Connective Tissue Vol. 30. 1998

Review

Cell Adhesion to Collagen - Is One Collagen Receptor Different from Another ?

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Received, 10 August 1998; accepted, 7 September, 1998.

Abstract: The integrins are a large family of cell adhesion receptors involved in cell-cell and cell-matrix interactions. At present, 23 different integrin heterodimers are known. Integrins participate in a complex apparatus which anchors cells to their surroundings and transduces signals into the cells. These signals regulate many important aspects of cell behavior, including growth, survival, differentiation, and phenotype. Integrins may also play a central role in the healing process of tissue injuries and in the progression of human cancer. This review is focused on integrins mediating cell adhesion to the collagens, the most important structural components of the connective tissue.

Key words: Collagen, Integrins, Cell adhesion, Collagen receptors

The Collagen Receptor Integrins

The existence of collagen-like proteins might have been essential for the development of the first metazoans around 600-800 million years ago[1]. In mammals at least 19 different collagen types are known. The major subgroups are 1) fibrillar collagens (types I, II, III, V, XI), 2) network forming collagens (types IV, VIII, X), 3) fibril-associated collagens (types IX, XII, XIV, XVI, XIX), 4) collagens forming beaded filaments (type VI), 5) collagens forming anchoring fibrils (type VII), and 6) transmembrane collagens (types XIII, XVII)[2]. The basic structure of collagen is highly conserved from marine sponges to vertebrates. Marine sponges, such as *Geodia cydonium*, have a fibril collagen and some of these metazoan species also have a network forming basement membrane type collagen[3]. In addition to the collagen types resembling the ones found in mammals many invertebrates have developed large numbers of unique collagen subtypes.

The integrin-type cell adhesion receptors are responsible for the attachment of the most cell types to their surroundings in all metazoans[4]. A typical integrin is composed of an α and a β subunit (the members of the integrin family are shown in Figure 1). Both subunits have a relatively short intracellular domain (with the exception of the hemidesmosome laminin receptor β4 subunit), one single transmembrane domain and a ligand binding extracellular domain. The two subunits are non-covalently linked together and inside the cell they are connected to cytoskeleton. In addition to anchoring, integrins mediate molecular signals into cells and regulate important cellular functions including migration and differentiation. Integrin ligands include many collagen types, laminins, fibronectin, and some members of the immunoglobulin super gene family.

The classical example of the cell-extracellular matrix interaction is the recognition of the arginine-glycine-aspartic acid (RGD) sequence[5] in fibronectin and in many other glycoproteins by integrins, such as α5β1, αIIbβ3, αVβ3, αVβ5, and αVβ6. Also type I collagen has an RGD motif but is seems to be recognized only in denatured or degraded collagen. Instead, two RGD-independent integrins, α1β1 and α2β1 heterodimers, mediate cell adhesion to native collagens. Integrin α1β1 is expressed on fibroblasts, smooth muscle cells, certain endothelial cells, chondrocytes, osteoblasts, and lymphocytes. Integrin α2β1 is expressed for example on epithelial cells, platelets, osteoblasts, chondrocytes, and fibroblasts. Integrin α1β1 may prefer the basement membrane type collagen (type IV) and α2β1 integrin seems to have larger affinity to fibrillar collagens. Most collagen subtypes have not been tested in the terms of integrin binding.

In addition to α1β1 and α2β1 integrins a third heterodimer, namely α3β1 has been shown to recognize distinct collagen types. In spite of the fact that in many cell types the adhesion to collagen leads to the localization of α3β1 integrin in focal adhesion sites, it is more probable that cells do not use it in primary adhesion to collagen but rather as an assisting receptor[6]. Interaction of other integrins, including the tenascin receptor α9β1, with collagen has also been suggested[7].

A recent paper reports the cloning of a new collagen

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receptor, α10 β1 integrin[8]. It is expressed on chondrocytes, but its biology is mostly unknown[8].

The fact that in tissues the collagen fibrils are often covered by glycoproteins and proteoglycans rises the question whether cells can at all bind directly to collagen molecules in them. Often it is more likely that proteins such as fibronectin mediate adhesion to collagen fibrils. Thus the role of the cellular receptors for native collagens might be limited to the recognition of newly synthesized molecules or to the binding of collagen sequences uncovered during tissue injury or inflammation.

