Matrix Biology

0040

ALPHA1-CT, A NOVEL PHYSIOLOGICAL INHIBITOR IN CONTROLLING CONVERSION OF PRO-MMP9 IN WOUND HEALING

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The factors leading to wound chronicity remain unknown. Excessive inflammation and activation of proteolytic enzymes, in particular MMP-9, have been reported by many investigators including ourselves to be important in this pathophysiology. Also, control of MMP-9 activation by conversion of 92-kDa zymogen into 82-kDa active proteinase, has been observed in chronic wounds. At previous WISH meetings, we identified that TNF-α potently induces the conversion into active MMP-9 in human skin through a tissue-associated chymotrypsin like proteinase.

Alpha-1-antichymotrypsin (α1-CT) is a liver secreted acute factor in response to trauma and can reach concentrations in plasma of 7-10 μM. In this report we show α1-CT is a critical factor in control conversion of pro-MMP-9. Wound fluids (n=6) from acute trauma have high levels of α1-CT and inactive pro-MMP-9. In contrast, α1-CT is degraded and non-functional in chronic wounds, while pro-MMP-9 is converted into active form. We then demonstrated that α1-CT (1 μM) effectively inhibited the TNF-α induced activation of pro-MMP-9 by organ-cultured human skin. Endogenous α1-CT in acute wounds could explain the documented lack of active MMP-9 in these wounds. In conclusion we believe α1-CT may play an essential role in the control of the MMP-9 activation during wound healing.

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FAK REGULATES SURVIVAL IN THE FIBROBLAST-POPULATED COLLAGEN MATRIX

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Background. Mechanical anchorage of the fibroblast-populated collagen matrix (FPCM) activates focal adhesion kinase (FAK) and promotes fibroblast survival. RNA interference (RNAi) of FAK produces specific inhibition of FAK in fibroblasts. We hypothesized that temporary knockout ("knockdown") of FAK with RNAi in the anchored FPCM would induce fibroblast apoptosis.

Methods. Anchored FPCMs were incubated for 72 hr prior to transfection with short interfering RNA (siRNA, 67 nM in 0.67% lipid transfection vehicle) specific for FAK. Lysate FAK was quantified with immunoblotting, and apoptosis in cytosin preparations of the FPCM was assayed with the TUNEL. Data (mean ± sd) was compared with ANOVA and the unpaired t-test.

Results. Transfection vehicle alone did not affect FAK expression; siRNA transfection resulted in a 50% decrease in FAK level at 2-4 days post-transfection (imunoblot data not shown). Cell death increased from zero to 7.3 ± 1.5% by day 4 post-transfection in siRNA-treated FPCM, while increasing only 1-2% in the vehicle-treated FPCM (see Figure; p < 0.05 compared to vehicle only).

Conclusions. Knockdown of FAK with RNAi in the FPCM induces cell death which is evident within 48 hr after transfection with the FAK-specific siRNA. Fibroblast survival in the anchored FPCM is dependent at least in part on the presence of FAK. The mechanism of anchorage-dependent survival in the FPCM involves signaling through FAK.

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IMPACT OF KERATINOCYTE-FIBROBLAST COCULTURE ON COLLAGEN AND CYTOKINE PRODUCTION

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Hypertrophic scars result from excessive collagen deposition at sites of cutaneous wounds. Regulation of collagen synthesis and deposition is a direct approach to the control of scar tissue formation. Previously we have demonstrated reduction in hypertrophic parameters in humans and rabbits by application of silicone dressings, which have become standard therapy for hypertrophic scars. Although the mechanism of action is unknown, our previous work led to the hypothesis that keratinocyte hydration modulates dermal fibroblast collagen production. To test this hypothesis, an in vitro coculture model was used to study the dependence of collagen expression on keratinocytes-fibroblast dependent production of TGF-α and KGF-2. Stratified normal young human epidermal keratinocytes (HEK) and confluent normal young human dermal fibroblasts (HDF) were cocultured in a serum-free insert system for 72 hours under hydrated conditions, which were compared with HDF and HDF alone. Extracted RNA of the different lysed cells was analyzed by real-time RT-PCR for collagen, TGF-α and KGF-2 expression. The RT-PCR shows a 37.5% decrease of collagen expression by cocultured fibroblasts compared to the controls. In contrast, TGF-α expression by cocultured HEK increased by 39.3% compared to HEK alone. Cultures stimulated an 87.9% increase in KGF-2 expression by HDF compared to HDF alone. These findings were confirmed by ELISA, which demonstrated a 4-fold increase in KGF2 secretion by cocultured HDF compared to control (456.63 ng/mL vs. 114.35 ng/mL, respectively). These results provide further evidence for a paracrine interaction between HEK and HDF that appears to play an important role in the regulation of collagen synthesis and may provide insight into the mechanism of action of silicone dressing in the treatment of hypertrophic scars.

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HYALURONAN MODULATION OF HUMAN FIBROBLAST GAP JUNCTION INTERCELLULAR COMMUNICATION

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In fetal scarless wound repair, hyaluronic acid (HA) concentrations remain elevated. During adult wound repair, the addition of HA optimizes granulation tissue collagen organization. It is proposed that HA optimizes collagen organization through gap junction intercellular communication (GJJC). Gap junctions are gated membrane channels whose structure is composed of connexin (Cx) proteins. Gap junctions allow rapid intercellular communication, enabling the synchronization of numerous cellular activities. Coupled fibroblasts show enhanced organization of collagen fibrils in vitro and in vivo. In addition, HA is reported to enhance Cx 43 expression in transformed fibroblasts. By scrape loading the coupling index for human dermal fibroblasts was 4.6 ± 0.2, while the coupling index for fibroblasts treated with HA more than doubled to 10.6 ± 0.7. Immuno-blotting showed no differences in the protein levels of Cx 43 or β-catenin, a cytoplasmic protein associated with Cx 43. By monolayer immuno-histology, both Cx 43 and β-catenin were evenly distributed throughout control fibroblasts. However, a co-localization of Cx 43 and β-catenin to the cell surface was found in HA treated fibroblasts. It is proposed that HA increases GJJC through the accumulation of Cx 43 and β-catenin on the cell surface, leading to enhanced collagen organization.