



Up-to-Date Pathologic Classification and Molecular Characteristics of Intrahepatic Cholangiocarcinoma

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Intrahepatic cholangiocarcinoma (iCCA) is an aggressive primary liver malignancy with an increasing incidence worldwide. Recently, histopathologic classification of small duct type and large duct type iCCA has been introduced. Both these types of tumors exhibit differences in clinicopathological features, mutational profiles, and prognosis. Small duct type iCCA is composed of non-mucin-producing cuboidal cells, whereas large duct type iCCA is composed of mucin-producing columnar cells, reflecting different cells of origin. Large duct type iCCA shows more invasive growth and poorer prognosis than small duct type iCCA. The background liver of small duct type iCCA often shows chronic liver disease related to hepatitis B or C viral infection, or alcoholic or non-alcoholic fatty liver disease/steatohepatitis, in contrast to large duct type iCCA that is often related to hepatolithiasis and liver fluke infection. Cholangiocarcinoma is a variant of small duct type iCCA composed of naïve-looking cuboidal cells forming cords or ductule-like structures, and shows better prognosis than the conventional small duct type. Fibrous tumor stroma, one of the characteristic features of iCCA, contains activated fibroblasts intermixed with innate and adaptive immune cells. The types of stroma (mature versus immature) are related to tumor behavior and prognosis. Low tumor-infiltrating lymphocyte density, *KRAS* alteration, and chromosomal instability are related to immune-suppressive tumor microenvironments with resistance to programmed death 1/programmed death ligand 1 blockade. Data from recent large-scale exome analyses have revealed the heterogeneity in the molecular profiles of iCCA, showing that small duct type iCCA exhibit frequent *BAP1*, *IDH1/2* hotspot mutations and *FGFR2* fusion, in contrast to frequent mutations in *KRAS*, *TP53*, and *SMAD4* observed in large duct type iCCA. Multi-omics analyses have proposed several molecular classifications of iCCA, including inflammation class and proliferation class. The inflammation class is enriched in inflammatory signaling pathways and expression of cytokines, while the proliferation class has activated oncogenic growth signaling pathways. Diverse pathologic features of iCCA and its associated multi-omics characteristics are currently under active

investigation, thereby providing insights into precision therapeutics for patients with iCCA. This review provides the latest knowledge on the histopathologic classification of iCCA and its associated molecular features, ranging from tumor microenvironment to genomic and transcriptomic research.

Keywords: intrahepatic cholangiocarcinoma, pathology, small duct, large duct, tumor microenvironment, genomics, transcriptomics

INTRODUCTION

Cholangiocarcinomas (CCAs) include intrahepatic CCA (iCCA), perihilar CCA, and distal CCA (1). Anatomically, iCCA, often called as “peripheral CCA,” is defined as a tumor located in the periphery of the second-order bile ducts, ranging from segmental bile ducts to smaller branches of the intrahepatic biliary tree. Perihilar CCA, also known as Klatskin tumor, is defined as a tumor that arises at the junction where the right and left hepatic ducts meet, with the insertion site of the cystic duct as its distal limit. CCAs involving more of the distal area, such as the common bile duct, is defined as distal CCA. This review mainly focuses on iCCA.

Recent evaluation indicates that iCCA comprises approximately 10–15% of primary liver malignancies (2, 3), and its incidence worldwide has increased over the past decades (4). However, changes in the nomenclature, classification, and the disease coding system of CCA have hampered the accurate estimation of the incidence of iCCA (5). Countries with the highest incidence include South Korea (2.8 per 100,000 people/year), where *Clonorchis sinensis* infection was prevalent in the past, and Thailand (2.2 per 100,000 people/year), which still is an endemic area for infections due to *Opisthorchis viverrini* (4, 6, 7). In other countries where parasites are not endemic, the incidence of iCCA is low, usually below or around 1 per 100,000 people/year. Moreover, there are proposed risk factors such as choledochal cyst, primary sclerosing cholangitis, chronic B or C viral hepatitis, and non-alcoholic fatty liver disease caused by obesity or metabolic syndromes. However, a significant proportion of patients with iCCA have no known risk factors (4, 8). Since these patients rarely present symptoms in the early stage, they are often diagnosed with advanced disease with a dismal prognosis and 5-year overall survival rate of approximately 10% even in developed countries (9–11).

