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Synergistic Signaling from Extracellular Matrix–Growth Factor Complexes

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Investigations on extracellular matrix (ECM) and growth factor (GF) complexes have revealed an underappreciated phenomenon: they can either negate GF activity or generate synergistic signals for cell function, in particular mitogenesis. ECM and pericellular matrix molecules were first recognized to complex with GFs and regulate GF activity by the seminal observations that basic fibroblast growth factor (bFGF or FGF-2) required binding to a cell-surface heparin sulfate proteoglycan and to its authentic cell-surface receptor for biological activity (Klagsbrun and Baird, 1991; Yayon *et al.*, 1991). Subsequently, numerous ECM–GF interactions that modulate GF activity were discovered; we have reviewed many of these findings (Macri *et al.*, 2007).

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In this issue, Upton *et al*. (2008) demonstrate that vitronectin (VN) complexes with insulin-like growth factor (IGF) and IGF-binding proteins (IGFBPs) could enhance migration of human keratinocytes *in vitro* and possibly *in vivo*. Given that some ECM–GF complexes, including VN–IGF–IGFBP, markedly enhance GF activity, such complexes might drive down the GF dose required for wound therapy to the nanogram level. Such a low GF dose—hundreds- to thousands-fold less than that currently needed—would potentially provide great monetary savings for wound treatments with GF products. Furthermore, the same investigators previously demonstrated that VN could form complexes with GFs other than IGF, such as epidermal growth factor (EGF). ECM molecules capable of binding a menu of GFs might provide breakthrough technology for wound treatment because such molecules might localize endogenously produced GFs to injured tissue and thereby solve the dilemma of which specific array of GFs should be added to wounds to promote healing.

We previously reported that the ECM proteins fibronectin (FN) and fibrin accumulate in injured tissue as provisional matrix molecules (Clark *et al.*, 1982). VN, osteonectin (SPARC), thrombospondin (TSP), and tenascin, as well as FN and fibrin, are now known to fit this category. Interestingly, all these ECM proteins have been demonstrated to bind GFs and to enhance their activity, except for SPARC, which binds platelet-derived growth factor (PDGF) and vascular endothelial cell growth factor (VEGF) but inhibits their GF activities (Macri *et al.*, 2007). Complexities arise with TSP because it either activates or inhibits GFs. For example, TSP-1 activates transforming growth factor-β (TGF-β) (Young and Murphy-Ullrich, 2004) but inhibits VEGF (Greenaway *et al.*, 2007). From these data it can be hypothesized that provisional matrix proteins bind and regulate GFs in injured tissue. In fact, one of the major problems in chronic wounds may be the absence of provisional matrix proteins, such as FN and perhaps VN (Herrick *et al.*, 1992), secondary to their degradation by the plethora of proteolytic enzymes present (Grinnell and Zhu, 1994, 1996).

Results from provisional matrix protein null mice support such a hypothesis, at least in part. For example, SPARC-null mice demonstrate enhanced cutaneous wound healing (Bradshaw *et al.*, 2002); in contrast, VN-null mice demonstrated impaired angiogenesis during cutaneous wound repair (Jang *et al.*, 2000). Mice overexpressing TSP have delayed wound healing and diminished angiogenesis (Streit *et al.*, 2000). A caveat is that provisional matrix proteins may affect cellular function directly and thereby modulate wound repair independent of GF interactions. Such may be the case with FN, which binds VEGF directly and IGF and TGF-β indirectly (Macri *et al.*, 2007), but in addition has a pronounced direct effect on cell migration during embryogenesis (George *et al.*, 1993) and presumably during wound repair (Greiling and Clark, 1997).

> ECM and GF complexes conspire to regulate function.

On a molecular level, GF receptors co-localize with ECM protein receptors, i.e., integrins, within the focal contacts that form at the cell membrane when cells bind to ECM proteins (Miyamoto *et al.*, 1996; Plopper *et al.*, 1995). Using magnetic microbeads coated with FN or an RGD-containing peptide, Ingber's group found that focal adhesion complexes, which formed upon contact with microbeads, contained GF receptors as well as integrins and multiple signaling molecules, including c-Src, focal adhesion kinase, phosphatidylinositol-3-kinase, phospholipase C-gamma, and Na^+/H^+ antiporter (Plopper *et al.*, 1995). Yamada's group demonstrated that EGF, PDGF-BB, and FGF-2 produced a marked, transient

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activation of extracellular signal-regulated kinase, a signal-transduction molecule that induces mitogenesis, only if integrins were both aggregated and occupied by FN or RGD-containing peptide ligands (Miyamoto *et al.*, 1996). These findings support the hypothesis that GFs and integrins cooperate in a coordinate fashion to elicit signals necessary for cell function, especially mitogenesis. Because signal-transduction pathways are propagated along nanoscale scaffolding complexes inside the cell, coordinate GF and ECM signals from the external milieu apparently require solid-state presentation in the same nanospace; otherwise their resultant signal transduction pathways would be unable to integrate.

Thus, based on this body of *in vitro* and *in vivo* data, GF–ECM complexes may well be the most effective and efficient method to stimulate cell proliferation, as well as tissue healing or regeneration, as proposed by Upton and colleagues (2008).

CONFLICT OF INTEREST

President of NeoMatrix Formulations, Inc.

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β-Papillomavirus Infection and Skin Cancer

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The development of highly sensitive PCR techniques and multiplex bead-based Luminex platforms has accelerated the search for a specific role of human papilloma viruses in the development of squamous cell carcinoma. Human papillomaviruses are most likely indirectly involved in this process by facilitating UV-related carcinogenesis via preventing UV-induced apoptosis or impairing DNA repair, but other mechanisms are also possible.

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It has been widely accepted that human papillomavirus (HPV) 16 and related HPV types play a crucial role in the development of cervical and anogenital carcinomas. The role of HPV in the development of cutaneous squamous cell carcinoma (SCC) is still controversial (Asgari *et al.*, 2008, this issue).

The first time that HPV infection was linked with the development of skin cancer was in the rare genetic disease epidermodysplasia verruciformis (EV). EV patients develop keratotic skin lesions that display a high rate of progression to SCC, mainly on sun-exposed skin. They also exhibit diminished cell-mediated immunity and high susceptibility to infection with β-papillomavirus (β-PV) types (formally called EV-HPV types; Asgari *et al.*, 2008).

Organ-transplant recipients (OTRs) have a greatly increased risk of developing warts and other keratotic skin lesions, soon followed by the development of

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