The Structural Basis of Collagen Recognition

As mentioned above, integrins α1β1, α2β1, α3β1 and α10 β1 are those found to function as collagen receptors in various cells. Integrin α1, α2 and α10 subunits belong to a group of eight α subunits having about 200 amino acids long inserted domain between the second and the third repeated sequence, close to the N-terminus. The collagen binding integrin α1, α2 and α10 subunits are the only matrix receptor integrins that have an I domain (Fig. 1). Thus the collagen receptors have structural similarities with the lymphocyte integrins and differ from e.g. fibronectin and laminin receptors. The integrin I domains (or A domains) show high homology to collagen binding A domains found in von Willebrand factor[9]. The important role of the I domain in ligand binding has been shown with many α subunits[10-16]. For collagen binding integrins it has been shown with function blocking antibodies[14,17], using recombinant proteins to bind to collagen[13, 18-21] and using mutagenesis studies to point out important amino acid residues[13,17].

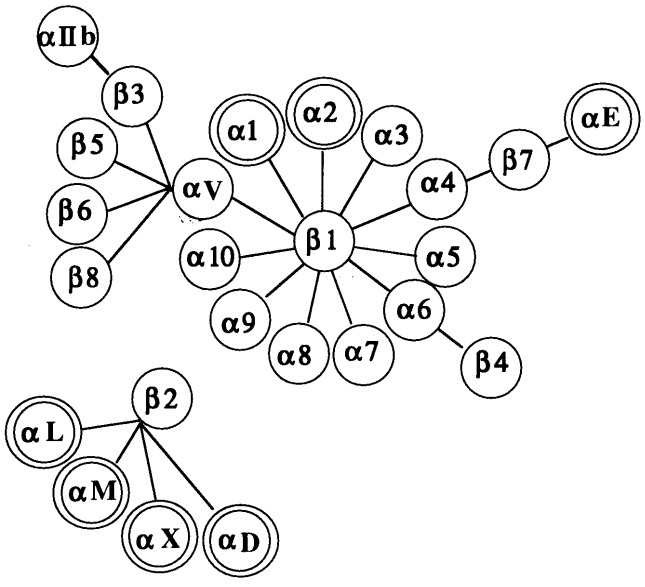

Fig. 1. The integrin family of cell adhesion receptors. At the present 17 α subunits and 8 β subunits are known. Together they form 23 different heterodimers (in the figure the heterodimer forming subunits have been connected with a line). Heterodimers containing β1, β3 , β4, or αV subunits are receptors for various extracellular matrix molecules. The integrin α subunits which have a ligand binding αI domain have been marked with a double circle. Note that the collagen receptor integrins α1, α2 and α10 are the only matrix receptors that have an αI domain.

The crystal structure of α2 I domain has been solved recently[22] and it is quite similar when compared to two other I domain structures, αM and αL[23,24] as well as to von Willebrand factor A3 and A1 domain structures[25-27]. The I domain consists of α-helices surrounding β-sheets buried inside the domain and the structure is called the 'Rossman fold'[28]. The I domain also has a binding site for divalent metal called MIDAS[23].

The role of the divalent metal ion for the function of the I domains is still somewhat uncertain. Based on αM I domain structure it has been proposed that the metal could be involved in ligand binding[29]. For α2 I domain there is no direct evidence indicating collagen binding to Mg^{++} in α2I domain. However, it is commonly expected that Asp or Glu in collagen would be important for the binding. These two amino acids have the potency to participate in the coordination of the metal ion. There is some controversy in the cation-dependency of collagen binding: two groups have shown that the phenomenon is dependent on the metal[19,21] and Kamata and Takada[13] have introduced metal-independent collagen binding. Also the possible structural arrangements by the metal in I domains are uncertain. In both von Willebrand A domain structures no metal is bound[25-27] and this was also shown experimentally for A3 domain[25].

Amino acids surrounding MIDAS in I domain have been proposed to be responsible for the ligand recognition/binding [23] and some evidence for this proposal has been presented [30,31]. The α2 I domain contains an extra α-helix (αC) very close to MIDAS and the DNA sequence alignment of α1 I and α2 I subunits predicts that α1I domain has that helix too[22]. Emsley *et al.*[22] manually docked a model of collagen triple helix into the structure of α2 I domain and proposed that ten amino acids around MIDAS could be involved in collagen binding. They also pointed out that αC-helix could have an important role in collagen binding.

