Abstract  Phosphoinositides (PIs) play pivotal roles in the regulation of many biological processes. The quality and quantity of PIs is regulated in time and space by the activity of PI kinases and PI phosphatases. The number of PI-metabolizing enzymes exceeds the number of PIs with, in many cases, more than one enzyme controlling the same biochemical step. This would suggest that the PI system has an intrinsic ability to buffer and compensate for the absence of a specific enzymatic activity. However, there are several examples of severe inherited human diseases caused by mutations in one of the PI enzymes, although other enzymes with the same activity are fully functional. The kidney depends strictly on PIs for physiological processes, such as cell polarization, filtration, solute reabsorption, and signal transduction. Indeed, alteration of the PI system in the kidney very often results in pathological conditions, both inherited and acquired. Most of the knowledge of the roles that PIs play in the kidney comes from the study of KO animal models for genes encoding PI enzymes and from the study of human genetic diseases, such as Lowe syndrome/Dent disease 2 and Joubert syndrome, caused by mutations in the genes encoding the PI phosphatases, OCRL and INPP5E, respectively.—Staiano, L., and M. A. De Matteis. Phosphoinositides in the kidney. J. Lipid Res. 2019. 60: 287–298.

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Phosphoinositides (PIs) are phosphorylated derivatives of phosphatidylinositol. The inositol ring can be phosphorylated on three positions (positions 3, 4, and 5) generating seven different species of PIs (PI3P; PI4P; PI5P; PI3,4P2; PI3,5P2; PI4,5P2; and PI3,4,5P3) as a result of single or multiple phosphorylation events (1). The seven PI species can be interconverted by the activity of a family of enzymes (more than 50) comprising PI kinases and PI phosphatases (2). The steady state amount of PIs in resting cells represents 10–20% (percent of moles) of cellular phospholipids with PI4P and PI4,5P2 accounting for 0.2–1% of total cellular phospholipids (3, 4).

PIs are distributed in different cell districts and their subcellular localization affects their function and downstream effects. The PI distribution is a snapshot of the PI distribution at steady state. In some cases, the appearance of these fast-evolving pools may occur by different mechanisms, such as lipid precursor delivery by lipid transfer proteins, which may operate at the level of membrane contact sites (8).

The effort the cell puts into keeping the PIs at the right place at the right time is justified by their importance as initiators, controllers, and terminators of several cellular functions. The number of PI enzymes (20 kinases and...
Changes in PI distribution are required for modification at the apical surface in a region that faces the nascent tubule. This signal engages an intracellular signal that is transmitted to the opposite membrane determination by laminin accumulation gener-
bly of laminin at the basement membrane (13). Basement cascade that involves Rac1 activation leading to the assem-
extracellular matrix. This interaction triggers a signaling

Indeed, mutations in single enzymes are causative of severe inherited human diseases. These diseases are in many cases characterized by the involvement of specific tissues and organs, a phenomenon that is far from being completely understood (2). In some cases, it can be explained by the differential intracellular targeting of en-
zymes with the same activity to particular subcellular sites. Evidently, some tissues can tolerate alterations of the PI system, while other tissues suffer when the system is altered.

Overall, 27 Mendelian disorders are known to be caused by mutations in 18 of the 54 PI enzymes, and recurrent target tissues in these diseases are principally the central and peripheral nervous system, but also muscle and kidney, indicating that correct PI metabolism is particularly crucial for these organs that provide important models to study the physiological roles of PIs (2).

Among the several functions exerted and controlled by PIs, we will discuss the roles of PIs in the kidney and in cell processes that are fundamental for kidney embryogenesis and function, such as determination of cell polarity (9–13), control of cytoskeletal rearrangements (14, 15), and initiation, progression, and termination of endocytosis (15–17). We will also discuss animal models and genetic diseases (Fig. 1) involving different domains of the kidney caused by the loss of function of PI en-
zymes, and we will highlight how the study of these disorders has been fundamental for understanding the role of PIs and for uncovering basic cellular and molecular mechanisms controlled by PIs. Finally, we will refer to PI metabolism as one of the pathways that is currently targeted to treat renal cancer.

