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Transdominant Regulation of Integrin Function: Mechanisms of Crosstalk

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Abstract

Transdominant inhibition of integrins or integrin-integrin crosstalk is an important regulator of integrin ligand binding and subsequent signaling events that control a variety of cell functions in many tissues. Here we discuss examples of integrin crosstalk and detail our current understanding of the molecular mechanisms that are involved in this receptor phenomenon. The cytoskeleton associated protein talin is a key regulator of integrin crosstalk. We describe how the interaction of talin and the cytoplasmic tail of β integrin is controlled and how competitive inhibitors of this binding play a role in integrin crosstalk. We conclude with a discussion of how integrin crosstalk impacts the interpretation of integrin inhibitor and knockdown studies in both the laboratory and clinical setting.

Keywords

Matrix adhesion; receptors; cytoskeleton; signaling; adaptor proteins; phosphorylation

1. Introduction

The term integrin refers to a member of a family of matrix and cell-cell adhesion receptor proteins that exists at the cell surface as a dimer composed of an α and β subunit. In mammals, 18 α and 8 β subunits have been identified[1,2]. The various combinations of α s and β s exhibit ligand specificity and interact with various matrix molecules including fibronectin, collagens, laminins, proteoglycans as well as intercellular adhesion molecules[2,3]. In addition to their role in adhesion, integrins are critical regulators of complex cellular processes such as adhesion, migration and proliferation. Receptor clustering occurs as a consequence of integrin ligand binding and this, in turn, results in recruitment of cytoskeletal and signaling adaptor proteins to integrin cytoplasmic tails[1,4].

Integrin subunits were initially identified and characterized by the use of antibodies generated against cell surface proteins. Moreover, some of the integrin antibodies that have been prepared over the years have been very useful in dissecting integrin functions since they have the ability to impede cell adhesion to ligands or other cells. Therein lies a conundrum. Most cells are now known to express more than one integrin heterodimer, some of which share ligands[2]. Why does inhibition of one integrin subunit or heterodimer in many instances also perturb ligand

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binding of a second and distinct integrin? An accepted explanation for this phenomenon is a process involving transdominant inhibition of integrin function, the topic of this review[5].

Transdominant inhibition of integrin function, or for convenience we will use the designation integrin crosstalk, is a mechanism in which one integrin regulates the activation state of a different integrin in the same cell. It is believed to play a central role in regulating integrin ligand binding in a number of in vivo situations[5,6]. In addition, the ability of integrin subunit-specific antibody antagonists to inhibit multiple integrins likely is both a bonus and potential detriment in their use in the treatment of a variety of diseases. Several mechanisms have been reported to mediate integrin crosstalk, with the cytoskeleton associated protein talin featuring prominently as a key regulator in most. Such mechanisms generally involve the regulation of the binding of intracellular proteins to integrin cytoplasmic tails and/or differential phosphorylation of residues within the integrin tail. In this review, we will not consider integrin crosstalk with growth factor receptors or other non-integrin adhesion receptors. We focus on integrin-integrin crosstalk and discuss a limited number of examples of tissue/cell systems in which crosstalk has been reported. We will detail what we know of the mechanism(s) underlying crosstalk and review the physiological and medical significance of the phenomenon. To begin, we will first briefly discuss integrin activation since the ability to regulate such activation is the molecular basis of crosstalk.

2. Integrin Activation

Integrins exist in an unfolded, active and folded, inactive conformation[3,7-9]. In the inactive state integrins do not bind ligand and fail to signal[3,7]. Integrins can be activated “outside-in”, following interaction with extracellular matrix ligands, or by “inside-out” signaling in which intracellular proteins bind to and induce separation of the cytoplasmic tails of integrins [1,7,9-13]. Activation of integrins in which the cytoplasmic tails become straightened increases ligand affinity and induces the formation of a signaling complex in the cytoplasm[3,7,9,11].

