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Integrin function in vascular biology: a view from 2013

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Abstract

Purpose of review—This review considers recent developments concerning the role of integrins in vascular biology with a specific emphasis on integrin activation, and the crosstalk between integrins and growth factor receptors.

Recent findings—Recent studies have shown leukocytes can mediate direct transfer of molecules into endothelial cells, how specific integrins can be used to transduce signaling events, in particular in vascular beds, and how endothelial cell integrins can be targeted with specific ligands for the delivery of therapeutics. Kindlin and talin are both essential for integrin activation based on in-vivo studies of mice and humans in which the genes encoding for these proteins have been inactivated. Recent studies have attempted to translate these in-vivo realities into in-vitro models with mixed results.

Summary—Mechanisms and consequences of integrin–ligand interactions on blood and vascular cells remain a major topic of hematological research. Crucial to the ligand binding function of integrins are two intracellular binding partners, talin and kindlin. In seeking to define the molecular basis for 'integrin activation', a mechanism must be envisioned in which both proteins talin and kindlin are required to produce a productive functional response, be it platelet aggregation or leukocyte extravasation. On endothelial cells, integrins and vascular endothelial growth factor receptor 2 influence the activation of one another by virtue of their direct physical interaction. It has been shown that this bidirectional communication is subject to regulation during angiogenesis.

Keywords

growth factor receptors; integrin activation; kindlin; talin

INTRODUCTION

Vascular cells, be they components of blood vessels themselves or circulating within the blood vessels, must be constantly poised to sense and respond to changes within their environment. A primary mechanism for such sensory perception is the utilization of

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receptors that engage ligands in the extracellular environment, such as components of the extracellular matrix (ECM), blood, or on other cells they encounter. One group of receptors that orchestrate rapid responses to environmental changes is the integrin family of cell adhesion receptors. Integrins were named for their capacity to integrate cells into their extracellular environment by virtue of their capacity to bind ligands to their extracellular domains and link to the actin cytoskeleton via their intracellular cytoplasmic tails [1]. By controlling these linkages, cells use integrins to adhere, change shape, and migrate, and thus respond to or relocate within their environment. They also use integrins to transmit bidirectional signals, either inside-out or outside-in signals, across the cell membrane.

Each integrin is a noncovalent heterodimer composed of an α and β subunit. Each subunit crosses the cell membrane once in a type I orientation (N-terminal outside the cell), making its large extracellular domain available to bind environmental ligands, and its short cytoplasmic tail to act as a transmitter and receiver of intracellular signals. In mammals, there are a total of eight β and 18 α subunits that combine to form 24 integrins [2]. Integrins are expressed on virtually every cell type. Even though mature erythrocytes, the most abundant circulating blood cells, do not express or have only very low levels of integrins, their precursor erythroid cells do, and integrin expression levels are modulated as the erythroid cells undergo maturation [3]. Other circulating blood cells, such as platelets, express five different integrins (α IIb β 3, α v β 3, α 2 β 1, α 5 β 1, and α 6 β 1). The four members of β 2 subfamily of integrins are restricted primarily to leukocytes [4]. Depending on their anatomical origin, endothelial cells may express up to 10 integrins [5,6].

INTEGRINS

Known to exist on blood and vascular cells before the name 'integrin' was first applied to the family, new discoveries continue to be made regarding integrins and their function. In 2013, there have already been more than 1670 citations added to the almost 50 000 citations on 'integrins.' The role of integrins in cancer continues to be a dominant theme of recent articles, reflecting the high expression of integrins on transformed cells and the continued exploration of integrins as targets for the delivery of cancer therapeutics and tumor imaging. Noteworthy articles focusing on integrins in vascular biology include the study by Jerke *et al.* [7] that showed that β 2 integrins could mediate the transfer of myeloperoxidase (MPO) from neutrophils into endothelial cells. The MPO transfer required direct contact between the two cell types, and the MPO entered the cytosol and nucleus of the endothelial cell, and was functionally active. Such a direct transfer provides an alternative to secretion and uptake of soluble effector molecules. In another interesting integrin-related study, Welser-Alves *et al.* [8] examined the expression of β 4 integrin in the central nervous system and peripheral organs. α 6 β 4 is a laminin receptor, but it is only expressed in a small fraction of the cerebral blood vessels. The authors found that these vessels are arterioles, where β 4 played a role in arteriolar remodeling under hypoxic conditions associated with transforming growth factor (TGF)- β signaling. As a third selected article of interest, we call attention to the work of Pollinger *et al.* [9], which used ligand modified quantum dots to target the integrin α v β 3 on micro-vascular endothelial cells in the posterior of the eye. Intravenous injection of nanoparticles coated with a cyclic RGD ligand of the integrin targeted to endothelial cells, but not to other cells, and targeting did not occur in the absence

of ligand. This approach offers a possibility for therapeutic intervention at a particular site, the posterior of the eye, which is difficult to access.

