Renal cilia display length alterations following tubular injury and are present early in epithelial repair

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Abstract

Background. Renal cilia are flow sensors that are required for the maintenance of normal kidney architecture. Defects in this organelle are frequently associated with polycystic kidney disease, but the role of renal cilia during acute tubular injury has not been investigated.

Methods. We have analysed the presence and dimensions of renal cilia following renal ischaemia-reperfusion and ureteral obstruction injury in the mouse, and related these results to injury and repair of the renal tubule. The expression of genes encoding cilium-localized proteins was measured following ischaemia-reperfusion injury.

Results. Ischaemia-reperfusion injury was demonstrated to affect the length of cilia in the renal tubule and duct. The average length of renal cilia in the proximal tubule decreases 1 day (2.8 ± 0.4 µm) and 2 days (3.0 ± 0.2 µm) after injury, as compared to the control uninjured proximal tubule (4.2 ± 0.3 µm). Later in the injury and repair process at 4 and 7 days, the average length of cilia increases in both the proximal (7 days = 6.2 ± 0.3 µm) and distal tubule/collecting duct (4 days = 4.4 ± 0.3 µm; 7 days = 5.5 ± 0.4 µm; control 2.5 ± 0.1 µm). The expression level of genes encoding cilium-localized products did not correlate with the increase in cilium length following ischaemia-reperfusion injury. Ureteral obstruction for 8 days also caused lengthening (8 days UUO = 5.8 ± 0.3 µm; control 2.5 ± 0.1 µm) of renal cilia in the distal tubule/collecting duct. During the repair process that follows ischaemia-reperfusion injury, cilia were present on the dedifferentiated cells that proliferate and adopt an epithelial phenotype to facilitate the repair of the ischaemic renal tubule.

Conclusions. We propose roles for the renal cilium in responding to changes in the renal environment caused by injury, and in the repair process that re-establishes the epithelial layer of the damaged renal tubule.

Keywords: Cilium; epithelial; ischaemia-reperfusion; polycystic kidney disease; ureteral obstruction

Introduction

Renal cilia are non-motile sensory organelles that are believed to detect fluid flow across the apical surface of the renal epithelial layer and initiate a cascade of events responsible for maintaining the architecture of the nephron and collecting duct [1,2]. Mutations affecting several cilium-localized proteins lead to polycystic kidney disease (PKD) [3], resulting largely from the dedifferentiation and over-proliferation of epithelial cells [4]. The behaviour of cilia following renal injury has not been investigated in detail, but we speculated that this organelle may also be involved in maintaining and repairing the epithelial layer following tubular injury.

Renal ischaemia-reperfusion injury (IRI) is caused by the transient interruption and reflow of blood supply to the kidney, and primarily damages epithelial cells of the renal proximal tubule [5]. This injury results in extensive epithelial death and dedifferentiation in the renal tubule, and an associated decrease in renal function [6]. Repair of the tubule following IRI is facilitated by a population of cells arising from the dedifferentiation and proliferation of surviving cells. These cells have a mesenchymal phenotype and re-epithelialize the damaged tubule by a process of migration, proliferation and redifferentiation [7,8]. Unilateral ureteral obstruction (UUO) is another cause of tubular injury involving the mechanical prevention of urine flow through the ureter. UUO results in dilatation of the collecting duct and distal nephron with accompanying epithelial cell death and interstitial expansion, and a dramatic reduction in glomerular filtration [9]. In contrast to IRI, the proximal tubule is resistant to damage by UUO [10].

With the exception of intercalated cells, every epithelial cell in the healthy renal tubule bears a cilium [11]. The fate of these cilia in the injured kidney and whether the cells that repopulate and repair the tubule bear a cilium has not been investigated. In this study, we followed the length and distribution of renal cilia in 7 days following severe...
renal IRI. Histology and immunostaining for dedifferentiation and cell proliferation were used to measure the status of injury and repair, and provide a framework for the interpretation of IRI cilia data. The post-ischaemic expression of four genes coding for cilium-localized proteins was also measured to determine whether the expression levels of these genes correlate to changes observed at the cellular level. The 8-day unilateral ureteral obstruction model was also used to further investigate the factors affecting renal cilia in a contrasting model of tubular damage.

