Tensegrin in context
Dual role of α8 integrin in the migration of different cell types

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Key words: integrin, migration, adhesion, mesenchymal cell, epithelial cell, vascular smooth muscle cell

Abbreviations: VSMCs, vascular smooth muscle cells; SM-α-actin, smooth muscle α-actin; ECM, extracellular matrix; PDGF, platelet-derived growth factor; TGFβ, transforming growth factor-beta

α8β1 integrin is highly expressed in cells with contractile function, such as mesangial cells of the kidneys and vascular smooth muscle cells (VSMCs). Although it promotes migration of neural crest cells and breast cancer cells, recent studies suggest that α8 integrin has a negative regulatory role in VSMC migration. In this Review, the question of why α8β1 integrin plays a dual role in cell migration is raised and discussed. It seems that cells require optimum contractility and balanced tensile forces for migration. α8β1 integrin promotes migration of cells that are initially in a less than optimal contractile state (e.g., neural cells) and reduces the migration of cells known as contractile cells. α8β1 integrin can be called “Tensegrin” as it fits perfectly into the tensegrity model (tensional integrity) and seems to play a prominent role in the integration of the tensile forces.

Introduction

Integrin cell adhesion receptors serve as integrators of the cell’s exterior and interior, after which property they are named. Each integrin has its own signaling properties.1 α8β1 integrin is one of the latest integrins to be discovered. The only partner of α8 integrin is the β1-subunit. In contrast to α5β1, whose only known ligand is fibronectin, α8β1 can bind to several matrix components, including fibronectin,2 osteopontin,3 vitronectin, tenascin-C,4,6 tenascin-W7 and nephronectin.8

α8β1 integrin is highly expressed during kidney and lung development and α8-deficient mice display abnormal renal development suggesting that α8β1 integrin plays a critical role in organogenesis.8,9 Using FISH and genomic database analysis, Ekwa-Ekoka et al. have shown that α8 gene maps to chromosome 10p13 and consists of >200 kbp organized into 30 exons.10

α8β1 integrin, is intensely expressed in vascular smooth muscle cells (VSMCs), visceral smooth muscle cells, kidney mesangial cells, liver stellate cells and lung interstitial cells.4 In the adult lung, α8 integrin is expressed in contractile interstitial cells, including alveolar myofibroblasts, lipid-containing fibroblasts and pericytes.11 It seems that α8β1 integrin is expressed in cells with contractile properties. Existing evidence indicates a link between α8β1 integrin expression and cardiac, lung, kidney and liver fibrosis.12-14 When we look at the pathological conditions in which α8 expression is increased, we see that there is one property in common. In these fibrotic organs, tensile forces are increased.

Using gain and loss of function strategies, we demonstrated that α8 integrin functions to retard vascular smooth muscle cell (VSMC) migration.15,16 These observations have become controversial, as some reports have implicated α8 integrin as a positive regulator of motility in different cell types. Although data about α8 integrin’s role in differentiated epithelial cells is sparse, what is worthy of attention is that α8 integrin is upregulated during the migration of neural and breast cancer cells.

In neuronal cells, α8 integrin is found to promote cell attachment, spreading and neurite outgrowth.2 In addition, α8 integrin promotes breast cancer cell migration.2 It is interesting that α8 integrin can positively and negatively control migration in different contexts. Therefore, it seems that the role of α8β1 integrin is depending on the cell types.

Although it is speculative, whether α8 integrin can promote or inhibit migration may depend on the initial or differentiated state of the cells. In cells, which are differentiated for contractile function, including mesangial cells of kidney and VSMCs, reduced α8 integrin expression heightens migration, whereas in cells which are not initially contractile (e.g., neural cells), α8 integrin upregulation may promote migration.

