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Scleroderma Keratinocytes Promote Fibroblast Activation Independent of TGF-ß

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Session Time: 9:00AM-11:00AM

Background/Purpose: Systemic sclerosis (SSc) is a devastating fibrosing disease that primarily involves the skin, but may have life-threatening effects on the heart, lungs, gastrointestinal tract, and kidney. Although the exact pathogenesis of SSc remains unclear, one identified mechanism is the excessive extra cellular matrix (ECM) produced by fibroblast activation. Keratinocytes have recently been shown to influence fibroblast function; thus, we hypothesized that SSc keratinocytes may contribute to fibroblast activation and subsequent ECM production.

Methods: All SSc subjects fulfilled the ACR/EULAR criteria for SSc and were classified as limited cutaneous (lc) SSc and diffuse cutaneous (dc) SSc. Keratinocytes were cultured from the isolated epidermis of two 4mm forearm punch biopsies for two passages, and conditioned media was collected at passage 2. Keratinocyte population purity was assessed by morphology. Studies were performed on at least 3 lcSSc, 3 dcSSC, and 4 normal samples for each assay. Normal primary fibroblasts were stimulated for 24 or 72 hours with conditioned keratinocyte media in the presence or absence of TGF-β neutralizing antibody. Fibroblast activation was measured by expression of αsmooth muscle actin (SMA) and type 1 collagen expression via RT-PCR and with αSMA staining via immunofluorescent microscopy. Affymetric Human Gene ST2.1 microarrays were completed on cultured SSc and control keratinocytes. Pathway analysis was performed using Genomatix software.

Results: Stimulation of normal primary fibroblasts with keratinocyte conditioned media from lcSSc and dcSSc samples yielded increased collagen (lcSSc 1.6-4.5 fold, $p<0.0001$; dcSSc 1.2-4.4 fold,$p<0.0002$)and αSMA mRNA expression (lcSSc 6.6-12.5 fold,$p<0.0001$; dcSSc 6.2-13 fold;$p<0.0001$). αSMA expression was also increased on immunofluorescent staining of normal

fibroblasts after exposure to scleroderma keratinocyte conditioned media. Microarray analyses and confirmation by RT-PCR demonstrated decreased TGF-β expression in both dcSSc and lcSSc keratinocytes. The addition of TGF-β neutralizing antibody to keratinocyte conditioned media did not decrease induction of αSMA or collagen expression by normal fibroblasts.

Conclusion: These results demonstrate that unstimulated scleroderma, but not control, keratinocytes are able to promote fibroblast activation, as measured by αSMA and collagen expression, independent of TGF-β production. Identification of the mediator of this activation may provide novel targets for prevention or treatment of fibrosis for SSC patients

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