PIs AND RENAL CELL POLARITY

PIs play a pivotal role in the polarization of epithelial cells. In vitro studies have shown that kidney cells (MDCK) grown in three dimensions are able to polarize and generate a tubule-like structure (10). The polarization is trig-
gered by the interaction of β1-integrin with collagen in the extracellular matrix. This interaction triggers a signaling cascade that involves Rac1 activation leading to the assembly of laminin at the basement membrane (13). Basement membrane determination by laminin accumulation generates an intracellular signal that is transmitted to the opposite side of the cell defining it as the apical surface. This signal affects the cytoskeleton with most of the actin accumulating at the apical surface in a region that faces the nascent tubule. Changes in PI distribution are required for modification of the actin cytoskeleton and thus contribute to the specific-
ation of the apical and basolateral regions of the cells. In nonpolarized cells, PI4,5P2 and PI3,4,5P3 are evenly distributed at the plasma membrane (with PI3,4,5P3 being far less abundant and generated only upon cell stimulation) (18). However, when polarization begins, the PI3,4,5P3-3-phosphatase, PTEN, is targeted to the apical domain and converts PI3,4,5P3 into PI4,5P2, which thus becomes the main PI at this site, while PI3,4,5P3 remains enriched at the basolateral domain of the cell (18, 19). Apical PI4,5P2 recruits annexin 2 (Anx2) that promotes Ca²⁺-dependent actin polymerization. To this end, Anx2 recruits the small 

PIs IN PODOCYTES

Podocytes are highly specialized cells that are key elements of the filtering unit in the nephron. The peculiarity of the podocyte epithelial monolayer is the presence of cell projections (i.e., foot processes) that interdigitate with foot processes emanating from the surrounding cells (22). Each foot process is able to generate additional and secondary expansions in order to make an arborized, interconnected, and zipper-like structure called the slit diaphragm (23, 24). Many proteins, such as nephrin, podocin, and Neph1, take part in the formation and the structural maintenance of the slit diaphragm by interacting with the actin cytoskele-
ton (25, 26). The relevance of the structural proteins of the slit diaphragm and also of the cytoskeletal proteins in-
volved in its formation for the filtering capacity has been confirmed by the appearance of focal segmental glomeru-
ulosclerosis and subsequent proteinuria when one, or more, of these proteins is mutated (27). Podocytes thus have a pivotal role in building up the filtration barrier of the nephron. However, in the late fifties, an active role of podocytes in internalizing plasma components was hypo-
thesized. Electron microscopy studies of podocytes revealed the presence of intracellular vesicles that increased under pathological conditions such as glomerulonephritis and lupus erythematosus (28). To date, the contribution of endo-
cytosis as an internalization route of plasma components in podocytes is still unclear; this role has been mainly associated
with the proximal tubule (PT) epithelial cells. Although endocytosis (where a clear role of PIs has been demonstrated) is not the main function of podocytes, several lines of evidence suggest a pivotal role of PIs and PI-metabolizing enzymes in podocyte biology and, in general, in glomerular functions. For example, the depletion of the PI4,5P2 5-phosphatase, synaptojanin (Synj)1, in mice results in severe proteinuria and failure in the formation of functional foot processes in podocytes (29). Studies of focal segmental glomerulosclerosis have highlighted the tight interconnection between Synj1, the endocytic machinery, the actin cytoskeleton, and the formation of a proper slit diaphragm. In particular, in the absence of Synj1, an increase of its substrate, PI4,5P2, triggers Arp2/3-dependent ectopic accumulation of nucleated actin at clathrin-coated pits, as also happens in dynamin-depleted podocytes (30). Dynamin is a GTPase whose activity is required to close the neck and allow the internalization of nascent clathrin-coated vesicles (CCVs) (Fig. 2). In the absence of dynamin, podocytes show an increase in undetached CCVs and actin accumulation at these sites and, as a global consequence, the mice develop proteinuria (29). These two examples (Synj1 and dynamin depletion in podocytes) show the tight interconnection between PI metabolism, endocytosis, and the formation of a functional glomerular filtration barrier. This is mainly due to the role of the endocytic machinery in the formation, stabilization, and maintenance of a functional slit diaphragm. The slit diaphragm is a modified and improved version of a tight junction representing the terminal and principal barrier for the retention of plasma macromolecules (23). Although it may appear as a static and fixed structure, the proteins composing the slit diaphragm undergo continuous cycles of endocytosis and recycling to be properly modified and fully functional. For instance, to improve the stability of the slit diaphragm, nephrin is phosphorylated and tightly interacts with podocin, stabilizing the whole complex. When nephrin is dephosphorylated, it interacts with β-arrestin and is then internalized via clathrin-mediated endocytosis. Defective endocytosis of nephrin results in altered podocyte morphology and hypofunctional glomeruli because the endocytic machinery coupled with lysosomal quality control is required for the physiological
turnover of nephrin (29). ZO-1 is another slit diaphragm protein that is regulated by the activity of endocytic proteins. In particular, interaction with the actin-based myosin motor protein, Myo1e, and with the PI4,5P2-interacting motor protein, Myo1c, is required to maintain localization of ZO-1 at tight junctions at the plasma membrane (31, 32). Myo1e has also been associated with the viability and adhesion of podocytes and with their endocytic capacity because its overexpression in vitro increases the internalization of transferrin (33). Intracellular membrane trafficking and, in particular, the endolysosomal pathway thus contribute to the quality control of the slit diaphragm that is, in turn, strictly connected with the vitality and functionality of podocytes. Additional evidence for the importance of PIs in podocyte biology comes from the observation of severe glomerulosclerosis in mice with podocyte-specific ablation of \( \text{Vps34} \), a PI3-kinase responsible for the generation of PI3P, whose role in the control of autophagy has been clearly demonstrated (34). Mice with podocyte-specific conditional KO of \( \text{Vps34} \) develop significant proteinuria at 3 weeks of age as a consequence of massive podocyte vacuolization with subsequent loss of foot processes and impairment of the glomerular filtration barrier (35). Furthermore, \( \text{Vps34} \) KO podocytes reveal gross trafficking defects, especially at early-to-late endosome maturation (35) (Fig. 2). Other evidence of defective PI metabolism that results in aberrant podocyte biology comes from the study of a mouse model genetically ablated of the class II PI3-kinase C2\( \alpha \) \( (\text{PIK3C2C2} \alpha) \), the enzyme that controls the conversion of PI3P to PI3,4P2.

