EVALUATION OF THE MODULATION OF GAS6/TAM SYSTEM IN FIBROSIS *IN VITRO*

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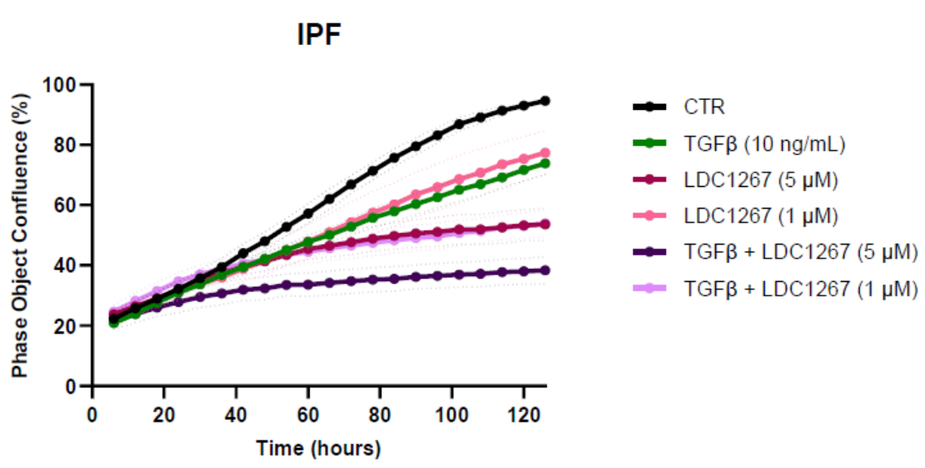
Background

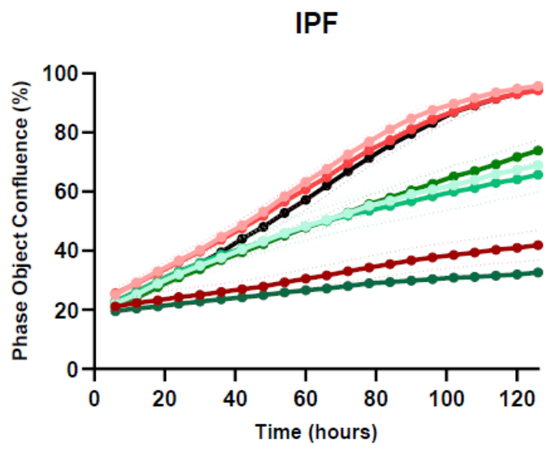
TAM receptors - Tyro3, Axl and Mer - and their main ligand Growth Arrest- Specific 6 (Gas6) represent a highly pleiotropic system implicated in the regulation of inflammation and development of fibrosis. Recently, it has been reported increased expression of Gas6 and Axl in lung samples and in fibroblasts culture from Idiopathic Pulmonary Fibrosis (IPF) patients compared with normal subjects.

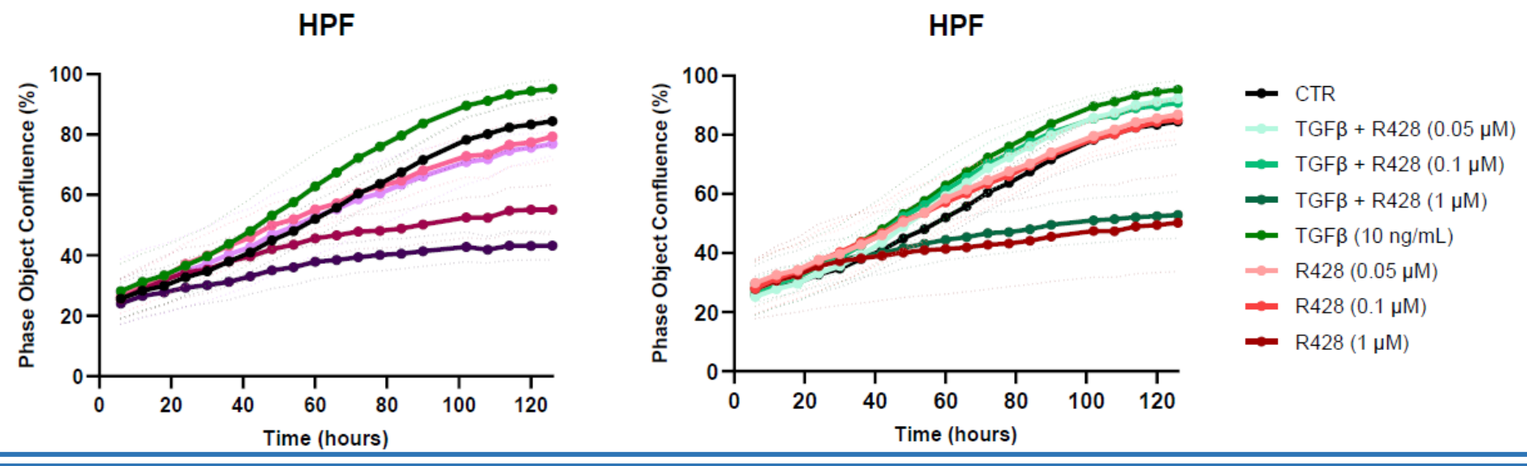
Aim of the study

The aim was to explore the involvement of Gas6/TAM in the fibrotic signaling and to evaluate the inhibition of this system in the modulation of fibroblast activity.