The binding sites in different collagen molecules are not known, but triple helical structure and Asp/Glu and Arg were shown to be important for the binding of α1β1 integrin to collagen IV[32]. There is evidence for multiple binding sites present in collagen I for α2β1[33] and in collagen IV for α1β1 and α2β1[34].

The region surrounding I domain in α2 subunit has been shown to be important for the ligand binding[21]. The α2 subunit contains three EF hand-like metal binding sites close to the I domain and by adding the first EF motif to recombinant I domain the collagen binding was significantly enhanced. Springer[35] predicted that all seven N-terminal repeats are forming a special fold called β-propeller domain and the experimental evidence was published just recently[36].

At the present it seems that in collagen receptor integrins α1β1 and α2β1 there are two major regions involved in ligand binding: αI domain and EF hand-like repeats around αI domain. Despite the fact that the αI domains can fold and recognize ligands independently the common β1 subunit may still participate in ligand binding by the collagen receptors or in the regulation of integrin activity.

Despite their structural similarities α1I and α2 I domains may have functional differences. There is some evidence that α1 I domain might have higher affinity to type IV collagen than to fibrillar collagens. It is therefore possible that cells

need distinct collagen receptors because of their differences in the recognition of various collagen types. More information is needed before this conclusion can be made.

Independent Regulation of Collagen Receptor Integrin Genes

Cellular integrin pattern changes during physiological and pathological conditions, such as cell differentiation[37,38], wound healing[39], and chronic and acute inflammation[40]. This is at least partly due to the action of different growth factors and cytokines.

The effect of growth factors on collagen receptor expression has been studied in different cell culture models. Integrin subunits α2 and β1 are upregulated by transforming growth factor-β (TGF-β) in osteogenic sarcoma cells and human lung fibroblasts[41,42]. In fibroblasts the expression of integrin α1 is enhanced by TGF-β, as well[43]. TGF-β may enhance tumorigenesis with concomitant increase in collagen receptor integrins[44]. TGF-β also induces the reorganization of collagen fibrils around cells, seen as contraction of floating collagen gels. This is due the increased expression of α2β1 integrins (45, see below).

Bone morphogenetic proteins (BMPs) are a group of peptide growth factors which are closely related to TGFs-β. Seven BMPs have been isolated from vertebrates, namely BMP-2-8[46,47]. BMPs are in addition to being initiators of cartilage and bone formation, also morphogens in other tissues. BMP-2 reduces the expression of integrin α2 subunit in a human keratinocyte cell line but has no effect on the α2 integrin levels in osteosarcoma cells, chondrocytes or fibroblasts[48]. In all these cell types BMP-2 reduces the expression level of α3 integrin[48].

Epidermal growth factor (EGF) and transforming growth factor-α (TGF-α) belong to the same family of growth factors[49]. TGF-α and EGF stimulate cell proliferation and angiogenesis. TGF-α is also involved in tumorigenesis[50]. It is expressed in pituitary gland, brain, skin and macrophages[51]. TGF-α induces the expression of α2, α3, and β1 integrin subunits in colon carcinoma cells[52]. Integrin subunit α2 is also upregulated in keratinocytes by TGF-α and EGF[53]. In fibroblasts EGF induces the expression of β1 integrin[54] and in epidermoid and squamous carcinoma cells it upregulates integrin α2 expression[55,56].

Fibroblast growth factor family consists of at least nine members, of which acidic fibroblast growth factor (αFGF) and basic fibroblast growth factor (βFGF) are known to regulate the expression of integrin subunits. FGFs are involved in chemotaxis, cell mitosis, neurite growth, and angiogenesis. The role of FGFs in angiogenesis can be at least partially explained by integrin upregulation. Basic FGF induces the expression of α2β1 integrin in human endothelial cells[57]. Acidic FGF upregulates the α1β1 integrin expression in PC12 cells[58].

Platelet-derived growth factor (PDGF) is a polypeptide growth factor, involved in inflammation, wound healing, atherosclerosis, and tumorigenesis[59]. PDGF increases the synthesis of α2 integrin subunit which leads to increased collagen gel contraction by fibroblasts[60]. Recently it has been shown that PDGF upregulates the expression of α2 integrin only when fibroblasts are adhered to collagen[61].

