FRI0410 TGFβ PROMOTES FIBROSIS BY MYST1-DEPENDENT EPIGENETIC REGULATION OF AUTOPHAGY

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Background: Autophagy is catabolic process allowing cells to degrade unnecessary or dysfunctional cellular organelles. Aberrant activation of autophagy has been also implicated into the pathogenesis of fibrotic diseases. Several stimuli present in fibrosis such as pro-fibrotic cytokines are known to activate autophagy. **Objectives:** The objective of this work is characterise the regulation of autophagy by TGFβ and analyse whether targeting of autophagy in fibroblasts may prevent their aberrant activation in fibrotic diseases.

Methods: To selectively disable autophagy in fibroblasts we generate *Atg7^{fl/fl} × Col1a2;CreER* mice. The role of the autophagy was investigated in the model of bleomycin- and TβRIact-induced dermal and pulmonary fibrosis. Overexpression of *Myst1* was achieved by adenovirus encoding for *Myst1*. Collagen release and protein expression were measure by Western blot. Target genes were analysed by RT-PCR. Co-immunoprecipitation and reporter assay were performed to study physical and functional interactions between MYST1 and SMAD3. To monitor the autophagic flux *in vitro* and *in vivo* we generate an adenovirus encoding for tandem fluorescent-tagged LC3 (mRFP-EGFP-LC3), defined as reliable autophagy maker.

Results: We provide evidence that transforming growth factor-β (TGFβ) activates autophagy by an epigenetic mechanism to amplify its profibrotic effects. TGFβ induces autophagy in fibrotic diseases by SMAD3-dependent downregulation of the H4K16-histoneacetyltransferase MYST1, which controls the expression of core components of the autophagy machinery such as ATG7 and BECLIN1. Activation of autophagy in fibroblasts promotes collagen release and is both, sufficient and required, to induce tissue fibrosis. Forced expression of MYST1 abrogates the stimulatory effects of TGFβ on autophagy and re-establishes the epigenetic control of autophagy in fibrotic conditions. Interference with the aberrant activation of autophagy inhibits TGFβ-induced fibroblast activation and ameliorates experimental dermal and pulmonary fibrosis. These findings link uncontrolled TGFβ signalling to aberrant autophagy and altered epigenetics in fibrotic diseases and may open new avenues for therapeutic intervention in fibrotic diseases.

Conclusions: We demonstrate that the epigenetic control of autophagy is disturbed by a TGFβ-dependent downregulation of MYST1. The increased activation of autophagy induces fibroblast-to-myofibroblast transition and promotes fibrotic tissue remodelling. Re-expression of MYST1 prevents aberrant autophagy, limits the profibrotic effects of TGFβ and ameliorates experimental fibrosis. Restoration of the epigenetic control of autophagy might thus be a novel approach to ameliorate fibrotic tissue remodelling.

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FRI0411 DUAL PRO-INFLAMMATORY AND ANTI-FIBROTIC ROLE OF IL-17A IN SYSTEMIC SCLEROSIS SKIN

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Background: Increased levels of IL-17A have been reported in systemic sclerosis (SSc) which role in fibrogenesis is still debated.¹ Furthermore, epithelial cells

are preferential targets of IL-17A and recent findings suggest that keratinocytes may participate in dysregulated extracellular matrix homeostasis.²

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: Primary human keratinocytes were primed with IL-17A and/or TGF-β and conditioned-media were used to stimulate healthy donors (HD) and SSc fibroblasts. Alternatively, organotypic cultures of HD full human skin were challenged 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 neutralisation assays. Changes in gene expression in full human skin after treatment with IL-17A and/or TGF-β were analysed by RNA sequencing (RNA-seq).

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. Priming of keratinocytes with IL-17A directly decreased Col-I production and significantly reduced Col-I induced by TGF-β both in SSc and HD fibroblasts. 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, as well as decreasing by 2-fold collagen production triggered by TGF-β (p=0.02). RNA-seq revealed that TGF-β induced the expression of many collagen genes, while this was not the case for IL-17A. However, IL-17A promoted a pro-inflammatory signature in the skin and strongly downregulated expression of serpin family members, known to be involved in fibrogenesis.

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, promoting pro-inflammatory and anti-fibrotic responses.

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FRI0412 B CELL DEPLETION AMELIORATES TISSUE FIBROSIS THROUGH REGULATING MACROPHAGE DIFFERENTIATION IN A BLEOMYCIN-INDUCED SYSTEMIC SCLEROSIS MODEL MOUSE

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Background: B cells play a critical role in pathogenesis of autoimmune diseases through various functions such as cytokine production and induction of other immune cell activation. Recent studies have shown the efficacy of B cell depletion therapy with rituximab, a human CD20 chimeric monoclonal antibody, in systemic sclerosis (SSc) patients. However, it still remains unclear why B cell depletion can be effective for SSc.

Objectives: The purpose of this study is to assess the role of B cell depletion in SSc. We evaluated the skin and lung fibrosis of bleomycin (BLM)-induced SSc model mice treated with B cell depletion. Furthermore, we investigated effects of B cell depletion on T cells and macrophages.

Methods: To generate BLM-induced SSc model mice, 200 µg of BLM was injected subcutaneously to C57BL/6 mice every other day for 4 weeks. Anti-mouse CD20 monoclonal antibodies, which can deplete mouse B cells, were also injected every 2 weeks from either one week before (the pre-depletion group) or 2 weeks after (the post-depletion group) BLM treatment. After 4 weeks of BLM treatment, skin and lung fibrosis was assessed histopathologically. To examine the effect of B cell depletion on T cells and macrophages, purified B cells were cultured with T cells or macrophages and then T cell cytokine production and macrophage phenotype were analysed.

Results: Skin and lung fibrosis increased in BLM-induced SSc mice. In the coculture experiments, B cells from BLM-induced SSc mice promoted differentiation of T cells producing fibrogenic cytokines such as interleukin-4 compared with control B cells, while they inhibited regulatory T cell (Treg) differentiation. Skin and lung fibrosis was inhibited in both pre- and post-depletion groups with the inhibition greater in the pre-depletion group than in the post-depletion group. Despite the finding that greater fibrosis remained in the post-depletion group than in the pre-depletion group, the post-depletion group showed significantly higher frequencies