Fibronectin Interaction and Enhancement of Growth Factors: Importance for Wound Healing

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Significance: This critical review focuses on interactions between cells, fibronectin (FN), and growth factors (GF).

Recent Advances: Initially, the extracellular matrix (ECM) was thought to serve simply as a reservoir for GFs that would be released as soluble ligands during proteolytic degradation of ECM. This view was rather quickly extended by the observation that ECM could concentrate GFs to the pericellular matrix for more efficient presentation to cell surface receptors. However, recent reports support much more complex interactions among GFs and ECM molecules, particularly FN, and the way these interactions can fine-tune cell responses to the microenvironment.

Critical Issues: Wounds that are unable to synthesize and sustain a functional ECM cannot optimally benefit from endogenous or exogenous GFs. Therefore, GF treatments have recently focused on utilizing ECM molecules as delivery vehicles. Thus, ECM can influence GF stability and activity, and GFs can modulate the ECM activity. Hence, both individually and together, ECM and GFs modulate cells that in turn control the type and level of GFs and ECM in the pericellular environment that ultimately results in new tissue generation. Although many ECM components are important for optimal tissue regeneration and wound healing, FN stands out as absolutely critical not only for wound healing and tissue regeneration but also for embryogenesis and morphogenesis.

Future Directions: Understanding ECM/GF interactions will greatly facilitate our understanding of normal wound repair and regeneration, the failure of wounds to heal, and how the latter can be salvaged with proper ECM/GF combinations.

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Submitted for publication November 13, 2014. Accepted in revised form January 30, 2015.

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SCOPE AND SIGNIFICANCE

IN THIS STUDY, WE REVIEW a multitude of physical and biological interactions between cells, fibronectin (FN), and growth factors (GFs) that have important implications for the treatment of wounds. The different ways in which cells, FN, and GFs can interact include the following: FN as a reservoir for GF; FN cryptic sites that bind and enhance GFs after exposure by cell tension forces or enzymatic release as soluble peptides;

FN regulation of GF and GF receptor (GFR) expression; FN- and GF-receptor hybrid adhesion complexes that integrate signaling; direct activation of GFR through FN receptors; FN preconditioning of cells for GF signaling; interaction of FN, heparin/heparin sulfate (HS), and GFs.

TRANSLATIONAL RELEVANCE

Disturbances in GFs and the extracellular matrix (ECM) account for

a long list of human diseases, including chronic wounds. We believe that better understanding ECM-GF interactions will lead to breakthrough therapies for large acute wounds that cannot be closed and nonhealing wounds that use a combination of ECM and GF or their components.

CLINICAL RELEVANCE

Chronic wounds differ from acute wounds not only by absence of cells but also by the paucity of provisional ECM, such as FN. The latter is a result of the imbalance between proteolytic enzymes and their inhibitors. Many clinical trials using GF have been largely disappointing. Recently, however, preclinical studies with GF and engineered ECM containing GF-binding domains show promise,^{1–4} presumably secondary to the critical dependence of GF on ECM to provide optimal signals required for rapid wound healing or regeneration.

BACKGROUND

The three-dimensional network of the ECM is a dynamic structure constantly undergoing cell-mediated reorganization. ECM is composed of structural proteins such as the collagens and elastin; adhesive glycoproteins, such as FN, vitronectin, and laminin; hyaluronan, highly hydrated glycosaminoglycans (GAGs); proteoglycans such as versican, syndecans, glycans, and perlecan; and matricellular proteins, such as secreted protein acidic and rich in cysteine (SPARK), thrombospondin 1 and 2 (TSP-1 and TSP-2), tenascin C and X, and osteopontin that modulate ECM interactions and cell functions.⁵ Besides providing structural support for organs, the entire organism, and adhesive sites for tissue cells and extravasated leukocytes, molecules of the ECM bind GFs and modulate their activity. Information in this newer dimension of ECM function has rapidly expanded and undoubtedly plays a critical role during embryogenesis, morphogenesis, wound healing, and regeneration. In this brief review, we will describe in detail FN, a critical component of the provisional ECM in embryogenesis, morphogenesis, wound repair, as well as FN receptors, and how their interactions with GFs can modulate cell function. Many other ECM molecules also bind and enhance GFs.^{5,6} Unfortunately, the space allotment for this review is not sufficient for an extensive review that would cover all ECM-GF interactions. Nevertheless, we have compiled a table, which addresses many of the interactions of GF with ECM proteins (Table 1). In addition, fibroblast growth factors (FGFs) 1–9, platelet-derived growth factors (PDGFs), hepatocyte growth factor (HGF), and

