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Therapeutic Targets in Polycystic Liver Disease

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Abstract

Polycystic liver diseases (PLD) are a group of genetic disorders initiated by mutations in several PLD-related genes and characterized by the presence of multiple cholangiocyte-derived hepatic cysts that progressively replace liver tissue. PLD co-exists with Autosomal Dominant Polycystic Kidney Disease (ADPKD) and Autosomal Recessive PKD as well as occurs alone (i.e., Autosomal Dominant Polycystic Liver Disease [ADPLD]). PLD associated with ADPKD and ARPKD belong to a group of disorders known as cholangiociliopathies since many disease-causative and disease-related proteins are expressed in primary cilia of cholangiocytes. Aberrant expression of these proteins in primary cilia affects their structures and functions promoting cystogenesis. Current medical therapies for PLD include symptomatic management and surgical interventions. To date, the only available drug treatment for PLD patients that halt disease progression and improve quality of life are somatostatin analogs. However, the modest clinical benefits, need for long-term maintenance therapy, and the high cost of treatment justify the necessity for more effective treatment options. Substantial evidence suggests that experimental manipulations with components of the signaling pathways that influence cyst development (e.g., cAMP, intracellular calcium, receptor tyrosine kinase, transient receptor potential cation channel subfamily V member 4 (TRPV4) channel, mechanistic target of rapamycin (mTOR), histone deacetylase (HDAC6), Cdc25A phosphatase, miRNAs and metalloproteinases) attenuate growth of hepatic cysts. Many of these targets have been evaluated in pre-clinical trials suggesting their value as potential new therapies. This review outlines the current clinical and preclinical treatment strategies for PLD.

Keywords

Cholangiocytes; cholangiociliopathies; cilia; hepatic cystogenesis; polycystic liver disease; therapies

1. Polycystic Liver Disease

The polycystic liver diseases (PLD) comprise a group of genetic disorders characterized by the progressive growth of cholangiocyte-derived fluid-field cysts that gradually replace liver tissue. PLD occurs in combination with two forms of Polycystic Kidney Disease (PKD) – Autosomal Dominant PKD (ADPKD) and Autosomal Recessive PKD (ARPKD) as well as alone (i.e., Autosomal Dominant PLD) [1-5]. PLD associated with ADPKD and ARPKD belongs to a group of disorders referred to as “cholangiociliopathies” since disease-causative

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and disease-related proteins are expressed in cilia of cholangiocytes [2, 5]. The primary cilium is a solitary, tubular, non-motile organelle protruding into the extracellular space from the plasma membrane of most eukaryotic cells. Cilia function as sensory organelles and signaling centers that recognize and transduce multiple stimuli associated with physiologically active substances present in the cell environment and the mechanical forces of the extracellular milieu. The sensory and signaling functions of cilia are linked to numerous receptors and ion-conducting channels localized to the ciliary membrane and to diverse signaling mechanisms [2, 6, 7]. Abnormal expression of ciliary-specific proteins affects their structural integrity and causes functional damage disturbing intracellular signaling pathways and promoting cystogenesis.

ADPKD is the most common inherited nephropathy and the second most common inherited syndrome. The estimated prevalence of ADPKD is 1:400; there are ~700,000 people with disease and ~6000 new cases arise annually [8, 9]. ADPKD is associated with mutations in two genes - *PKD1* and *PKD2* encoding polycystin-1 and polycystin-2, respectively [10]. Mutations in the *PKD1* account for 85% of cases; the remaining 15% linked to mutations in *PKD2*. Polycystin-1 is a large trans-membrane receptor that regulates several cellular functions and signaling pathways. Polycystin-2 acts as a calcium-permeable channel forming functional complexes with polycystin-1. Cyst formation in ADPKD requires initial germline mutations in either *PKD1* or *PKD2* followed by a second loss-of-function mutation in the functional gene copy [10]. Also, a threshold model of cystogenesis in ADPKD has been proposed. According to this model, levels of polycystin-1 or polycystin-2 that fall below the threshold limit initiate cyst growth [9].

ARPKD is a childhood-onset disorder that occurs with a frequency of 1:20,000 live births. Approximately half of ARPKD neonates die shortly after birth due to pulmonary hypoplasia [11]. Mutations in the *PKHD1* gene encoding the protein fibrocystin (also known as polyductin) are causative in ARPKD. While renal insufficiency and end-stage renal diseases are prominent features in the neonatal period, enlarged livers with ductal plate malformations, dilated intrahepatic bile ducts and progressive fibrosis of portal tracts resulting in congenital hepatic fibrosis (CHF) are the major features in adult survivors [12].

