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Adhesion receptors and cell invasion: mechanisms of integrin-guided degradation of extracellular matrix

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Abstract. The integrins are a large family of heterodimeric cell adhesion receptors mediating cell-matrix and cell-cell adhesion. They seem to play a central role in cell migration and invasion and are therefore essential in processes such as healing of tissue injuries and the progression of human cancer. Integrins function in cell invasion by mediating cell movement on matrix molecules and also by regulating the expression of matrix-degrading enzymes, namely the matrix metalloproteinases. Here we review recent findings on the mechanisms by which integrins regulate matrix degradation. A novel, multistep model of integrin-guided collagen degradation is proposed.

Key words. Integrins; cell adhesion; signaling; matrix metalloproteinases; cell invasion; collagen.

The integrins are a large family of cell adhesion receptors, which mediate cell-matrix and cell-cell adhesion. Their functions have been implicated in processes like development, the immune response, hemostasis, and maintenance of tissue integrity. Integrins also participate in a number of pathological conditions, such as chronic inflammation, invasion of cancer cells, and metastasis. They are heterodimers composed of a larger α and a smaller β subunit. Both contain a large extracellular domain, a membrane-spanning region, and a relatively small cytoplasmic domain. The extracellular parts of the two subunits associate noncovalently to form a functional receptor. At present, 24 $\alpha\beta$ heterodimers formed by 8 different β and 18 α subunits are known. Eighteen of these receptors recognize and bind to matrix proteins while others have cell surface proteins as their counter-receptors. Some integrins can mediate both interactions. The $\beta 1$ subunit is found in 12 different $\alpha\beta$ heterodimers, the αV subunit has five β partners, and there are four $\beta 2$ integrins. However, most integrin subunits participate in only one or two complexes (fig. 1). The integrins participate in cell movement and invasion in several different ways. The formation of new cell matrix contact sites is an essential phenomenon in cell migration. Here, we want to review the evidence suggesting that integrins concomitantly recognize the structure of the surrounding matrix and mediate messages to cells leading to integrin-guided degradation of molecular barriers obstructing invasion.

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Integrin structure

A general theme in structural biology is that proteins are composed of common structural motifs or modules that, like building blocks, are combined to form a functional protein. Two distinct combinations of these modules are found in the α subunits. All α subunits have in their amino terminus seven approximately 60 amino-acid repeats that have been predicted to adopt a four-stranded β-sheet structure and in the model, the seven repeats adopt an N-terminal structure referred to as the β propeller [1]. In the integrins, which lack the additional structures, this domain forms the ligand-binding pocket together with the β subunit (fig. 2). Nine of the known 18 α subunits have an additional highly conserved, autonomously folded $\sim$200 amino-acid I domain inserted between the third and fourth repeat of the β propeller. This is probably situated on top of the globular integrin head formed by the β propeller of the α subunit and the amino terminus of the β subunit. This

inserted domain is responsible for the ligand binding of the I-domain integrins [2–5]. They include the four collagen-binding $\beta 1$-associated α subunits, namely $\alpha 1$, $\alpha 2$, $\alpha 10$, and $\alpha 11$, and the $\beta 2$ leukocyte integrins αL, αX, αD, and αM. A number of I-domain structures have been solved and in all of these, the I domains adopt an $\alpha\beta$ fold with six central β strands surrounded by seven α helixes [6–8]. Within the I domain lies a highly conserved metal ion-dependent adhesion site (MIDAS) that coordinates magnesium [6]. No structural data are available on the β subunit, but models predict that the N terminus may adopt an I domain-like structure that would then bind to the β propeller of an α subunit forming a ligand-binding site. A number of studies have also suggested the role of amino acids in the β subunit I-like domain to be critical for ligand binding in the integrins lacking the αI domain. Unlike the autonomously folded I domain, both the β propeller and the β subunit N-terminal structure may require the presence of the other subunit for appropriate folding [9].

