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POLYCYSTIN-1 C TERMINUS CLEAVAGE AND ITS RELATION WITH POLYCYSTIN-2, TWO PROTEINS INVOLVED IN POLYCYSTIC KIDNEY DISEASE

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Abstract Autosomal dominant polycystic kidney disease (ADPKD), a most common genetic cause of chronic renal failure, is characterized by the progressive development and enlargement of cysts in kidneys and other organs. The cystogenic process is highly complex and involves a high proliferative rate, increased apoptosis, altered protein sorting, changed secretory characteristics, and disorganization of the extracellular matrix. ADPKD is caused by mutations in the genes encoding polycystin-1 (PC-1) or polycystin-2 (PC-2). PC-1 undergoes multiple cleavages that intervene in several signaling pathways involved in cellular proliferation and differentiation mechanisms. One of these cleavages releases the cytoplasmic C-terminal tail of PC-1. In addition, the C-terminal cytoplasmic tails of PC-1 and PC-2 interact *in vitro* and *in vivo*. The purpose of this review is to summarize recent literature that suggests that PC-1 and PC-2 may function through a common signaling pathway necessary for normal tubulogenesis. We hope that a better understanding of PC-1 and PC-2 protein function will lead to progress in diagnosis and treatment for ADPKD.

Key words: polycystin-1 (PC-1), polycystin-2 (PC-2), C-terminal tail (CTT) cleavage of polycystin-1, primary cilium, mTOR, cAMP, Ca^{2+}

Resumen ***Clivaje del C-terminal de policistina-1 y su relación con policistina-2, dos proteínas involucradas en la poliquistosis renal***. La poliquistosis renal autosómica dominante (ADPKD por sus siglas en inglés) es una causa genética muy común de falla renal crónica que se caracteriza por el progresivo desarrollo y agrandamiento de quistes en los riñones y en otros órganos. El proceso de cistogénesis comprende incrementos en la proliferación y muerte celular por apoptosis, así como alteraciones en la distribución intracelular de proteínas, el movimiento transcelular de solutos y organización de la matriz extracelular. ADPKD es causada por mutaciones en los genes que codifican para policistina-1 (PC-1) o policistina-2 (PC-2). PC-1 puede sufrir múltiples clivajes y los fragmentos generados intervienen en diferentes cascadas de señalización involucradas en mecanismos de proliferación y diferenciación celular. Uno de estos clivajes libera el extremo C-terminal citoplasmático de la PC-1. Se ha demostrado que los extremos C-terminal citoplasmático de PC-1 y PC-2 pueden interactuar tanto *in vitro* como *in vivo*. El propósito de esta revisión es resumir la literatura más reciente que sugiere que PC-1 y PC-2 pueden funcionar a través de una cascada de señalización común necesaria para la tubulogénesis normal. Creemos que una mejor comprensión de los mecanismos moleculares de acción de PC-1 y PC-2 contribuirán al progreso en el diagnóstico y tratamiento de ADPKD.

Palabras clave: policistina-1 (PC-1), policistina-2 (PC-2), clivaje del extremo C-terminal (CTT) de policistina-1, cilia primaria, mTOR, cAMP, Ca^{2+}

Polycystin 1 and 2: structure, localization and cleavage

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common monogenic disorders, and globally is the third most common cause of end stage kidney disease in humans. It is characterized by the slow development of multiple bilateral cysts in the kidneys, resulting in progressive renal failure requiring the need for renal replacement therapy in 50% of the patients by the time they are 60 years old. Furthermore, ADPKD is a multisystem disease that also causes cysts in the liver, pancreas, and other organs, aneurysms of the cerebral artery, cardiac valvular insufficiency and colonic diverticula[1]. Most cases of ADPKD are caused by mutations in two separate genes, *PKD1* and *PKD2*, which encode the polycystin-1 (PC-1) and polycystin-2 (PC-2) proteins, respectively[2, 3].

