Stefanadis, *Vascular wall shear stress: basic principles and methods.* Hellenic J Cardiol, 2005. **46**(1): p. 9-15.
35. Karamatic Crew, V., et al., *CD151, the first member of the tetraspanin (TM4) superfamily detected on erythrocytes, is essential for the correct assembly of human basement membranes in kidney and skin.* Blood, 2004. **104**(8): p. 2217-23.

36. Kagan, A., et al., *Occurrence of hereditary nephritis, pretibial epidermolysis bullosa and beta-thalassemia minor in two siblings with end-stage renal disease.* Nephron, 1988. **49**(4): p. 331-2.
37. Testa, J.E., et al., *Eukaryotic expression cloning with an antimetastatic monoclonal antibody identifies a tetraspanin (PETA-3/CD151) as an effector of human tumor cell migration and metastasis.* Cancer Res, 1999. **59**(15): p. 3812-20.
38. Zijlstra, A., et al., *The inhibition of tumor cell intravasation and subsequent metastasis through the regulation of in vivo tumor cell motility by the tetraspanin CD151.* Cancer cell, 2008. **13**(3): p. 221-234.
39. J.F. Haeuw, L.G., C. Bailly, N. Corvaia, *Tetraspanin CD151 as a target for antibody-based cancer immunotherapy.* Biochem Soc Trans, 2011. **39**(2).
40. Voss, M.A., et al., *Tetraspanin CD151 is a novel prognostic marker in poor outcome endometrial cancer.* Br J Cancer, 2011. **104**(10): p. 1611-1618.
41. Yue, S., W. Mu, and M. Zöller, *Tspan8 and CD151 promote metastasis by distinct mechanisms.* European Journal of Cancer, 2013. **49**(13): p. 2934-2948.
42. Takeda, Y., et al., *Deletion of tetraspanin Cd151 results in decreased pathologic angiogenesis in vivo and in vitro.* Blood, 2007. **109**(4): p. 1524-32.
43. Takeda, Y., et al., *Diminished metastasis in tetraspanin CD151-knockout mice.* Blood, 2011. **118**(2): p. 464-72.
44. Deng, X., et al., *Integrin-Associated CD151 Drives ErbB2-Evoked Mammary Tumor Onset and Metastasis.* Neoplasia, 2012. **14**(8): p. 678-IN3.
45. Deng, X., et al., *Integrin-associated CD151 drives ErbB2-evoked mammary tumor onset and metastasis.* Neoplasia, 2012. **14**(8): p. 678-89.
46. Cordes, N. and C.C. Park, *beta1 integrin as a molecular therapeutic target.* Int J Radiat Biol, 2007. **83**(11-12): p. 753-60.
47. Akiyama, S., K. Olden, and K. Yamada, *Fibronectin and integrins in invasion and metastasis.* Cancer and Metastasis Reviews, 1995. **14**(3): p. 173-189.
48. Brakebusch, C., et al., *Genetic analysis of beta1 integrin function: confirmed, new and revised roles for a crucial family of cell adhesion molecules.* J Cell Sci, 1997. **110 (Pt 23)**: p. 2895-904.
49. Zetter, B.R., *Adhesion molecules in tumor metastasis.* Semin Cancer Biol, 1993. **4**(4): p. 219-29.
50. Fujita, S., et al., *Alteration of expression in integrin beta 1-subunit correlates with invasion and metastasis in colorectal cancer.* Cancer Lett, 1995. **91**(1): p. 145-9.
51. Elliott, B.E., et al., *Anti-beta 1 integrin IgG inhibits pulmonary macrometastasis and the size of micrometastases from a murine mammary carcinoma.* Cell Adhes Commun, 1994. **1**(4): p. 319-32.
52. Cannistra, S.A., et al., *Expression and Function of β1 and αvβ3 Integrins in Ovarian Cancer.* Gynecologic Oncology, 1995. **58**(2): p. 216-225.
53. Strobel, T. and S.A. Cannistra, *Beta1-integrins partly mediate binding of ovarian cancer cells to peritoneal mesothelium in vitro.* Gynecol Oncol, 1999. **73**(3): p. 362-7.
54. Chen, M.B., et al., *Abstract 306: Role of tumor beta-1 integrin in the tumor cell extravasation cascade.* Cancer Research, 2015. **75**(15 Supplement): p. 306.