The integrin family

The functions of the various integrins are overlapping. Multiple receptors can bind to the same ligand and most integrins are considered promiscuous, meaning that they have several ligand molecules. The first integrin recognizion site identified in molecular detail was the already classical integrin-binding motif arginine-glycine-aspartic acid (RGD) found in fibronectin [10]. This binding motif has now been identified in a wide range of extracellular matrix molecules, virus proteins, (snake venom) toxins, and growth factors [11–13]. Most integrins however do not recognize this sequence. Other motifs in fibronectin can also serve as an integrin recognition sequence and most integrin ligands do not contain a functional RGD site. A common theme has arisen from the various integrin recognition sites identified so far. Many of the physiological integrin-binding motifs, both in matrix proteins and cell surface receptors, are short linear sequences and seem to contain an acidic residue. The collagens are an exception and in these the integrins recognize triple-helical motifs [14]. Snake venom proteins blocking the function of the collagen receptor integrins may also use a distinct binding strategy [15].

Integrins $\alpha 1\beta 1$ and $\alpha 2\beta 1$ have been considered as the major collagen receptors of the cell. Integrin $\alpha 1\beta 1$ prefers type IV collagen over type I collagen and also binds laminin-1. Integrin $\alpha 2\beta 1$ binds collagens, laminin-1, tenascin, and a snake venom protein called jararhagin. It is also the only integrin-type collagen receptor found on platelets and it plays an important role in collagen-induced platelet aggregation [16]. Integrin $\alpha 3\beta 1$ also binds collagen but in an assisting manner. The major ligand for $\alpha 3\beta 1$ is a basement membrane component, laminin-5; affinity to laminin-1 and fibronectin have also been demonstrated. Integrin $\alpha 4\beta 1$ is an important leukocyte receptor recognizing the vascular cell adhesion molecule (VCAM-1) on endothelial cells. $\alpha 4\beta 7$ also binds to a cell surface molecule, MadCAM. Both $\alpha 4$ heterodimers bind fibronectin, but to a motif distinct from the RGD. The

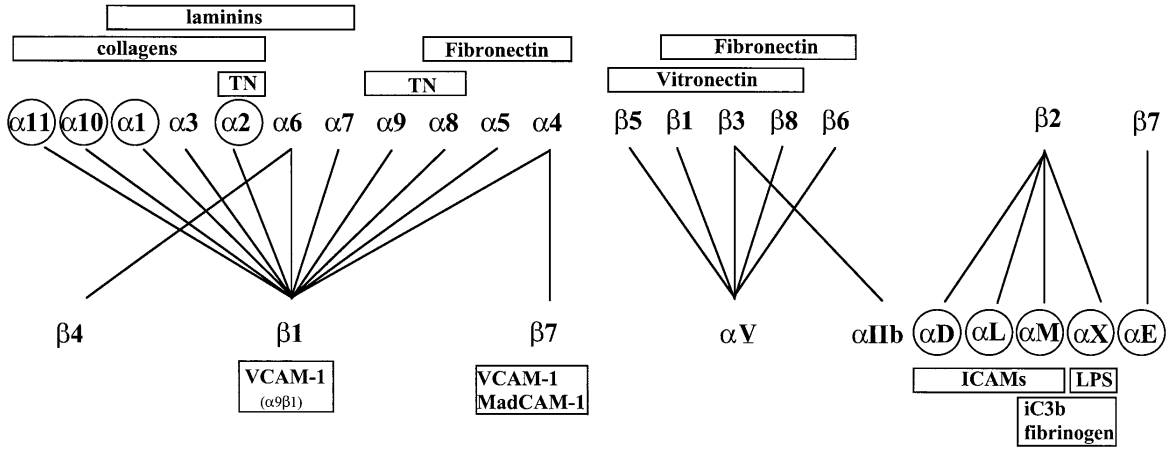

Figure 1. The integrin family of cell adhesion receptors. At present, 24 $\alpha\beta$ combinations are known. The bars at the top indicate the extracellular matrix ligands of the receptor and the bars below, the plasma component ligands and cell surface counter-receptors. The α subunits containing the I domain are circled.

