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Review

Mechanisms and Consequences of Integrin Mediated Cell Adhesion to Collagen

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Abstract: The four collagen receptor integrins are structurally and functionally distinct when compared to other members of the integrin family. The collagen receptors have partially overlapping expression pattern but they have differences in their ability to recognize collagen subtypes and also in their signaling function. The collagen receptor knockout mice have been used to confirm the *in vivo* relevance of some of the cell culture observations.

Key words: integrin, collagen, signaling

The Collagen Receptor Integrins Are Widely Expressed in Human Tissues

The integrins are a large family of cell surface receptors, which mediate cell adhesion to extracellular matrix. They are composed of one α and one β subunit that form a noncovalently bound dimer. In man there are eight β and eighteen α subunits that can form 24 different combinations. Integrins can be divided into three subcategories, namely (i) fibronectin and vitronectin receptors, which recognize an RGD-motif, or other similar short motifs, in their ligands, (ii) laminin receptors, and (iii) integrins that have a special inserted-domain (I-domain) in their α subunit (Fig. 1). While the two first integrin subgroups are expressed in all Metazoans, the I-domain integrins have only been found in Chordates (includes vertebrates), but not in Nematodes or Arthropods[1]. Four out of nine I-domain integrins, namely $\alpha1\beta1$, $\alpha2\beta1$, $\alpha10\beta1$ and $\alpha11\beta1$, are collagen receptors[2-5]. Collagens are the most abundant extracellular matrix proteins. Twenty-seven collagen subtypes (types I-XXVII) are known at the moment[6-10]. The collagens can be grouped into subclasses according to their structural details. Many collagen subtypes (namely I, II, III, V, and XI) have a long continuous triple-helix and they can form large fibrils. In other collagens the triple helix has interruptions. Some collagens form networks (types IV, VIII, X) or beaded filaments (type VI). Other collagen subclasses include: the fibril-associated collagens with short interruptions in triple helices (FACIT or FACIT-like collagens, types IX, XII, XIV, XVI, XIX), anchoring fibrils forming collagens (type VII), and transmembrane collagens (XIII, XVII, XXIII, XXV). Collagen types XV and XVIII are found in association to basement membranes and they are called the Multiplexins. Collagen receptor integrins can mediate cell adhesion to different collagen subtypes. However, they only bind to specific recognition sites and the four receptors shown different binding preferences (see the next chapter and Fig. 2).

In addition to collagens the collagen receptor integrins can recognize certain other extracellular matrix and basement membrane proteins, such as laminins[11] and tenascins[12]. In man all four collagen receptor integrins have different expression pattern. Integrin $\alpha1\beta1$ is expressed in many mesenchymal cell types, including smooth muscle cells, endothelial cells, fibroblasts and chondrocytes[13]. It is also found on certain lymphocytes and monocytes. Based on information from $\alpha1$ deficient knockout mice[14], $\alpha1\beta1$ integrin is not critical for normal development, but it may participate in the regulation of cancer-related angiogenesis[15], fibrosis[16], chronic inflammation[17-19], and bone fracture healing[20]. Integrin $\alpha2\beta1$ is expressed on epithelial cells, platelets, endothelial cells, fibroblasts, chondrocytes[21], lymphocytes, mast cells[22], and neutrophilic granulocytes[23]. Integrin $\alpha2\beta1$ deficient knockout animals are viable, but their platelets do not react to stimulation with collagen[24,25]. In animal models $\alpha2\beta1$ also seems to participate in cancer-related angiogenesis[26,27] and chronic inflammation[7]. Epidemiological studies have indicated that in man high level of $\alpha2\beta1$ integrin on platelet surface is a risk factor for cerebrovascular stroke and myocardial infarction[28,29]. Both $\alpha1\beta1$ and $\alpha2\beta1$ are expressed on variable cancer cell types. Integrin $\alpha2\beta1$ has been connected to invasion and progression of melanoma[30], ovarian cancer[31], prostate cancer[32], and gastric cancer[33]. Integrins $\alpha10\beta1$ and $\alpha11\beta1$ have been found quite recently and little is known

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about their biological function. Their expression patterns suggest that they may participate in the metabolism of bone and cartilage[5,34].