Table 1. Growth factor binding to ECM proteins

Growth factor	ECM protein association	Released from ECM active/latent
TGF- β	Type IV collagen, betaglycan and decorin, heparin/HS, FN, fibrinogen, fibrin	Latent
HGF	Type I, III, IV, V, and VI collagen, heparin/HS, FN, fibrinogen, fibrin	Latent
CSF	Heparin/HS	Latent
IGF	Insulin-like growth factor-binding protein	Latent
PDGF	SPARC (secreted protein acidic and rich in cysteine), heparin/HS, FN, fibrinogen, fibrin	Active
VEGF	Collagen, heparin/HS, FN, fibrinogen, fibrin	Active
FGF	heparin/HS, FN, fibrinogen, fibrin	Active

CSF, colony stimulating factor; ECM, extracellular matrix; FGF, fibroblast growth factor; FN, fibronectin; HGF, hepatocyte growth factor; HS, heparin sulfate; IGF, insulin-like growth factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.

vascular endothelial growth factors (VEGFs) all bind heparin and HS.

The ECM directly affects a variety of cellular functions, including proliferation, differentiation, migration, survival, cytoskeletal organization, and contractility.⁷ The physical connection between the cells and the ECM is established through transmembrane cell adhesion receptors called integrins. Composed of two noncovalently associated transmembrane glycoprotein subunits, 18 distinct α - and 8 unique β -subunits have been identified *in vivo*. The extracellular components of integrins bind to the ECM through Ca^{2+} and Mg^{2+} cation-dependent mechanisms. The intracellular components interact with the cytoskeleton through adaptor proteins, namely talin, α -actinin, and filamin.

Anchorage to the ECM is required for normal cells to enter the S phase, even in the presence of GFs. Furthermore, detachment from the matrix often promotes apoptosis known as anoikis, observed in epidermal keratinocytes detaching from the basement membrane.⁸ The bidirectional flow of information between cells and the ECM allows for a feedback loop of constant regulation of cell activity known as dynamic reciprocity, a term first coined by Mina Bissell, where integrin or other ECM receptor triggered signals initiate a cascade of events through the cytoskeleton as well as classic signal cascade systems into the nucleus, resulting in the expression of specific genes that may alter the production of ECM molecules, ECM degradation enzymes and/or their inhibitors, and ECM receptors.^{9,10} Thus, cells and their products degrade old, synthesize new, and remodel existing ECM by changing its organization, conformation, and elasticity. ECM degradation and remodeling are often mediated by a family of enzymes known as matrix metalloproteases (MMPs), which are

upregulated during tissue restoration, angiogenesis, re-epithelialization, and leukocyte influx.¹¹ MMPs have also been shown to degrade GFs, their receptors, and angiogenic factors.

To add further complexity to this system, ECM can bind GFs serving as a reservoir system by protecting them from protease degradation and holding them inaccessible to their receptors until enzymatic processing of ECM releases them.¹² On the other hand, ECM-bound GFs can be presented more efficiently to their receptors and often demonstrate enhanced activity through a variety of mechanisms, as discussed for FN in the following paragraphs and in a recent review.¹³

RECENT ADVANCES

ECM and GFs

Initially, positively charged GFs were found to bind negatively charged ECM GAGs (Table 2) and were thought to be released by enzymatic degradation of the GAGs.¹⁴ From this framework, it was proposed that GAGs may act as a reservoir for GFs that were released from the solid phase to function as soluble ligands. Since that time, this understanding has been greatly expanded, as there have been multiple reports of GFs binding to ECM proteins without the involvement of GAGs.⁵ The association of GFs with the ECM allows storage of large quantities of signaling molecules in a readily available form. GFs stored within the ECM may aid in the maintenance or recovery of organ structure and function.¹⁵ This understanding offers new insights into the dynamics of tissue processes, such as healing, development, and regeneration.