Tumor necrosis factor-α (TNF-α) is a cytokine which is mainly expressed by monocytic cells, but also by B and T lymphocytes, natural killer cells, glial cells, and adipocytes[62,63]. TNF-α affects cell growth, adhesion, and differentiation. TNF-α upregulates α1 integrin in osteogenic sarcoma cells, skin fibroblasts, melanoma cells, endothelial cells, and in normal synoviocytes[64-67]. It also upregulates the expression of α2 integrin in epidermal keratinocytes[68].

Interferon-γ (IFN-γ) regulates MHC class I and II protein expression on the cell surface of immunosystem cells. It also functions in the induction of cell surface receptors, integrins and ICAM-1. Interferon-γ is produced by T lymphocytes and natural killer cells. The production of interferon-γ is regulated by other cytokines, TNF-α[69] and interleukin-2 (IL-2)[70]. Interferon-γ upregulates the expression of α1 integrin in human synoviocytes[71].

All these examples show that the genes of the collagen binding integrin α subunits are regulated independently. However, very little is known about the molecular details of integrin gene regulation in different conditions. The 5'-flanking region of α2 integrin subunit gene has been cloned[72]. This 961-base pair fragment lacks TATA and CAAT boxes but contains six Sp1 binding sites. Consensus binding sites for AP-1 and AP-2 complexes, a GATA box, Pu.1 box, and two palindromic motifs with the potency to bind the estrogen receptor are also present.

It has been shown that nuclear factor-κB (NF-κB) is a potential regulator of α2 integrin subunit. Normal adult human dermal fibroblasts grown in a three-dimensional collagen lattice increase mRNA level of α2 integrin[61]. Collagen gel also elevates DNA binding activity of NF-κB and PKC-ζ activation precedes this[73]. Collagen lattice induces the nuclear translocation of p50, one member of NF-κB family, and the degradation of NF-κB inhibitor protein, I-κB-α. A region located between -549 and -351 bp in the promoter of integrin α 2 gene confers the inducibility by three-dimensional collagen lattice[74].

There are two Sp1 binding sites in the promoter region of α2 integrin gene. These sites and a potential AP-2 motif are located at the core region of the α2 integrin promoter (between -30 and -92 bp). This region plays essential role in α2 integrin promoter activity[75]. It has been shown that both Sp1 sites are required for full promoter activity and for DNA-protein complex formation[76].

The regulation of α1 integrin subunit gene at transcriptional level has been studied in chicken smooth muscle cells. Integrin α1 expression was down-regulated during serum-induced dedifferentiation. Promoter analyses have revealed that the 5'-upstream region, -516 to +281, is sufficient for transcriptional activation in differentiated smooth muscle cells or chick embryo fibroblasts. The promoter region of integrin α1 gene, like α2 and other α integrin promoters, lacks TATA and CCAAT boxes and contains putative binding sites for AP1 and AP2. It differs from other α integrin subunit promoters in that the regulatory element of α1 integrin gene contains CArG box-like motif. In differentiating smooth muscle cells this is an essential cis-element for transcriptional activation, unlike the binding sites for AP1 and AP2[77].

Integrins α1β1 And α2β1 Have Distinct Cellular Functions

Integrin α2β1 is the prominent collagen receptor on platelets and epithelial cells, whereas smooth muscle cells seem to use α1β1 in collagen binding. Many cell types express concomitantly the both collagen receptors and several *in vitro* assays indicate that they might have distinct functions.

The function of collagen binding integrins *in vitro* has been studied by assessing the ability of cell cultures to bind to different matrices. In recent years the conventional adhesion assays have been somewhat replaced with a collagen gel contraction assay which not only measures the ability of cells to cope with a three dimensional collagen environment but also the ability of the integrins expressed on the cell surface to function as organizers of the matrix. Two different types of collagen gels are commonly used as a model for matrix reorganization[78]. The gels in both experimental models are prepared from soluble type I collagen preparation which occasionally includes traces of other types of collagens as well. The cells studied are added in the neutralized solution of collagen which initially, when placed in 37°C, gelates and traps the cells inside a three dimensional fibrillar collagen network. When the gels are detached from the sides of the cell culture wells and growth media is added, the gel starts to shrink in size. As the cells are adhering to the proximal collagen fibers trying to migrate on them the areas of the gels are diminished and the collagen fibers form a dense tissue like structure[79a,80a]. When fibroblasts are used this type of floating collagen gel is thought to mimic the formation of dermis, whereas the anchored type in which the gel is attached to the cell culture plastic, resembles scar formation[78]. Type I collagen is a major constituent in bone matrix and therefore in addition of wound healing the collagen contraction assay may be used as a model of bone fracture healing. Addition of different growth factors such as TGF-β and PDGF increases the ability of many cell types to contract collagen gels[81a, 82a].

