Regulation of fibroblast activity by keratinocytes, TGF-β and IL-1α
- studies in two- and three dimensional in vitro models

av

Anita Koskela von Sydow

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Opponent: Professor Hans Törnä
Institutionen för Medicinska Vetenskaper/Dermatologi och Venereologi/Uppsala universitet

Örebro universitet
Institutionen för Medicinska Vetenskaper
701 82 ÖREBRO
Abstract
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Dysregulated wound healing is commonly associated with excessive fibrosis. Connective tissue growth factor (CTGF/CCN2) is characteristically overexpressed in fibrotic diseases and stimulated by transforming growth factor-β (TGF-β) in dermal fibroblasts. Reepithelialisation and epidermal wound coverage counteract excessive scar formation. We have previously shown that interleukin-1α (IL-1α) derived from keratinocytes counteracts TGF-β-stimulated CTGF-expression. The aim of this thesis was to further explore the effects of keratinocytes and IL-1α on gene and protein expression, as well as pathways, in TGF-β stimulated fibroblasts. Fibroblasts were studied in vitro by conventional two dimensional cell culture models and in a three dimensional keratinocyte-fibroblast organotypic skin culture model.

The results showed that IL-1 suppresses basal and TGF-β-induced CTGF mRNA and protein, involving a possible TAK1 mechanism. Keratinocytes regulate the expression of fibroblast genes important for the turnover of the extracellular matrix. Most of the genes analysed (11/13) were regulated by TGF-β and counter regulated by keratinocytes. The overall results support a view that keratinocytes regulate fibroblasts to act catabolically (anti-fibrotic) on the extracellular matrix.

Transcriptional microarray and gene set enrichment analysis showed that antagonizing effects of IL-1α on TGF-β were much more prominent than the synergistic effects. The most confident of these pathways was the interferon signaling, which were inhibited by TGF-β and activated by IL-1α. A proteomics study confirmed that IL-1α preferentially counteracts TGF-β effects. Six new fibroblast proteins involved in synthesis/regulation were identified, being regulated by TGF-β and antagonized by IL-1α. Pathway analysis confirmed counter-regulation of interferon signaling by the two cytokines. These findings have implications for understanding the role of fibroblasts for inflammatory responses and development of fibrosis in the skin.

Keywords: Fibroblast, Keratinocyte, TGF-β, IL-1α, coculture, fibrosis CTGF/CNN 2, dermal, organotypic culture.

Anita Koskela von Sydow, School of Medicine Örebro University, SE-701 82 Örebro, Sweden, anita.koskela-von-sydow@regionorebrolan.se