Materials and methods

Induction of renal ischaemia-reperfusion and ureteral obstruction injury

Animal experiments were approved in advance by a Monash University Animal Ethics Committee and adhere to the ‘Australian Code of Practice for the Care and Use of Animals for Scientific Purposes’. To induce renal IRI, male C57bl/6j mice (6–8 weeks, 20–25 g) were anaesthetized with inhaled 1–4% isofluorane, and a flank incision was made to expose the left kidney. The left renal artery and vein were clamped for 60 min using a vessel clamp (S & T Fine Science Tools, Foster City, CA, USA). Following removal of the clamp, incisions were sutured and mice were removed from anaesthesia for recovery. Sham control mice underwent surgery in the same manner but no clamp was applied. Three mice were used per time point for histology, immunofluorescence, cilium measurement and proliferating cell nuclear antigen (PCNA) staining. Six experimental and three sham animals were used per time point for real-time PCR. For UUO, the procedure was used as for IRI but the clamp was placed on the ureter of the left kidney and remained there following recovery. UUO injured kidneys were collected for immunohistochemistry 8 days after surgery.

Histology and immunohistochemistry

Mice used for histology and immunohistochemistry were perfusion fixed with 4% paraformaldehyde in phosphate buffered saline. Kidneys were removed, embedded in paraffin and sectioned. Periodic acid-Schiff (PAS) staining was used to visualize the cellular extent of renal injury. Immunofluorescence staining used proteinase K or citrate buffer antigen retrieval and primary antibodies against acetylated α-tubulin (Sigma, St Louis, MO, USA), aquaporin-1 (Chemicon, Temecula, CA, USA) and vimentin (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Anti-mouse Alexa Fluor 488, anti-rabbit Alexa Fluor 555 and anti-goat Alexa Fluor 647 secondary antibodies (all from Molecular Probes, Eugene, OR, USA) were used to visualize acetylated α-tubulin, aquaporin-1 and vimentin respectively. Nuclei were stained with DAPI, specimens mounted in Prolong Gold medium (Molecular Probes) and examined on a Provis fluorescence microscope (Olympus, Tokyo, Japan) or a Fluoview 1000 confocal microscope (Olympus) with FV10-ASW software version 1.3c (Olympus).

Measurement of cilium length

Cilia were visualized on the Provis microscope using anti-acetylated α-tubulin as described above. This antibody produces intense staining of the acetylated microtubules of the cilia and does not stain unacetylated α-tubulin or unacetylated cytoplasmic microtubules [12]. Images of cilia oriented parallel to the plane of focus captured from randomly chosen high-power fields (100× objective, 92 × 69 µm) in the cortex and outer medulla. Aquaporin-1 staining of the proximal tubule brush border was used to distinguish the proximal tubule from the distal tubule and collecting duct. AnalySIS version 5.0 software (Olympus) was used to trace and measure the length of cilia in captured images. Twenty proximal and 20 distal tubule/collecting duct cilia were measured from each of the three mice per time point, giving a total of 60 proximal and 60 distal tubule/collecting duct cilia per time point.

Quantification of proliferation

Anti-PCNA (DAKO, Glostrup, Denmark) was used on paraffin sections with boiling 10 mM citric acid buffer antigen retrieval and an anti-mouse Alexa Fluor 488 secondary antibody (Molecular Probes). Sections were immunostained with anti-aquaporin-1, nuclei stained with DAPI and mounted as described above. PCNA staining was observed using fluorescence microscopy and images of at least five fields (40× objective, 231 × 173 µm) captured from the cortex and outer medulla of each kidney in the IRI and control groups (three mice per time point). The percentage of PCNA-positive nuclei was calculated for the proximal tubule and distal tubule/collection duct based on at least 1000 nuclei for each time point.

Real-time PCR

RNA was isolated from kidneys using the Rneasy Mini Kit (Qiagen, Doncaster, Australia) and cDNA synthesis was with multiscribe and random hexamers (Applied Biosystems, Foster City, CA, USA). Real-time PCR primer sequences for the mouse Inversin (Invs), polycystic kidney disease 1 (Pkd1), polycystic kidney disease 2 (Pkd2) and Intraflagellar transport 52 (IFT52) genes were obtained from primer bank [13] and checked with sequence from GenBank (http://www.ncbi.nlm.nih.gov/Genbank/) to ensure that a primer or the amplicon spanned an intron. The primer bank ID numbers for primers used were 31982787a1 for Invs, 7305389a1 for Pkd1, 31543487a1 for Pkd2 and 31981999a1 for IFT52. Gene expression levels were determined relative to 18S rRNA by the comparative dCT method using Sybr green and the ABI PRISM 7700 sequence detector system (Applied Biosystems).