INTEGRIN LIGANDS

The repertoire of integrin ligands is extensive and likely to continue expanding. Integrin ligands relevant to vascular biology include many ECM proteins. These are recognized by integrins on vascular cells, including both on the luminal and abluminal surface of endothelial cells, and by integrins on the surface of circulating blood cells. Prominent ECM ligands for integrins include fibronectins, collagens, laminins, and thrombospondins. Although these are named, it is only a partial list. Moreover, many of these ligands can bind to multiple integrins (e.g. fibronectin binds $\alpha 4\beta 1$, $\alpha 5\beta 1$, and $\alpha v\beta 3$; collagens bind $\alpha 1\beta 1$, $\alpha 2\beta 1$, and $\alpha 3\beta 1$). Integrins on blood cells can recognize ligands on the surface of endothelial cells [intercellular adhesion molecule 1 (ICAM 1), vascular cell adhesion molecule 1 (VCAM 1)], and thereby participate in the adhesion of circulating blood cells to endothelium and subsequent transmigration. Expression of endothelial integrin ligands can be rapidly modulated by cytokines, for example tumor necrosis factor (TNF)- α , thereby augmenting attachment and transmigration of blood cells into inflamed tissues.

A common feature of most natural ligands of integrins is their multivalency. Many are composed of multiple subunits, and each subunit presents an integrin recognition site. In many ligands, the individual subunits contain more than one nonidentical integrin recognition site. Furthermore, many of the ligands are capable of self-association and can densely pack themselves within the ECM. As a result, the molecules present to the integrins as ligands that can bind simultaneously to and cross-link several integrins, and such clustering may be important in transmitting intracellular signaling across integrins.

INTEGRIN ACTIVATION: A FUNCTIONAL PERSPECTIVE

In order for integrins to efficiently engage ligands on circulating cells, they must undergo activation. Although this term has been used extensively in the integrin literature for more than 30 years, it remains loosely defined. In many studies, integrin activation is equated with affinity modulation and is the outcome of inside-out signaling. Affinity modulation is dependent upon conformational change within a single integrin molecule, allowing it to increase its affinity for a monovalent integrin ligand. It is distinguished from avidity modulation, which depends upon the binding of multivalent ligands to clustered integrins. The distinction between avidity and affinity modulation can be conceptualized and distinguished with monovalent versus polyvalent ligands, and the primacy of each process has been discussed extensively in the corresponding literature [10–14]. However, in biological systems, where most ligands (other than designed drugs and snake venoms) are multivalent, both affinity and avidity modulation do occur. From the perspective of $\alpha IIb\beta 3$ on platelets, where the activation of this integrin is necessary for hemostasis and the absence of integrin activation leads to spontaneous bleeding episodes, the activation of this integrin has been shown to involve both avidity and affinity modulation [15,16]. Thus, these two events, affinity and avidity modulation, are inextricably linked and both events occur during productive integrin activation. Indeed, it seems more biologically relevant to define integrin activation from a functional perspective; these changes are necessary to result in a

functionally productive response, be it the formation of an aggregate of platelets or an arrest of leukocytes on endothelium.

