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REVIEW

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ABOUT COVER

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REVIEW

Cholangiocarcinoma and liver transplantation: What we know so far?

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Abstract

Cholangiocarcinoma (CCA) is a type of cancer with increasing prevalence around the world that originates from cholangiocytes, the epithelial cells of the bile duct. The tumor begins insidiously and is distinguished by high grade neoplasm, poor outcome, and high risk for recurrence. Liver transplantation has become broadly accepted as a treatment option for CCA. Liver transplantation is expected to play a crucial role as palliative and curative therapy for unresectable hilar CCA and intrahepatic CCA. The purpose of this study was to determine which cases with

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CCA should be subjected to liver transplantation instead of resection, although reported post-transplant recurrence rate averages approximately 20%. This review also aims to highlight the molecular current frontiers of CCA and directions of liver transplantation for CCA.

Key Words: Cholangiocarcinoma; Liver transplantation; Primary sclerosing cholangitis; Neoadjuvant chemoradiotherapy

Core Tip: Currently, there are many controversial hypotheses concerning liver transplantation in cholangiocarcinoma (CCA) and risk factors and molecular pathogenesis of CCA, with a focus on primary sclerosing cholangitis. Here, we mainly review the current advances in classification and treatment of CCA.

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INTRODUCTION

Cholangiocarcinomas (CCA), also known as bile duct cancer, constitute a diverse group of biliary epithelial tumors affecting the intrahepatic CCA (iCCA), perihilar CCA (pCCA), and distal bile duct CCA (dCCA)[1]. CCA is the second leading cause of liver malignancy after hepatocellular carcinoma (HCC), and the overall incidence and mortality rates of CCA have increased progressively worldwide in the last 4 decades [2]. Primary sclerosing cholangitis (PSC) as a chronic liver disease can increase the risk for CCA reaching approximately 10% or 398-fold *vs* with the general population[3,4]. CCA has remained the common cause of death at the global level among PSC patients, whereby 30% of all CCAs are recognized annually after diagnosing PSC[5,6]. CCA is generally considered to be one of the contraindications in relation to liver transplantation characterized by poor prognosis. CCA patients have a median survival of 2 years following diagnosis. The only potentially curative treatment chance is surgery, depending on the stages of disease[7]. It has been shown that neoadjuvant therapy with liver transplantation as a novel treatment exhibits better survival rates with fewer recurrence in comparison with conventional resection for localized, node-negative hilar cholangiocarcinoma (hCCA)[8]. CCAs are a highly aggressive epithelial malignancy, and many patients represent advanced stages of disease[9]. Early detection of CCA still remains a challenge owing to its 'silent' clinical feature (most patients in the initial stage at the time of diagnosis are asymptomatic) and difficult to reach anatomical sites[10]. It seems that the use of liver transplantation for the treatment of CCA can influence clinical outcomes in patients around the world. This class of tumor driving from the bile duct epithelial cells is clinically malignant, and its occurrence and prognosis are mostly associated with its anatomic location within the biliary tree and its chance to achieve complete resection with negative margins[11]. This review summarizes the risk factors and molecular pathogenesis of CCA, with a focus on PSC and liver transplantation along with advances in classification and treatment.

CCA CLASSIFICATION

CCA may be originated from the different cell types of the biliary tract, including cholangiocytes, the epithelial cells lining of the biliary surface epithelium, the epithelial cells of the peribiliary glands, hepatic progenitor cells, or any other mature hepatocytes that have become malignant. In this regard, CCA could also be classified

in terms of anatomical, histological, and molecular aspects[12].

Anatomical classification of CCA

According to anatomical location of the tumor, CCA will most commonly be classified into three sub-groups: (1) iCCA; (2) pCCA; and (3) dCCA (Table 1)[12,13]. Given the tumor location, iCCA typically arises from the intrahepatic biliary tract including segmental bile ducts to smaller branches of the intrahepatic biliary system. Thus, this subtype of CCA occurred in the periphery of the second-order bile ducts[13]. Also, iCCA represents approximately 20% of all CCA reported cases[14]. pCCA arises around the hepatic ducts and their junctions[15]. Finally, dCCA refers to the malignancy that occurs in the common bile duct, *i.e.* originated from Vater's ampulla [14,16].

Histological classification of CCA

From the histological point of view, characteristics of pCCA and dCCA that can be considered as extrahepatic CCA (eCCA) subtypes are conventionally mucin-producing adenocarcinomas or papillary tumors. On the other hand, iCCAs are more heterogeneous than two other subtypes of CCA. Histological studies showed that the adenocarcinoma is formed by columnar to cuboidal epithelial cells in the tubular structures, acini formation, and micropapillary architecture with variable morphological aspects, which are the most common types of iCCA[12].

Moreover, it has been suggested that, according to the level or size of the displayed bile duct, iCCA is classified into two main histological subtypes. First, the small bile duct iCCA that presents as small-sized tubular or acinar adenocarcinoma. These tumors commonly originated from small intrahepatic bile ducts, progenitor cells, and mature hepatocytes[17]. In contrast, large bile ducts iCCA derive from large intrahepatic bile ducts and/or associated peribiliary glands. Moreover, depending on the origin of the large bile duct iCCA, the histological aspects of this subtype of iCCAs are partly similar to pCCA and dCCA. However, the gross examination is not sufficient for accurate tumor classification, and further histological, molecular, and clinical investigation is required[18].

Molecular classification of CCA

First, it needs to be explained that, due to some differences in the characteristics of the existing studies, including different molecular detection methods and diversity in the selection of populations, there is still no consensus on the molecular characteristics of CCA classification[17]. However, it is possible to establish an acceptable relationship between the anatomical and molecular aspects of CCA subtypes. Integrative molecular analyses not only provided the functional information for CCA classification but also were used to understand the pathogenesis and signaling pathways underlying the CCA carcinogenesis and progression[14].

Mutation-based classification is the main approach of CCA molecular classification. For instance, the isoforms 1 and 2 of isocitrate dehydrogenase (IDH1 and IDH2) and *NRAS* mutations are the main molecular manifestation of iCCA, whereas eCCA typically showed *TP53*, *KRAS*, and *BRAF* mutations[14,19]. Also, it has been reported that *IDH1/2* and *BAP1* mutations and fibroblast growth factor receptor 2 (FGFR2) fusions are the main molecular characteristics of iCCA, while protein kinase CAMP-activated catalytic subunit alpha (PRKACA) and AT-rich interactive domain-containing protein 1B mutations are more common in eCCA. Besides, *KRAS*, *GNAS*, and *TP53* mutations are shared between iCCA and eCCA[19]. Interestingly, FGFR2 pairs with PRKACA in iCCA, as well as PRKACB in eCCA[20]

On the other hand, previous molecular studies also have attempted to connect the morphological CCA subtypes with specific molecular-based patterns. In this regard, the large-duct type iCCAs have a specific molecular property such as high mutation frequency of oncogenes and tumor suppressor genes and lack other gene mutations that are typically seen in small-duct iCCA. It has been reported that *KRAS* and *TP53* are two prominent genes with high mutation frequency in the large-duct type iCCAs as well as lack of *IDH1/2* mutations and *FGFR2*-fusions, which are molecular characteristics of small-duct iCCA[21].