In this work, we reviewed literature regarding α8β1 integrin’s role in different cell types with a stronger focus on VSMC function. Lessons from α8β1 integrin function in VSMCs may shed light on its dual role in different situations.
α8β1 Integrin Promotes Cell Migration

α8β1 integrin was first identified in the chick embryo nervous system.17 Zhang et al. have shown that α8 integrin is upregulated during development of the chicken optic tectum.18 α8 integrin promotes the migration of immature neurons during this process. It is noteworthy that neurite outgrowth is also driven by tension19,20 and application of tensional forces through the ECM directly promotes axon elongation.21

Another condition with which α8 integrin upregulation is associated is heightened migratory activity in human tumors,22 especially in more malignant tumors. Mammary tumors have high exogenous tension compared to normal mammary glands.23 The gradient in exogenous tension is high in tumors and low in surrounding tissues. Interestingly, α8 integrin is increased in the more invasive and migratory regions of tumors.7

α8 Integrin as a Differentiation Marker and a Negative Regulator of Migration

The main function of VSMCs is contraction to maintain vascular tone. Thus, VSMCs are differentiated for contractile function. After vascular injury, VSMCs are modulated from the contractile to the less contractile phenotype, which is a prerequisite for their migratory activity. Then, VSMCs migrate from the tunica media toward the intima, resulting in neointima formation.8

After balloon injury concomitant with loss of the contractile phenotype, α8β1 integrin is downregulated in the tunica media.15 α8 integrin gene silencing evokes the downregulation of VSMC contractile markers and the upregulation of de-differentiation markers.24 Moreover, α8 integrin gene silencing results in the VSMC changes from spindle to polygonal shape and stress fiber fragmentation into short bundles that are moved to the perinuclear region.25 These changes are characteristics of the de-differentiation state of VSMC and leads to the heightened VSMC migration,15 while α8 integrin overexpression in de-differentiated VSMCs attenuates migratory activity.16 Interestingly, it has been reported that α8β1 integrin is upregulated during the differentiation of mesenchymal cells from the kidney and lung.23 Therefore, α8β1 integrin seems to be upregulated during the differentiation of cells with contractile abilities and can serve as a differentiation marker of these cells.

Taken together, α8 integrin in all these conditions has a positive relationship with contractile state. Therefore, the question is: why does α8 integrin promote migration in cancer, while inhibiting it in VSMCs and mesangial cells? The answer resides in the concept that cells require adjustment for optimal adhesion and contractility to migrate.

Migration and Contractility

Cell movement is a complicated process involving dissolution of the cell’s contacts with other cells and the extracellular matrix (ECM), the formation of lamellipodia and new contacts with the environment and the contraction of actin filaments in the trailing edge, eliciting movement of the cell body.7 On an optimally-stiff surface, cells form adhesions and assemble actin structures that are sufficient to permit attachment and generate enough tractional force for movement, yet not so adhesive or contractile as to inhibit the release of adhesions at the trailing edge necessary to translocate the cell body.26

Because their primary function is contraction, VSMCs are in a highly contractile state. In this phenotype, the tensile forces exerted at the connecting point between the cell-ECM result in the development of focal adhesions and the assembly of parallel actin stress fibers.27 The consequence of this feature of cell-ECM interaction is excessive adhesiveness and contractility accompanied by reduced migratory activity.27

As mentioned earlier, cells require optimum adhesiveness and contractility to migrate. It has been shown that integrins may contribute to an increase in migration if adhesion is initially less than optimal.28 However, if cells are initially in an optimal adhesion state, integrins may decrease migration. It also appears that cells require optimum contractility to migrate. If cells are initially in a prestress situation and contractile mode (e.g., VSMCs and mesangial cells), the increase in contractility reduces migration. On the other hand, in cells, which are not initially contractile in nature (e.g., neuronal cells and ductal epithelial cells of breast) increase in contractility promotes migration (Fig. 1). In this context, α8 integrin may provide tensile forces required for optimal contractility and adhesiveness.

Therefore, the initial state of the cells and the balance between tensile forces outside and inside the cells can account for the different modes of α8 integrin action in different cell types.

Tensegrity and Integrins

In an attempt to explain the balance between internal and external forces, Donald Ingber introduced the tensional integrity model, which proposes that the whole cell is a prestressed...
structure. In this model, tensional forces are balanced by forces that resist compression. He explains how individual filaments can have dual functions and, therefore, exert either tension or compression in different structural contexts. The efficiency of mechanical coupling between these forces depends on the type of molecular adhesion complex that forms on the cell surface. We know that tension in the environment surrounding the cell is distributed by integrins. Integrins regulate cellular tension by triggering actin cytoskeleton organization. The tensegrity model holds that changes in the balance of mechanical forces across integrins can provide additional signaling to regulate cell function. By showing that with the same growth factors and integrin signaling different outcomes could result, depending on whether the cell is spread or round, Ingber emphasized the situation in cells before applying tensile forces. However, the question that can be raised is whether this tensegrity role is a general role attributed to all integrins or restricted to a small group of integrins?