Osteogenic cells being *in vivo* surrounded by type I collagen make a useful tool to study cell-collagen interaction. Addition of TGF-β, which may also be involved in the healing processes of bone matrix, to these cells, increases the magnitude of collagen gel contraction[45]. Among other things TGF-β is a potent regulator of integrin expression[83a,42]. Indeed, to be able to contract the collagen gels the cells need to have a proper collagen binding integrin pattern on their surface[45], an intact cytoskeleton[78] and also the presence of serum is required[78].

Studies using antibodies against collagen binding integrins and transfections with sense and antisense integrin cDNA constructs have shown the relevance of the α2β1 integrin as a reorganizer of the collagen matrix[79b,45]. Overexpression of this collagen receptor on the cell surface enhances contraction and downregulation of α2β1 with antisense strategies abrogates contraction[45]. The experiments suggest that α2β1 integrin may participate in physiological phenomena, such as scar contraction. There are however few reports that demonstrate that the role of α1β1 integrin is essential in the contraction process with smooth muscle cells[80b] and liver myofibroblasts[81b]. Either of the cell types do not express α2β1 integrin *in vivo*.

With many cell types the collagen α1(I) mRNA levels are reduced inside collagen gels when compared to monolayer cultures[82b], whereas the expression of matrix metalloproteinase-1 (MMP-1) mRNA is induced[78,83b,84]. The cells seem to sense that no excess collagen is required rather a proteinase is perhaps useful in the context of reorganizing the matrix. Also these phenomena are regulated by collagen binding integrins. Studies on α2β1 integrin have shown that not only does it act as a collagen receptor but also as a sensor of the environment signaling to the cell interior and triggering the expression of MMP-1[83b, 84]. On the other hand there is evidence that the downregulation of collagen inside collagen gels is mediated through α1β1 integrin[83b, 84]. Thus it is becoming clear now that the two collagen binding integrins α1β1 and α2β1 have clearly distinct missions (Fig. 2).

The ability of cells to move on collagen is important during development, tissue healing and also in invasion. The migration rate of cells can be altered by changing the expression pattern of integrins on the cell surface[85]. The migration of osteogenic cells as well as the invasion of them through collagen gels can be enhanced by overexpressing α2β1 integrin on their surface[85]. Similarly the movement of keratinocytes and melanoma cells on collagen is dependent on the presence of α2β1 integrin[53,86,87].

Three-dimensional collagen network has for long been used as a growth environment which supports the formation of differentiated structures such as glands by colon carcinoma cells [88] or duct like structures by mammary tumor cells[89]. First clues from the involvement of integrins came from studies in

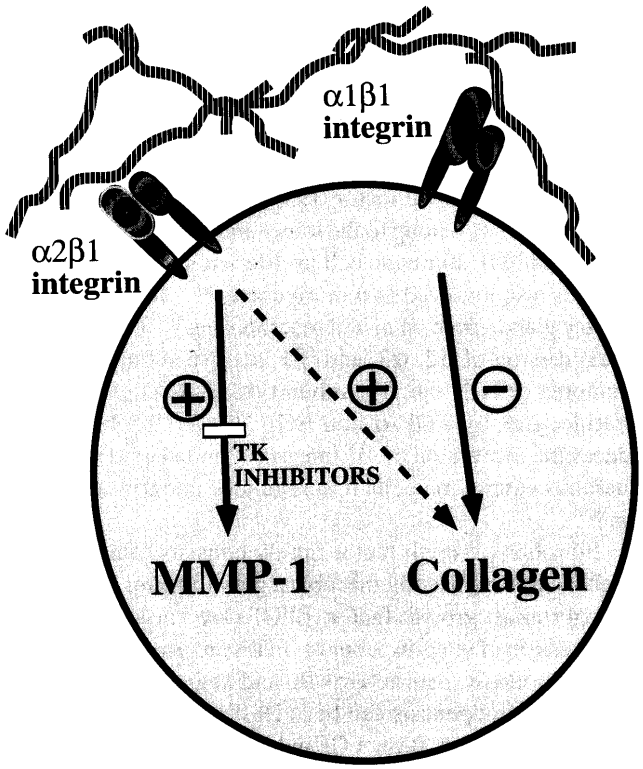

Fig. 2. Both α1β1 and α2β1 collagen receptor integrins seem to be involved in the regulation of collagen accumulation but by different mechanisms. Integrin α1β1 seems to be a negative feed-back regulator of collagen synthesis, whereas α2β1 integrin induces collagenase-1 (MMP-1) gene expression. In the absence of α1β1 integrin α2β1 might even enhance the collagen synthesis.