Some GFs exist constitutively in their biologically active form, such as PDGF-BB, VEGF, and FGF-2, while other GFs occur constitutively in latent forms having ~1% of the biological activity, such as HGF, transforming growth factor- β 1, 2, 3 (TGF- β 1-3), and colony stimulating factor-1 (CSF-1). As initially proposed, any ECM-bound GFs may be latent or hidden within the matrix and become active only after ECM degradation. In addition, intrinsic domains within ECM proteins might act as ligands for GFRs, such as epidermal growth factor (EGF)-like domains in laminin. These EGF-like repeats in ECM molecules can bind and activate GFRs in a direct manner, leading to cell proliferation.¹⁶ Furthermore, binding of ECM-associated GF to its receptor may prevent endocytosis and result in prolonged, intense, and localized signaling by the ECM-GF/GFR complex. The plethora of outcomes from ECM-GF interactions will be further elucidated through our focus on FN and its GF associations.

Table 2. Biological activity of growth factors

Growth factor	Function
IGF-1, 2	<ul style="list-style-type: none"> Stimulates cell mitogenesis, differentiation, and survival. Amplifies activity through engagement of integrins (IGFBP-2, -3, -5) or ECM glycosaminoglycans heparin-binding domains (IGFBP-6); affecting cell adhesion and migration.^{78,79}
TGF- β 1, 2	<ul style="list-style-type: none"> Modulates cell growth and differentiation. Stimulates the synthesis of collagen, FN, and other ECM components, including hyaluronic acid, TSP, tenascin. Increases production of protease inhibitors while reducing synthesis and secretion of proteases.^{6,15,80} TGF-β1 and 2 promote scar formation, while TGF-β3 aids in matrix organization to limit scarring through improved neostromal architecture.
EGF	<ul style="list-style-type: none"> Stimulates epithelial cell proliferation. Regulates a subset of G1 cell-cycle events. Elevates levels of EGFR or mutant versions have been detected in epithelial cell-derived tumors.⁶⁰
HGF	<ul style="list-style-type: none"> Stimulates matrix remodeling and epithelial regeneration. Inhibits fibrosis. Inactive form found in organs such as liver, spleen, and kidney and activated after injury in the specific organ. Activated after tissue damage.⁸¹
FGF-2	<ul style="list-style-type: none"> Induces fibroblast proliferation and angiogenesis during wound healing.¹⁰ Oligomerized FGFs, potentiated by HS, prolong the stimulation by providing storage and protecting it from proteolysis and endocytosis.⁶
PDGF-BB, AB	<ul style="list-style-type: none"> Regulates angiogenesis.⁸² Occurs in five isoforms -AA, -AB, BB, CC, and DD.⁸³ Chemotactic for fibroblasts and monocytes. Accelerates granulation tissue formation and ECM deposition
VEGF-A	<ul style="list-style-type: none"> Controls blood vessel formation and growth.⁶ Binds to the heparin-binding sequences in FN to synergistically promote endothelial cell proliferation.⁸⁴

EGF, epidermal growth factor; EGFR, EGF receptor; IGFBP, insulin-like growth factor binding protein; TSP, thrombospondin.

FN and GFs

FN is a multifunctional protein most abundantly found in the ECM under dynamic remodeling conditions such as wound healing, development, and the progression of disease such as cancer and atherosclerosis. FN, in acute wound repair is initially deposited from blood plasma in the context of a fibrin clot, whereas later it is deposited by activated tissue cells, including epidermal cells, fibroblasts, and endothelial cells.¹⁷ Through its fibrillar structure, FN reinforces perimatrix formation, where it serves as a biological glue mediating interaction between cells and other ECM proteins⁶ and as a pericellular structure for collagen matrix deposition.¹⁸ Cell-derived FN is polymerized into ECM fibrils through a cell-dependent process that can be rapidly up- or downregulated depending on the local signals.¹⁹

Within the ECM, FN is organized as an extensive network of elongated branching fibrils that range from 5 to 25 nm in diameter. FN fibrils can be stretched to four times their length by cells through slippage of individual fibrils along each other.²⁰ A similar slippage mechanism involving the actin cytoskeleton and integrin receptors is used intracellularly.¹⁹