3. Examples of Integrin Crosstalk

3.1 Integrin Crosstalk in the Immune System

In the immune system, ligand binding of α II β 3 integrin in platelets inhibits α 2 β 1 integrin mediated adhesion to collagen[14]. Failure of such crosstalk may be the cause of the genetic disorder, Glanzmann's thrombasthenia, a disease characterized by defective platelet aggregation and severe bleeding[14,15]. In patients afflicted with the disease, serine residue 752 in the cytoplasmic domain of the β 3 integrin subunit is mutated to proline[16]. This mutation prevents activation of platelet α II β 3 integrin by inside-out signaling, resulting in a reduction in platelet binding to plasma proteins and, consequently, impedes platelet aggregation[15]. In addition, the same mutation inhibits the ability of α II β 3 integrin to prevent ligand binding of α 2 β 1 integrin and, thus, promotes platelet adherence to vessel walls[14].

Integrin crosstalk also plays a role in the regulation of lymphocyte extravasation from the circulation into sites of inflammation during an immune response[17]. In human lymphocytes, α L β 2 (LFA-1) integrin binding to ICAM-1 decreases adhesion of α 4 β 1 integrin to VCAM-1 and fibronectin, facilitating detachment of α 4 β 1 integrin from the apical surface of endothelial cells[17]. Moreover, decreased α 4 β 1 integrin activity leads to an enhancement of α 5 β 1 integrin mediated migration on fibronectin, a process that promotes transmigration through an endothelium[17].

3.2 Integrin Crosstalk in Angiogenesis

In an adult, angiogenesis is the formation of new blood vessels from a pre-existing vasculature [18]. It contributes to tissue remodeling and wound healing. Pathological angiogenesis occurs

in, but is not limited to, psoriasis and age-related macula degeneration. In the latter, aberrant blood vessel formation results in tissue damage and tissue failure. Pathological angiogenesis also plays a role in cancer where it is required for the development of tumors larger than a few millimeters in size[19]. With regard to integrins, $\alpha v\beta 3$ and $\alpha 5\beta 1$ integrin are now recognized as key players in angiogenesis[20-25]. Not only do the levels of $\alpha v\beta 3$ and $\alpha 5\beta 1$ integrin protein increase in angiogenic endothelial cells, but also inhibiting $\alpha v\beta 3$ or $\alpha 5\beta 1$ integrin function individually with antibodies or small compounds results in perturbation of angiogenesis[24, 25](Fig. 1). These results at first glance are somewhat unexpected. One might imagine that inhibition of $\alpha v\beta 3$ integrin function would have little effect on angiogenesis since $\alpha 5\beta 1$ integrin should compensate and vice versa, particularly since these two integrin share a common matrix ligand (fibronectin). It was even more of a surprise that loss of $\alpha v\beta 3$ integrin expression in the mouse did not affect pathological angiogenesis[26], despite the anti-angiogenic ability of small molecule $\alpha v\beta 3$ integrin inhibitors and antibody antagonists [26]. Moreover, it is intriguing to note that in vivo, if expression of $\alpha v\beta 3$ integrin is lost, the activity of $\beta 1$ subunit-containing integrins appears enhanced, so much so, that pathological angiogenesis is promoted[26]. Rather, it is now clear that there is a complex crosstalk between $\beta 1$ and $\beta 3$ subunit containing integrins during angiogenesis and in various in vitro and in vivo models. Numerous examples are detailed in the literature. For example, in our own studies, we have demonstrated that in vitro in endothelial cells crosstalk between $\beta 1$ and $\beta 3$ is bidirectional such that when $\alpha v\beta 3$ integrin is blocked using antibodies there is a concomitant inhibition in ligand binding of $\alpha 3\beta 1$ and $\alpha 6\beta 1$ integrin[27]. In the same cells, a combination of antibodies that functionally inhibit $\alpha 3$ and $\alpha 6$ integrin block ligand binding of $\alpha v\beta 3$ integrin[27]. Studies by Ly and colleagues reveal that expression of $\alpha 5\beta 1$ integrin in CHO cells inhibits $\alpha v\beta 3$ mediated adhesion and migration on fibrinogen[28]. Both adhesion and migration require a high affinity state of $\alpha v\beta 3$ integrin for ligand. In the absence of $\alpha 5\beta 1$ integrin, $\alpha v\beta 3$ integrin is present in a high affinity state in CHO cells. However, expression of $\alpha 5\beta 1$ integrin in CHO cells inhibits $\alpha v\beta 3$ mediated cell adhesion and migration[28]. A constitutively active $\beta 3$ integrin which is believed to switch $\beta 3$ integrin to a high affinity state is resistant to the inhibitory effect of $\alpha 5\beta 1$ integrin[28]. Some other examples of the crosstalk between integrins involved in angiogenesis are described below where we discuss molecular mechanisms that regulate this phenomenon.

