Identification of a myofibroblast-specific expression signature in skin wounds

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Abstract

After skin injury fibroblasts migrate into the wound and transform into contractile, extracellular matrix-producing myofibroblasts to promote skin repair. Persistent activation of myofibroblasts can cause excessive fibrotic reactions, but the underlying mechanisms are not fully understood. We used SMA-GFP transgenic mice to study myofibroblast recruitment and activation in skin wounds. Myofibroblasts were initially recruited to wounds three days post injury, their number reached a maximum after seven days and subsequently declined. Expression profiling showed that 1749 genes were differentially expressed in sorted myofibroblasts from wounds seven days post injury. Most of these genes were linked with the extracellular region and cell periphery including genes encoding for extracellular matrix proteins. A unique panel of core matrisome and matrisome-associated genes was differentially expressed in myofibroblasts and several genes not yet known to be linked to myofibroblast-mediated wound healing were found (e.g. Col24a1, Podnl1, Bvcan, Tinagl1, Thbs3, Adamts16, Adamts19, Cxcl's, Ccl's). In addition, a complex network of G protein-coupled signaling events was regulated in myofibroblasts (e.g. Adcy1, Plbc4, Gnas). Hence, this first characterization of a myofibroblast-specific expression profile at the peak of *in situ* granulation tissue formation provides important insights into novel target genes that may control excessive ECM deposition during fibrotic reactions.

Introduction

Fibroblasts are central effector cells in skin repair. Due to changes in the extracellular matrix (ECM) and the release of inflammatory signals after injury, dermal fibroblasts transform from quiescent cells embedded within an organized ECM in the intact skin into migrating, proliferating cells leaving the ECM niche. These cells enter the wound and differentiate into contractile, alpha smooth muscle actin^{+} (SMA^{+}) myofibroblasts [1]. Here, myofibroblasts pull the wound edges together to close the wound [2] and lay down a substantial amount of ECM consisting of collagens, fibronectin, and other matrix components to stabilize the granulation tissue [3,4]. In addition, myofibroblasts are a source for signal molecules that coordinate the recruitment and activation of other cells within the wound. Later, during the remodeling phase of wound repair, myofibroblasts undergo apoptosis or revert to an

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inactive phenotype [5,6]. Hence, myofibroblasts are crucial to restore skin integrity after injury. Any imbalances in myofibroblast homeostasis can result in abnormal healing responses and ECM deposition as seen in fibrosis and hypertrophic scarring [7].

There have been earlier attempts to identify gene networks expressed in differentiated skin myofibroblast in order to determine the molecular mechanisms of normal and pathological myofibroblast activation. Previously, genome scale gene expression profiling of cultured human dermal and chicken myofibroblasts [8–10] or skin biopsies from scleroderma patients [11] was performed to outline conserved gene networks. However, *ex vivo* cultures do not mimic the complex microenvironment in the wound and the heterogenous cellular composition of scleroderma biopsies may hamper the identification of myofibroblast-specific networks. Approaches to identify a myofibroblast-specific transcriptome in skin wounds have been lacking.

Here, we used transgenic SMA-GFP mice [12] to determine the course of myofibroblast differentiation in skin wounds and to define the transcriptome at the peak of myofibroblast formation. We found that myofibroblasts are particular abundant in wounds between five and seven days after injury when they express a unique gene signature linked to ECM production, cell-matrix interactions, cell proliferation and communication. This unique gene expression network therefore defines the transcriptional activation of skin wound myofibroblasts and identifies factors and pathways that may be targeted to inhibit myofibroblast activation, ECM production and fibrotic reactions *in vivo*.

