

DELETING TGF- β RECEPTOR IN RENAL TUBULAR CELLS STIMULATES COLLAGEN PRODUCTION BY FIBROBLASTS

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Although TGF- β promotes tubulointerstitial fibrosis, we observed a paradoxical increase in fibrosis and TGF- β signaling in mice with the TGF- β type II receptor (TBRII), necessary for TGF- β activity, deleted specifically in the collecting system following injury. We hypothesized that TBRII deletion in collecting duct epithelial cells increases TGF- β signaling by neighboring fibroblasts, resulting in greater production of collagen I. This hypothesis was tested in vitro.

Using our TBRII floxed mice, we created inner medullary collecting duct (IMCD) cells and deleted TBRII with in vitro adeno-Cre infection. We examined how the conditioned media (CM) of TBRII null IMCD cells might alter matrix production by fibroblasts by incubating fibroblasts with conditioned media from IMCD cells, with and without a blocking TGF- β antibody (clone 2G7), for 48 hrs. Then we created lysates of the fibroblasts and underlying matrix and performed immunoblots for collagen I. A similar experiment was conducted using a transwell system in which filters containing confluent IMCD cells were placed upon wells plated with fibroblasts and co-incubated for 48 hrs. To test whether deleting TBRII in IMCD cells alters the surrounding levels of TGF- β , we measured total and active TGF- β in the CM of IMCD cells with and without TBRII. We assessed total TGF- β using the R&D Quantikine ELISA and bioactive TGF- β using PAI-1 cells with a truncated TGF- β responsive element in the PAI-1 promoter joined with a luciferase reporter gene.

The CM of TBRII $-/-$ IMCD cells increased fibroblast production of collagen I, an effect largely blocked by the TGF- β neutralizing antibody and replicated using transwells. CM of TBRII null IMCD cells had less total but more active TGF- β compared to the wild type. In conclusion, deleting TBRII in IMCD cells in vitro results in greater active TGF- β in the CM which partially mediates the increased collagen I production by fibroblasts. The increased active TGF- β in CM of TBRII null IMCD cells may result from activation of latent TGF- β , and mechanisms whereby this could occur are being studied.