ADPLD is characterized by the presence of cysts mainly in the liver with no or very few renal cysts. The prevalence of this PLD is 1:100,000. ADPLD is linked to mutation in two genes, *PRKSCH* and *SEC63*, which encode hepatocystin and SEC63, respectively. Both proteins are residents of the endoplasmic reticulum and expressed in hepatocytes and cholangiocytes [5, 13]. How these proteins are involved in hepatic cystogenesis, remains unknown; secondary somatic mutations (i.e., a second hit) in liver cysts of patients with heterozygous germline mutations in *PRKSCH* are likely contributing factors [5, 13, 14]. A recent study also identified *LRP5* as a novel gene associated with hepatic cystogenesis in patients clinically diagnosed with PLD [15]. LRP5 is a single-span transmembrane protein that is involved in Wnt signaling functioning as a co-receptor of the Frizzled protein family members.

The hepatic cystogenesis in all the PLDs is sex-dependent [3]. Females often have a more severe disease and higher total liver volume [10]. In all PLD patients, the number of cysts

and total liver volume increase with age, growing in the range of 0.9%-3.2% annually [16]. Most patients are asymptomatic; however extreme hepatomegaly causes serious abdominal symptoms and complications. Approximately half of the patients with advanced disease have cyst hemorrhage, rupture or infection. Current non-pharmacologic therapies for PLD include cyst aspiration, sclerosis, or fenestration, as well as hepatic resection and liver transplantation. These therapies are partially effective, do not change the natural course of the disease, and are associated with high rate of reoccurrence, as well as significant morbidity and mortality [10, 17]. The only currently available drug treatment for PLD patients involves somatostatin analogs that halt disease progression and improve quality of life [18, 19]. However, the modest clinical benefits, required long-term maintenance therapy, and high cost emphasize the need for more effective therapeutic options.

2. Mechanisms Of Hepatic CystogenesiS

Substantial evidence suggest that hepatic cystogenesis is linked to: (i) defective ductal plate remodeling; (ii) altered sensory functions of cholangiocyte primary cilia; (iii) structural ciliary and centrosomal abnormalities; (iv) enhanced cell proliferation; (v) altered fluid secretion; (vi) cell cycle dysregulation; (vii) increased levels of intracellular cAMP; (viii) decreased levels of intracellular calcium; (ix) cell-matrix remodeling; (x) aberrantly expressed and mislocalized cellular proteins; (xi) altered levels of microRNAs (miRNAs); and (xii) increased expression of cytokines and growth factors. The detailed description of these mechanisms has been recently reviewed [1, 5]. Advances in our understanding of the pathophysiology of hepatic cystogenesis catalyzed the search for new therapeutic targets. Most promising targets are described below and summarized in the Table 1.

3. Therapeutic Targets In Pld

3.1. G-protein Coupled Receptors Linked to cAMP Signaling

Intracellular cAMP is considered as one of the major driving forces of hepatic cystogenesis [1, 4, 5, 16, 20]. Levels of cAMP are elevated in cystic cholangiocytes [21] influencing cellular pathways known to be defective in PLD. Several examples include: (i) cell proliferation involves cAMP down-stream effectors, such as EPAC1, EPAC2, PKA and ERK1/2 [21, 22]; (ii) expression of the cell cycle proteins is cAMP-regulated [23]; (iii) fluid secretion into cystic lumen is enhanced in response to cAMP elevation and requires the water channel AQP1 and the ion transporters, CFTR and AE2 [24]; and (iv) cilia in cystic cells lack the components of the cAMP signaling pathway (e.g., adenylyl cyclase 5/6, A kinase anchoring protein 150 (AKAP150), PKA and phosphodiesterase 4C) subsequently interrupting cross-talk between cAMP and intracellular calcium; as a result cholangiocyte proliferation and cyst growth are increased. Importantly, a number of studies suggest that targeting the components of the cAMP machinery in cystic cholangiocytes attenuates hepatic cystogenesis.

