Integrin Modulation by Lateral Association*

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Integrins are a major family of cell-matrix and cell-cell receptors. Bidirectional signal transmission between the extracellular matrix or other integrin ligands and the submembranous cytoskeleton and associated adaptor proteins is now being resolved (1–6). This review will concentrate on an emerging area of study: how the type of adhesion and the signaling following integrin ligation may be modulated by lateral interactions with other membrane components. This may occur through direct or indirect interactions. Two major groups of transmembrane proteins will form the focus of this review. One group, the tetraspans or TM4SF proteins (7–9), is composed of transmembrane proteins implicated in regulation of cell migration and invasion. They can interact directly with the extracellular domain of the α chain of specific integrins. The second group, the syndecans (10–15), has not been shown to bind integrins directly but to bind to separate domains within integrin ligands. These also modify integrin-based adhesion, migration, invasiveness, and matrix assembly. The similarities in integrin modulation will be discussed.

Integrin-binding Transmembrane Proteins

Because of space constraints, integrin interactions with other transmembrane proteins will only be briefly mentioned. The first integrin-associated transmembrane protein (IAP, CD47) was cloned in 1993 (16). This is a receptor for the cell-binding domain of thrombospondin (17), and it can regulate vitronectin binding to αβ3 (16, 18) and activate α1β1 through thrombospondin binding (19). Mice lacking IAP have decreased resistance to bacterial infection probably because of delayed neutrophil migration to the site of infection and defective activation (20). IAP may also interact with αβ3 integrin in vascular smooth muscle cells (21). Second, integrin and growth factor signaling pathways intersect, and a subset of transmembrane proteins implicated in regulation of cell migration and invasion. They can interact directly with the extracellular domain of the α chain of specific integrins. The second group, the syndecans (10–15), has not been shown to bind integrins directly but to bind to separate domains within integrin ligands. These also modify integrin-based adhesion, migration, invasiveness, and matrix assembly. The similarities in integrin modulation will be discussed.

Integrin-Tetraspan/TM4SF Interactions

Tetraspans/TM4SFs are a large family of transmembrane proteins, which have four transmembrane domains, short N- and C-terminal cytoplasmic tails, and two extracellular loops (reviewed in Refs. 7–9). The transmembrane domains are the most highly conserved between family members, particularly the inclusion of polar amino acids, and the sequences of hydrophobic residues. There are also 2–3 conserved charged residues in the 4-amino acid cytoplasmic loop between transmembrane domains 2 and 3, including a glutamic acid residue. These, together with a PXSC motif and the conserved placement of 3 cysteines in the large extracellular domain form the structural basis for the family classification (7–9). The extracellular domains of tetraspans/TM4SFs are otherwise divergent between each family member and, except for some conservation of glycosylation sites, are ~80% identical between species. An exception to this is the tetraspan/TM4SF (NAG-2), which is 90% conserved between mouse and human (27).

Tetraspan/TM4SF Proteins

Tetraspans/TM4SFs are very widely expressed and can interact with themselves, other tetraspans/TM4SFs, and a range of other cell surface components. Because of the multicomponent complexes they participate in, they have been termed “adaptor” or “facilitator” molecules (7, 8). Some are nearly ubiquitously expressed (e.g. CD9, CD63, and CD81), whereas others are limited to platelets, immune cells, or neuronal cells (reviewed in Refs. 7–9). Many were originally identified as tumor-associated proteins and implicated in changes in adhesion, motility, metastatic potential, or proliferation (7–9). For example, CD9 transfection into carcinoma cells reduced motility and metastasis, and reduced CD9 expression accompanies poor prognosis in breast carcinoma (29). They also may play role(s) in development. For example, the Drosophila Ibr gene is needed for normal formation of neuromuscular junctions, CD9 is transiently expressed during neuronal development, and the complement of tetraspans/TM4SFs expressed by T and B cells differs with the developmental stage (reviewed in Refs. 7–9).

Tetraspan/TM4SF Interactions with Integrins

Recently (30) tetraspans/TM4SFs were found to interact with integrins. The association is constitutive, e.g. in resting platelets (31), and independent of adhesion because it occurs in cells in suspension (32). Most studies have used co-immunoprecipitation under low stringency conditions and surface cross-linking and have identified integrin-tetraspan/TM4SF interactions with αβ2, αβ5, ααβ4, and ααβ6 with cell type-specific or contradictory reports for αβ1, αβ3, or αβ2 (reviewed in Refs. 7–9). Although deletion/chimeric transfection studies indicate tetraspans/TM4SFs interact through the extracellular domain of the α subunit, ~30% of hydrophobic interactions may play some role (31), and the β subunit may also contribute because, although αδβ1 does interact, αδβ2 does not (30, 34).