Although FN comes from a single gene,²¹ the resulting protein can exist in twenty different isoforms.²² Depending on alternative splicing of a single 8 kb mRNA, yielding subunits range in size from 230 to 270 kDa. FN is a dimeric glycoprotein with the two monomers linked at their carboxy terminus by two interchain disulfide bonds, for a total molecular weight of ~500 kDa. Each monomer is composed of 12 type I, 2 type II, and 15–17 type III domains (the latter depends on exon usage and can result in two extra type III domains).^{22,23} FN I and II domains are ~40–60 amino acids in length, respectively, while type III domains are ~90 amino acids in length, composed of seven beta-strands arranged in antiparallel β -sheets connected by flexible loops without disulfide bonds.²² The presence (FN I and II domains) or absence (FN III domains) of intra molecular disulfide bonds provides differential structural integrity or flexibility in these domains, respectively, in response to applied forces.¹⁹ FN modules, grouped as compact domains interconnected by flexible strands, present numerous binding sites for cells, other ECM proteins, and GFs.

The coordinated stepwise process of the FN matrix assembly begins when FN binds to the cell surface through the FN integrin receptor $\alpha 5\beta 1$. Subsequent FN–FN interactions cause conformational changes that promote self-association and stabilization of the ECM FN fibrils. Further self-assembly sites are cryptic in FNIII₁, FNIII₂, and FNIII₁₀ domains and play an important role in fibrillogenesis.^{20,24–26} They are exposed as cell tension forces that pull open the domains and thereby are utilized in the FN matrix assembly. A number of ECM proteins depend on FN fibrils for matrix deposition either through direct or indirect association, such as collagens, fibrillin, fibulin, latent TGF- $\beta 1$ binding protein (LTBP), and tenascin C.^{18,27} FN possesses binding sites for collagen, heparan sulfate proteoglycans, and fibrin. The fibrin binding sites identified in FN are FNII_{1–5} and FNI_{10–12},²⁸ which play an important role in wound healing by mediating cell attachment and migration on fibrin clots.^{22,29}

FN as a reservoir for GF

Increasing evidence shows that GFs can bind to multiple sites in FN.^{30,31} Thus, FN can serve as a reservoir for GFs increasing their local bioavail-

ability. HGF binds FN and forms complexes with the HGF receptor (HGFR) and integrins, leading to enhanced cell migration.³² Using recombinant FN domains, the C-terminal heparin-II domain of FN (FNIII_{13–14}) was identified as a key VEGF-binding site. Mutation of the heparin-binding residues on FNIII_{13–14} abolished VEGF binding, and peptides corresponding to the heparin-binding sequences in FNIII_{13–14} inhibited VEGF binding to FN. These ECM associations with VEGF synergize to promote cell proliferation.^{33,34}

Martino and Hubbell (2010) found that FN III12–14 binds most of the GFs from the PDGF/VEGF and FGF families and some GFs from the transforming GF- β and neurotrophin families.³ Furthermore, smooth muscle spheroid sprouting was greatly enhanced when PDGF-BB was added to fibrin matrices functionalized with FN III12–14 compared to the native fibrin. In addition, we have shown that FN-GF-binding domains (FNIII₁, FNIII_{13–14}, and IIICS) enhance PDGF-BB survival signals in FN-null fibroblasts, only when they were surface bound.³⁰ These FN domains contain sequence similarities that bind PDGF-BB³¹ as well as FGF-2, and TGF- $\beta 1$ (Lin *et al.*, manuscript in preparation). These observations are similar to substrate-bound EGF being more potent than soluble EGF.³⁵ EGF presented in the solid phase is not internalized and degraded, but instead provides sustained signaling. On the other hand, FN-sequestered GFs can ultimately be released by controlled enzymatic digestion of ECM, as occurs in wound healing and other tissue regenerative processes.¹² Thus, localized, prolonged, intense GF activity can occur naturally without *de novo* synthesis.³⁶

