

Proteomic analysis of TGF- β 1-induced fibrogenesis and mechanisms of action of antifibrotics in renal fibroblasts

Aim: We aim to establish a proteomic view on TGF- β 1-induced *in-vitro* model of fibrosis in NRK-49F normal rat kidney fibroblasts and develop a comparative proteomic approach to differentiating the mechanisms of action of three antifibrotic entities: Scutellariae Radix (SR), a commonly used herb documented in Chinese, European and US herbal pharmacopoeias; baicalein, a SR-derived flavonoid; and IN1130, a TGF- β type I receptor inhibitor.

Methods: Antifibrotic activities were examined by quantitative analysis of total collagens and fibrogenic molecular markers. Cell lysates and conditioned media were harvested for TMT-labelling MS-based proteomic analysis. Proteins of interest were validated by ELISA.

Results: TGF- β 1-induced fibrogenesis in NRK-49F cells was characterised by increased ribosomal proteins and reduced proteins involved in multiple metabolism pathways, including highly significantly reduced catalase, an important scavenger of reactive oxygen species. The dysregulated ribosome and metabolism pathways were linked by increased Impdh2, a druggable target. Secretomic analysis indicated that TGF- β 1-induced fibrosis was mediated by dysregulation of key regulators of matrix degradation (increased Serpine1 and repressed Mmp3), signalling mediators (increased Ccn1, Ccn2 and Tsku, and repressed Ccn3) and an increased collagen crosslinker Plod2, and was coupled with increased chemokines Ccl2 and Ccl7. In this model, we found that methanolic SR (SRM), which contained 8-fold higher flavonoids than SR decoction, had 4-fold higher antifibrotic activities. Among five SR flavonoids commonly used for SR quality control, baicalein showed the highest antifibrotic activity and little toxicity. Comparative pathway analyses of conditioned media and cell lysates indicated that SRM, baicalein and IN1130 all significantly regulated the ribosome pathway; SRM and baicalein, but not IN1130, regulated the lysosome pathway, while they differentially regulated metabolism pathways. They all reversed TGF- β 1-induced Serpine1, Plod2, Ccn2, Ccl2 and Ccl7. Baicalein and IN1130, but not SRM, reversed TGF- β 1-induced Ccn1 and Tsku. Only baicalein reversed TGF- β 1 repression of Mmp3, and only IN1130 reversed TGF- β 1 induction of Impdh2 and repression of Ccn3. Among all proteins in cell lysates, Enpp1 was most dramatically induced by TGF- β 1 and it was also the most dramatically repressed by SRM, baicalein and IN1130; Aldh3a1 was the most dramatically repressed by TGF- β 1 and this was only reversed by IN1130.

Conclusion: TGF- β 1-induced fibrogenesis in rat kidney fibroblasts involves dysregulation of multiple secreted proteins involve in regulation of collagen synthesis, crosslinking and degradation, and is characterised by dysregulation of intracellular metabolism. It is inherently coupled with increased secretion of chemokines Ccl2 and Ccl7, suggestive of a pro-inflammatory role for myofibroblasts. Comparative proteomic analysis has uncovered overlapping mechanisms of the three antifibrotics, especially supporting baicalein as a promising multi-target antifibrotic drug. Besides known antifibrotic targets Serpine1 and Ccn2, the values of intracellular and extracellular proteins Enpp1, Aldh3a1, Impdh2, Ccn1, Ccn3, Mmp3, Plod2 and Tsku as targets for developing new antifibrotics deserve further investigation.