Editorial Comments

Ciliary function of polycystins: a new model for cystogenesis

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Introduction

Autosomal dominant polycystic kidney disease results from loss-of-function mutations in either polycystin-1 (Pc-1) or polycystin-2 (Pc-2). These transmembrane proteins directly interact through cytosolic domains of their C-termini. Pc-1 has been implicated in cell–extracellular matrix (ECM) interactions at focal adhesion contacts, but also in cell–cell interactions at tight junctions, adherens junctions and desmosomes [1]. Pc-2, located to the plasma membrane and/or to the endoplasmic reticulum [2,3], was shown to increase the intracytosolic calcium from both ER stocks and extracellular milieu [4]. The variability of polycystin localization within the cell may be explained by differences in antibody characteristics, cell type, degree of confluence, developmental stage, but also raises the hypothesis of multiple biological functions [1]. The large extracellular domain of Pc-1 is thought to transduce signals from the environment into the cells, through Pc-1-dependent signalling pathways and through activation of PC-2 calcium channel properties. However, the search for polycystin ligands and functions at sites of cell–cell or cell–ECM interactions has remained elusive to date. In fact, the first recently reported biological function of polycystins depends on an additional and unexpected polycystin subcellular localization—the renal primary cilia—and is triggered by an unexpected ligand, the urinary flow.

Renal primary cilia

Primary cilia are cell organelles originating from the basal body and related to the centriole. Their basic structure consists of a central axoneme composed of nine doublets of microtubules and a ciliary membrane continuous with the plasma membrane [5]. One or two primary cilia are present on the surface of most vertebrate cells. With the exception of intercalated cells, every epithelial cell lining the nephron carries at least one primary cilia, which is usually long (2–30 μm) and thin (0.25 μm in diameter). Renal primary cilia protrude into the tubular lumens and are in contact with the urine [5,6]. Although they may have some motility in lower vertebrates, renal primary cilia in mammals do not propel urine. Many consider primary cilia as a vestigial structure from a motile ancestor and totally useless for the cell. This hypothesis was recently challenged by the fact that several proteins implicated in the biology of polycystic kidney diseases were located in the renal cilia (Figure 1).

Polycystins are located in renal primary cilia

In the nematode Caenorhabditis elegans, Lov-1, pkd-2 and OSM-5 (respective orthologues of the human proteins Pc-1, Pc-2 and polaris) were shown to colocalize in specialized cilia, named sensory neurons [7]; these proteins also act in a non-redundant manner through a common transduction pathway [8], and their mutations alter the structure and/or the sensory function of cilia, finally compromising reproduction of the male worm [8,9]. These studies raised the question of a possible link between polycystic kidney disease and cilia in mammals, which was subsequently confirmed by studies conducted in mice (compare with Table 1). Disruption of the Tg737 gene (Tg737Δrepk or Tg737Δdelta2-3betaGal mice) induces PKD and other anomalies, including altered left–right axis determination [10,11]. Tg737 encodes polaris, a protein normally present at the basal body and along the axoneme of primary cilia, which is indispensable for cilia assembly through ascendent intraflagellar transport of particles [11]. The laterality defects observed in this murine model were attributed to the absence of cilia on the ventral node cells during embryogenesis [12]. Similarly, disruption of the inv gene (inv/inv mice) induces a cystic phenotype and...
left–right axis anomalies, both corrected by re-expression of inversin, a protein encoded by inv and located in the primary cilium [13,14]. Spontaneous mutations of Cys-1 (in Cpk/cpk homozygous mice) induce a recessive form of PKD, and although no left–right patterning defects or cilia structural anomalies were demonstrated, it appears that cystin-1, the protein encoded by Cys-1, is also localized in cilia [15]. Finally, several laterality defects were found in one model of Pkd2 inactivation in mice [16]. Taken together, these findings suggested that the polycystins could be localized in the monocilia of nodal cells, but also in primary cilia of renal epithelial cells.

