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Review

Regulation of Tissue Injury Responses by the Exposure of Matricryptic Sites within Extracellular Matrix Molecules

George E. Davis,* Kayla J. Bayless,*
Michael J. Davis,† and Gerald A. Meininger†

From the Departments of Pathology and Laboratory Medicine and Medical Physiology,† Texas A&M University Health Science Center, College Station, Texas*

Extracellular matrix (ECM) is known to provide signals controlling cell shape, migration, proliferation, differentiation, morphogenesis, and survival. Recent data shows that some of these signals are derived from biologically active cryptic sites within matrix molecules (matricryptic sites) that are revealed after structural or conformational alteration of these molecules. We propose the name, matricryptins, for enzymatic fragments of ECM containing exposed matricryptic sites. Mechanisms regulating the exposure of matricryptic sites within ECM molecules include the major mechanism of enzymatic breakdown as well as others including ECM protein multimerization, adsorption to other molecules, cell-mediated mechanical forces, and ECM denaturation. Such matrix alterations occur during or as a result of tissue injury, and thus, the appearance of matricryptic sites within an injury site may provide important new signals to regulate the repair process. Here, we review the data supporting this concept and provide insight into why the increased exposure of matricryptic sites may be an important regulatory step in tissue responses to injury. ***(Am J Pathol 2000, 156:1489–1498)***

The extracellular matrix (ECM) contains signals that control cell shape, migration, proliferation, differentiation, morphogenesis, and survival.[1–3] It appears that ECM signals act in concert with other signaling pathways, such as those initiated by growth factors, to regulate cell behavior. Cells use a series of receptors for ECM including integrins, cell surface proteoglycans, and a newly described class of cell-surface-expressed tyrosine kinase receptors with direct affinity for ECM.[3–6] The components of ECM include insoluble ECM proteins (ie, collagens, laminins, fibronectin, proteoglycans), matricellular ECM proteins that modulate cell-matrix interactions and other cellular responses such as cell proliferation (ie, SPARC, thrombospondins, osteopontin, tenascin)[7,8] and ECM-associated proteins such as growth factors.[9] Recent reviews discuss the unique properties of these individual insoluble or matricellular ECM proteins in detail.[3,8,10–15] During tissue injury, the composition of ECM and its cellular recognition sites are altered in a number of significant ways (Figure 1). Increased vascular permeability results in the recruitment of plasma-derived proteins (eg, fibronectin, vitronectin, fibrinogen) into ECM, whereas cells in the injury site are induced to release or synthesize new ECM components (eg, osteopontin, SPARC, thrombospondins, tenascins, alternatively spliced fibronectins) which regulate tissue repair.[16–19] Furthermore, tissue injury may result in alterations in existing ECM proteins within tissues or in recruited ECM that reveal cryptic biologically active (matricryptic) sites that provide important signals within the injury site. Recent work has implicated the potential importance of these matricryptic sites.[20–25] In this review, we discuss the data supporting this concept and provide insight into why increased exposure of matricryptic sites may be a critical step during tissue injury responses.

Matricryptic Sites and Matricryptins Regulate Cell and Tissue Responses

Matricryptic sites are defined here to be biologically active sites that are not exposed in the mature, secreted form of ECM molecules (including both proteins and car-

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Address reprint requests to George E. Davis, M.D., Ph.D., Department of Pathology and Laboratory Medicine, Texas A&M University Health Science Center, 208 Reynolds Medical Building, College Station, TX 77843-1114. E-mail: gedavis@tamu.edu.

