Abstract

During wound healing increased mechanical stress, the release of TGF-β and the formation of a specialized extracellular matrix stimulate the recruitment of fibroblasts from the intact dermis in the granulation tissue and promote their differentiation into myofibroblasts. Those cells secrete extracellular matrix proteins and contract the wound during the granulation phase. MicroRNAs are small non-coding RNA molecules, which act as guiding molecules for the post-transcriptional regulation. They can bind target mRNAs, regulate their expression and influence almost all biological processes. However, their function in the regulation of myofibroblast homeostasis is not well understood.

In this study, a method to isolate myofibroblasts from murine wound tissue was established. For this purpose, an α-SMA-GFP transgenic mouse model was used, which expresses the green fluorescent protein (GFP) under the control of the promoter of the actin isoform alpha smooth muscle actin (α-SMA). Histological and immunohistological analysis confirmed that GFP and α-SMA are produced in myofibroblasts. These GFP^+ myofibroblasts were highly enriched in the granulation tissue seven days post wounding and could be isolated by flow cytometry. The myofibroblast phenotype of the cells was confirmed by global mRNA transcriptome analysis and the expression of 690 miRNAs was analysed by miRNA microarray analysis. In total 40 miRNAs were differentially expressed compared to fibroblasts. Among those miR-127-3p was seven fold upregulated, stimulated the expression of α-SMA/GFP and an increased cell size as well as inhibited the proliferation by G1/G0 arrest in cell culture experiments with isolated fibroblasts. Fibroblasts with increased miR-127-3p concentration adhered more slowly to plastic substrates and induced a stronger contraction of collagen gels. Out of 20 predicted target mRNAs, Chst15, Dlk1, Kif3b, Scn8a, Setd8, Slc12a4, Wnt7a, Ypel5 and Zc3h4 were identified as target mRNAs using luciferase reporter studies. Transfection of miR-127-3p resulted in a decreased mRNA amount for Chst15, Kif3b, Setd8 and Zc3h4. Preliminary analysis of target mRNA function by siRNA-mediated regulation in fibroblasts demonstrated that this leads to a similarly decreased cell proliferation and increased GFP intensity. However, the phenotype was not as strong as after transfection with miR-127-3p mimic. In summary, a method to isolate myofibroblasts from wound tissue was established. The mRNA as well as the miRNA transcriptome was analysed and based on these data a myofibroblast inducing miRNA was identified and the underlying molecular mechanism determined.