In addition to mutation and sequence alterations, epigenetic study based on the methylation profiles of CCA subtypes can be used for CCA classification. For example, CCA has been related to hypermethylation at the promoter of tumor suppressor genes, such as *DAPK*, *P14* (ARF), and *ASC*[22]. Moreover, despite the different patterns of methylation in GC-rich regions (CpG islands) in the CCA subtypes-related genes, it has been revealed that there is an alteration in CpG methylation that belonged to WNT, transforming growth factor-β, phosphatidylinositol 3 kinase, mitogen-activated

Table 1 Summary of anatomical, histomorphological, and molecular characteristics of cholangiocarcinoma subtypes

Anatomical classification		Histomorphological classification	Molecular specification (gene alterations)
iCCA	Small intrahepatic bile ducts iCCA	Mass forming tumors[17]	IDH1/2, FGFR2, EPHA2, BAP1[14,19]
	Large intrahepatic bile ducts iCCA	Mass forming, periductal, or intraductal mucinous tumors[17,18]	EPHA2, BAP1, KRAS, TP53, GNAS, NRAS, MRAS, SMAD4[12,14,21]
eCCA	Perihilar CCA	Intraductal mucinous tumors[12,17]	KRAS, TP53, GNAS, NRAS, MRAS, SMAD4, ARID1B, PRKACA, BRAF[14,19,24]
	Distal CCA	Periductal mucinous tumors[12,17]	KRAS, TP53, GNAS, NRAS, MRAS, SMAD4, ARID1B, PRKACB, BRAF[14,19,24]

CCA: Cholangiocarcinomas; iCCA: Intrahepatic CCA; eCCA: Extra-hepatic CCA; EPHA2: Ephrin type-A receptor 2 precursor; FGFR2: Fibroblast growth factor receptor 2; BAP1: BRCA1 associated protein-1; NRAS: Neuroblastoma RAS viral [v-ras] oncogene homolog; KRAS: Kirsten rat sarcoma virus; TP53: Tumor protein; PRKACA: Protein kinase cAMP-activated catalytic subunit alpha.

protein kinase, and NOTCH signaling pathways[14]. Furthermore, the results of various studies showed that molecular characteristics of CCA subtypes consisting of sequence and copy number alterations, gene expression, and DNA methylation can be categorized into different clusters, but the details of this issue are beyond the scope of this article[23].

In addition to the mentioned above, another recent large cohort of CCA suggested that according to whole-gene expression data, chromosomal aberrations, and signaling pathway activation, CCA can be divided into two molecular subgroups: (1) inflammation class; and (2) proliferation class, which accounts for 38% and 62% of CCA cases, respectively[24]. The inflammation class of CCA has been characterized by the activation of inflammatory response and overexpression of T helper 2)-related cytokines and down-regulation of Th1-related cytokines. Moreover, it has been reported that several oncogenic pathways were enriched in the proliferation class that is accompanied by activation of receptor tyrosine kinase pathways (*i.e.* epidermal growth factor, RAS, AKT, MET, angiogenesis-related vascular endothelial growth factor, and platelet-derived growth factor) and Kirsten rat sarcoma viral oncogene homolog mutations[14,24].

Despite all of the before-mentioned data about molecular CCA classification, many other studies provide more useful information about molecular characteristics of CCA subtypes, such as the information derived from the noncoding RNA alteration, proteomics, and radiogenomic studies, which should be discussed in a separate article focusing on molecular classification of CCA[24].

Molecular pathogenesis of CCA

Cholangiocarcinogenesis is linked not only with genetic and epigenetic alterations but also with major changes in the microenvironment of the tumor. These modifications contribute to the triggering of different signaling pathways that are able to drive the initiation and progression of tumors[25]. Chronic inflammation contributes to increased exposure of cholangiocytes to Wnt inflammatory mediators, interleukin-6, cyclo-oxygenase-2, and tumor necrosis factor-alpha, leading to progressive mutations in some critical cancer-related genes including tumor suppressors, proto-oncogenes, and DNA mismatch-repair[26]. Increased apoptosis, decreased pH, and activation of extracellular signal-regulated kinase 1/2, Akt, and nuclear factor-kappa B signaling pathways following the accumulation of bile acids from cholestasis lead to promotion of survival, cell proliferation, and migration. Vascular endothelial growth factor, transforming growth factor-β, hepatocyte growth factor, and other microRNAs (miRNAs) are other mediators that are upregulated in CCA. Tumor development, angiogenesis, and migration are triggered by increased expression of the glucose transporter protein type 1, the cell surface receptor c-Met, and the sodium iodide symporter. The composition of the extracellular matrix and macrophage/fibroblast recruitment result in stromal shifts that establish a microenvironment to promotes cell survival, invasion, and metastasis[25,27-29]. The major signaling pathways involved in CCA are illustrated in Figure 1.

Genetic factors in the pathogenesis of CCA

Few studies have described chromosomal abnormalities in CCA, and, due to the limited number of samples and large genetic variation between the population groups

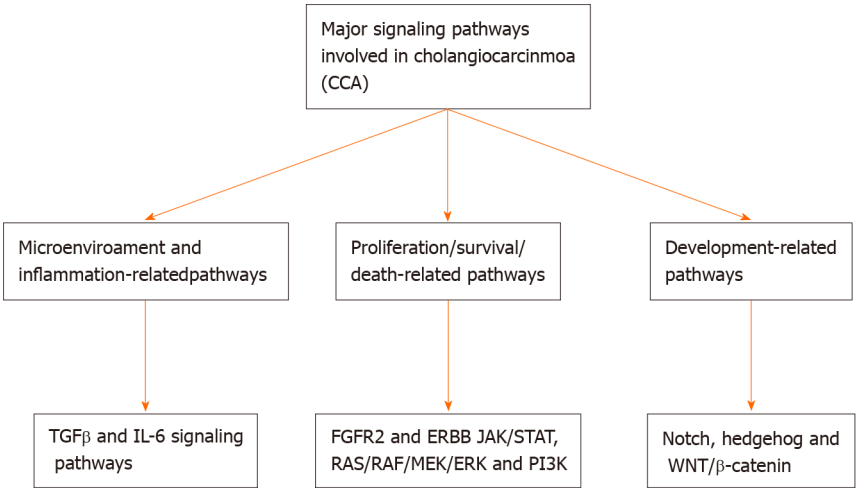

Figure 1 Major signalling pathways involved in cholangiocarcinoma. The major signaling pathways involved in cholangiocarcinomas could be categorized into three main class: (1) Microenvironment and inflammation-related pathways; (2) Pathways related to proliferation/survival/death; and (3) Development-related pathways. TGF-β: Transforming growth factor-β; CCA: Cholangiocarcinoma; IL: Interleukin; FGFR2: Fibroblast growth factor receptor 2; PI3K: Phosphatidylinositol 3 kinase; ERK: Extracellular signal-regulated kinase; JAK/STAT: Janus kinase and signal transducer and activator of transcription.