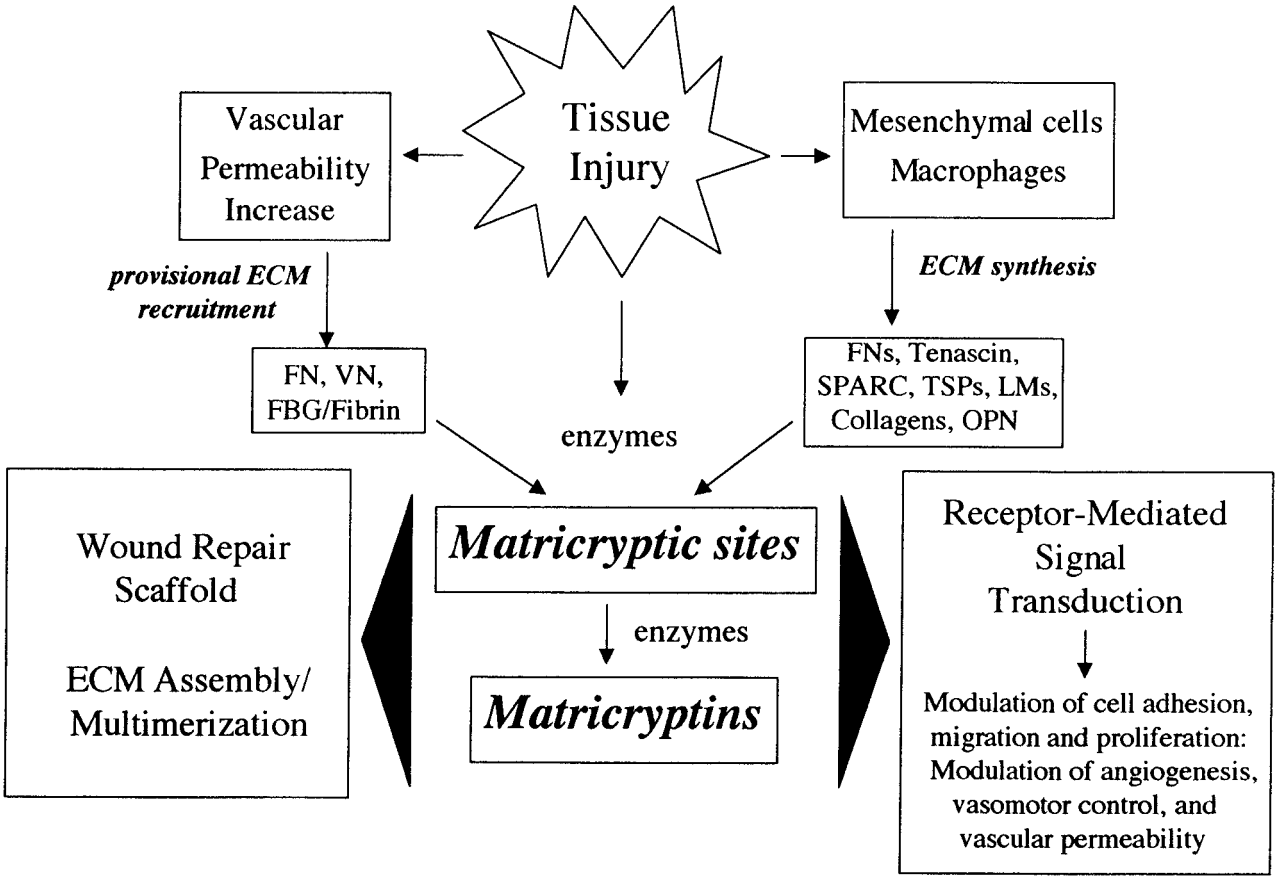

Figure 1. Schematic diagram illustrating how tissue injury leads to the generation of matricryptic sites and matricryptins which participate in the regulation of key aspects of tissue injury responses. FN, fibronectin; VN, vitronectin; FBG, fibrinogen; OPN, osteopontin; LM, laminin; TSP, thrombospondin.

bohydrates such as glycosaminoglycans), but which become exposed after structural or conformational alterations. These sites can be derived from insoluble ECM molecules that are deposited in tissues, matricellular ECM proteins, and plasma-derived ECM molecules. The term is limited to those sites derived directly from ECM molecules and does not refer to sites derived from other ECM-associated molecules such as proteases, protease inhibitors, or growth factors. We propose the term matricryptins to refer to biologically active fragments from ECM molecules (as defined above) which expose functional matricryptic sites (Figure 1). This term refers specifically and is limited to biologically active ECM fragments that contain a cryptic domain that is not normally exposed in the intact molecule.

An accumulating number of studies have suggested that matricryptic sites exist within ECM molecules and that these sites regulate biological phenomena such as ECM matrix assembly, formation of a wound repair scaffold, and receptor-mediated signal transduction which can induce a variety of important biological effects (Table 1 and Figure 1). Matricryptic sites and matricryptins have been reported within protein components of ECM as well as in glycosaminoglycans such as hyaluronic acid. Listed in Table 1 are ECM molecules with known matricryptic sites or which contain biologically active matricryptins. In addition, information is provided concerning the known activities and structure of individual matricryptic sites as well as mechanisms involved in their generation.

Some examples of matricryptic sites and matricryptins include plasmin-derived fibrin fragments which increase vascular permeability,[50,51] and collagen, fibronectin, and elastin fragments that 1) stimulate directed cell migration (eg, leukocytes),[37,42,43,61] 2) affect cell proliferation,[39] 3) induce focal contact disassembly,[46] and 4) induce arteriolar vasodilation.[22] In addition, the N-terminal thrombin fragment of osteopontin as well as proteolytic fragments of laminin or tenascin all contain cryptic cell adhesion sites that are not exposed in the intact molecules.[48,59,60] Also, ECM fragments or peptides derived from SPARC, hyaluronate, and collagen type XVIII (ie, endostatin) have been shown to affect important biological processes such as angiogenesis.[24,25,52,53,55] Furthermore, recent studies have shown that proteolysis of laminin can stimulate directed cell migration of either epithelial tumor cells or epithelial cells undergoing morphogenesis, and also can stimulate hemidesmosome formation.[23,47,62] Overall, these studies show that alterations of ECM molecules can generate new signals for cells that influence important cellular events.