Exposure of FN cryptic sites that bind and enhance GFs

ECM undergoes constant turnover and remodeling, most obviously during development, wound healing, and other states of new tissue formation. Some ECM molecules are flexible and extendable, particularly FN. The flexibility and extensibility of FN provide mechanisms by which GF-binding sites on relaxed FN in the absence of GAGs can be cryptic. For example, two classes of VEGF-binding sites on FN have been identified, one constitutively available and the other modulated by a FN conformational state in response to heparin and other GAGs.³⁷ In addition, cell-driven extension of FN can expose cryptic sites, while disrupting normally exposed sites.²⁰ In fact, tension on FN is required to expose cryptic sites within the second and tenth type III repeats (FNIII₂ and FNIII₁₀) for FN fibrillogenesis.^{20,24–26} Such cell tension on FN requires $\alpha 5\beta 1$ binding to the FN central cell-binding domain

(FNIII₉₋₁₀)³⁸ that is readily available when FN is in the relaxed state.²⁰ Using monoclonal antibodies to the PDGF-BB enhancing P12 peptide in FNIII₁ (see below), we have demonstrated that P12 residues in the context of intact FN can only be detected when FN is under tension from cell traction forces. Furthermore, cells that cannot generate sufficient traction forces fail to expose P12 residues and survive and grow poorly in the presence of PDGF-BB (Lin *et al.*, unpublished observations).

Soluble FN-derived peptides as GF cofactors

Our group first demonstrated that FN fragments could increase cell migration in response to the GF-abundant serum.³⁹ Later, proteolytically derived FN fragments that were 30 and 120 kDa were shown to increase microvascular, but not macrovascular, cell proliferation.⁴⁰ Recently, our group identified a FN peptide P12 from FNIII₁ (Fig. 1) that can bind PDGF-BB with high affinity (10–100 nM Dissociation Constant) and promote cultured human dermal fibroblast survival in the context of PDGF-BB and limit burn-induced dermal injury progression *in vivo* (Fig. 2).³¹ Moreover, P12 alters PDGF-BB/PDGF receptor (PDGFR) endocytosis and survival signals, that is, Akt phosphorylation.⁴¹ Results suggested that P12 shifts cell trafficking of PDGF-BB/PDGFR from clathrin-mediated (Fig. 3a–c) dynamin-dependent endocytosis (Fig. 3d) to a macropinocytosis-like pathway and thereby prolongs PDGFR signaling.⁴²

Hybrid FN- and GF-receptor adhesion complexes that integrate signaling

FN-integrin interactions play a key role in cellular behaviors, including cell migration, vascular development, and cohesion. One of the most studied sequences in FN is Arg-Gly-Asp (RGD), which is located in FNIII₁₀. This tripeptide binds many cell surface integrins, including $\alpha v \beta 3$ and $\alpha 5 \beta 1$.⁴³ In addition, a second amino acid sequence, PHSRN, in FNIII₉ stabilizes $\alpha 5 \beta 1$ integrin ligation to RGD.⁴⁴ FN ligation induces integrin clustering

and translocation of FN-bound integrins within the cell membrane as well as assembly of actin bundles through adaptor proteins, talin, α -actinin, vinculin, and filamin, and signal transduction through the focal adhesion kinase (FAK), Src kinase, and other molecules.^{19,45}

Although upstream signals initiated by integrin ligation are distinct from those stimulated by activated GFRs, downstream signal transduction pathways often converge, for example, the Ras-MAPK pathway, PI3K-Akt pathway, Rho family GTPases.^{46,47} FAK, which is associated with focal adhesions, hence its name, is one of the converging points between ECM and GF signaling.⁴⁸ Integrin-mediated cell attachment to ECM triggers FAK activation and is required for integrin-stimulated signaling⁴⁹ and in some cases, FAK is required to integrate integrin and GF signals.⁵⁰ Cells deprived of FAK are refractory to motility signals from EGF, while re-expression of FAK rescues these defects. These studies, however, did not directly demonstrate that integrin and GFR needed to be in close proximity.

