was analysed by qPCR and ELISA. CD4+ T cells were stimulated with anti-CD3/anti-CD28 beads (ratio 1 bead: 5 cells) alone or in combination with recombinant human Sem4A (200 ng/ml), in the presence or absence of neutralising anti-NRP1 or PlexinD1 antibodies, and the expression of PlexinB2, PlexinD1, NRP-1 and the production of Th17 cytokines was analysed by qPCR, ELISA and flow cytometry.

**Results:** Plasma levels of Sem4A were significantly higher in SSc patients compared to healthy controls (HC) and positively correlated (r=0.611) with the skin disease severity. Sem4A and PlexinB2 expression was significantly higher in monocytes and CD4+ T cells from SSc patients, respectively. Moreover, Poly IC and CXCL-4 significantly up-regulated the expression and secretion of Sem4A in monocytes from SSc patients, and CD4+ T cells stimulation with anti-CD3/anti-CD28 beads increased the expression of PlexinB2 and NRP-1 in both HC and SSc patients. Finally, functional assays showed that Sem4A significantly enhanced the expression of Th17 cytokines induced by CD3/CD28 in CD4+ T cells from both HC and SSc patients, and the blocking of the Sem4A signalling using neutralising antibodies anti-PlexinD1 and anti-NRP-1 significantly reduced this expression. Importantly, the Sem4A-induced IL-17 secretion was significantly higher in stimulated CD4+ T cells from SSc patient compared to HC.

**Conclusions:** Sem4A signalling is deregulated in SSc patients and plays an important role in Th17 skewing. Therefore, Sem4A and its receptors could be promising therapeutic targets for the treatment of SSc.

**Disclosure of Interest:** None declared

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**AB0195**

**PROTEOMIC APPROACH IDENTIFIES DIFFERENTIAL PROTEIN EXPRESSION IN CULTURED FIBROBLASTS UNDER STIMULATION WITH TGF-β1**

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**Background:** Fibroblasts (Fb) are key effectors cells in systemic sclerosis (SSc). TGF-β1 stimulation with TGF-β1 is usually considered as the positive control in studies assessing the fibrogenesis in SSc. Yet, the lack of standardisation of TGF-β1 stimulation might be responsible for discrepancies in experiments performed in different conditions. Proteomic approach allows the analysis of differential expression of the whole proteins (proteome) in Fb, and appears an interesting approach to compare different culture conditions.

**Objectives:** We designed this study to compare the whole protein expression in Fb stimulated by TGF-β1 in different conditions.

**Methods:** At fifth passage, primary culture of human Fb from healthy subjects (ATCC; PCS-201-012) were stimulated or not with different concentrations of recombinant human active TGF-β1 (0.04, 1 and 5 ng/ml) (R and D Systems; 240-B-002) during 24, 48 and 72 hours. Proteins were extracted and analysed using an eFASP LC-MS/MS approach on an Orbitrap mass spectrometer (Thermo Scientific). Quantification was performed by Maxquant and statistical analysis by Perseus using ANOVA and principal component analysis (PCA).

**Results:** A total of 3267 proteins were identified, of which 1957 showed differential expression using ANOVA analysis. PCA revealed several clusters of differential proteins expression (figure 1). There were clear clusters of protein expression related to (i) unstimulated and stimulated conditions, (ii) between the three different times of stimulation and (iii) to TGF-β1 concentrations used. Although the expression of proteins in Fb exposed to 0.04 and 1 ng/mL of TGF-β1 during 72 hour were rather close, there was a unique proteins profile related to the condition with 5 ng/ml of TGF-β1 during 72 hour. Figure 1: PCA representation of differential proteins expression in different conditions.

**Conclusions:** This study highlights a variation of proteins expression depending on both stimulation time and TGF-β1 concentrations in Fb culture. The identification of protein differentially expressed will provide insights in the impact of TGF-β1 on Fb physiology under stimulation conditions. These data underline the need of standardisation of culture conditions to allow inter-data comparisons using non-sensitive “omic” approaches.

**REFERENCES:**


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