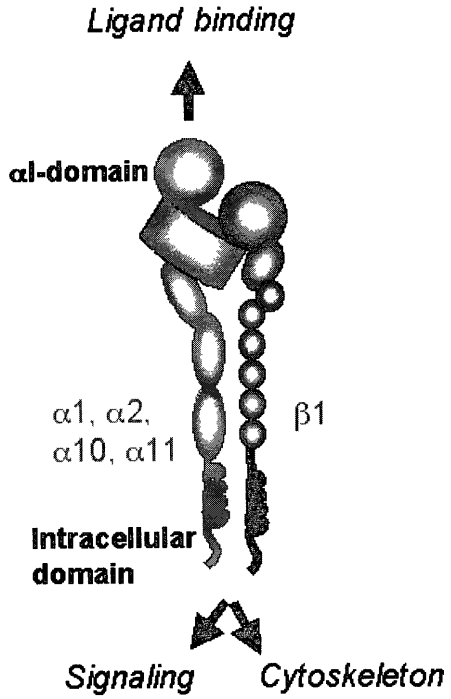

Fig. 1. The αI-domains mediate the binding of the collagen receptor integrins to their ligands.
The four collagen-binding αI-domains are structurally similar, but small structural differences may explain their distinct ability to recognize collagen subtypes. The short intracellular domains of the α subunits have no similarities, suggesting that they may have distinct signaling function.

An Inserted I-domain in the α Subunit Mediates Collagen Binding

The collagen receptor integrins use their αI-domains in ligand recognition and binding (Fig. 1). The αI-domain is built up of β-sheets surrounded by amphipathic α-helices[35]. I-domain is homologous to the A-domain found in von Willebrand factor and also in cartilage matrix protein, in some collagen subtypes, and in the components of the complement system. The A- or I-domains are commonly involved in molecular interactions and they are responsible for the collagen binding activity of von Willebrand factor and collagen receptor integrins[36-38]. Human recombinant αI-domains have been used to analyze to molecular details of the bind mechanism[39]. The top of the I-domain, where a ligand binds, resides a metal ion in a conserved coordination site called the MIDAS (metal ion-dependent adhesion site)[40]. Five amino acid side chains bind the magnesium ion and a glutamate from a collagenous ligand may complete the coordination. The MIDAS is centered on a groove restricted on one side by a helix called αC-helix. This αC-helix can only be found in collagen binding integrin αI-domains. Ligand binding induces a conformational change in the α2I-domain resulting in unwinding of the αC-helix and opening of the binding site[39]. Recombinant α2I-domain missing the αC-helix has been shown to have altered kinetics in the binding to type I collagen[41].

In all four collagen binding αI-domains (termed as α1I, α2I, α10I, α11I) the basic structure is very similar[42]. However, αI-domain binding assays have indicated that their ligand binding mechanisms and, for example, their ability to bind to different collagen subtypes is

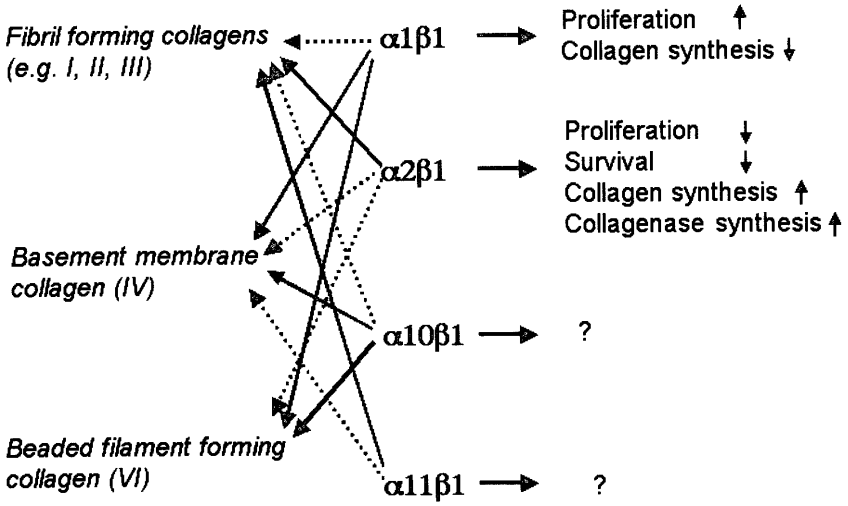

Fig. 2. Integrins α1β1 and α10β1 prefer basement membrane (type IV) and beaded filament forming (type VI) collagens when compared to fibril forming collagens.
Integrins α2β1 and α11β1 prefer fibril forming collagens. In fibroblasts that are cultured inside three dimensional collagen gels the signaling functions of α1β1 and α2β1 seem to be opposite. Note that in other cell type and in cells that are in contact with non-fibrillar collagen the consequences of integrin signaling might be different (see the text for more details and for the references).

