

## **Substrate Stiffness Regulates Renal Epithelial Cell Cilia Formation via Autocrine TGF $\beta$ Signaling**

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### **Background:**

The primary cilium senses the extracellular environment. Malformation of primary cilia has been shown to result in kidney disease. Although much is known regarding ciliogenesis, the role of mechanical features of the microenvironment in cilia formation is poorly understood.

### **Methods:**

Human primary proximal tubule epithelial cells (HRECs) and LLC-PK1 cells were plated on collagen-coated polyacrylamide gels with different stiffness (0.5 KPa, 1 KPa, 10 KPa and 40 KPa) and grown for 5 to 7 days. Cilia formation was analyzed by immunofluorescence staining of AC-tubulin followed with microscopy imaging. Protein expression was measured by immunoblotting. Recombinant TGF $\beta$  1 and an inhibitor of TGF $\beta$ RII SB431542 were used for TGF $\beta$  signaling modulation. LiCl was utilized to stimulate P-GSK3b.

### **Results:**

Stiff hydrogels (10 KPa and 40 KPa) gave rise to higher ciliary density than compliant gels (0.5 KPa and 1KPa) did (60% vs 20%). Furthermore, this phenotype could be altered by manipulation of transformation growth factor beta (TGF $\beta$ ) signaling: addition of TGF $\beta$  1 could increase the cilia frequency even when the cells were on compliant gels; similarly, inhibition of TGF $\beta$  receptor II impairs cilia formation in the context of stiff substrates. Further supporting a role for TGF $\beta$ , substrate stiffness was associated with increased SMAD2 and GSK3B phosphorylation. Incubation with LiCl also increased GSK3B phosphorylation and cilia formation independent of TGF $\beta$ .

### **Conclusions:**

Substrate stiffness determines cilia formation through TGF $\beta$  signaling via downstream GSK3 $\beta$  phosphorylation. This work highlights that substrate mechanical properties have a strong influence on markers of differentiation. This has relevance for tissue engineering efforts and also suggests a mechanism of disease progression in the scarred kidney. This may broaden researches on kidney disease and contribute to disease therapy.