

Review

Why Integrin as a Primary Target for Imaging and Therapy

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Abstract

Integrin-mediated cell adhesion is involved in many essential normal cellular and pathological functions including cell survival, growth, differentiation, migration, inflammatory responses, platelet aggregation, tissue repair and tumor invasion. 24 different heterodimerized transmembrane integrin receptors are combined from 18 different α and 8 different β subunits. Each integrin subunit contains a large extracellular domain, a single transmembrane domain and a usually short cytoplasmic domain. Integrins bind extracellular matrix (ECM) proteins through their large extracellular domain, and engage the cytoskeleton via their short cytoplasmic tails. These integrin-mediated linkages on either side of the plasma membrane are dynamically linked. Thus, integrins communicate over the plasma membrane in both directions, i.e., outside-in and inside-out signaling. In outside-in signaling through integrins, conformational changes of integrin induced by ligand binding on the extracellular domain altered the cytoplasmic domain structures to elicit various intracellular signaling pathways. Inside-out signaling originates from non-integrin cell surface receptors or cytoplasmic molecules and it activates signaling pathways inside the cells, ultimately resulting in the activation/deactivation of integrins. Integrins are one of key family proteins for cell adhesion regulation through binding to a large number of ECM molecules and cell membrane proteins. Lack of expression of integrins may result in a wide variety of effects ranging from blockage in pre-implantation to embryonic or perinatal lethality and developmental defects. Based on both the key role they played in angiogenesis, leukocytes function and tumor development and easy accessibility as cell surface receptors interacting with extracellular ligands, the integrin superfamily represents the best opportunity of targeting both antibodies and small-molecule antagonists for both therapeutic and diagnostic utility in various key diseases so far.

Key words: Integrin, inside-out signaling, outside-in signaling, cell adhesion molecule, angiogenesis.

1. INTRODUCTION

Adhesion of cells to the surrounding extracellular matrix (ECM) and to other cells is essential for tissue integrity, which have been crucial for the evolution from protozoans to metazoans. Integrin-mediated cell adhesion to ECM plays a central role

in the formation and remodeling of tissues and organs in multicellular organisms [1-2]. Integrins mediate cell-to-cell and cell-to-ECM adhesion, providing adhesion for stationary cells, traction during cell movement and, importantly, the promotion of many

signaling pathways that regulate diverse processes including proliferation, migration, cell survival, differentiation, tumor invasion and metastasis [3-4]. As a family of heterodimeric transmembrane receptors consisting of an α and a β subunit, 18 different α and 8 different β subunits are known in mammals, which can combine to 24 different integrin receptors [5-6]. Some subunits can combine with several different partners, adding to the structural complexity of integrin receptors. All these integrins can be segregated into two groups, one containing and the other lacking an extra von Willebrand factor type A domain (vWFA, known as α A or α I in integrins) in their α -subunits (Fig. 1). The composition and morphology of integrin-dependent adhesions (focal complexes/podosomes, focal adhesions and fibrillar adhesions) vary with the cell type, matrix and integrin, and may switch from one adhesion contact to another, contributing to cell advancement, tissue remodeling and repair [7]. Based on their important biological and pathological function, integrins have been intensively investigated as imaging or therapy targets, which will be discussed in details in the following chapters of this book. The purpose of this chapter is to give an overall description of the structure, expression pattern, and biological/pathological function of various integrins.

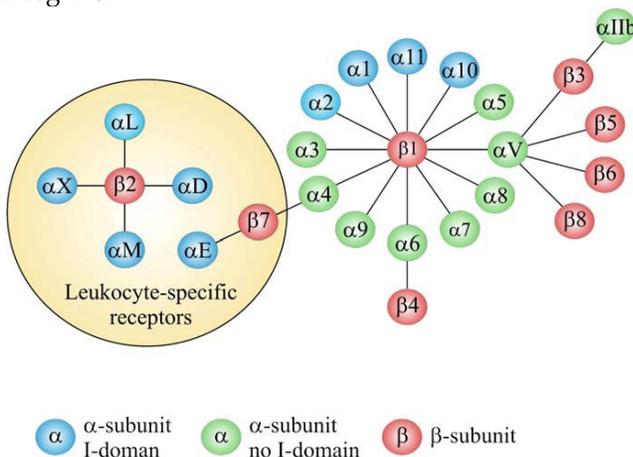


Figure 1. The integrin superfamily. The integrins can be subdivided according to their β chains but note that some α chains can combine with several β chains. 24 different integrins are present in humans [8].

2. INTEGRIN STRUCTURE

Each integrin subunit contains a large extracellular domain (N-terminus of >700 residues), a single transmembrane domain (>20 residues) and a usually short cytoplasmic domain (C-terminus of 13-70 residues) [9]. The I (inserted) domain is the most im-

portant integrin domain in ligand recognition [10]. For integrins, the α I domain presents in approximately half of all α subunits and the β I domain presents in all β subunits. The α I domain is the primary ligand binding region in the integrins which have this domain. Whereas in integrins which lack the α I domain, the binding site in the integrin "head" is formed by structural contributions of both the α and β chains [11]. α I is a GTPase-like domain in which the catalytic site at the apex is replaced with a conserved metal-ion-dependent adhesion site (MIDAS), which is occupied by a divalent cation. All of the integrin-ligand interfaces analyzed thus far have had at their center a coordinate bond between the integrin MIDAS and a carboxylate from the ligand and Mg^{2+} is the physiological metal that comprises integrin MIDAS [12]. α I domains have been crystallized in three distinct conformations, termed closed, intermediate, and open, with coordination of the metal in the MIDAS, arrangement of the β 6- α 7 loop, and axial disposition of the C-terminal α 7-helix along the side of the I domain [13-14]. Change in coordination at the MIDAS of α I domains is coupled to backbone movements of loops that bear the coordinating residues to increase complementarities to ligands (Figure 2) [15].

In the α I domain-collagen triple helical peptide structure [16], the stiff collagen rod docks onto a shallow trench on top of the I domain, with MIDAS situated at the center, coordinated by a glutamate residue of collagen. Another major α I ligand class is comprised of cell surface molecule intercellular cell adhesion molecules (ICAMs). These are recognized by a β 2 class of integrins that are exclusively expressed on leukocytes. This interaction is crucial in enabling leukocyte migration into sites of inflammation. Based on the structure of the ICAM-3- α I domain solved by Song et al. [17], and the important structural determinants for the interaction are distributed across a rigid scaffold composed of a β sheet and a structurally constrained loop.

When present, α I domain is situated at the top of the heterodimer and forms clear protrusions [18-19], whereas the β I domain folds together intimately with the β -propeller domain of the α subunit, constituting half of the large globular head [20]. The crystal structure of the ectodomain from the α I domain-lacking integrin, α v β 3, was also determined in both unliganded (in the presence of Ca^{2+} or Mn^{2+}) and ligand-bound states [11, 21]. As shown in Figure 3, α v consists of four domains: an N terminal seven-bladed β -propeller, an Ig-like Thigh domain and two large β -sandwich domains, Calf-1 and Calf-2. The β -subunit comprises an N-terminal cysteine-rich PSI

(Plexin-Semaphorin-Integrin) domain in which an Ig-like “Hybrid” domain is inserted in its c-terminal loop (the Hybrid domain contains in turn an α A-like domain (β A domain) inserted between its two β -sheets). PSI is followed by four EGF-like domains and a membrane proximal novel tail domain (β -TD). The propeller and β A domains assemble into a “head” structure, which accounts for formation of the $\alpha\beta$ heterodimer. The integrin head sits on top of an α and a β “legs”, formed respectively of the Thigh and Calf domains from the α V-subunit and the PSI, Hybrid, EGF1-4 and β TD domains from the β 3-subunit [11].