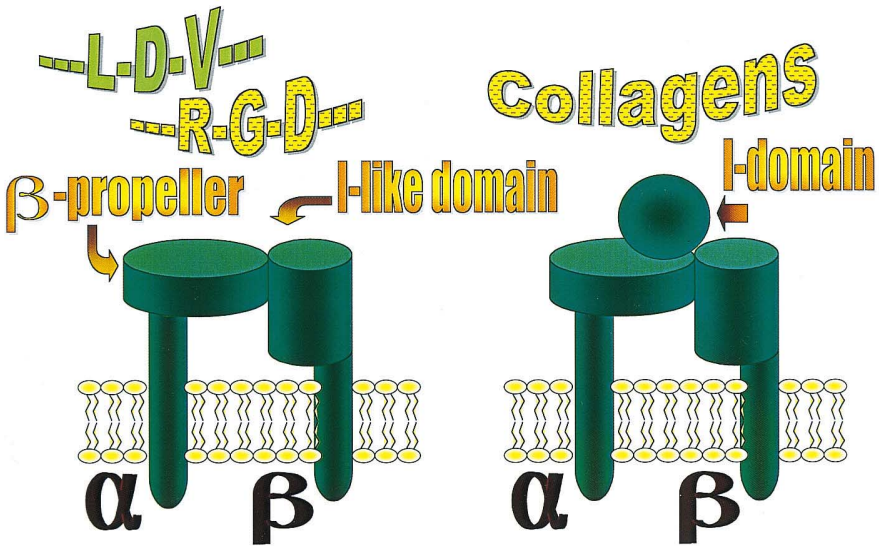

Figure 2. Integrin structure. On the left is the current model of an integrin lacking the αI domain. The proposed ligand-binding pocket lies between the globular heads of the two subunits. Loops on the top of the β propeller are important for ligand binding in some of these integrins. On the right is a model of an I domain integrin. The ligand-binding αI domain is situated on the top of the proposed β propeller.

first fibronectin receptor originally described to bind to RGD is in the present nomenclature the $\alpha5\beta1$ integrin. Integrins $\alpha6\beta1$ and $\alpha7\beta1$ are receptors for the laminins. Another fibronectin-binding receptor is $\alpha8\beta1$ that also binds tenascin. $\alpha9\beta1$ is a receptor for tenascin and VCAM-1 and contributes with $\alpha4\beta1$ to transendothelial neutrophil migration [17]. The two latest members of the family, $\alpha10\beta1$ and $\alpha11\beta1$, are collagen receptors recently found in cartilage and muscle, respectively. In addition to the $\beta1$ integrins, another group of receptors share a common subunit: these are the αV integrins. All αV integrins recognize the RGD motif in their ligands. $\alpha V\beta5$ and $\alpha V\beta6$ bind only to vitronectin or fibronectin, respectively, while the other αV integrins ($\alpha V\beta1$, $\alpha V\beta3$, and $\alpha V\beta8$) bind both ligands. Some integrins are found only in certain cells or distinct cellular structures. The platelet integrin $\alpha IIb\beta3$ is found on platelets where it mediates RGD-dependent binding to fibrinogen, fibronectin, and vitronectin. Integrin $\alpha6\beta4$, on the other hand, is an essential component of hemidesmosomes in which it binds laminin-5. The $\beta2$ integrins are found on leukocytes where they mediate cell-cell adhesion and transmigration of leukocytes to the vascular endothelium. $\alpha E\beta7$ binds to E-cadherin on epithelial cells.

More than glue, the integrins signal

When first identified, integrins were considered simply as 'glue' that binds the cell to its surroundings, since these receptors lack intrinsic kinase activity. Today, integrins are commonly appreciated as versatile receptors interacting with both the exterior and the interior of the cell. Integrins transduce signals from the outside of the cell to control cell movement, morphology, cell growth, and gene expression. Integrins can also be controlled from the inside of the cell to modulate their binding affinity to ligands [18, 19]. Both inside-out and outside-in signaling require the receptor to bind cellular components and the number of proteins shown to interact with the integrin transmembrane or cytoplasmic domains is growing rapidly. Proteins interacting with integrins include components of the cell cytoskeleton and various kinases involved in signaling pathways previously identified for growth factors and oncogenes [for review see ref. 20].