Intrahepatic cholangiocarcinoma is an epithelial neoplasm with biliary differentiation, and usually presents with abundant fibrous tumor stroma containing cancer-associated fibroblasts (CAFs), innate and adaptive immune cells, etc. Recently, iCCAs have been classified into two subtypes namely, small duct type and large duct type (3, 12). Furthermore, the molecular characteristics of iCCA are under active investigation owing

to technological advances in nucleotide sequencing and the availability of massive data sources on cancer, such as The Cancer Genome Atlas (TCGA) and cBio Cancer Genomics Portal (13, 14). The combination of histopathological and multi-omics data have provided novel insights into understanding the molecular pathology and thereby, developing therapeutic options for iCCA.

This review aims to provide the latest knowledge on the histopathologic classification of iCCA based on the fifth edition of the World Health Organization (WHO) classification of digestive system tumors. In addition, we discuss the associated molecular features based on tumor microenvironment, genomic and transcriptomic research results presented so far.

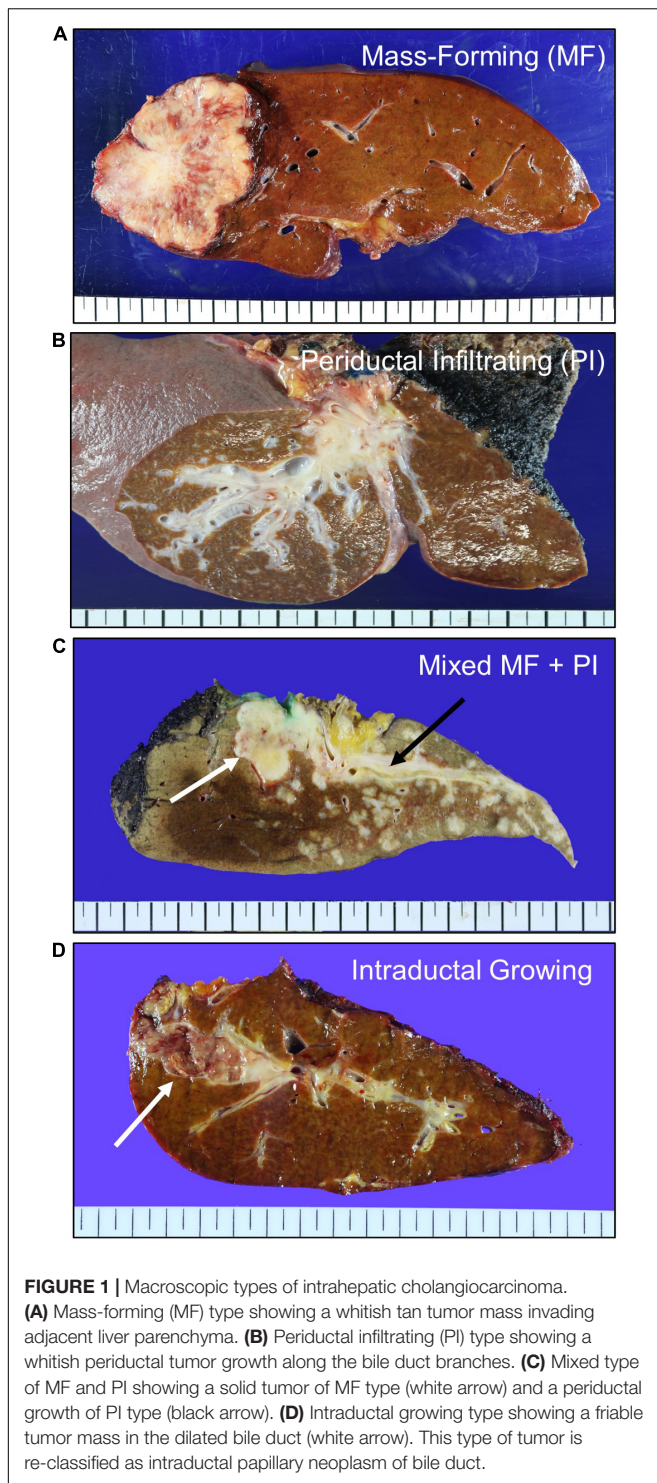
PATHOLOGICAL FEATURES OF INTRAHEPATIC CHOLANGIOCARCINOMA

Intrahepatic cholangiocarcinoma is an adenocarcinoma arising in the intrahepatic biliary tree. Fibrous tumor stroma is one of the characteristic features of iCCA, and the fibrous stroma is various in amount and distribution. The tumor center is usually more fibrotic than the tumor periphery, showing proliferating tumor cells invading into the surrounding liver. Lymphovascular and perineural invasion are often detected even at an early stage.