The structure of liganded $\alpha\beta$ 3 ectodomain was determined after diffusing the high affinity cyclic pentapeptide, cilengitide, which contains the prototypical Arg-Gly-Asp (cRGD), into preformed integrin

crystals in the presence of 5 mM Mn^{2+} . RGD inserts into a crevice between the propeller and β A domains, with the residues R and D side chains exclusively contacting the propeller and β A domains, respectively, and bringing these two domains closer together [21]. The activating inward movement of α 1 helix and rearrangement of the F/ α 7 loop seen in liganded α I are also observed in liganded β I, leading to similar although much less dramatic changes in the three surface loops coordinating MIDAS and the SDL loop. Metal coordination at ADMIDAS changes significantly, such that it no longer locks the α 1 helix to the F/ α 7 loop, allowing the activating movements in both. The inward movement of α 1 carries ADMIDAS with it, moving it closer to MIDAS, thus helping stabilize the new metal ion at MIDAS [21].

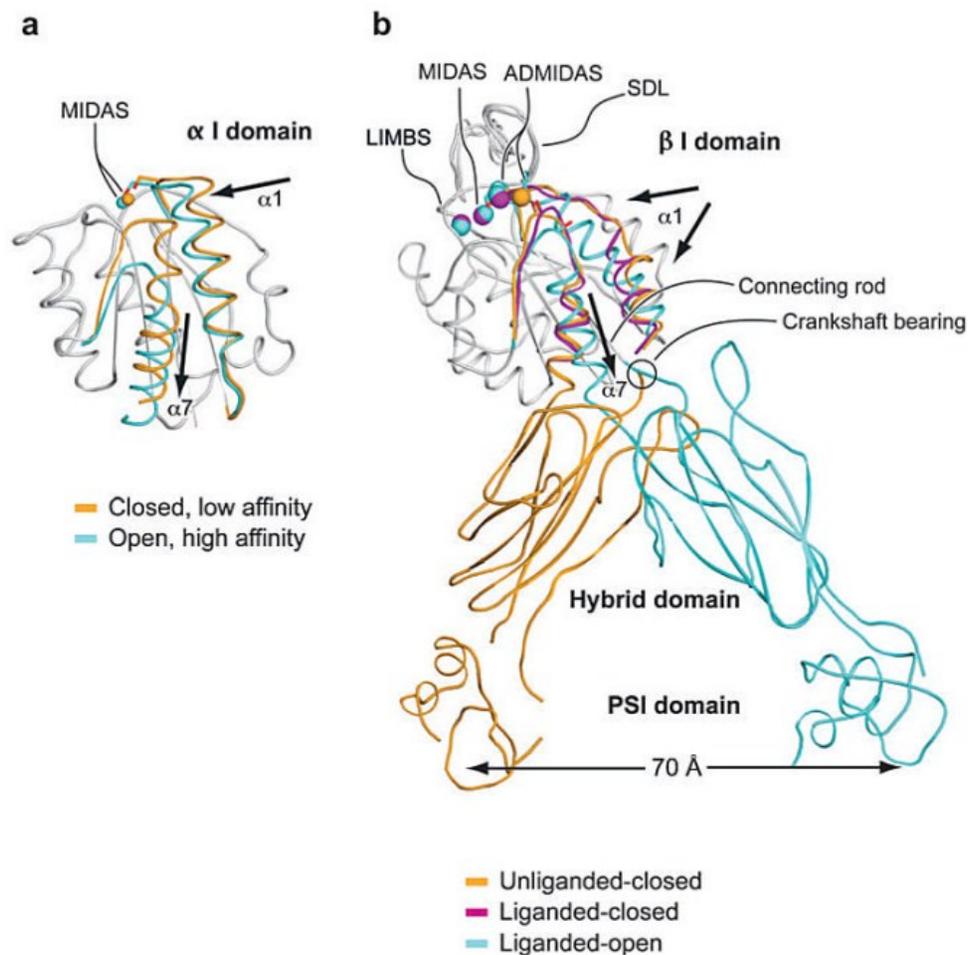


Figure 2. Conformational change and transmission of allostery by α and β I domains. (a) The α I domain. Nonmoving segments of the backbone are shown as a gray worm. The moving segments of the backbone and the MIDAS metal ions are closed (gold) and open (cyan). The direction of movement is indicated with arrows. (b) The β I domain and its linkage to the hybrid and plexin/semaphorin/integrin (PSI) domain. Nonmoving segments of the β I backbone are shown as a gray worm. Moving segments and metal ions are color coded as shown. Directions of α 1- and α 7-helix movements are shown with arrows [18].

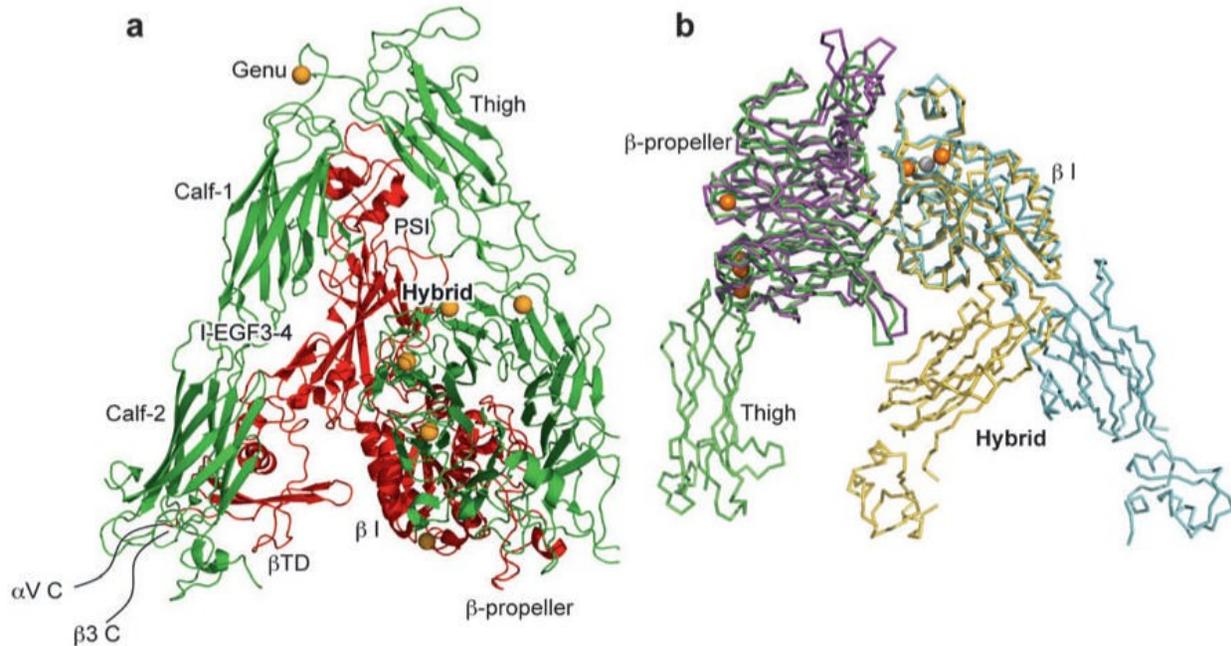


Figure 3. Crystal structures of integrins $\alpha V\beta 3$ and $\alpha IIb\beta 3$. (a) The structure of $\alpha V\beta 3$ in the bent conformation. The αV and $\beta 3$ subunits are colored in green and red, respectively. (b) Superposition of liganded-open $\alpha IIb\beta 3$ and unliganded-closed $\alpha V\beta 3$ headpieces. The α and β subunits are colored in green and yellow in $\alpha V\beta 3$ and in purple and light blue in $\alpha IIb\beta 3$. Calcium and magnesium ions in $\alpha IIb\beta 3$ only are gold and gray spheres, respectively [18].