Regulated intramembrane proteolysis (RIP) is an evolutionarily conserved process by which transmembrane proteins are cleaved to release cytoplasmic domains or luminal/extracellular domains. Released cytosolic domains can translocate to the nucleus to regulate transcription

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of genes involved in apoptosis, inflammation, lipid metabolism and neurogenesis. To date, numerous integral membrane proteins have been shown to release a soluble cleavage fragment, and included on this list is PC-1[4]. PC-1 is an integral membrane protein of 4,302 amino acids with a large extracellular region containing sequences with homology to domains that are usually involved in protein–protein or protein–carbohydrate interactions[5, 6]. This segment is followed by 11 transmembrane (TM) domains and a short cytoplasmic C-terminal domain. Moreover, PC-1 undergoes an autocatalytic cleavage at a site that is homologous to a G-protein coupled receptor proteolytic site (GPS)[7]. This GPS motif is localized in the extracellular domain immediately proximal to the first TM domain. This cleavage essentially removes the entire extracellular N-terminus of the protein (~350 kDa fragment, NTF) and generates a ~150 kDa C-terminal fragment that is composed of all of the TM domains and the C-terminal tail of PC-1 (CTF). Furthermore, the entire C-terminal tail (CTT) of PC-1 can be proteolytically released from the membrane and can translocate to the nucleus, where it may be able to regulate the expression of genes involved in cellular proliferation and differentiation[7-10] (Fig. 1). Finally, Low et al. observed a 17 kDa cleavage product, which is produced by a different cleavage event in the C-terminal half of the PC1 tail. This small fragment is released from the plasma membrane and targets the nucleus in association with STAT transcription factors[9] (Fig. 1). In summary, the CTT of PC-1 undergoes two different cleavages, each of which generates fragments that participate in dissimilar signaling processes. These processes will be reviewed below.

PC-2 is a 968 amino acid protein that contains six TM domains with an ion conducting pore that resides between the TM5 and TM6 domains. Both the N- and C-termini are predicted to be intracellular[12] (Fig. 2). Immunocytochemical colocalization, subcellular fractionation and endoglycosidase H analyses have established that epithelial cells express a large fraction of PC-2 in the membranes of the endoplasmic reticulum (ER)[13]. It has been demonstrated that the cytoplasmic tail of PC-2 contains signals that are necessary for ER retention[13] and in the ER, PC-2 can act as a Ca^{2+}-activated Ca^{2+}-release channel[14].

Role of polycystin 1/polycystin 2 interaction

Patients with mutations in *PKD1* and those with mutations in *PKD2* genes have a similar clinical phenotype, and both *PKD1*-/- and *PKD2*-/- mice develop kidney and liver cysts resembling the human phenotype[15, 16]. These studies suggested that PC-1 and PC-2 are implicated in common cellular signaling pathways. Experiments conducted both *in vitro* and *in vivo* have shown that PC-2 directly interacts with PC-1[17-20] (Fig. 2). It has been described that the C-