Gross Features of Intrahepatic Cholangiocarcinoma

Macroscopically, iCCAs can be classified into three types: mass-forming, periductal infiltrating, and intraductal growing. Based on gross appearance, the mass-forming type is most common, and often exhibits mixed features (**Figure 1**) (15). The mass-forming type shows a definite round tumor mass with invasive border. The cut surface is usually white, pale tan or yellowish in color with firm consistency due to fibrous tumor stroma. The periductal infiltrating type shows a growth pattern that extends along the bile duct, exhibiting a whitish fibrotic and thickened bile duct wall. The intraductal growing type grows into the lumen of the bile duct, forming single or multiple soft papillary masses attached to the bile duct wall. The tumor mass and resultant obstruction often dilate the bile duct and make the tumor symptomatic. Since most of the intraductal growing type iCCA cases are now being considered as malignant transformations of intraductal papillary neoplasm of the bile duct (IPNB), in the latest fifth edition of WHO classification, gross morphologic types of iCCA include mass forming, periductal infiltrating and mixed type of these two (3, 16).

Abbreviations: CCA, cholangiocarcinoma; iCCA, intrahepatic cholangiocarcinoma; CAFs, cancer-associated fibroblasts; TCGA, The Cancer Genome Atlas; WHO, World Health Organization; MUC, mucin; CRP, c-reactive protein; EBV, Epstein-Barr virus-encoded small RNA; CKs, cytokeratins; FGB, fibrinogen; B, B; CLC, cholangiocarcinoma; HCC, hepatocellular carcinoma; DPM, ductal plate malformation; IPNB, intraductal papillary neoplasm of the bile duct; BilIN, biliary intraepithelial neoplasia; IPMN, intraductal papillary mucinous neoplasm; TAMs, tumor-associated macrophages.



Classification of Small Duct Type and Large Duct Type

Conventional iCCA can be further classified into two histopathological types according to the level or size of the affected duct. Recently, small duct type and large duct type iCCA have been introduced in the WHO classification (3).

Small duct type iCCA, which has been reported as a peripheral, ductular, and cholangiolar type, accounts for 36–84% of iCCA (12, 17–19). Small duct type iCCA shows small-sized tubular growth of cuboidal or low-columnar tumor epithelial cells. There is little or no mucin production, and occasional areas of growth exhibit a pattern resembling ductular reaction with slit-like glandular lumen (**Figures 2A,B**). Background liver of small duct type iCCA often shows chronic liver disease related to B viral hepatitis, C viral hepatitis, alcoholic hepatitis, and non-alcoholic steatohepatitis (12, 17, 20).

Large duct type iCCA, which has been reported as bile duct type or perihilar type, arises in large intrahepatic bile ducts and comprises 8–60% of iCCA cases (12, 17–19). Large duct type iCCA is composed of mucin-producing columnar cells forming irregular shaped-and-sized tubules or gland-like structures. This type usually shows a highly invasive growth pattern accompanied by a desmoplastic reaction (**Figures 2D,E**) (3). Pathological examination of the background liver of large duct type iCCA often reveals chronic bile duct injury due to hepatolithiasis, parasitic infection in bile ducts, or primary sclerosing cholangitis (8, 20).

Furthermore, histopathological features of small duct type and large duct type iCCA are related to their gross appearance. The periductal infiltrative type of iCCA is exclusively large duct type, whereas the mass-forming type is more heterogeneous, including small duct and large duct types (17, 21). Mass-forming type iCCA with small duct type histology showed better prognosis than other types (17).

Putative Cells of Origin

Small duct type iCCA occurs in smaller intrahepatic bile ducts compared to large duct type. Canals of Hering, which are histological structures that link hepatic canaliculi and the biliary tree, cuboidal cholangiocytes of bile ductules, and interlobular bile ducts are considered as the putative cells of origin (22, 23). In contrast, large duct type iCCA might be derived from columnar biliary epithelium producing mucin or peribiliary glands around them (8, 20). However, the cellular origin of iCCA is still controversial, since various lineage tracing animal studies showed mixed results indicating hepatic stem or progenitor cells, cholangiocytes or hepatocytes as the cells of origin of iCCA (24, 25). Further research is required to conclusively define the origin of small duct and large duct type iCCAs.