Results

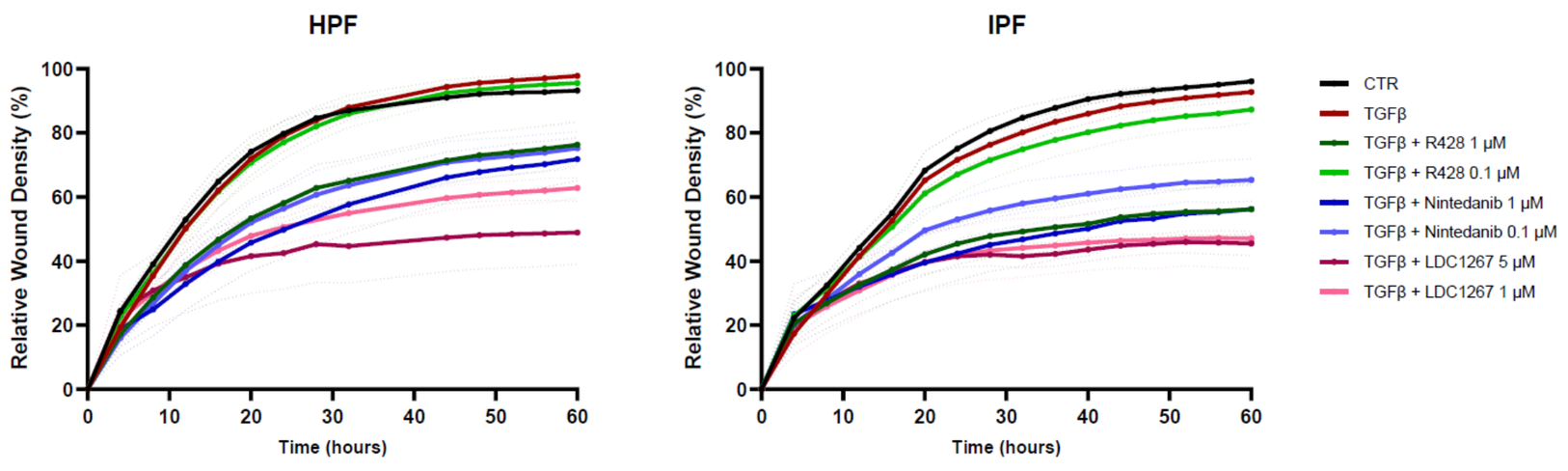


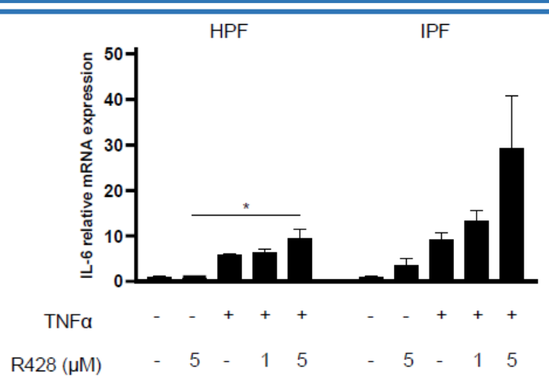


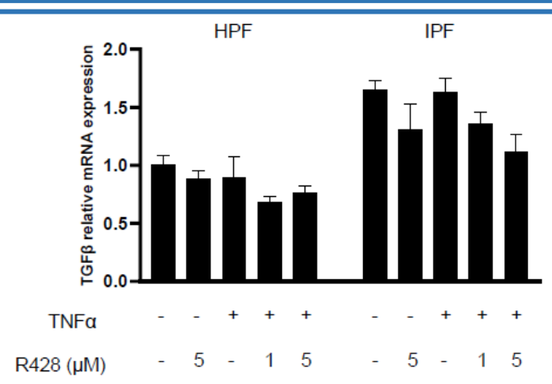


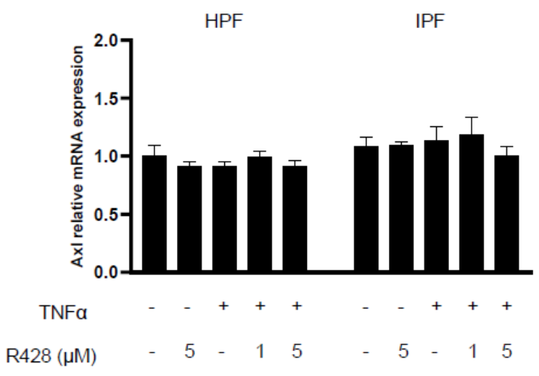
The proliferation of the fibroblasts treated with TGF-β1 (10 ng/mL), the drugs R428 and LDC1267, and the combination of TGF-β1 and drug, over 126 hours showed: in HPF, TGF-β1 stimulated cell proliferation compared to controls, whereas in IPF FB it showed growth inhibitory effects. For LDC1267 only minor differences in cell confluence were detected between IPF FB and HPF, R428 at higher concentration (1 μM) seemed to have a higher inhibitory impact on IPF FB.