3.1.1. Somatostatin Receptors—Somatostatin is a powerful but short-lived (~ 3 minutes) antagonist of the cAMP signaling pathway in cholangiocytes; the peptide binds to five somatostatin receptors (SSTR1-5) decreasing cAMP, suppressing fluid secretion, inhibiting cell proliferation and release of several hormones (insulin, glucagon, gastrin,

secretin, cholecystokinin) and growth factors (IGF-1 and VEGF) [25, 26]. All SSTRs are present in healthy cholangiocytes and aberrantly expressed in hepatic cysts [27]. More stable and selective synthetic analogs of somatostatin (octreotide, lanreotide and pasireotide) have been developed for clinical use. Octreotide and lanreotide bind with high affinity to SSTR2 and SSTR3, and with moderate affinity to SSTR5. Pasireotide has a broader binding spectrum (i.e., SSTR1-3 and SSTR5) and longer half-life (12 hours compared with 2 hours for octreotide). Octreotide and pasireotide decrease cAMP, suppress cell proliferation and cyst growth *in vitro* in cultured rat and human cystic cholangiocytes [21, 27]. In pre-clinical studies, both drugs decreased hepatic and renal cystic and fibrotic areas in rodents with PLD (i.e., PCK rats and *Pkd2*$^{WS25/-}$ mice) with pasireotide being more effective than octreotide [21, 27]. These preclinical studies provided a strong rationale for assessing the potential therapeutic value of somatostatin analogs in humans. Several clinical trials tested octreotide-LAR or lanreotide in patients with ADPKD and ADPLD and showed their therapeutic effectiveness with a reasonable similar result across the studies [17, 18, 28-32]. The total liver volumes were decreased by 4%-6% and quality of life was improved. Moreover, octreotide and lanreotide overall are well tolerated. An ongoing clinical trial (NCT01670110) tests the effects of pasireotide in patients with ADPKD and ADPLD.

3.1.2. Secretin Receptor—Secretin is considered to be a major cAMP agonist in cholangiocytes and exerts its biological effects through the secretin receptor located on their basolateral domains. By increasing cAMP synthesis, secretin regulates secretion of water and electrolytes in cholangiocytes, induces phosphorylation of PKA, opens CFTR channels, activates Cl^-/HCO^{3-} exchanger, AE2, and mediates the microtubule-dependent insertion of AQP1 into the apical membrane of cholangiocytes [25, 33]. In humans with ADPKD, administration of secretin increased fluid secretion by hepatic cystic epithelia [4, 34].

Subcutaneous administration of exogenous secretin had negligible effects on cyst growth in PCK rats and *Pkd2*$^{WS25/-}$ mice, and the severity of hepatic and renal cystogenesis was not affected in *Pkd2*$^{WS25/-}$; *SCTR*$^{-/-}$ double mutant mice which are deficient in functional secretin receptors [35]. Therefore, it is unlikely that secretin plays a significant role in the pathogenesis of PLD and could be a useful therapeutic target.

3.1.3. Follicle-Stimulating Hormone Receptor (FSHR)—FSH, which is required for granulosa cell differentiation and follicular growth, executes its action by binding to FSHR, activating G_s proteins and increasing cAMP production. Given that FSH stimulates cholangiocyte proliferation via phosphorylation of ERK1/2 and ELK-1 [36], the involvement of FSH and FSHR in liver cyst growth was evaluated. The study demonstrated that cholangiocytes isolated from hepatic cysts of ADPKD patients expressed both FSH and FSHR; the latter was co-localized with phospho-ERK. Stimulation of FSH increased cell proliferation and cAMP while silencing of FSH abolished these effects [37]. While these data implicate FSH in proliferation of ADPKD cholangiocytes, further studies are necessary to elucidate its pathophysiological and therapeutic role in PLD.

3.1.4. Bile Acid Receptor TGR5—TGR5 (GPBAR1) is a recently discovered, bile acid-responsive GPCR linked to cAMP signaling. TGR5 is expressed in many tissues, regulates multiple cellular processes, and has emerged as a therapeutic entity in metabolic,

inflammatory and digestive diseases [38-40]. TGR5 is expressed in normal cholangiocytes being localized to different cellular compartments including primary cilia, apical membrane, intracellular vesicles and the nuclear membrane [39, 41]. The cellular response to TGR5 activation in healthy cholangiocytes is ciliary-dependent [41]. Specifically, in cholangiocytes with fully-developed primary cilia TGR5 agonists decrease cAMP and cell proliferation; in contrast, in non-ciliated cholangiocytes, activation of TGR5 results in the opposite effects (i.e., increased cAMP and enhanced proliferation) [41]. Importantly, TGR5 is over-expressed in cystic cholangiocytes of animal models and humans with PLD. Thus, given that: (i) TGR5 is linked to cAMP signaling [39, 41]; (ii) cAMP are increased in cystic cholangiocytes [26, 42]; (iii) cellular response to TGR5 activation is ciliary-dependent [41]; and (iv) cilia are functionally and structurally abnormal in cystic cholangiocytes [43], we have suggested that TGR5 might have therapeutic potential in PLD. Currently, additional studies on the role of TGR5 in hepatic cystogenesis are in progress in our laboratory.