which the RGD peptide could partially block the differentiation of colorectal tumor cells grown inside collagen gel[90,91]. Later on function blocking antibody experiments on capillary forming endothelial cells[92] and colon carcinoma cells[93] showed that α2β1 integrin function is crucial for the differentiation of these cells to form three dimensional functional structures.

The Two Collagen Receptor Integrins May Be Connected to Distinct Signaling Pathways

Integrin-mediated anchoring to the components of the extracellular matrix has an essential role in regulating gene expression, cell differentiation, survival and proliferation. This is due to the fact that integrins are not only responsible for mediating cell adhesion but they also activate intracellular signaling pathways. Many of the pathways previously described to be involved in growth factor and oncogene signal transduction have now been shown to be utilized by integrins too. In addition to activating intracellular signaling integrins also have dramatic effects on the actin-containing cytoskeleton. The Figure 3 shows some of the known principles of integrin signaling.

The initiation of integrin signaling involves binding of the extracellular ligand to the integrin. This is believed to result in conformational changes in integrins observed in both the globular head region and the membrane proximal regions of the β subunit[94,95]. However, one must remember that in cultured cells the integrins do not function as isolated signal transducing receptors but are found in a complex structure termed focal adhesion. These structures involve both structural proteins and signaling molecules. The structural components include proteins that are able to directly bind to integrins, namely talin and α-actinin[96,97,98] and bind other structural proteins like vinculin, paxillin and tensin leading to reorganization of actin filaments. The signaling components involve a number of kinases and transmembrane adaptor molecules (Fig. 3).

The clustering of integrins in response to ligand molecule binding leads to increased tyrosine phosphorylation of several proteins[99,100]. Today these events have been intensively studied and numerous participating molecules have been identified. Integrin signaling induces the activation of many membrane-proximal kinases that interact either directly with the cytoplasmic domain of the β subunit[101,102] or indirectly via other proteins found in focal adhesions. Focal adhesion kinase (FAK) is a nonreceptor protein-tyrosine kinase that unlike other cytoplasmic tyrosine kinases is devoid of the Src homology (SH)2 or SH3 domains. It contains a central tyrosine kinase domain flanked by amino-terminal and carboxy-terminal domains. Activated FAK is autophosphorylated at tyrosine residue 397 which creates a binding site for the SH2 domain of the Src family kinases Src or Fyn[103]. Src and Fyn can phosphorylate paxillin and tensin, which are involved in the regulation of focal adhesions and also the multidomain docking protein Cas[104]. Once phosphorylated Cas may interact with adaptor proteins Crk and