Mice depleted of the \( \text{Pik3c2c2} \alpha \) gene develop severe proteinuria and podocyte foot process damage resulting in chronic renal failure. In addition, the absence of PIK3C2\( \alpha \) also affects the expression and localization of nephrin, synaptopodin, and desmin with a deleterious impact on podocyte morphology and physiology (36). The importance of the endocytic machinery in podocytes is far from being just a mechanism to control the quality and the localization of the proteins of the slit diaphragm. As stated above, while active reabsorption of plasma proteins by endocytosis is mainly a feature of PT cells (PTCs), podocytes can reabsorb and degrade proteins that have been filtered through the glomeruli (30). Albumin, for instance, has been observed inside podocytes and has been shown to be targeted to lysosomes (37).

### PIs IN KIDNEY TUBULE CELLS

The main function of the kidney tubule is the active reabsorption of proteins, hormones, vitamins, and ions that are present in the glomerular ultrafiltrate, but have to be absent or at very low amounts in urine. Of the different segments of the tubule, the PT is the main station for protein reabsorption. The epithelial cells lining this part of the nephron (PTCs) are characterized by an apical surface that faces the tubule lumen and a basolateral surface that faces the surrounding cells (lateral) and the blood vessels (basal). The polarization of the PTCs that, as highlighted above, is controlled by PIs is instrumental for their function and
endosomes with reduced sorting of receptors to the plasma membranes impact on the trafficking of the receptors out of the early endosomes (16). These cytoskeletal rearrangements result in the differential distribution of transporters and receptors. Once polarity has been established in PTCs, the apical side facing the lumen is enriched in ion transporters and receptors that are key players and regulators of the main function of PTCs: the maintenance of body fluid and solute homeostasis by reabsorption from the glomerular ultrafiltrate (38). PTCs are able to reabsorb large amounts of plasma proteins (several grams per day) such as albumin and transferrin, hormones such as insulin and growth hormone, and vitamins such as retinol and vitamin D. The PT reabsorbs ~80% of proteins and peptides from the ultrafiltrate, thus providing a protein-free environment for the following segments of the nephron. The uptake of proteins by the PT relies mainly on clathrin-mediated endocytosis (39). Megalin and cubilin are the two main multiligand receptors that control the uptake of proteins. Megalin is a member of the low density lipoprotein receptor family, whereas cubilin is a membrane-associated protein that lacks a transmembrane domain and relies on megalin for its internalization and intracellular trafficking (38). The amnionless protein is another protein that indirectly participates in the uptake of proteins from the ultrafiltrate by controlling the processing, trafficking, and anchoring of cubilin to the apical plasma membrane (40, 41). The binding of ligands to these receptors at the plasma membrane induces their internalization via clathrin-mediated endocytosis. The pivotal role of PIs in the initiation, control, and progression of clathrin-mediated endocytosis highlights the importance of an efficient PI system in the physiology of PTCs. In PTCs, the binding of a protein to be reabsorbed to its cognate receptor induces internalization and the receptor-ligand complex enters the cell in CCVs. The assembly of CCVs is triggered by the PI4,5P2-dependent recruitment of adaptor proteins (APs) and clathrin (42). The CCVs containing the receptor-ligand complex then progress through the endocytic pathway and, after conversion of PI4,5P2 to PI3P (via PI3,4P2), which stimulates uncoating (dissociation of the clathrin coat from the vesicle), the receptor-ligand complex reaches the apical endosomal compartment (17, 43). This compartment is extremely important in polarized PTCs because the ligand dissociates from the receptor in this tubular-shaped structure, thanks to the acidification of the lumen via the vacuolar ATPase, and is recycled back to the apical membrane (44). The conversion of PIs during CCV internalization and the arrival of endocytosed cargo-receptor complexes in a PI4,5P2-depleted/PI3P-enriched endosomal compartment is fundamental for the following phases of intracellular trafficking, such as recycling of the receptors and the lysosomal targeting of the cargoes (7, 16). The PI4,5P2 5-phosphatase, OCRL (the protein mutated or absent in Lowe syndrome), is a key regulator of this step (16). In the absence of OCRL, its substrate accumulates ectopically at endosomal membranes, one of the sites where OCRL resides. This PI4,5P2 accumulation stimulates Arp2/3-dependent actin polymerization and a resultant increase in actin fibers around the endosomes (Fig. 3) (16). These cytoskeletal