In fact, GFRs and integrins can form signaling complexes through various mechanisms,^{51,52} including an ECM physical link that brings GFRs and integrins together.^{30,33,53} For example, using either FNIII₉₋₁₀ or FNIII₈₋₁₁ for the $\alpha 5 \beta 1$ cell-binding domains and FNIII₁, FNIII₁₂₋₁₄, or FNIII₁₂₋₁₅ for a GF-binding domain, synergistic effects on human dermal fibroblasts were only demonstrated when the cell- and GF-binding domains were coupled or presented on the same surface. These results strongly support the contention that GF and FN synergy under these conditions requires the cell-binding and GF-binding domains in close proximity outside the cell and may be organized, in part, by FN domains from adjacent FN molecules in a FN fibril. Proximity of such FN domains could act to organize focal adhesion complexes that contain GFRs as well as integrins in the plane of the membrane^{54,55} and promote integration of transduced signals from the two receptors types.^{16,30}

Figure 1. Schematics of FN and the FN first type III repeat that contains P12. **(a)** FN schematic showing type I repeats as *small rectangles*, type II repeats as *ovals*, and type III repeats as *large rectangles*. Arrows indicate locations of P12 peptide residues in the first type III repeat (FNIII₁) and central cell-binding domain RGD residues in the 10th type III repeat (FNIII₁₀). **(b)** Structural schematic showing the immunoglobulin sandwich β -pleated sheet secondary structure and location of P12 residues. FN, fibronectin (from Markus Seeliger, PhD, Department of Pharmacological Sciences, with permission). To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

Figure 2. P12 intravenous infusion 1 and 24 h after the burn inhibited injury progression in the rat hot comb burn model. (a and b) Burns at 7 days after injury without treatment. (c and d) Burns at 7 days after injury in animals that had been treated with 10 mg/kg P12. (e) Necrotic interspaces 2, 5, and 7 days after injury in rats treated or not treated with P12. (f) Necrotic interspaces 7 days after injury determined by histologic analysis. 0, infusion with lactated Ringer's buffer. Variance of interspace necrosis among rats within a specific treatment arm was no more than variance among interspaces on a given rat; therefore, interspace data for all five rats per treatment arm were pooled. $n = 30$ interspaces (six interspaces/rat) in five rats per treatment arm. Bar = 10 mm. Reprinted by permission from Lin *et al.*³¹ To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

FN regulation of GF and GFR expression

ECM signaling can regulate GF expression. For example, the spliced variant of FN that includes the so-called extra domain A increases VEGF-C expression in colorectal carcinoma.⁵⁶ The fact that ECM signaling can regulate GFR expression was exemplified by the study of Wang *et al.*, demonstrating that antibody blocking of $\beta 1$ -integrin caused a downregulation of the EGF receptor (EGFR) expression level in mammary epithelial cells.⁵⁷ EGFR expression was also downregulated when cells were detached from ECM.⁵⁸ Cell adhesion to ECM can also reduce GFR degradation, as demonstrated by increases in PDGFRs when cells were attached to the ECM compared to being in suspension.⁵⁹ Furthermore, an RGD-mimetic promoted VEGF-driven angiogenesis by increasing VEGF receptor (VEGFR-2) recycling.¹

Direct activation of GFRs through integrins

Although the binding of integrins and GFR is a distinct event,⁵⁴ it has been observed that a wide variety of receptor tyrosine kinases (RTKs) can be activated by integrins in the absence of GFR ligands⁶⁰ and leads to a subset of specific signaling molecules and regulation of the downstream function. Among the RTKs transmembrane receptors activated by integrins are EGFR, PDGFR, VEGFR, HGFR, and others.⁶⁰ It is unclear whether the cross talk between integrins and GFs is a result of mem-

brane proximal interactions or cooperation in the downstream signal transduction pathways or both.¹⁶

FN conditioning of cells for GF signaling

In addition to the ability of ECM to regulate GF or GFR expression and active GFRs directly, ECM can condition cells for GF signaling. Classically, this conditioning has been called anchorage dependence, which refers to the requirement of cell adhesion for survival.^{61,62} Loss of adhesion in normal cells usually results in growth arrest (often called anoikis) secondary to impaired GF responsiveness.^{63,64} Furthermore, different ECM can condition cells to generate different responses to the same GF. For example, under the same GF conditions, cell binding to FN through $\alpha 5 \beta 1$ activates Rac and cell-cycle progression, whereas $\alpha 2 \beta 1$ -mediated adhesion to laminin results in growth arrest.^{65,66}