To address this question I will discuss the role of different integrins in VSMC, which is a cell type with a prominent contractile function.

**Integrin or Integrins?**

Each integrin $\alpha\beta$ combination has its own signaling properties and different functions. It is likely that different integrins recruit different signaling molecules and differentially control cell signaling and cellular tension. Hence, we should avoid using the term “integrin” in general, which sounds as if all members of the group act synonymously. For instance, the pattern of integrin expression changes during VSMC phenotype modulation from contractile to non-contractile state. Some of the integrins that are poorly expressed in contractile VSMCs, especially $\alpha_2\beta_1$, $\alpha_3\beta_1$ and $\alpha_v\beta_3$, become more prominent in non-contractile state and mediate VSMC migration. $\alpha_2\beta_1$ integrin, a collagen receptor, has been implicated in platelet-derived growth factor (PDGF)-induced VSMC migration. It has been reported that stress fiber disassembly by fibroblast growth factor may promote the differential utilization of $\alpha_2\beta_1$ integrin for VSMC motility. Bix et al. have demonstrated that the interaction between $\alpha_2\beta_1$ integrin and endorepellin triggers a unique signaling pathway that leads to the disassembly of focal adhesions and stress fibers. $\alpha_5\beta_1$ and $\alpha_6\beta_1$ are poorly expressed in contractile VSMCs. However, they are upregulated in phenotype-modulated VSMCs and promote migration. $\alpha_v\beta_3$ is one of the most-studied integrins. $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins both mediate VSMC migration. Moreover, endothelin1, which is known to enhance contractility, inhibits $\alpha_v$ expression. Therefore, it appears that $\alpha_v$ integrin is downregulated in conditions of increased contractile forces. On the other hand, some other integrins are more related to the contractile state of VSMCs including $\alpha_8\beta_1$, $\alpha_1\beta_1$, and $\alpha_7\beta_1$ integrins. $\alpha_8\beta_1$ integrin is one of the integrins that is intensely expressed in VSMCs. It has been reported that $\alpha_1$ integrin is also another integrin involved in contraction. Downregulation of some other integrins, including $\alpha_7$ and $\alpha_1$, has been shown to be associated with the VSMC noncontractile phenotype. Therefore, it is plausible to divide integrins into two groups: contractile and less contractile integrins. However, there is always a balance between the expressions of different integrins. In our work, we observed that when $\alpha_8$ integrin is knocked down, $\alpha_1$ integrin is downregulated, whereas integrins which are poorly expressed in differentiated VSMCs, including $\alpha_2$, $\alpha_5$ and $\alpha_v$, are upregulated. Moreover, concomitant with loss of the VSMC-differentiated phenotype after several passages, $\alpha_8\beta_1$ integrin is downregulated while $\alpha_v\beta_3$ is upregulated. It has been demonstrated that $\alpha_v\beta_3/\beta_5$ and $\alpha_5\beta_1$ integrins were found to be elevated on the lumen side of the neo-intima and their expression was lower on the medial side, while our data disclosed that $\alpha_8\beta_1$ integrin was expressed and distributed more on the medial side of the neo-intima. Interestingly, the lack of $\alpha_1$ integrin is accompanied by an increase in $\alpha_v$ and $\alpha_5$ integrins. Therefore, it appears that the integrins involved in contractile function counterbalance other integrins. Taken together, it seems that among different members of the integrin superfamily, $\alpha_8$ integrin’s role is prominent in the inducing of tensile forces and its expression is always accompanied with an increase in contractility.