rearrangements impact on the trafficking of the receptors out of the endosomes with reduced sorting of receptors to the plasma membrane (megalin), to the trans-Golgi network (mannose 6-phosphate receptor), and to lysosomes (EGFR) (Fig. 3) (16). In PTCs, fast and efficient recycling of receptors to the apical membrane is necessary for the continuous reabsorption of ligands that would otherwise be lost with the urine. This recycling depends on the local and spatiotemporally controlled synthesis of PI4,5P2 that is required for the recruitment of effector proteins to guarantee the completion of this step, because PI4,5P2 is responsible for the recruitment of coat proteins and of proteins involved in actin polymerization (which functions in tubule elongation and vesicle budding from the apical endosomal compartment). Along with PIs, the small GTPases of the

Fig. 3. OCRL dysfunction causes alterations of PTCs. Schematic representation of a healthy PTC (left) and a PTC from a Lowe syndrome patient with dysfunctional OCRL (right). The absence of OCRL results in an increase in clathrin-coated vesicles that do not lose their clathrin coat and that display the presence of actin comets as a consequence of the persistent presence of PI4,5P2. PI3P-positive early endosomes are normally free of PI4,5P2 (healthy). The absence of OCRL induces an increase in PI4,5P2 levels at the early endosome with the induction of actin polymerization at these sites. This actin polymerization impairs the trafficking of receptors through the early endosomes, resulting in reduced recycling back to the plasma membrane, reduced retrograde trafficking to the Golgi complex and reduced lysosomal sorting of cargos. Furthermore, in the absence of OCRL, PI4,5P2 accumulates on autolysosomes resulting in defective autophagic flux and an accumulation of autophagosomes. The PC undergoes structural and functional changes when OCRL is absent.
Rab family are other key players in these important pathways for PTC and kidney physiology (45). By interplaying with PIs, Rab5 has a pivotal role in the early endocytic compartment, whereas Rab4 and Rab11 mainly control the recycling pathway (46). After dissociation from the receptors, the ligands are trafficked through the early endosomal compartment toward the lysosomal compartment for degradation. Of note, a large amount of endocytosed proteins is transferred to the blood circulation by transcytosis to the basolateral membrane, bypassing the endolysosomal compartment (47). PTCs rely on lysosomal function for the proper processing of endocytosed materials but also for general cellular homeostasis via the autophagy pathway. Indeed, PTCs have a high rate of basal autophagy that is required to counteract the excess of proteins they have to deal with (48). It has been reported recently that, in vitro and in vivo, the autophagy pathway is impaired in PTs of proteinuric mice characterized by high amounts of albumin in the urine (48). A reduction in the number of autophagosomes and a decrease of the autophagic flux were observed in these conditions that are causative of progression toward chronic kidney disease. This underscores the need for a fully functional autophagic-lysosomal pathway in PTCs to handle large amounts of protein. We have recently discovered that PIs (in particular PI4,5P2) and PI-metabolizing enzymes [phosphatidylinositol-4-phosphate 5-kinase (PIP5Ks) and OCRL] can also control this trafficking step. In particular, in PTCs, where a sustained autophagic flux is necessary (48), a high level of autophagosome-lysosome fusion is required (49). To this end the PTCs activate a response, the lysosomal cargo response, which ensures that the protein and lipid composition of the lysosomal membrane is optimal to sustain the rate of fusion events (7). The fusion of autophagosomes with lysosomes and the delivery of autophagic cargo triggers the lysosomal cargo response by activating Toll-like receptor 9 (TLR9), which senses mitochondrial DNA present within the lysosomes (7, 50). The activation of TLR9 induces the recruitment of PIP5K1α and PIP5K1β to autolysosomes that synthesize PI4,5P2 (51). The local synthesis of PI4,5P2 is required for the recruitment of the AP, AP2, and of clathrin to induce the budding of vesicles from the autolysosomes. These vesicles are needed for the retrieval of membrane proteins, such as the autophagosomal SNARE, syntaxin 17 (52), to new autophagosomes that are then ready to undergo a new fusion cycle. The ectopic synthesis of PI4,5P2 at the autolysosome has to be limited in time and space, a function undertaken by OCRL at autolysosomes where it degrades PI4,5P2 and shuts down the response. In the absence of OCRL, the excess of PI4,5P2 has a deleterious effect on lysosomal fusogenic capacity (Fig. 3) because it inhibits TRPML1 (53, 54), the ion channel that, when dysfunctional, severely reduces the rate of autophagosome-lysosome fusion (7, 55).