examined, the findings have been difficult to interpret. Data have revealed gains at 1q, 7p, 8q, 17q, and/or 20q and losses at 1p, 3p, 4q, 6q, 8p, 9pq, 13q, 14q, 17p, 18q, and/or 21q[24,30]. Curiously, genetic heterogeneity may be correlated with CCA in cells other than cholangiocytes. Natural killer cells and T-lymphocytes, for instance, express the natural killer group 2D receptor that plays an important role in cytotoxicity and tumor surveillance regulated by cells. One study indicated that the risk of experiencing CCA ranged significantly in patients with PSC, according to the patient's natural killer group 2D alleles; some were protective and others more than doubled the risk[31]. As potential risk factors for CCA, host genetic factors, alone or combined with environmental factors, have been investigated. For polymorphic variants that may be correlated with greater vulnerability to CCA, genes coding for xenobiotic detoxification, multidrug resistance, enzymes responsible for carcinogen metabolism, DNA repair, folate metabolism, and inflammation have been investigated. However, due to the inclusion of gallbladder and ampullary cancers in their evaluation in some of these reports and the lack of replication in separate cohorts, no conclusive conclusions can be taken. Multiple gene polymorphisms have been correlated with greater and reduced danger of experiencing CCA in many hospital-based, case-control studies. Due to the different populations of the sample and the lack of replication of the study in separate cohorts, it is hard to draw definite conclusions about these results. Table 2 summarizes genetic mutations and polymorphisms associated with CCA.

Epigenetic alterations in CCA

By the advent of array-based and deep sequencing techniques, technological advances have taken epigenetics into the omics-age, emphasizing the role of the epigenome in the human carcinogenesis process, including DNA CpG methylation, histone modifications, and non-coding RNA organisms. Only few systematic CCA epigenomic reports have been conducted, and data on abnormal CpG promoter methylation have mainly focused on individual genes in the CCA regulation[32]. In various important cancer-associated genes in CCA, abnormal epigenetic modulation such as promoter hypermethylation, was reported[32,33]. Studies examining these modifications to existing prognostic and predictive gene signatures have not yet been investigated in CCA to predict the therapeutic benefits of agents targeting the cancer epigenome. In CCA, the well-studied epigenetic process is DNA methylation. The promoter regions of tumor suppressor genes are highly methylated (promoter hypermethylation) in CCA tumorigenesis, which contributes to gene silencing. The promoter hypermethylation of genes involved in the repair of DNA, cell cycle, apoptosis, metabolism of carcinogen/drugs, and cell adhesion has been documented in CCA[33,34]. Some of the most frequent epigenetic events reported in CCA by methylation is summarized in Table 3.

Table 2 Genetic mutations and polymorphisms associated with cholangiocarcinoma

Gene (Full name)	Protein (Full name)	Normal function(s)	Ref.
ATP8B1 (ATPase Phospholipid Transporting 8B1)	FIC1 (Familial Intrahepatic Cholestasis type 1)	Transmembrane phospholipid transfer	Wadsworth *et al*[88], 2011
ABCB11 (ATP Binding Cassette Subfamily B Member 11)	BSEP (Bile Salt Exporter Pump)	Transport of cholate conjugates from hepatocytes to bile	Wadsworth *et al*[88], 2011
ABCC2 (ATP Binding Cassette Subfamily C Member 2)	MRP2 (Multidrug resistance-associated protein 2)	Transport of endogenous and xenobiotic compounds from hepatocytes to bile	Hoblinger *et al* [89], 2009
ABCB4 (ATP Binding Cassette Subfamily B Member 4)	MDR3 (MHC class I polypeptide-related sequence A)	Transport of lipids from hepatocytes to bile	Khabou *et al* [90], 2019
COX-2 (Cyclooxygenase 2)	COX-2 (Cyclooxygenase 2)	Inflammatory cytokine	Kim *et al*[91], 2002
CYP1A2 (Cytochrome P450 1A2)	CYP1A2 (Cytochrome P450 1A2)	Xenobiotic metabolism	Prawan *et al* [92], 2005
KLRK1 (Killer Cell Lectin Like Receptor K1)	NKG2D (NKG2-D type II integral membrane protein)	Tumor surveillance	Melum *et al* [93], 2008
MTHFR (Methylenetetrahydrofolate Reductase)	MTHFR (5,10-Methylenetetrahydrofolate reductase)	DNA methylation	Ko *et al*[94], 2006
NAT2 (N-Acetyltransferase 2)	ARY2(Arylamine N-acetyltransferase 2)	Drug and carcinogen metabolism	Prawan *et al* [92], 2005
PTGS2 (Prostaglandin-endoperoxide synthase 2)	PTGS2 (Prostaglandin G/H synthase 2)	The key enzyme in prostaglandin biosynthesis, and acts both as a dioxygenase and as a peroxidase	Sakoda *et al* [95], 2006
XRCC1 (X-ray repair cross complementing 1)	XRCC1 (DNA repair protein XRCC1)	Involved in DNA single-strand break repair by mediating the assembly of DNA break repair protein complexes	Huang *et al* [96], 2008
GSTO1(Glutathione S-transferase omega-1)	GST01 (Glutathione S-transferase omega-1)	Detoxification of endogenous and xenobiotic compounds	Marahatta *et al* [97], 2006
MICA (MICA PERB11.1)	MICA (MHC class I polypeptide-related sequence A)	Stress-induced self-antigen and Ligand for the KLRK1/NKG2D receptor	Melum *et al* [93], 2008
NR1H4(Nuclear Receptor Subfamily 1 Group H Member 4)	BAR (FXR) (Bile acid receptor (Farnesoid X receptor)	Negative feedback inhibitor of bile acid synthesis	Wadsworth *et al*[88], 2011
TYMS (Thymidylate Synthetase)	TYMS (Thymidylate synthase)	DNA repair	Razumilava *et al*[61], 2014
XRCC1 (X-Ray Repair Complementing Defective Repair in Chinese Hamster Cells 1)	XRCC1 (DNA repair protein XRCC1)	DNA repair	Gong *et al*[98], 2015
APC (Adenomatous polyposis coli)	APC (Adenomatous polyposis coli)	Tumor suppressor	Kang *et al*[99], 1999
ARID1A (AT-Rich Interaction Domain 1A)	ARID1a (AT-rich interactive domain-containing protein 1A)	Transcription factor	Razumilava *et al*[61], 2014
BAP1 (BRCA1 Associated Protein 1)	BAP1 (Ubiquitin carboxyl-terminal hydrolase BAP1)	Regulates cell growth	Yoshino *et al* [100], 2020
BCL-2 (B cell Lymphoma-2)	Bcl-2 (B-cell lymphoma 2)	Regulates apoptosis	Fingas *et al* [101], 2010
BRAF (B Rapidly Accelerated Fibrosarcoma)	B-Raf (B-Rapidly Accelerated Fibrosarcoma)	Proto-oncogene	Sia *et al*[24], 2013
BRCA1 (Breast Cancer 1)	BRCA1 (Breast cancer type 1 susceptibility protein)	Tumor suppressor and DNA repair	Paradiso *et al* [102], 2020
BRCA2 (Breast Cancer 2)	BRCA2 (Breast cancer type 2 susceptibility protein)	DNA repair	
CCND1(Cyclin D1)	CCND1 (G1/S-specific cyclin-D1)	Regulates cell growth	Yoshino *et al* [100], 2020
CDH1(Cadherin 1)	E-cadherin (Epithelial cadherin)	Tumor suppressor, cell adhesion	Ross *et al*[103], 2014
CDK6 (Cyclin-Dependent Kinase 6)	CDK6 (Cyclin-Dependent Kinase 6)	Controls cell cycle and differentiation	