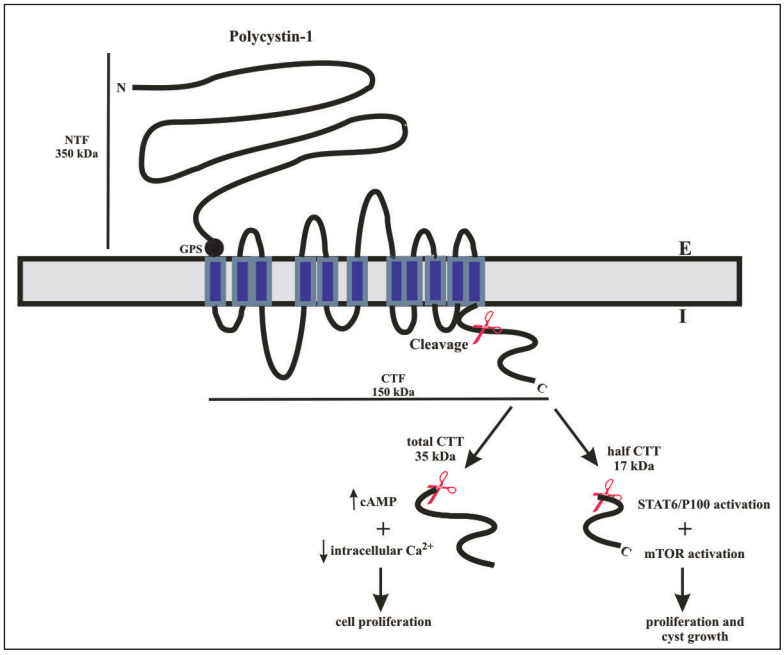

Fig. 1.– The predicted topology of polycystin-1 (PC-1). PC-1 has a large extracellular region (NTF) with a highly conserved G protein–coupled receptor proteolytic cleavage site (GPS). This segment is followed by 11 transmembrane (TM) domains and a short cytoplasmic C-terminal domain (CTF). The CTT of PC-1 undergoes two different cleavages, each of which generates fragments that participate in dissimilar signaling processes. E: extracellular space, I: intracellular space. The thick gray rectangle indicates the membrane bilayer.

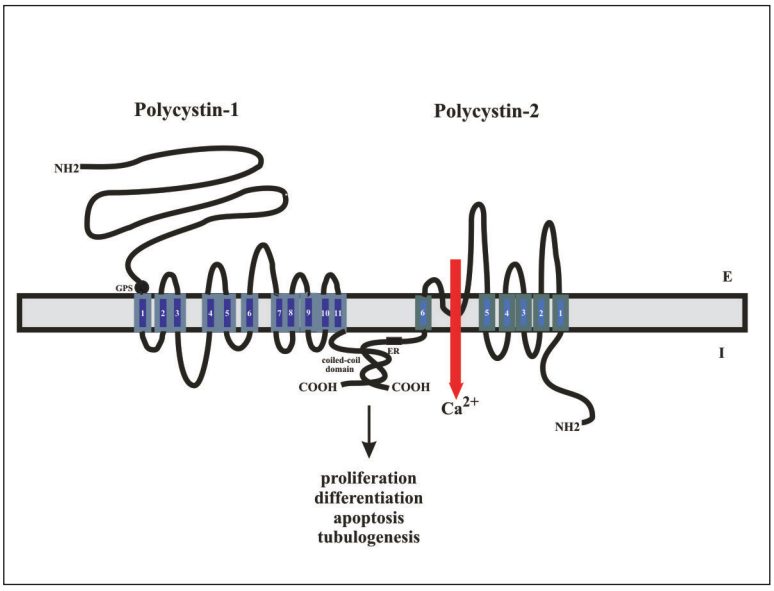

Fig. 2.– The predicted topology of polycystin-2 (PC-2). PC-2 has an ER endoplasmic reticulum retention signal (ER). The short cytoplasmic tail of PC1 contains a coiled-coil region that couples with the COOH-terminal region of PC-2. The PC-1/PC-2 interaction was implicated in common cellular signaling pathways. Proteins are not drawn to scale. The numbers represent the predicted transmembrane domains. E: extracellular space, I: intracellular space. The thick gray rectangle indicates the membrane bilayer.

terminal domain of PC-1 contains a coiled-coil motif that binds to the C-terminal domain of PC-2. Hanoaka et al., have provided evidence that the co-expression of PC-1 and PC-2 in mammalian cells results in the appearance of a unique non-selective Ca^{2+}-permeable cation channel on the plasma membrane[18, 21], and that the deletion of the carboxyl-terminus of either protein destroys the PC-1/PC-2 interaction and fails to generate Ca^{2+} currents. In artificial lipid bilayers, recombinant PC-2 can reconstitute Ca^{2+} channel activity in the absence of PC-1, but PC-1 appears to stabilize or activate PC-2 channel activity in this system[22]. In addition, co-expression and co-assembly of PC-2 with PC-1 have been shown to dislodge PC-2 from the ER and to allow its relocalization to the cell surface in mammalian cells[18]. Whether PC-1 can reconstitute channel activity by itself by forming part of a channel pore and/or act as a chaperone protein remains unclear.