PIs AND THE PRIMARY CILIUM

The PI-Rab-dependent regulation of the endocytic compartment is also fundamental for the formation and activity of another important organelle of the polarized PTC: the primary cilium (PC) (56, 57). The PC is a microtubule-based sensory organelle that can sense and integrate extracellular stimuli and transmit the information by triggering signaling cascades, such as Hedgehog, Wnt, Notch, G protein-coupled receptors (GPCRs), and receptor tyrosine kinases (58). By controlling such a large set of signaling cascades, dysfunctions of the PC by mutations of ciliary genes result in diseases belonging to the ciliopathy class. The membrane surrounding the PC is in continuity with the plasma membrane. However, a domain at the ciliary base, known as the transition zone, blocks the diffusion of proteins and lipids from the plasma membrane (59). The ciliary membrane thus has a unique protein and lipid composition. It follows that the PIs at the PC differ from those of the plasma membrane, and this difference has a great impact on the functions of this sensory and signaling organelle (59). In particular, the main PI at the PC membrane is PI4P, while PI4,5P2 is confined to the ciliary base (59). The presence of PI4P is guaranteed by the recruitment of the 5-phosphatase, INPP5E (59, 60), the protein mutated in Joubert syndrome (JBTS) (see below), belonging to a family of diseases known as ciliopathies (61–63). The absence of PI4,5P2 at the PC ensures reduced actin levels (because PI4,5P2 has a crucial role in driving actin polymerization), which is fundamental for the properties of the PC as a signaling antenna that necessitates not only a more stable structure but also a membrane that is less subjected to the continuous remodeling that occurs during endocytic and exocytic events at the plasma membrane (64). The PI composition of the PC is particularly important for the Hedgehog signaling pathway that controls cell proliferation and differentiation (65). Hedgehog signaling is transduced at the PC by the GPCR protein, Smoothened, that has been reported to bind to PI4P (66). The local production of PI4P mediated by INPP5E-mediated PI4,5P2 dephosphorylation ensures a hub for Smoothened binding and signal transduction. In addition, the accumulation of PI4,5P2 at the ciliary base is instrumental in the binding of TULP3, a protein that regulates the entry into the PC of a GPCR, Gpr161, that acts as a negative modulator of Hedgehog signaling (67, 68). In addition to the role of PIs and PI enzymes in the identity of the PC and in the regulation of ciliary signaling, it emerged recently that PI-controlled vesicular trafficking is required for the maintenance of the ciliary structure. The PI4,5P2 5-phosphatase, OCRL, has been shown to control trafficking to the PC by interacting with Rab5 and Rab8, which are involved in the arrival of vesicles to the PC from the plasma membrane and from the trans-Golgi network, respectively (69, 70). Depletion of OCRL reduces targeting of ciliary rhodopsin that occurs by both Rab5- and Rab8-dependent trafficking (69). Furthermore, in the absence of OCRL, ciliary length is reduced in fibroblasts and retinal pigmented epithelial cells and increased in kidney cells (MDCK) (70, 71). This suggests an active role of OCRL in providing key building blocks for the morphogenesis of the PC (Fig. 3). Interestingly, depletion of Ocrl in zebrafish leads to the appearance of phenotypes, such as underdeveloped eyes and brain, laterality defects,
and cystic kidneys, similarly to other zebrafish models of ciliopathies (69). At the cellular level, Ocrl depletion reduces the length of cilia in the pronephros, the embryonic equivalent of the kidney in zebrafish (69). Thus, PI compartmentalization and PI-regulated trafficking at the PC are fundamental for the structural and signaling functions of this organelle. It has been reported recently that the depletion of Pik3c2a, the PI3,4P2-producing enzyme, also results in an impairment of the Rab8-mediated trafficking of the ciliary signaling component, polycistrin-2 (72). Alteration in the PI composition of the PC, for example in the absence of INPP5E and Ocrl, causes defects in ciliogenesis and ciliary functions and results in pathological manifestations (61).