Table 1. Matricryptic Sites and Matricryptins in Extracellular Matrix Molecules

ECM molecule	Matricryptic site(s)	Matricryptic site function	Mechanism(s) of matricryptic site exposure	References
Fibronectin	Type III (1st and 10th) repeats	FN matrix assembly	Cell-mediated mechanical forces, FN multimerization	10, 26–33
	Type III repeat (10th)-RGD site	Cell adhesion	Cell-mediated mechanical forces, adsorption	34–36
	120-kd cell-binding domain, 40-kd gelatin-binding domain	Stimulates cell migration	Enzymatic degradation	37, 38
	N- and C-terminal heparin-binding fragments	Inhibits cell proliferation	Enzymatic degradation	39
Vitronectin	RGD site	Cell adhesion ($\alpha v\beta 3$, $\alpha v\beta 5$)	VN multimerization, adsorption	40
Collagens	RGD site(s)	Cell adhesion ($\alpha v\beta 3$), arteriolar vasoactivity	Enzymatic degradation (MMP-1, elastase), thermal denaturation	20–22, 41
	$(Pro\text{-}Pro/Hyp\text{-}Gly)_5$ synthetic peptides	Stimulates cell migration	Enzymatic degradation (Cathepsin G, bacterial collagenase)	42, 43
	Unknown	FN, MMP-2, MMP-9 binding	Enzymatic degradation, thermal denaturation	44, 45
	Unknown	Decreased VSMC adhesion and focal contacts	Enzymatic degradation (bacterial collagenase)	46
Laminins	LM-5, $\gamma 2$ chain fragment	Stimulates tumor cell motility	Enzymatic degradation (MMP-2)	23, 47
	RGD site	Cell adhesion	Enzymatic degradation	48
Collagen XVIII	C-terminal globular domain (~20 kd)	Angiogenesis inhibitor (endostatin)	Enzymatic degradation (MMPs)	24
Fibrinogen/fibrin	γ chain, D domain	Fibrin polymerization	Enzymatic degradation (thrombin), fibrinogen multimerization	49
	Fragment D and fibrinogen B chain (β peptides)	Increased vascular permeability	Enzymatic degradation (plasmin)	50, 51
Hyaluronic acid	Fragments of 3–16 disaccharide units in length	Stimulation of angiogenesis, increased metalloelastase production	Enzymatic degradation (hyaluronidase)	52–54
SPARC	KGHK site	Stimulation of angiogenesis, copper binding	Enzymatic degradation	55
	Helix loop A	Increased affinity for collagens	Enzymatic degradation (MMPs)	56, 57
Thrombospondin	Unknown	ECM interactions with FN and heparin, increased protease resistance	Heterotypic binding to FN or heparin	58
Osteopontin	N-terminal fragment	Cell adhesion ($\alpha 9\beta 1$)	Enzymatic degradation (thrombin)	59
Tenascin	RGD site	Cell adhesion ($\alpha 8\beta 1$)	Enzymatic degradation	60
Elastin	VGVAPG sites	Stimulates cell migration	Enzymatic degradation	61

Abbreviations: VSMC, vascular smooth muscle cell; MMP, matrix metalloprotease; FN, fibronectin; VN, vitronectin; LM, laminin.

Matricryptic Sites Are Revealed in Extracellular Matrix after Enzymatic Degradation, Heterotypic Binding to Other Molecules, Multimerization, Cell-Mediated Mechanical Forces, or Denaturation

The mechanisms regulating the exposure of matricryptic sites may constitute an important step in the control of many biological processes (Figure 2 and Table 1). At least five mechanisms may play a role in generating these new sites and all share a common step that involves a change in ECM molecule structure or conformation. These changes can be induced in ECM molecules after 1) enzymatic degradation, 2) heterotypic binding to other molecules (adsorption), 3) multimerization (ie, self-assembly), 4) cell-mediated mechanical force, and 5) denaturation.

Heterotypic binding of ECM molecules to other molecules as well as their adsorption to surfaces is known to alter protein conformation.[34,36,49,58,63,64] These interactions can lead to the exposure of matricryptic sites which likely play a role during deposition of ECM proteins after synthesis and secretion as well as in the generation of the provisional ECM that occurs after increases in vascular permeability and the recruitment of plasma-derived proteins into the ECM. This mechanism is particularly prominent during acute inflammatory responses or during tumorigenesis where provisional ECM formation containing fibrin, fibronectin, and other ECM proteins is observed.[10,16,34,49] Other studies have revealed how new epitopes are revealed in ECM proteins after binding to cell-surface receptors. These sites, termed receptor-induced binding sites by Plow and colleagues,[65,66] were identified in fibrinogen after its binding to the platelet integrin, $\alpha IIb\beta 3$. These sites are believed to facilitate platelet aggregation and fibrinogen polymerization to stabilize developing fibrin-platelet clots.[65,66] Thus, matricryptic sites are revealed in several instances of heterotypic binding of ECM molecules to other components of ECM or to cell-surface receptors.