The primary cilium is a nonmotile hair-like structure localized in the apical surface of most tubular epithelial cells. It has been observed that both PC-1 and PC-2 localize to the primary cilium of renal epithelial cells and vascular endothelial cells[23-25]. Recent advances support the hypothesis that in the primary cilium the large extracellular N-terminal domain of PC-1 functions as a sensing antenna for mechanical[26] or chemical stimuli[27]. Mechanical stimuli in the cilia, in response to flow rates comparable to those observed in renal tubules, may induce conformational changes of PC-1, therefore activating its binding partner PC-2, which could mediate Ca^{2+} entry into the cell. This calcium entry could then amplified by Ca^{2+} release from IP3- and/or ryanodine-sensitive stores and spread to neighboring cells through gap junction communication[23].

The CTT of PC-1 and PC-2 have also been linked to other signaling pathways. The C-terminus of PC-1 has been shown to induce *JNK*/ activator protein-1 (AP-1) transcription via the activation of heterotrimeric G proteins and PKC α[28], while PC-2 expression upregulated JNK/AP-1 upon activation of PKC ε[29]. Additionally, PC-1 activates the Janus kinase (JAK2) and Signal Transducers and Activators of Transcription (STAT)1/3 pathway in a process that requires PC-2 and the up-regulation of $p21^{waf1}$, leading to cell cycle arrest in G0/G1[30]. The PC1/PC2 complex also interacts with the adherens junction protein E-cadherin and its associated cytoplasmic catenins in lateral membranes of renal tubular cells[31, 32]. Thus, a delicate relationship between PC-1 and PC-2 may regulate processes involved in the control of different cellular programs such as proliferation, differentiation, apoptosis[29], tubulogenesis[31], adhesion and maturation[32], which are all essential steps of kidney morphogenesis.

Distal proteolytic cleavage of CTT and its role in ADPK

As mentioned above, PC-1 undergoes another proteolytic cleavage in the distal half of the CTT[9]. The same group proposed that under normal luminal fluid flow conditions,

PC-1 localizes to primary cilia, where it is in a complex with P100 and STAT6. Renal injury, during which the luminal fluid flow is strongly decreased or absent, will trigger cleavage of the second half of PC1 tail by a yet unknown mechanism, and this is accompanied by activation of STAT6 by its tyrosine phosphorylation, translocation of the PC-1 tail/STAT6/P100 complex from the cilia to nuclei and induction of gene expression[9] (Fig. 1). The same authors showed that the cleaved nuclear PC-1 tail is also prominent in ADPKD cysts together with an upregulation of STAT6 and P100[9].

The mTOR (mammalian target of rapamycin) is a conserved serine/threonine kinase that is involved in cell growth regulation, proliferation, regulation of the cellular cytoskeleton and cell survival[33-35]. The mTOR can be inhibited by the fungal metabolite rapamycin. The two major regulators of the mTOR are *TSC1* and *TSC2,* the genes that encode hamartin and tuberin, respectively and are mutated in Tuberous Sclerosis Complex (TSC)[36]. The mTOR is activated by the small GTPase Rheb when it is in a GTP-bound state. The tuberin/hamartin complex favors a GTP-to-GDP conversion of Rheb, which results in the inactivation of Rheb and the inhibition of mTOR[35]. Disruption of the tuberin-hamartin complex signaling, leads to relief of this inhibition and therefore mTOR activation.