3.3 Integrin Crosstalk in Keratinocytes

Integrin crosstalk is also observed, at least in vitro, during the adhesion and the migration of skin cells (keratinocytes). Keratinocytes express multiple integrins, two of which, $\alpha 6\beta 4$ and $\alpha 3\beta 1$ integrin, bind laminin-332 in the extracellular matrix[29,30]. When keratinocytes are plated in vitro onto laminin-332 they initially bind via $\alpha 3\beta 1$ integrin, as demonstrated by antibody blocking studies[30]. However, very rapidly after plating, $\alpha 6\beta 4$ integrin displaces $\alpha 3\beta 1$ integrin from their common matrix ligand and, at least in some instances, induces stable adhesion to substrate[30]. This displacement can be inhibited by antibodies that block the function of $\alpha 6\beta 4$ integrin (Fig. 2). Moreover, there is evidence that $\alpha 6\beta 4$ integrin can inhibit haptotactic migration driven by $\alpha 3\beta 1$ integrin, resulting in stable adhesion[31]. This has led to the proposal that $\alpha 6\beta 4$ integrin negatively regulates activation of $\alpha 3\beta 1$ integrin. However, during wound healing in vivo, the functions and interactions of $\alpha 6\beta 4$ and $\alpha 3\beta 1$ integrin may be even more complex since data also exist indicating that $\alpha 6\beta 4$ integrin supports signaling pathways that drive migration, while $\alpha 3\beta 1$ integrin has recently reported to inhibit motility of keratinocytes[32].

4. Mechanisms of Integrin Crosstalk

To date, mechanisms that regulate integrin crosstalk generally involve the integrin β integrin cytoplasmic tail. With the exception of $\beta 4$ integrin, the cytoplasmic domains of β integrins are

short, composed of approximately 50 amino acids[33]. This tail acts as a docking site for over 40 proteins with the regulation of such interactions being key to integrin function and crosstalk [11,13,33]. The features of the $\beta 3$ integrin tail serve as a model for other integrins, except the $\beta 4$ integrin subunit. There are three domains that provide the major binding sites for cytoskeleton and signaling proteins: the membrane proximal NPxY motif containing regulatory tyrosine residues, a membrane distal NxxY motif and an intervening sequence bearing serine and threonine residues. We will next discuss how the regulation of protein binding to and the differential phosphorylation of the β integrin tail both likely play roles via which integrin crosstalk is mediated.

4.1. Talin

Talin is a large intracellular molecule of over 200kD, shown to induce the activation of integrins by increasing the affinity of an integrin for its ligand[12,34-37]. The amino-terminal FERM domain of talin interacts with integrins[12,36-38]. It is divided into three subdomains, termed F1, F2, and F3. The latter subdomain contains a phosphotyrosine binding (PTB)-like domain fold that exhibits high affinity binding for β integrin tails and is sufficient to activate the $\beta 3$ integrin[12,36-38]. It is believed that the F3 domain of talin initially engages the membrane distal region of the β integrin tail[10,37]. Integrin activation is triggered by the interaction of the PTB-like domain of talin with the membrane proximal helix-forming domain of the $\beta 3$ integrin tail[12,36,37,39]. There are some differences in the sequences required for talin activation of β integrin tails. For example, talin mediated activation of $\beta 1$ integrin requires the N-terminal and F1 domains as well as F3[36].

As mentioned previously, talin is a central molecular switch that regulates integrin crosstalk [11,12,39-41]. Indeed, Calderwood and colleagues have demonstrated that cells that over-express β integrin cytoplasmic tails defective in talin binding are unable to mediate crosstalk [41]. Moreover, integrin crosstalk can be reversed by over-expressing integrin binding and activating fragments of talin, while expression of a non-integrin binding protein that can sequester talin, such as PIPK1 γ 90, inhibits integrin activation[41].

