255 MICROARRAY ANALYSIS MAPS GLOBAL EFFECTS OF EPIGENETIC BROMODOMAIN INHIBITOR JQ1 ON GENE EXPRESSION IN TRANSFORMING GROWTH FACTOR-β-STIMULATED ADULT LUNG FIBROBLASTS

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Background: Pulmonary fibrosis represents one of the most prevalent and serious disease complications in SSc, affecting >70% of patients, and currently has no effective treatment. Chronic paracrine and autocrine TGF-β signalling to fibroblasts is believed to be a central driver of fibrotic processes in SSc and can serve as an *in vitro* disease model of activated fibroblasts to identify suitable therapeutic strategies. Recently, epigenetic regulation and aberrant histone modification has been implicated in fibrosis. Bromodomain and extraterminal (BET) proteins, including BRD2, 3 and 4, are epigenetic readers recognising histone acetyl marks and thus regulate gene transcription. Here we examine the global effect on gene expression in human activated lung fibroblasts of the bromodomain inhibitor JQ1, a new antifibrotic compound, which has demonstrated potential beneficial effects on fibroblast phenotype and limited gene expression *in vitro*, and in the murine bleomycin model of lung fibrosis.
Methods: Adult pulmonary fibroblasts were obtained by explant culture from histologically determined unaffected lung from patients undergoing cancer resection surgery and maintained under standard culture conditions. Fibroblasts were treated with 2 ng/ml TGF-β and 500 nM $JQ1^+$ or its inactive enantiomer $JQ1^-$ for 24 h in triplicate. RNA from cell lysates was subjected to analysis on Affymetrix Human Exon 1.0 ST arrays. Hybridisation signals were normalized for each array by Affymetrix software and GeneSpring software was used to determine differentially expressed (DE) genes, with DE < 1.3-fold filtered out. A number of DE genes were verified by quantitative RT-PCR.
Results: Preliminary analysis revealed 731 genes (401 upregulated and 330 downregulated) responsive to TGF-β. In the presence of TGF-β, 509 genes were significantly regulated by $JQ1^+$ compared with $JQ1^-$. Of the genes regulated by TGF-β, 218 were significantly affected by $JQ1^+$ and 516 genes were not. Of these genes, 132 genes upregulated by TGF-β were downregulated by JQ1, i.e. expression was normalized to varying degrees ranging from 60 to 167% of TGF-β-induced expression levels. This group included *IGF-1*, *IL-6*, *EGR2*, *EDN1* and *NOX4* genes. A total of 79 genes downregulated by TGF-β

were upregulated by JQ^+, including *HGF* and *PLAT*. Interestingly, 7 genes downregulated by TGF-β were further downregulated by $JQ1^+$. Among the 516 genes regulated by TGF-β but unaffected by JQ1 are also fibrosis-related genes such as *CTGF* and *SERPINE1* (PAI-1) dysregulated in SSc fibroblasts.

Conclusion: These data provide greater insight into the effects of JQ1 on fibroblast gene expression, which is essential in understanding its impact on phenotype, and may help in developing this and related drugs for use in diseases like SSc.

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