Growth Factor–Extracellular Matrix Interactions Regulate Wound Repair

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Background: Wound healing is a process made up of several phases, including hemostasis/inflammation, proliferation, and scar formation/remodeling. An array of growth factors is produced during each of these phases that helps direct the repair process.

The Problem: Most of the information we have about the biological actions of growth factors are from studies examining a growth factor in isolation. However, growth factors are known to interact with the extracellular matrix (ECM), and these associations influence cell behavior. Details about these interactions within the complex and continuously changing wound environment are not well understood and are likely to be very important during repair.

Basic/Clinical Science Advances: Several types of growth factor/ECM interactions have been described that could impact wound healing. The ECM can interact directly with growth factors, offering protection from degradation and controlling bioactivity of the growth factor. Portions of the ECM can bind to growth factor receptors, and cell–ECM binding can influence growth factor receptor signaling. Growth factors can also control production and degradation of the ECM; therefore, the relationship between growth factors and ECM is bidirectional.

Clinical Care Relevance: New information about the relationship between growth factors and ECM could be used to optimize growth factor-based therapies or lead to the development of novel treatment strategies for wound care.

Conclusion: Growth factor–ECM interactions likely have a strong impact on the rate and quality of healing. A better understanding of the relationship between these classes of molecules and how it can be exploited to enhance healing is needed.

BACKGROUND

THE RESPONSE TO INJURY involves a series of complex and highly regulated events that, if successful, lead to complete healing of the wound. The repair process is generally divided into phases of hemostasis/inflammation, proliferation, and scar formation/remodeling.^{1,2} Immediately after injury, resident inflammatory cells become activated, and circulating inflammatory cells traffic to the wound site, where they clear cellular

debris and destroy potential pathogens. Keratinocytes are responsible for restoring the epidermal barrier, whereas endothelial cells and fibroblasts respond in the dermis by taking part in angiogenesis and producing new extracellular matrix (ECM), respectively. Eventually, fibroblasts remodel the new dermal matrix, and mature scar tissue is formed. Various signals direct the cellular responses during each phase of healing, but growth factors are among the most

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Abbreviations and Acronyms

2D	= two-dimensional
3D	= three-dimensional
DDR	= discoidin domain receptor
ECM	= extracellular matrix
EGF	= epidermal growth factor
EGFR	= epidermal growth factor receptor
FGF-2	= fibroblast growth factor 2
FGFR	= fibroblast growth factor receptor
HSPGs	= heparan sulfate proteoglycans
MMPs	= matrix metalloproteinases
PDGF	= platelet-derived growth factor
TGF- β 1	= transforming growth factor beta 1
TSP	= thrombospondin
VEGF	= vascular endothelial growth factor
VEGFR-2	= vascular endothelial growth factor receptor 2

important. An array of growth factors is produced at different times, eliciting diverse effects on multiple target cells. For effective repair of a wound, the correct growth factors should be presented within the proper framework. The ECM serves as the framework and together, the combination of growth factors and ECM shape cellular responses and the ultimate outcome of repair.

TARGET ARTICLE

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CLINICAL PROBLEM ADDRESSED

In the majority of cases, damaged skin proceeds smoothly through the stages of healing, leading to effective repair of the wound. However, for many patients, the wound fails to advance through the phases appropriately, thus leading to clinical problems such as chronic, nonhealing wounds or excessive scarring. It is very likely that inappropriate growth factor–ECM interactions, which then negatively impact cellular responses, are at the root of these issues. More detailed information about the relationship between growth factors and the ECM could be useful for developing novel wound healing therapies and for enhancing the effectiveness of current therapies.

RELEVANT BASIC SCIENCE CONTEXT

Many growth factors involved in wound healing interact with the ECM, including platelet-derived growth factor (PDGF), fibroblast growth factor 2 (FGF-2), vascular endothelial growth factor (VEGF), and transforming growth factor beta 1 (TGF- β 1). The ECM is made up of many molecules that are in constant contact with both growth factors and neighboring cells. In addition to providing a structural support system, the ECM can act as a foundation for cell adhesion and a storage site for growth factors.³ There are several broad classes of ECM molecules that play a part in wound healing, including structural proteins, adhesive glycoproteins, glycosaminoglycans/proteoglycans, and matricellular proteins.^{4–6}

Structural proteins are an important group of ECM molecules in the context of cutaneous wound healing, because they are responsible for maintaining the integrity of the skin. Collagen and elastin belong to this group of proteins. Collagen is

one of the most abundant dermal ECM components, and it should be deposited, appropriately aligned, and cross-linked to increase the tensile strength of the injured area. An overproduction of collagen, though, can lead to the formation of abnormal or excessive scars.