In addition, matricryptic sites are revealed during ECM self-assembly or multimerization which has been

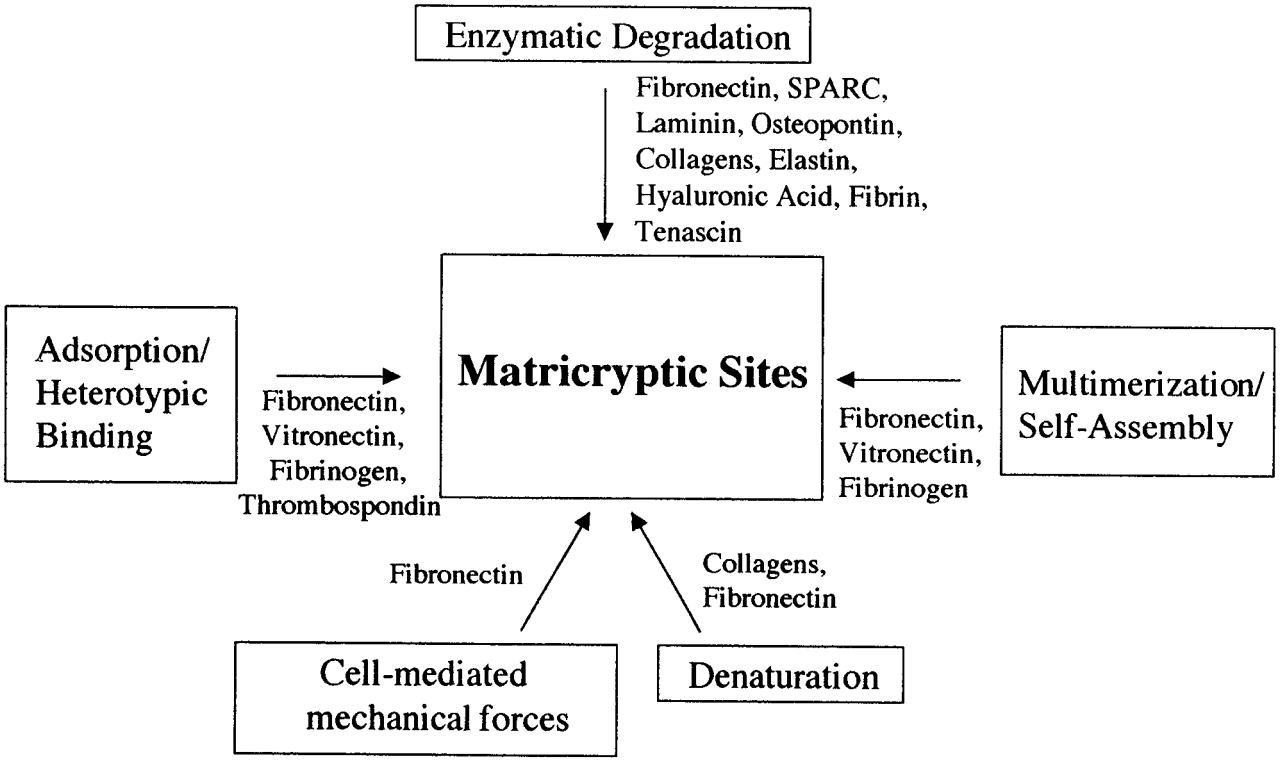

Figure 2. Mechanisms for the generation of matricryptic sites during tissue injury responses. Individual ECM proteins and known mechanisms for the generation of matricryptic sites are indicated.

observed with fibrinogen, vitronectin, and fibronectin.[10,26–33,40,49,67] As many recent studies have indicated, ECM protein multimerization is a major mechanism regulating ECM assembly.[10] In some cases, matricryptic sites may catalyze the multimerization process to stimulate the deposition of insoluble ECM.[29,49] For example, the addition of a recombinant 14-kd fibronectin fragment to intact fibronectin induces multimerization of fibronectin through a self-assembly mechanism involving covalent disulfide cross-links.[29] The disulfide-exchange reaction that participates in this multimerization event[10,29] has recently been attributed to an additional matricryptic site (ie, a Cys-X-X-Cys motif) in fibronectin which possesses disulfide isomerase activity.[33] A further example of these concepts is that monoclonal antibodies, which bind only to surface-adsorbed fibrinogen and not to soluble fibrinogen, were found to inhibit fibrin polymerization indicating that matricryptic sites are exposed and directly contribute to fibrin multimerization.[49] An important extension of these findings is that ECM multimerization increases matrix valency (perhaps by increasing the exposure of matricryptic sites) which then enhances cell-matrix interactions.[29,68]

