TGF-β pathway activation by IPF fibroblast-derived soluble factors is mediated by IL-6 trans-signaling

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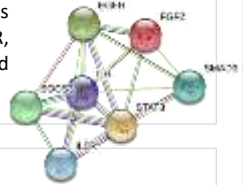
INTRODUCTION

Fibrotic diseases, such as IPF and systemic sclerosis (SS), are characterized by uncontrolled activation of fibroblasts. This activation was shown to be caused by increased inflammatory cytokines, (e.g. TNFα, IFNγ and IL-6), which are usually considered to be secreted by inflammatory cells (e.g. macrophages).

Tocilizumab (TCZ), an anti-human IL-6R neutralizing antibody, which prevents binding of IL-6 to IL-6R, inhibits both classic and trans-signaling pathways.



OBJECTIVES

The SENSCIS™ trial for SS associated interstitial lung disease (SS-ILD) showed that the treatment that is already proven effective for IPF (i.e. nintedanib) could also be effective for SS-ILD. We hypothesized that it could also be vice versa.

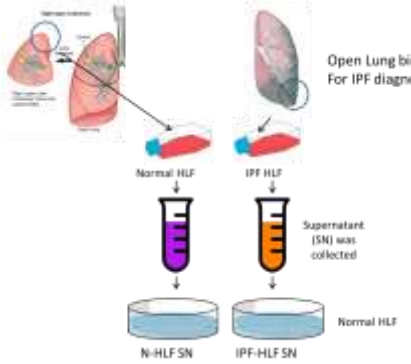
Since the microenvironment is known to affect cell behavior, activated fibroblasts can in turn activate healthy neighboring cells.

METHODS

Supernatants (SN) from primary human lung fibroblast (HLF) cultures from IPF (IPF-HLF) and control donor (N-HLF) lung tissues were collected and then added to N-HLFs for further culture.

Interleukin-6 (IL-6) and TGF-β-related signaling factors (e.g. STAT3, Smad3) were evaluated by western blot and qPCR. IL-6 levels were measured by ELISA. IL-6 signaling was blocked by Tocilizumab (TCZ) (10ng/ml).

IPF soluble microenvironment model



RESULTS

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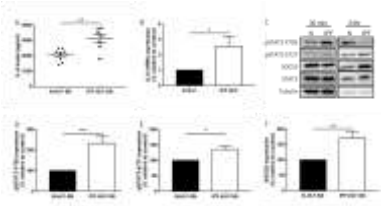
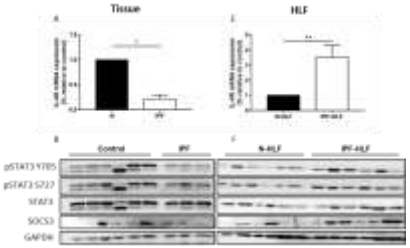
IPF-HLFs secrete high levels of IL-6 and activate the STAT3 pathway in normal HLFs

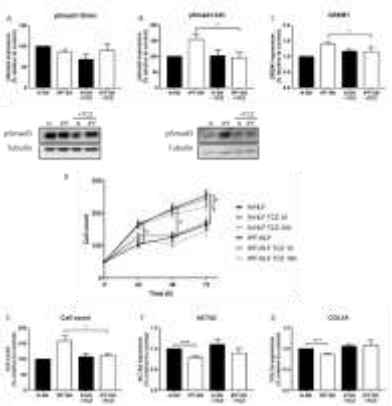


Human lung fibroblasts from patients with IPF (IPF-HLF) or control donors (N-HLF) were cultured and their supernatants (SN) were collected. IL-6 levels in the SN(A). IL-6 mRNA levels from N-HLF and IPF-HLF cells by qPCR (B). SN from IPF-HLFs was added to N-HLF for further cultures. The effect of the IPF-HLF-SN on N-HLF pSTAT3-Y705 (30 min, C-D), pSTAT3-S727 (24hr, E) and total protein levels of SOCS3 (24hr, F) were analyzed by western blotting. (C) Representative western blots for figures D-F. *p <0.05, **p< 0.01, and ***p< 0.001. (n≥4).

The IL-6 pathway is overexpressed in IPF-HLFs



IPF secreted factors activate Smad3 and induce cell proliferation via IL-6 trans-signaling



Effects of the IPF-HLF-SN with/ without Tocilizumab (TCZ, 10 ng/ml) on pSmad3 protein levels and GREM1 mRNA levels were tested by Western Blot (A-B) and qPCR (C), respectively.

Lung fibroblasts derived from patients with IPF (IPF-HLF) or from control donors (N-HLF) were cultured with/ without TCZ, 10 and 100 ng/ml. Cell growth was monitored at 24, 48, and 72hr. At 24h, culture media was changed and TCZ was added. Values are means ± SE (D). One-way ANOVA was used for each time point, with the main effect of IPF-HLF versus N-HLF. *p≤ 0.05, n=5.

The effect of IPF-HLF-SN with/ without TCZ (10 ng/ml) on cell number was tested at 24h (E). The effect of IPF-HLF-SN with/ without TCZ (10 ng/ml) on mRNA levels of αSMA (ACTA2) and Collagen1a (COL1A) were tested by qPCR at 24h (F-G). ***p< 0.001

CONCLUSIONS

IPF-HLF paracrine signaling leads to IL-6R overexpression, which in turn, affects N-HLF survival.
The IL-6/STAT3/Smad3 axis facilitates cellular responses that could potentially promote fibrotic disease.
This interplay was successfully blocked by Tocilizumab.

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