3.2. PKA-RAS-RAF-MEK-ERK Cascade

The down-stream effectors of cAMP (i.e., PKA, RAS, RAF, MEK and ERK1/2) play an important role in cyst growth [22, 44]. In mice deficient in polycystin-2, hepatic cystogenesis is associated with altered intracellular calcium homeostasis and activated RAS-RAF-MEK-ERK machinery [44]. Activation of the RAS-RAF-MEK-ERK1/2 axis also triggers HIF1α-dependent secretion of VEGF which in turn stimulates cell proliferation and cyst vascularization [45, 46].

Recent preclinical study tested how the targeting of B-RAF affects hepatic cystogenesis. Unexpectedly, the tyrosine-kinase inhibitor, sorafenib, accelerated cyst progression in Pkd2cKO mice by activating RAF-1 in a cAMP-dependent manor while combinational administration of sorafenib and octreotide abolished the paradoxical effects of sorafenib [44].

Also, the potential therapeutic value of targeting the PKA-MEK-ERK pathway was supported by *in vitro* studies demonstrating that PKA activation in cystic cholangiocytes enhances cell proliferation and cyst growth in a MEK-dependent fashion [22]; PKA and/or MEK inhibitors abolished these effects emphasizing the importance of this pathway in hepatic cystogenesis and its potential therapeutic value.

3.3. Cholangiocyte Primary Cilia

In the liver, cholangiocytes but not hepatocytes possess primary cilia providing biliary epithelia with functionally important sensory and signaling mechanisms [7]. The linkage of cholangiocyte primary cilia to intracellular calcium and cAMP signaling occurs via several ciliary-associated proteins including: (i) a cell surface receptor, polycystin-1; (ii) a non-selective cation channel with a high permeability to Ca^{2+}, polycystin-2; (iii) the TRPV4 channel that is activated by extracellular hypotonicity and inhibited by extracellular hyper tonicity; (iv) a nucleotide receptor, $P2Y_{12}$; (v) a bile acid receptor, TGR5; and (vi) the AKAP-signaling complex consisting of AKAP150, adenylyl cyclases 4, 6 and 8 (AC4, AC6 and AC8), protein kinase A regulatory subunits RI-β and RII-α (PKA RI-β and PKA RII-α), and EPAC2 [6]. Polycystin-1 and polycystin-2 form a mechano-sensory complex,

activation of which increase intracellular calcium [42]. The initial increase in $[Ca^{2+}]_i$ originates from the entry of extracellular Ca^{2+} into cholangiocytes via polycystin-2 which in turn stimulates the release of Ca^{2+} from the intracellular stores via activation of the IP3 receptors (IP_3R). Activation or inhibition of TRPV4 also results in changes in $[Ca^{2+}]_i$ and in cholangiocyte functional response such as bicarbonate secretion [42, 47].

In PLD, cholangiocyte cilia are abnormal, reduced in length and/or malformed and do not express polycystin-1, polycystin-2 and fibrocystin, i.e., are characterized by impaired mechanisms of sensory and transducing functions [6, 20, 42, 48]. These abnormalities result in inhibition of calcium signaling and activation of cAMP signaling affecting the functions of biliary epithelia. In particular, a low calcium level activates the Ca^{2+}-inhibitable AC6 resulting in cAMP overproduction that in turn induces cholangiocyte hyperproliferation and cysts growth via the PKA-MEK-ERK pathway [6, 42].

The importance of the altered sensory and transducing functions of cholangiocyte cilia in PLD suggests that the progression of this disease could be affected therapeutically by targeting the structurally and functionally impaired primary cilia, i.e., by “ciliotherapy”. The term “ciliotherapy” was proposed by Surya Nauli based on his observation that activation of a ciliary-associated dopamine receptor-5 (DR5) increases ciliary length in endothelial cells of PKD mice and partially rescues the altered mechano-sensitive function of primary cilia. As a result, the increased blood pressure, a characteristic of PKD, was reduced [49].