CTNNB1 (Catenin Beta 1)	B-catenin	Proto-oncogene	O'Dell *et al* [104], 2012
EGFR (ERBB1) (Epidermal Growth Factor Receptor)	EGFR (ErbB-1) (Epidermal Growth Factor Receptor)	Proto-oncogene	
ERBB2 (HER2) (Avian Erythroblastosis oncogene B2)	ErbB-2 (HER2) (Receptor tyrosine-protein kinase erbB-2)	Proto-oncogene	
FBXW7 (F-Box and WD Repeat Domain Containing 7)	FBXW7 (F-box/WD repeat-containing protein 7)	Component of proteasomal protein degradation pathway	Ross *et al*[103], 2014
FGF19 (Fibroblast Growth Factor 19)	FGF19 (Fibroblast Growth Factor 19)	Regulation of bile salt synthesis	
FGFR2 (Fibroblast Growth Factor Receptor 2)	FGFR2 (Fibroblast Growth Factor Receptor 2)	Cell surface receptor regulating cell proliferation, differentiation, migration and apoptosis	
IDH1 (Isocitrate dehydrogenase 1)	Isocitrate de-hydrogenase 1 (Isocitrate dehydrogenase (cytoplasmic))	Glucose metabolism, indirectly mitigates oxidative stress	Nabeshima *et al*[105], 2020
IDH2 (Isocitrate dehydrogenase 2)	Isocitrate de-hydrogenase 2 (Isocitrate dehydrogenase (mitochondrial))	Glucose metabolism, indirectly mitigates oxidative stress	
Keap1 (Kelch-like ECH-associated protein 1)	KEAP1 (Kelch-like ECH-associated protein 1)	Prevents Nrf2-driven transcription	Ma *et al*[106], 2020
KRAS (Kirsten Rat Sarcoma)	K-Ras (Kirsten Rat Sarcoma)	Proto-oncogene	Tannapfel *et al* [107], 2000
MDM2 (Mouse Double Minute 2)	Mdm2 (E3 ubiquitin-protein ligase Mdm2)	Proto-oncogene, p53 inhibitor	Ross *et al*[103], 2014
MYC (Avian myelocytomatosis virus oncogene cellular homolog)	Myc (Myc proto-oncogene protein)	Proto-oncogene	Zhou *et al* [108], 2019
NF1 (Neurofibromin 1)	NF1 (Neurofibromin)	Stimulates Ras activity	Ross *et al*[103], 2014
PBRM1 (Polybromo 1)	PBRM1 (Protein polybromo-1)	Negative regulator of cell proliferation	Luchini *et al* [109], 2017
PIK3CA (Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha)	PIK3CA (Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform)	Generates PIP3 that activates signalling cascades for cell growth, survival and motility	Xu *et al*[110], 2011
PTEN (Phosphatase and Tensin Homolog)	PTEN (Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase PTEN)	Tumor suppressor	Zhu *et al*[111], 2014
RAD51AP1 (RAD51 Associated Protein 1)	RAD51AP1 (RAD51 Associated Protein 1)	DNA damage repair	Liu *et al*[112], 2021
RASSF1A (Ras association domain family 1 isoform A)	RASSF1A (Ras association domain-containing protein 1 isoform A)	Tumor suppressor	Chen *et al*[113], 2005
SMAD4 (Small Mothers Against Decapentaplegic 4)	SMAD4 (Small Mothers Against Decapentaplegic 4)	Tumor suppressor, transcription factor	Yoshino *et al* [100], 2020
SOCS3 (Suppressor of Cytokine Signaling 3)	SOCS3 (Suppressor of Cytokine Signaling 3)	Signal transduction inhibitor	Andersen *et al* [114], 2012
TP53 (Tumor Protein 53)	p53 (Protein 53)	Tumor suppressor	O'Dell *et al* [104], 2012

NKG2D: Natural killer group 2D.

Non-coding RNAs changes in CCA

MiRNAs are a type of small non-coding RNA that is involved in the post-transcriptional regulation of gene expression. The upregulation/downregulation in multiple miRNAs have been reported in CCA, wherein dysregulated miRNAs led to mitosis, increased cell survival, and metastasis[35]. However, whether the alteration in miRNA expression in CCA is part of the process of carcinogenesis or the consequence of established CCA remains to be fully understood[36]. Long non-coding RNAs (lncRNAs) widely transcribed in the genome are evolving as key cancer regulators and play crucial roles in almost every facet of cell biology, including tumorigenesis. *Via* their association with DNA, proteins, and RNA, lncRNAs control cells' malignant transformation. The molecular mechanisms of lncRNA involved in CCA tumorigenesis may therefore be promising targets for therapeutic intervention and diagnostic applications in the battle against cancer[37,38]. The majority of upregulated genes are

Table 3 DNA methylation in the genomic sequences of specific genes that are associated with the pathogenesis of cholangiocarcinoma

