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P347 -Proteomic landscape of an in-vitro model of TGF-β1-induced fibrogenesis in renal fibroblasts

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Introduction: Transforming growth factor (TGF)-β and (myo)fibroblasts are crucial mediators of fibrosis. To understand the intracellular and extracellular events underpinning TGF-β1-induced fibrogenesis in renal fibroblasts, we analysed the proteomic profiling of cell lysates and conditioned media of NRK-49F normal rat kidney fibroblasts, one of the best characterised renal fibroblast cell line, and compared it with that of NRK-49F cells undergoing TGF-β1-induced fibrogenesis.

Methods: Conditioned media and cell lysates of NRK-49F cells treated with and without 5 ng/ml TGF-β1 for 48h were subjected to collagen assays to confirm successful induction of fibrogenesis in the TGF-β1-treated group, followed by isobaric labelling-based mass spectrometric (MS) analysis of proteomic profiling, bioinformatic analysis and ELISA validation of proteins of interest.

Results: TGF-β1 significantly induced accumulation of total collagens in cell lysates and soluble collagens in conditioned media ($p<0.001$, $n=4$). MS analysis identified 1438 and 725 proteins in cell lysates and conditioned media, respectively, among which 628 (43.7%) and 62 (8.6%) were significantly regulated by TGF-β1 ($p<0.05$). No protein was detected only in either control or TGF-β1 group. TGF-β1-regulated proteins in cell lysates were significantly enriched in 44 cellular component clusters (cytoplasm, extracellular exosome, nucleus, membrane, mitochondrion and cytosol, etc), 24 biological processes (translation, oxidation-reduction process, response to drug, cell-cell adhesion and fatty acid β-oxidation, etc) and 27 molecular functions (poly(A)RNA binding, protein binding, ATP binding, structural constituent of ribosome, protein homodimerisation activity, etc). TGF-β1-regulated proteins in conditioned media were significantly enriched in 20 cellular component clusters (cytoplasm, extracellular exosomes, space, region and matrix, etc), 40 biological processes (cell adhesion, angiogenesis, proteolysis, cellular response to interleukin-1, positive regulation of ERK1 and ERK2 cascade, etc) and 7 molecular functions (binding of heparin, calcium ion, integrin, proteoglycan, insulin-like growth factor, kininogen and CCR2 chemokine receptor). In cell lysates, TGF-β1-treated group was characterised by increased ribosomal proteins and dysregulation of proteins involved in multiple metabolic pathways, and was featured with most significantly repressed catalase, a scavenger of reactive oxygen species, most robustly supressed Aldh3a1 and most robustly induced Enpp1, inviting further studies on roles for these intracellular pathways and proteins in fibrogenesis. In conditioned media, TGF-β1-treated group was characterised by dysregulation of matrix degradation regulators (most robustly induced PAI-1 and significantly repressed Mmp3), signalling mediators (most robustly supressed Nov/Ccn3, significantly induced Cyr61/Ccn1, Ctgf/Ccn2 and Tsku) and significantly induced collagen crosslinker (Plod2), and was unexpectedly featured with significantly induced chemokines Ccl2 and Ccl7.

Conclusion and discussion: For the first time, the proteomic landscape of TGF-β1-induced fibrogenesis has been established in a renal fibroblast cell line. It suggests that TGF-β1-induced fibrogenesis in fibroblasts may be essentially an intracellular metabolic disorder, leading to dysregulation of multiple secreted proteins involved in fibrogenesis, and may be inherently coupled with inflammation mediated by increased secretion of chemokines. Further studies in human renal fibroblasts and in-vivo studies are warranted to establish

human relevance of our findings, to further examine the discovered novel biological mechanisms and to test the identified novel targets and pathways for anti-fibrotic and anti-inflammatory drug discovery.