Potential targets in inflammatory fibrotic conditions in SSC

**OP0289 MICRONRNAS AS POTENTIAL REGULATORS OF MONOCYTE DIFFERENTIATION AND FUNCTION IN HEART FIBROSIS IN SYSTEMIC SCLEROSIS**

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**Background:** Heart involvement in patients with systemic sclerosis (SSc) resembles the inflammatory dilated cardiomyopathy (iDCM) phenotype with predominance of inflammation, fibrosis, vasculopathy and heart dysfunction. Animal studies of iDCM indicated bone marrow originated cells as a major source of pathological myofibroblasts. MicroRNAs are key regulators of immune cell function and are involved in many cardiac pathological processes. However, their roles in monocyte differentiation and fibrogenesis are unclear.

**Objectives:** To determine the role of circulating monocytes and microRNAs in the onset and progression of myocardial fibrosis in SSc, we examined monocyte differentiation and their microRNAs expression profile in SSc.

**Methods:** Endomyocardial biopsies from SSc/iDCM patients and healthy controls were screened by immunohistochemistry. CD14+ monocytes isolated from peripheral blood of SSc patients and healthy donors were differentiated towards the myofibroblast phenotype by stimulation with TGF-β1, IL-4, IL-10 and IL-13. In addition, CD14+ monocytes were co-cultured with dermal fibroblasts originated from SSc patients and healthy subjects. After 7 days, myofibroblast gene expression and cytokines secretion profile were evaluated. MicroRNA candidates were selected using DIANA-TarBase v7.0 and TargetScan Release 7.0 and further analysed by qPCR.

**Results:** Myocardium of SSc/iDCM patients (n=10) revealed extensive fibrosis and accumulation of inflammatory cells including CD14+ monocytes. Moreover, fibrotic myocardium from SSc patients exhibited the presence of CD14+Fra-2+ monocyte-derived fibroblast-like cells. Bioinformatical analysis indicated several potential microRNAs being involved in monocyte differentiation, and qPCR confirmed predicted candidates. We observed decreased basal levels of let-7i (Mann-Whitney U test, p=0.005), miR-155 (p=0.002), miR-199a (p=0.008) and miR-544a (p=0.002), and increased levels of miR-29b (p=0.03) in circulating CD14+ monocytes isolated from SSc patients (n=10) compared to healthy controls (n=4). Stimulated monocytes acquired a myofibroblast-like phenotype with increased expression of collagen I, fibronectin, α smooth muscle actin and Fra-2 in comparison to untreated cells. Similarly, CD14+ monocytes exposed to dermal fibroblasts acquired myofibroblast features. CD14+ monocytes from SSc patients were characterised by higher production of IP-10, MIP-3α, LIF and NT-3. The process of monocyte to myofibroblast differentiation employed Fra-2/TGF-β signalling. Inhibition of the canonical SMAD-dependent pathway with TGFRI inhibitors resulted in the abrogation of monocyte-to-myofibroblast differentiation.

**Conclusions:** Here we demonstrated the capability of peripheral blood monocytes to differentiate towards the myofibroblast phenotype, indicating these cells as one of the potential sources of pathological tissue myofibroblasts in SSc. Different miRNA expression profiles in SSc monocytes indicate a primary activation state. Further studies of miRNAs regulation pathways might lead to novel treatment strategies, particularly for heart involvement.

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