**PIs IN MONOGENIC KIDNEY DISEASE**

**Lowe syndrome and Dent disease 2**

Lowe syndrome, first described in 1952 (73), is an inherited X-linked disease characterized by ocular, cerebral, and renal involvement with a prevalence of 1:500,000 to 1:1,000,000 (2, 74, 75). These features are summarized in the nomenclature of the disease that is also known as oculocerebro-renal Lowe syndrome (OMIM 309000). In 1992, Ocrl was identified as the gene causative of Lowe syndrome (76). Although primarily affecting males, Lowe syndrome can also occur in females as a consequence of balanced X chromosome:autosome translocation or nonrandom X chromosome inactivation (77, 78). The renal pathology in Lowe syndrome is characterized by the loss of solutes with the urine, a condition known as Fanconi syndrome that develops in the first months of life in almost all patients, but with variable severity. Renal Fanconi syndrome in Lowe syndrome is considered incomplete because it is characterized by low molecular weight proteinuria and albuminuria, whereas glycosuria is rarely observed (79–81). Aminociduria and lysosomal enzymuria are always present along with renal bicarbonate and phosphate wasting (81). In approximately 50% of patients, hypercalciuria has been observed with subsequent onset of nephrocalcinosis (81). All children with Lowe syndrome present dense bilateral cataracts and severe hypotonia at birth. The eye involvement also includes the nystagmus, in almost all patients, and the development of glaucoma in 50% of patients (79). The neurological symptoms include mild to severe intellectual disabilities and behavioral abnormalities, such as compulsive, aggressive, irritable, and stereotyped behavior (79, 80). All patients experience growth retardation that could be mostly related to the Fanconi syndrome. However, patients with no renal symptoms display growth retardation, suggesting a bone dysfunction related to Ocrl loss of function. The life expectancy of Lowe patients has increased over recent decades and currently is about 40 years. In most cases, kidney failure represents the cause of death. Whereas surgery is the common procedure for the cataracts, a therapy for renal Fanconi syndrome has to be adapted to the needs of each patient. In some cases, Lowe patients are treated with oral supplementation of water, bicarbonate, citrate, phosphate, salts, and vitamin D to compensate for the urinary loss of these compounds. In a few selected cases, the worsening of kidney function in Lowe syndrome requires dialysis and kidney transplantation.