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different[42-44] (Fig. 2). The best characterized difference between $\alpha 1\beta 1$ and $\alpha 2\beta 1$ is that $\alpha 1\beta 1$ prefers basement membrane type IV collagen over fibril forming collagens, whereas the preference of $\alpha 2\beta 1$ integrin is opposite[45]. In addition, $\alpha 1\beta 1$ is a receptor for beaded filaments forming type VI collagen[42,46] and transmembrane type XIII collagen[43]. The collagen binding pattern of $\alpha 10$I-domain is similar to $\alpha 1$I-domain, but differences between different collagen subtypes are smaller than in the case of $\alpha 1$I-domain[42]. The ligand binding pattern of $\alpha 11$I-domain resembles $\alpha 2$I-domain[44]. All collagen subtypes may not be ligands for the collagen receptor integrins, since the largest collagenous domain (COL15) of transmembrane type XVII collagen can not be recognized by them[47].

The first high affinity integrin binding site in type I collagen was found to be a triple helix formed by GFOGER (O is hydroxyproline) sequence[48,49] or other very similar sequences. Recent studies have also suggested that $\alpha 1\beta 1$ may recognize the same sites in type I collagen as $\alpha 2\beta 1$[50]. It remains to be shown, whether other collagen subtypes have unrelated binding sites. This is possible, because collagen receptor integrins have significant differences in the recognition of different collagen subtypes.

Recently, we have analyzed the ligand binding patterns of the collagen receptors in correlation to their atomic structures and based on the experiments with mutated αI domains we have suggested that D219 in the $\alpha 2$I-domain and the corresponding amino acid R218 in $\alpha 1$I-and $\alpha 10$I-domain is one of the critical residues in the determination of the binding specificity[42]. These amino acids are located on the known collagen binding surface of the αI-domain, close to the MIDAS.

The Collagen Receptor Integrins Have Distinct Signaling Functions

Due to the fact that the short intracellular domains of the collagen binding α subunits are very different, it has been presumed that the four receptors may have distinct signaling functions after ligand binding (Fig. 1). This has been evident in the case of $\alpha 1\beta 1$ and $\alpha 2\beta 1$ integrins, that often have opposite effects on cells (Fig. 2). The mechanism of signaling through the collagen receptors has been studied by deletions, mutations and swaps of the $\alpha 2$ subunit cytoplasmic domain[51-54]. These observations suggest that the cytoplasmic domains of the collagen receptor α subunits regulate the function of cellular signaling pathways.

The analysis of $\alpha 1$ integrin deficient knockout mice[14] has suggested that $\alpha 1$ might promote cell proliferation; since the dermis of the animals seems to be hypocellular[55] and their bone marrow-derived mesenchymal stem cells proliferate more slowly than those of control animals[20]. This can be explained by the fact that $\alpha 1\beta 1$ is among the integrins that can activate Shc in a process requiring caveolin-1 and Fyn[55,56]. Shc activation subsequently leads to activation of Ras and the growth promoting mitogen activated protein kinases (MAPKs), such as extracellular signal regulated kinase (ERK). The $\alpha 1$ null mice may also have defects in the regulation of the collagen gene expression. The collagen synthesis in enhanced in their dermis while a simultaneous increase in matrix metalloproteinase (MMP) expression prevents the accumulation of collagen[16]. Based on cell culture experiments, several lines of evidence support the idea that $\alpha 1\beta 1$ integrin is a negative regulator of collagen synthesis, especially in cells that are surrounded by a three-dimensional collagenous matrix. Early observations using osteosarcoma cells with a low expression level of $\alpha 1\beta 1$ indicated that these cells do not down-regulate collagen synthesis inside collagen[57]. This was later confirmed by experiments performed with cells derived from $\alpha 1$ integrin deficient knockout animals[16]. Furthermore, experiments utilizing functional anti-integrin antibodies have led to the same conclusion[58].

The deficiency of $\alpha 2$ integrin in mice has no obvious effects on development or viability[24,25]. In addition to the inability of their platelets to bind collagen[25], mammary gland branching morphogenesis is slightly affected[24]. In cell culture experiments the overexpression of $\alpha 2\beta 1$ integrin enhances collagen production. Increased collagen synthesis can be prevented by selective inhibitors of the α isoform of p38 MAPK, which is known to be activated by $\alpha 2\beta 1$ after contact which collagen[51]. The activation of the p38 pathway seems to be one of the most pronounced events observed after $\alpha 2\beta 1$-collagen interaction since it has been observed to take place in several different cell lines and experiment models[51,59-61]. Cell contact to three-dimensional collagen activates the expression of collagenase-1 (matrix metalloproteinase-1, MMP-1), as well. Other MMPs regulated by either $\alpha 1\beta 1$ or $\alpha 2\beta 1$ include stromalysin-1 (MMP-3)[62] and collagenase-3 (MMP-13)[61]. In skin fibroblasts that are inside collagen, collagenase-1 (MMP-1) expression seems to be activated by a pathway involving protein kinase (PK) C-ζ and nuclear factor (NF) KB[63,64], but the p38 pathway may also participate in the process[60]. The p38 pathway seems to mediate the upregulation of MMP-13 by $\alpha 2\beta 1$ integrin[61]. Other matrix genes activated by $\alpha 2\beta 1$ integrin include osteocalcin and bone sialoprotein[65]. The activation of bone-related genes has been linked to $\alpha 2\beta 1$-dependent activation of Osf2 transcription factor in osteoblasts[65].

