DISCUSSION

This study demonstrates tyrosine phosphorylation of 5 major protein bands in FRF, obtained from a time when repair is scarless (13 days' gestation), but not in the ARF cell lysates, at the given concentration of protein lysate. We speculate that these bands represent tyrosine kinase receptors that may have the potential to control cellular responses to the extracellular matrix and their secondary signaling molecules. Thus, signals received by the fetal fibroblasts may be processed and modulated within an intracellular signaling cascade that are inherently different than that of adult fibroblasts. Based on these data, we hypothesize that these unique signaling proteins may lead to the differences in dermal scarring phenotype between fetal and adult fibroblasts. Furthermore, because scar formation is determined by the lack of organization of wound collagen, fetal fibroblasts are the key effector cells in scarless repair.[3,4] Thus, fetal fibroblasts may perceive and respond to collagen differently. Additional studies that may be critical to understanding the extensive tissue turnover and remodeling needed for scarless repair are under way to characterize the biomolecular role of tyrosine phosphorylated proteins in fetal fibroblasts.

REFERENCES

1. Mackool RJ, Rowe NM, Mehrara BJ, et al: Excisional wound healing in the fetal rat. Surg Forum 49: 463–464, 1998

2. Schlessinger J: Direct binding and activation of receptor tyrosine kinases by collagen. Cell 91:869–872, 1997

3. Gallivan K, Alman BA, Moriarty KP, et al: Differential collagen I gene expression in fetal fibroblast. J Pediatr Surg 32:1033–1036, 1997

4. Lorenz HP, Lin RY, Longaker MT, et al: The fetal fibroblast: The effector cell of scarless fetal skin repair. Plast Reconstr Surg 96:1251–1259, 1995

HUMAN FIBROBLASTS IN A MECHANICALLY STRESSED MATRIX UNDERGO APOPTOSIS AFTER STRESS REMOVAL

Mark A. Carlson, MD, Meifang Zhu, BS, John M. Abrams, PhD, and Fred Grinnell, PhD

KNOWLEDGE OF THE MECHANISM of granulation tissue regression would have therapeutic implications in fibrotic disease. Fibroblasts within granulation tissue are abundant and are under mechanical stress, as demonstrated by their spindle morphology, orientation along stress lines, and intracellular stress fibers.[1] Fibroblasts in both scar and normal dermis are scarce and do not appear to be under stress, having stellate morphology and no stress fibers. Granulation tissue (and its fibroblast population) regresses and is replaced with scar as the wound closes. It has been demonstrated in a granulation tissue model (below) that mechanically stressed fibroblasts proliferate and unstressed fibroblasts do not.[2] The contrast in phenotype between granulation tissue and scar/dermal fibroblasts and the fact that a mechanically unstressed fibroblast population regresses led us to hypothesize that fibroblasts

From the Departments of Surgery and Cell Biology, University of Texas Southwestern Medical Center, Dallas, TX. *Supported by National Institutes of Health grant GM 2168132.*

switched from mechanically stressed to unstressed conditions undergo apoptosis and that cellular events occurring in response to loss of stress are involved in the mechanism.

MATERIALS AND METHODS

Our granulation tissue model is a collagen matrix populated with fibroblasts.[2] Bovine dermal collagen (3.0 mg/mL) is mixed with early passage fibroblasts (from human foreskin), and an aliquot (0.2 mL = 2×10^5 cells) is placed into a plastic well with a 12-mm circular score. Polymerization into a gel (matrix) occurs after a 1-hour incubation, after which the matrix is covered with serum-supplemented medium. Fibroblasts under these conditions will organize and contract the collagen matrix. If the matrix is left attached to the immovable plastic well, the fibroblasts develop mechanical stress (as seen in granulation tissue) as contraction is attempted. If the matrix is detached (released) from the plastic (with a spatula) so that the matrix floats in the medium, contraction in all dimensions ensues, shrinking the matrix to less than 25% of its starting volume in 24 hours. The fibroblast phenotype after release evolves to scar/dermal phenotype, that being stellate without stress fibers. The released contracted matrix appears mechanically relaxed compared with the attached matrix. Matrix fibroblasts were recovered after enzymatic digestion of collagen, and analyzed with TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling) and fluorescent microscopy.

RESULTS

The first set of experiments was performed to determine if apoptosis was occurring in the detached matrix. Attached matrices developed stress for 24 hours, after which some matrices were released and allowed to float in the medium for 24 or 48 hours, controls remained attached for a total of 72 hours. TUNEL data from a representative experiment (performed 4 times with similar results) are given as follows as percentage of apoptotic cells ± SD: attached matrix, 0.5 ± 0.9; 24-hour released matrix, 11.3 ± 0.6; 48-hour released matrix, 15.7 ± 1.3 ($P < 0.05$, attached vs. released). Therefore, release of an attached matrix results in apoptosis in 10%–15% of the fibroblasts detectable at 24 and 48 hours.