Loss-of-function mutations in the **Ocrl** gene are also causative of Dent disease 2, an X-linked proximal tubulopathy characterized by low molecular weight proteinuria, hypercalciuria, and progressive renal failure (82). Approximately 15% of patients with Dent disease show mutations in **Ocrl** (Dent disease 2) (83, 84), whereas the remaining 85% are characterized by mutations in the endosomal chloride channel **Clcn5** (Dent disease 1) (85). The phenotypic spectrum is comparable in Dent disease 1 and 2 with a slightly higher incidence of extrarenal symptoms in Dent disease 2 (82, 86). Renal manifestations in Dent disease 2 are milder than those observed in Lowe syndrome, especially regarding the presence of nephrocalcinosis and the urinary wasting of amino acids, bicarbonate, phosphate, or potassium (75). The **Ocrl** gene, located on chromosome Xq 25-26, codes for one of the 10 inositol polyphosphate 5-phosphatases present in eukaryotic cells (2, 87). Ocrl is present in two isoforms: the a isoform is ubiquitously expressed, whereas the b isoform is expressed in all tissues with the exception of the brain. Compared with other enzymes with similar 5-phosphatase activity, such as SYNJ1 and SYNJ2, SKIP and INPP5B, Ocrl has the highest activity toward PI4,5P2 and Ins1,4,5P3. It is also active, although to a lesser extent than INPP5E (see below), toward PI3,4,5P3 (88). Ocrl is a 110 kDa multi-domain protein that, in addition to its 5-phosphatase catalytic domain, contains a pleckstrin homology domain (which is however unable to bind PIs) (89), an ASPM SPD-2 Hydin domain [where most of the interactions with Rab GTPases occur (characteristic of proteins that localize to centrosomes and primary cilia)] (90–93), and a RhoGAP-like domain (which does not exhibit any GTPase activating function, but mediates the interaction of Ocrl with Cdc42 and Rac1) (91, 94–96). Ocrl also has one AP2 binding site and two clathrin binding domains (97). Pathogenetic mutations are present throughout the gene with most mutations that cause Lowe syndrome located in exons 8-23 and those causing Dent disease 2 in exons 1-7 (75). Lowe-causing mutations fall in the 5-phosphatase, ASPM SPD-2 Hydin, and Rho-GAP domains. Mutations falling in the pleckstrin homology-like domain mainly cause Dent disease 2 and may result in the expression of splicing variants that retain some biological activity (98). A clear genotype-phenotype correlation is not evident; indeed, milder variants of Lowe syndrome have been found to be associated with mutations causing complete loss of function of the protein. Thus, it seems probable that genetic background impacts on the clinical manifestations of the disease (99, 100). Interestingly, three missense mutations in the 5-phosphatase domain (821T>C, 952T>C, and 1568A>G) are causative of Lowe syndrome in some cases and Dent disease 2 in others, suggesting the presence
of modifier genes. No cure is currently available for Lowe syndrome or Dent disease 2. As such, animal models are fundamental not only to gain new insights into the pathogenesis of the diseases but also to develop new therapeutic strategies. The first mouse model of Lowe syndrome (Ocrl KO) was generated in 1998, but did not show any of the eye, brain, or kidney defects observed in humans (101). It was subsequently discovered that the absence of clinical phenotypes was due to the compensatory ability of Inpp5b, a 5-phosphatase with the same catalytic activity as OCRL and with 45% sequence identity and a very similar domain organization at the protein level (102, 103). The reasons why Inpp5b can compensate for Ocrl loss in mice, but not in humans, is not fully known, although it has been observed that, in mice, Inpp5b is more expressed in the affected tissues compared with humans. Furthermore, Inpp5b is differentially spliced in mice and humans because mouse Inpp5b contains an alternative promoter and an alternative transcription start site in exon 7 (103). For these reasons, a new mouse model was generated in 2011 by deleting Ocrl and substituting mouse Inpp5b with human INPP5B. The Ocrl−/−/Inpp5b−/− INPP5B mice display proximal tubular dysfunction with low molecular weight proteinuria and aminoaciduria, but do not present extrarenal symptoms (102). In 2016, a new mouse model for Lowe syndrome was generated by the conditional KO of Inpp5b in the kidney of Ocrl−/− mice (104). The absence of Ocrl and Inpp5b in mouse kidney leads to defects in PT reabsorption as a consequence of impaired endocytosis (104).

With the availability of animal models and with the steadily increasing knowledge on the cell biology of Lowe syndrome, the main challenge will be to discover new potential strategies for the treatment of Lowe syndrome. Because most of the cellular phenotypes with an impact on tissue and organ physiology are caused by the accumulation of the substrate of OCRL (PI4,5P2), a substrate reduction approach could be exploited by targeting the kinases (PI4Ks and PIP5Ks) responsible for PI4,5P2 synthesis. Along with a drug repositioning approach, the development of new compounds able to act on PIP5Ks and selectively interfere with PI4,5P2 synthesis may be promising avenues to find a treatment for Lowe syndrome.