Crystal structures of the $\alpha V\beta 3$ ectodomain in complex with the cyclic RGD peptide [21], as well as another RGD-type integrin ($\alpha IIb\beta 3$) complexed with ligand mimetic compounds [22] have revealed that the minimal RGD set provides two essential anchorpoints: one is the Asp carboxylate, which coordinates directly the β MIDAS; the other is the Arg side chain (or basic moiety), which forms a salt bridge to an acidic residue in the b-propeller domain of the $\alpha V/\alpha IIb$ subunit. In addition, these anchorpoints are located at the bottom of the narrow cleft or 'well', where the small ligand compounds are well accommodated, burying more than half of the molecular surface at the interface. $\alpha V\beta 3$ integrin shows little preference in its choice among the many RGD-containing proteins. Surprisingly, even the strict RGD sequence requirement rule can be bent [23]. For example, cells from homozygous knock-in mice carrying the RGD→RGE mutation in fibronectin exhibited normal fibronectin assembly in an $\alpha V\beta 3$ -dependent manner [24]. This observation indicated that the positioning of both an acidic and basic residue in proper geometrical proximity is sufficient for meaningful affinity. The well-type pocket containing β MIDAS seems specifically designed to accommodate 'loop ligand'. Residues adjacent to RGD seem to provide some specificity toward certain in-

tegrins, both by making extra direct contacts and by changing the 'width' of the RGD segment [25-26].

Although α MIDAS and β MIDAS normally function in a tandem fashion in αI domain-containing integrins, they each could potentially engage two independent external ligands simultaneously. For example, semaphorin 7A, a GPI-anchored membrane protein that contains an RGD motif, was reported to promote axon outgrowth through $\beta 1$ integrin(s), though the α subunit responsible for this activity was not determined [27]. Purified $\alpha 1\beta 1$ does recognize semaphorin 7A in an RGD-dependent manner [28]. It is therefore tempting to speculate that RGD binds to the β MIDAS, leaving the α MIDAS unoccupied. Laminins are major basement membrane proteins composed of three different polypeptide chains, termed a, b, and g. Till date, five a, three b, and three g chains are known to make up at least 15 isoforms of heterotrimeric laminins in mammals [29]. It has been reported that integrins exhibited a distinct laminin isoform preference [30]. Mutational and antibody mapping studies have revealed that C-terminal laminin globular (LG) domains of the a chain, particularly LG1-3, are important for integrin recognition [31]. However, recombinant fragments corresponding to this region have not shown significant cell adhesion activity comparable to that of intact heterotrimeric

laminins. Full integrin-binding activity of LG1-3 requires the heterotrimeric C-terminal portion of the coiled-coil domain, including the most distant C-terminal region of the b and g chains [32].

Accumulating evidence supported that the structure changes of integrins are the basis of the integrin signaling. Indeed, negative stain electron microscopy with image averaging of integrins has shown three overall conformations of the extracellular domain and these correspond to a low affinity, bent conformation (as in the $\alpha\beta3$ structure), an intermediate affinity, extended form with a closed headpiece, and a high affinity, extended form with an open headpiece, which is induced by ligand mimetic compounds [22]. In integrins that lack the I-domain, the ligand binds directly to the β I-like domain, and causes a downward movement of its $\alpha7$ helix, similarly to what was described above for the integrin α I-domains. This shift results in a swing-out movement of the β chain hybrid and PSI domains and these domains act as a rigid lever that transmits and amplifies the motion, resulting in a separation of the α and β legs and integrin extension [22]. On the other hand, the swing-out movement of the upper β leg could readily occur if it were preceded by extension of both legs.

Several models have been proposed to integrate the structural changes of integrins and consequent biochemical findings [33]. For example, a "switch-blade" model proposed that the head structure with the large ($\sim 80^\circ$) Hybrid swing-out represents the high affinity state of βA . To provide the necessary space for the Hybrid swing-out, the model suggests that the integrin first fully extend its knees, converting the bent into a linear asymmetric conformation, described as such in 2D EM images [19]. An alternate "dead-bolt" model suggest that elastic deformation of the F/ $\alpha7$ loop is possible with only modest quaternary changes at the β TD interface with βA and possibly Hybrid domains [34]. It has been found that upon binding of ICAM-5 to the LFA-1 I-domain, a remarkable outward movement of the $\alpha7$ helix was observed, which resulted in the replacement of the corresponding $\alpha7$ helix from a neighboring I-domain into the $\alpha7$ helix position, but in an upside-down configuration. This $\alpha7$ helix replacement was further propagated, resulting in a large I-domain/ICAM-5 cluster. In this way, a weak initial interaction between the integrin and a ligand can result in the formation of large ligand/receptor aggregates [35].

3. INTEGRIN SIGNALING

Integrins bind ECM proteins through their large extracellular domain, and engage the cytoskeleton via

their short cytoplasmic tails. These integrin-mediated linkages on either side of the plasma membrane are dynamically linked. The cytoskeleton controls affinity and avidity of the integrin extracellular domain to modulate the ECM, and integrin binding to ECM changes the shape and composition of the cytoskeleton beneath [36]. Thus, integrins communicate over the plasma membrane in both directions, i.e., outside-in and inside-out signaling [18]. In outside-in signaling through integrins, ligands bind to extracellular integrin domains, where a conformational change occurs so that the signal is transmitted into the cell. Integrin clustering may also occur. Inside-out signaling originates from non-integrin cell surface receptors or cytoplasmic molecules and it activates signaling pathways inside the cells, ultimately resulting in the activation/deactivation of integrins. In this case, adhesion may be regulated both by conformational changes in the integrin and by valency change (integrin clustering). In fact, both signaling events may occur simultaneously and reinforce each other [8].

Changes in interactions and/or in the structures of the cytoplasmic domains of integrins within the membrane-proximal regions play crucial roles in integrin activation via inside-out signaling [37-38]. Conformational changes of integrin induced by ligand binding on the extracellular domain altered the cytoplasmic domain structures to elicit various intracellular signaling pathways. A prominent biochemical event in integrin outside-in signaling is protein tyrosine phosphorylation due to activation of Src and FAK family protein tyrosine kinases [39-40]. Src and its inhibitory Csk are constitutively bound to the integrin β -tails. Integrin binding to ligand induces Src activation through Csk dissociation, Src transphosphorylation and/or recruitment of tyrosine phosphatases (e.g. receptor tyrosine phosphatase RPTP α , or PTP-1B [39]. However, the spatiotemporal and structural basis of the interaction of integrin cytoplasmic-tails with the growing list of signaling molecules including cytosolic and receptor kinases (Src, FAK, Syk, ILK and PKC) and phosphatases remains to be determined [9].

