**K-Cadherin Protein is a Novel Basalolateral Sensor Involved in the Trans-Nephron Signalling for BMP-7 Release**

Cadherins are structural trans-membrane proteins that maintain epithelial integrity. Cadherin proteins are usually found on the lateral surface of cells classically forming junctions with neighbouring cells through homophillic binding. In the human kidney K-Cadherin (CDH6) is exclusively expressed in proximal tubular epithelial cells (PTEC) as opposed to the mouse, where K-Cadherin is only expressed in embryonic tubular cells. K-Cadherin exhibits low homology to E- (35%) & N- cadherin (38%). The loss of K-Cadherin in the kidney and the subsequent appearance of the full-length molecule in urine is associated with progression of diabetic kidney disease. Loss of K-Cadherin would suggest injury and recovery of the intact full-length molecule might also suggest regulated release in vesicles possibly indicating a signal of injury at a very precise location. This would be an excellent mechanism for signalling down the tubule to distal tubule cells which in turn are ideally located to supply reparative growth factors by basal release to the injured PTEC.

**Methods:** Biopsies of human kidney taken from 3 months post-transplant patients and biopsies from prospective organ donors were probed for K-Cadherin by immunohistochemistry. HKC clone 8 tubular human epithelial transformed cell line which do not have detectable K-cadherin protein were grown on collagen IV coated coverslips and transfected with human K-cadherin pcDNA3.1 expression vector (1µg) using Fugene 6. After 48h cells were fixed and immunostained for K-cadherin. Cells were visualized by using a 3D deconvolution microscope. Epithelial cells isolated from adult mice kidneys which also do not have detectable K-cadherin protein were separated into predominantly distal and proximal fractions and cultured to confluence. Conditioned human PTEC media taken from confluent PTEC cells and subjected to an exosomal isolation protocol was added to mice cells and allowed to incubate for 24h; cells treated with conditioned media were compared to cells treated with control media. Media was analysed for anti-fibrotic factor Bone Morphogenetic Protein 7 (BMP-7) and cells were lysed for western blot analysis.

**Results:** Analysis of human biopsy tissue: confirmed the proximal tubule expression of K-Cadherin in tissue from ‘donor’ kidneys, with basolateral expression; in the protocol biopsies K-Cadherin expression was disrupted in some PTEC regions and vesicular K-Cadherin was noted in some epithelial cells of distal tubules. In the transfected HKC8 cells K-cadherin immunostaining was found to be localised both in vesicular Golgi-type structures as well as near the cell periphery which were more prominent in the basal layer compared to the apical layer.

In the media of primary human PTEC cultures we identified the presence of full length K-Cadherin; the K-cadherin was still detected after performing the exosomal isolation. We subsequently investigated the effects of conditioned media from human PTEC on two preparations of mice primary cultures which were (a) “predominantly distal” or (b) “predominantly proximal” tubular epithelial cells and (c) murine collecting duct cells, IMCD. Significant *de novo* K-Cadherin protein was detected in distal mice cells treated with PTEC media compared to treatment with control media (*p*<0.05, n=6), K-cadherin in mice proximal cells (megalin positive) treated with PTEC media was not as pronounced. No K-cadherin uptake was detectable in mice collecting duct cells. BMP-7 was released from both mice distal and proximal tubular cell cultures treated with PTEC media but not with control media.

**Conclusions:** K-cadherin localisation in transfected HKC8 cells and healthy tissue is consistent with both a basement membrane sensor of the changes of matrix and a constituent of vesicles prepared for secretion. The up-take of K-cadherin by the treated murine distal cells is also consistent with our findings in protocol biopsy tissue from transplant recipients. This and the associated BMP-7 secretion strongly support our hypothesis of a previously unrecognised trans-nephron communication system.