When binding to ligands, the integrins move laterally in the plane of the membrane to form clusters called focal adhesion sites. These sites are rich in both cytoskeletal proteins and signaling molecules. Most of the current knowledge on integrin outside-in signaling is derived from in vitro studies where integrin-ligand

interactions take place at the focal adhesions even though the corresponding in vivo structures may be somewhat different. Some integrin signaling complexes are never found in focal adhesion sites [21]. The occupancy of an integrin by a natural ligand leads to receptor clustering and, further, to the organization of the cytoskeleton and recruitment of signaling molecules to the focal adhesion. The events essential for integrin outside-in signaling can be divided into various stages corresponding to different steps in the formation of the mature, actively signaling focal adhesion complex [for a review see ref. 22]. Ligand occupancy by a monomeric ligand results in integrin recruitment to the focal adhesion site where the clustering of the receptors results in the accumulation of cytoskeletal proteins like tensin, α-actinin, talin, vinculin, and focal adhesion kinase (FAK). The next step is tyrosine phosphorylation, which triggers an accumulation and activation of both signal transduction molecules such as Src-type kinases, Src substrates, Ras, the mitogen-activated protein (MAP) kinases, i.e., ERK, JNK, and p38, and a large number of other signaling molecules and cytoskeletal proteins like F-actin and paxillin.

FAK was among the first proteins shown to be activated by cell adhesion via integrins. Binding of an integrin to the extracellular matrix (ECM) triggers FAK phosphorylation. 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. FAK kinase has been shown to interact with the integrin β-subunit cytoplasmic tail, which would provide a mechanism for FAK activation upon integrin clustering. The C terminus of this kinase contains the focal adhesion targeting (FAT) domain and binding sites for both cytoskeletal proteins and downstream signaling molecules. The biological role of FAK remains somewhat unclear. Since activation of FAK involves the recruitment of the Grb2/Sos complex and integrin-mediated adhesion triggers activation of MAP kinases, downstream effectors of the Ras pathway [23, 24], it could well be that FAK activation might serve to activate Ras and downstream pathways. However, recent data have provided evidence of MAP kinase activation in response to integrin ligation that is independent of FAK [25]. Other important functions have also been demonstrated. Integrin signaling must function in synergy with signals initiated by other receptors, such as the growth factor receptors. The growth factors are able to trigger MAP kinase activation more efficiently in adherent cells and this function depends on FAK activity [26, 27]. This provides an elegant way to control cell growth. Signaling via FAK is also important in controlling anoikis [28] and cell motility [29].

All ECM-binding integrins activate FAK and pathways downstream of it. However, since the various integrins have distinct functions, integrins must also be able to activate subunit-specific pathways in addition to the common pathways described above. How is this specificity in signaling established? Two possible mechanisms are emerging. Integrins can either associate with transmembrane adaptor proteins like caveolin and the transmembrane-4 superfamily proteins (TM4SF), or cellular components may interact with the cytoplasmic domain of the α subunit. The first illustration of how integrin-specific signaling is achieved was provided by Wary et al. [25], who showed that a subset of integrins ($\alpha1\beta1$, $\alpha5\beta1$, $\alpha6\beta4$, and $\alpha V\beta3$) promote cell proliferation via recruiting Shc and activating the Ras-ERK pathway using caveolin as an adaptor. They also showed that other integrins, such as $\alpha2\beta1$, $\alpha3\beta1$, and $\alpha6\beta1$, fail to activate this pathway. Furthermore, $\alpha2\beta1$ can regulate a distinct MAP kinase pathway leading to the activation of p38α [30, 31]. Later, the identification of association between TM4SF proteins showed how integrin-specific signaling might function outside the focal adhesions. The TM4SF proteins interact with integrins, such as $\alpha3\beta1$ and $\alpha6\beta1$, and activate at least phosphatidylinositol 4-kinase (PI-4K) and possibly also other signaling molecules [32]. Studies on naturally occurring splice variants of integrin α subunits have on the other hand demonstrated that the cytoplasmic tail may also induce signaling [33].