The integrin cytoplasmic domains play a central role in integrin activation [41-42]. However, the short integrin cytoplasmic tails lack enzymatic activity, and so depend on recruitment of adaptor molecules and signaling proteins for effector functions [36]. Talin is a major cytoskeletal protein that co-localizes with and binds to integrins and to actin and actin-binding proteins such as vinculin [43]. It has been demonstrated that binding of talin to the β cytoplasmic domain is a crucial, final step in activation of several classes of integrins [44-45]. Knockdown of talin expression in

CHO (Chinese-hamster ovary) cells inhibits the activation of both $\beta 1$ and $\beta 3$ integrins without altering integrin expression [44]. NMR studies suggest that direct disruption of α - β tail interaction by the talin head domain result in integrin activation [46]. Proposed a two-step activation model, the talin head domain first recognizes the high-affinity binding site in the membrane-distal region, which provides a strong linkage between the talin and the integrin β tail, and subsequently binds to a second lower-affinity membrane proximal site that is involved in α - β association, triggering separation of the tails and integrin activation [47-48]. Although talin is an important player in integrin activation mechanisms, the possibility remains that other integrin tail-binding proteins can substitute for talin. For example, many other PTB-domain-containing proteins including Numb, Dok-1, ICAP-1 α (integrin cytoplasmic domain-associated protein-1 α) and Kindlins bind to integrin β tails in a similar fashion to talin [49].

Integrin activation requires transmission of conformational rearrangements from the cytoplasmic face to the extracellular domain. The interaction between the membrane-proximal regions of the α and β subunits is believed to be stabilized by a salt bridge between a conserved arginine residue in the α tail and an aspartate residue in the β tail and by the hydrophobic residues immediately N-terminal to the arginine and aspartate residues [50]. Mutations that disrupt this 'clasp' lead to integrin activation, and integrins can be constitutively activated by deletion or mutations in these membrane-proximal sequences [42]. These data together strongly indicate that this membrane-proximal interaction plays an essential role as a stabilizer in the association of the α and β membrane proximal regions, maintaining the integrins in a low-affinity state. In addition, mutational analysis and computational modeling together suggest that a helical interface between the integrin α and β subunit transmembrane domains stabilizes the inactive state, and suggest that disruption of this helical transmembrane interface leads to activation [51] [52].

4. LIGANDS OF INTEGRINS

The majority of integrin ligands are protein components of ECM (Table 1). ECM is assembled from components synthesized and deposited outside the cell surface that provide structural and functional integrity to connective tissues and organs [53]. The ECM can also serve as a reservoir or provisional ma-

trix for growth factors and plasma proteins found in the blood [54]. The protein components of ECM can be divided into three major classes: (1) structural proteins such as the collagens and elastin (although collagen can also have functional properties); (2) multidomain adhesive glycoproteins such as fibronectin, vitronectin, and laminin; and (3) matricellular proteins such as secreted protein acidic and rich in cysteine (SPARC; also known as osteonectin and BM-40), thrombospondin 1 (TSP1) and 2 (TSP2), tenascin C and X, and osteopontin [53]. Among them, collagen primarily provides tensile strength to the tissue and elastin is responsible for the elastic recoil. Fibronectin, an adhesive glycoprotein, is a multifunctional protein that can act as a structural molecule owing to its fibrillar architecture, a biological glue that mediates interactions between cells and other ECM proteins, or a bridge forming cell to cell contacts [55]. Matricellular proteins are secreted macromolecules that interact with cell-surface receptors, ECM, growth factors, and proteases but do not function as structural molecules *per se* [56]. These glycoproteins regulate interactions between cells and ECM. For instance, SPARC, TSP1, and tenascin C disrupt cell-matrix interactions (i.e., are counteradhesive) – a critical component of angiogenesis and tissue remodeling [57].

All five αV integrins, two $\beta 1$ integrins ($\alpha 5$, $\alpha 8$) and $\alpha IIb\beta 3$ share the ability to recognize ligands containing an RGD tripeptide active site, which is shared by the RGD binding integrins are among the most promiscuous in the family, with $\beta 3$ integrins in particular binding to a large number of ECM and soluble vascular ligands including fibronectin, fibrinogen, vitronectin, vWF, LAP-TGF β and Del-1. Although many ligands are shared by this subset of integrins, the rank order of ligand affinity varies, presumably reflecting the preciseness of the fit of the ligand RGD conformation with the specific α - β active site pockets [58].

Collagens are a major family of structural ECM proteins and characterized by the presence of triple helical regions. Cells employ four different αI domain containing integrins including $\alpha 1$, $\alpha 2$, $\alpha 10$, and $\alpha 11$ to maintain holds with them. Immunoglobulin superfamily members also act as ligands for several integrins, and the best characterized integrin ligands are VCAM-1 (Vascular Cell Adhesion Molecule) and the ICAMs. VCAM-1 binds to $\alpha 4\beta 1$, $\alpha V\beta 3$ and $\alpha 4\beta 7$, whereas the leukocyte-specific CD11/CD18 integrins (including LFA-1 and Mac-1) bind to ICAMs [59].

Table 1. Ligands and distribution of integrins

Integrins	Ligands	Cellular and tissue distribution	Potential implications
$\alpha\text{v}\beta 1$	Ln, Fn, Opn, Vn	SMCs, fibroblasts, osteoclasts, tumor cells	Vascular disorders
$\alpha\text{v}\beta 3$	Fg, Vn, Tn, Opn, Tsp, Fn, PECM, MMP2, VEGF-A, Fibrillin-1, vWF	Endothelial cells, SMCs, osteoclasts, platelets, fibroblasts, tumor cells, epithelial cells, leukocytes, chondrocytes	Angiogenesis, restenosis, vascular disorders, osteoporosis
$\alpha\text{v}\beta 5$	Opn, Fg, Vn, Fn, Tsp	Endothelial cells, SMCs, osteoclasts, platelets, epithelial cells, leukocytes, chondrocytes	Angiogenesis, vascular disorders
$\alpha\text{v}\beta 6$	Fn, Fg, Vn, Tn, LAP-TGF β , Fibrillin-1	Epithelial cells, carcinoma cells	Promotion of cell migration, control of cell proliferation, activation of TGF β s, suppression of apoptosis, modulation of protease activity and mediating invasion of carcinoma cells.
$\alpha\text{v}\beta 8$	Vn	Melanoma, kidney, brain, ovary, uterus, placenta	Proper brain blood vessel development
$\alpha 1\beta 1$	Cn VI, Cn I, Ln, Semaphorin 7A	Chondrocytes, endothelial cells	Induction of laminin-1 expression
$\alpha 2\beta 1$	Cn I, Cn IV, Ln, Echovirus 1, Tenascin	Keratinocytes, chondrocytes, platelets endothelial cells	Wound healing
$\alpha 3\beta 1$	Ln, VEGF-A, Tsp	Keratinocytes	Branching morphogenesis in mammary epithelia, tumorigenesis and metastasis
$\alpha 4\beta 1$	Fn, VCAM-1, MadCAM-1	Leukocytes, endothelial cells	Adhesion and extravasation of lymphocytes, monocytes and progenitor cells tumor homing
$\alpha 5\beta 1$	Fn, Fibrillin-1, Tsp	Chondrocytes, endothelial cells	Angiogenesis
$\alpha 6\beta 1$	Ln, Tsp, CYR61	Chondrocytes, endothelial cells	Angiogenesis
$\alpha 7\beta 1$	Ln,	Differentiated muscle cells	Unclear
$\alpha 8\beta 1$	Fn, LAP-TGF β	Smooth muscle cells	Fibrotic response
$\alpha 9\beta 1$	Tn, VCAM-1, VEGF-A, VEGF-C, VEGF-D, and HGF	Keratinocytes, endothelial cells	Lymphangiogenesis
$\alpha 10\beta 1$	Cn,	Chondrocytes,	Unclear
$\alpha 11\beta 1$	Cn,	Mesenchymal non-muscle cells	Embryonic development
$\alpha 6\beta 4$	Ln	Endothelial cells	Keratinocyte migration, carcinoma invasion, keratinocyte survival
$\alpha \text{IIb}\beta 3$	Fg, Fn, vWF	Platelets	Stable clot formation and hemostasis
$\alpha \text{E}\beta 7$	E-cadherin	Leukocytes	
$\alpha \text{X}\beta 2$	ICAM-1, Fg, iC3b, factor X, heparin	Leukocytes	Leukocyte adhesion deficiencies (LAD),
$\alpha \text{L}\beta 2$	ICAMs 1–5	Leukocytes	Multiple sclerosis and psoriasis, LAD
$\alpha \text{M}\beta 2$	ICAM-2, Fg, Fn, iC3b, factor X, heparin	Leukocytes	LAD
$\alpha \text{D}\beta 2$	VCAM-1, Vn, Plasmonogen	Leukocytes	LAD
$\alpha 4\beta 7$	MAcCAM-1	Leukocytes	Multiple sclerosis and psoriasis

