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Polycystic liver diseases: from molecular basis to development of effective treatments

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Polycystic liver diseases (PLDs) comprise a heterogeneous group of congenital genetic disorders that mainly affect bile duct epithelial cells, known as cholangiocytes. Patients with PLD usually present with bile duct dilatation and/or progressively develop intrahepatic, fluid-filled biliary cysts (more than 10), which is the main cause of morbidity (1). Cysts may arise isolated in the liver in patients with autosomal dominant PLD (ADPLD), affecting approximately 1 per 100,000 inhabitants (2). On the other hand, liver cystogenesis may more frequently develop in parallel with renal cysts in patients with autosomal dominant or autosomal recessive polycystic kidney disease (ADPKD, around 68:100,000 inhabitants, and autosomal recessive polycystic kidney disease (ARPKD), around 1:20,000 inhabitants, respectively) (3,4). PLD clinical presentation is very heterogeneous and a remarkable variation in symptomatology may be observed (1). Considering the reduced number and size of liver cysts observed in up to 80 % of patients, a remarkable proportion of patients remain asymptomatic for quite a long period of time. Still, among patients with obvious clinical manifestations, most common symptoms include abdominal discomfort, local pressure with back pain, gastro-esophageal reflux, dyspnea, and early satiety, mostly as a result of massive cyst growth and hepatomegaly, in parallel with cyst-related complications such as cyst hemorrhage, infection and/or rupture (5). Current therapeutic approaches are usually applied to patients with remarkable symptomatology aiming to treat symptomatic cysts. Percutaneous (*i.e.*, aspiration sclerotherapy) and/or surgical (*i.e.*, hepatic cyst fenestration, liver resection) procedures, in parallel with chronic

somatostatin analogue administration (octreotide, pasireotide, lanreotide), are the most common therapeutic approaches used for patients with PLD but exhibit short-term and modest benefits, hence liver transplantation remains the only potentially curative option (6). Therefore, novel therapeutic strategies based on a deeper understanding of the molecular mechanisms driving cystogenesis are required.

PLD develops as a result of germline mutations in distinct genes. Up to now, mutations in 12 different genes have been related to the development of PLDs. Specifically, mutations in seven of these genes (*i.e., PRKCSH, SEC63, LRP5, GANAB, ALG8, SEC61B*, and *PKHD1*) have been associated with ADPLD. Besides, ADPKD has been associated with mutations in six genes (*i.e., PKD1, PKD2, GANAB, LRP5, DNAJB11* and *ALG9*), while mutations in *PKHD1* and *DZIP1L* have been related to the development of ARPKD. Although several genes were already associated with the development of PLDs, the full genetic landscape is not yet completely understood, particularly in ADPLD, since the causative genes are not identified in up to 50 % of cases (7).

PLD-related cystogenesis is defined by several functional alterations in cholangiocytes, which are directly mediated by increased levels of cyclic adenosine monophosphate (AMP): cAMP, and decreased intracellular calcium ($_{i}Ca^{2+}$) levels. Events responsible for the pathobiology of PLDs include cholangiocyte hyperproliferation and hypersecretion, elevated matrix metalloproteolytic activity, changes in microRNAs expression patterns, autophagy and morpho-functional alterations of the primary cilium (8). In the past years, novel mechanisms of pathogenesis were added to this list, allowing the identification of novel therapeutic targets for PLDs (Fig. 1). In this regard, since 75 % of the identified PLD-related genes are known to encode for endoplasmic reticulum (ER)-related proteins, involved in Ca^{2+} homeostasis and in the post-translational modification of proteins, increased ER stress was hypothesized to contribute to disease pathogenesis. In line with this, the expression levels of unfolded protein response (UPR) sensors (*i.e.*, *ATF6*, *IRE1α* and *PERK*) and their downstream effectors (i.*e., GRP78, XBP1* and *CHOP*), which are usually increased in response to ER stress, were shown to be upregulated in liver tissue from patients with PLD and in human and rat cystic cholangiocytes in culture (9). Proteomic analysis of cystic cholangiocytes in culture in comparison to normal human cholangiocytes revealed alterations in protein synthesis,

folding, trafficking and degradation. Additionally, an enlargement of ER lumen was observed in cystic cholangiocytes in comparison to healthy cholangiocytes, in parallel with proteasome hyperactivation, all these features being indicative of aberrant proteostasis, accumulation of unfolded/misfolded proteins, and ER stress (9). Of note, chronic treatment of PCK rats (animal model of PLD) with the chemical chaperone 4-phenylbutyric acid (4-PBA), known to reduce ER stress, diminished total liver weight as well as liver and cystic volumes, when compared to control rats, by normalizing aberrant proteostasis and impacting on cholangiocyte hyperproliferation, apoptosis, and vascular endothelial growth factor (VEGF) secretion (9). Overall, restoration of aberrant proteostasis and decreasing ER stress with 4-PBA was proposed as a novel therapeutic strategy to treat hepatic cystogenesis that now deserves future clinical evaluation.