Enzymatic degradation of ECM and ECM protein denaturation are additional mechanisms that reveal matricryptic sites. Conformational changes in ECM secondary to denaturation are known to occur after proteolysis, oxidant damage, and thermal injury.[69–77] Cell types that are particularly efficient in inducing ECM denaturation during acute and chronic injury include leukocytes such as neutrophils and monocytes/macrophages (see below) and malignant tumor cells.[70,73,78,79] An excellent model of ECM denaturation is the well-described unfolding of triple helical collagen molecules that occurs after cleavage of triple-helical collagen by collagenases such as MMP-1 and MMP-8.[69,78,80] The triple helix of cleaved collagen molecules then undergoes thermal melting at 37°C. Thermal denaturation of intact collagen molecules similarly unfolds the triple helix at temperatures greater than 42°C,[69] a phenomenon that occurs *in vivo* after thermal injury to the skin.[76,77] Also, the unfolding of collagen renders its individual chains susceptible to broad spectrum proteases that can create biologically active collagen fragments (see below).[69]

Leukocytes, such as neutrophils and macrophages, cause ECM denaturation by the action of secreted proteases (eg, elastase, collagenases, gelatinases) and oxidants.[73,78] Interestingly, neutrophils and monocytes express the integrins, $\alpha M\beta 2$ (Mac-1), $\alpha X\beta 2$ (p150,95), and $\alpha 4\beta 1$, which can interact with conformationally modified or denatured proteins.[81–84] Although the nature of the Mac-1, p150,95, and $\alpha 4\beta 1$ binding sites within denatured proteins remains unknown, the majority of proteins seem to possess them. Thus, leukocyte-derived ECM degrading agents may create leukocyte-integrin binding sites in their surrounding ECM. The ability of leukocytes to denature and degrade ECM could allow them to migrate into essentially any tissue site and may facilitate their ability to phagocytose diverse tissue or matrix debris. In related studies, we have recently found that the leukocyte integrins, $\alpha 4\beta 1$, $\alpha M\beta 2$, $\alpha X\beta 2$, and $\alpha LRI\beta 3$, are all capable of binding osteopontin [85](Bayless et al, unpublished obser-

vations), an Arg-Gly-Asp (RGD)-containing protein that is induced during injury and which is secreted in substantial amounts by macrophages. This may indicate a special role for osteopontin in leukocyte functions such as migration, tissue recruitment, and phagocytosis. Thus, osteopontin, an ECM protein known to be markedly induced during tissue injury, contains multiple integrin-binding sites (and perhaps matricryptic sites)[17,59,85] capable of regulating leukocyte and other cellular behaviors during wound repair responses.

Matricryptic Sites Bind Integrins and Regulate Extracellular Matrix Assembly

Recent data suggests that heterotypic binding, multimerization, or adsorption of ECM molecules can promote the exposure of matricryptic sites with affinity for integrins. The RGD integrin- and cell-binding site of vitronectin has recently been shown to be cryptic, in that it is not exposed in plasma vitronectin unless it adsorbs to surfaces or multimerizes.[40] This property may allow the RGD site of vitronectin to be exposed only when it is needed (ie, after increases in vascular permeability and binding of vitronectin into the ECM of injured tissues). A related and interesting question is whether the RGD sites within soluble plasma fibronectin are cryptic. A number of studies suggest that this is the case because monoclonal antibodies, which bind near the RGD site, bind weakly to fibronectin in solution but strongly bind after its adsorption.[34] Also, the 120-kd RGD-containing and cell-binding domain fragment of fibronectin can induce monocyte chemotaxis whereas the intact protein does not.[37] Structural analysis shows that the RGD site is present in a flexible loop which seems to be sensitive to cell-mediated mechanical forces.[35] In contrast to the above studies, this structural analysis suggested that appropriate forces applied to fibronectin molecules may actually decrease the accessibility of the RGD site to integrin binding. The above data together suggests that the RGD sites are present within a flexible domain that could be regulated (perhaps in a reversible manner) to expose or hide the sites depending on the biological situation. Other types of molecular interactions involving fibronectin heterotypic or self-assembly binding events may induce the exposure of its RGD sites (like those described for adsorption above) such as when fibronectin covalently cross-links into fibrin matrices, binds heparan sulfate proteoglycans, binds denatured collagen, or polymerizes during fibronectin matrix assembly.[26–30,32,34–36] During this latter event, cells bind secreted fibronectin and exert tension on these molecules. This tension reveals matricryptic sites which promote fibronectin-fibronectin binding and assembly of a fibronectin matrix.[26,27] Experiments using a fibronectin-green fluorescent protein chimera have demonstrated that cell-bound fibronectin fibrils exhibit elasticity in response to changes in cell movement and shape.[28] These studies support the concept that cell-mediated mechanical forces can generate and perhaps regulate the exposure of matricryptic sites in ECM to effect ECM assembly and subsequent cellular responses. It is also well known that cell-mediated mechanical forces on ECM can regulate complex processes such as cellular morphogenesis[25] and some of these effects might be mediated by matricryptic sites.