Immunohistochemical Markers for Small Duct Type and Large Duct Type Intrahepatic Cholangiocarcinoma

Examination of a panel of immunohistochemical (IHC) markers is useful to differentiate small duct type and large duct type. In large duct type iCCAs, high expression of mucin (MUC) core protein 5AC, MUC6, and S100 calcium-binding protein P (S100P) has been reported (26, 27), whereas neural cell adhesion molecule (NCAM, also known as CD56) and N-cadherin have been found to be highly expressed in the small duct type. NCAM and N-cadherin are normally expressed in cholangioles (28, 29). Intra- and/or extracellular mucin, detected by mucicarmine or Alcian blue staining, is abundant in the large duct type

in contrast to its scarcity or absence in the small duct type. Examining a panel of these markers, including S100P, N-cadherin, NCAM, and Alcian blue, has been reported to be more effective in differentiating small duct and large duct types (12). Additionally, c-reactive protein (CRP) was recently found to be an effective marker for the diagnosis of small duct type iCCA (30) (**Figures 2C,F**). Biliary cytokeratins (CKs) such as CK7 and CK19 are useful for confirming biliary differentiation or biliary origin. However, their ability to differentiate between small duct type and large duct type iCCA is limited (31, 32).

Histopathological features of hematoxylin and eosin-stained slides usually provide insight to distinguish small duct and large duct type iCCA. In addition, application of immunohistochemical (IHC) markers (S100P, NCAM, and N-cadherin) and special stain for mucin is useful to support the diagnosis of iCCA subtypes, especially when the tissue is limited in biopsies.

Comparison of Prognosis and Treatment Response Between Small Duct Type and Large Duct Type Intrahepatic Cholangiocarcinoma

The prognosis of small duct type iCCA is generally favorable compared to that of large duct type iCCA (12, 17, 33, 34). Accordingly, inflammation-related markers [CRP and fibrinopeptide B (FGB)] and proliferation-related markers [extracellular signal-regulated kinases (ERK) 1/2 and Ki-67] are highly expressed in small duct type iCCA and large duct type iCCA, respectively (17). It has also been reported that the response to conventional chemotherapy is better with small duct type than with large duct type iCCA (35).

Differential Diagnosis of Intrahepatic Cholangiocarcinoma in a Biopsied Tissue

For liver primary tumors, diagnosis of iCCA requires differentiation from combined hepatocellular-cholangiocarcinoma (cHCC-CC), since both components of cHCC-CC may not clearly present due to the limitations of the biopsied tissue. Application of IHC markers for hepatocellular carcinoma (HCC) is helpful to identify portions of hepatocytic differentiation. Hepatocyte paraffin-1 (Hep Par 1) and arginase-1 (ARG1) are highly sensitive and specific (both exceeding 80%) markers, and addition of glypican-3 (GPC) is shown to be useful for the diagnosis of poorly differentiated areas of hepatocytic differentiation with sensitivity over 80% (36).

Since an iCCA is histopathologically an adenocarcinoma, it is necessary to differentiate it from metastatic adenocarcinoma from other organs. Application of the following IHC markers is helpful. Caudal-type homeobox 2 (CDX2) is a widely used marker for the diagnosis of metastatic colorectal adenocarcinoma with sensitivity over 90%. Since its specificity is relatively low (70%) (37), combination with CK7 and CK20, which are usually negative and positive in colorectal adenocarcinoma, respectively, is recommended (32). Adenocarcinoma of the lung and ductal carcinoma of the breast, which can be differentiated by IHC staining with antibodies of thyroid transcription factor-1 (TTF-1; 75% sensitivity and specificity) (38, 39) and GATA binding protein 3 (GATA-3; over 90% sensitivity and specificity),

respectively (38, 40, 41). Paired box 8 (PAX8) is a sensitive marker for ovarian and endometrial carcinomas, as well as for renal cell carcinomas with sensitivity approaching 90% (40). Metastatic prostate adenocarcinoma is usually positive for the antibodies against prostate specific antigen (PSA) and prostate specific acid phosphatase (PSAP), with sensitivity and specificity exceeding 95% (40, 42). However, in cases of metastatic adenocarcinomas originating from organs adjacent to the liver including gallbladder, pancreas, and stomach, etc., it is difficult to differentiate iCCA from these tumors, due to the lack of specific IHC marker. Some potentially promising markers have been introduced, and filamin A was reported to show high positivity (63%) by immunohistochemistry on iCCA (43). Recently, an *in situ* hybridization assay for albumin RNA was reported to show 90% sensitivity and 100% specificity for iCCA, particularly for the differentiation of small duct type iCCA and metastatic tumors (44–46).