There are as yet no reports on “ciliotherapy” in PLD or PKD. However, we believe that “ciliotherapy” may be considered as a novel therapeutic approach for the treatment of liver pathology associated with structurally and functionally aberrant cholangiocyte cilia that are responsible for erroneous regulation of cholangiocyte functions.

3.4. Intracellular Calcium

Experimental evidence supports the concept that decreased intracellular calcium in cystic cholangiocytes has cAMP/PKA-dependent permissive effects on cell proliferation and cysts growth *in vitro* while calcium restoration with ionophore, A23187, inhibits these processes via the PI3K/AKT pathway [22]. Activation of TRPV4 increases calcium levels subsequently suppressing cell proliferation and cyst growth in culture by activating AKT and inhibiting B-RAF-ERK signaling pathway. Importantly, TRPV4 activator, GSK1016790A, also reduced cystogenesis *in vivo* [50].

In addition, other PLD-causative proteins might be involved in regulation of calcium homeostatic in cystic cholangiocytes. Hepatocystin is known to interact with the C-terminal domain of polycystin-2 preventing its degradation [51]. Hepatocystin binds to IP_3R increasing the intracellular calcium release and maintaining the basal rate of cell proliferation while depletion of IP_3R from cholangiocytes enhances their growth [10]. Fibrocystin was also shown to interact with the calcium-modulating cyclophilin ligand [52] and to trigger the calcium signaling induced by mechano-sensation [53]. Together these studies support the notion that intracellular calcium the restoration might cease cyst progression in PLD.

3.5. Cell Cycle

The cell cycle profiles of cystic cholangiocytes are different from control cells likely resulting from the over-expression of the master cell cycle regulator, Cdc25A [23]. Cdc25A overexpression is associated with an increased proportion of cholangiocytes that have atypical centrosomal positioning, supernumerary centrosomes, multipolar spindles and extra cilia [43]. Depletion of Cdc25A decreased proportion of cells in the G1/0 and S phases and the number of centrosomes, mitotic spindles and cilia per cholangiocytes. As a result, repressed cell proliferation and cyst growth *in vitro* was achieved. Genetic elimination of Cdc25A from cystic cholangiocytes by cross-breeding of $Cdc25A^{+/-}$ mice with $Pkhd1^{del2/del2}$ mice (an animal model of ARPKD) reduced hepatic cystogenesis in $Cdc25A^{+/-};Pkhd1^{del2/del2}$ double mutants [23]. Therefore these data provided the rationale for testing the effects of Cdc25A inhibition on disease progression in the pre-clinical trial using PCK rats and $Pkd2^{WS25/-}$ mice. As predicted, two Cdc25A inhibitors, menadione and PM-20, attenuated hepatic and renal cystic and fibrotic scores in rodents by suppressing activity and expression of Cdc25A [23].

3.6. Histone Deacetylase 6 (HDAC6)

HDAC6, a regulator of cell cycle progression and ciliary disassembly, is over-expressed in cystic livers of human and animals with PLD and in cultured rat and human cystic cholangiocytes. Inhibition of HDAC6 by Tubastatin-A, Tubacin, and ACY-1215 decreased cholangiocyte proliferation in a dose- and time-dependent manner, and inhibited cyst growth in three-dimensional cultures. Moreover, ACY-1215 diminished liver cyst growth and hepatic fibrosis in PCK rats [54].

3.7. Extracellular Matrix (ECM) and Metalloproteinases (MMP)

Interactions between cholangiocytes and the ECM play an important role in ductal plate formation and are likely involved in hepatic cyst progression [1]. ECM remodeling in PLD is associated with the over-expression and hypersecretion of metalloproteinases (MMP), with the latter being influenced by IL-6, IL-8 and 17β-estradiol [10]. IL-6, IL-8 and 17β-estradiol are present in cholangiocytes and cystic fluid of ADPKD patients and known to increase cell proliferation [4, 10, 55, 56]. Oral administration of the MMP inhibitor marimastat decreased hepatic cystogenesis in PCK rats [56].