Matricryptic RGD Sequences in Collagens and the Probable Importance of RGD as a Wound Signal

After the seminal discovery of the RGD cell-binding sequence in fibronectin and vitronectin,[86,87] it was recognized that collagens have multiple copies of this sequence. However, it became clear that cell attachment to native collagen substrates did not appear to involve RGD. Anti-integrin antibodies and RGD peptides which block the function of RGD-binding integrins failed to interfere with cell binding to native collagen substrates.[20,41] In contrast, other investigators showed that cell attachment to denatured collagen was RGD-dependent.[20,21,41] Davis[20] originally proposed that the exposure of RGD sites, which occurs during the transition from native to denatured collagen, might constitute a wound signal for cells. Furthermore, the $\alpha v\beta 3$ integrin was shown to bind strongly to denatured collagen and minimally to native collagen in affinity chromatography experiments suggesting that the RGD sites in native collagen are cryptic.[20] In addition, the $\alpha 2\beta 1$ integrin which binds native collagen showed minimal binding to denatured collagen.[20,88] These results suggested the possibility that cells might use this information as part of a wound recognition system. Considerable recent work has provided support for this idea. For example, melanoma tumor cells are unable to proliferate and form a tumor without the $\alpha v\beta 3$ integrin which is a receptor for denatured collagen.[21] Matrix metalloprotease (MMP)-1-treated collagen was also shown to bind melanoma cell $\alpha v\beta 3$.[21] Furthermore, during normal bone resorption by osteoclasts, it was shown that denatured collagen and $\alpha v\beta 3$ were present at cell matrix contact sites, whereas in adjacent sites, native collagen was present.[89] These data provide direct evidence for the potential importance of integrin-denatured collagen interactions in biologically important phenomena. Local collagen denaturation can result in the generation of matricryptic RGD signals delivered to cells through the $\alpha v\beta 3$ integrin and may potentially eliminate or alter signals delivered through $\alpha 2\beta 1$ or a recently described native collagen-binding tyrosine kinase receptor.[5,6] A recent article reports that denatured type I collagen fragments induce focal contact disassembly in vascular smooth muscle cells in a manner that is not dependent on RGD sequences and that may involve $\alpha 2\beta 1$.[46] The above data supports the concept that collagen-derived matricryptic sites include RGD as well as non-RGD sites. Also, a recent report shows that cells are able to bend collagen fibrils[90] raising the possibility that cell-mediated mechanical forces exerted on collagen fibrils, like those mentioned above concerning cell-fibronectin interactions, might generate collagen-derived matricryptic sites.

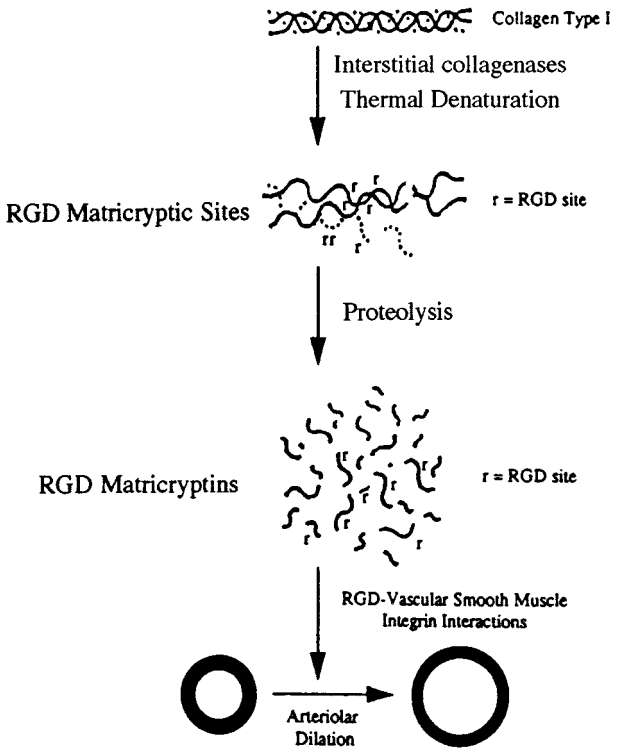

Figure 3. Generation of RGD-containing matricryptic sites and matricryptins from collagen type I regulates arteriolar vasomotor activity.