As a consequence of sustained ER stress, alterations in protein dynamics in cystic cholangiocytes may appear. Post-translational modifications (PTMs) are critical for proper protein function, and disturbances in PTMs were related to aberrant proteostasis in cystic cholangiocytes (10,11). Specifically, several players involved in SUMOylation (*i.e.*, *UBE2I, SAE1, UBA2* and *SUMO1*) and NEDDylation (*i.e.*, *NAE1, UBA3* and *NEDD8*) were found to be upregulated in the liver tissue from patients and rats with PLD, when compared to healthy livers, this being associated with increased hepatic cystogenesis (10,11). Importantly, targeting the SUMOylation pathway with S-adenosylmethionine (SAMe), a natural inhibitor of UBC9, restored hyperSUMOylation levels in cystic cholangiocytes. Moreover, SAMe administration to human cystic cholangiocytes reduced proteosome hyperactivity and proliferation, while inducing stress-related apoptosis, attenuating the growth of 3D cystic cholangioids *in vitro*. Outstandingly, chronic treatment of PCK rats with SAMe decreased hepatic cystogenesis and reduced liver fibrosis, hence further clinical evaluation in patients is warranted in the next years (10). Similarly, pevonedistat, a first-in-class inhibitor of the NEDDylation pathway, decreased proliferation and induced apoptosis in human cystic cholangiocytes in culture (11). This compound deserves now pre-clinical assessment in animal models of PLD.

Since ER stress and altered proteostasis may increase autophagy, cystic cholangiocytes were also shown to have alterations in the autophagic flux, presenting more autophagosomes, lysosomes and autolysosomes in different experimental *in vitro* and *in vivo* settings (12). Furthermore, the observed upregulation of autophagy-related proteins, including ATG5, beclin 1, ATG7 and LC3B, in cystic cholangiocytes suggested autophagy as a direct promoter of hepatic cystogenesis (12). In this sense, targeting the autophagic flux with bafilomycin A_1 or hydroxychloroquine reduced the proliferation of cystic cholangiocytes *in vitro* and the growth of hepatic cysts in 3D cultures, ultimately decreasing hepatic cystogenesis in PCK rats (12). Thus, autophagy emerges as a potential therapeutic target for PLD treatment that deserves attention in the future.

Structural and/or functional abnormalities (elongation, shortening or absence) in the cholangiocyte primary cilium are common alterations in cystic cholangiocytes (13,14). In fact, histone deacetylase 6 (HDAC6) overexpression was reported in human and rat cystic cholangiocytes, inducing α-tubulin deacetylation and consequent ciliary disassembly (15). Therefore, HDAC6 arose as novel potential therapeutic target in PLD. Recently, a new single molecule-based strategy (16) was used to combine selective HDAC6 inhibitors with the endogenous, hepatoprotective bile acid (BA) ursodeoxycholic acid (UDCA), which was shown to inhibit hepatic cystogenesis in experimental models (17) and in patients with advanced PLD (18). In this regard, a family of UDCA synthetic conjugates with selective HDAC6 inhibitory activity (UDCA-HDAC6is) were prepared and tested in experimental models of PLD (16). Particularly, chronic administration of UDCA-HDAC6i #1 to PCK rats effectively reduced PLD-related hepatomegaly and nephromegaly, halting liver cystogenesis and increasing the hepatic levels of free UDCA. Additionally, HDAC6 activity in treated rats was confirmed by observing a significant increase in the levels of acetylated α-tubulin and in the length of the primary cilium, when compared to untreated rats. *In vitro* experiments showed that UDCA-HDAC6i #1 inhibited the proliferation of cystic cholangiocytes and the growth of cystic cholangioid 3D cultures, in an ERK1/2-dependent manner, exerting an anti-proliferative effect superior to the isolated or combined effects of the pharmacologically active elements (UDCA and HDAC6 inhibitory arm) (16). Of note,

using this new family of compounds that have a dual therapeutic effect by combining UDCA and HDAC6 inhibition constitute a promising therapeutic strategy for PLDs that will now be evaluated in a clinical setting.