In addition to integrin outside-in signaling, cells can modulate the affinity of integrins to their ligands by inside-out signaling. In this process, unidentified cytoplasmic signals change the ligand-binding affinity of the receptor from a low-affinity state to a high-affinity receptor [34]. The current model for this suggests the existence of a salt bridge between the two integrin subunits. The receptor is held in an inactive conformation by this bond and mutation of the charged amino acid residues involved results in a constitutively active integrin [35]. In the cell, this similar affinity-state switch is thought to be regulated by proteins such as calreticulin [36], serine/threonine kinases, and small GTPases [37, 38]. In addition to the salt bridge, hydrophobic interactions are also involved since mutations in conserved hydrophobic residues in the integrin cytoplasmic domains abolish integrin activation [35].

Integrins in vivo

A constantly increasing number of transgenic animals lacking a certain integrin have provided valuable in vivo data on the function of these receptors. However, a number of surprises have emerged. Depletion of some integrins results in severe phenotypes, where the em-

bryos die early in development. These include the $\beta 1$ subunit, which could be predicted since $\beta 1$ is involved in the majority of integrin heterodimers. A similar effect was anticipated for deletion of the αV subunit since the αV integrins have been implicated in many developmental processes. However, although αV-null is lethal, the animals show considerable development such that 20% of the mice are born alive [39]. In some cases, the lack of either the receptor or the ligand results in different phenotypes suggesting overlapping functions of integrins. One example of this is the difference seen between the fibronectin-null animals and those lacking the fibronectin receptor $\alpha 5\beta 1$ [40]. Both knockouts give an 'embryonic lethal' phenotype but the defects of the $\alpha 5$-null embryos are less severe, probably because αV integrins can replace some of the functions of $\alpha 5\beta 1$ [41]. However, in some cases, the integrins for the same ligand cannot replace each other. The depletion of collagen receptor $\alpha 1\beta 1$ has only minor effects [42, 43], while preliminary observations suggest that mice lacking $\alpha 2\beta 1$ have an 'early embryonic lethal' phenotype (H. Gardner, personal communication). Some integrin-null animals have been surprisingly normal even though the missing integrin has been connected to some important function. One example is the $\alpha 7$-null mouse. It is viable and fertile even though antibody data have demonstrated the essential role of $\alpha 7$ integrin in myogenesis [44]. At birth, these animals are healthy suggesting that myogenesis during development can take place in the absence of $\alpha 7$ integrin. However, the importance of this integrin is evident since later in life these $\alpha 7$-null mice develop a form of muscular dystrophy. In conclusion, it can be emphasized that all integrins have distinct roles in the organism, but when necessary these functions may in many cases be replaced by an overlapping function of another integrin.

Integrins control cell invasion through extracellular matrix via regulating the expression and activation of matrix metalloproteinases

Matrix metalloproteinases (MMPs) are a family of structurally related zinc-dependent endopeptidases that collectively are able to degrade most ECM components. At present, 18 members of the human MMP gene family are known and these can be divided into subgroups of collagenases, gelatinases, stromelysins, membrane-type (MT)-MMPs, and novel MMPs (see Johansson et al. in this multi-author review). In general, MMPs are not constitutively expressed by cells in vivo. Instead, their expression is induced by growth factor or cytokine stimulation. Furthermore, alterations in the cell matrix interactions regulate MMP expression [45]. This suggests for integrins an important role as the regulators of the MMPs. Indeed, to date, six integrins have been shown to influence MMP expression. Integrins can regulate the expression of the MMP genes and they have also been shown to influence the secretion of the granule-stored MMPs and the activation of the latent protease at the cell surface. These data are summarized in table 1. All the data suggest that recognition of matrix molecules by cell surface integrins and the following degradation of the matrix are important mechanisms in cell invasion. This process could be called integrin-guided proteolysis.