RGD Sites May Be a Fundamental Matricryptic Signal

Many studies throughout the years suggest that RGD sites may represent a fundamental matricryptic signal within ECM proteins. A very important unanswered question concerns the normal density of RGD integrin-binding sites in noninjured tissues. We speculate that very few exposed RGD sites exist in normal tissue and that after tissue injury, a marked increase in the density of RGD sites will occur. Thus, RGD sequences may be an important mediator in tissue repair responses along with other mediators that regulate inflammatory or wound repair phenomena. The potential sources of this RGD signal include: 1) plasma-derived proteins, fibronectin, vitronectin, and fibrinogen; 2) alteration of pre-existing ECM proteins such as collagens; and 3) proteins synthesized in the wound site such as osteopontin, fibronectin, and tenascin. The abundance of collagens, and the fact that most collagens contain multiple RGD sequences (ie, 7- type I, 11- type IV, 11- type VI), strongly suggests that these proteins may constitute a biologically significant source of RGD sequences for cellular injury responses.

An important question is whether the exposure of RGD sites within tissue injury sites is required for appropriate responses of cells to initiate and propagate the wound-repair response program. A related consideration is whether RGD sites are necessary for malignant tumor growth and progression because the tumor microenvironment is reminiscent of wounds with a prominent provisional ECM containing fibrin and other RGD-containing proteins such as fibronectin and osteopontin.[16,91] Major cellular responses within wounds include proliferation, migration, phagocytosis, as well as differentiation and apoptosis.[92] All of these responses are known to be regulated by RGD sequences and by RGD-binding integrins, such as $\alpha v\beta 3$.[93] To illustrate this point further, one can consider the conditions used to propagate cells in tissue culture which mimic a wound environment. Two major proteins from serum that are critical to cell adhesion during growth in tissue culture are vitronectin and fibronectin,[86,87,93] both RGD-containing ECM proteins. Also, certain cell types such as endothelial cells proliferate more readily when the substrate is first coated with denatured collagen,[94] a protein containing multiple RGD sites. Denatured collagen substrates also strongly adsorb serum-derived fibronectin as well as fibronectin secreted from cells.[44] Intriguingly, heparin is also typically added to endothelial cells to enhance proliferation.[95] Thus, three components of a putative wound scaffold matrix are present (denatured collagen, fibronectin, heparin) to optimize endothelial cell growth (see later on). It would seem that optimal tissue-culture conditions include RGD-containing ECM substrates. What remains unclear is whether RGD signals are actually required for the growth of adherent normal or transformed cells. We speculate that the recruitment of RGD-containing proteins from plasma, the exposure of cryptic RGD sites from existing ECM, and the synthesis of RGD containing ECM proteins within wound sites provides evidence for an RGD signal requirement for appropriate cellular responses during tissue injury *in vivo*.

Other studies demonstrate the importance of RGD-mediated signaling in microvascular responses such as arteriolar vasomotor activity and the regulation of angiogenesis.[17,22,96–98] The $\alpha v\beta 3$ integrin expressed on vascular smooth muscle cells was shown to regulate arteriolar vasodilation in response to RGD peptides[22,98] and this receptor is markedly induced on endothelial cells during angiogenesis.[96,99] Reagents which interfere with $\alpha v\beta 3$ function can inhibit angiogenesis and induce vessel regression through an apoptotic mechanism.[97] In addition, $\alpha v\beta 3$ has been reported to directly interact with MMP-2 on the cell surface to modulate both adhesive and proteolytic function.[100] One interesting aspect of this finding is that both $\alpha v\beta 3$ (through RGD sites) and MMP-2 (through its fibronectin-like domain) have direct binding affinity for denatured collagen. This affinity may facilitate the interaction between $\alpha v\beta 3$ and MMP-2 on the cell surface. Also, MMP-2 is a potent enzyme that degrades denatured collagens[70,100] and MMP-2, like other enzymes, should be capable of generating biologically active RGD-containing fragments from denatured collagen degradation. Such interactions may play an important role in the control of proteolytic balance which is a critical regulator of tissue morphogenesis and regression as well as tumorigenesis.[2,70,79,101,102]

Matricryptins Induce Signals, Alter Cell Behavior, and Alter Microvascular Responses

Many studies throughout the years have strongly implicated the role of ECM breakdown in the generation of new biological signals. Enzymes that are either known to generate such fragments or which may participate in their generation include MMPs, serine proteases (ie, plasmin and neutrophil elastase), and enzymes that degrade glycosaminoglycans (ie, hyaluronidase and heparinase).[70–73,78] Here, we have proposed the name, matricryptins, to describe these biologically active fragments of ECM. Matricryptins are derived from molecular domains that are cryptic and enzymatic breakdown is required to expose the new biologically relevant activity. A series of biological activities can now be attributed to matricryptins such as effects on cell proliferation, vascular permeability, cell migration, arteriolar vasoreactivity, stimulation and inhibition of angiogenesis, ECM assembly, and focal contact stability (Table 1).

