Identification of efficacy biomarkers based on a molecular functional signature associated to the active fibrosis process

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Introduction

Rodent models are commonly used to study the development of fibrosis. To optimize their predictability for the human situation there is a need to identify which key molecular mechanisms are related to new collagen formation, a key process in active fibrosis.
The current study integrates molecular and functional data to 1) identify genes and proteins associated to the active fibrosis process, 2) identify which of these genes and proteins are regulated *in vitro* and *in vivo* upon anti-fibrotic treatment, 3) identify efficacy biomarkers related to the molecular functional signature.

Methods

Fibrosis development was induced using bleomycin in the case of lung fibrosis studies and unilateral obstruction of the ureter (UUO) in case of kidney fibrosis. The transcriptome was determined using gene microarray analysis. New collagen formation was quantified using a proteomics approach in which deuterated-water in the drinking water was used to label newly formed proteins. Cellular proteome data from *(normal human lung fibroblasts; NHLF)* was determined using LC-MS/MS profiling. Data were integrated according the workflow in figure 1.

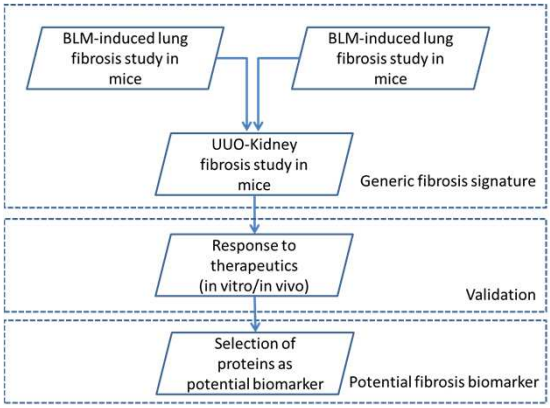


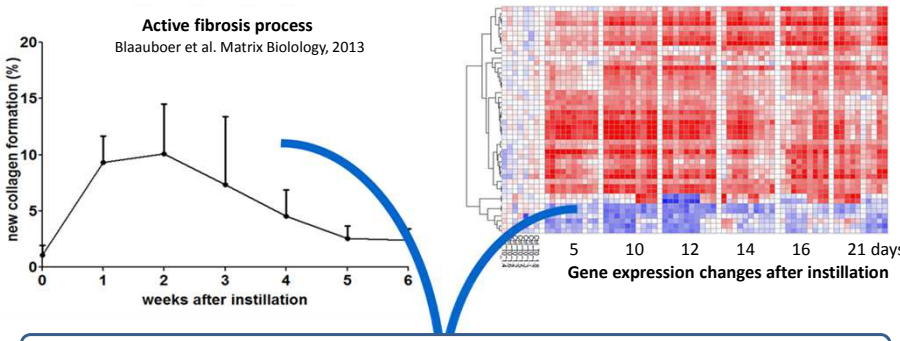
Figure 1. Workflow molecular signature related to active fibrosis process.

Conclusion:

- **Data integrative methods elucidate a functional gene-signature which is strongly correlated to the active fibrosis process.**
- **Applied to both *in vitro* and *in vivo* studies we selected a subset of the functional molecular signature to be regulated in disease development and responsive to anti-fibrotic treatment.**
- **In all, our method suggests identification of biomarkers for fibrosis development and subsequently allows use of gene expression profiling as readout for drug efficacy testing.**

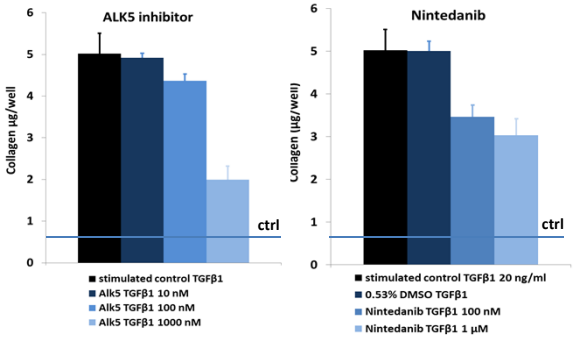
Results:

Generic fibrosis signature: Integrative approach to identify a core set of genes (microarray) that correlate (r=0.75; P<0.001) to newly formed collagen(determined by D_2O incorporation).

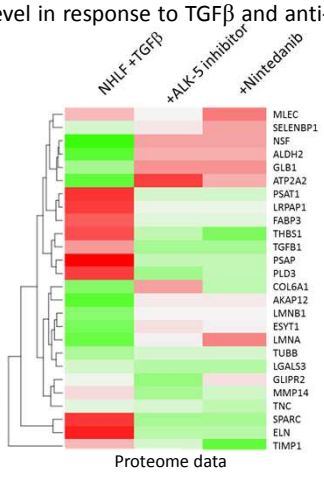


Set of 235 genes is associated to the active fibrosis process. These are used in validation studies.

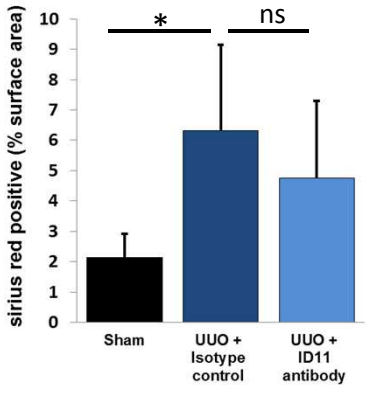
Validation (in vitro): NHLF+TGFβ were exposed to anti-fibrotic compounds. A subset of the functional signature (49 proteins) are regulated on protein level in response to TGFβ and anti-fibrotic treatments. ctrl= non-stimulated cells

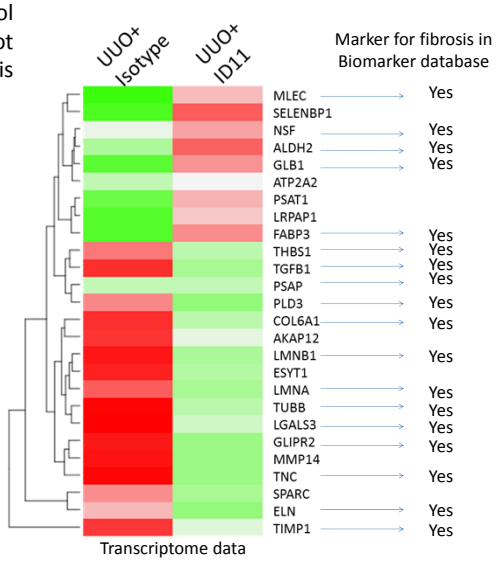


ALK5 inhibitor and Nintedanib reduce total collagen levels in TGFβ - stimulated NHLF. The heatmap shows protein regulation of a subset of fibrosis molecules (green=down; red = up)



Validation (in vivo): In a therapeutic protocol the efficacy of ID-11 (anti-TGFβ) was not significantly effective on histological fibrosis score (using Sirius Red staining).





Efficacy biomarkers: In the absence of efficacy, the transcriptome data of our functional molecular signature indicates an anti-fibrotic response of ID-11 treatment. In addition, the proteins are part of the Thomson Reuters Integrity database in relation to fibrosis.

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