55. Schwartz, M.A. and A.R. Horwitz, *Integrating Adhesion, Protrusion, and Contraction during Cell Migration.* Cell, 2006. **125**(7): p. 1223-1225.
56. Horwitz, R. and D. Webb, *Cell migration.* Current Biology, 2003. **13**(19): p. R756-R759.
57. Lauffenburger, D.A. and A.F. Horwitz, *Cell Migration: A Physically Integrated Molecular Process.* Cell, 1996. **84**(3): p. 359-369.
58. Huttenlocher, A., R.R. Sandborg, and A.F. Horwitz, *Adhesion in cell migration.* Current Opinion in Cell Biology, 1995. **7**(5): p. 697-706.
59. Caswell, P. and J. Norman, *Endocytic transport of integrins during cell migration and invasion.* Trends in Cell Biology, 2008. **18**(6): p. 257-263.
60. Yauch, R.L., et al., *Highly Stoichiometric, Stable, and Specific Association of Integrin α3β1 with CD151 Provides a Major Link to Phosphatidylinositol 4-Kinase, and May Regulate Cell Migration.* Molecular Biology of the Cell, 1998. **9**(10): p. 2751-2765.
61. Sterk, L.M., et al., *Association of the tetraspanin CD151 with the laminin-binding integrins alpha3beta1, alpha6beta1, alpha6beta4 and alpha7beta1 in cells in culture and in vivo.* J Cell Sci, 2002. **115**(Pt 6): p. 1161-73.
62. Serru, V., et al., *Selective tetraspan-integrin complexes (CD81/alpha4beta1, CD151/alpha3beta1, CD151/alpha6beta1) under conditions disrupting tetraspan interactions.* Biochemical Journal, 1999. **340**(Pt 1): p. 103-111.
63. Nishiuchi, R., et al., *Potentiation of the ligand-binding activity of integrin α3β1 via association with tetraspanin CD151.* Proceedings of the National Academy of Sciences of the United States of America, 2005. **102**(6): p. 1939-1944.
64. Scales, T.M., et al., *alpha3beta1 integrins regulate CD151 complex assembly and membrane dynamics in carcinoma cells within 3D environments.* Oncogene, 2013. **32**(34): p. 3965-79.
65. Zoller, M., *Tetraspanins: push and pull in suppressing and promoting metastasis.* Nat Rev Cancer, 2009. **9**(1): p. 40-55.
66. Devbhandari, R.P., et al., *Profiling of the Tetraspanin CD151 Web and Conspiracy of CD151/Integrin β1 Complex in the Progression of Hepatocellular Carcinoma.* PLoS ONE, 2011. **6**(9): p. e24901.
67. Vigetti, D., et al., *Matrix metalloproteinase 2 and tissue inhibitors of metalloproteinases regulate human aortic smooth muscle cell migration during in vitro aging.* FASEB J, 2006. **20**(8): p. 1118-30.
68. Wang, C.M.X.-F., *In Vitro Assays for the Extracellular Matrix Protein-Regulated Extravasation Process.* Cold Spring Harb Protoc, 2008.
69. Zhang, Q., T. Liu, and J. Qin, *A microfluidic-based device for study of transendothelial invasion of tumor aggregates in realtime.* Lab Chip, 2012. **12**(16): p. 2837-42.
70. Holliday, D.L. and V. Speirs, *Choosing the right cell line for breast cancer research.* Breast Cancer Research : BCR, 2011. **13**(4): p. 215-215.
71. Shepro, D., *Microvascular Research: Biology and Pathology*. 2005: Academic.
72. Sakaue-Sawano, A., et al., *Visualizing spatiotemporal dynamics of multicellular cell-cycle progression.* Cell, 2008. **132**(3): p. 487-98.

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