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Caroli disease: an update on pathogenesis

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Caroli disease (CD) is a rare congenital disorder characterized by segmental dilatation of the intrahepatic bile ducts.[1] The simple CD is rare, with the majority of patients complicated by congenital liver fibrosis. Current treatments for CD mostly target complications but do not prevent disease progression. Surgical resection and liver transplantation are effective treatment options, but both have limitations.[2] Therefore, fully elucidating the pathogenesis of CD to identify therapeutic targets to delay disease progression is a priority.

Genetics of CD: CD falls into the clinical spectrum of autosomal recessive polycystic kidney diseases (ARPKD), which are caused by mutations in *PKHD1*.[3]*PKHD1* encodes fibrocystin, which is expressed in the kidneys, bile ducts, pancreatic ducts, heart, large vessels, testes, trachea, and sympathetic ganglia in animal models. Although the complete function remains unclear, fibrocystin might be involved in cellular proliferation, differentiation, cell-matrix interactions, and regulation of cell polarity.

Abnormal proliferation and differentiation of cholangiocytes: Polycystic kidney (PCK) rats are homozygous animal models with *Arpkd*-mutation replicating the slow progressive phenotype of ARPKD and CD with congenital hepatic fibrosis. Cholangiocytes in PCK rats have a higher proliferation rate. Several signaling pathways might participate in the abnormal proliferation of cholangiocytes [Table 1].

The cyclic adenosine monophosphate (cAMP) pathway is overactivated in cholangiocytes of PCK rats, leading to hyperproliferation and CD pathogenesis, which can be reversed by intraperitoneal injection of octreotide.[4] Epidermal growth factor (EGF) pathway overactivation can inhibit developmentally regulated apoptosis, leading to the formation of renal cysts in ARPKD patients. EGF can activate the tyrosine kinase activity of EGF receptor (EGFR) and promote cholangiocyte hyperproliferation in PCK rats through the mitogen/mitogen-activated protein kinase 5 (MEK5)/extracellular signal-regulated protein kinase 5 (ERK5) pathway. Gefitinib, an EGFR tyrosine kinase inhibitor, and small-interfering (si) RNAs targeting MEK5 can inhibit excessive proliferation of cholangiocytes in PCK rats.[5] The expression of Hedgehog (Hh) pathway components and downstream effectors are increased in PCK rats. Intraperitoneal injection of cyclopamine, an Hh antagonist, decreases serum alanine aminotransferase, alkaline phosphatase, and total liver and kidney cyst volumes, yet not liver fibrosis degree, in PCK rats.[6] The mammalian target of rapamycin (mTOR) forms two different signaling complexes, mTORC1 and mTORC2, which activate different downstream signaling pathways. mTOR expression is increased in liver and kidney tissues of PCK rats and ARPKD patients. Although rapamycin and everolimus (inhibitors of mTORC1) could not inhibit bile duct cyst formation in PCK rats, NVP-BEZ235 (an inhibitor of both mTORC1 and mTORC2) inhibited cholangiocyte proliferation, reduced bile duct dilatation, and ameliorated liver fibrosis.[7] Yes-associated protein (YAP) and its target gene products are overexpressed in cholangiocytes of PCK rats and liver tissues of ARPKD patients. The YAP inhibitor verteporfin, as well as short hairpin RNAs targeting YAP, inhibited the abnormal proliferation of cholangiocytes in PCK rats. In 2019, Tsunoda *et al*[8] established human induced pluripotent stem (iPS) cells with *PKHD1* knockout via CRISPER/Cas9 technology, and these iPS cells were able to differentiate into cholangiocyte-like cells in 3D cell culture. The expression of interleukin-8 (IL-8) significantly increased in *PKHD1*-knockout iPS cells. IL-8, via an autocrine effect, promoted cholangiocyte proliferation and expression of connective tissue growth factor, which promoted the progression of liver fibrosis.

Liver fibrosis in CD: Transforming growth factor-β1 (TGF-β1) is overexpressed in livers of PCK rats promoting liver fibrosis. cAMP-PKA pathway is activated in cholangiocytes in *Pkdh1*-mutant mice, promoting secretion of

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Table 1: Molecular pathways and possible therapeutic targets for CD.