The relationship between integrin activation and MMP gene expression has mainly been studied in two ways. Both ligation of integrins on the cell surface with subunit-specific anti-integrin antibodies, and culturing cells on or inside various matrix components can alter cellular MMP expression (table 1). Cell-fibronectin interaction has highly divergent effects on MMPs depending on cell type and the integrins involved. Rabbit synovial fibroblasts express basal levels of MMP-1, MMP-3, and MMP-9 when cultured on intact fibronectin in vitro. However, when cultured on the RGD-containing region of fibronectin, the MMP levels are increased [46]. Two fibronectin-binding integrins recognizing distinct regions in fibronectin cooperate in MMP regulation. $\alpha 4\beta 1$ adhesion to the CS-1 fraction of fibronectin downregulates the metalloproteinases, while $\alpha 5\beta 1$ recognizing the RGD site on fibronectin has an opposite effect. In moderately invasive melanoma cells, the perturbation of $\alpha V\beta 3$ integrin results in a concomitant increase in the invasiveness and expression and secretion of MMP-2, suggesting that $\alpha V\beta 3$ upregulates MMP-2 in these cells [47]. In highly invasive melanoma cells, a switch in integrin expression has been described. These cells express relatively high levels of $\alpha 5\beta 1$ while $\alpha V\beta 3$ expression is low. The switch in integrin expression leads to even higher MMP-2 production, which correlates with the higher invasion capacity of these cells [48]. Metalloproteinase expression in cells may change in response to altered integrin expression or altered ligand concentration. In addition, the structure of the ECM is important. Osteogenic cells expressing the collagen-binding integrins $\alpha 1\beta 1$ and $\alpha 2\beta 1$ show marked changes in gene expression in response to three-dimensional collagenous matrix. Most of the changes cannot be seen in cells plated on two-dimensional type I collagen. In this model, the ligation of $\alpha 2\beta 1$ leads to an increase in MMP-1 mRNA levels while $\alpha 1\beta 1$ mediates downregulation of collagen [49]. Fibroblasts derived from $\alpha 1\beta 1$-deficient mice on the other hand have lost the negative feedback regulation of cellular collagen production and show increased MMP-13 production [43], which is possibly an $\alpha 2\beta 1$ integrin-mediated effect [30]. The existence of integrin-guided proteolysis in vivo could be studied by concomitant localization of inte-

grins, matrix proteins, and MMPs. Only a few of such studies have been published. Pilcher et al. [50] showed that during wound healing, migrating keratinocytes express MMPs only when in contact with collagen. This is in good agreement with the suggestions that $\alpha 2\beta 1$ integrin regulates MMP-1 expression and we would like to propose that similar mechanisms might be quite common in other processes as well.

A hypothesis of stepwise integrin-guided degradation of fibrillar collagen

The data provided above on integrins and metalloproteases lead to a tempting model in which proteolysis of collagens is guided by the integrins in a stepwise manner (fig. 3). Fibrillar collagen matrix is first degraded by collagenases and denatured to gelatin whose further degradation is due to gelatinase activity. According to this hypothesis, ligation of $\alpha 2\beta 1$, when cells are in contact with three-dimensional collagen, leads to production of MMP-1. This metalloproteinase can cleave native fibrillar collagen at a defined site to yield gelatin. In this partially degraded collagen, an RGD site is exposed [51]. Integrin $\alpha V\beta 3$ is known to recognize the RGD site and this leads to upregulation of gelatinase-A (MMP-2) expression [47, 52]. MMP-2 finishes the degradation of collagen.