Recent work from our laboratories[22] has shown that proteolytic fragments of denatured collagen type I induce arteriolar vasodilation in a manner identical to that of synthetic RGD peptides (Figure 3). In both cases, this vasodilatory response involved the $\alpha v\beta 3$ integrin expressed by the arteriolar vascular smooth muscle. Blocking antibodies directed to $\alpha v\beta 3$ were shown to inhibit these responses. Increased blood flow to injured tissues is a well-known consequence of tissue injury and may in part be controlled by this RGD matricryptin-mediated mechanism. Further studies using patch-clamp recordings have shown that soluble RGD peptides, when added to isolated vascular smooth muscle cells, decrease Ca^{2+} current through L-type Ca^{2+} channels.[98] This decreased Ca^{2+} current, which lowers intracellular Ca^{2+}, is known to induce a vasodilatory response which is consistent with our results showing RGD peptide-induced vasodilation of intact arterioles.[22,103] Interestingly, this decreased Ca^{2+} current occurred equally well in vascular smooth muscle cells when the RGD signal was supplied in either a soluble or insoluble manner.[98] A previous study revealed an $\alpha v\beta 3$ integrin signaling response to soluble ligands in endothelial cells.[104] In contrast, the $\alpha 5\beta 1$ integrin was found to increase Ca^{2+} current through L-type channels in vascular smooth muscle cells, but it could only signal when its ligands were presented in an insoluble form.[98] These data suggest that $\alpha v\beta 3$ may possess a special ability among the integrin family of receptors to signal when ligands are presented in a soluble form. This property may allow this receptor to serve as a wound or RGD sensor to detect RGD locally or after diffusion from distant sites which could be an important component in a wound recognition and response system.

Matricryptic Sites May Participate in the Formation of a Wound Repair Scaffold

As mentioned above, matricryptic sites exist within ECM molecules such as fibrinogen and fibronectin which are major components of the provisional ECM that forms within injured tissues after increases in vascular permeability. Evidence suggests that matricryptic sites play a role in both fibrin and fibronectin matrix assembly (Table 1) and because these two molecules have affinity for each other, it is possible that they also play a role in the formation of a fibrin-fibronectin scaffold. In addition, fibronectin has the ability to selectively adsorb to denatured collagen.[44,45] This interaction has formed the basis for one of the steps in the purification of fibronectin from human plasma.[44,105] Although fibronectin does bind native collagens, it shows a markedly increased affinity for denatured collagen.[44,45] Because collagen denaturation occurs within areas of tissue injury,[70–74,78,80,102] this affinity may allow fibronectin to preferentially adsorb to these areas. An intriguing possibility is that fibronectin-denatured collagen complexes present RGD or other matricryptic sites in such a way that provides a signal unique to an injured tissue compared to a normal tissue. Further consideration suggests the possibility that heparin, released from mast cells during tissue injury, could bind fibronectin-denatured collagen complexes to stabilize this interaction or affect the presentation of matricryptic sites. Previous studies indicate that heparin increases the affinity of fibronectin for denatured collagen.[44,45] The complex of fibronectin/denatured collagen/heparin might serve as a nucleation center for the selective entrapment of molecules involved in wound repair such as growth factors, ECM proteins, proteases, and protease inhibitors. Many growth factors and ECM components have affinity for heparin or other components of this trimolecular complex.[9,17,44,58,87] Thus, the generation of denatured collagen after tissue injury could serve as a component of a nucleation center for the accumulation of repair molecules precisely within the wound site. The recruitment of plasma-derived ECM such as fibrinogen/fibrin, which also directly interacts with fibronectin, might serve as the structural scaffold (ie, fibrin clot) on which the assembly of this wound repair apparatus could occur. In support of these concepts, low-density lipoprotein uptake by macrophages was found to be markedly enhanced by the concomitant presence of fibronectin/denatured collagen/heparin complexes.[106] This data suggests that, in addition to serving as a nucleation center, the complex may directly stimulate cellular functions (eg, phagocytosis) necessary for proper wound repair responses.

Summary

In summary, it is clear that matricryptic sites and matricryptins represent an important class of biological information that is revealed to cells during tissue injury responses. One of the most significant matricryptic sites is the RGD sequence, which appears to be a cryptic site in many, if not all, ECM proteins that possess the sequence. This sequence seems to play a special role in microvascular signaling by regulating arteriolar vasoactivity as well as angiogenic responses. The RGD sequence also appears to play a fundamental role in cell phenomena critical to the repair of injured tissue such as cell prolif-

eration, migration, survival, morphogenesis, and phagocytosis. In addition to RGD sites, many recent studies have identified new non-RGD matricryptic sites that are currently being molecularly characterized. A wide variety of biological activities can now be attributed to these matricryptic sites and include effects on cell proliferation, migration, ECM matrix assembly, development of a wound repair scaffold, arteriolar vasoreactivity, focal adhesion stability, and morphogenesis. Thus, the regulated exposure of matricryptic sites represents a fundamental step during tissue repair responses and acts in concert with other mediators to control these events.

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