Results

To determine the distribution of myofibroblasts after skin injury full thickness wounds were inflicted on the

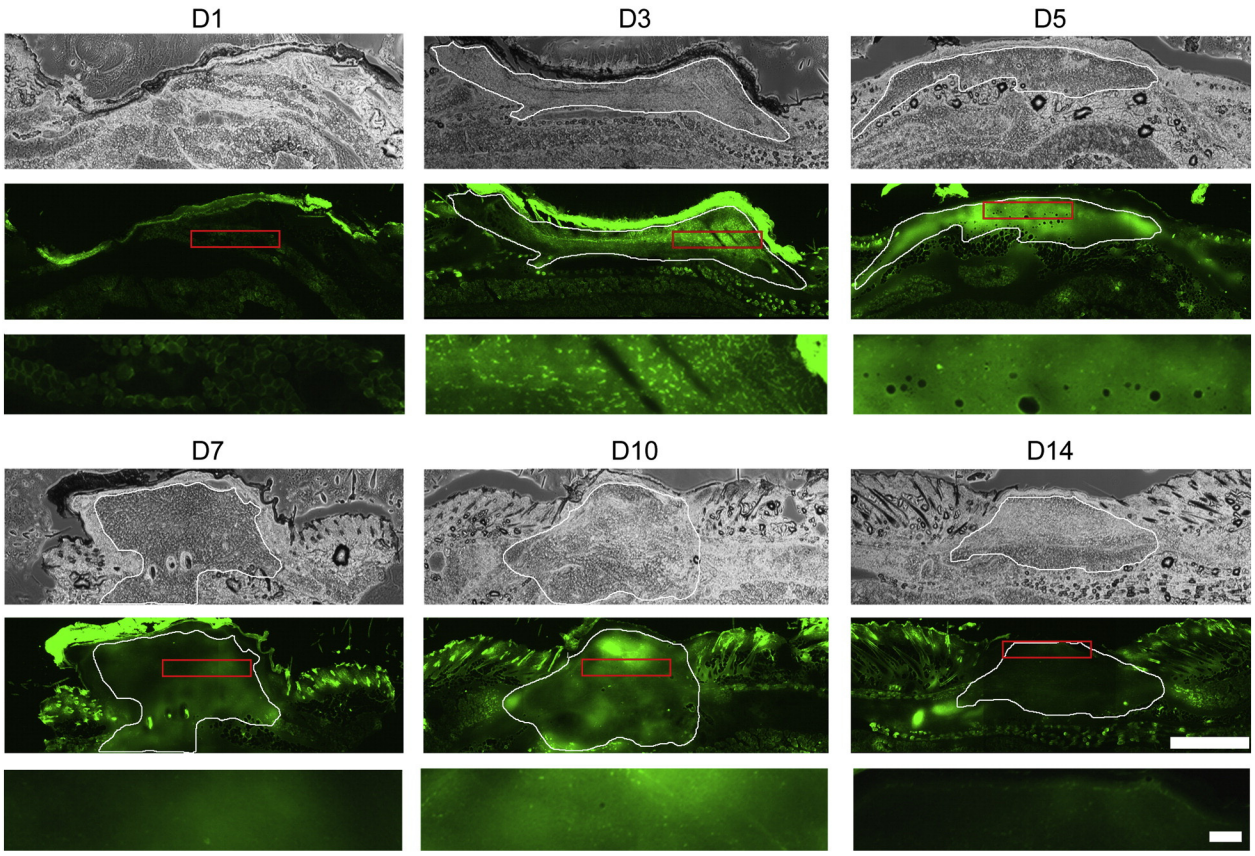

Fig. 1. Characterization of the SMA-GFP expression in wound repair. Representative unprocessed cryosections of full thickness wounds from the back skin of SMA-GFP transgenic mice one (D1), three (D3), five (D5), seven (D7), ten (D10) and 14 (D14) days post injury were analyzed by light (top row) and fluorescence (central row) microscopy. Higher magnifications of the wound are shown (bottom row). Three images were merged to generate an overview of an individual wound. The granulation tissue (white line) and the magnified area (red box) are marked. Bar: 400 μm (central row), 100 μm (bottom row). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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