LOSS OF EXTRACELLULAR MATRIX ANCHORAGE RESULTS IN FAK DEPHOSPHORYLATION AND APOPTOSIS IN FIBROBLASTS

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CONTROL OVER FIBROBLAST SURVIVAL has therapeutic potential for diseases in which fibrosis is a prominent component. Previously we demonstrated that fibroblast survival in a 3-dimensional collagen matrix (the fibroblast-populated collagen matrix [FPCM]) is dependent on matrix attachment to tissue culture plastic—ie, a rigid substratum.¹ ² Focal adhesion kinase (FAK) promotes adhesion-dependent survival in other systems, and Akt is a downstream effector of FAK; both proteins are activated by phosphorylation.³ We hypothesized that FPCM attachment promotes phosphorylation of both FAK and Akt and that detachment results in dephosphorylation of both proteins.

MATERIALS AND METHODS

Primary human fibroblasts (5 × 10⁵/mL, matrix concentration) were cultured for 48 hours in an FPCM (0.2 mL matrix volume forming a discoid structure 12 mm in diameter) constituted with type I bovine collagen (1.5 mg/mL), which was attached to tissue culture plastic and covered with 10% FBS/DMEM. Matrices were then mechanically detached (using a spatula) from the plastic, and TUNEL (on Cytospin preparations) and immunoblotting (with the indicated antibodies on 7% SDS-PAGE-resolved lysates) were performed 24 to 48 hours later. Protein loading for the immunoblots was equilibrated using LDH activity and verified with subsequent Coomassie staining of the blot membranes.

![Att vs Det FAK and p-FAK immunoblots](image)

Fig 1—Focal adhesion kinase (FAK) immunoblot in the attached (Att) vs detached (Det) fibroblast-populated collagen matrix.

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RESULTS

The apoptotic rate in the attached matrix at 48, 72, and 96 hours after matrix constitution (defined as $t = 0$) is 0% to 1%; detaching the matrix at $t = 48$ hours increased the rate to 5% and 10% at 72 and 96 hours, respectively ($^*P < .05$; ANOVA), as previously demonstrated.\(^2\) Immunoblotting for phosphorylated FAK (p-FAK) at $t = 72$ hours (ie, 24 hours after detachment) using an antibody that recognizes phospho-Tyr-397 (the FAK autophosphorylation site crucial for activity) demonstrated that FAK was phosphorylated in the attached matrix and dephosphorylated in the detached matrix (Fig 1). At the same time point, immunoblotting for phosphorylated Akt using an antibody that recognized phospho-Ser-473 (a phosphorylation site crucial for Akt activity) demonstrated that Akt was phosphorylated in the attached matrix and dephosphorylated in the detached matrix (Fig 2).

CONCLUSIONS

In other systems, attachment-dependent survival is defined in the context of whether the cell is attached to a substratum; for example, detachment of epithelial cells from a basement membrane results in rapid epithelial cell apoptosis.\(^4\) In the FPCM, we have shown that fibroblast survival is regulated in the context of matrix attachment to a substratum. Detachment of the FPCM from the tissue culture plastic produced fibroblast apoptosis even though the cells were not removed from the matrix and were not deprived of serum. This suggests that something specific to matrix attachment is essential for cell survival in the FPCM; one possibility is mechanical tension, which will develop in the FPCM if the matrix is attached to a rigid substratum\(^5\) and presumably dissipates if the matrix is detached. Mechanical tension is known to activate FAK through an integrin linkage,\(^3\) and both FAK and
Akt are well-known modulators of survival. We have shown that detachment of a FPCM from its rigid substratum was associated with dephosphorylation of FAK and Akt. Taken together, these data are consistent with a hypothesis that detachment of the FPCM results in apoptosis secondary to a loss of mechanical stimulus, with subsequent downregulation of FAK and Akt.

REFERENCES


ACCELERATED ACUTE FASCIAL HEALING IS ASSOCIATED WITH INCREASED FIBROBLAST PCNA AND p21 EXPRESSION

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FASCIAL WOUND HEALING FAILURE results in 400,000 incisional hernias a year in the United States and 200,000 repeated operations to repair the defects.1 We previously reported that normal fascial wounds should regain tensile strength faster than dermal incisions in rats.2 The mechanism for accelerated fascial repair involved increased fascial fibroblast kinetic activity as measured by increased contraction of fascial fibroblast-populated collagen lattices and earlier fascial wound collagen production. Whether fascial versus dermal fibroblast cellular heterogeneity exists following wounding is not known. It has been observed in nonhealing dermal ulcers that the cellular contribution to repair may depend on wound fibroblasts capable of emerging from quiescence, repairing DNA, and undergoing cell division.3 The present study was designed to measure whether normal fascial healing is associated with increased wound fibroblast cell-cycle progression compared with a simultaneous dermal wound.

MATERIALS AND METHODS

Sixteen Sprague-Dawley rats underwent elevation of a ventral skin flap and isolated midline fascial incision using a previously described model of fascial repair.2 Two additional rats served as unwounded controls. Rats were killed on postoperative days (PODs) 1, 7, 14, and 21, and the wounds were biopsied, fixed in formalin, and embedded in paraffin. Fibroblast cell cycling was measured in situ by immunofluorescent staining using antibodies against markers of DNA synthesis (proliferating cell nuclear antigen [PCNA]) and cell-cycle arrest (p21) (Santa Cruz Bio-