Gene (location)	Function	Epigenetic modification/effect	Outcome	Ref.
p16INK4A or CDKN2A (9p21)	Tumor suppressor gene Regulates cell proliferation and oncogenesis	Promoter region hypermethylation of the p16 ^{INK4A} results in gene inactivation. Common event in PSC-associated CCA	More frequent in ECC cases. More commonly observed in tumors with vascular invasion. Poor clinical outcome	Ueki *et al* [115], 2004
p14ARF (9p21)	Encoded by the β transcript of CDKN2A (p16/CDKN2A)	Methylation of p14^{ARF} MF = 38 and 25% (32.35); 40.2% liver fluke CCA (37)	Increased tumorigenesis in CCA	Kim *et al* [116], 2007
p15INK4b or p15 (9p21)	Effecter of TGF-β-mediated cell cycle arrest	Promoter hypermethylation of p15 gene	Increased tumorigenesis in CCA	Yang *et al* [117], 2005
p73 gene (1p36.3)	Tumor suppressor gene and related to the p53 gene	Promoter region hypermethylation increased tumorigenesis	Increased tumorigenesis in CCA	
TMS1/ASC (16p11.2)	Tumor suppressor gene	Aberrant methylation of the TMS1/ASC cause inactivation of gene	Associated with CCA	Liu *et al* [118], 2006
FHIT (3p14.2)	Tumor suppressor gene	Promoter hypermethylation of the FHIT gene results in epigenetic silencing of the FHIT promoter region	Development of intrahepatic CCAs	Foja *et al* [119], 2005
RASSF1A (3p21.3)	Tumor suppressor gene induces cell cycle arrest by inhibiting the accumulation of cyclin D1	Hypermethylation of its CpG island promoter region results in inactivation	Promoter methylation is more common in ECC than	Wong *et al* [120], 2002
hMLH1 (3p21.3)	DNA mismatch repair gene	Promoter methylation/hypermethylation of the hMLH1 gene	Methylation frequencies vary in sporadic CCA, biliary papillary, neoplasms, and liver fluke-related CCA. Associated with poorly differentiated subtype of CCA with vascular invasion	Yang *et al* [117], 2005
APC (5q21–q22)	Tumor suppressor gene Controls cell division, cell-cell interactions and cell migration and invasion, and conservation of chromosomal number during cell division	APC gene hypermethylation	Worse clinical outcome in CCA	Yang *et al* [117], 2005
RAR-β (or *HAP, RRB2* and *NR1B2*) (3p24)	Mediates cellular signaling in embryonic morphogenesis, cell growth and differentiation by regulating gene expression	Gene silencing by promoter region hypermethylation Results in increased tumorigenesis	Increased tumorigenesis in CCA	
Epithelial (*E*) *cadherin gene* (16q22.1)	Tumor suppressor gene	Hypermethylation of the promoter region of E gene Results in loss of function and contribute to progression of cancer by increasing proliferation, invasion and metastasis	Development of intrahepatic CCA	Lee *et al* [121], 2002
DAPK (9q34.1)	Tumor suppressor gene Positive mediator of interferon-γ (IFN-γ)-induced programmed cell death	DAPK gene hypermethylation	Associated with poorly differentiated CCAs and with a poor prognosis	Tozawa *et al*[122], 2004
CHFR gene (12q24.33)	Tumor suppressor gene Delays the entry into the metaphase	Gene silencing by promoter hypermethylation	Increased tumorigenesis in CCA	
RUNX3 gene (Ip36)	Tumor suppressor gene Regulate proliferation of the biliary tract epithelium	Methylation of RUNX3 results in gene silencing	Associated with poorer survival	
GSTP gene (1q43)	Regulate drug and xenobiotic. metabolism	Promoter region hypermethylation	Hypermethylation more frequent in ICCA than in ECC	Lee *et al* [121], 2002
MGMT gene (10q26)	Responsible for repairing alkylation. DNA damage inhibits estrogen receptor-mediated cell proliferation	Methylation of discrete regions of the MGMT CpG island, results in heterochromatinization of the MGMT transcription start site and silencing of the gene	Increased frequency of GC to AT transitions in oncogenes and tumor suppressor genes and a poor prognosis	Koga *et al* [123], 2005
BLU gene (3p21.3)	Tumor suppressor gene	Gene methylation	Increased tumorigenesis in CCA	Tischoff *et al*[124], 2005
SEMA3B (3p21.3)	Tumor suppressor gene by inducing apoptosis. Plays a critical role in the guidance of growth cones during neuronal development	Methylation of SEMA3B gene	Increased tumorigenesis in CCA	
TIMP3 gene	Plays a role in the induction of	CpG island methylation of TIMP3 gene	Associated with worse survival	Lee *et al*

(22q12.3)	apoptosis			[121], 2002
RIZ1	Tumor suppressor gene	Methylation of RIZ1 Results in chromatin compaction and gene silencing MF = 38% liver fluke CCA (47)	Increased proliferation and migration of CCA cell line	Khaenam *et al*[125], 2010
OPCML	Tumor suppressor gene	Hypermethylation of OPCML	Increased tumorigenesis in CCA	Sriraksa *et al*[126], 2011
GSTP1	Tumor suppressor gene	Methylation of GSTP1	Increased tumorigenesis in CCA	Yang *et al* [117], 2005
COX-2/PTGS2 (1q25.2–q25.3)	Acts both as a dioxygenase and as a peroxidase	Methylation of COX-2 gene	Increased tumorigenesis in CCA	Lee *et al* [121], 2002
THBS1 gene (15q15)	Mediates cell-to-cell and cell-to-matrix interactions and play roles in platelet aggregation, angiogenesis and tumorigenesis	Hypermethylation in the promoter region of THBS1 gene	Increased tumorigenesis in CCA	Tischoff *et al*[124], 2005
SOCS3	responsible for sustained IL-6/STAT-3 signaling and enhanced Mcl-1 expression in cholangiocarcinoma	Hypermethylation in the promoter region of SOCS3 gene	Increased tumorigenesis in CCA	Zhang *et al* [127], 2012

TGF-β: Transforming growth factor-β; CCA: Cholangiocarcinomas; IL: Interleukin; TIMP3: Tissue inhibitors of metalloproteinase 3; SOCS3: Suppressor of cytokine signaling 3; STAT-3: Signal transducer and activator of transcription; THBS1: Thrombospondin 1; SEMA3B: Semaphorin 3B; GSTP1: Glutathione S-transferase pi gene; OPCML: Opioid binding protein/cell adhesion molecule-like gene; MGMT: O6-methylguanine DNA methyltransferase; RIZ1: Retinoblastoma protein-interacting zinc finger gene 1.

involved in carcinogenesis, diseases of the hepatic system, and transduction of signals. The miRNAs and lncRNAs related to the promotion of the pathogenesis of CCA are indicated in Tables 4 and 5.

EPIDEMIOLOGY

Several publications have shown that PSC has an annual incidence rate of 0.77 *per* 100000 persons. PSC is more prevalent in adults between 25-years-old and 45-years-old; the median age of diagnosis of PSC is 41 years. Patients with PSC have a considerably higher risk of CCA, with an estimated incidence rate ranging from approximately 0.5% to 1.5% annually and lifetime incidence of 20%[4,39,40]. The estimated prevalence of CCA in patients with PSC ranges from 6.5% to 13.3%[4,41,42]. A recent cohort study on 7121 patients from 37 countries showed the prevalence of CCA in patients with PSC to be 8.3%[43]. In high prevalence regions, such as Scandinavian countries, PSC is the most common indication for liver transplantation[44]. Death attributed to PSC is increased nearly four-fold as compared to the general population, in part because of end-stage liver disease; however, more than 40% of deaths in PSC patients have been attributed to cancer development[4].