Variants of Intrahepatic Cholangiocarcinoma

Cholangiocarcinoma (CLC) is a variant of iCCA that belongs to the small duct type. It is defined as an iCCA with more than 80% of the tumor area showing cholangiolocellular differentiation without hepatocellular differentiation. The prefix “cholangiolo” implies histopathological similarity to the cholangiole or canals of Hering (47). CLCs show small cuboidal cells forming cords or tubular structures with antler-like growth resembling the ductular reaction of non-tumorous liver (48). Often, the lumina of tumor cords are inconspicuous, the atypia or pleomorphism of tumor epithelial cells is minimal, and regularly spaced intervening stroma is also a characteristic feature (**Figure 3A**).

CLC is thought to arise at the bile ductule, containing hepatic stem or progenitor cells, and canals of Hering. It was previously classified as a subtype of combined hepatocellular-cholangiocarcinoma (49), however, molecular profiling studies favor the classification of CLC as part of iCCA (50). According to the current WHO classification, CLC without components of HCC or intermediate carcinoma is an iCCA and is not considered as combined hepatocellular-cholangiocarcinoma.

CLC is distinguished from conventional small duct type iCCAs based on its excellent outcome, which shows significantly higher overall and disease-free survival (49, 51). Even iCCAs with cholangiolocellular differentiation (>10% of the tumor area) were found to have a better prognosis than those without (17, 52). A transcriptomic profiling study reported that iCCA with cholangiolocellular differentiation correlated with inflammation class, while iCCA without cholangiolocellular differentiation correlated with proliferation class (the molecular classification is discussed in more detail later in the genomic-transcriptomic profiles section) (52, 53).

Intrahepatic Cholangiocarcinoma With Ductal Plate Malformation Pattern

Ductal plate malformation (DPM) refers to a developmental anomaly characterized by pathologically existing embryonic bile duct structures (“ductal plates”). The percentage of iCCAs