Certain proteins that compete with talin for β integrin tail binding regulate integrin activation and therefore likely play an important role in the way talin mediates integrin crosstalk[11,33, 42,43]. For example, DOK, a downstream of kinase signaling proteins, is a PTB containing protein that binds to the membrane distal region of the β integrin tail[37,44]. Since DOK fails to bind to the membrane proximal region of the β integrin tail, it cannot activate an integrin but can inhibit activation by competing for integrin binding with talin. Other PTB containing proteins, including Shc and NUMB, likely function in the same way by regulating talin-integrin interaction[33]. There is also evidence that talin-integrin interaction is inhibited when 14-3-3 proteins bind to phosphorylated serine/threonine residues in β integrin tails[33,45]. Such is the case when T-cells are activated and threonine residue 758 in the $\beta 2$ integrin tail is phosphorylated[45]. Another protein that may play an important role in regulating talin-integrin interaction and, hence, integrin activation is filamin, an actin cross linker[42]. Filamin binds to unphosphorylated serine/threonine residues within $\beta 7$ integrin and prevents binding of talin[42].

The precise roles of PTB containing proteins such as DOK, filamin and 14-3-3 proteins in regulating talin-mediated integrin crosstalk should be an interesting avenue of future study. However, it is also clear that a variety of signaling enzymes modulate the binding of both talin and talin competitors to integrins via their ability to directly or indirectly phosphorylate key residues in the cytoplasmic tails of β integrins. We will discuss these next.

4.2. Protein Kinase A

There are several reports that demonstrate integrin ligand binding or clustering of $\beta 1$ integrin with anti- $\beta 1$ integrin antibodies leads to the activation of the cAMP dependent protein kinase A (PKA), a serine/threonine kinase which, in turn, mediates integrin crosstalk[46-48]. For example, Varner and colleagues have presented evidence that addition of $\alpha 5\beta 1$ integrin function-blocking antibodies inhibits $\alpha \nu\beta 3$ -mediated cell migration and angiogenesis in vivo in a PKA-dependent fashion[47].

PKA has also been demonstrated to play a role as a mediator of integrin crosstalk and a negative regulator of $\alpha \nu\beta 3$ integrin function in endothelial cells subject to shear stress[48]. Orr and colleagues have established that activation of $\alpha 2\beta 1$ integrin by shear stress inhibits the activation state of both $\alpha \nu\beta 3$ integrin and $\alpha 5\beta 1$ integrin in endothelial cells plated on collagen [48]. Blocking PKA with a pharmacologic inhibitor restores ligand affinity of $\alpha \nu\beta 3$ integrin but not $\alpha 5\beta 1$ integrin. In contrast, in endothelial cells plated onto fibronectin or fibrinogen, flow activates $\alpha 5\beta 1$ and $\alpha \nu\beta 3$ integrin, eliciting activation of protein kinase C (PKC) which suppresses $\alpha 2\beta 1$ integrin function[48]. These same workers also have presented evidence that talin is central to the integrin crosstalk they detail since its overexpression overrides integrin inhibition in endothelial cells subject to shear, regardless of substrate.

We recently demonstrated that in endothelial cells, $\beta 1$ integrin function blocking antibodies inhibit $\alpha \nu\beta 3$ integrin mediated adhesion via a pathway that involves PKA and inhibition of serine/threonine phosphatase I (PP1) via the inhibitor-1 pathway[46]. Inhibition of PP1 activity correlates with an increase in serine phosphorylation of the $\beta 3$ integrin cytoplasmic tail[46]. Moreover, mutating serine 752 of $\beta 3$ integrin to aspartic acid, a phosphomimetic, inhibits the ability of $\alpha \nu\beta 3$ integrin to mediate adhesion in CHO cells[46]. Based on these studies, we have hypothesized that $\beta 1$ integrin clustering results in activation of PKA which phosphorylates inhibitor-1, an endogenous inhibitor of serine/threonine phosphatase I inhibiting its activity. PP1 activity is required to maintain $\beta 3$ integrin in an unphosphorylated state. Since overexpressing an activating form of talin surprisingly fails to overcome the inhibition on $\alpha \nu\beta 3$ mediated cell adhesion by $\beta 1$ integrin function blocking antibodies, it is unlikely that PKA is acting on talin directly[46]. Instead, we have speculated that PKA may regulate talin-integrin interaction indirectly by altering the ability of talin to associate with $\beta 3$ integrin. We base this speculation on the literature and the numerous studies, some of which have been discussed above, that demonstrate that phosphorylation state of integrins can regulate the association of many intracellular proteins that alter the activation state of the integrin by modulating talin binding[33,45].