3.8. Proto-Oncogene Tyrosine-Protein Kinase Src (c-Src)

c-Src is involved in phosphorylation of specific tyrosine residues of different proteins and is associated with several types of cancers promoting angiogenesis and proliferation. Increased c-Src activity was found to correlate with liver cyst progression in PCK rats [57]. Inhibition of c-Scr with the pharmacologic inhibitor, SKI-606, ameliorated biliary duct abnormalities. However, a high incidence of drug-related gastrointestinal adverse events may limit its future use in clinic for the PLD treatment [58].

3.9. mTOR Signaling

mTOR plays an important role in PLD by integrating a spectrum of pathways, including growth factor signaling [46]. Cystic cholangiocytes of animal models and patients with PLD

over-express mTOR, VEGF, VEGF-R2, IGF1 and IGF-R1 [59, 60]. VEGF and IGF-1 and their cognate receptors stimulate proliferation of cystic cholangiocytes and enhance angiogenesis around cysts by autocrine-paracrine mechanism [46]. Furthermore, IGF-1 and VEGF signaling converge via mTOR pathway in a PI3K-AKT-MEK-ERK-dependent fashion. MTOR inhibitor, rapamycin, blocks secretion of VEGF repressing cell proliferation and cyst growth *in vitro*. The treatment of Pkd2cKO mice with rapamycin reduced hepatic cystogenesis, density of cystic microvasculature and PCNA expression [46]. Though, rapamycin failed to attenuate kidney and liver disease progression in PCK rats [61].

The effects of mTOR inhibitors of hepatic and renal cystogenesis were also evaluated in clinical trial. In ADPKD patients with kidney transplants, sirolimus reduced liver volume by 11.9% [62]. However current mTOR inhibitors have a narrow therapeutic window [58, 63] and due to their side effects a higher doses might be unsuitable especially for a long-term treatment.

3.10. Cytokines and Growth Factors

As discussed above, a number of cytokines, growth factors and their cognate receptors (i.e., IL-6, EGF, VEGF, IGF-1, angiopoietin-1, VEGFR2 and Tie-2) are present in cystic cholangiocytes and cystic fluid promoting cell proliferation, cyst expansion and angiogenesis [1, 4, 60]. Therefore, they all might be therapeutic targets in PLD. Indeed, inhibition of VEGF production or targeting of VEGFR-2 with SU5416 decreased hepatic cystogenesis in mice which are deficient for polycystin-2 [45, 64].

3.11. Peroxisome Proliferator Activated Receptor(PPAR)-γ

PPAR-γ, a member of the nuclear hormone receptor superfamily, is a ligand-activated transcriptional factor that influences inflammation, cell proliferation and fibrosis. Several studies suggest that full (i.e., pioglitazone) or partial (i.e., telmisartan) agonists of PPAR-γ ameliorate hepatic cystogenesis in PCK rats by decreasing TGF-β expression in cholangiocytes [12, 65].

3.12. MiRNA Dysregulation

MiRNAs, are small non-coding RNAs that regulate protein expression. The contribution of miRNAs in PLD pathogenesis was recently shown [66, 67]. MiRNA profiles are globally affected in cystic cholangiocytes with the majority of them being down-regulated. Computational analysis revealed that some of these miRNAs target proteins that belong to pathways crucial for hepatic cystogenesis [68]. In particular, in cystic cholangiocytes, miR-15a (levels of which are decreased) targets the cell cycle regulator, Cdc25A (levels of which are increased), affecting cell proliferation and cyst growth *in vitro* [66]. Moreover, experimental restoration of miR-15a in cystic cholangiocytes decreased cell proliferation and cyst expansion suggesting that miR-NAs might be considered as therapeutic tool in PLD.

3.13. Autophagy

Autophagy is the major intracellular degradation system by which outdated or damaged cytosolic proteins and/or organelles are sequestered into autophagosomes for degradation

after fusion with the lysosome [69]. Recent evidence revealed a link between autophagy and ADPLD demonstrating that the loss of wild type endogenous hepatocystin or the presence of pathogenic hepatocystin mutants induces autophagy *in vitro* [70]. However, the pathogenic and therapeutic role of autophagy in of PLD has still to be elucidated.

3.14. Combinational Therapies

Given that numerous affected signaling pathways and cellular functions contribute to hepatic cystogenesis, simultaneous manipulations of several different pathways or targeting different components of a single pathway to achieve the synergistic efficacy is an attractive concept. Indeed, beneficial effects of combinational administration of sorafenib and octreotide on hepatic cystogenesis were demonstrated in Pkd2KO mice [44]. Unfortunately, another study that evaluated the role of simultaneous administration of everolimus and octreotide to PLD patients were ineffective. After 48 weeks, the reduction in liver volume was similar between patients treated with both drugs together or with octreotide alone [71].