INTEGRIN ACTIVATION: A STRUCTURAL PERSPECTIVE

An alternative approach in defining integrin activation is to entirely consider it on a structural basis. With the insights into the structure of integrins provided over the past decade, it currently appears feasible to provide such a definition. Accordingly, integrin activation begins with the unclasp of the complex between the cytoplasmic tails of the α and β subunits. In turn, this separation unclasp the association of the transmembrane helices between the two subunits and allows transmission of a conformational change into the extracellular domain to 'expose the ligand binding site.' The precise molecular details of these events have been discussed in several reviews [10,12,17–19]. The prevailing view is that the extracellular headpiece of the integrin (see Fig. 1) resides in close proximity to the cell membrane and is in a closed conformation, which provides limited access to high molecular weight ligands. Conformational changes in the extracellular domain include extension of the stalks or legs to move the headpiece away from the membrane, referred to as a 'switchblade motion,' and swing out of the hybrid domain to open up the headpiece and permit greater ligand access. This model allows the integrin to exist in an intermediate activation state in which the integrin legs are extended, but the headpiece still limits ligand access. There has been functional [20] and structural evidence [21–22] that both of these intermediate states can exist. However, we must always keep in mind that all of these states are in equilibrium, and ligand binding and integrin clustering are also equilibrium shifting events. Thus, as emphasized with G-protein coupled receptors, the energy landscape is constantly changing [23], and ligand and receptor encounters may occur and reverse on the way to leading to productive interactions.

ROLES OF TALIN AND KINDLIN IN INTEGRIN ACTIVATION

Talin is a cytoskeletal protein composed of a FERM domain containing head (talin-H) and a large rod domain (talin-R) (see Das *et al.* [24] for recent review). Evidence that talin plays a key role in integrin activation began to accumulate at the turn of the 21st century, and it is now broadly accepted that this role is indispensable. Talin's contribution to integrin activation depends on its binding via its talin-H to the cytoplasmic domain of the β subunit of integrin, and displacing it from its complex with the α subunit [25]. An NMR structure showed how the F3 subdomain within talin-H bound to the integrin $\beta 3$ subunit was reported in 2007 [26]. A recent crystal structure [27] revealed how a C-terminal segment within a specific segment of talin-R masks the integrin binding site in talin-H, and this structure also showed that the negatively charged surface in the talin-R segment prevents talin-H from binding to the cell membrane, an interaction that is vital to integrin activation. When talin becomes activated by PIP₂, the membrane strongly attracts talin-H and simultaneously repels the negatively charged talin-R. This 'pull-push' model differs from the previously accepted model for PIP₂-induced FERM domain activation.

With the heavy emphasis on the contribution of talin to integrin activation, it is often overlooked that the original discovery and function of talin arose from its linkage to the actin [28]. Yet, there has been a steady series of structural studies characterizing the

interaction of talin with actin and other cytoskeletal constituents, such as vinculin [29,30]. In one of the most recent contributions, Goult *et al.* [31■] showed that some of the vinculin binding sites in talin-R also bound Rap1-interacting adapter molecule (RIAM), a Rap1 binding protein also implicated in integrin activation. RIAM and vinculin interaction with these talin-binding sites was mutually exclusive, which could account for the differences in their distribution in adhesion structures in cells.

Kindlins are a three-member family of FERM domain containing proteins, and they are composed of the F1, F2, and F3 subdomains that typify FERM domains. A distinguishing characteristic of kindlins is the presence of a pleckstrin homology domain, which transects the F2 subdomain. Kindlins also contain an F0 subdomain at their N-terminus. Kindlins bind to integrin β subunits via their F3 subdomain. Talin also binds to integrin β subunits via its F3, but kindlin and talin can bind simultaneously to integrins [32■] and primarily to different sites in the integrin β subunit. A deficiency of each kindlin has profound pathogenic consequences in mice and/or humans. Kindler syndrome, a skin disease with frequent intestinal involvement in humans, arises from a kindlin-1 deficiency, and the phenotype is recapitulated in mice that are deficient in kindlin-1 [33,34]. Kindlin-2 deficiency is embryonically lethal in mice [35]. Particularly, impressive evidence for the role of kindlins in integrin function comes from the studies of kindlin-3. The implication of kindlin-3 in platelet aggregation is based not only on murine knockout studies [36,37], but most convincingly on the discovery of a new human disease, leukocyte adhesion deficiency III (LADIII). When kindlin-3 was missing as a consequence of mutations, patients presented with episodic bleeding, and blunted platelet aggregation in response to various agonists and an absence of α IIB β 3 activation [38,39]. LADIII patients are also susceptible to frequent infections and osteopetrosis (increased bone density) [39–41]. These symptoms, as well as abnormally shaped erythrocytes, were noted in mice in which the gene for kindlin-3 had been inactivated [42,43]. In a recent patient with LADIII, abnormal red cell shape was also noted for the first time [44■]. Altogether, the described abnormalities associated with kindlin deficiencies attest to their importance in integrin activation, and thus lead to the unequivocal conclusion that kindlins are essential for functionally productive integrin activation.