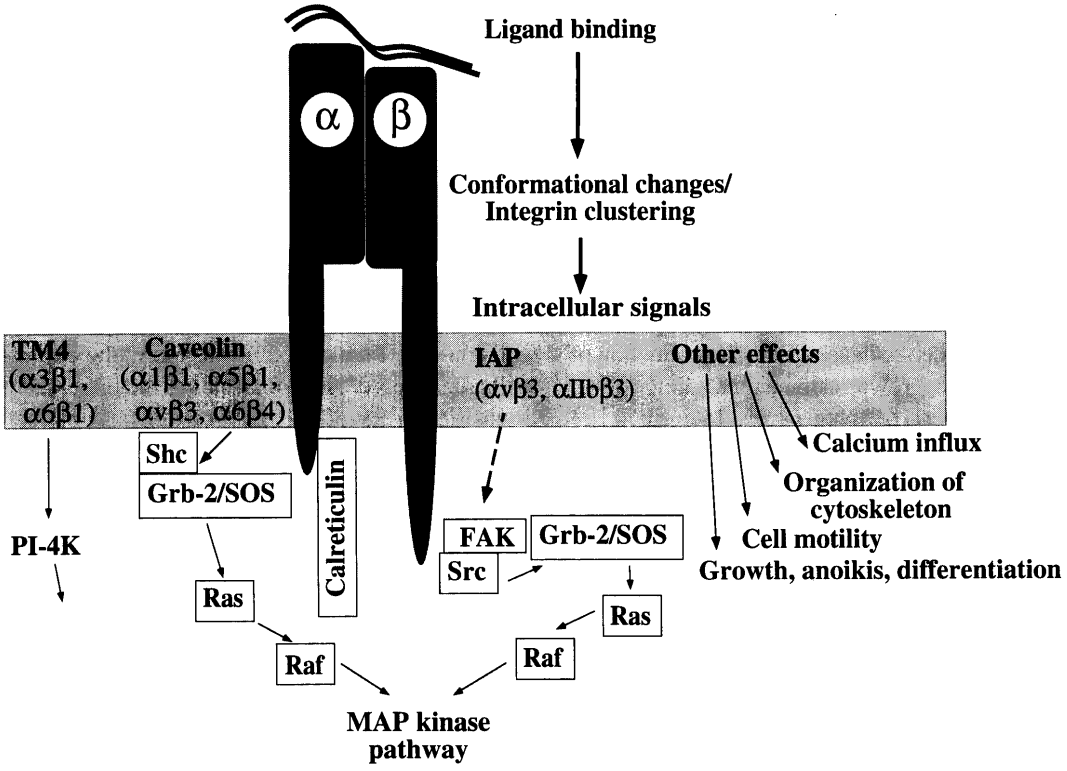

Fig. 3. Overview of integrin signaling. Integrin ligation results in outside-in integrin signaling where the binding of the extracellular matrix ligand triggers conformational changes and clustering of the receptor. Different integrin subunits bind to either transmembrane adaptor proteins that in turn transmit the signal to membrane proximal kinases involved in signal transduction or interact directly with these kinases via their cytoplasmic tails. Calreticulin is a calcium regulatory protein shown to bind the conserved sequence GFFKR found in all α subunits. IAP (integrin associated protein) has been shown to bind to some β subunits and to activate FAK (focal adhesion kinase). See the text for discussion of the other pathways shown in this figure.

Nck and therefore also regulate the MAP kinase pathway[105]. Binding of the Src family kinases to Tyr397 leads to further phosphorylation of FAK at Tyr925 which leads to recruitment of Grb2/Sos adaptor protein-exchange factor complex[106]. Finally phosphorylated FAK can associate with and activate phospho-inositide 3-kinase (PI-3K)[107].

The biological role of FAK remains some what unclear. Since activation of FAK involves the recruitment of the Grb2/Sos complex and integrin mediated adhesion triggers the activation of MAP kinases[108,109] it can well be that FAK activates Ras and downstream pathways. However recent data has provided evidence of MAP kinase activation in response to integrin ligation that is independent of FAK[110]. FAK activation has been shown to be important in controlling anchorage-dependent cell survival[111] and cell motility[112].

It is now generally accepted that integrin ligation leads to activation of ERK via Ras signaling[113] and something is also known about the mechanisms by which some integrins activate Ras. Integrins α6β4, α1β1, α5β1, and αvβ3 are able to recruit adaptor protein Shc either directly[114] or in an indirect manner involving transmembrane adaptor proteins, possibly caveolin[110]. Other integrins, like α2β1, α3β1, α6β1, appear to be unable to activate Ras. This Ras signaling pathway involves the downstream kinase Raf which in turn activates the MAP kinase cascade. While FAK does not seem to be required for Ras activation it can activate PI-3K which is necessary for activation of Raf by Ras in response to integrin ligation[115]. The activation of the MAP kinase cascade in response to cell adhesion has been shown to be important in a number of cellular processes, such as the control of cell cycle progression[110], cell migration[116] and gene transcription[117].

Ligation of different integrins sharing a common β subunit but having different α subunits seem to have different effects on cell behavior[83,118]. An important and still unanswered question is how is this specificity in signaling established? A tempting explanation would be the specific interactions of some integrins with transmembrane adaptor proteins[110,119,120]. The collagen binding integrins α1β1 and α2β1 have different effects on gene expression. Integrin α2β1 regulates the level of matrix metalloproteinase 1 (MMP-1) expression in osteogenic cells while the decrease in the production of type I collagen, seen in cells grown inside a three-dimensional collagen gel, is mediated by α1β1 integrin[83,84]. Integrin α1β1 is able to activate Ras by binding to transmembrane adaptor protein caveolin that in response to adhesion recruits Shc whereas α2β1 integrin seems to unable to interact with caveolin. It is not known whether this explains the different signaling through these receptors. In addition the third collagen binding integrin, α3β1, is also unable to interact with caveolin but instead has been shown to bind to tetraspan transmembrane proteins CD63 and CD81. These proteins associate with phosphatidylinositol 4-kinase (PI-4K) and may therefore control the first step in the biosynthesis of PIP2 in response to cell adhesion[121].

