

Abstracts of Short Talks

'Smart' matrix (engECM), composed of hyaluronan and recombinant fibronectin functional domains, promotes robust en masse migration of adult human dermal fibroblasts and *in vivo* wound repair.

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Injured tissue heals when tissue cells receive the proper signals for regeneration or repair. A plethora of engECM formulations have utilized synthetic or semisynthetic polymers rendered biologically active by tethered RGD, a cell binding tripeptide. Although transformed cell lines attach, spread and proliferate on or within these biomaterials, there is a paucity of data on their ability to influence primary human cell strains. Even less information exists regarding the ability of engECM to facilitate cell migration, a process critical for wound repair. Here, we report a novel 3D engECM that promotes robust en masse migration of adult human dermal fibroblast. Hydrogels were formulated by coupling three recombinant fibronectin functional domains, previously shown to be necessary and sufficient for optimal human fibroblast migration on a 2D surface, to thiolated hyaluronan using a polyethylene divinylsulfone crosslinker. En masse migration of adult human dermal fibroblast on engECM decorated with the three recombinant fibronectin functional domains was a function of recombinant fibronectin functional domains type and dose. In contrast, adult human dermal fibroblast failed to spread and migrate on synthetic RGD-tethered engECM. The en masse migration of adult human dermal fibroblasts on our engECM *in vitro* is similar to that observed during rapid granulation tissue formation in healing wounds. Our engECM also promoted such response *in vivo*. These data suggest that recombinant fibronectin functional domains polyethylene divinylsulfone-hyaluronan may be effective in treatment of non-healing wounds.

Effect of elevated polyamine biosynthesis in cutaneous wound healing.

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Characterization of the ornithine decarboxylase (ODC)/ER2-inducible transgenic mouse, in which ODC is expressed *de novo* in the adult suprabasal epidermis, has revealed that induction of ODC activity following topical treatment with the inducer 4-hydroxytamoxifen stimulates both epidermal proliferation and differentiation resulting in only moderate hyperplasia. Although ODC activity is directed to suprabasal epidermal cells, cells in the dermis are activated as well. Following 4-hydroxytamoxifen treatment, deposition of extracellular matrix proteins such as tenascin-C and collagen increases in the dermis of ODC/ER2 transgenic mice accompanied by a diminution of the fat layer and increased vascularization. These results suggest that *de novo* suprabasal epidermal induction of ODC activity activates both keratinocytes and dermal components in a manner similar to skin undergoing wound healing. Skin abrasion experiments with 4-hydroxytamoxifen-treated ODC/ER2 mice demonstrate sustained epidermal hyperplasia, stromal activation, and increased vascularization 2 weeks following wounding compared to similarly abraded 4-hydroxytamoxifen-treated normal mice and ethanol-treated ODC/ER2 transgenic mice. As most tumors possess elevated ODC levels, it is likely that the wound healing phenotype induced by increased polyamine biosynthesis, as seen in ODC/ER2 transgenic mice, creates a more permissive microenvironment for the expansion of dormant, genetically altered epithelial cells to a malignant phenotype. (Supported by NIH grant CA070739.)

A keratinocyte wound-related hypermotility and p16^{INK4A} growth arrest response activated by the γ2 precursor form of laminin 5 and regulated by the TGF-β receptor.

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We have reported previously that the replicative lifespan of primary human keratinocytes in culture is limited by a telomere-unrelated, stochastic induction of the cell cycle inhibitor p16^{INK4A} (p16) and that senescent, p16-positive keratinocytes also display an increased expression of the basement membrane protein Laminin 5 (Lam5) and a remarkable directional hypermotility. We report here that that coordinate expression of Lam5 and p16 occurs at the migrating front of keratinocytes in skin wounds *in vivo*, wounds made in organ-cultured skin, and scratch wounds in confluent keratinocyte cultures grown on plastic. Lam5/p16 expression is also induced by plating keratinocytes on surfaces coated with the γ2 chain precursor form of Lam5 (Lam5γ2pre). Under these conditions, cells immediately exhibit a persistent, directional hypermotility of ~150 μm/hour and subsequently express p16 and become irreversibly growth-arrested. Cells plated on control surfaces in the presence of transforming growth factor-beta (TGF-β) exhibit hypermotility after a 12–24 hours lag period. Plating cells on Lam5γ2pre triggers nuclear translocation of smad2/3-downstream effectors of TGF-β receptor activation. A TGF-β receptor I kinase-specific drug inhibitor blocks smad2/3 translocation, hypermotility, and growth arrest in response to TGF-β and to Lam5γ2pre. A collagen I-coated surface induces a vigorous directional hypermotility response but does not trigger smad2/3 translocation or growth arrest. Expression of a smad4-RNAi blocks smad2/3 nuclear translocation but does not block Lam5γ2pre-induced growth inhibition or hypermotility. In contrast, drug inhibitors of several smad-independent signal pathway kinases downstream of TGF-β receptor activation, including p38^{MAPK}, p160^{ROCK}, and fyn, reduce or prevent Lam5γ2pre-induced hypermotility and growth inhibition. These results provide the fundamental molecular characterization of a coordinated hypermotility and growth arrest response that is activated in keratinocytes during wound healing and reveal a cross-talk between adhesion- and TGF-β-stimulated signal pathways in this cell type.