*Fg, fibrinogen; Fn, fibronectin; Ln, laminin; Opn, osteopontin; Tn, tenascin; Tsp, thrombospondin; Vn, vitronectin; Cn, Collagen; vWF, von Willebrand factor

5. BIOLOGICAL AND PATHOLOGICAL FUNCTION OF INTEGRINS

Cell adhesion is involved in many essential normal cellular and pathological functions including cell survival, growth, differentiation, migration, inflammatory responses, platelet aggregation, tissue repair and tumor invasion. Integrins are one of key family proteins for cell adhesion regulation through binding to a large number of extracellular matrix molecules and cell membrane proteins. Using knockout mice it has become evident that the integrins possess both redundant and nonredundant functions, and that lack of expression may result in a wide variety of effects ranging from blockage in pre-implantation to embryonic or perinatal lethality and developmental defects.

β 1 Integrin. The largest subgroup of integrins is formed by the β 1 subunit containing integrins which consist of 12 members with different ligand binding properties. Besides ECM proteins, some β 1 integrin containing receptors also interact with cellular receptors. For example, α 4 β 1 and α 9 β 1 bind to VCAM-1 and α 4 β 1 to MadCAM-1 [60]. During early embryogenesis totipotent embryonic cells differentiate and polarize to form endoderm, basement membrane (BM), and ectoderm. However, embryoid bodies derived from β 1-deficient ES cells develop endoderm, but do not form a BM, polarized ectoderm and a cavity [61]. β 1-integrin null embryos die by embryonic day (E)9.5–10.5 in utero owing to implantation defects [62–63]. The major function of β 1 integrin during early development may be to induce laminin-1 expression since exogenous addition of laminin 1 allows BM formation also in the absence of β 1 integrins, though the signaling pathway mediating the regulation is still unclear [64]. β 1 integrins are crucial for the attachment of basal keratinocytes to the BM, since loss of β 1 leads to widespread blisters between the epidermis and the BM. In addition, loss of β 1 integrin in skin decreases the proliferation of basal keratinocytes by about 70% [65]. Similarly to skin, β 1 integrins are important for the attachment to and maintenance of the BM in brain [66]. Although β 1 integrins are important ECM receptors, they are not generally required for cell migration. While some cells, such as keratinocytes or primordial germ cells, are highly dependent on β 1 integrin, other such as myoblasts, neurons and lymphocytes migrate normally also in the absence of β 1 integrin. Tumor cells expressing β 1 integrin formed significantly larger primary tumors and had a dramatically increased metastasis into liver and lung [67]. Moreover, ablation of the β 1 integrin gene in mammary epithelium dramatically impairs

mammary tumorigenesis in mice [68]. However, in human patients, no clear correlation between tumor formation, invasion and β 1 integrin expression could be identified yet.

Integrins α 1 β 1 and α 2 β 1 are expressed on lymphatic endothelium in healing wounds in response to VEGF. Inhibition of these integrins blocked lymphangiogenesis in these wounds [69]. In normal animals, VEGFA treatment upregulates expression of both α 1 β 1 and α 2 β 1 on vascular endothelial cells. Function-blocking antibodies directed against both integrins reduced VEGFA-induced angiogenesis in vivo and reduced tumor growth and angiogenesis [70]. Tumors grown in α 1 β 1-deficient mice grew more slowly and exhibited less angiogenesis, indicating an important role for this integrin in angiogenesis [71]. However, mice lacking α 2 β 1 integrins exhibit increased B16F10 melanoma growth and angiogenesis when compared with wild-type mice [72]. By contrast, Lewis lung carcinoma (LLC) tumors did not exhibit increased angiogenesis or growth in α 2-null mice. The different response resulted from different level of a VEGFR1 binding placental growth factor (PLGF) expression which was high from B16F10 melanoma cells and low from LLC cells. Enhanced angiogenesis was attributed to an increase in VEGFR1 expression and function on α 2-null endothelial cells [72].

Integrin α 4 β 1, a receptor for VCAM and fibronectin containing the CS1 region, is best known as a lymphocyte integrin involved in adhesion and extravasation of lymphocytes by binding to VCAM1 which is expressed on inflamed endothelial cells. Loss of integrin α 4 during development leads to defects in placentation, heart development and coronary artery development, causing lethality between E10.0 and E12.0 [73]. It has been reported that integrin α 4 β 1 was expressed on neo-vasculatures in both murine and human tumors and responded to VEGF, bFGF, IL1 and TNF. Antagonists of α 4 β 1 blocked tumor neo-vascularization by induction of cell death of both endothelial cells and pericytes and decreased tumor growth in chick and murine models of tumor growth [74]. It has been proposed that integrin α 4 β 1-VCAM1 interactions facilitated close proximity of the endothelial cells and pericytes, which promoted the survival of both endothelial cells and pericytes during angiogenesis [74]. Moreover, integrin α 4 β 1 selectively promotes the homing of both endothelial progenitor cells and monocytes to neovascular tissue [75]. Treatment of mice bearing LLC tumors with antagonists of integrin α 4 β 1 significantly suppressed the number of monocytes and progenitor cells found within tumors and reduced blood vessel density [76], indicating that the suppression of monocyte and

progenitor cell homing to tumors by integrin $\alpha 4\beta 1$ antagonists could be a useful supplementary approach to suppress tumor angiogenesis and growth.

Embryonic deletion of the integrin $\alpha 5$ subunit induces early mesenchymal abnormalities, leading to lethality of $\alpha 5$ -null embryos [77]. Further studies using $\alpha 5$ -null ES cells to grow teratocarcinomas showed decreased proliferation, increased apoptosis and decreased vascularization in teratocarcinomas derived from $\alpha 5$ -null ES cells compared with controls [78]. Integrin $\alpha 5\beta 1$ expression is induced in response to a variety of angiogenic stimuli, such as bFGF, IL8 and the ECM protein DEL1 (also known as EDIL3), but not by VEGF [79]. Integrin $\alpha 5\beta 1$ -mediated adhesion promotes endothelial cell migration and survival *in vivo* and *in vitro* by suppressing the activity of protein kinase A (PKA) [80]. Integrin $\alpha 5\beta 1$ is poorly expressed on quiescent endothelium but its expression is significantly upregulated on endothelium during tumor angiogenesis in both mice and humans [81]. In some types of cancer, integrin $\alpha 5\beta 1$ is also expressed on tumor cells and plays a direct role in tumor cell migration, invasion, and survival [82-84]. A small peptide antagonist (amino-acid sequence PHSCN) to integrin $\alpha 5\beta 1$ significantly reduced neovascularization and formation of metastases in an animal model of prostate carcinoma that expresses integrin $\alpha 5\beta 1$ [83, 85-86]. Expression of $\alpha 5\beta 1$ is also upregulated during corneal angiogenesis [87].