Integrins can be cellular receptors of MMPs

In most cases, metalloproteinases have been studied as secreted soluble proteases produced by the cells. A new emerging idea is that cell surface receptors can target proteolysis to a limited pericellular area. The MT-MMPs activate latent MMPs at the cell surface [53–55]. In fibrosarcoma cells, the adhesion of $\alpha 5\beta 1$ to fibronectin leads to induction of MMP-2 secretion and concomitant upregulation of MT1-MMP, a membrane-bound metalloprotease shown to activate latent MMP-2 at the cell surface [54]. The membrane-bound MMP-2, not the secreted soluble form, was shown to be critical for ECM remodeling by fibrosarcoma cells in vitro [56]. In melanoma cells, ligation of $\alpha V\beta 3$ with vitronectin also resulted in an increase in membrane-bound MMP-2 and a concomitant increase in invasion [57]. Integrins can also target adhesion and proteolytic activity to the same site. MMP-2 localizes in a proteolytically active form to the surface of invasive cells due to interaction with $\alpha V\beta 3$ integrin [58]. Similar connections may exist between other integrins and the metalloproteinases they regulate, since binding of the protease to the receptor that regulates its expression could be an excellent way to target proteolysis to the right areas in the pericellular space.

Table 1. Regulation of metalloproteinase expression and activation by integrins

MMP	Integrin	Ligand/antibody	Cell type	Reference
MMP-1; ↑	$\alpha 2\beta 1$	type I collagen	osteogenic cells, human	49
			fibroblasts, human	59
			primary keratinocytes, human	50
	$\alpha 4\beta 1$	fibronectin (RGD)	synovial fibroblasts, rabbit	46
MMP-1; ↓	$\alpha 4\beta 1$	fibronectin (CS-1)	synovial fibroblasts, rabbit	46
MMP-2; ↑	$\alpha 2\beta 1$	anti-$\alpha 2$ mAb	rhabdomyosarcoma cells, human	60
	$\alpha 3\beta 1$	anti-$\alpha 3$ mAb	rhabdomyosarcoma cells, human	60
	$\alpha 3\beta 1$	anti-$\alpha 3\beta 1$ mAb	glioma cells, human	61
	$\alpha 5\beta 1$	anti-$\alpha 5\beta 1$ mAb	glioma cells, human	61
	$\alpha 5\beta 1$	anti-$\alpha 5\beta 1$ mAb	melanoma cells, human	48
	$\alpha V\beta 3$	anti-$\alpha V\beta 3$ mAb	melanoma cells, human	47
		vitronectin	melanoma cells, human	47
	$\alpha V\beta 3/\beta 5$	fibronectin, vitronectin	osteogenic cells, human	52
MMP-2; ↑✂	$\alpha 4\beta 1$	fibronectin (CS-1)	autoreactive T cells, mouse	62
MMP-2; ✂, MT1-MMP↑	$\alpha 5\beta 1$	fibronectin, mAbs	fibrosarcoma cells	63
MMP-3; ↑	$\alpha 5\beta 1$	fibronectin (RGD)	synovial fibroblasts, rabbit	46
MMP-3; ↓	$\alpha 4\beta 1$	fibronectin (CS-1)	synovial fibroblasts, rabbit	46
MMP-9; ↑	$\alpha 3\beta 1$	anti-$\alpha 3$ and $\beta 1$ mAbs	mucosal keratinocytes, human	65
	$\alpha 5\beta 1$	fibronectin (RGD)	synovial fibroblasts, rabbit	46
		fibronectin	myeloid leukemia cells, human	64
MMP-9; ↓	$\alpha 4\beta 1$	fibronectin (CS-1)	synovial fibroblasts, rabbit	46
MMP-13; ↑	$\alpha 1\beta 1$	type I collagen	fibroblasts, human	30
	$\alpha 2\beta 1$	type I collagen	fibroblasts, human	30

↑, upregulation of expression; ↓, downregulation of expression; ✂, activation of pro-MMP; mAb, monoclonal antibody

