**The Hyaluronan Synthase-1 (HAS1) isoenzyme promotes differentiation to a distinct subset of myofibroblasts that limit fibrosis progression.**

**Introduction:** Chronic kidney disease (CKD) is common, rising in prevalence and associated with high morbidity and mortality. Renal interstitial fibrosis is a key determinant of CKD progression, and increased synthesis of the glycosaminoglycans, Hyaluronan (HA), in renal tissue correlates with fibrosis and renal outcomes. Factors that regulate HA synthesis therefore influence CKD progression. HA is synthesized by three HA Synthase enzymes (HAS1, HAS2 and HAS3), and our work has specifically shown that increased HAS2 expression is causally linked with fibrosis *in vivo* and is a critical mediator of pro-fibrotic cell phenotype *in vitro*. The influence of the HAS1 isoenzyme has previously not been studied. Research from our group and others indicates that the anti-fibrotic growth factor BMP7 (Bone Morphogenetic Protein-7) prevents and reverses pro-fibrotic cell phenotype and renal fibrosis in murine models. Our recent data indicates that BMP7 also significantly increases HAS1 expression, indicating a possible protective role for this enzyme in fibrosis. In this study, we compare the role of HAS1 versus HAS 2 in the regulation of pro-fibrotic cell phenotype and in prevention/reversal of fibrosis.

**Methods:** Studies were performed on human proximal tubular epithelial cells (PTEC) and in our established library of scarring *versus* non-scarring primary fibroblasts. Alongside this, genetic and histological analysis of murine kidneys from HAS1, HAS3 and HAS1/3 double knockout mice and mice with Ischaemia Reperfusion Injury (IRI)-induced renal fibrosis were studied. Custom-designed siRNA and plasmid constructs were utilised for knockdown and forced over-expression of HAS1 versus HAS2. HA levels were assessed and correlated with inflammatory and fibrosis profiles using ELISA, RT-qPCR, immunohistochemistry and confocal microscopy and FACS.

**Results:** In response to the pro-fibrotic cytokine TGF-β1, cells primarily expressing the HAS2 isoenzyme assembled large pericellular HA coats, and these coats were tethered to the principal HA receptor CD44-standard (CD44s) at the cell surface. The cells were significantly alpha-smooth muscle actin positive and had a contractile phenotype. In addition, they expressed significant levels of ED-A Fibronectin and generated increased levels of type I collagen matrix. In comparison, TGF-β1 stimulation in cells expressing primarily the HAS1 isoenzyme demonstrated negligible HA pericellular coats and had enhanced cell-surface expression of the CD44 variant isoform, CD44v7/8 (linked to prevention/reversal of fibrosis in our published studies). These cells had a limited alpha-smooth muscle actin response to TGF-β1, but instead demonstrated increased expression of the cell-surface protease Fibroblast Activated Protein (FAP). Moreover, they had attenuated levels of EDA-Fibronectin and type I Collagen matrix; and demonstrated a limited contractile response. Instead they laid down different fibronectins and were pro-migratory cells in response to TGF-β1. Kidneys from mice with IRI have elevated levels of both HAS1 and HAS2 in acute early injury, and HAS2 in late injury; whilst kidneys from HAS1/3 DKO mice only expressing the HAS2 isoform are predisposed to pro-fibrotic renal injury. Specifically, mice kidneys only expressing HAS2 and no renal injury have enhanced basal levels of alpha-smooth muscle actin, attenuated ECadherin and attenuated CD44v7/8 expression.

**Conclusion:** This study demonstrates that the different HAS isoenzymes have distinct and likely conflicting roles in response to pro-fibrotic stimuli. Whilst HAS2 promotes a pro-fibrotic contractile cell phenotype associated with tissue fibrosis, HAS1 appears to limit pro-fibrotic phenotypes and instead promotes differentiation to a distinct subset of myofibroblasts that are FAP positive and pro-migratory. *In vivo* data suggests that HAS1-driven HA synthesis may be involved in reparative processes that limits fibrotic injury, potentially through regulation of cell-surface CD44 variant expression.