In Western countries, PSC is the most common known predisposing factor for CCA. The risk of CCA development *per* year among patients with PSC is 0.5% to 1.5%, with estimated lifetime prevalence of 5%–10%[45]. Several potential risk factors for CCA in PSC patients have been evaluated; smoking and alcohol consumption are increasingly recognized as risk factors for CCA[46].

Epidemiologic data studies regarding CCA mortality risk indicate that age-adjusted death rate for iCCA is increasing while trend mortality from pCCA and dCCA is expected to decrease worldwide[47]. Although the recorded rise in the incidence of CCA during the past 30 years has been observed as an increase in iCCA, it might be due to potential misclassification of perihilar tumors as iCCAs[48]. The age-adjusted incidence rate according to the United States database for iCCA enhanced from 0.59 *per* 100000 population in 1990 to 0.91 in 2001. Subsequently, the age-adjusted incidence rate decreased to 0.6 *per* 100000 population by 2007. Contrarily, the incidence rate among pCCA plus dCCA patients remained approximately 0.8 *per* 100000 population until 2001 then steadily increased to 0.97 until 2007. Perihilar CCA was identified as iCCAs before 2001 and subsequently was recognized as pCCA after releasing the 3rd edition of Classification of Tumors. This amendment plausibly affected the aforementioned alterations in specific incidence rates of both CCA subtypes[49].

Table 4 Unique microRNAs that were identified to promote the pathogenesis of cholangiocarcinoma

miRNAs	Target gene	Correlation with CCA tumorigenesis	Upregulated/downregulated	Ref.
miR-26a	GSK-3b	Tumor growth	Upregulated	Zhang *et al*[127], 2012
miR-24	MEN1(11q13)	Tumor suppressor gene	Upregulated	Ehrlich *et al*[128], 2017
miR-29b	MCL-1	Tumor suppressor gene	Downregulated	Stutes *et al*[129], 2007
let-7a	NF2	Tumor suppressor gene	Upregulated	
miR-148a	DNMT-1	Regulate methyltransferase	Downregulated	Braconi *et al*[130], 2010
miR-124	SMYD3	Migration and invasion of CCA cells	Downregulated	Zeng *et al*[131], 2012
miR-21	PTEN	Tumor suppressor gene	Upregulated	Meng *et al*[132], 2006
miR-152	DNMT-1	Regulate methyltransferase	Downregulated	Braconi *et al*[130], 2010
miR-200b	PTPN12	Tumor suppressor gene	Upregulated	Meng *et al*[132], 2006
miR-429	CDH-6	Tumor suppressor gene	Upregulated	Goeppert *et al*[133], 2016
miR-122, miR-145, miR-200c, miR-221, and miR-222	Multiple	Associated with tumorigenesis of ICCA	Downregulated	Karakatsanis *et al*[134], 2013
miR-21, miR-31, and miR-223	Multiple	No association with clinic-pathological parameters of CCA	Upregulated	
miR-370	MAP3K8	Tumor suppressor gene	Downregulated	Stutes *et al*[129], 2007
miR-141	CLOCK	Tumor suppressor gene	Upregulated	Meng *et al*[132], 2006
miR-214	Twist	Oncogene	Downregulated	Li *et al*[135], 2012

CCA: Cholangiocarcinoma; CXCR4: C-X-C chemokine receptor type 4; MAP3K8: Mitogen-Activated Protein Kinase Kinase Kinase 8; PTEN: Phosphatase and TENsin homolog deleted on chromosome 10; GSK-3b: Glycogen synthase kinase 3 beta; SMYD3: SET and MYN-domain containing 3; MCL-1: myeloid cell leukemia-1; NF2: Neurofibromatosis type 2.

iCCA is a primary carcinoma of the liver with rare entity, accounting for about 3% of global gastrointestinal cancers[50]. iCCA comprises 8%–10% of all CCA and has a distinguished disease course, incidence, and prevalence of disease from hilar and eCCA[51]. In addition, in spite of the fact that iCCA has been historically mistaken for other HCC[52], previous studies have shown that ICC accounts for 10%–20% of primary liver malignancies[53]. iCCA is uncommon in individuals under 40 years of age; it occurs primarily at an old age with the peak incidence in the 5th and 7th decade of life[54]. In the United States it is estimated a slight male predominance in iCCA cases (1.5 fold) over women[54].

Despite the low frequency of iCCA *vs* HCC, the incidence of iCCA appears to be increasing worldwide[55]. This increased risk of incidence rate is independent of tumor size and staging, and it is implausibly secondary to earlier diagnosis[55]. In the United States the incidence of iCCA during the past 30-year period enhanced 165% to 0.95 cases/100000 population[55]. A similar rise in iCCA incidence rate has also been reported in the United Kingdom, Japan, and Crete[56].

Globally, there is a certain disparity incidence of iCCA, with markedly lower rates of iCCA reported in Western nations when compared to East Asian countries[50]. This demographic variation is explained mainly by the prevalence of risk factors for iCCA in these East Asian countries[57].

In addition, Hispanic-Americans (1.22 *per* 100000 population) were considered to be significantly susceptible to high incidence of iCCA compared to other ethnic groups; for instance, African-Americans have a low rate of incidence (0.3 *per* 100000 population). The researchers have shown that this disparity may reflect genetic diversity, cultural differences, and socio-economic status in iCCA susceptibility[58].

Several risk factors implicated in iCCA pathogenesis have demographical prevalence. A previous report indicated that approximately 40% of iCCA patients will have no detectable risk factor, suggesting the need to be explored for further research in this regard[59].

Table 5 Upregulated long non-coding RNAs that are reported in cholangiocarcinomas