In conclusion, much progress has been made over the past few decades leading to a better understanding of the pathophysiology of PLD. Novel deregulated processes and signaling pathways have been identified, which allowed the discovery of novel pharmacological drugs that were tested at the pre-clinical level. Therefore, improving ER stress and aberrant proteostasis with 4-PBA, SAMe and/or pevonedistat, inhibiting autophagy with bafilomycin A_1 or hydroxychloroquine, or using UDCA-HDAC6is as a novel single molecule-based therapy might be revealed as promising and effective therapeutic strategies for patients with PLD deserving subsequent clinical evaluation. Therefore, their clinical efficacy, alone or in combination with somatostatin analogues, should be assessed prospectively in large and well-defined cohorts of patients with PLD. In addition, novel, interesting emerging fields are evident in the study of PLD pathogenesis, and the role of metabolism is yet to be unveiled. While metabolomic reprogramming and mitochondrial dysfunction have already been studied in the context of polycystic kidney disease (PKD) (19-21), their dysregulation in and impact on PLD pathogenesis remain unknown, although they may be of great relevance. For instance, the administration of fenofibrate, a peroxisome proliferator-activated receptor-α (PPARα) agonist, to mice with PLD boosted fatty acid β-oxidation and reduced hepatorenal cystogenesis (22). Likewise, the PPARγ (peroxisome proliferator-activated receptor-γ) agonist telmisartan (23), the anti-diabetic drug metformin (24), and long-term moderate exercise (25) were shown to diminish liver fibrosis and hepatic cystogenesis in PCK rats, confirming once more the relevance of lipid metabolism in PLD pathogenesis. The field of energetic metabolism and mitochondrial biology has progressed substantially in recent years, and has yielded numerous opportunities to translate discoveries into clinical practice; therefore, novel effective therapies may emerge for PLD in the upcoming years.

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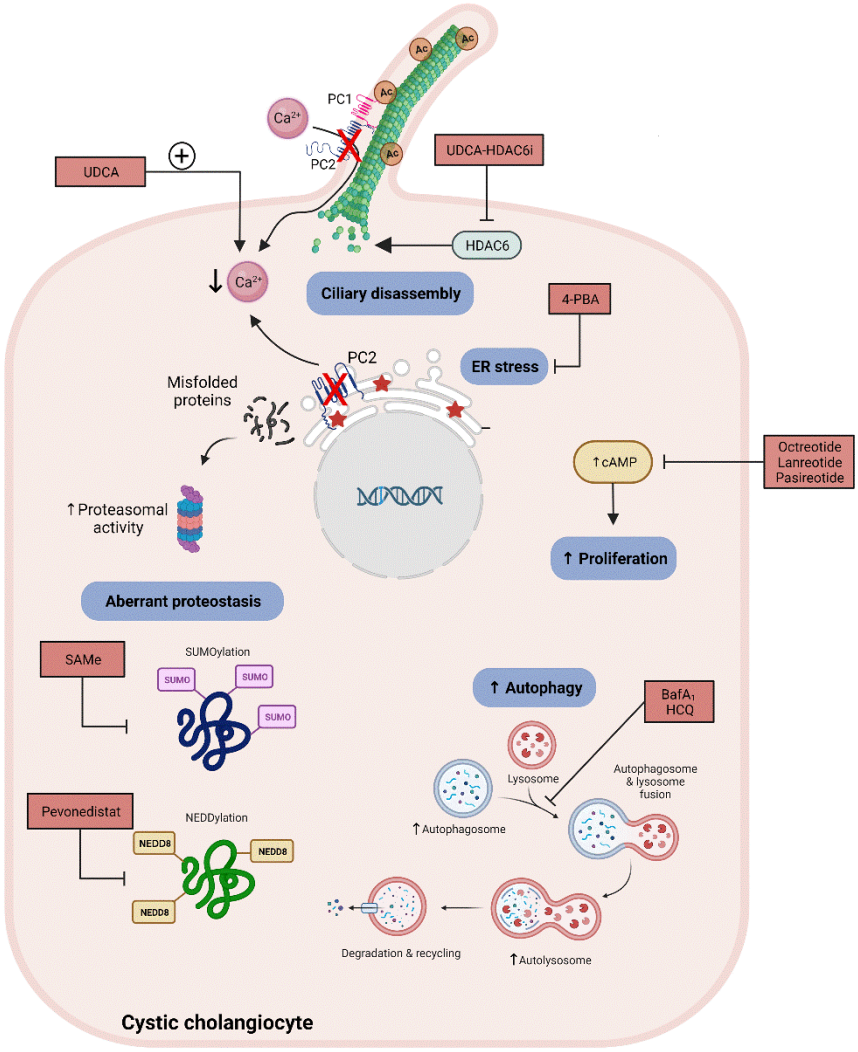

Figure 1. Events contributing to hepatic cystogenesis in PLD. Several signaling pathways and aberrant processes stimulate the hyperproliferative phenotype of cystic cholangiocytes. For instance, ER stress, aberrant proteostasis and ciliary disassembly as a result of HDAC6 overexpression stimulate hepatic cystogenesis progression and development. These heterogeneous processes are therapeutically targeted by different drugs, which have been already tested in a preclinical level. 4-PBA, 4-phenylbutyric acid; Ac, acetyl; $BafA_1$, bafilomycin A_1; cAMP, cyclic AMP; HCQ, hydroxychloroquine; HDAC6, histone deacetylase 6; Ca^{2+}, calcium; PC1, polycystin 1; PC2, polycystin 2; SAMe, S-adenosylmethionine; UDCA, ursodeoxycholic acid; UDCA-HDAC6i, UDCA-HDAC6 inhibitor.