Molecular analysis has revealed that, the *PKD1* and *TSC2* genes are located very close to each other on the human chromosome 16[37]. Large deletions of chromosome 16 affecting both the *TSC2* and the *PKD1* gene produce more severe and earlier onset polycystic kidney disease than is seen in patients with *PKD1* mutations alone[37, 38] and this new syndrome is called TSC/PKD[37]. In addition, it has been observed that cells that are null for tuberin exhibit a defect in the trafficking of PC1 to the plasma membrane[39]. These observations suggest that PC1 and tuberin have a functional link and may act together in the same pathway. It has been recently reported that the CTT of PC1 interacts, directly or indirectly, with tuberin and also with mTOR[40]. In addition, the same authors showed that mTOR is upregulated in the cystic epithelia of ADPKD tissues and PC1-null model[40]. Treatment of "orpk" mice (a mouse model with a defect in the ciliary polaris gene) or treatment of "bpk" mice (with a defect in the ciliary bicaudal-C gene) with rapamycin resulted in a dramatic inhibition of renal cyst growth and preservation of renal function[40]. ADPKD patients who underwent renal transplantation and were receiving rapamycin to prevent rejection exhibited a significant reduction in the renal volume of the polycystic kidney that had not been removed[40]. Interestingly, the damage produced by ureteral obstruction, during which fluid flow essentially ceases and the damage is caused by the proliferative and profibrotic response of the renal epithelial cells, can be prevented by treating the animals with rapamycin[41]. Lieberthal et al. found that ischemia-reperfusion injury induces dramatic upregulation of mTOR activity in the kidney and that rapamycin treatment prevented the proliferation and induction of apoptosis[42]. All of these results are consistent with the following model:

1) In normal renal tubule epithelial cells, luminal fluid flow stimulates primary cilia and PC1 remains intact and localized to primary cilia and the lateral plasma membranes. Under those conditions, PC-1 is a constitutive partner of the tuberin/hamartin complex that is required for the inactivation of Rheb and the repression of mTOR[43]. As long as PC-1 forms a complex with P100 and STAT6 in the primary cilia, mTOR and STAT6 activities are suppressed, resulting in a normal cell proliferation.

2) During ischemic injury or ureteral obstruction, fluid flow essentially ceases. An important function of primary cilia is to sense the lack of luminal fluid flow associated with renal injury. The lack of ciliary mechano-stimulation leads to proteolytic cleavage of the second half of PC1 tail, which translocates to the nucleus. This disruption of PC1 would lead to the inability of CTT PC1 to assemble with tuberin/hamartin inhibitory complex, leading to dysregulation of mTOR. PC1 tail cleavage also results in the cessation of STAT6 inhibition through its retention on the cilia, resulting instead in its translocation to the nuclei and induction of gene expression. The constitutive activation of the mTOR and STAT6 pathways produce cyst growth and an aberrant proliferation required for injury repair[9, 40].

Association between the PC1 CTT and both intracellular cAMP and Ca^{2+} in ADPK

The 'PKD phenotype', which includes enlargement and formation of renal cysts as a consequence of net fluid secretion into the cysts and increased cell proliferation in the cyst wall, were simulated by the overexpression of the entire cytosolic C-terminal tail of PC1 in mouse cortical collecting duct cells[44]. There is a considerable body of literature providing evidence that cAMP-stimulated chloride transport is the driving force for the fluid secretion associated with cyst filling[45-47]. Besides, it was demonstrated that cAMP is involved in stimulating the abnormal proliferation of cystic epithelial cells. Studies from Wallace's laboratory demonstrated that primary cells from various ADPKD cysts had significantly lower basal levels of intracellular calcium. These ADPKD cells responded to cAMP with increased cell proliferation. This response to cAMP could be normalized by re-introducing calcium to these cyst epithelial cells[48]. Additionally, Yamaguchi et al. observed that a combination of restriction in intracellular Ca^{2+} and elevated cAMP in normal renal epithelial cells can lead to abnormal cell proliferation[49] (Fig. 1). These authors observed that in immortalized M-1 collecting duct cells, the calcium restriction caused an inhibition of the phosphatidylinositol 3-kinase/Akt pathway, which relieves the

