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P348 -The role of GATA3 in bone morphogenetic protein 7 (BMP7) dependent prevention and reversal of myofibroblast phenotype

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Introduction: In progressive renal fibrosis, accumulation of scar-forming myofibroblasts results from differentiation of resident fibroblasts and pericytes which share a common renal stromal progenitor expressing forkhead box D1 (FOXD1) (1). We have recently shown that the transcription factor GATA3 is expressed by all FOXD1 lineage cell types and is critical for their differentiation. Moreover, GATA3 expression is lost in myofibroblasts suggesting a role in regulating myofibroblast phenotype. The glycosaminoglycan, hyaluronan (HA), is a key regulator of myofibroblast phenotype. Accumulation of peri-cellular HA promotes transforming-growth-factor-β1 (TGFβ1)-driven myofibroblast differentiation, while bone morphogenetic protein-7 (BMP7) prevents and reverses this differentiation by internalising peri-cellular HA through the actions of nuclear hyaluronidase 2 (HYAL2) and an alternatively-spliced variant isoform of the HA receptor, CD44 (CD44v7/8) (2-3). In this study we investigated the role of GATA3 in HA-mediated prevention and reversal of myofibroblast differentiation.

Methods: Primary human fibroblasts were used to test BMP7 antagonism of TGFβ1-driven myofibroblast differentiation (3). Custom-designed siRNA and plasmid constructs were utilised for knockdown and forced over-expression of CD44v7/8, HYAL2 and GATA3. HA levels were assessed and correlated with fibrosis profiles using ELISA, RT-qPCR, immunofluorescence and confocal microscopy. Alongside these in vitro studies, histological analyses of kidneys from control and ischaemia-reperfusion injured (IRI) rats were performed to examine the expression of GATA3, CD44v7/8, alpha-smooth-muscle-actin (α-SMA) and HA by immunohistochemical and immunofluorescence methods.

Results: Fibroblasts treated with BMP7 increased GATA3 expression in a time- and dose-dependent manner, with a maximal fold increase after 72 hours. This increase was HYAL2-dependent: treating cells with siRNA targeting HYAL2 resulted in attenuated BMP7-driven GATA3 expression indicating that nuclear HYAL2 regulates expression of GATA3. In a model of BMP7-driven antagonism of TGFβ1-driven myofibroblast differentiation, GATA3 expression showed a 10-fold increase ($p<0.01$) with a concomitant attenuation in α-SMA (ACTA2) expression and increased HA internalisation compared to cells treated with TGFβ1 alone. Transfection of fibroblasts with siRNA targeting GATA3 resulted in a 2-fold reduction ($p<0.03$) of the CD44 variant 7/8 expression, which we have previously shown to have an anti-fibrotic role by mediating HA internalisation. Histological analysis of rat kidney sections immunostained for GATA3 and CD44v7/8 showed co-localisation in a subset of peritubular fibroblasts in control animals. However, expression of both GATA3 and CD44v7/8 was attenuated in cortical perivascular fibrotic regions containing myofibroblasts that strongly co-stained for α-SMA and HA in the 28-day post-IRI rat model.

Conclusion: Our studies reveal a previously undescribed role for GATA3 in renal interstitial fibroblasts and show that GATA3 is a crucial mediator of BMP7-driven antagonism of TGFβ1-driven myofibroblast differentiation through its actions on CD44v7/8 expression. We propose that GATA3 is required to maintain the fibroblast phenotype and its loss contributes to myofibroblast differentiation, thus identifying a new potential therapeutic target for intervention in fibrosis.