Adhesive glycoproteins, including fibronectin and laminin, are ECM components present in the dermis as well as in basement membranes surrounding blood vessels and separating the epidermal and dermal layers of the skin.^{4–6} Among other functions, fibronectin and laminin play important roles in cell migration.

Glycosaminoglycans are polysaccharides, and proteoglycans are polysaccharides that are linked to a protein core. Hyaluronan is a glycosaminoglycan, whereas versican, syndecan, chondroitin sulfate, and heparan sulfate are proteoglycans. These molecules help maintain tissue hydration by binding water, and some, particularly heparan sulfate, are important for modulating growth factor activity.⁴

Finally, matricellular proteins interact with structural ECM proteins and cell surface receptors. Thrombospondins (TSPs), tenascin, and osteopontin are matricellular proteins. Unlike some of the other ECM molecules, matricellular proteins are thought to play more of a regulatory role, rather than a structural role. TSP-1, for example, is capable of converting latent TGF- β 1 to the active form, thereby regulating its activity.⁷ Since TSP-1 levels increase in healing wounds,⁸ it could contribute to the activation of TGF- β 1 during wound healing and, as a result, impact the overall outcome of the repair process.

EXPERIMENTAL MODEL OR MATERIAL: ADVANTAGES AND LIMITATIONS

When the skin is damaged, cells, growth factors, and the ECM work in concert to effectively repair the wound. The continuous, multi-directional interactions between cells, growth factors, and ECM components are referred to as *dynamic reciprocity*.^{4,9} This concept was initially used to describe the effect of ECM on endothelial cell activity and on gene expression in cells,^{10,11} but the idea is highly relevant to wound healing as well. The give-and-take relationship between cells and the growth factor signals and ECM components surrounding them is apparent at each stage of wound repair. Unfortunately, these relationships are quite complicated and are difficult to recapitulate in an experimental system. Often, the response of a particular cell type to a growth factor, using read-outs of receptor activation, signaling, or various

cellular responses, is tested in two-dimensional (2D) cell culture systems. This type of approach can generate useful information in some cases, but it does not mimic the conditions under which a cell would normally interact with a growth factor or the ECM. Three-dimensional (3D) cell culture models could be used to more accurately replicate the *in vivo* environment. Alterations in the behavior of cells in 2D versus 3D culture, which includes differences in the response to growth factors, is well documented.¹² Knockout animals have been utilized to study growth factor–ECM interactions *in vivo*.¹³ This offers an advantage over cell culture systems by allowing one to examine the effects of deleting a single growth factor or ECM component in a biologically relevant setting, but as with any knockout mouse model, there are disadvantages. Perhaps most importantly, there is a risk of inadvertently disrupting multiple growth factor–ECM interactions by eliminating a single growth factor or ECM component. The development of new systems for studying complex growth factor–ECM interactions may be needed if we are to understand them completely.

DISCUSSION OF RELEVANT LITERATURE

Growth factors can interact with the ECM in multiple ways, and these interactions control the behavior of surrounding cells. The interactions can either be direct or indirect, and both types are important for the wound repair process. Several general categories of growth factor/ECM interactions with some specific examples are discussed next.

Growth factors interact directly with the ECM

Many growth factors interact directly with ECM components, which, in turn, dictate cellular responses. The ECM can bind to and sequester growth factors. Through this binding, the ECM can protect growth factors from degradation or help form concentration gradients, which are important for directing the migration of cells to a certain site within the tissue. The ECM can serve as a storage place for growth factors, which can later be released, free to diffuse through the tissue until it comes into contact with its cognate growth factor receptor. In addition to acting as soluble mediators, growth factors can also be presented by the ECM to their receptors in a way that facilitates activation and signaling (see Summary Illustration section). In this way, ECM binding can regulate cellular responses to growth factors. Growth factor–ECM binding is actually required for FGF-2 signaling.