In mesenchymal cells, including smooth muscle cells and fibroblasts, fibrillar collagen seems to prevent proliferation[66,67]. Similar results have been reported with melanoma cells[68]. In fibroblasts growth arrest may require that the cells are inside floating and contracting collagen gels[67]. In smooth muscle and melanoma cells as well as in fibroblasts, growth arrest has been connected to $\alpha 2\beta 1$ function and the accumulation of cyclin/cdk inhibitor, $p27^{kip}$ in the cells[66-68]. In murine mammary gland-derived epithelial cells, $\alpha 2\beta 1$ has been reported to increase proliferation, when tested in monolayer cultures on non-fibrillar collagen[69]. Indeed, the organization of the collagenous matrix maybe critical for the action of $\alpha 2\beta 1$. It has been speculated that clustering of $\alpha 2\beta 1$ by antibodies or non-fibrillar collagen may actually pro-

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mote proliferation, while fibrillar collagen prevents $\alpha2\beta1$ clustering and therefore inhibits proliferation[68].

Release of mechanical tension in a three-dimensional collagen gel model triggers apoptosis in fibroblasts[70-72]. In these conditions $\alpha2\beta1$ mediates cell adhesion to collagen and is essential for contraction. However, a large number of studies indicate that $\alpha2\beta1$ can actually protect cells from apoptosis[73-76]. As in the case of $\alpha2\beta1$-related regulation of cell proliferation the results might reflect altered $\alpha2\beta1$ function when the receptor is bound to monomeric instead of fibrillar collagen. Apoptosis may be regulated by the ERK pathway and Akt/Protein kinase B (PKB). In fibroblasts and osteosarcoma cells, protein phosphatase 2A is activated in a process that requires the presence of $\alpha2$ cytoplasmic domain and Cdc42 activity[77]. Activation of PP2A leads consequently to dephosphorylation of Akt/PKB[77], a well-known promoter of cell survival.

It is possible to speculate that the opposite effects of $\alpha1\beta1$ and $\alpha2\beta1$ on many cellular functions may partially be due to the alterations in the balance between p38 and ERK pathways. It is not known, whether $\alpha10\beta1$ or $\alpha11\beta1$ binding to collagen leads to alterations in cell behavior.

Fibroblast Integrins $\alpha1\beta1$ and $\alpha2\beta1$ in Would Healing —A Hypothetical Model

Based on the data reviewed in this paper it is possible to propose a model of collagen receptor action during wound healing, in which the expression of $\alpha1\beta1$ and $\alpha2\beta1$ integrins is differentially regulated. Fibroblasts that first migrate to the site of injury express $\alpha1\beta1$[78], while later the situation is reversed by the induction of $\alpha2\beta1$ and the down-regulation of $\alpha1\beta1$[78,79]. The expression of $\alpha1\beta1$ and $\alpha2\beta1$ integrins is regulated by growth factors, such as transforming growth factor-β[80] and platelet-derived growth factor[79]. Published studies suggest that cells binding to collagenous matrix with $\alpha1\beta1$ express only low levels of type I collagen[16,58,81] and have the potency to proliferate[55]. The induction of $\alpha2\beta1$ expression may change the cell behaviour. Integrin $\alpha2\beta1$ has the potency to block proliferation[66], to induce collagen synthesis[57,58] and to activate matrix metalloproteinases, which may help matrix remodeling[57,61]. Subsequently, $\alpha2\beta1$ integrin may promote matrix contraction[81,82]. When both $\alpha1\beta1$ and $\alpha2\beta1$ are present at the same time, like on primary skin fibroblasts, $\alpha2\beta1$-related signals on growth regulation seem to dominate, since integrin $\alpha2\beta1$ has better affinity to type I collagen than $\alpha1\beta1$. Finally, $\alpha2\beta1$ may promote the apoptosis[71-73] seen in scar fibroblasts[83]. In $\alpha1$ deficient mice, wound healing is not delayed, indicating that other mechanisms, such as growth factor action, can compensate the lack of $\alpha1$ function in the regulation of cell proliferation[16]. No defects in the would healing have been found in $\alpha2$ integrin knockouts[24]. Therefore, it is probable that collagen receptors support, but not dictate the progression of wound healing.

In general the analysis of cellular signaling *via* the collagen receptors has revealed their interaction with many distinct signaling pathways. In some cases it has been possible to confirm the relevance of the observations *in vivo*. However, in many tissues the biological function of the collagen receptor integrins is still unknown.

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