Two recently published papers indicate that in addition to integrins two orphan-type receptors, namely discoidin domain receptors 1 and 2 (DDR1 and DDR2) bind to collagen[122,123]. Although it is not probable that these receptors mediate actual cell attachment to collagen they may have an important contribution to collagen-related signaling in cells.

Collagen Receptors in Cancer

Malignant transformation can affect the cell surface integrin expression, including the expression of collagen receptors. Chen *et al.*[124] have shown that a single biopsy of a human lung tumor consists of cell populations with different integrin patterns, but those showing high α1β1 and α2β1expression levels also display the most effective metastatic capacity *in vivo* in scid mice. The expression of α1β1 integrin can be upregulated in Ewing's sarcoma[125], in neuroblastoma[126], and in melanoma cells[65]. However, in most cases the expression levels of α1β1 integrin are unaltered after malignant transformation. Recently it has been suggested that the interplay of α1β1 and TNF-α mediated signaling is associated to the favourable diagnosis of IFN-α treated melanoma patients[127].

The role of α2β1 integrin in cancer initiation can be bipolar depending on the type of tissue. In some tissues, as in normal mammary gland cells, the function of α2β1 can be in the maintenance of the normal tissue structure. Poorly differentiated mammary carcinoma cells show restored ability to differentiate into gland-like structures and reduced tumorigenicity when made to express α2β1 integrin[128]. However, the increased expression of α2β1 integrin often correlates with the malignant behavior of cells. For example, melanoma cells use both α1β1 and α2β1 integrins as collagen receptors[129], but the expression of α2β1 integrin can be associated to melanoma tumor progression[130,131]. The increased expression of α2β1 integrin mediates reorganization of collagen fibrils by aggressive melanoma cells[79] and leads to enhanced migration of squamous carcinoma cells on type I collagen[56]. Chan *et al.*[132] have shown that the expression of α2β1 integrin in human rhabdomyosarcoma cells enhances experimental metastasis, without affecting the tumor growth rate. In human osteosarcoma cells the expression of α2 integrin is induced by malignant transformation[133]. In another osteosarcoma cell line the expression of α2 integrin could be induced by tumor promoters, namely 12-*o*-tetradecanoylphorbol 13-acetate (TPA) and okadaic acid[134]. TPA is an activator of protein kinase C and okadaic acid is an inhibitor of serine/threonine protein phosphatases. TPA also induces the spreading and adhesion of these cells on type I collagen. Furthermore, the expression of α2β1 collagen receptor is responsible for the increased migration and invasion ability of malignant osteosarcoma cells in *in vitro* model[85]. In some other experimental models the role of α2β1 integrin has also been discussed. Lang *et al.*[135] have shown that epithelial cells derived from malignant prostate tissue adhere to bone marrow via α2β1 integrin. This suggests a role for α2β1integrin in the formation of bone metastasis. The preferential adhesion of epithelial ovarian carcinoma cells to type I collagen is mediated by α2β1 - a phenomenon which can be important in intraperitoneal dissemination of ovarian carcinoma[136].

Conclusions

The two major cellular collagen receptors, integrins α1β1 and α2β1, share many structural similarities, but still many cell types seem to express both receptors on their surfaces. The existence of two distinct collagen receptors may be required

for several reasons. The present knowledge suggests that α1β1 and α2β1 integrins differ at least in three points: 1) in their ability to recognize distinct collagen types, 2) in their ability to activate signal transduction pathways inside cells, and 3) in the regulation of their gene expression.

The fact that mice deficient of α1 integrin subunit are viable suggests that α1 integrin is not required for normal development[137]. These animals will however provide an excellent model for future studies on α1 integrin function in different tissues and organs.

Integrin α2β1 seems to participate in a large variety of cellular functions. It may be an important receptor in phenomena such as collagen-induced platelet aggregation, wound healing, organ formation and cancer invasion. In the lack of a transgenic mouse model it is not possible to speculate in which of the phenomena other adhesion receptors may be able to replace it and whether there are cellular functions in which α2β1 integrin is absolutely required.

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