4.3. Calcium/calmodulin dependent kinase II

Our own data indicate that $\beta 1$ clustering or inhibition triggers signaling that inhibits ligand binding of $\alpha \nu\beta 3$ integrin[46]. However, the reverse is also the case. For example, more than ten years ago, it was demonstrated that in leukocytes, ligation of $\alpha 5\beta 1$ integrin enhances calcium/calmodulin dependent kinase II (CaMKII) which is required for $\alpha 5\beta 1$ mediated phagocytosis and migration[49]. In contrast, ligation of $\alpha \nu\beta 3$ integrin inhibits the ability of $\alpha 5\beta 1$ integrin to support phagocytosis and migration in the same cell[49]. Moreover, the ability of $\alpha \nu\beta 3$ integrin to modulate the activities of $\alpha 5\beta 1$ integrin involves serine residue 752 in the $\beta 3$ integrin tail. Mutating this residue to alanine has no effect on $\alpha \nu\beta 3$ integrin ligand binding but affects the ability of $\alpha \nu\beta 3$ to inhibit $\alpha 5\beta 1$ integrin functions[49].

How does CaMKII regulate $\alpha 5\beta 1$ integrin function in the above crosstalk scenario? Bouvard and colleagues have reported that in CHO cells activation of CaMKII or blocking calcineurin, a calcium calmodulin dependent protein phosphatase inhibits $\alpha 5\beta 1$ interaction with its ligand fibronectin[50]. This same group identified integrin-cytoplasmic-domain-associated protein 1

(ICAP-1 α) as the target for CaMKII activity and a regulator of $\alpha 5\beta 1$ integrin affinity for ligand [51]. ICAP-1 α has been shown to interact with the cytoplasmic tail of $\beta 1A$ integrin and binding is dependent on the NPxY integrin motif[51]. ICAP-1 α contains several putative phosphorylation sites for PKC, PKA, PKG and CaMKII. Mutating threonine 38 residue to alanine generates an ICAP α protein that cannot be phosphorylated by CaMKII but can rescue CHO cell adhesion to fibronectin[51]. In contrast, mutating threonine 38 to aspartic acid, a phosphomimetic, results in a strong defect in cell spreading that cannot be overcome by inhibiting CaMKII activity. In other words, the balance of serine/threonine kinase activity and serine/threonine phosphatase activity regulates the activation state of $\alpha 5\beta 1$ integrin by a process that does not involve phosphorylation of the integrin cytoplasmic tail but, instead, acts by altering the ability of the adaptor protein, ICAP α to associate with the $\beta 1$ integrin cytoplasmic tail and compete with talin for binding[52,53]. Indeed, ICAP α is a good example of an adaptor protein involved in integrin crosstalk. Its binding to integrin is regulated by serine/threonine kinases and serine/threonine phosphatases and it inhibits integrin function by negatively regulating the ability of talin to bind to β integrin tails[52].

5. Summary, Future Directions and Implication of Crosstalk for Integrin-based Treatment of Human Disease

As we have discussed, integrin-integrin crosstalk is a complex process involving cytoskeleton and signaling adaptor proteins. Clearly, talin is a key regulator of such crosstalk although we are only just beginning to understand how it modulates integrin affinity and how talin binding to integrin cytoplasmic tails is controlled. A number of competitors of talin-integrin binding have been identified but the number of these will surely grow. The role of phosphorylation of various residues in the β integrin tail is also key to the regulation of binding of both talin and its competitors and more study in this area is necessary. In addition, there is some evidence in the literature that integrin crosstalk may be at the level of regulation of the stability of integrin subunit mRNA[54]. Specifically, in one model system, it has been demonstrated that $\beta 1$ integrin protein expression can down regulate $\alpha \beta 3$ integrin expression by decreasing the stability of the mRNA encoding the $\beta 3$ integrin subunit[54].