Statistical analyses

Values are expressed as mean ± SEM. Statistically significant differences were defined as $P < 0.05$. Cilium length and PCNA data from IRI were analysed using a one-way ANOVA with an accompanying Tukey’s post hoc test performing inter-group comparisons. Real-time data were
analysed using a one-way ANOVA with an accompanying Dunnett’s post hoc test. Cilium length data from UUO was analysed using an unpaired $t$-test.

Results

Histopathology of ischaemia-reperfusion damage and repair

PAS staining was used to observe the extent of tubular damage and repair in the corticomedullary region (Figure 1A–F). This region of the kidney contains the S3 segment of the proximal tubule that is particularly susceptible to IRI. Compared to control (Figure 1A), 1 day (not shown) and 2 days (Figure 1B) after IRI there was a loss of the proximal tubule brush border, widespread cell death and protein cast formation in the tubular lumen, and an interstitial inflammatory cell infiltrate was evident. At 4 days and 7 days resolving protein casts (Figure 1C and E) were frequently surrounded by spindle-shaped cells attached to the luminal side of the tubular basement membrane (Figure 1D and F). An increased interstitial inflammatory cell infiltrate was associated with mild extracellular matrix deposition and interstitial expansion at 7 days (Figure 1F).

Fig. 1. The histopathology of IRI. Control kidney (A), 2 days (B), 4 days (C and D) and 7 days (E and F) after IRI are shown. Kidneys from IRI mice exhibited a loss of the brush border, widespread cell death and tubular cast formation at 2 days (B, asterisks). Resolving tubular casts were evident 4 days after IRI (C, asterisks). (D) A high-power image of spindle-shaped cells (arrow) lining the tubular basement membrane 4 days after induction of IRI. (E) 7 days post-ischaemic injury kidneys showed resolving tubular casts (asterisk). (F) A high-power image shows the casts were lined by spindle-shaped cells (arrow) or cuboidal epithelium (open arrows). Scale bars: $E = 25 \mu m$ and A, B and C are at the same magnification; $F = 10 \mu m$ and D is at the same magnification.

Cilium length is altered in the ischaemic kidney

An antibody against acetylated $\alpha$-tubulin detected renal cilia on epithelial cells throughout the nephron and collecting duct in the control kidney (Figure 2A, C, E and G). Cilia were visible in all but the most severely damaged nephron segments of the ischaemic kidney at 1 days and 2 days (not shown). Some cilia were observed in extensively damaged regions of the proximal tubule at 4 days and 7 days time points and were borne by dedifferentiated, spindle-shaped cells as discussed below. Most cilia at 7 days were present on cells that had a normal cuboidal or columnar epithelial phenotype and an aquaporin-1-positive brush border if located in the proximal tubule (Figure 2B, D, F and H). Many cilia in the 7 days kidney appeared longer than those observed in control kidneys (Figure 2A–H). Measurement of

Fig. 2. Cilium lengthening 7 days after IRI. Cilia (arrows) were stained with anti-acetylated $\alpha$-tubulin (green), the brush border of the proximal tubule with anti-aquaporin-1 (red) and nuclei with DAPI (blue in A, B, E and F). Cilia were viewed with standard fluorescence microscopy (A, B, E and F) or using confocal reconstruction (C, D, G and H). Normal length cilia were visible in the proximal (A and C) and distal tubule/collection duct of control tissue (E and G). Longer cilia were observed in the proximal tubule (B and D) and distal tubule/collection duct (F and H) of IRI kidneys at 7 days. Scale bars: $F = 10 \mu m$ and A, B and E are at the same magnification; $H = 5 \mu m$ and C, D and G are at the same magnification.
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Fig. 3. Cilium lengths in IRI damaged kidneys. The length of cilia in the proximal tubule was significantly decreased at the 1 day and 2 days time points (A). However, with resolving renal injury there was a significant increase in cilium length in both the proximal and distal tubule/collecting duct (A and B). In the proximal tubule this cilium length increase occurred at 7 days while in the distal tubule/collecting duct cilium length was significantly increased relative to 0 day control at both the 4 days and 7 days time points (B). Note that the 1 day and 2 days distal tubule/collecting duct cilium lengths in B are significantly different ($P < 0.05$) to each other but not to 0 day control. (C) and (D) show the frequency distribution of cilium lengths in control (open bars) and 7 days post-ischaemic kidneys (black bars). Cilia in the proximal tubule of the 7 days kidney show a shift towards the longer length classes (C). There are many cilia in the 7 days distal tubule/collecting duct longer than the 4–6 µm maximum found in the control kidney (D). Values in A and B with different letters are significantly different ($P < 0.05$).