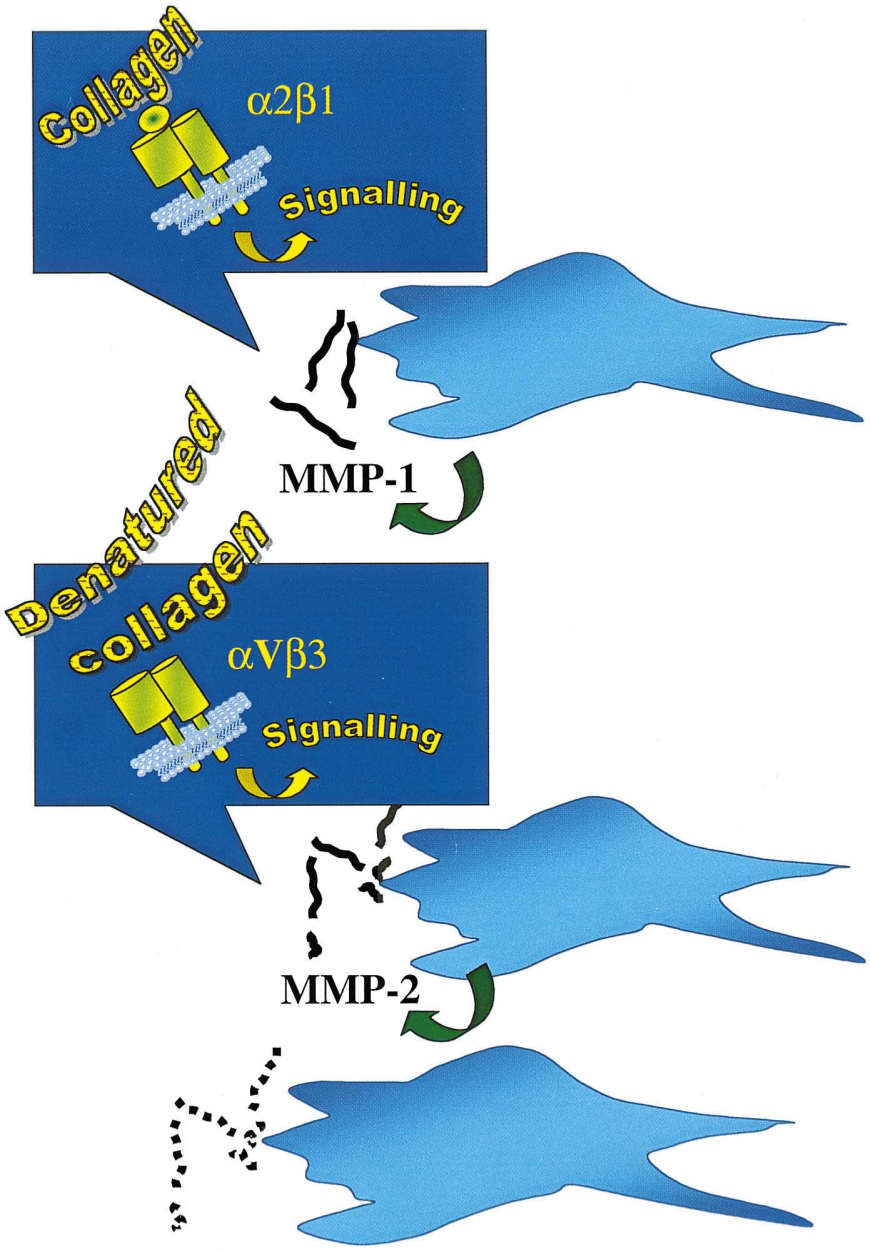

Figure 3. A hypothesis of stepwise integrin-guided degradation of fibrillar collagen. Integrin $\alpha 2\beta 1$ recognizes native collagen and this results in the upregulation of MMP-1 production. MMP-1 cleaves the native collagen at a specific site yielding gelatin and exposing the RGD sequence in gelatin. Integrin $\alpha V\beta 3$ recognizes the RGD sequence and this results in integrin-mediated upregulation of MMP-2. MMP-2 degrades the gelatin matrix resulting in complete collagen matrix turnover.

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