Integrins and tetraspans/TM4SFs co-localize in clusters, particularly at leading lamellae of cells or in “footprints” left after cell detachment (28, 30–32, 34) or at intercellular contact sites (35). However, tetraspans/TM4SFs seem to be excluded from focal ad-
hensions (28, 30–32, 34, 36). Indeed, in CHO co-transfection experiments, CD9 colocalized with αmβ3 integrin only in clusters at the leading edge of lamellae, whereas αmβ3 was additionally present in focal adhesions in cells seeded on fibrinogen (31). Furthermore, CD9 transfection of CHO cells did not alter focal adhesion formation (31). However, transfection with CD9 did alter actin cytoskeleton organization in HT1080 cells (36), and the effect varied with substrate. Most studies point to a role for tetraspans/TM4SFs and their associated integrins (e.g. αβ3 and αβ1) in migration rather than adhesion per se (8, 35, 37). This is highlighted by one study (33) that confirmed a stable, specific interaction of one tetraspan/TM4SF (CD151) with integrin αβ3 by co-immunoprecipitation under stringent conditions. Unlike previous studies, where only subsets of tetraspan/TM4SF or integrin molecules interacted, most (70–90%) of αβ3 associated with CD151 and no additional cell surface components were co-immunoprecipitated. Antibodies against either CD151 or αβ3 dramatically reduced neutrophil migration, confirmed in a separate study (35). Anti-αβ1 (but not anti-αβ3) or anti-CD151 (but not anti-CD9) specifically inhibited neurite outgrowth, with no effect on adhesion. It remains to be seen whether the association of αβ3 with CD151 is responsible for the previously observed association of αβ3 with CD9, CD63, CD81, and NAG-2 (33).

**Tetraspan/TM4SF Signaling**

Tetraspan/TM4SF interaction with integrins may provide indirect association of integrins, which do not have intrinsic enzymatic activity, with enzymes or other signaling molecules. Src family tyrosine kinase and lesser amounts of serine/threonine kinase activities associate with CD63-β3 integrin complexes (37), and CD53 binds an unknown tyrosine phosphatase (38). Conventional protein kinase C (cPKC) isoforms can associate with CD81 and CD151 (9) after activation. Phosphatidylinositol 4-kinase (PI4-K; probably type II) associates constitutively with a multicomponent complex containing the tetraspans/TM4SFs CD63, CD81, and αβ3 (32) and with αβ3 complex (33). PI4-K activity was associated with αβ3, through CD151 with only minor amounts through CD63 and CD81. The enzyme associates with the tetraspan/TM4SF protein rather than αβ3 (32, 33). Interestingly, clustering of the αβ3-CD63-CD81 complex with antibodies to either tetraspan/TM4SF protein did not induce tyrosine phosphorylation of FAK or pp130ζζ′, whereas clustering with anti-integrin did. HT1080 cells containing CD9 do, however, differ in FAK phosphorylation from wild type, and again this depends on which substrate is used for adhesion (36). Furthermore, antibodies against CD63, CD82, or CD151 all potentiated FAK phosphorylation when present as a mixed substrate with collagen (36), but adhesion to these antibodies alone reduced the level of FAK phosphorylation below that even seen in suspended cells. Thus, signaling through the tetraspans/TM4SF proteins appears complex. This recent study also demonstrated that tetraspan/TM4SF proteins are only weakly associated with the actin cytoskeleton (36). Thus, interactions of tetraspan/TM4SF proteins with their ligands has been suggested as an alternate signaling pathway leading to weak or transient cell-matrix-cytoskeleton interactions suitable for lamellipodial extension and retraction. CD9, CD63, CD81, CD82, and CD151 colocalize at punctate structures at the cell periphery, rather than focal adhesions, and although some colocalization of talin and FAK occurs, they do not appear to colocalize with vinculin- or Paxillin-containing structures (36). Integrins are inserted at the leading edge of cells (reviewed in Ref. 39), P14-K is implicated in vesicular transport (40), and CD63 has an internalization motif (41), which allows for lamellipodial colocalization of integrin/CD63 and rapid re-internalization of αβ3 if complexes are not stabilized.