LncRNA	Possible mechanism	Clinical relevance	Ref.
AFAP1-AS1	(1) Decreasing the expression of c-Myc, Cyclin D1, MMP-2 and MMP-9; and (2) Decreasing the AFAP1 expression and promoting cell stress filament integrity	Unfavorable prognostic biomarker; potential therapeutic target	Lu *et al*[136], 2017
CCAT2	-	Unfavorable prognostic biomarker; potential therapeutic target	Xu *et al*[137], 2018
HULC	Activating CXCR4 by sponging to miR-372/miR-373 as ceRNA	Potential therapeutic target	Wang *et al*[138], 2016
ASAP1-IT1	Interacting with hedgehog signaling pathway	Unfavorable prognostic biomarker; potential therapeutic target	Guo *et al*[139], 2018
CPS1IT1	Coexpressed with host gene CPS1	Unfavorable prognostic biomarker; potential therapeutic target	Lu *et al*[136], 2017
EPIC1	Directly interacting with Mys	-	Li *et al*[140], 2018
H19	Activating IL-6 by sponging to let-7a/let-7b as ceRNA	Unfavorable prognostic biomarker; potential therapeutic target	Xu *et al*[141], 2017
CCAT1	Sponging to miR-152 as ceRNA	Independent prognostic factor; potential therapeutic target	Jiang *et al*[142], 2017
LINC01296	Modulating MYCN transcription by sponge miR-5095 as ceRNA	Potential therapeutic target	Jiang *et al*[142], 2017
PCAT1	Enhancing Wnt/β-catenin signaling through miR-122 repression and WNT1 expression	Potential therapeutic target	Zhang *et al*[143], 2017
SNHG1	Modulating cancer-related gene like CDKN1A by co-operating with chromatin-modifying enzymes as EZH2	Unfavorable prognostic biomarker; potential therapeutic target	Yu *et al*[144], 2018
MALAT1	(1) Activating PI3K/Akt pathway; and (2) miR-204-dependent CXCR4 regulation as ceRNA	Unfavorable prognostic biomarker; potential therapeutic target	Tan *et al*[145], 2017
PVT1	Binding to epigenetic modification complexes, adjusting the expression of ANGPTL4	Potential therapeutic target	Yang *et al*[146], 2018
UCA1	(1) Facilitating apoptosis *via* Bcl-2/caspase-3 pathway; (2) Activating AKT/GSK-3β/CCND1 axis; and (3) Upregulating MMP-9	Unfavorable prognostic biomarker; potential therapeutic target	Xu *et al*[147], 2017
SPRY4-IT1	Recruiting EZH2, LSD1 or DNMT1 *via* sponging to miR-101-3p	Unfavorable prognostic biomarker; potential therapeutic target	Xu *et al*[148], 2018
T-UCRs	Downstream of Wnt pathway and sponging to miR-193b	Unfavorable prognostic biomarker; potential therapeutic target	Carotenuto *et al*[149], 2017

LncRNA: Long non-coding RNAs; ceRNA: competing endogenous RNAs; MMP9: Matrix metallopeptidase 9; EZH2: Enhancer of zeste homolog 2; PI3K: Phosphoinositide 3-kinase; LSD1: lysine-specific demethylase 1; DNMT1: DNA (cytosine-5)-methyltransferase 1; CCND1: cell cycle proteins, cyclin D1; ANGPTL4: Angiopoietin-like protein 4.

It is believed that PSC is a predisposing factor for the development of iCCA. Both biliary inflammation and subsequent chronic proliferative activation of hepatic stem cells potentially predispose to iCCA formation[60]. It has been reported that PSC patients possess a lifetime incidence of CCA from approximately 5%–10%, while 50% of cases are recognized during 2 years of the course of PSC[61]. Additionally, researchers showed a predisposing risk of iCCA (odds ratio: 2.2; 95% confidence interval: 1.2–3.9) in ulcerative colitis patients[59]. The iCCA arose in PSC patients earlier, despite most individuals diagnosed between the ages of 30 and 50.

Recent results indicated that cancer risk is higher among patients with primary biliary stones and chronic biliary tract inflammation. Furthermore, incidence risk of iCCA has been found to be approximately 7% in hepaticolithiasis patients[61]. Another Asian study demonstrated that hepaticolithiasis in CCA patients followed by surgical resection is nearly 70% in Taiwan[60].

Furthermore, congenital anomalies of biliary tree, like Caroli's disease and fibrocystic hepatic disorder, reveal approximately 15% lifetime risk factors of iCCA following the 2nd decade of life[60]. Caroli's disease is a rare inherited disorder characterized by cystic widening of ducts in the liver, usually in a bilobar pattern. iCCA risk has been shown to be rising among subjects with bile stasis, cholangitis, and chronic inflammation[62].

CCA represents approximately 3% of all gastrointestinal cancers. The total incidence rate of CCA appears to have increased dramatically over the past 30 years[49]. The 5-

year overall survival rates after diagnosis remained at 10% during this span of time [46].

LIVER TRANSPLANTATION

CCA is a highly fatal malignancy tumor due to late clinical presentation[57]. While it is generally believed that standard of care is resection, most patients who present with metastatic disease are deemed unresectable[63]. The liver transplantation outcomes alone for unresectable conditions have been disappointing[64]. A previous study examined the effectiveness of a novel modality combining neoadjuvant chemoradiotherapy followed by liver transplantation. Survival outcomes from a combination of neoadjuvant chemoradiotherapy and liver transplantation for CCA are considerably excellent in comparison with resection[65]. Thus, even if transplantation may be a useful cure for unresectable iCCA, survival output remains poor. Orthotopic liver transplantation utilization is increasing within the United States and appears promising because it may obviate complications to achieve surgical margins into the liver. Unfortunately, efforts during the past decades were poor. In addition, according to the registry between 1968 and 1997, researchers have reported a 28% 5-year survival rate with a 51% risk of tumor recurrence rate after liver transplantation[64]. Furthermore, during the first 2 years, 84% of recurrences were identified and can occur in up to 47% of candidates of liver allograft. Other surgery centers in Europe reported a similar result; the 3-year survival for 36 patients was 30%[66]. Accordingly, most liver transplant centers historically consider CCA a contraindication for liver transplantation[67].

There are many benefits for liver transplantation *vs* conventional resection to acquire complete elimination of tumor. There is some difficulty in evaluating hepatic duct tumor involvement before resection, and this is the most frequent reason for failure towards the achievement of an R0 resection. This problem is considerably obviated by liver transplantation. Liver transplantation promotes extirpation of all adjacent tissue and resection of the caudate. Liver transplantation facilitates arterial and portal venous inflow preservation to the remaining liver. Liver transplantation provides wide local excision and higher patient survival than what could be achieved with conventional resection[8].

Researchers conclude that neoadjuvant supportive treatment therapy in combination with liver transplantation presently appears to have fared far better than resection for selected patients with regional lymph node negative hCCA. Surgical staging information is essential; 23% of patients had localized lymph node metastases and concomitant extrahepatic disorder, which increased subsequent risk for transplantation. In a quarter of patients with underlying PSC, pancreatoduodenectomy may be required to obtain complete removal of the patient's tumor with biliary tract involvement at the time of transplantation. Liver transplantation in combination with neoadjuvant treatment should be considered as an alternative option to surgical resection for patients with hCCA[8].