inhibition of B-Raf and allows cAMP to activate PKA, which activates Ras. Ras can then activate the B-Raf/MEK/ERK pathway, causing an increased rate of cell proliferation. M-1 cells stably overexpressing polycystin-1 CTT exhibited a cAMP growth-stimulated phenotype involving B-Raf and ERK activation and it was reversed by the addition of a calcium ionophore.

Interestingly, in ADPKD cyst cells, Xu et al. observed equivalent whole-cell levels of PC1 and PC2 to those found in normal epithelial cells, but the primary cilium in the cyst cells lacked any detectable PC1 and presented reduced PC-2 expression. ADPKD cysts cells also exhibited no flow-sensitive elevation of intracellular Ca^{2+} concentration at any level of shear stress and were characterized by reduced endoplasmic reticulum Ca^{2+} stores and reduced capacitative Ca^{2+} entry[50]. Igarashi´s group demonstrated that primary cilia of renal epithelial cells contain a cAMP-regulating protein complex, including PC2 and PDE4C. Under normal conditions, PDE4C promotes the hydrolysis of cAMP, and PC2 acts as a Ca^{2+} entry channel and mediates the local accumulation of Ca^{2+} that inhibits the activity of Ca^{2+} -sensitive adenylyl cyclase 5/6[51]. These findings support the concept that ADPKD is associated with a disruption in calcium homeostasis and accumulation of intracellular cAMP and these processes also have a central role in the primary cilium.

Similarly to renal epithelial cells, vascular endothelial cells sense blood flow, which induces an increase in intracellular Ca^{2+} concentration followed by the endothelial release of nitric oxide leading to vasodilation[52]. Nauli´s group showed that the primary cilia of endothelial cells respond to shear stress and expresses PC-2 and PC-1. Furthermore, the same group observed that endothelial cells from *Pkd2*-knockout or *Pkd1*-knockout mice exhibit impairments in the increase in intracellular Ca^{2+} concentration and release of nitric oxide induced by flow[24, 53]. In addition, the PC-1 protein expressed in endothelial cells undergoes a C-terminal cleavage, resulting in loss of receptiveness of cells to high fluid shear[24]. This study suggests that in patients with high blood pressure, the cilia may be not able to sense changes in blood pressure because PC-1 is proteolytically inactivated by high fluid-shear stress, which may in turn produce a malfunction in the autoregulation of the local circulatory system and may increase the possibility of the localized blood vessel injuries characteristic of ADPKD.

Presence of PC-2 activates PC1 CTT cleavage and modulates cAMP and Ca^{2+} -independent signaling pathway

In addition to the findings of Weimbs' group, Chauvet et al. observed that blocking the sensing of the urinary flow *in vivo*, either by occluding a ureter in WT animals or by genetically abolishing cilia formation in renal cyst of epithelial cells, led to an increase in total CTT cleavage, as evidenced by the accumulation of the CTT fragment in the nuclei of renal epithelial cells[8]. In addition, several groups have shown that PC-2 is expressed at higher levels in cyst-lining epithelium of ADPKD kidneys[54-56]. As mentioned previously, PC-1 and PC-2 are involved in common signaling pathways and they interact physically with each other[17-20]. Nevertheless, it still remains unclear whether high levels of PC-2 and/or the interaction of PC-2 with PC-1 may regulate the cleavage and nuclear translocation of the PC-1 CTT.