Recent studies have sought to assign specific roles to talin and kindlins in integrin activation. In one such study, integrin α IIB β 3 was reconstituted into nanodiscs, and it was found that talin was sufficient to induce integrin 'activation', whereas kindlin was not [45]. This conclusion would seem to ignore the severe symptoms and deficits in the activation of multiple integrin subclasses associated with kindlin-3 deficiency in LADIII patients, despite normal levels of talin. Thus, from a number of functional studies, it is absolutely clear that talin alone is not sufficient to induce productive integrin activation in physiological systems, that is to induce the binding of naturally occurring ligands exemplified by fibrinogen [35] or to cause leukocyte arrest on endothelium [33]. The differences in these results may revolve around the definition of 'integrin activation' as discussed above. One must also consider that the nanodiscs lack essential membrane components that are critical for kindlin function.

It was recently demonstrated that neutrophils and T-cells are able to weakly bind their ligands and adhere to endothelium in a transient fashion in the absence of kindlin-3 [46■].

This kindlin-independent process occurred only when endothelial cells expressed high levels of integrin ligands VCAM-1 and ICAM-1. However, firm adhesion of T cells and their arrest on immobilized integrin ligands or endothelium required the presence of kindlin-3. Thus, talin alone may be able to mediate the initial transition of integrin to a partially activated or 'intermediate conformation' enabling integrins to weakly and transiently interact with their ligands (see Fig. 1). However, further transition of the integrin to a high affinity state, which supports stable ligand binding known to be essential for T-cell arrest and extravasation, requires kindlin-3 [46■■]. Because of the multimeric nature of physiological integrin ligands, it is possible that this final kindlin-dependent stage of integrin activation and stabilization of integrin–ligand bonds is reciprocally linked to integrin clustering [41,42]. This evolving model is also consistent with a recent publication of Lefort *et al.* [20], in which the authors concluded that talin induced a first step of integrin activation, that is the extension of $\beta 2$ integrins, while kindlin-3 was necessary to open up the head piece, and both events were necessary for leukocyte extravasation. Thus, it is clear that productive integrin activation requires two consequent steps where both talin and kindlin are required, and neither is sufficient.

REGULATION OF INTEGRIN FUNCTION BY INTERACTIONS WITH OTHER CELLULAR RECEPTORS

Aside from integrin activation by inside-out signaling, integrin function can be modulated by their direct or indirect interactions with other transmembrane proteins.

For endothelial cells, growth factor receptors are integrins' most important partners. It is quite well established that growth factors, vascular endothelial growth factors (VEGFs) in particular, are able to activate various integrins by the means of inside-out signaling [47].

However, the relationship between the integrin and growth factor receptor was shown to be more complex than unidirectional activation [48]. Integrin ligation was shown to augment growth-factor-induced signalling and, vice versa, stimulation with growth factors leads to modulation of integrin activity. Thus, this crosstalk is clearly reciprocal in nature [49]. The best known example of this partnership is the cross-talk between VEGF receptor 2 (VEGFR2) and $\alpha v\beta 3$ integrin, which was shown to control adhesive and migratory responses of endothelial cells during vascular development and VEGF-induced angiogenesis [49–52]. Treatment with VEGF or integrin ligation induces a complex formation between these two receptors, which can be disrupted either by mutations within $\beta 3$ integrin cytoplasmic domain or by cell-permeable peptides corresponding to the key sequences within $\beta 3$ integrin [49,53■■]. With the disruption of the complex between receptors, endothelial adhesion, migration, and angiogenesis are all suppressed. Importantly, not only individual receptors but also their crosstalk or complex represent a target for additional regulation during angiogenesis.

A recent study by Ravelli *et al.* [54■■] identified the bone morphogenic protein antagonist gremlin as a novel noncanonical ligand for VEGFR2 and a strong inducer of angiogenesis. Gremlin binds to VEGFR2 and stimulates its interaction with $\alpha v\beta 3$ integrin as shown by both co-immunoprecipitation and fluorescence resonance energy transfer analysis. This

interesting study further emphasizes the importance of the functional crosstalk between integrin and VEGFR2. Another study by Veron *et al.* [55] showed that similar crosstalk between VEGFR2 and $\alpha v\beta 3$ integrin is operational in the glomerulus. VEGF knockdown in podocytes disrupted integrin activity leading to the disruption of the glomerular filtration barrier, which in turn resulted in proteinuria and ultimate acute renal failure.