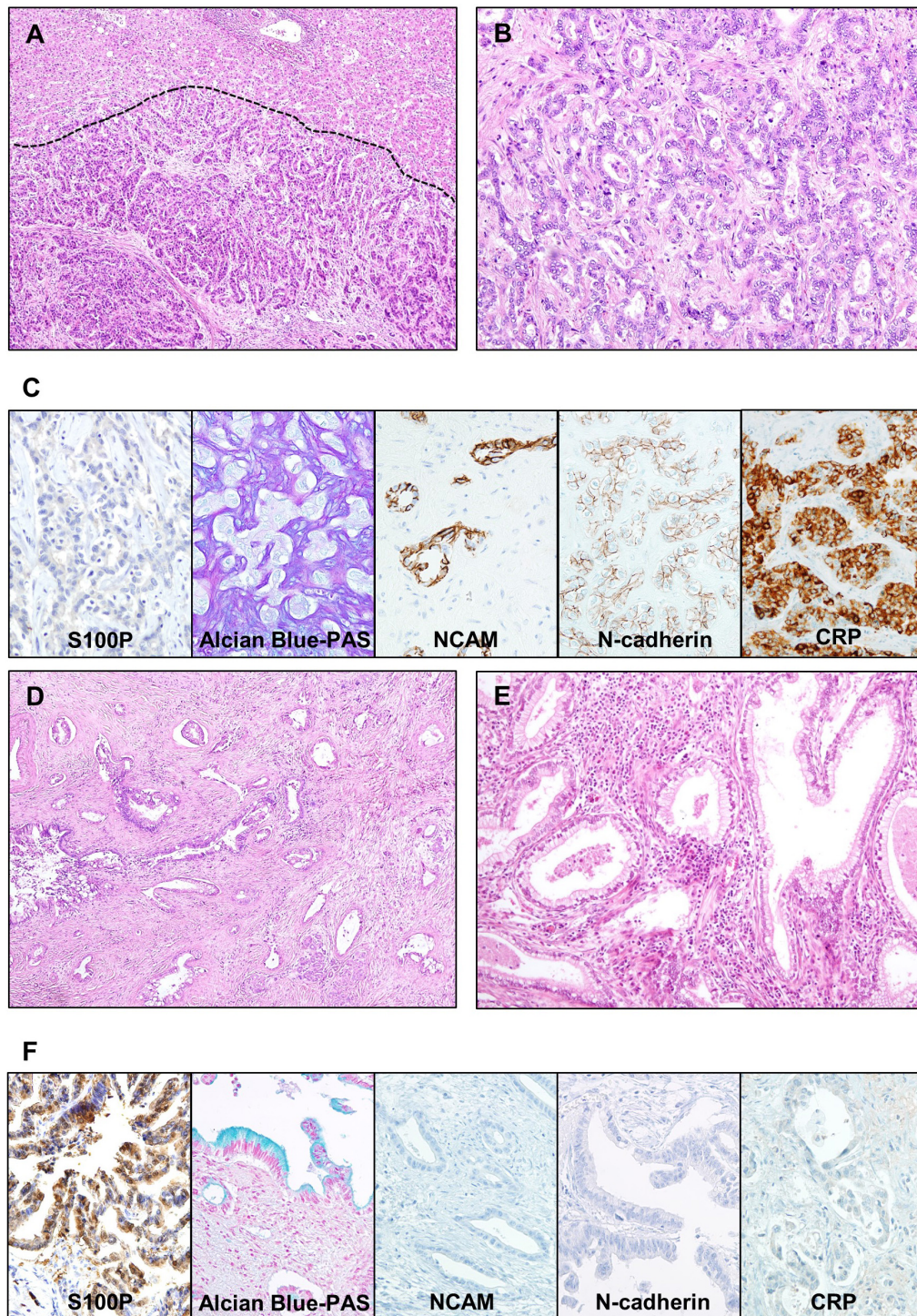


FIGURE 2 | Representative microscopic images of small duct type and large duct type intrahepatic cholangiocarcinoma (iCCA). **(A–C)** Small duct type iCCA. **(A)** A low-power view showing uniform-shaped tumor glands replacing hepatocellular trabeculae at the border (indicated by dashed line). **(B)** A higher magnification image showing the growth of cuboidal cells forming cords and small glandular structures, without intra- or extracellular mucin. **(C)** Microscopic images of special and immunohistochemical panel staining for small duct type iCCA; positive expression of NCAM, N-cadherin and CRP, negative expression of S100P, and absence of mucin in the Alcian blue staining is characteristic. **(D–F)** Large duct type iCCA. **(D)** A low magnification image shows infiltrative growth of adenocarcinoma with rich fibrous stroma. **(E)** A higher magnification image showing columnar cells with intracellular mucin forming irregular glandular spaces. **(F)** Microscopic images of special and immunohistochemical panel staining for large duct type iCCA; positive expression of S100P, presence of mucin in the Alcian blue staining, and negative expression of NCAM, N-cadherin, and CRP is characteristic. Original magnification: 40× for **(A,D)**, 100× for **(B,E)**, 200× for **(C,F)**. S100P, S100 calcium-binding protein P; NCAM, neural cell adhesion molecule; CRP, c-reactive protein; PAS, periodic acid–Schiff.