Not surprisingly, several groups have now confirmed that Pc-1 and Pc-2, along with polaris, cystin-1 and inversin, are co-localized in primary cilia of renal epithelial cells [4,17,18]. One may wonder why such a peculiar localization of polycystins has not been discovered sooner. Cilia are not easily visualized by optic microscopy, and their in vitro development requires specific culture conditions, which include high confluency and complete polarization [18,19]. As suggested above, it is possible that in different conditions of cell culture, Pc-1 may localize at different cell membrane compartments, such as the cell–cell lateral contacts or at the basal pole of cells. As for Pc-2, its subcellular localization to the endoplasmic reticulum may be used as a reservoir for proteins secondarily exported to the cilia membranes.
Ciliary function of polycystins

Three putative functions have initially been evoked for cilia in tubular lumens: reabsorption of solutes, presentation of membrane receptors to urinary ligands and mechano-sensorial rheostat to urinary flow. Recently, this last function was demonstrated by two studies of outstanding interest. In response to a lateral fluid flow, cilia of epithelial monolayers bend with subsequent rise in intracellular calcium [20]. Using different cell lines, Nauli et al. [4] further demonstrated that the fluid-induced cellular influx of calcium is abolished when Pc-1 is mutated, or upon ligation of the extracellular domains of Pc-1 or Pc-2 to blocking antibodies. These in vitro results indicate that the kidney primary cilium is a mechanoreceptor, and strongly suggest that the urinary flow may well be the long-term searched main ligand for the large extracellular domain of Pc-1. Interestingly, some properties of the extracellular domain of Pc-1 could contribute to the sensitivity to ciliary bending, such as cis homophilic interactions between PKD domains [21] and/or extracellular cleavage [22]. Pc-2 combines with Pc-1 and acts as a calcium channel, which is responsible upon activation of an acute increase in intracellular calcium, from both extracellular sources and intracellular stocks. However, whether Pc-2 triggers intracellular calcium release through InsP3 or ryanodine receptors (or both) and the kinetics of intracellular calcium rise are controversial and will require further clarification.

Currently, the main challenge is to identify the other consequences of polycystin complex mechanoreceptor stimulation: downstream transduction pathways, target genes and ultimately effects on cell or tissue fate. Of note, all the polycystin-interacting molecules and polycystin-dependent transduction pathways identified to date were discovered using in vitro systems not designed to express cilia: will these results hold true if polycystins are allowed to induce their more physiological ciliar expression? In this perspective, it will be particularly interesting to determine the link between cilia, polycystins and the main Pc-1-dependent signalling pathways identified so far, including JAK2-STAT1-p21 [23], G proteins [24] and Wnt-β catenin pathways [25].

Cilia and cystogenesis

According to the ‘double-hit theory’, the initial and critical molecular event underlying cyst formation is the occurrence of a ‘second hit’ (a somatic PKD1 or PKD2 mutation), which superimposes on the initial inherited germinal PKD1 or PKD2 mutation [26]. This theory accounts for the focal nature of cysts, but does not explain why tubular cells dedifferentiate, proliferate and ultimately form continuously growing cysts.

In mice with homozygous invalidation of either Pc-1 or Pc-2, the first renal cysts are detectable after 15–16 days of gestation, i.e. after the onset of a glomerular filtration flow [27]. This observation indicates that polycystins are not mandatory for the initial steps of tubulogenesis, but also suggest that mutated polycystins may fail to transmit to the tubular cells a ‘stop signal’ normally generated by the urinary flow [28]. Tubular cells unable to perceive the ciliary ‘stop signal’ may become hyperproliferative, dedifferentiated and initiate an aberrant tubular growth leading to the formation of a cyst. Thus, if fluid-flow perception controls the diameter and differentiation state of renal tubules through cilia, any anatomic and/or functional anomaly of cilia (induced by mutations of polycystins, polaris, cystin or inversin) may induce renal cystogenesis. Altered perception of cilia bending may also account for other clinical features of PKD, such as liver and pancreatic cysts, colonic diverticuli or vascular aneurysms, but this hypothesis will require further investigation.

The identification of cilia dysfunction as a key feature of polycystic kidney diseases will also encourage new therapeutic strategies. For instance, taxanes, which promote microtubule assembly (one of the putative functions of cystin), were shown to ameliorate renal cystic disease in cpk/cpk mice [29]. We thus speculate that any other pharmacological agent able to improve tubular ciliary functions or to stimulate polycystin complex mechanoreceptors will be a strong candidate for PKD treatment.

Conflict of interest statement. None declared.

References