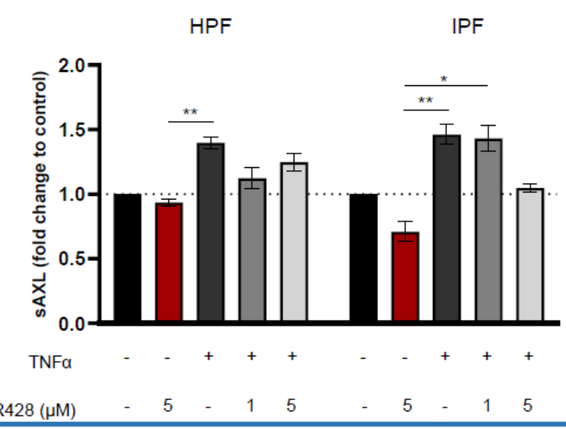
Regarding cell migration, the fibroblasts treated with LDC1267 exhibited slower wound closure. IPF FB were, in general, more affected by the different treatments and showed lower cell migration than HPF. R428 (1 μM) treatment led to a relative wound closure of 76% in HPF, but only 56% in IPF FB, by the end of 60 hours.





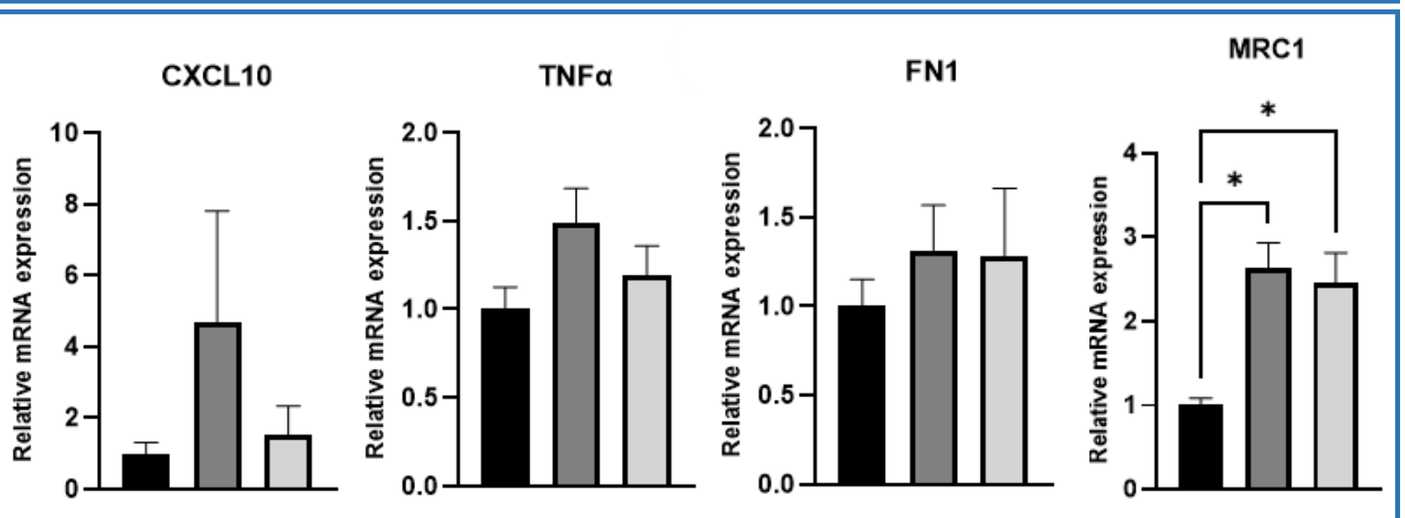






The pre-treatment of the fibroblasts with R428 prior to the TNF-α treatment had a cumulative effect in the upregulation of IL-6, but induced the reduction of TGF-β expression. In this same experiment, while Axl mRNA showed no significant differences among conditions and cell types, sAxl was elevated by the TNF-α treatment and these levels were moderately diminished by the pre-treatment with R428. *p<0.05

The co-culture of HPF and IPF FB with monocyte-derived macrophages (MDM) from healthy donors led to a significantly increased expression of MRC1 (M2 marker), while the expression of FN1 (M2 marker) and TNF-α, CXCL10 (M1 markers) was moderately increased. *p<0.05



Conclusions

Collectively, these results suggest that R428 and LDC1267 impact the proliferation and migration of activated fibroblasts, and may influence the fibrotic process through the modulation of TAM signaling. Moreover, these preliminary findings underline the influence of fibroblasts on macrophage polarization, which confirms the importance of cell cross-talk during fibrotic pathologies.