To pursue these results further, we decided to address some outstanding and fundamental questions: 1) is PC-2 able to modulate the PC-1 CTT cleavage? 2) is the PC-1/PC-2 interaction necessary for the increase in CTT cleavage? And finally 3) is the PC-1 CTT cleavage modulated by the extracellular and/or intracellular calcium and cAMP levels? In order to study the CTT cleavage of the PC-1 protein we generated a fusion protein in which a chimera of the Gal4 transcription factor and the VP16 transcriptional activator was inserted into the full length PC-1 sequence downstream of the intracellular CTT domain (PKDgalvp). Our strategy included transfecting COS-7 cells with PKDgalvp together with a Gal4-driven Firefly luciferase reporter gene. We expected that if the CTT of PC-1 is cleaved, it will translocate to the nucleus, and there it would drive the expression of the luciferase gene that is under the control of the Gal4 promoter (Fig. 3). The magnitude of luciferase expression can be measured using a quantitative luminescence assay[57]. To determine whether PC-2 increases CTT cleavage and its nuclear traslocation, we coexpressed PC-2 and the PKDgalvp construct in COS-7 cells. We showed that both luciferase activity and the expression of CTT were significantly increased in the presence of PC-2 without changes in PC-1 full length expression.

To answer our second question, we used a series of truncated mutant constructs of PC-2 that were generated by introducing premature termination codons into the C-terminus of the cDNA encoding PC-2. The PC-2 mutant constructs which were incapable of associating with PC-1[19] stimulated PC-1 CTT cleavage to the same extent as full length PC-2, suggesting that the interaction between PC-1 and PC-2 is not necessary to elicit PC-1 CTT cleavage.

Finally, as we mentioned above, Ca^{2+} homeostasis and intracellular cAMP are connected to the CTT PC-1 cleavage in renal epithelial and vascular endothelial cells. Therefore, we wanted to determine whether the Ca^{2+} channel function of PC-2, Ca^{2+} and/or cAMP intracellular concentrations are important for CTT cleavage regulation. Thus, we altered extracellular and intracellular Ca^{2+} levels through several different treatments but we were unable to detect any changes in luciferase activity. Furthermore, when we increased the intracellular levels of cAMP with