Conclusion

The recent advances in the understanding of the pathobiology of hepatic cystogenesis have already identified promising drugs (i.e., somatostatin analogs) for clinical use in spite of the fact that the drug effects on hepatic cystogenesis in animal models are not quantitatively translated to humans. The reasons for this discrepancy might be linked to the onset of therapeutic intervention. Indeed, many pre-clinical trials start the treatment of rodents at early stages of disease while in clinical trials the majority of patients have advanced PLD. In addition, different approaches are applied to analyze drug efficacy in rodents and humans. In animal models, changes in hepatic cystic areas (calculated using thin liver sections) between treated and untreated groups are the standard approach. In humans, relative changes of total liver volume before and after treatment (assessed by MRI or CT scan) are the endpoint of drug efficacy.

Despite the progress that has been made, the search for effective, safe and affordable long-term therapies for patients with PLD is ongoing. Extensive experimental data have shown that many intracellular signaling pathways are abnormal in cystic cholangiocytes and thus could theoretically represent potential therapeutic targets. Special attention should be given to the targets that already been tested in preclinical trials.

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List of Abbreviations

cAMP	Cyclic adenosine monophosphate
Cdc25A	phosphatase, Cell division cycle 25 homolog A
PKHD1	Polycystic kidney and hepatic disease
PRKSCH	Protein kinase C substrate 80K-H
LRP5	Low-density lipoprotein receptor-related protein 5
EPAC	Guanine nucleotide exchange factor
PKA	Protein kinase A
ERK	Extracellular signal-regulated kinases
AQP1	Aquaporin 1
CFTR	Cystic fibrosis transmembrane conductance regulator
AE2	Anion exchange
IGF	Insulin growth factor
VEGF	Vascular endothelial growth factor
PCK	Polycystic kidney
RAS	Rat sarcoma
MEK	Mitogen-activated protein kinase kinase
HIF1	Hypoxia induced factor 1
IL	Interleukin

Table 1

Therapeutic targets in PLD

Target	Drug	Evaluated		Therapeutic effects	Reference
		clinically	pre-clinically		
cAMP signaling (SSTRs)	octreotide		√	Reduced hepatic cystogenesis and fibrosis in PCK rats and *Pkd2*$^{WS25/-}$ mice	[21,27]
	octreotideLAR	√		Reduce total liver volume in ADPKD and ADPLD patients	[17,18,28-32]
	pasireotide		√	Reduced hepatic cystogenesis and fibrosis in PCK rats and *Pkd2*$^{WS25/-}$ mice	[27]
		√		ongoing clinical trial (NCT01670110)	
Calcium signaling (TRPV4)	GSK1016790A		√	Marginally reduced hepatic cystogenesis in PCK rats	[50]
Cell cycle (Cdc25A)	Vitamin K3 and PM-20		√	Reduced hepatic cystogenesis and fibrosis in PCK rats and *Pkd2*$^{WS25/-}$ mice	[23]
Primary cilia (HDAC6)	ACY-1215		√	Reduced hepatic cystogenesis and fibrosis in PCK rats	[54]
Extracellular matrix (Metalloproteinase)	marimastat		√	Reduced hepatic cystogenesis, fibrosis and inflammation in PCK rats	[56]
c-Src	SKI-606		√	Reduced hepatic cystogenesis in PCK rats	[57]
Growth factors (VEGFR-2)	SU5416		√	Reduced hepatic cystogenesis in *Pkd2KO* and *Pkd2*$^{WS25/-}$ mice	[45,64]
PPAR-g	pioglitazone and telmisartan		√	Reduced hepatic cystogenesis in PCK rats	[12,65]
mTOR signaling (mTOR)	sirolimus		√	Reduced hepatic cystogenesis in *Pkd2KO* mice	[46]
				No effects on hepatic cystogenesis in PCK rats	[61]
		√		Decrease total liver volume in ADPKD patients with kidney transplant	[62]
B-RAF and cAMP signaling (B-RAF, SSTRs)	sorafenib + octreotide		√	Reduced hepatic cystogenesis in *Pkd2cKO* mice	[44]
mTOR and cAMP signaling (mTOR, SSTRs)	everolimus + octreotide	√		Reduction in total liver volume was similar between patients treated with both drugs together or octreotide alone	[71]

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