Liver transplantation as an important therapeutic option for iCCA is still debated. It has been reported that iCCA recurs within 5 years of liver transplantation among 70% of patients[68]. Locoregional interventions, such as radiofrequency ablation and transarterial chemoembolization, have garnered attention as a therapeutic alternative for localized, unresectable iCCA patients[69]. The standard treatment for patients with advanced-stage iCCA is the most common combinations, which includes systemic chemotherapy regimen of gemcitabine and cisplatin. According to a recent clinical study, liver transplantation could be a treatment choice for patients with early detected unresectable iCCA (*i.e.* ≤ 2 cm), with better survival results compared with those of HCC[70].

iCCA remains a contraindication for liver transplantation in most clinical centers around the world because of very poor prognosis, with a 2-year overall survival rate of approximately 30%[71]. The lack of standardization due to different patients' selection and the absence of neoadjuvant treatments are expected to change outcomes[72]. The best survival was achieved in hCCA thanks to careful patient selection for neoadjuvant radiotherapy. Results from cohort studies after 2014 confirmed promising results after liver transplantation for iCCA[70]. The significance of proper patient selection criteria was first evaluated in a global multicentric report among iCCA patients who underwent liver transplantation[70]. The only curative treatments available for pCCA are surgical resection and neoadjuvant chemoradiation therapy after liver transplantation. Owing to the existence of parenchymal liver disease, PSC patients in

most cases need liver transplantation as the preferred choice when compared to surgical resection[1]. Besides this, recent studies confirm that in PSC patients, intense immunosuppression ensuing liver transplantation increased risk of disease recurrence [73]. Liver transplantation for pCCA patients following neoadjuvant chemoradiation treatment establishes a proper long-term survival rate in a group of selected candidates with unresectable early stage pCCA and patients with PSC–related pCCA. Commitment to appropriate selection criteria, heavy neoadjuvant intervention, operative staging before liver transplantation, and specified technical procedures throughout the transplant process are required for success[74].

The research evidence shows that neoadjuvant therapy for liver transplantation is an effective treatment for unresectable early stage pCCA and pCCA occurring in the setting of PSC[75]. Recently, programmed cell death protein 1 inhibitors are noticed as a promising therapeutic option for CCA. Chimeric antigen receptor T cells, oncolytic viruses cancer vaccines and bispecific antibodies, show a remarkable ability to achieve satisfactory results.

Furthermore, the combinations of immunotherapy with other immunotherapeutics such as conventional therapies display some efficacy, and various studies have provided new insights into their administration in antitumor therapy[76]. The main barrier to successful liver transplantation and effective treatment is the availability of donor organs[75]. According to data from the Mayo Clinic and several other centers, from the start of therapy, a promising survival rate between 5-10 years was reached [77]. Post-transplant survival is approaching 50% at 5 years for both pCCA–related PSC and *de novo* pCCA, and these findings rationalize the use of both deceased and living donors.

After onset of this therapy in 1993, significant increases were observed in the time elapsed between the end of neoadjuvant treatment and liver transplantation. This interval can differ widely between patients by blood type compatibility, transplant center address, and availability of living donor organs. It has been shown that longer time elapsed between neoadjuvant therapy and liver transplantation results in reducing local recurrence[78]. Selection of patients with prolonged intervals and better oncologic biography, who are less susceptible to advancing the disease following neoadjuvant treatment, are less prone to develop recurrence post-transplantation. However, patients with radiation-induced fibrosis and longer intervals can significantly complicate the staging and transplant operations. Living donor liver transplant (LDLT) may solve these problems by removing the need to waitlist for a deceased donor and help physicians for optimal timing of liver transplantation. Recent findings based on clinical study demonstrated that LDLT and deceased donor liver transplant (DDLT) outcomes for pCCA-associated PSC are similar. In addition, LDLT for *de novo* pCCA shows a recurrence tendency and slightly worse patient survival outcomes *vs* DDLT. Despite these minor differences, researchers have been looking into possible mechanisms of disease progression following neoadjuvant treatment for a period to choose those patients who are at risk due to disease progression in order to prevent post-transplant disease recurrences[79]. A previous report indicated that liver transplantation is more effective and achieved better survival and less recurrence than surgical resection, and that the indications for liver transplantation and neoadjuvant treatment should advocate for resectable pCCA patients. According to these favorable findings, physicians have advocated for this viewpoint for patients with pCCA-associated PSC and transplanted many such patients at many transplant centers.

The role of neoadjuvant chemoradiation therapy and liver transplantation remains a consideration though, especially in *de novo* pCCA patients. Earlier studies were equivocal and unable to detect whether a subset of patients with *de novo* pCCA may benefit from liver transplantation *vs* surgical resection[80]. In 2015, American Hepato-Pancreato-Biliary Association recommended that surgical resection can be standard curative treatment for patients with resectable *de novo* pCCA[81]. Recent reports have suggested that liver transplantation *vs* surgical resection for hCCA patients who may need a liver transplant had better prognosis than those found after resection[82]. Analysis of results obtained from multicenter study between 2000 to 2015 showed that patients with pCCA not associated with PSC continued to show superiority of transplant compared to resection with promising post-transplant survival outcomes at 3 and 5 years (54% *vs* 44%, $P = 0.03$; 54% *vs* 29%, $P = 0.03$)[82]. Additionally, researchers pointed out 5-year estimated overall survival of 41% for patients enrolled onto clinical trials of neoadjuvant treatment/transplant procedure *vs* 27% among those patients who underwent surgical resection[83]. This discrepancy (14%) is too minor to approve the use of a donor liver for resectable non-PSC related pCCA[83]. In France a multi-center randomized clinical trial evaluating neoadjuvant chemoradiation and liver transplantation in comparison with resection will further elucidate pivotal details

on these equivocal results.

In brief, over the past 2 decades liver transplantation has been currently considered the proven treatment of unresectable early stage pCCA and pCCA associated with PSC. Outstanding findings can be attained by stringent adherence to patient selection criteria and clinical management, application of high-dose neoadjuvant radiation therapy, and clinical staging before liver transplantation. Liver transplantation in combination with neoadjuvant treatment can obtain outcomes similar to surgical resection for unresectable early stage pCCA patients, and this is the treatment of choice administered for patients with pCCA arising in the setting of PSC[84]. Approximately 5% of all cases affected by pCCA require liver transplantation under the Mayo eligibility criteria. If the liver does not work properly, without transplantation, a median survival time is approximately 1 year. pCCA is reported as the most common malignancy and aggressive type of the biliary duct and arises from biliary lining the liver hilum[85]. The Mayo Clinic and other international centers are recently selecting the optimal subgroup to treat patients with locally advanced pCCA by neoadjuvant chemoradiation in combination with liver transplantation[65,86]. Outcome of patients treated according to this guideline, a 5-year survival rate of 53%, marginally improves the survival rate of patients after surgery for resectable type of disease[86,87].

CONCLUSION

It is most important to understand oncological suitability, donor liver organ availability, as well as ability to obtain appropriate long-term results in patients with CCA with or without PSC. In pCCA not associated with PSC, liver transplantation seems to provide promising survival. In resectable types of pCCA patients, neoadjuvant chemoradiotherapy and liver transplantation by strict selection criteria may improve the survival rate of patients compared to unresectable early stage pCCA patients. Owing to the shortage of available organs, it still remains unknown whether liver transplantation and neoadjuvant chemoradiotherapy should be increasingly considered for other classifications of CCA. Imbalance between organ supply and demand further conducts a need for stringent indications and contraindications in recognizing liver transplantation proper status. It is also essential for doctors to stay up to date with the general indications for liver transplantation and to consider when it is suitable or unsuitable to refer patients for transplant evaluation.

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