**Cell Surface Proteoglycans**

Cell surface proteoglycans can modify cell adhesion and migration, similar to the tetraspans/TM4SFs (reviewed in Refs. 10–15). Several early studies indicated a need for interaction of both heparin binding and “cell” binding motifs for the development of stress fibers and focal adhesions in a variety of anchorage-dependent cells (reviewed in Ref. 14). This has been confirmed recently (42, 43). Cell attachment and spreading appears to be integrin-mediated, whereas later cytoskeletal organization appears to be heparan sulfate proteoglycan-mediated (14), with concomitant activation of PKC (44). A study monitoring CHO responses through transfected αmβ3 to fibrinogen substrates (45) showed that antibody-coated substrates, whether integrin-activating or not, induced only limited spreading; full spreading required additional activation of PKC, and stress fiber formation followed Rho activation. The roles of the G proteins Rac, Rho, and Cdc42 in cell spreading and stress fiber/focal adhesion formation have been recently reviewed (46). Thus, there appear to be three sets of signaling involved in focal adhesion assembly: tyrosine phosphorylation events associated with integrin ligation, PKC activation associated with cell surface proteoglycan interactions, and Rho-GTP signaling.

**Syndecans in Cell Adhesion**

There is one report of a syndecan being coimmunoprecipitated with CD9 tetraspan/TM4SF, which also associates with integrin β3 (47). Direct interactions of syndecans with integrins have not been reported, but co-immunoprecipitation under low stringency such as used for tetraspan/TM4SF-integrin interactions has not been described. Syndecans have a single transmembrane domain, a short cytoplasmic domain, and a larger extracellular domain that bears 3–5 glycosaminoglycan chains, mostly heparan sulfate (10–15). The four mammalian syndecans have cell and developmental expression specificity (48). Briefly, syndecan-1, -2, and -3 are the major syndecans of epithelial, fibroblastic, and neuronal cells, respectively, whereas syndecan-4 is unusual, appearing as a minor component of most cells. Syndecan core proteins range in size from ~20 kDa (syndecan-4) to ~45 kDa (syndecan-1) as deduced from sequencing, which is not unlike that of tetraspans/TM4SFs (Table 1). Like the tetraspans/TM4SFs, syndecan core proteins have high homology in the transmembrane domain. In addition, however, syndecans have two cytoplasmic regions, proximal and distal to the membrane, that are also highly conserved. We have termed these C1 and C2, respectively (14). In between these constant regions is a small cytoplasmic sequence (denoted V) unique to each syndecan but conserved between species. This has led to suggestions of syndecans having common and unique functions (14, 15). The extracellular domains are highly divergent except for the glycosaminoglycan attachment sites (10–15), even for the same syndecan of different species.

Syndecans can bind a wide range of extracellular matrix molecules, growth factors, lipoproteins, and enzymes through their heparan sulfate chains (10–15). This has led to them being regarded as “co-receptors” for cell surface binding and internalization of a number of ligands (10). However, their precise roles in binding, presentation, and signaling are only now being revealed. Transfection experiments with syndecans have indicated that they can affect adhesion and/or migration. Syndecan-1 was the first to be cloned and has been the most studied. Expression of syndecan-1 in lymphocytes results in decreased invasion of collagen gels (49), whereas reduction of syndecan-1 in epithelial cells results in the cells appearing fibroblastic and invading collagen gels (50). This correlates with a loss of E-cadherin, and conversely, down-regulation of E-cadherin results in down-regulation of syndecan-1 (51). Syndecan-1, like tetraspan/TM4SFs, can be a prognosis marker for tumor progression (52). When expressed in Schwann cells,
Syndecan-1 led to increased cell spreading and stress fiber formation, and the transfected syndecan-1 codistributed with microfilaments during spreading but was not inserted into the focal adhesions that ultimately formed (reviewed in Ref. 12). Syndecan-1 and -3 can both codistribute with the microfilament system if clustered with antibodies against the ectodomain (12). Syndecan-2 overexpression also results in increased spreading (53) but does not increase focal adhesion formation nor is it a focal adhesion component in transfected cells or normal fibroblasts (54).

Syndecan-4, unlike integrin-associated tetraspans/TM4SFs, is localized to focal adhesions on a variety of substrates (54, 55), codistributed with α5β1 or α6β1 integrins in fibroblasts on fibronectin or vitronectin substrates, respectively (54). Its presence, therefore, is not limited to one specific integrin, but association may be limited to those integrins involved in focal adhesion formation. Primary fibroblasts will attach and spread on substrates coated with the "cell-binding" domain of fibronectin but do not form focal adhesions or stress fibers (42, 43, 56). Addition of clustering antibodies against syndecan-4 ectodomain can promote focal adhesion formation in cells prespread on the cell-binding domain of fibronectin (43).