Since growth factor receptors on the fibroblast plasma membrane become desensitized after release of an attached collagen matrix,[3] and the cell actin cytoskeleton becomes disrupted,[4] experiments were carried out to learn if treatment of cells in the attached matrix by growth factor withdrawal and/or cytoskeleton disruption would result in apoptosis comparable with that observed in the released matrix. Incubation of the attached matrix in serum-free medium (SF) for 24 hours and addition of cytochalasin (cyt), 10 μM, were used to mimic the above effects. The TUNEL data are as follows (performed twice with similar results): control, 0.9% ± 0.7%; SF, 5.0% ± 1.8%; cyt: 3.4% ± 2.9%; SF + cyt, 15.4% ± 2.5% ($P < 0.05$, SF + cyt vs. others). Therefore, treatment of fibroblasts in an attached collagen matrix with a combination of SF medium and cytochalasin resulted in the same degree of apoptosis observed in the released collagen matrix.

DISCUSSION

Removal of stress from the extracellular matrix in this model results in apoptosis of the fibroblasts populating the matrix. Treatment of cells in stressed matrices by growth factor withdrawal and actin cytoskeleton disruption results in a similar degree of apoptosis observed after stress removal. These data support the hypothesis that fibroblast apoptosis occurs after a reduction in mechanical stress and that the sig-

naling mechanism(s) involved depends on growth factor withdrawal combined with actin cytoskeleton disruption. Removal of stress at the completion of healing of an open dermal wound is likely an important factor in regression of the granulation tissue.

REFERENCES

1. Desmoulière A, Gabbiani G: The role of the myofibroblast in wound healing and fibrocontractive diseases, in Clark RAF (ed): The Molecular and Cellular Biology of Wound Repair. New York, Plenum Press, 1996, pp 391–423
2. Nakagawa S, Pawelek P, Grinnell F. Long-term culture of fibroblasts in contracted collagen gels: Effects on cell growth and biosynthetic activity. J Invest Dermatol 93:792–798, 1989
3. Lin YC, Grinnell F: Decreased level of PDGF-stimulated receptor autophosphorylation by fibroblasts in mechanically relaxed collagen matrices. J Cell Biol 122:663–672, 1993
4. Mochitate K, Pawelek P, Grinnell F: Stress relaxation of contracted collagen gels: Disruption of actin filament bundles, release of cell surface fibronectin, and down-regulation of DNA and protein synthesis. Exp Cell Res 193:198–207, 1991

KELOID FIBROBLASTS PRODUCE A REDUCED AMOUNT OF TRANSFORMING GROWTH FACTOR β_3 COMPARED WITH TRANSFORMING GROWTH FACTOR β_1 AND TRANSFORMING GROWTH FACTOR β_2 ISOFORMS

Steve Lee, MD, Dorothy Chau, MD, Norman M. Rowe, MD, George K. Gittes, MD, and Michael T. Longaker, MD

KELOIDS ARE AN EXAMPLE of pathologic scarring characterized by an excessive accumulation of extracellular matrix (ECM) and occluded microvessels. Keloid formation may be mediated by transforming growth factor β (TGF-β), which induces collagen and matrix production during wound healing. Three isoforms of TGF-β (β_1, β_2, and β_3) exist in mammals, with only TGF-β_3 thought to possess an anti-scarring effect. We hypothesized that the biology promoting keloid formation may be attributable to a relatively decreased production of TGF-β_3. The purpose of this study was to quantitatively compare the production of TGF-β_1, -β_2, and -β_3 by keloid fibroblasts (KFs) with that by normal human dermal fibroblasts (NHDFs).

MATERIALS AND METHODS

Three different KF and NHDF cell lines were grown by standard tissue culture techniques. After incubation in serum-free DMEM for 3 days, the media were collected from the 6 different cell lines. Mink lung epithelial cells (MLECs), permanently transfected with a plasminogen activator inhibitor-1 promoter construct linked to a luciferase reporter gene, were plated at 1.6×10^4 cells per well in a 96-well plate. This bioassay is sensitive to picomolar amounts of all 3 isoforms of TGF-β. Media collected were heated at 80°C and applied to the MLECs to yield total TGF-β (latent and active). Application of neutralizing antibodies to TGF-β_1

From the Laboratory of Developmental Biology and Repair, Department of Surgery, New York University Medical Center, New York, NY.