To further highlight the unique properties of $\alpha_8$ integrin, it should be noted that $\alpha_8\beta_1$ integrin binds to the RGD site in ECM proteins through mechanisms that are distinct and separate from $\alpha_5$ and $\alpha_v$ integrins. The cytoplasmic domain sequence of $\alpha_8$ integrin is distinct from all other known $\alpha$-subunit cytoplasmic domains, including $\alpha_1$ and $\alpha_5$, $\alpha_v$ and $\alpha_5$ along with $\alpha_1\beta_2$ are the $\alpha$-subunits most closely related to $\alpha_8$ (42–43% amino acid identity).

**$\alpha_8$ Integrin and Contractile Ability in VSMC**

In vitro studies have confirmed that $\alpha_8\beta_1$ integrin is upregulated in the VSMC contractile state while downregulated during phenotype modulation. It has been shown that transforming growth factor-beta (TGF-beta) can revert the phenotype of less contractile VSMCs to the contractile phenotype. However, in the presence of siRNA-$\alpha_8$ integrin, TGF-beta stimulation fails to induce VSMC re-differentiation. Moreover, TGF-beta-induced myofibroblastic features are impaired in $\alpha_8$ knocked down fibroblasts. On the other hand, in VSMCs that exhibit a less contractile phenotype, high-passage cells, $\alpha_8$ integrin overexpression elicits the restoration of contractile phenotype characteristics. Therefore, $\alpha_8$ integrin seems to exert a prominent role on both sides of VSMC phenotypic transition. Moreover, $\alpha_8$ integrin as well as SM $\alpha$-actin are upregulated in the neo-intima during constrictive remodeling concomitant with the late lumen loss. It seems likely that $\alpha_8$ integrin downregulation can shut down the mechanisms responsible for the VSMC contractile phenotype. It is well-documented that RhoA activity is critical for controlling the VSMC contractile phenotype. The assembly of actin stress fibers and focal adhesions. To be fully functional, RhoA needs to be anchored to the cell membrane. However, the membrane-associated molecules with which RhoA interacts remain uncharacterized. Interestingly, there is an interaction between $\alpha_8$ integrin and RhoA in VSMCs and $\alpha_8$ integrin gene silencing...
leads to reduced membrane-anchored RhoA, a hallmark of RhoA activity.\(^5\) (Fig. 2A).

**Tension-Dependent Growth and \(\alpha_8\) Integrin**

Another example of a biological process, in which tensile forces are increased, is proliferation. Proliferation requires augmented tension and contractility.\(^5\) Although the cellular mechanism involved is not clear, Rho proteins may play an important role in tension-dependent growth control,\(^5\) as they regulate cytoskeletal contractility and G\(_1\) progression.\(^5\) It has been suggested that expression of the proliferation genes is associated with the expression of contractile marker genes.\(^5\) The relationship between enhanced VSMC contractility and accelerated proliferation also seems to be reasonable according to studies by Ingber et al.\(^5\) who demonstrated that the cell’s ability to respond to surrounding mitogens is enhanced by increased contractility.

Interestingly, \(\alpha_8\) integrin is upregulated in proliferating VSMCs and its gene silencing reduces DNA synthesis,\(^5\) disassembly of actin stress fibers and dislocation of vinculin from focal adhesion sites (Fig. 2B). Moreover, siRNA-\(\alpha_8\) leads to the reduced membrane associated-RhoA (Fig. 2A). It appears that after \(\alpha_8\) gene silencing, RhoA cannot be anchored to the plasma membrane, thus, it leads to the disassembly of focal adhesions and stress fibers as well as the shape alteration, which are all characteristics of phenotype-modulated VSMCs. Although it seems that \(\alpha_8\) integrin and RhoA may be closely intertwined, our knowledge of \(\alpha_8\) integrin signaling is insufficient; hence, further clarification is fundamentally important.

**Lessons from Parallel Universes**

Cell types with contractile function share many characteristics with VSMCs and could be considered as parallel universes. Patterns of expression and function of \(\alpha_8\) integrin in these cells and also in pathological conditions where \(\alpha_8\) is upregulated could further elucidate its tensional role.