Both FGF-2 and the FGF receptor (FGFR) interact directly with extracellular heparan sulfate proteoglycans (HSPGs). A single HSPG molecule can bind multiple FGF-2 proteins and multiple FGFR molecules. As a result, HSPGs are thought to not only facilitate binding of FGF-2 to FGFR, but also assist in dimerization of two FGFR molecules.¹⁴ As is the case with most growth factor receptors, dimerization is an important initial step in receptor activation and subsequent signaling. Under some circumstances, ECM binding can inhibit the activity of a growth factor. In the case of TGF- β 1, binding to the proteoglycans decorin, betaglycan, and biglycan restricts its activity.¹⁵

ECM domains act as “matrikines”

Matrikines are portions of ECM molecules that are capable of binding to and activating cell surface receptors (see Summary Illustration section).¹⁶ Matrikines fall into one of two major categories. Natural matrikines are capable of signaling in their native form, whereas matricryptins (or cryptic matrikines) require a conformational change or proteolytic cleavage to become a functional matrikine.¹⁷ The epidermal growth factor (EGF)-like repeats present in tenascin-C function as a natural matrikine and bind to the epidermal growth factor receptor (EGFR). Similar to tenascin-C, laminin-332 (previously laminin-5) also contains EGF-like repeats that can signal through EGFR; however, laminin-332 is considered a matricryptin, because it is cleaved by matrix metalloproteinases (MMPs) to expose the EGF-like repeats. Although these two matrikines have weak EGFR binding affinity, they are able to stimulate EGFR signaling.¹⁷ These signals appear to be important for the migration of keratinocytes and fibroblasts.¹⁶ A substantial increase in the levels of these matrikines, as observed during wound healing,¹⁷ is likely necessary for effective signaling due to the low binding affinity to EGFR. In addition to EGFR, discoidin domain receptors (DDRs) can bind ECM components. The binding of DDR2 to collagen, for example, regulates fibroblast proliferation and migration and induces the transcription of MMPs.¹⁸

Contact between the cells and the ECM mediates growth factor signaling and activation

In some instances, cells mediate indirect growth factor–ECM interactions through integrin molecules (see Summary Illustration section). Integrins are cell surface receptors that regulate the attachment of cells to ECM. α V β 3 integrins on endothelial cells play an important role in angiogenesis, or new blood vessel growth. Binding of these integrins to the glycoprotein vitronectin can enhance endothelial cell

responsiveness to VEGF, and integrin signaling can result in activation of VEGF receptor 2 (VEGFR-2).¹⁹ $\alpha V\beta 6$ integrin, which is upregulated in keratinocytes after injury,²⁰ is another integrin that can interact with growth factors. This integrin is able to cause local activation of latent TGF- $\beta 1$ by binding to latency-associated peptide.¹³

Growth factors regulate the ECM

The relationship between growth factors and the ECM is bidirectional. The ECM can regulate growth factor production and signaling, and growth factors can also alter the composition of the ECM. Several growth factors play a prominent role in regulating the ECM, either by stimulating the production of ECM components or stimulating the production of molecules that break down the ECM (see Summary Illustration section). Although PDGF and other growth factors are known to stimulate the production of structural ECM proteins such as collagen, TGF- $\beta 1$ is one of the most important regulators of the ECM. TGF- $\beta 1$ regulates the production of multiple ECM components, including collagen, fibronectin, hyaluronic acid, TSP, and tenascin.⁴ TGF- $\beta 1$ also influences the ECM by inhibiting the production of proteases and increasing the synthesis of protease inhibitors.⁴

INNOVATION

Given the importance of growth factor–ECM interactions in regulating the repair process, it is likely that these interactions are disrupted when wounds do not heal properly. Topical growth factors have been used as a treatment strategy for poorly healing wounds. Unfortunately, the addition of growth factors alone may not be effective, considering the impact that the ECM has on whether or not a growth factor will activate signaling in a target cell. For example, chronic, nonhealing wounds contain higher than normal levels of proteases that alter the structure of the ECM. This can modify the ability of growth factors such as FGF-2 to bind to its receptors on the surface of cells. In addition, proteases are known to inactivate or degrade growth factors such as VEGF, PDGF, and TGF- $\beta 1$.⁴ Supplementing a chronic wound with protease-resistant growth factors has been proposed to overcome this obstacle.²¹

TAKE-HOME MESSAGE

Basic science

- Growth factors are important regulatory molecules in all phases of wound repair. They can bind receptors as soluble ligands or as solid-phase ligands when bound to the ECM.
- Bidirectional interactions exist between growth factors and ECM molecules that are important for wound healing.
- The ECM can store and release growth factors, protect them from degradation, or aid in the formation of concentration gradients, whereas growth factors can regulate the production and degradation of ECM components.
- Important categories of growth factor–ECM interactions include direct growth factor–ECM binding, binding of ECM domains (matrikines) to cell-surface receptors, integrin-mediated stimulation of growth factor signaling, and regulation of ECM composition by growth factors.