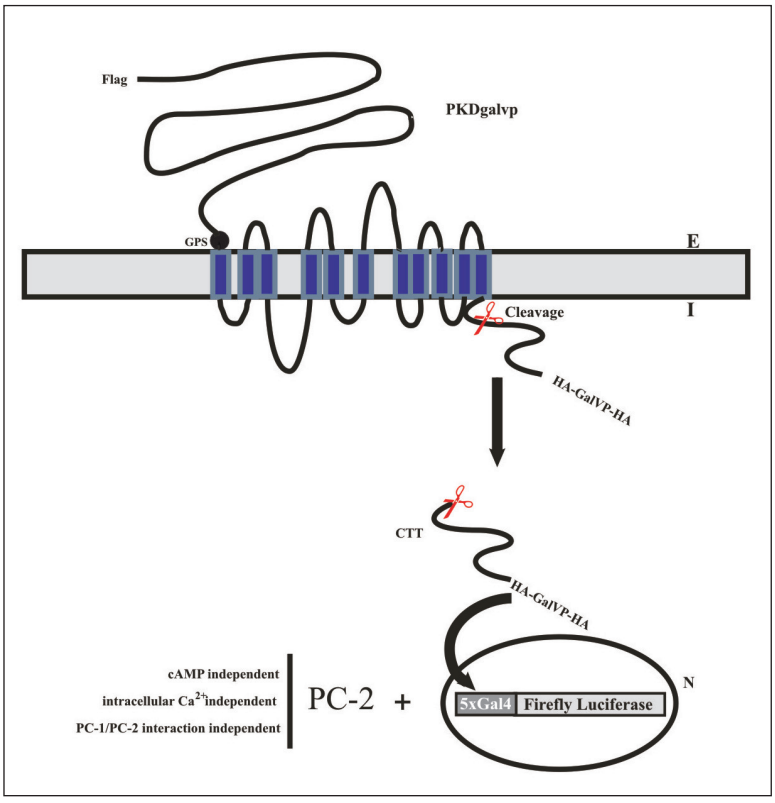

Fig. 3.– Schematic representation of the PC-1 tagged at its C-terminal tail (CTT) with HA-epitope/galvp and at its N-terminus with a Flag epitope (PKDgalvp), and schematic representation of the luciferase reporter assay. Proteolytic cleavage of the PC-1 fusion protein releases the CTT with the Gal4-VP16, which translocates to the nucleus (N) and activates a Gal4-responsive luciferase reporter gene. Therefore, luciferase expression correlates with PC-1 CTT cleavage. The CTT cleavage requires the presence of PC-2, however, it does not involve the PC-2/PC-1 interaction nor is it regulated by the presence of cAMP nor Ca^{2+}.

forskolin, an adenylate cyclase activator, IBMX, a phosphodiesterase inhibitor or *8-Br-cAMP,* an analogue of cAMP, we observed that the PC-2-enhancement of luciferase activity was not altered by any of these treatments (Bertuccio and Caplan, unpublished data).

Taken together, these data suggest a role for PC-2 in the regulation of the cleavage and nuclear translocation of the CTT of PC-1. Although this cleavage requires the presence of PC-2, it does not involve the PC-2/PC-1 interaction nor is it directly regulated by the presence of cAMP nor Ca^{2+} (Fig. 3), suggesting that the release of the CTT occurs through a novel mechanism that has not been described before.

Discussion

A key event of cystogenesis is the high rate of proliferation and poor differentiation of renal epithelial cells. However, up to now, no specific treatment is available to prevent these processes in ADPKD. The goal in developing therapies for ADPKD is to find treatments that slow cyst enlargement and preserve the renal parenchyma surrounding the cyst, thus conserving kidney function. Several studies have shown that the treatment with rapamycin alleviates cyst enlargement in murine models. Unfortunately, the use of mTOR inhibitors in clinical trials failed to show a clinical benefit in ADPKD patients[58, 59].

How the primary cilium, along with PC-1 and PC-2, transmit signals to the nucleus to modulate DNA replication and cell division is not completely understood. We believe that studying and identifying the role and function of PC-2 in this process may help in the understanding of the molecular mechanism responsible for this disease and may help to identify novel therapeutic strategies in the treatment of ADPKD in the future.

Conflicts of interest: No conflicts of interest, financial or otherwise, are declared by the authors.

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Las mismas promesas de la medicina son increíbles, porque han de atender a diversos accidentes y males que con frecuencia nos hostigan a la vez y que tienen relación casi necesaria, como el calor del hígado y la frialdad del estómago. En este caso los médicos nos quieren persuadir de que [con sus menjunjes] uno de los ingredientes nos calentará el estómago y otro nos refrescará el hígado. Un tercero irá a los riñones y la vejiga sin obrar sobre las demás partes ni perder su virtud en tan largo y accidentado camino, merced a sus propiedades ocultas; un cuarto secará el cerebro y un quinto humedecerá los pulmones. Si de tal amasijo se hace ¿no es una fantasía esperar que sus virtudes se distribuyan y dividan, dejando esa confusión para ir a los lugares que les están destinados? Por mi parte mucho me temo que pierdan o cambien sus propiedades y únicamente perturbación produzcan. ¿Quién puede suponer que en esa líquida confusión las facultades de sus componentes no han de corromperse, confundirse y mudamente alterarse? Y, para colmo la composición de esa receta queda a merced de una persona desconocida a cuya fe abandonamos nuestra vida.

Michel de Montaigne (1533-1592)

Ensayos (Essais, 1580,1588, 1595, póstumos). *Del parecido de sus hijos a los padres*. Libro II., XXVII, p 381.Traducción de Juan G. de Luaces. Buenos Aires: Hyspamérica, 1984