Cilium length determined that cilia initially shorten in the proximal tubule after IRI before becoming significantly longer in both the proximal and distal tubule/collecting duct (Figure 3A and B). There was a significant shortening ($P < 0.05$) of cilia on proximal tubule epithelial cells 1 day ($2.8 \pm 0.4 \mu m$) and 2 days ($3.0 \pm 0.2 \mu m$) after IRI, compared to the uninjured control proximal tubule ($4.2 \pm 0.3 \mu m$) (Figure 3A). With resolving injury and tissue remodelling after IRI, there was a significant lengthening ($P < 0.05$) of the cilia in the proximal tubule at 7 days ($6.2 \pm 0.3 \mu m$) (Figure 3A) and in the distal tubule/collecting duct at 4 days ($4.4 \pm 0.3 \mu m$) and 7 days ($5.5 \pm 0.4 \mu m$) compared to control ($2.5 \pm 0.1 \mu m$) (Figure 3B). Cilia were assigned to length classes spanning 2 µm and the percentage of cilia in each class plotted for control and 7 days IRI kidney (Figure 3C and D). Cilium lengths in the proximal tubule 7 days after injury were shifted towards the longer categories and were predominantly 4–10 µm in length, while most cilia from the control proximal tubule were 2–6 µm in length (Figure 3C). In the distal tubule/collecting duct, many cilia from the 7 days time point were in excess of 6 µm, a cilium length that was not observed in control kidneys (Figure 3D). Confocal 3D reconstructions such as those shown in Figure 2C, D, G and H allowed us to visualize cilia that were not parallel to the plane of focus.

These confocal images confirmed that the images obtained by standard fluorescence microscopy were representative in most cases. However, the time-consuming nature of confocal reconstruction and obtaining cilium measurements from these reconstructions made this technique impractical for sampling large numbers of cilia.

Proliferation in the renal tubule and duct following IRI

Proliferation was investigated in the proximal tubule and distal tubule/collecting duct following IRI. Immunostaining for PCNA detected only the occasional positive nucleus in the control kidney (Figure 4A). Many PCNA-positive nuclei were detected in the in the proximal tubule at 2 days (Figure 4B) and 4 days (not shown). The average percentage of proliferating cells per field in the proximal tubule was highest at 2 days (56%) and 4 days (46%) and was subsiding by 7 days (14%) (Figure 4C). Levels of proliferation were markedly less in the distal tubule/collecting duct than in the proximal tubule at all time points following injury (Figure 4C).

Expression levels of genes encoding cilium-localized proteins following IRI

Real-time PCR quantification from samples of the kidney was used to determine the expression levels of Pkd1, Pkd2,
Fig. 4. Cell proliferation in the renal tubule and duct following IRI. Proliferation was measured by immunostaining for PCNA (green, white arrows) as seen in control (A) and at 2 days post IRI (B). Proximal tubules were identified by aquaporin-1 staining (A and B, red) and nuclei were stained with DAPI (blue). There was a visible increase in PCNA-positive nuclei in the proximal tubule (white arrows) and the interstitium (open arrow) at 2 days, while PCNA staining in the nuclei of aquaporin-1-negative tubules and ducts (*) remained infrequent (B). The percentage of proliferating cells was dramatically elevated at 2 days and 4 days in the proximal tubule, but remained low in the distal tubule/collecting duct at all time points following IRI (C). Scale bar in B = 10 \( \mu \)m and A is at the same magnification.

Fig. 5. Relative gene expression of Pkd1, Pkd2, Invs and Ift52 after IRI. Real-time PCR demonstrated that Pkd2 (B) and Invs (C) expression was significantly upregulated (*\( P < 0.05 \)) 2 days and 1 day after ischaemic injury respectively. Pkd1 (A) and Ift52 (D) expression was not upregulated in the kidney following IRI. Values show gene expression relative to 0 day control.

Ureteral obstruction results in cilia lengthening in the distal tubule/collecting duct

Eight days of UUO did not significantly alter the average cilia length in the aquaporin-1-positive proximal tubule (Figure 6A and D). UUO did however cause a dramatic increase in the average cilia length in aquaporin-1-negative distal tubules and collecting ducts (2.5 \( \pm \) 0.1 \( \mu \)m to 5.8 \( \pm \) 0.3 \( \mu \)m; \( P < 0.05 \)) (Figure 6B and D). The proximal tubule was not visibly dilated after 8 days of UUO while aquaporin-1-negative structures representing the distal tubule/collecting duct were frequently dilated (Figure 6C).