Adenosine A_{2A} receptors and dermal fibrosis: a pathogenic role for adenosine in diffuse dermal fibrosis.

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Adenosine is a potent endogenous regulator of inflammation and tissue repair. As adenosine A_{2A} receptors stimulate dermal wound healing, in part, by stimulating collagen matrix production, we examined whether adenosine A_{2A} receptors also contribute to the pathogenesis of dermal fibrosis. Collagen production by primary human dermal fibroblasts was analyzed by ¹⁴C-proline incorporation. Intracellular signaling of dermal collagen production upon adenosine A_{2A} receptor stimulation was investigated using inhibitors of MEK-1 and by direct demonstration of stimulated extracellular signal-regulated kinase phosphorylation. The role of the adenosine A_{2A} receptor in dermal fibrosis was studied in a bleomycin-induced (1 mg SQ, on alternate days for 18 days) model of scleroderma in adenosine A_{2A} receptor-deficient, wild-type littermate mice and C57BL/6 mice, as well as C57BL/6 mice treated with an adenosine A_{2A} receptor antagonist (ZM241385, 50 mg/kg in dimethylsulphoxide/cremophore, administered intraperitoneally twice a day). Skin thickness, skin-fold thickness, skin breaking tension, and dermal collagen (hydroxyproline) content were determined as measures of dermal fibrosis in control or bleomycin-treated mice. Adenosine A_{2A} receptor occupancy promoted collagen production by primary human dermal fibroblasts and this process was blocked by an antagonist to the adenosine A_{2A}, but not A₁ or A_{2B} receptor. Adenosine A_{2A} receptor ligation stimulated extracellular signal-regulated kinase phosphorylation and A_{2A} receptor-mediated promotion of collagen production by dermal fibroblasts was blocked by inhibitors of MEK-1. Adenosine A_{2A} receptor-deficient mice and adenosine A_{2A} receptor antagonist-treated mice were completely protected from development of dermal fibrosis following exposure to bleomycin.

In conclusion, these results demonstrate that adenosine A_{2A} receptors play an active role in the pathogenesis of dermal fibrosis and suggest a novel therapeutic target in the treatment and prevention of dermal fibrosis in diseases such as scleroderma.

EGF modulates slug expression during cutaneous wound re epithelialization

The Slug transcription factor is essential for effective cutaneous wound reepithelialization both *in vitro* and *in vivo*.

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Enhanced expression of Slug at cutaneous wound margins is associated with decreased cell:cell adhesion and enhanced keratinocyte motility. The role that Slug plays in cutaneous wound healing is reminiscent of its role in epithelial mesenchymal transformation during embryogenesis. During development, expression of Slug is modulated by a variety of growth factors, including transforming growth factor-β and basic fibroblast growth factor; therefore, we investigated the role of growth factors in modulating Slug expression during cutaneous wound reepithelialization. Human keratinocytes were treated with a variety of growth factors, and expression levels of Slug and the related transcription factor Snail were determined by quantitative reverse transcription-PCR. Several growth factors, including epidermal growth factor (EGF), keratinocyte growth factor, IGF-1, and basic fibroblast growth factor, enhanced Slug expression. Of the growth factors tested, EGF was the most effective at enhancing Slug expression and was the only one that enhanced Snail expression. We further examined the importance of EGF-stimulated Slug expression in cutaneous wound healing in skin explants from wild-type mice, mice homozygous for an inactivated Slug gene (Slug-lacZ), and mice heterozygous for the Slug-lacZ allele. Administration of exogenous EGF accelerated epithelial outgrowth from explants and enhanced Slug expression in migrating cells. In the presence of the EGF receptor inhibitor AG1478, explant outgrowth and Slug expression were markedly inhibited. There was no outgrowth from Slug knockout explants, even in the presence of exogenous EGF. These findings suggest that EGF enhances cutaneous wound reepithelialization by stimulating expression of the Slug transcription factor.