Integrin $\alpha 6\beta 1$ is expressed at high levels in capillary endothelial cells *in vivo* [88]. Unlike $\alpha 6\beta 4$, which shows a preference for laminin 5, integrin $\alpha 6\beta 1$ binds most laminin isoforms as well as other ECM proteins including CYR61, thrombospondin 1 and thrombospondin 2 [89]. As $\alpha 6$ integrin antagonists and $\alpha 6$ small interfering RNA constructs inhibit angiogenesis, it is possible that integrin $\alpha 6\beta 1$ promotes angiogenesis. However, as these agents can block the function of both integrins $\alpha 6\beta 1$ and $\alpha 6\beta 4$, it is not yet clear what part integrin $\alpha 6\beta 1$ plays in tumor angiogenesis [88]. Antibody inhibitors of $\alpha 6$ integrin prevented endothelial cell tube formation *in vitro*, suggesting a role for $\alpha 6$ in the angiogenic process [88].

Integrin $\alpha 9\beta 1$ is structurally similar to integrin $\alpha 4\beta 1$. However, unlike $\alpha 4\beta 1$, it is a receptor for a number of ECM proteins and cell surface receptors including tenascin C, thrombospondin, osteopontin, IIICS fibronectin, VCAM1 and other ligands [90]. Integrin $\alpha 9\beta 1$ is expressed on epithelia, osteoclasts, smooth muscle cells and also endothelial cells. $\alpha 9\beta 1$ promotes VEGFA-stimulated angiogenesis by directly binding to VEGFA and blocking antibodies to $\alpha 9\beta 1$ suppress VEGFA-induced angiogenesis [91]. However, other studies found that $\alpha 9\beta 1$ also binds directly to

the N terminus of thrombospondin and blocking antibodies to $\alpha 9\beta 1$ inhibited angiogenesis induced by this thrombospondin fragment [92]. Integrin $\alpha 9\beta 1$ also has a role in growth factor-mediated lymphangiogenesis as PROX1, a lymphatic endothelial cell-selective transcription factor, coordinately upregulates integrin $\alpha 9\beta 1$ and VEGFR3 expression and endothelial cell motility *in vivo* [93]. This integrin promotes VEGFC- and VEGFD-stimulated cell migration by directly binding these growth factors and antagonism of $\alpha 9\beta 1$ suppresses VEGFC-induced motility [94]. Taken together, these studies indicate that $\alpha 9\beta 1$ has unique and crucial roles in lymphangiogenesis. Indeed, integrin $\alpha 9\beta 1$ -null mice die 8-12 days after birth owing to lethal defects in development of the lymphatic system; however, they do not exhibit obvious defects in development of blood vessels [95].

$\alpha v\beta 3$ Integrin. The integrin $\alpha v\beta 3$ is the first of the αv integrins to be characterized and shown to regulate angiogenesis [96-97]. It binds with multiple components of the ECM including vitronectin, fibronectin, fibrinogen and osteopontin since they are all RGD-containing proteins. It has been found in 1994 that integrin $\alpha v\beta 3$ was widely expressed on blood vessels in human tumor biopsy samples but not on vessels in normal human tissues. Its expression on endothelial cells is stimulated by angiogenic growth factors such as bFGF, TNF α and IL8 and it is upregulated on endothelium in tumors, wounds and sites of inflammation $\alpha v\beta 3$ integrin has a key role in endothelial cell survival and migration during angiogenesis [96] [98]. Antagonists of $\alpha v\beta 3$ inhibited angiogenesis and tumor growth in a variety of animal models of cancer and blocked corneal as well as choroidal angiogenesis in animal models of ocular disease [98-99]. In addition, integrin $\alpha v\beta 3$ antagonists induce endothelial cell apoptosis through a caspase 8-dependent cell death program [100]. *In vitro* studies revealed that agonists of endothelial cell $\alpha v\beta 3$, but not other integrins, suppressed p53 activity, blocked p21WAF1/CIP1 expression, and increased the bcl-2/bax ratio, thereby promoting cell survival [101]. Ligation of endothelial $\alpha v\beta 3$ integrin has also been shown to activate mitogen-activated protein kinase (MAPK), FAK and Src, among other kinases, resulting in cell proliferation, differentiation and migration [102].

Based on the sizable body of evidence addressed above, it is reasonable to draw the conclusion that integrin $\alpha v\beta 3$ promotes angiogenesis and endothelial cell survival and that antagonism of this integrin suppresses angiogenesis by inducing endothelial cell apoptosis *in vitro* and *in vivo*. Indeed, antagonists of $\beta 3$ integrin are being used in clinical trials as an-

ti-angiogenic therapy, including the humanized monoclonal antibody Vitaxin and the RGD mimetic Cilengitide [103-104]. However, these agents did not appear to be as successful at inhibiting angiogenesis in clinical trials for cancer treatment as originally hoped [105]. Recently, it has been reported that low concentrations of RGD-mimetic integrin inhibitors promote VEGF mediated angiogenesis by altering $\alpha\beta3$ integrin and vascular endothelial growth factor receptor-2 trafficking, thereby stimulating tumor growth and angiogenesis [106].

More importantly, a series of genetically Knock-out studies forced researchers to reconsider the role of integrin $\alpha\beta3$ and $\alpha\beta5$ in angiogenesis. For example, genetic ablation of the α integrin subunit did not appear to affect developmental angiogenesis [107]. Even conditional deletion of α integrin in Tie2-expressing endothelial cells is not sufficient to affect angiogenesis either [108]. Furthermore, $\beta3$ null and $\beta3/\beta5$ doubly deficient mice produce vascular networks without obvious defects during developmental angiogenesis, and more surprisingly, display enhanced tumor growth and pathological angiogenesis [109-110]. However, male mice lacking $\beta3$ integrin exhibit coronary capillaries of irregular endothelial thickness, with endothelial protrusions into the lumen, and expanded cytoplasmic vacuoles. These defective coronary vessels can be normalized by administration of inhibitors of VEGF or VEGF receptor 2 (VEGFR2, also known as KDR), suggesting that enhanced VEGF signaling might compensate for the loss of $\beta3$ integrin [111]. In addition, with a mouse knockin model, it has been demonstrated that the phosphorylation of Tyr747 and Tyr459 in $\beta3$ integrin participates in enhancing pathological angiogenesis [112].

All considered, the above studies suggest that $\alpha\beta3$ integrin may have both positive and negative roles during angiogenesis. Indeed, $\alpha\beta3$ can bind a plethora of factors that are thought to enhance angiogenesis including VEGFR2, vitronectin, fibronectin, Del1, ANGPTL3, CYR61, bone sialoprotein, thrombin, and can also bind to several factors that are thought to induce anti-angiogenic effects including thrombospondin, angiostatin, and tumstatin [113]. Another explanation is that functioning as a biosensor, $\alpha\beta3$ may promote angiogenesis in the ligated state, but when ligands are absent or ligand binding is antagonized, $\alpha\beta3$ may activate a death pathway to inhibit angiogenesis. Thus, depending on the microenvironment and the ligation state, integrins promote or inhibit angiogenesis by positively or negatively regulating cell survival [114]. In this way, integrin $\alpha\beta3$ appears to function as a regulator of angiogenesis by balancing opposing signals in the tumor microenvironment.