Cilia are present on dedifferentiated cells in the renal tubule following IRI

Staining with an antibody against vimentin was used to detect surviving tubular cells that had dedifferentiated in response to IRI. Vimentin-positive cells with a spindle-shaped morphology were detected lining the basement membrane of damaged proximal tubules at 4 days (Figure 7A–J) and 7 days (Figure 7K–T) after injury. Cilia projecting into the lumen were detected on a number of these vimentin-positive cells, some of which also co-expressed aquaporin-1, thereby
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C. increase (D). Scale bars: inducible factor-I injury. We investigate the behavior of renal cilia following tubular reperfusion and ureteral obstruction models were used to study the effect of renal ischemia-reperfusion damage and associated cellular proliferation. The role of renal cilia in maintaining renal architecture is well established [3,14,15]. Given the importance of the renal cilia in the normal kidney, we hypothesized that renal cilium in the normal kidney, we hypothesized that the role of renal cilia in maintaining renal architecture is well established [3,14,15]. Given the importance of the renal cilia in the normal kidney, we hypothesized that renal cilium in the normal kidney, we hypothesized that the role of renal cilia in maintaining renal architecture is well established [3,14,15]. Given the importance of the renal cilia in the normal kidney, we hypothesized that renal cilium in the normal kidney, we hypothesized that

demonstrating the reappearance of the brush border (Figure 7F–J and P–T). At both the 4 days and 7 days time points, cilia were present on aquaporin-1-negative, vimentin-positive, spindle-shaped cells in tubules that could be identified as proximal by the presence of cells that had already formed an aquaporin-1-positive brush border (Figure 7A–E and K–O). This result indicates that the presence of an apical cilium precedes the formation of the proximal tubule brush border.

Discussion

The role of renal cilia in maintaining renal architecture and the relationship of cilia-related defects to PKD is well established [3,14,15]. Given the importance of the renal cilia in the normal kidney, we hypothesized that this organelle may also be involved in re-establishing renal architecture following injury. The mouse renal ischaemia-reperfusion and ureteral obstruction models were used to investigate the behavior of renal cilia following tubular injury.

We have demonstrated that renal cilium length is altered in response to severe IRI. An observed initial decrease in cilium length in the proximal tubule is consistent with hypoxia inducible factor-Iα (HIF-Iα)-mediated regulation of ciliary assembly described for cultured renal carcinoma cells [16]. HIF-Iα inhibits the assembly of cilia and its abundance increases rapidly in the proximal tubule after ischaemic renal injury [17,18]. The initial shortening of cilia in the proximal tubule is followed by a lengthening of cilia in both the proximal tubule and distal tubule/collecting duct. Ischaemia-reperfusion damage and associated cellular proliferation are most pronounced in the proximal tubule, but cilium lengthening is not correlated to these factors and is actually greater in the distal tubule/collecting duct. Cilium lengthening also occurs at 7 days in the proximal tubule which is after the peak in the percentage of PCNA-positive cells. Cilium lengthening is an energy-dependent process that uses a kinesin- and dynein-based mechanism referred to as intraflagellar transport [19]. Thus cilium lengthening during epithelial injury is likely to be a directed response, rather than the result of simple metabolic disruption. Real-time PCR studies of IRI kidneys suggest that it is difficult to monitor cilium assembly by measuring the expression of genes coding for cilium-localized products. Pkd2 and Invs code for polycystin-2 and inversin respectively, proteins involved cilium-mediated signalling, and their expression was significantly increased shortly after IRI. However, expression of these genes had returned to normal levels by the time cilium length increases were detected. Polycystin-2 and inversin localize to other regions of the cell in addition to the cilium [15] and Pkd2 and Invs are likely to be upregulated to fulfill extracellular roles. Pkd1 and Ifit32, whose products function in cilium-mediated signaling and cilium assembly roles, respectively, were not upregulated by IRI to a statistically significant degree. Microarray analysis of UUO, which also exhibits cilium lengthening as discussed below, failed to demonstrate significant upregulation of Pkd1, Pkd2 and Invs at 7 days (data not shown). These results parallel a previous study showing that the expression level of the Tg737 gene coding for a cilium assembly component was not significantly different in MDCK cultures with and without cilia [20]. Thus, in genetically normal renal epithelia, cilium length appears to be linked to the regulation of assembly rather than the abundance of cilial precursors and assembly machinery.