\(\alpha_8\) integrin is expressed in mesangial cells of the glomerulus.\(^1\) Stellate cells of the liver, lung alveolar myofibroblasts, and lung interstitial cells are other cell types with intense \(\alpha_8\) integrin...
expression in which contraction is a common feature. It has been shown that α8-deficient mice have a defect in sensory hair cells of the inner ear.59 The role of tension in the morphogenesis and function of these cells has also been reported.60 When we look at the pathological conditions in which α8 expression is increased, for example, after injury in models of pulmonary and hepatic fibrosis, in carotid constrictive remodeling after angioplasty or glomerulonephritis13,61 there is one property in common. In these fibrotic organs, tensile forces are increased. The other avenues where a significant role for α8 is observed are in development, in which tension is a central factor,62 especially in the later stages of morphogenesis. In these situations, cytoskeletal tension rises within nearby cells.63 Expectedly, α8 integrin is a marker for lung mesenchymal cells, starting early in development, and plays a role in branching morphogenesis.35 α8 integrin is critically important for epithelial-mesenchymal interactions during kidney morphogenesis.8 In a recent study, Benjamin et al.64 have verified a role for α8 integrin in lung development using α8-null mice. By using in vivo and in vitro studies they demonstrated that α8 integrin-null fetal lung mesenchymal cells fail to form stable adhesions and have increased migration. They suggested a critical role for α8 integrin in lung morphogenesis by regulating mesenchymal cell adhesion and migration.

Altogether, α8 integrin expression in the pathological and physiological conditions in which tensile forces required led us to propose an important tensile role for this integrin.

**Conclusion**

α8β1 integrin seems to play a critical role in regulating cell migration. α8β1 integrin reduces the migration of cells known as contractile cells. However, it promotes the migration of breast tumor cells as well as neural cells, which are initially in a less than optimal contractile state. As mentioned earlier, cells require optimum contractility to migrate. If cells are initially in a contractile mode (e.g., VSMCs and mesangial cells) the increase in contractility by α8 integrin reduces migration. On the other hand, in cells that are not initially contractile in nature (e.g., neuronal cells and dural epithelial cells of breast) α8 integrin may provide tensile forces required for optimal contractility and migration. Therefore, α8β1 integrin is upregulated when tension and contractility are required and its effect on cell migration may depend on the overall contractile nature of the cells and their environment.

α8 integrin properties seems to be different from those of other integrins. To the best of our knowledge, α8 integrin is the only integrin that has been reported to inhibit VSMC migration. Does α8 integrin induce conformational changes in the β1-subunit that are different from those of the other α-subunit partners of β1 integrin? The uniqueness of α8 signaling compared to other integrin subunits is an exciting issue that needs to be investigated.

One of the easiest ways to elucidate α8 integrin role in cell migration is to silence it in the different cell types (Mesangial cells, as an example of contractile cell and neural cell, as an example of non-contractile cell) and see how migration occurs. My speculation is that α8 integrin downregulation might promote migration in mesangial cells, while reducing migration in neural cells. To verify if the effect of α8 is due to the increase of tensile forces we can pretreat cells with RhoA activators and inhibitors.

Pretreatment of mesengial cells with Rho-kinase inhibitor Y-27632 can reduce tensile forces.65 Then, we can overexpress α8 integrin and examine whether it can increase tensile forces leading to the reduced migration or not. On the other hand, we can increase tensile forces in neural cells by activating actin fibers assembly or by RhoA activators and examine the migratory activity. After this step, siRNA-α8 can be applied to verify if reducing α8 inhibits migratory activity.

Another important experiment would be to verify if the tensile effect of α8 integrin is unique to α8 or applies to other contractile integrins (e.g., α1, α7) as well. The suggested experiment examines whether overexpression of α1 integrin in VSMCs can overcome the reduced tensile forces due to α8 integrin gene-silencing.

Doubtless, further studies, especially in vivo experiments, are necessary to unveil some elements of α8β1 integrin’s mode of action.

Finally, α8 integrin fits perfectly into the tensegrity model (tensional integrity). Integrins serve as integrators of the cell’s exterior and interior and α8 integrin seems to play a more prominent role in the integration of tensile forces. Therefore, inspired by the tensegrity model of Donald Ingber, I coin the name “Tensegrin” for α8β1 integrin. I hope that future investigations may shed light on its mysterious role in cell biology.

**Acknowledgements**

Hereby, I acknowledge the comments of Dr. Alan Hall. I also thank Dr. Gaetan Thibault for everything he provided during the years of my research on α8 integrin.

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