ECM stiffness by generating increased focal adhesion complexes and cytoskeletal organization can also condition cells to generate different responses.⁶⁷ For example, ECM of differing stiffness differentially regulate stem cell differentiation.^{2,68} Besides mechanical signals effecting cell differentiation, substrate stiffness alterations can modulate the response of cells to GF stimulation. For example, a stiff substrate sensitizes epithelial cells to EGF, as demonstrated by the ability of a 4.5-fold substrate stiffening to reduce the amount of EGF needed to override contact inhibition.⁶⁹ ECM stiffness also

Figure 3. P12 rescued PDGF survival activity in CHC knockdown and dynamin-inhibited AHDF. **(a)** CHC siRNA was employed to knock down CHC expression in AHDF cells. The protein level dropped significantly after two consecutive transfections (36 h apart) with Hiperfect transfection reagents. **(b)** Mock cells were treated with 1 nM PDGF-BB $\pm$ 10 μ M P12 (sP12) in HBSS for 3 days, and cell metabolism was measured with the XTT assay. Experiment was repeated thrice (asterisk, $p < 0.05$ compared to PDGF alone, $n = 6$). Error bar indicates SD. **(c)** CHC knockdown cells were treated with 1 nM PDGF-BB $\pm$ 10 μ M P12 (sP12) in HBSS for 3 days, and cell metabolism was measured with the XTT assay. Experiment was repeated thrice (asterisk, $p < 0.05$ compared to PDGF alone, $n = 6$). Mean $\pm$ SD. **(d)** AHDFs were preincubated for 1 h in a serum-free medium containing 80 μ M dynasore, a dynamin inhibitor, and then treated with 1 nM PDGF-BB $\pm$ 10 μ M P12 in HBSS. Western blots were performed with antibodies against phospho-Akt, phospho-JNK, phospho-ERK1/2, phospho-SHP2, and eIF4e. GAPDH was used as a loading control. Data are representative of three independent experiments. AHDF, adult human dermal fibroblast; CHC, clatherin heavy chain; HBSS, Hank's balanced salt solution; PDGF, platelet-derived growth factor. Reprinted by permission from Zhu *et al.*⁴²

primes the TGF- β 1 pathway to promote chondrocyte differentiation.⁷⁰

FN, heparin/HS, and GFs

In addition to integrin binding, one of the most commonly observed interactions between FN and the ECM occurs through either heparin or HS binding. Heparin, a highly sulfated member of the HS family can be stored in granules of connective tissue mast cells and released at sites of injury and/or inflammation.⁷¹ To date, four heparin-binding modules have been identified on FN (FNI₁₋₅, FNIII₁, FNIII₅, FNII₁₃₋₁₄).⁷² The importance of FN-heparin/HS interactions was observed in fibroblasts that attach, but fail to spread due to the lack of focal contact formation on HepII-null FN fragments (FNII₁₂–FNII₁₅).⁷³ Binding between heparin/HS and FN or heparin/HS and GF is mostly charge-mediated since heparin/HS is highly negatively charged, while GFs, such as FGF-2,

Figure 4. FN matrix or peptides enhance GF activity on tissue cells to enhance new tissue formation or tissue remodeling. FN can influence GF stability and activity, and GFs can modulate FN activity. Hence, both individually and together, FN and GFs modulate cells that in turn control the type and level of GFs and ECM in the pericellular environment, which ultimately results in new tissue generation. ECM, extracellular matrix; GF, growth factor.

PDGF-BB/AB and HGF, are highly positively charged as are the FN heparin-binding domains. Thus, in the presence of heparin, complexes of GF-heparin-FN may form to facilitate signaling.

Studies using an engineered glutathione -S-transferase-tagged fusion protein containing a portion of FNIII₁ with an exposed cryptic R⁶¹³R⁶¹⁵K⁶¹⁷ heparin-binding site FNIII1H directly coupled to the central cell-binding domain (FNIII₈₋₁₀) revealed that this construct (GST/FNIII1H, 8–10) stimulates cell spreading, growth, contractility, and migration similar to FN.⁷⁴ The finding suggests that exposure of the cryptic heparin-binding site in FNIII₁ mediates ECM-FN effects on cell behavior. *In vitro*, the sequence induces cell proliferation,⁷¹ migration, and contractility, where *in vivo* it has been shown to induce vasodilation.⁷⁵