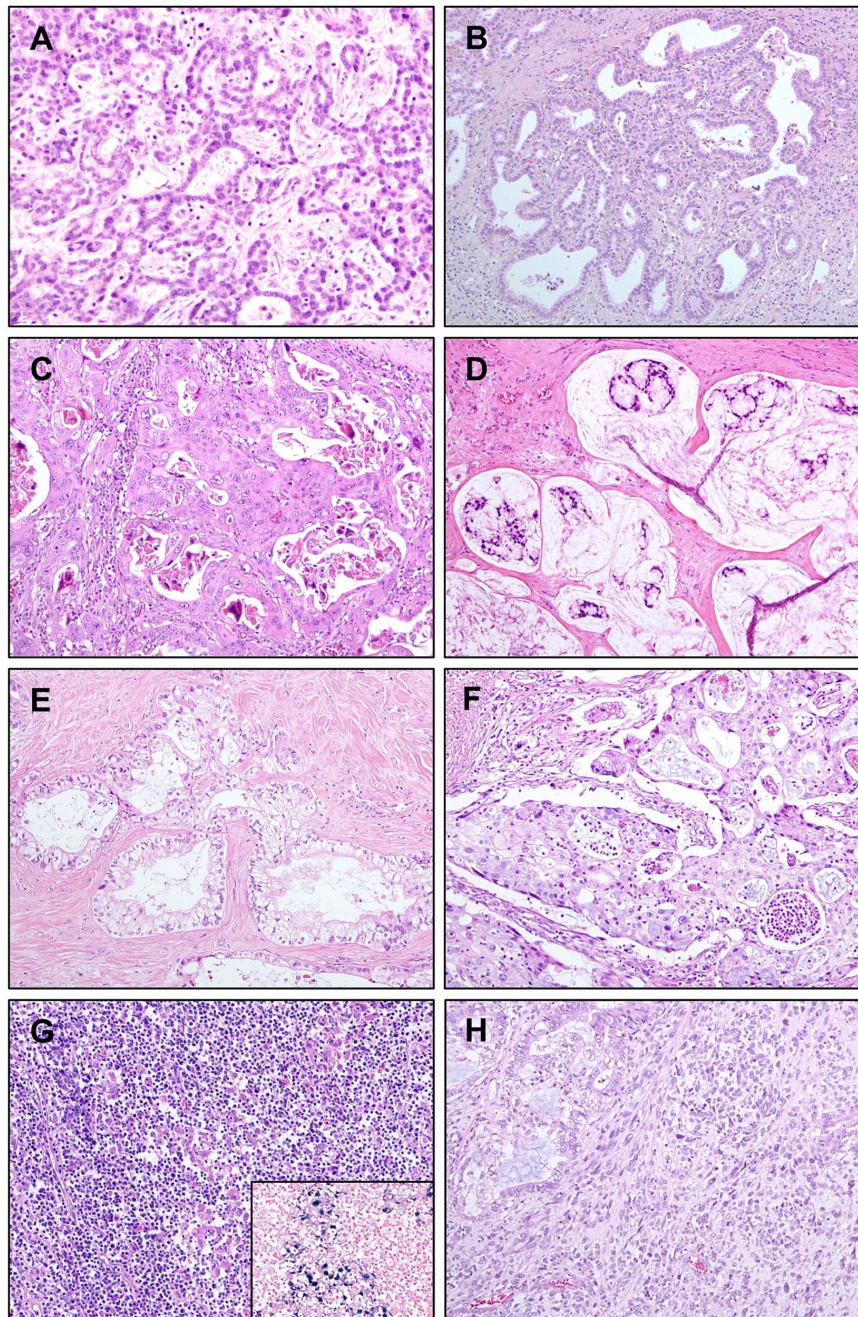


FIGURE 3 | Variants of intrahepatic cholangiocarcinoma (iCCA). **(A)** Cholangiocarcinoma. Bland-looking small cuboidal tumor epithelial cells are forming cords or ductules, with antler-like branching pattern. **(B)** iCCA with ductal plate malformation pattern. Cuboidal tumor cells are forming irregularly dilated and coalesced spaces, resembling developmental anomaly of ductal plate. **(C)** Adenosquamous carcinoma showing both of gland-forming portion and portions with squamous differentiation. **(D)** Mucinous carcinoma. Mucin-producing tumor cell clusters are floating in mucin pools. **(E)** Clear cell carcinoma. Tumor cells have large clear cytoplasm with eccentric nuclei. **(F)** Mucoepidermoid carcinoma showing squamoid tumor cells intermixed with mucin-producing cells. **(G)** Lymphoepithelioma-like carcinoma showing marked lymphocytic infiltration into tumor epithelial component. Tumor epithelial cells are positive for Epstein–Barr virus (EBV), detected by *in situ* hybridization of EBV-encoded small RNA (inset). **(H)** Sarcomatous iCCA showing mainly pleomorphic spindle cells, with adenocarcinoma components in the upper left corner. Original magnification: 100 \times .

that are diagnosed as iCCAs with DPM pattern is very small, approximately 2.9% of cases in a cohort of 175 resected iCCAs (54). Histopathologically, the tumor epithelial cell lining is

usually benign-looking cuboidal cells without mucin production, and they form glandular structures that are elongated, tortuous, and coalesced, mimicking ductal plates (**Figure 3B**) (55). Genetic

alterations in iCCA with DPM pattern include point mutations in *FGFR2*, *PTPRT*, *ARID1A*, and *CDKN2A*, and fusion of *FGFR2* (54, 56). Patient survival seems better than that of conventional small duct type iCCAs (54).