Overexpression of syndecan-4 in CHO cells leads to an increase in spreading, accompanied by increased formation of focal adhesions and stress fibers (53). Expression of antisense cDNA for syndecan-4 or of a core protein with a truncated cytoplasmic domain, in contrast, leads to a decrease in spreading, focal adhesion, and stress fiber formation (53), confirming that signaling through syndecan-4 (see below) is fundamentally involved in cytoskeletal organization. Promotion of focal adhesion formation appears to be through clustering of the core protein, because this will occur if syndecan-4 core protein is overexpressed in mutant CHO cells that cannot add heparan sulfate chains and do not normally form focal adhesions (57). Syndecan core proteins spontaneously oligomerize (reviewed in Ref. 12).

**Syndecan-based Signaling**

Syndecan-4 cytoplasmic domain can bind PKCs and its constitutively active catalytic fragment PKM in vitro (58). It also associ-ates in vivo with PKC as shown by co-clustering, co-immunoprecipitation, and affinity chromatography. Furthermore, interactions require prior activation of PKC, presumably to translocate PKC from the cytoplasm to the membrane (58). The site in syndecan-4 that binds PKC is its unique cytoplasmic V region, comprising the sequence LGKKPIYKK (58). The maximal activity of PKC is induced by phospholipid mediators, or of PKM, is enhanced in vitro by interaction with syndecan-4 core protein or with synthetic peptides encompassing the V region sequence (58). This requires multimerization of the core protein (54), which is a property of all syndecans (10–15). Phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P2) promotes the oligomerization of the cytoplasmic domain of syndecan-4 (59, 60). PIP2 is itself an activator of cPKC isoforms and does not need to be cleaved by phospholipase C to diacylglycerol to achieve activation. Furthermore, syndecan-4 potentiates PKC activity by interaction with syndecan-4 core protein or with synthetic pep-tides encompassing the V region sequence (58). This requires mul-timerization of the core protein and adaptation signaling domains.

**Common Features of Integrin Response Modifiers**

Despite different structural characteristics, tetraspans/TM4SFs and syndecans have some strikingly convergent properties, some of which are listed in Table I and shown schematically in Fig. 2. They...
have similar protein sizes, very small cytoplasmic domains, and larger, but poorly conserved, ectodomains. Possibly structural motifs are conserved, whereas their constituent primary sequences are not. Perhaps most remarkable is the property of CD51, CD151, and syndecan-4 to bind activated cPKC (reviewed in Refs. 9 and 14). Direct interactions of PKC isoforms with transmembrane molecules are rare. PKC binding to tetraspans/TM4SFs may lead to signal transduction through regulation of integrin α5 and α6 subunits (9), and the role of PKC in cytoskeletal organization is now emerging as a major field of study (e.g. Ref. 4 and references therein). Syndecan-4 may localize PKCs to form focal adhesions, promote stable adhesion, and thereby reduce cell migration. Conversely, the same PKC bound to tetraspans/TM4SFs may enhance cell migration, because tetraspans/TM4SFs appear to be uniformly excluded from focal adhesions. How this system is balanced in cells is intriguing and may explain why PKC activation in different cell types can either promote adhesion or migration (4).

Another common connection is with phosphoinositide metabolism. Syndecan-4 cytoplasmic domain dimers are stabilized by interactions withPIP2 (60, 61). Synthesis of this phospholipid is enhanced by integrin ligation and Rho and Rac activation (reviewed in Ref. 46). Many actin-associated protein functions are regulated by phosphoinositide metabolism and growth, apoptosis, and matrix assembly. Tetraspans/TM4SFs and kinases intracellularly, with effects on focal adhesion disassembly, do not have intrinsic kinase activity, and that activity may be the result of PKC in cytoskeletal organization is now emerging as a major activity being potentiated by PKC isoforms with transmembrane molecules, possibly structural motifs. We thank colleagues for interesting discussions and constraints. We thank Dr. Weontae Lee and Donghan Lee (Yonsei University, Seoul, Korea) for Fig. 1.

Conclusions

The emerging paradigm is that lateral interactions of the tetraspans/TM4SFs, syndecans, and perhaps other transmembrane molecules act as response modifiers for integrin function. Integrins do not have intrinsic kinase activity, and that activity may be the “collaborator” function to control signaling. Integrins associate with tyrosine kinases, e.g. FAK, pp130, Src, and integrin-linked kinase intracellularly, with effects on focal adhesion disassembly, growth, apoptosis, and matrix assembly. Tetraspans/TM4SFs and syndecans may optionally contribute finetuning of cytoskeletal organization through regulation of phosphoinositide metabolism and PKC location and activity.

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