$\alpha\beta5$ integrin. All though integrin $\alpha\beta5$ binds with similar ECM components with $\alpha\beta3$, the related angiogenic promoting pathway is distinct from that regulated by $\alpha\beta3$. For example, anti- $\alpha\beta3$ antibodies blocked angiogenesis induced by bFGF, whereas antibodies that target $\alpha\beta5$ blocked the angiogenesis induced by VEGF in both the rabbit corneal eye pocket and the chick chorioallantoic membrane assay [115]. The VEGF- $\alpha\beta5$ pathway is dependent on Src kinase and protein kinase C30. *In vivo* angiogenesis assays showed $\alpha\beta5$ is required for TGF- β and VEGF-mediated angiogenesis and VEGF acted through $\alpha\beta5$ to promote vascular permeability [116], indicating $\alpha\beta5$ may also promote tumor metastasis [117]. However, integrin $\alpha\beta5$ -null mice exhibit completely normal development and normal angiogenesis [118], indicating that this integrin is not required for vascular development.

Genetic ablations of the α subunit, which eliminate expression of integrins $\alpha\beta1$, $\alpha\beta3$, $\alpha\beta5$, $\alpha\beta6$ and $\alpha\beta8$ suggest that, in some mice, α integrins are not required for blood vessel development in most tissues. Twenty percent of embryos lacking α integrins survive to birth with normal blood vessels in many tissues. However, 80% of mice die *in utero* between E10.5 and E11.5 with defective placental blood vessels. The remaining 20% of α -null mice die shortly after birth with severe brain and intestinal haemorrhage, with distended and leaky vessels in these tissues [107]. Thus, α integrins appear to have key roles in embryonic development of blood vessels in tissues such as placenta and brain.

$\alpha\beta6$ integrin. The $\alpha\beta6$ receptor is the only known integrin that is restricted exclusively to epithelial cells [119]. The $\alpha\beta6$ integrin is expressed primarily during embryonic development and is not expressed constitutively in healthy epithelia. However, $\alpha\beta6$ is upregulated during tissue remodeling, including wound healing and carcinogenesis [120]. $\alpha\beta6$ facilitates keratinocyte adhesion and migration on fibronectin, tenascin and vitronectin, all of which are components of the early wound matrix [121]. Using mouse $\beta6^{-/-}$ keratinocytes, Huang *et al.* found that $\alpha\beta6$ expression promotes keratinocyte migration through a pathway involving protein kinase C [122]. Binding of $\alpha\beta6$ to its ligand upregulated secretion of the pro-enzyme form of type IV collagenase, MMP-9, and exogenous activation of MMP-9 further increased keratinocyte migration [123]. Thomas *et al.* demonstrated that binding of $\alpha\beta6$ to the TGF β 1 latency associated peptide (LAP) promotes cell migration and MMP-9 expression, and also that cells will invade towards soluble LAP in an $\alpha\beta6$ -dependent manner [124]. In addition, TGF β 1 induces de novo expression

of $\alpha\beta6$ in normal human keratinocytes [125] and on the non-transformed keratinocyte cell line, HaCaT [126].

To date, $\alpha\beta6$ expression has been reported in carcinomas of the lung, breast, pancreas, stomach, colon, ovary, salivary gland as well as oral and skin squamous cell carcinoma [120, 127-131]. Elevated $\alpha\beta6$ expression facilitated the invasion and dissemination of colon carcinoma cells *in vivo* [132-133]. The $\beta6$ overexpressing cell line was significantly more invasive, which was, in part, through $\alpha\beta6$ -dependent upregulation of the type IV collagenase MMP-9 [134-135]. High $\alpha\beta6$ expression correlated with elevated uPA, uPAR and MMP-9 in ovarian carcinoma cell lines [136]. It has also been proposed that contribution of $\alpha\beta6$ for promoting cancer progression *in vivo* is related to the mechanism of epithelial-mesenchymal transition (EMT) by activating latent TGF- β [137]. The upregulation of $\alpha\beta6$ related with the poor prognosis [132], suggesting that $\alpha\beta6$ expression may be useful in predicting malignant transformation. Indeed, the integrin $\alpha\beta6$ now emerges as an attractive new candidate as a therapeutic target for metastatic colon carcinoma. However, epithelial cells in samples of lichen planus (a chronic mucocutaneous disease) have also been shown to express $\alpha\beta6$ suggesting that $\alpha\beta6$ expression per se is insufficient to drive malignant progression [138]. The net effect on tumor behavior is likely to depend on the stage of tumor development. In the later stages of tumor development, as cells become refractory to growth inhibition, then the role of LAP in promoting both $\alpha\beta6$ -dependent cell movement and MMP-9 expression may become prominent, culminating in a pro-oncogenic effect of TGF β [139].

$\alpha6\beta4$ Integrin. $\alpha6\beta4$ plays a key role in the formation and stabilization of junctional adhesion complexes called hemidesmosomes (HDs) that are connected to the intermediate filament (IF) system, as well as in the regulation of a variety of signaling processes. Increased expression of $\alpha6\beta4$ and changes in its distribution were then correlated with increased aggressiveness of tumors and poor prognosis [140]. $\beta4$ subunit has an unusual size of approximately 200 kDa, which is due to an unusually long cytoplasmic domain of over 1,000 amino acids [141]. This domain contains two pairs of type III fibronectin (FNIII) domains, separated by a connecting segment (CS) [142]. $\beta4$ knockout mice developed severe blistering of the skin [143]. Moreover, a mutation in the $\beta4$ gene is responsible for the pyloric atresia associated with junctional epidermolysis bullosa (EB) syndrome in humans. It has been found that interaction between $\alpha6\beta4$ and plectin is critical for proper HD assembly and at

least two sites on plectin and three on $\beta4$ mediate their interaction. The actin binding domain (ABD) of plectin binds to the first pair of FNIII domains and part of the CS of $\beta4$ [144]. Two other HD components, BP180 and BP230, bind to the third FNIII domain of $\beta4$ and to a region comprising the C-terminal end of the CS and the second pair of FNIII domains, respectively [145]. The $\alpha6$ subunit of $\alpha6\beta4$ interacts with the tetraspanin CD151 [146].

Apart from promoting keratinocytes migration, studies on the invasion of carcinoma cells revealed a role for $\alpha6\beta4$ -dependent activation of phosphatidylinositol (PI)-3 kinase, which is an upstream regulator of Rac1 [147]. Since growth factors activate enzymes that initiate the disassembly of HDs, the presence of overactive growth factor receptors may explain why $\alpha6\beta4$ is unable to initiate HD formation in many carcinoma cells. In fact, $\alpha6\beta4$ cooperates with several different tyrosine kinases in carcinoma cells to promote invasion, including c-met (hepatocyte growth factor receptor), ErbB2 and ErbB1 [148]. There is evidence that proteins other than tyrosine kinases can cooperate with $\alpha6\beta4$ to promote invasion. For example, invasion of breast carcinoma cells is stimulated by promoting Rab11-mediated vesicular trafficking under hypoxic conditions [149].