Although crosstalk between the integrins expressed in skin cells has been proposed, the $\beta 4$ subunit is very different from other β integrin subunits and lacks talin binding motifs. In other words, $\beta 4$ integrin cannot sequester talin from $\alpha 3\beta 1$ integrin in order to inhibit the ligand binding of the latter. How precisely $\alpha 6\beta 4$ integrin crosstalks to $\alpha 3\beta 1$ integrin is another potential area of research for the future.

At the cell biological level, integrin crosstalk complicates analyses where an antibody integrin or inhibitor is used. The same is true in instances where integrin subunit expression is manipulated. Any resulting phenotype might result not only from the inhibition or loss of the targeted integrin but may also result from effects on the ligand binding or signaling of the integrins with which it functionally interacts via crosstalk.

A number of integrin antibody and small molecule inhibitors have been or are in the process of undergoing clinical evaluation for the treatment of human disease, including age related macula degeneration and certain cancers such as glioblastoma[55,56]. Some of these inhibitors are designed to target $\beta 1$ subunit-containing integrins while some others target $\alpha \beta 3$ integrin [55,57]. These inhibitors work as a consequence of integrin crosstalk since, as we have already detailed, inhibition of $\beta 1$ integrin perturbs the function of $\alpha \beta 3$ integrin and vice versa. However, there may be downsides to this phenomenon. An antibody inhibitor of $\alpha 4$ integrin has been used to treat Crohn's disease where it inhibits migration of leukocytes into the gut and reduces inflammation[31]. The same integrin inhibitor has been evaluated for the treatment of patients with multiple sclerosis. However, adverse effects of such treatment have been

reported, possibly due to the effects of the inhibitor on the activity of integrins with which $\alpha 4\beta 1$ integrin cross talks. Thus, understanding the molecular mechanisms that are involved in regulating the activation state of distinct integrins in a particular tissue may provide additional targets for the development of new therapeutic agents with greater specificity.

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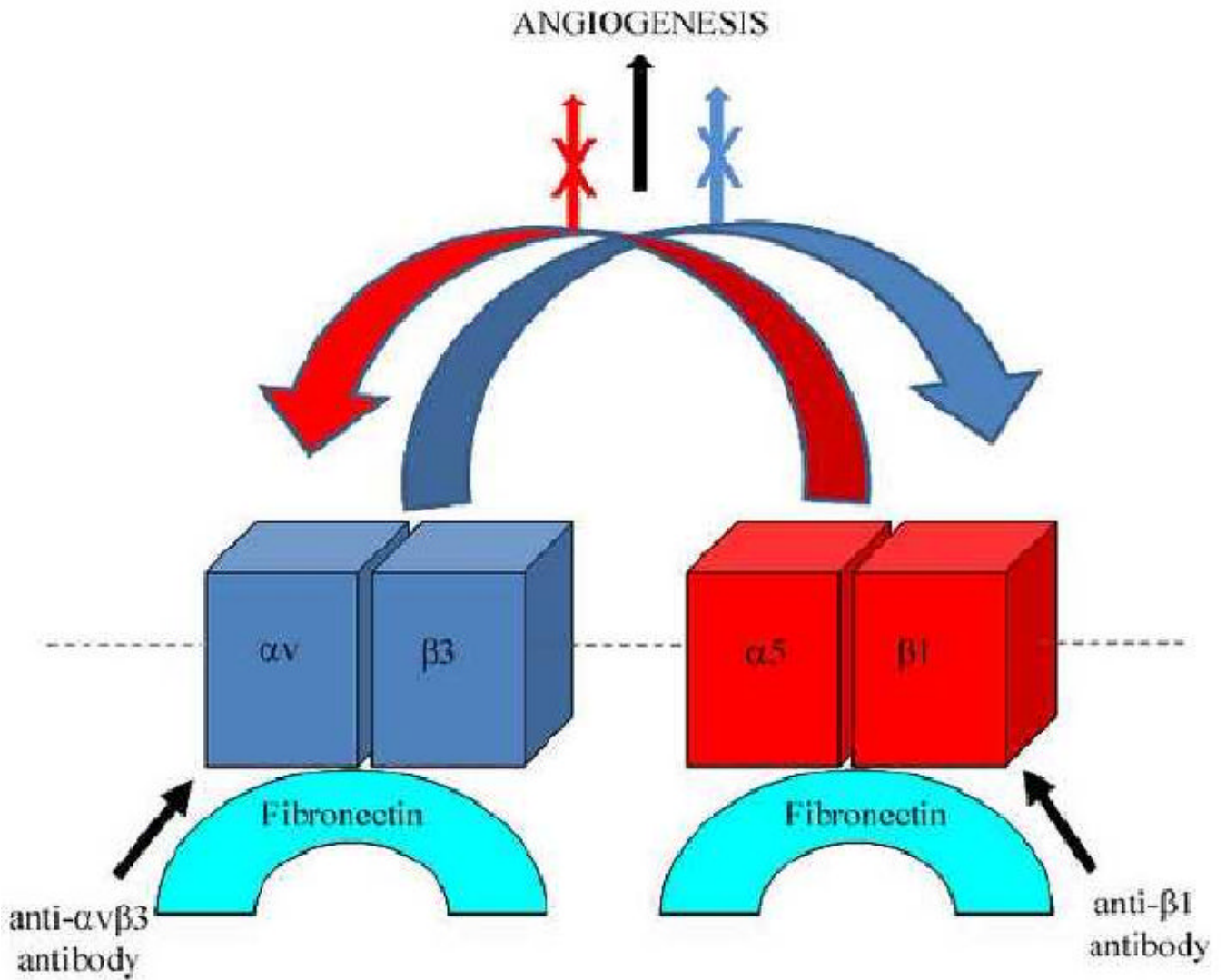