Molecular pathway	Pathophysiology	Therapeutic target	Therapeutic agents under study	Therapeutic effect known
cAMP pathway	Hyperproliferation	cAMP	Octreotide in PCK rats	↓liver weights and cyst volumes, liver fibrosis and mitotic indices in PCK rats
EGF/MEK5/ERK5 pathway	Inhibition of developmentally-regulated apoptosis, hyperproliferation	EGFR	Gefitinib in PCK rats	↓excessive proliferation of cholangiocytes in PCK rats
		MEK5	siRNA targeting MEK 5 in PCK rats	
Hh pathway	Hyperproliferation	Hh	Cyclopamine in PCK rats	↓serum ALT, ALP, and total liver and kidney cyst volumes in PCK rats
mTOR pathway	Hyperproliferation, cytoskeleton malformation	mTORC1 and mTORC2	NVP-BEZ235 in PCK rats	↓cholangiocyte proliferation, bile duct dilatation, and liver fibrosis in PCK rats
Hippo pathway	Hyperproliferation	YAP	Verteporfin in PCK rats shRNA targeting YAP in PCK rats	↓proliferation of cholangiocytes in PCK rats
TGF-β1 pathway	Liver fibrosis	Macrophage activation	Clodronate in *Pkhd1*-mutant mice	↓liver fibrosis and cyst volume in Pkhd1-mutant mice
		RAS	Telmisartan in PCK rats	↓liver fibrosis, Ki-67, and TGF-β1 expression in PCK rats
		PPAR-γ	*Pioglitazone in PCK rats	↓liver fibrosis and TGF-β1 in PCK rats

* Pioglitazone can also reduce cholangiocyte proliferation in PCK rats by inhibiting the MEK5/ERK5 pathway. ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; CD: Caroli disease; cAMP: Cyclic adenosine monophosphate; EGFR: EGF receptor; EGF: Epidermal growth factor; ERK5: Extracellular signal-regulated protein kinase 5; Hh: Hedgehog; mTOR: Mammalian target of rapamycin; MEK5: Mitogen-activated protein kinase 5; PPAR-γ: Peroxidase proliferator-activated receptor γ; PCK: Polycystic kidney; RAS: Renin-angiotensin system; si: Small-interfering; TGF-β1: Transforming growth factor-β1; YAP: Yes-associated protein; ↓: Decrease.

CXCL10 into portal microenvironment to recruit macrophages that secrete TGF-β1. The TGF-β1 further promotes its own secretion leading to liver fibrosis. Macrophage scavenger clodronate inhibits liver fibrosis and reduces cyst volume in *Pkhd1*-mutant mice. Activation of the renin-angiotensin system can also promote liver fibrosis through the TGF-β1 pathway, and angiotensin receptor antagonist telmisartan can reduce liver fibrosis index, Ki-67, and TGF-β1 expression in PCK rats.[9] TGF-β1 pathway is also regulated by the peroxidase proliferator-activated receptor γ (PPAR-γ) pathway. Although the PPAR-γ agonist pioglitazone can reduce cholangiocyte proliferation in PCK rats by inhibiting the MEK5/ERK5 pathway, it also reduces liver fibrosis index in PCK rats by downregulating the expression of TGF-β1.[10]

Abnormal cilia structure and function: Fibrocystin is a component of primary cilia in cholangiocytes. In PCK rats, cholangiocyte primary cilia are significantly shortened and malformed. Planar cell polarity (PCP) proteins that guide correct centrosome localization to promote mitosis along the tube axis are regulated by the primary ciliary structure. ARPKD tissues are deficient in PCP proteins, thereby disordering mitosis and resulting in duct dilatation and cyst formation.

Potential research directions and possible therapeutic targets: Current evidence about the pathogenesis of CD is mainly from model animals. While differences between species are an unavoidable limitation of animal models, the development of CRISPR/Cas9 and other technologies is making it easier to study the disease in human cells (such as iPS cells). It is expected that future studies in human cells will provide further clues about the pathogenesis of CD.

CD is associated with abnormal signal transduction, which might be targeted with multiple therapeutic agents (such as octreotide, gefitinib, cyclopamine, and mTORC1 and mTORC2 inhibitors), albeit mostly in animal models. It is expected that new technologies such as big data modeling will be helpful to screen potential drug targets. Further clinical research is clearly needed to promote the treatment of CD.

Conclusions: CD is caused by loss-of-function mutations in *PKHD1*. The deficient expression of the *PKHD1* product, fibrocystin, leads to abnormal development and cystic dilatations of intrahepatic bile ducts. Current evidence suggests that fibrocystin might play important roles in cellular proliferation, differentiation, cell-matrix interactions, and the regulation of cell polarity. Future studies that take advantage of new technologies, such as

CRISPR/Cas9, might pave the way for novel and improved therapeutic strategies for patients with CD.

Conflicts of interest

None.

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