Leukocytes specific integrins. The arrest of leukocytes rolling on target vascular beds involves rapid formation of shear-resistant adhesions by specialized leukocyte integrins. This arrest can be mediated by integrins that contain the $\beta2$ subunit, e.g. $\alpha L\beta2$ (leukocyte function-associated antigen-1 (LFA-1)) or $\alpha4$ subunit, e.g. $\alpha4\beta1$ (very late antigen-4 (VLA-4)). In leukocytes, integrins are involved in slow rolling, adhesion strengthening, and transendothelial migration [150-151]. Lymphocyte and myeloid cells cease rolling and arrest on lymph node high endothelial venules as well as on peripheral tissues upon activation of at least one of the four major leukocyte integrins: VLA-4, $\alpha4\beta7$, LFA-1, and the myeloid-specific integrin (integrin $\alpha M\beta2$ (Mac-1)) [152]. In addition, these integrins transmit signals that facilitate respiratory burst, complement-mediated phagocytosis, cytokine production, proliferation, survival, differentiation, degranulation, and cellular polarization. Loss or reduced $\beta2$ on cell surface, defects in $\beta2$ signaling, mutations in $\beta2$ will cause leukocyte adhesion deficiencies (LAD) [153].

Most circulating leukocytes maintain their integrins in largely low affinity state [154]. Leukocyte integrins must undergo *in situ* modulation to develop high avidity for their endothelial ligands to establish shear-resistant adhesion and firm leukocyte arrest on the target endothelial site [155]. For most leukocytes,

this dramatic change in integrin affinity is triggered when the rolling leukocyte encounters and rapidly responds to a proper chemoattractant signal presented on the apical endothelial surface [156]. The distribution of LFA-1 integrins to the leading edge of a polarized leukocyte is reported to proceed through a signaling cascade involving Rap1 and its downstream effectors RAPL and the threonine-serine kinase, Mst1 [157]. Integrins play key roles in cellular migration acting both as adhesive molecules that maintain locomotion over the apical endothelial surface and as signaling molecules, which, together with chemokine signals, maintain polarity and motility [158].

Leukocyte integrins are recognized therapeutic targets in various diseases, and integrin blocking monoclonal antibodies (natalizumab against $\alpha 4$ integrins and efalizumab against LFA-1) are already used in the clinic against multiple sclerosis and psoriasis [159-160]. Leukocyte adhesion cannot be inhibited by compounds containing the RGD sequence, but longer peptides to recognition sites in ICAM molecules or microbe-derived ICAM-1 inhibitors do show inhibitory activity [161-162]. It has been reported that promatrix metalloproteinases (proMMPs), particularly proMMP-9, are potent ligands of the leukocyte $\beta 2$ integrins and translocating proMMP-9/ $\alpha M\beta 2$ complex is part of the cell surface machinery guiding neutrophil migration [163], suggesting that a promising therapeutic approach could be to disrupt the association between the matrix metalloproteinases and integrins. It has also been found that the Del-1 protein is an important endogenous inhibitor of leukocyte adhesion [164]. The Del-1 protein is a secreted protein expressed by endothelial cells in immunoprivileged tissues, such as the brain, the eye, and the lung. Although a secreted molecule, Del-1 is absent from plasma and is rather localised to endothelial cells and/or the extracellular matrix [164]. In fact, it binds to the $\beta 2$ integrins (LFA-1, Mac-1) and when coated on plastic, leukocytes adhere to the protein. However, leukocyte binding to ICAM-1 is inhibited when both Del-1 and ICAM-1 are present. Del-1 knockout mice show a strong activation of adhesion and of inflammatory cell recruitment [164].

6. PERSPECTIVES OF INTEGRINS AS IMAGING AND THERAPY TARGETS

Integrins are ideal pharmacological targets based on both the key role they played in angiogenesis, leukocytes function and tumor development and easy accessibility as cell surface receptors interacting with extracellular ligands [1]. So far, the integrin superfamily represents the best opportunity of targeting both antibodies and small-molecule antagonists for

both therapeutic and diagnostic utility in various key diseases [165]. Preclinical studies and clinical trials showed that quite a few integrin targeting antibodies were effective to block tumor growth and metastasis. Most of studies focused on αv integrins including MEDI-522 (vitaxin) against $\alpha v\beta 3$ [98], CNTO 95 against both $\alpha v\beta 3$ and $\alpha v\beta 5$ [166], 17E6 against $\alpha v\beta 3$, $\alpha v\beta 5$ and $\alpha v\beta 1$ [167]. Besides, antibodies targeting to other integrins have been developed. For example, Tysabri (natalizumab), an antibody which blocks $\alpha 4$ integrins and inhibits the $\alpha 4$ -mediated adhesion of leukocytes to their counter receptor was used for treatment of patients with relapsing forms of multiple sclerosis (MS) [168]. Small molecular antagonists, mainly based on RGD containing peptides and RGD peptidomimetic [169-171], also showed potent inhibition of angiogenesis. More detailed information can be found in several recently published review articles [1, 172].

Integrin ligands are also under investigation as tumor endothelium-targeted diagnostic agents and integrin targeting is proving useful in tumor imaging. Both peptide and antibody-based diagnostic agents targeting RGD-binding integrins or αv integrins have been evaluated in animal models of cancer after ^{18}F or ^{64}Cu labeling. Tumor xenografts with positive integrin $\alpha v\beta 3$ expression were visualized clearly on PET images [173-175]. Other imaging modalities besides PET have also been investigated for integrin $\alpha v\beta 3$ targeted imaging. For example, integrin αv -targeted ultrasound microbubbles preferentially bound neovasculature at the periphery of experimental tumors [176]. The clinical significance of molecular imaging with integrin-targeting agents may be useful for lesion detection and grading. In addition, imaging will help identify appropriate patients for integrins related therapy and monitor the therapeutic responses. More important, integrin-targeting agents might also be useful in selectively delivering chemotherapeutics or gene therapeutic agents to tumors, thus minimizing systemic toxicities. For example, after fused with a RGD targeting moiety, RGD4C-TNF showed improved tumor accumulation and therapy effect in a human breast tumor model [177].

With regard to activity, signaling and regulation of integrins, evidences are accumulating with more crystal structures deciphered and new models established. However, the integrin pathways are still not fully understood due to extreme complexity. Besides their ligands, increasing number of biomolecules has been identified interacting with integrins including VEGF/VEGFR [178], HER2 [179], MMPs [180], uPAR [181]. Moreover, it has been found that ligand-bound integrins also actively suppress the activation of other

integrins in the same cell utilizing different signaling pathways. For example, when $\alpha 2\beta 1$ is ligated, both $\alpha 5\beta 1$ and $\alpha V\beta 3$ will be suppressed through PKA. When $\alpha 5\beta 1$ is ligated, $\alpha 2\beta 1$ will be suppressed through PKC α [182]. Integrins are also known to have transdominant roles over other integrins and can thus control overall cell behavior [183]. After epithelial integrity is restored in wounds, $\alpha V\beta 6$ expression will be strong upregulated. However, mutating the $\beta 6$ cytoplasmic domain by removing the terminal 11 amino acids prevents this density-dependent upregulation of $\alpha V\beta 6$ and replaces it by upregulating $\alpha V\beta 5$. This provides evidence that the expression of these integrins is linked and expression may be switched from one to the other [135].

So far, a lot of breakthroughs in integrin research have been made with purified integrins, or I-domains and their ligands. However, it is becoming increasingly apparent that integrin mediated cell adhesion is unusually complex. Thus, it is critical to study the events occurring at the cellular and organism levels in a temporal manner to make more meaningful conclusions. It is very promising for molecular imaging of living cells or animal models to meet the requirement. For example, microscopic imaging with FRET could identify integrin-integrin interaction and integrin-other proteins interaction in living cells [184] [185]. PET imaging with positron emission radioisotopes labeled integrin-targeting probes could visualize expression and regulation of integrins in animal models in a real-time mode [186]. We believe integrating data from both microscopic and macroscopic methods will quicken our steps on the long scientific journey to decipher the integrins related pathways.

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CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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