HS proteoglycans and heparin in the ECM act as reservoirs for bound GFs. However, HS proteoglycans in the cell membrane, for example, syndecans, can also act as cofactors for GFs like FGF-2 to bind their authentic signaling receptors.⁷⁶ HS is a helical polymer of the glucosamine-uronic acid disaccharide unit with a unique structure depending on the tissue of origin.^{15,77} The proteoglycan core protein may determine the presence of HS-binding domains to specific GFs. The GF protein sequences for heparin binding have been identified. In HGF and FGF-2 the determinants are discontinuous; however, PDGF-A- and VEGF HS-binding sequences are localized to linear ~20 amino acid stretch rich in basic residues, while TGF- β 1 binds heparin through a 60 kDa accessory protein.¹⁵

Binding to HS is reversible and has a fast off-rate, therefore HS-bound GFs can be released by competition with other GF-binding proteins or other heparin-binding ECM proteins. Heparin binding induces a local change in FNIII₁₂₋₁₄ that increases VEGF binding, which has been shown to be critical for angiogenesis.⁶ HS potentiates FGF signaling or prolongs its activity by protecting it from proteolysis. HSPGs modify the response to FGF by either degrading or redirecting ligand–receptor complexes to cell–cell or cell–matrix adhesions.⁶

ACKNOWLEDGMENTS AND FUNDING SOURCES

Funded by the U.S. Army Medical Research and Materiel Command (USAMRMC), the Office of Naval Research (ONR), the Veterans Administration (VA), and the National Institutes of Health

TAKE HOME MESSAGES

- Multiple interactions of cells, FN (and ECM molecules in general), and GFs are required for optimal healing (Fig. 4).
- Understanding these interactions may lead to breakthrough therapies for large wounds that cannot be closed for nonhealing ulcers.

(NIH). This award is supported by contract number W81XWH-08-2-0034.

AUTHOR DISCLOSURE AND GHOSTWRITING

Richard Clark, MD, is President of NeoMatrix Formulations, Inc., which is developing cyclized P12 for treatment of burn injury progression. No ghostwriting or technical writer was used in preparing this article.

ABOUT THE AUTHORS

Professor Richard A.F. Clark, MD, has made many seminal observations regarding the sources and significance of fibronectin protein in wound healing. Recently, he has discovered bioactive peptides within the fibronectin structure that enhance the activity of growth factors and act as survival factors for tissue cells. He is now applying that knowledge to the development of systemic and topical treatments to prevent burn injury progression and to promote scarless healing. He has 130 original publications and 81 reviews or book chapters to his credit. **Katarzyna M. Sawicka, PhD**, is a recent graduate of the Biomedical Engineering graduate program at Stony Brook University. Dr. Sawicka joined the Clark lab as a postdoctoral researcher and has been involved in carrying out the preclinical studies of the novel bioactive peptide discovered in the laboratory and investigated by NeoMatrix Formulations, Inc. Her graduate work focused on the development of a novel immunization platform that she has successfully shown effective in *in vitro* and *in vivo* models. **Markus Seeliger, PhD**, is an Assistant Professor and structural biologist in Pharmacological Sciences at Stony Brook University. **Tagai Musaev, BA**, is a medical student at the Albert Einstein School of Medicine in New York City. **Lauren K. Macri, PhD**, is an Associate Member of the New Jersey Center for Biomaterials.

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Abbreviations and Acronyms

CSF = colony stimulating factor
 ECM = extracellular matrix
 EGF = epidermal growth factor
 EGFR = EGF receptor
 FAK = focal adhesion kinase
 FGF = fibroblast growth factor
 FN = fibronectin
 GAGs = glycosaminoglycans
 GF = growth factor
 GFR = GF receptor
 HGF = hepatocyte growth factor
 HGFR = HGF receptor
 HS = heparin sulfate
 IGF = insulin-like growth factor
 MMP = matrix metalloproteases
 PDGF = platelet-derived growth factor
 PDGFR = PDGF receptor
 RTK = receptor tyrosine kinase
 TGF- β = transforming growth factor- β
 TSP = thrombospondin
 VEGF = vascular endothelial growth factor
 VEGFR = VEGF receptor