**JBTS**

JBTS (OMIM 213300) is a rare autosomal recessive disease with a prevalence of 1:100,000 live births. It is characterized by brain malformations of the cerebellum and brainstem, retinal dystrophy, renal cystic disease, liver fibrosis, and polydactyly (105). JBTS belongs to the family of diseases known as ciliopathies, caused by malfunctioning of the PC (see above) (62, 63, 105–109). In the kidney, primary cilia sense fluid movements and transduce the signal in order to balance proliferation and apoptosis (110). In patients with JBTS, renal cysts form as a consequence of the hyperproliferation of tubular cells. JBTS can be caused by mutations in more than 30 genes, most of them playing a role in the structural maintenance and function of primary cilia (105). One of the genes mutated in JBTS is the PI5,4,5P3 and PI4,5P2 5-phosphatase, INPP5E, mostly active in the dephosphorylation of PI3,4,5P3 (62, 111, 112). INPP5E is a 72 kDa 5-phosphatase characterized by two domains: a N-terminal proline-rich domain with two immunoreceptor tyrosine-associated motifs and interaction sites for SH3 domain-containing proteins and a 5-phosphatase catalytic domain. A C-terminal CAAX box mediates membrane anchoring after prenylation (112, 113). To date, 16 pathogenetic mutations in INPP5E have been identified, according to the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/), with most of them falling in the 5-phosphatase catalytic domain and affecting highly conserved basic amino acid residues (109). These mutations reduce the 5-phosphatase activity of INPP5E and also affect its protein-protein interaction network impacting on its subcellular localization. Interestingly, mutations in INPP5E have been described as causative of MORM syndrome, a ciliopathy characterized by mental retardation, truncal obesity, retinal dystrophy, and micropenis, observed in 14 consanguineous families from a Northern Pakistani family (114). Of note, the main pathogenic mutation in INPP5E associated with MORM syndrome is a homozygous nonsense 1879C-T transition in exon 10 that results in the production of a premature stop codon leading to a deletion of the 18 C-terminal amino acids and loss of the highly conserved CAAX motif (63). As consequence, INPP5E, although enzymatically functional, is no longer distributed along the ciliary axoneme, but is confined at the proximal part of primary cilia. Renal involvement has been observed in about 30% of JBTS patients and is characterized by tubulointerstitial defects with the thickening of the tubular epithelium basal membrane and progressive interstitial fibrosis. The presence of small cysts at the cortico-medullary junction has been described. The glomeruli are often normal, although in some cases some of them display fibrosis. As regards the renal pathology, young patients with cystic kidneys are often asymptomatic or show mild polyuria and polydipsia until the second decade of life when chronic renal insufficiency manifests. Kidney and liver complications represent the major causes of death in JBTS patients (109).

**THERAPEUTIC STRATEGIES TARGETING PIs**

Unfortunately, genetic diseases affecting the kidney caused by mutations in PI enzymes are currently devoid of a cure. However, PI metabolism is targeted by current strategies for the treatment of kidney cancer. Among the cancers affecting the kidney, renal cell carcinoma (RCC) has gained increasing attention in recent years for its asymptomatic onset and high tendency to evolve to metastatic RCC (mRCC) (115). This implies that most RCC diagnoses are made after the development of metastasis. Pharmacological treatment of mRCC is difficult considering the very low responsiveness to standard therapies, such as chemotherapy and radiation (115). However, the study of the cell biology of mRCC has highlighted the presence of signaling pathways that can be targeted to reduce the aggressiveness and the spreading of tumor cells. The angiogenesis pathway is one of the most activated in mRCC and is partially
due to the hyperactivation of the von Hippel-Lindau (VHL) gene by DNA hypomethylation (116). Hyperactive VHL induces the accumulation in cells of a transcription factor, hypoxia inducible factor (HIF), that results in an increase of the pro-angiogenic response (116, 117). Targeting this pathway, mainly through the inhibition of vascular endothelial growth factor receptor (VEGFR), has been the elected therapeutic strategy for many patients (118). However, many patients display intrinsic or adaptive resistance to these therapies (119). This led to the development of combined therapies acting on VEGFR and the Akt/mTOR pathway that was discovered to play a pivotal role in conferring resistance to anti-angiogenic treatment (120). A second strategy was thus characterized by the combined use of anti-angiogenic compounds and mTOR inhibitors (everolimus and temsirolimus) (120). Unfortunately, it turned out that, in RCC cells, these inhibitors, which inhibit mTORC1, activate mTORC2 (121). mTORC2 has as a downstream effect the activation of HIF-2α that contributes to the onset of resistance to these compounds (122). Furthermore, mTORC1 inhibition has revealed a potential positive feedback loop on PI3K/Akt signaling abolishing any potential beneficial activity. For these reasons, in the last years, many clinical trials using PI3K inhibitors [i.e., BMK-120 (Clinical-Trials.gov identifier NCT01283048) or RP6530 (Clinical-Trials.gov identifier NCT02017613)] began and some are still ongoing. Acting on the PI3,4,5P3-producing enzymes (123). [Image 239x407 to 248x414]

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