Clinical science

- Chronic, nonhealing wounds do not progress through the normal phases of repair.
- Disrupted growth factor–ECM interactions could be an underlying cause of insufficient healing in chronic wounds.
- The addition of growth factors to wounds has been used with limited success to accelerate healing in chronic wounds, but it is not known whether these exogenous growth factors interact properly with the ECM. This could significantly influence the therapeutic efficacy of the growth factors.

Relevance to clinical care

- Growth factor therapy for the treatment of chronic wounds may be optimized by taking advantage of their relationship with the dermal ECM.
- Protease-resistant growth factors could increase the effectiveness of treatments compared with growth factors that are not bound to and being protected from proteolytic degradation by the ECM.
- Incorporating ECM components into growth factor therapies may enhance cellular responses to the growth factor and result in a greater clinical response.
- Scaffolds that allow for controlled delivery of growth factors to the wound could offer new therapeutic options.

Another strategy for increasing the effectiveness of growth factor therapy is to deliver these molecules to wounds together with ECM fragments.²² A study by Upton and colleagues showed that growth factors complexed with vitronectin stimulated keratinocyte responses *in vitro* and accelerated reepithelialization *in vivo*, thus illustrating the utility of delivering growth factors with ECM components.²³ The timing of growth factor delivery may also be important, and scaffold-based delivery systems are being developed that allow for slow release of a single growth factor or even temporal release of multiple growth factors.²⁴ These new methods have the potential to refine

traditional growth factor-based therapies, thus making them more effective for wound care.

SUMMARY ILLUSTRATION

There are several ways that growth factor–ECM interactions can affect the behavior of surrounding cells, and ultimately, the outcome of wound healing. **(a)** Growth factors can bind directly to the ECM, which presents the growth factor in the proper context to activate growth factor receptor signaling. Heparin sulfate proteoglycans facilitate both the binding of FGF-2 to its receptor and receptor dimerization, thereby promoting downstream FGFR signaling. **(b)** Domains present within ECM molecules called *matrikines* can directly bind to and induce signaling of cell surface receptors. For example, EGF-like repeats within the ECM molecule tenascin-C are capable of binding to and activating EGFR. **(c)** Indirect interactions between growth factors and the ECM are also important, with integrins acting as a bridge between these two molecules. Under these circumstances, binding of integrins to the ECM can activate a downstream signaling cascade, which, in turn, stimulates growth factor receptor signaling. This type of interaction occurs when $\alpha V\beta 3$ integrins present on the surface of endothelial cells bind vitronectin, which then leads to VEGFR-2 signaling and enhanced responsiveness to VEGF. **(d)** Finally, growth factors can influence the ECM by increasing the synthesis of ECM components and/

or by inhibiting the production of proteases that break down the ECM. TGF- $\beta 1$ is a potent pro-fibrotic factor that stimulates the production of collagen and other ECM proteins by fibroblasts and also suppresses the production of ECM-degrading proteases.

CAUTION, CRITICAL REMARKS, AND RECOMMENDATIONS

Despite the importance of growth factor–ECM interactions on the behavior of the cells, the scope of these interactions within the complex wound environment is not well understood. Growth factors are often studied in isolation, in both basic science and clinical studies, despite the fact that these molecules act very differently within the framework of the ECM. This may, at least in part, explain why growth factor therapies have not been as successful as predicted for the treatment of chronic wounds.

FUTURE DEVELOPMENT OF INTEREST

Taking advantage of interactions between growth factors and their surrounding ECM may be key for optimizing growth factor-based treatments. For example, it may be important to alter the ECM before applying growth factors⁴ or growth factors may have to be delivered in combination with ECM components^{22,23} to achieve optimal results. Alternatively, the timing of growth factor release

or the delivery of multiple growth factors at different times by using innovative scaffolds and delivery systems may increase the clinical response to growth factors.²⁴ In any case, a greater understanding of the interactions between growth factors and the ECM and details about how they regulate each aspect of healing are needed. New information about these interactions could be useful for developing new treatments for recalcitrant wounds.

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