Fig. 1.

This diagram represents a simple scheme of transdominant inhibition of integrins expressed by endothelial cells. In the example shown $\alpha 5 \beta 1$ and $\alpha v \beta 3$ integrin both bind to fibronectin ligand and, upon ligand activation, they support signaling processes leading to angiogenesis (black arrow). However, upon antibody inhibition of $\beta 1$ integrin, not only is $\alpha 5 \beta 1$ integrin-ligand interaction perturbed but also $\alpha v \beta 3$ integrin binding to ligand is impeded (red curved arrow), resulting in a block in angiogenesis (X through red arrow). Likewise, upon antibody inhibition of $\alpha v \beta 3$ integrin, both $\alpha v \beta 3$ and $\alpha 5 \beta 1$ integrin-ligand binding (curved blue arrow) are perturbed as is angiogenesis (X through blue arrow).

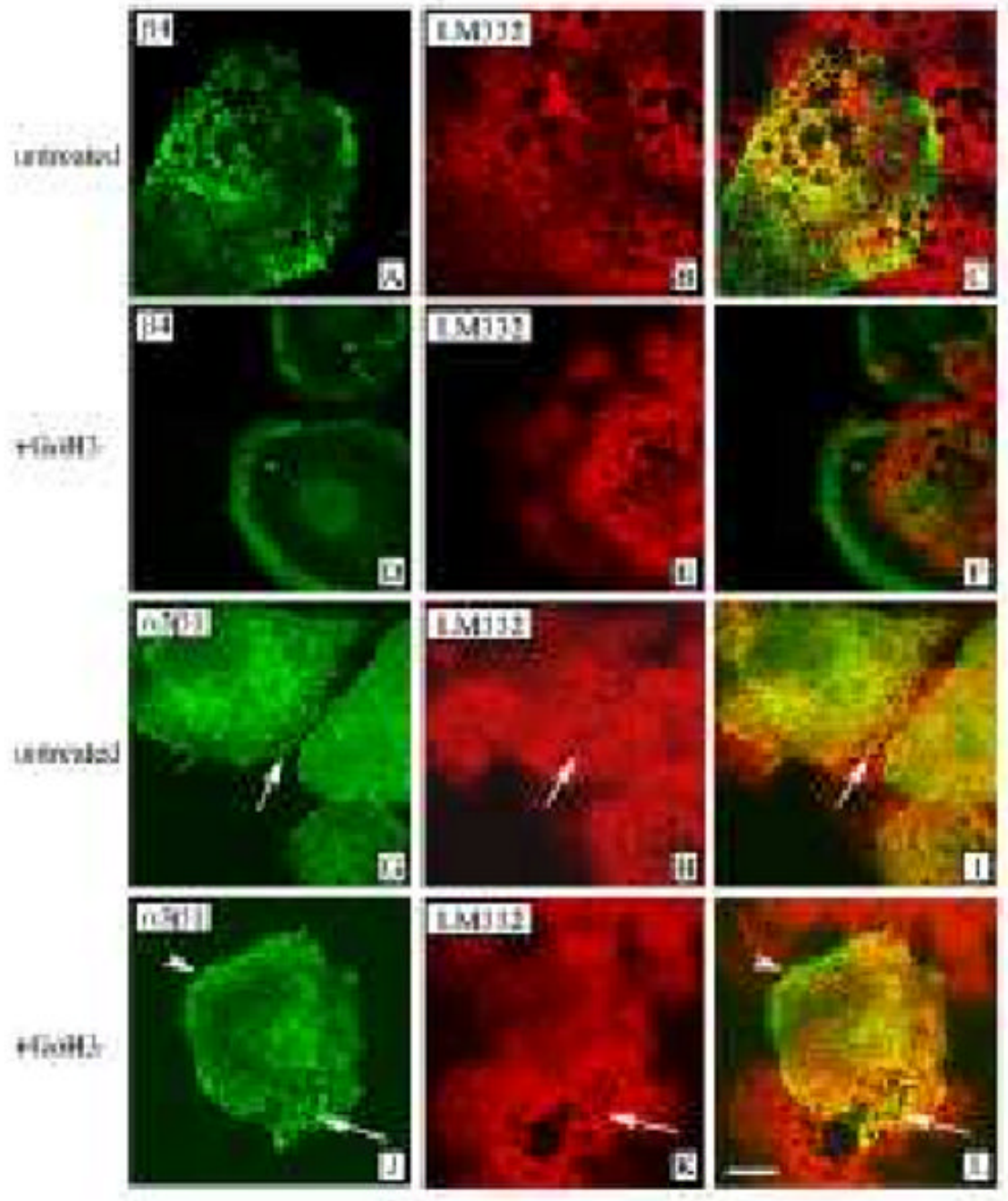


Fig. 2. Human keratinocytes (SCC12 cells) were plated onto the laminin-332-rich matrix of rat bladder 804G cells in the absence (A-C, G-I) or presence of GoH3, a function-inhibiting $\alpha 6$ integrin antibody (D-F, J-L). At 2 hours after plating, cells were prepared for double label immunofluorescence using a combination of antibodies against either $\beta 4$ integrin and laminin-332 or $\alpha 3\beta 1$ integrin and laminin-332 as indicated. Cells were viewed by confocal microscopy with the focal plane being as close as possible to the cell-substratum interface. Overlays of the sets of images are shown in C, F, I, and L. Note that the laminin-332 antibodies in B, E, H, and K generate a “Swiss cheese-like” pattern on the surface to which the SCC12 cells adhere. The $\beta 4$ integrin subunit in the cell in A shows colocalization with the laminin-332

in the matrix immediately underlying it (B). Colocalized antigens appear yellow in C. In the presence of GoH3 antibodies, the $\beta 4$ integrin no longer clusters in the cell in D over the laminin-332 matrix (H). Arrow in G-I indicates a cell region where $\alpha 3\beta 1$ integrin is clustered at the cell periphery, but is not organized into a Swiss cheese-like pattern. In J, in the presence of GoH3, clusters of $\alpha 3\beta 1$ indicated by the arrow are found codistributed with the Swiss cheese organization of laminin-332 in the matrix (K). The colocalized antigens appear yellow in L. The arrowhead in J indicates $\alpha 3\beta 1$ integrin found at the cell periphery in a cell region, sitting on an area of substrate deficient in laminin-332. Bar, 10mm.