Although multiple groups identified positive regulators of integrin–VEGFR2 crosstalk in angio-genesis, a manuscript from Gong *et al.* [56] truly stands out by showing that this crosstalk can also be negatively regulated. This novel mechanism involves Sprouty4 in endothelial cells. Overexpression of Sprouty4 leads to deficient angiogenesis *in vivo*, whereas its deletion has an opposite effect. Sprouty4 exhibits c-src activation, $\alpha v\beta 3$ integrin phosphorylation and its crosstalk with VEGFR2, and as a result endothelial cell migration during angiogenesis. Therefore, the complex and partnership between integrin and VEGFR2 is subject to elaborate regulation, which further emphasizes the physiological importance of this mechanism.

During angiogenesis, endothelial cells encounter various ECM proteins and engage more than one integrin during their migration induced by VEGF/ VEGFR2. Therefore, it is not surprising that VEGFR2 is involved in a similar type of interplay with $\beta 1$ integrin. Several studies demonstrated a synergism between $\beta 1$ integrin-dependent adhesive functions and downstream VEGFR2 signaling [57,58]. Mechanistically, this synergism is mediated by tetraspanin CD63, which interacts with both $\beta 1$ integrin and VEGFR2, and serves as a critical regulator of the complex between these two signaling components. Silencing of CD63 disrupts the $\beta 1$ integrin/VEGFR2 complex leading to deficient VEGFR2 activation and impaired endothelial adhesion and migration [58].

Another study demonstrated that integrin-associated protein (CD47) associates with $\alpha 4\beta 1$ and $\alpha L\beta 2$ integrins (also known as VLA-4 and LFA-1, respectively) and regulates their function required for leukocyte adhesion to endothelium and transmigration [59]. T cells lacking CD47 exhibited defects in recognition of endothelial ligands represented by ICAM-1 and VCAM-1, and, as a result, the T cells displayed reduced adhesion to TNF- α activated endothelium.

Thus, it is clear that many integrins and growth factor tyrosine kinase receptors on endothelium are a part of a complex and highly dynamic network on the cell surface. Clustering of different types of receptors leads to assembly of large signaling complexes, containing prominent intracellular adapter proteins and kinases, thereby creating favorable conditions for direct and indirect cross-activation of receptors. One of the most prominent players in this convergence of integrin and growth factor signaling pathways is c-src kinase [49,56,58]. Considering that each individual integrin or growth factor receptor has its own spectrum of binding partners, the numbers of molecules within these complexes might increase exponentially, thereby multiplying the possibilities of cross-activation and/or cross-inhibition of these receptors.

CONCLUSION

Integrin function on vascular and blood cells is absolutely essential for a number of physiological processes, including but not limited to the development, stability, and reorganization of vasculature, hemostasis, and inflammation. Integrin activation, defined as a transition to the state where integrin can productively bind its physiological ligand is more than a one step process. Although the first step enabling transient binding to ligands seems to be dependent on talin, the completion of integrin activation resulting in stable integrin–ligand bonding requires kindlin. Both molecules are essential for functional integrin activation and neither one is sufficient to complete the process on the cell surface. More studies are needed to define how exactly these two molecules are recruited to integrin complexes upon stimulation, and what the spatiotemporal dynamics and functional consequences are of interactions between integrins and their multiple binding partners. Integrins seem to function as a dynamic platform for an assembly of regulatory multimolecular complexes, thereby creating a possibility of therapeutic targeting of these partnerships rather than individual receptors.

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KEY POINTS

- It is reasonable to define integrin activation based on functional readouts, that is stable binding of naturally occurring ligands, which serves as a prerequisite for physiologically important functional consequences such as platelet aggregation or firm leukocyte adhesion and arrest.
- Functional integrin activation occurs in more than one step, and for naturally occurring integrin ligands affinity and avidity modulation are likely to be tightly and reciprocally linked.
- Integrin activation on blood and vascular cells requires both talin and kindlin.
- Apart from inside-out signaling, integrin function can be positively or negatively regulated via interactions with multiple transmembrane receptors.

FIGURE 1.
Mechanisms of integrin activation.