Cilium length changes during tubular injury were further investigated in the UUO model of hydronephrosis. UUO for 8 days caused injury and dilation of the aquaporin-1-negative distal tubule and collecting duct that was accompanied by a dramatic increase in average cilium length. No significant change in cilium length was observed in the undilated proximal tubule. Taken together, results of IRI and UUO experiments suggest that epithelial insults cause changes to urine flow and/or composition that trigger cilium lengthening in damaged and downstream regions of the nephron and collecting duct. Potential mediators of this effect include reductions in urine production and/or composition caused by severe IRI and UUO [21,22], and compounds or cellular fragments released into the urine by injured or dying epithelial cells. The established sensory capacity of the cilium may implicate it in the detection of the mediators discussed above, although direct detection by the cilium is not necessarily a prerequisite for the ability to influence cilium length. A lack of cilium lengthening in the proximal tubule after UUO suggests that the inflammatory environment of the injured kidney is not causing a non-specific increase in renal cilium length.

![Cilium length in UUO](image-url)
Fig. 7. Cilia on dedifferentiated cells in the IRI proximal tubule. Dedifferentiated cells in the damaged proximal tubule bear cilia at the 4 days (A–J) and 7 days (K–T) time points. Cilia (white arrows) were stained with anti-acetylated α-tubulin (green, A, F, K and P), the brush border of the proximal tubule (open arrows) with anti-aquaporin-1 (red, B, G, L and Q), dedifferentiated cells with vimentin (purple, C, H, M and R), and nuclei with DAPI (blue, D, I, N and S). Note that cast material stains for aquaporin-1 (G). Overlays are shown in E, J, O and T with insets (×3 zoom) showing cilia in E and J. At the 4 days and 7 days time points, cilia were present on dedifferentiated, vimentin-positive cells that lack aquaporin-1 expression (4 days, A–E; 7 days, K–O) as well as cells that are aquaporin-1 positive (4 days, F–J; 7 days, P–T). Scale bar in T = 10 µm and A–S are at the same magnification.

Regardless of the mechanism causing cilium assembly following injury, the lengthening of renal cilia has the potential to impact on epithelial differentiation. Studies of PKD suggest that flow-induced deflection of the renal cilium is required for the maintenance of epithelial phenotype in the renal tubule and duct [2, 14]. Cilium length and sensitivity to flow have previously been shown to be linked in the Oak Ridge polycystic kidney mouse which has shortened renal cilia and reduced sensitivity to flow [23]. Modelling studies also suggest that longer cilia experience greater the shear forces and bending in response to flow [24]. Moreover, evidence from other systems suggests that the cilium is a generalized antenna structure used for a variety of sensory purposes including the detection of chemicals, osmolarity and ligands involved in cellular signalling [25]. A longer cilium clearly has a larger surface area in contact with the external environment and is likely to be a more sensitive antenna. We propose that the lengthening of the renal cilium on sublethally injured cells increases their sensitivity to flow and other cilium-detected factors that promote epithelial phenotype, and may represent a compensatory response that counteracts dedifferentiation.

The sensory ability of the renal cilium is also likely to be important in badly damaged regions of the renal tubule where there is a requirement for cell replacement. We have demonstrated the presence of an apical cilium on the spindle-shaped cells with a mesenchymal phenotype that repopulate the denuded basement membrane and differentiate to facilitate epithelial repair [26]. The possession of a cilium would allow these cells to sense flow and other signals that may promote appropriate epithelial division and differentiation in the repairing tubule. The ability to sense flow has been proposed to specify the correct spatial orientation of cell division required to recellularize the tubule without distorting its architecture [27]. Cilium-mediated flow sensing has also been implicated in the regulation of Wnt signalling that allows epithelial differentiation during renal development [28] and may also be relevant during epithelial repair.

In summary, we have characterized the behaviour of cilia in response to IRI and propose roles for this organelle in compensating for the changes caused by injury and in the reepithelialization of the renal tubule. Given the possible involvement of renal cilia in cellular processes occurring after epithelial injury, it follows that renal cilium defects may compromise the ability of the kidney to respond to and repair tubular damage. This raises the possibility that PKD patients, whose condition is largely caused by cilium-related defects, may also have a reduced capacity to tolerate renal injury.

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