O019 IL-17A AT CROSSROAD BETWEEN KERATINOCYTES AND FIBROBLASTS IN HUMAN SKIN WITHIN SYSTEMIC SCLEROSIS

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Introduction Increased levels of IL-17A have been reported in systemic sclerosis (SSc) but its role in fibrosis development is still debated.[1] Recent findings suggest a role for keratinocytes in the development of fibrosis.[2] Of interest, epithelial cells are preferential targets of IL-17A.
Objectives Our aim was to investigate the interactions between epidermis and dermis in the presence of IL-17A, taking into perspective the fibrotic process.
Methods Conditioned-media of primary human keratinocytes primed with IL-17A and/or TGF-β were used to stimulate healthy donors (HD) and SSc fibroblasts. Alternatively, organotypic cultures of HD full human skin were treated with these cytokines. Responses were assessed by quantifying inflammatory mediators and type I collagen (Col-I) levels. The factors produced by keratinocytes were identified by a proteomic approach and their contribution was evaluated by their neutralisation. MicroRNA expression was examined by μParaflo technology platform.
Results Unstimulated HD- and SSc-derived keratinocyte-conditioned media (KCM) promoted collagen production by fibroblasts to a similar extent and in a dose-dependent manner. Cytokine array analysis and neutralising assays showed that TGF-β was, at least in part, responsible for the pro-fibrotic effect of KCM. Although priming of keratinocytes with IL-17A alone did not influence Col-I, it significantly decreased Col-I production induced by TGF-β by fibroblasts ($p=0.02$).

In full human skin, IL-17A promoted pro-inflammatory responses by inducing 2- to 4-fold increase of IL-8, IL-6, MCP-1 and MMP-1 levels, while showing direct anti-fibrotic effects and decreasing by 2-fold collagen production triggered by TGF-β ($p=0.02$). The combined injection of IL-17A and TGF-β in the full human skin resulted in a distinct pattern of miRNA expression, particularly driven by miR-4343, when compared to the expression induced by the separate injection of IL-17A and TGF-β.
Conclusions Keratinocytes profoundly influence dermal fibroblast responses, which are further modulated in the presence of IL-17A. These data support a role for keratinocytes in the pathogenesis of SSc. IL-17A acts as a potent anti-fibrotic factor in the model of keratinocyte – fibroblast interactions, as well as in the full human skin, which mechanisms are currently explored.

REFERENCES

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O020 LONG NONCODING RNA H19X IS A MASTER REGULATOR OF EXTRACELLULAR MATRIX PRODUCTION IN SYSTEMIC SCLEROSIS

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Introduction We have recently identified a novel, yet undescribed lncRNA, H19X, which was upregulated in the skin and lung of patients with SSc in a TGFβ dependent manner.
Objectives To characterise the function and the molecular mechanism of H19X in Systemic Sclerosis (SSc).
Methods The function of H19X was investigated in skin fibroblasts by knocking down H19X with locked nucleic acid oligonucleotides (LNA GapmeRs) and by using the following methods microarray analysis, immunofluorescence, Sircol, contraction assay, ELISA, Western blot (WB), *in situ* hybridization using Stellaris FISH probes and Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq).
Results Microarray analysis ($n=5$) showed that after H19X silencing collagen catabolic process, extracellular matrix organisation and extracellular matrix disassembly were among the pathways with highest number of enriched genes. Sircol assay for pan-collagen production ($n=5$, $p<0.05$), ELISA for procollagen Iα1 ($n=5$, $p<0.05$) and WB analysis for fibronectin ($n=7$, $p<0.05$) confirmed the importance of H19X in the regulation of extracellular matrix components. Additionally, silencing of H19X significantly impaired αSMA fibre formation, stress fibre formation as well as cell contractility strongly suggesting an important role of H19X in the development of the myofibroblast phenotype. Cell fractionation showed that TGFβ induced expression of H19X is localised mainly into the nucleus. *In situ* hybridization confirmed H19X localization as mainly nuclear and within a defined spot indicating that H19X could influence gene expression by interacting directly with the chromatin ($n=4$). TFAP2a was identified as the transcription factor with the strongest difference of occupied binding sites after H19X downregulation.
Conclusions The novel lncRNA H19X appears to be a *condition sine qua* non for the profibrotic effects of TGFβ. Mechanisms of action of the profibrotic effects of H19X point to epigenetic regulation of the transcription factor. LncRNA open new perspectives in the pathogenesis of fibrotic diseases.
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