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POS0327

INACTIVATION OF ALDEHYDE DEHYDROGENASE 3A2 INHIBITS FIBROBLAST ACTIVATION AND TISSUE FIBROSIS

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Background: The aldehyde dehydrogenase (ALDH) superfamily composes a group of 20 enzymes that catalyze aldehyde oxidation. Within this enzyme family, ALDH3A2 stands out for its central role in the oxidation of long-chain aldehydes. Of particular interest, the substrates of ALDH3A2 include also profibrotic lipid mediators such as sphingosine 1-phosphate or leukotrienes, which have been reported to be deregulated in the context of SSc.

Objectives: We aimed to investigate the role of ALDH3A2 in fibrotic tissue remodeling in SSc.

Methods: Fibroblast-to-myofibroblast transition was analyzed by quantification of ACTA2/ α SMA, by assessment of stress fiber formation and mRNA and protein levels of type I collagens. *ALDH3A2/Aldh3a2* siRNAs were employed to specifically knockdown ALDH3A2 in dermal fibroblasts both *in vitro* and *in vivo*. Overexpression of ALDH3A2 was achieved by *ALDH3A2*-pcDNA transfection. The role of ALDH3A2 was investigated in three different mouse models: Bleomycin- and cGvHD-induced dermal fibrosis as well as fibrosis induced by overexpression of a constitutively active TGF β receptor I (TBRI^{CA}). Target genes of *ALDH3A2* in fibroblasts were identified by RNA sequencing.

Results: The expression of ALDH3A2 was modestly reduced in dermal fibroblasts of SSc skin as compared to matched healthy controls. This reduction in ALDH3A2 expression was phenocopied by activation of TGF β signaling, whereas selective inhibition of TGF β signaling prevented the downregulation of ALDH3A2 in experimental fibrosis. ALDH3A2 overexpression promoted fibroblast-to-myofibroblast transition with increased levels of α SMA, enhanced formation of stress fibers and reduced collagen release. In contrast, knockdown of ALDH3A2 in dermal fibroblasts inhibited fibroblast activation and collagen release. Moreover, *in vivo* knockdown of ALDH3A2 in the skin of mice ameliorated dermal thickening, myofibroblast differentiation and collagen deposition in three different murine models of skin fibrosis: Bleomycin-induced skin fibrosis and sclerodermatous GvHD-as models of inflammatory stages of SSc and TBRI^{CA}-induced fibrosis as an inflammation-independent model of SSc. RNA sequencing of ALDH3A2-knockdown fibroblasts demonstrated that ALDH3A2 regulates the activity of a network of profibrotic developmental pathways including TGF β , Wnt, Notch, and Hedgehog signaling.

Conclusion: We demonstrate that ALDH3A2 regulates a network of profibrotic pathways to control fibroblast activation and tissue fibrosis. ALDH3A2 is modestly downregulated in SSc fibroblasts as result of an endogenous, TGF β -driven feedback loop. Although this modest downregulation is not sufficient to counterbalance the aberrant fibroblast activation in SSc, augmentation of this endogenous regulation by knockdown of ALDH3A2 demonstrates potent antifibrotic potential in experimental dermal fibrosis, thereby providing first evidence for ALDH3A2 as a target for antifibrotic therapies.

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ENGRAILED 1 COORDINATES CYTOSKELETAL ORGANIZATION TO PROMOTE MYOFIBROBLAST DIFFERENTIATION AND FIBROTIC TISSUE REMODELING

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Background: Engrailed 1 (EN1) is a homeodomain-containing transcription factor with essential roles in embryonic development. In most cell types, the expression of EN1 is restricted to embryonic development. However, under pathological conditions, EN1 can be re-expressed to promote phenotypical adaptation. EN1 is transiently expressed in the developing dermis of murine embryos in a distinct fibroblast lineage and silenced before birth (1). Former EN1-expressing cells give rise to a subpopulation of fibroblasts that has a high capacity for extracellular matrix production in adult murine skin. The role of EN1 in systemic sclerosis (SSc) was previously not explored.

Objectives: To study the role of EN1 in the pathological activation of fibroblasts in tissue fibrosis.

Methods: Bulk RNA-Seq and EN1 or SP1 ChIP-Seq were performed from cultured human dermal fibroblasts. The expression of EN1 was inhibited by siRNA. Cytoskeletal drugs paclitaxel, vinblastin and ROCK inhibitor (Y27632) were used to modulate the cytoskeleton in EN1 knockdown or overexpressing dermal fibroblasts. The role of EN1 in fibroblast activation was evaluated by functional experiments with EN1 knockdown or overexpression in standard 2D culture systems as well as in 3D skin equivalent models. The role of EN1 in skin fibrosis was further studied in En1^{fl/fl} X Col6Cre mice, with fibroblast-specific knockout of En1 in three complementary mouse models: overexpression of a constitutively active TGF β -receptor I (TBRI^{CA}), bleomycin-induced skin fibrosis and TSK1 mice.

Results: Pathologically activated dermal fibroblasts from SSc patients express higher levels of EN1 compared with age and sex matched healthy individuals in the skin and *in vitro*. TGF β induces EN1 expression in fibroblasts in a SMAD3-dependent manner both in cultured fibroblasts and in murine skin. Knockdown of EN1 prevents TGF β -induced fibroblast activation, whereas overexpression of EN1 fosters the pro-fibrotic effects of TGF β with increased expression of α SMA, stress fibers and collagen. RNA sequencing demonstrates that EN1 induces a pro-fibrotic gene expression profile functionally related to cytoskeleton organization and ROCK activation. *In silico* analyses of the promoters of En1 target genes coupled with siRNA-mediated knockdown demonstrated that EN1 regulates these pro-fibrotic target genes by modulating the activity of regulatory modules that contain transcription factors of the specificity protein (SP) family. Functional experiments with selective modulators of ROCK and of microtubule polymerization confirm the coordinating role of EN1 on ROCK activity and the re-organization of cytoskeleton during myofibroblast differentiation in both conventional culture systems and 3D skin equivalents. Consistently, mice with fibroblast-specific knockout of En1 demonstrate impaired fibroblast-to-myofibroblast transition, reduced dermal thickening and impaired collagen deposition in the TBRI^{CA}, bleomycin-induced and TSK1 models.

Conclusion: We characterize the homeodomain transcription factor EN1 as a molecular amplifier of TGF β signaling in myofibroblast differentiation that coordinates cytoskeletal organization in a SP-dependent manner. EN1 might thus be a novel candidate for molecular targeted therapies to interfere with myofibroblast differentiation in fibrotic diseases.

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