Adenosquamous Carcinoma/Squamous Carcinoma

Adenosquamous carcinoma of the liver has both squamous epithelial and glandular components (**Figure 3C**), and its incidence is rare (57). Squamous carcinoma, showing squamous differentiation in the entire tumor is extremely rare. This type of variant iCCA is reported to be correlated with chronic cholangitis caused by liver flukes or hepatolithiasis (58). The prognosis of adenosquamous carcinoma of the liver is usually poor, with a median survival of approximately 6 months (57).

Mucinous Carcinoma/Signet Ring Cell Carcinoma

Mucinous carcinoma is a variant that belongs to large duct type iCCA. It contains an overwhelming amount of extracellular mucin in the luminal space of tumor glands, usually over 50% of the total tumor volume by convention (59), often causing tumor epithelial cells to float in the mucin pool (**Figure 3D**). This type of tumor usually occurs due to the malignant transformation of the IPNB. Signet ring cell carcinoma occasionally presents as a mucinous carcinoma with varying distribution; however, pure signet ring cell carcinoma of the liver is extremely rare. The absence of ovarian-like stroma differentiates this variant of iCCA from mucinous cystic neoplasm (60).

Clear Cell Carcinoma

Clear cell carcinoma is characterized by bulky cytoplasmic clearing and eccentrically located nuclei in most tumor epithelial cells with glandular and trabecular growth patterns (**Figure 3E**) (58, 61). Primary clear cell carcinoma of the liver can be differentiated from HCC with clear cell change, metastatic clear cell carcinoma of the kidney, and metastasis from other gastrointestinal tract tumors by IHC staining for hepatocyte paraffin 1 (HepPar-1), CD10, and CK20, respectively (62).

Mucoepidermoid Carcinoma

Primary mucoepidermoid carcinoma of the liver shows features similar to those in other organs, including the salivary glands. It reveals a more intimate mixture of epidermoid or squamous and mucin-secreting elements, compared to adenosquamous carcinoma where mucin-secreting cells and foci of squamous differentiation exist separately (**Figure 3F**) (63). There have been only a few reports, and most of them have shown a poor prognosis (64).

Lymphoepithelioma-Like Carcinoma

Lymphoepithelioma-like carcinoma is characterized by dense lymphoid stroma around the tumor epithelial cells, often forming lymphoid follicles. Tumor epithelial cells show an undifferentiated or gland-forming pattern, rarely with well-differentiated or bland-looking glands (**Figure 3G**). Almost all cases are Epstein–Barr virus-encoded small RNA (EBER) positive and usually have favorable outcomes (65, 66).

Sarcomatous Intrahepatic Cholangiocarcinoma

Sarcomatous iCCA usually shows mixed features of conventional iCCA and undifferentiated components of cells with spindle or rhabdoid features (**Figure 3H**). When a conventional iCCA component is not present, a definite diagnosis is difficult, since the sarcomatoid component is often negative for epithelial markers by IHC staining (67). Sarcomatous iCCA usually has a worse prognosis than conventional iCCAs (68).

Precursor Lesions

Biliary Intraepithelial Neoplasia

Biliary intraepithelial neoplasia (BilIN), a precursor lesion of CCA, occurs at the epithelium of intra- and extrahepatic bile ducts and in the peribiliary glands. Large duct type iCCA, but not small duct type, is often accompanied by BilIN (17, 21). BilIN is virtually invisible upon gross examination, although it may be associated with subtle changes such as mucosal thickening. Microscopically, BilIN consists of flat or micropapillary (less than 3 mm in height) epithelial lesions that are graded as low-grade or high-grade (carcinoma *in situ*) based on the highest degree of cytoarchitectural atypia (69, 70). This two-tiered classification replaces the former three-tiered classification, wherein the former BilIN-1 and BilIN-2 are now classified as low-grade, and the former BilIN-3 is now classified as high-grade.

Low-grade BilIN shows mild cytoarchitectural atypia, including flat pseudopapillary and/or micropapillary growth pattern, nuclear stratification, hyperchromatic nuclei, and increased nuclear-cytoplasmic ratio; however, nuclear polarity is preserved. High-grade BilIN is characterized by moderate to severe cytoarchitectural atypia, including more complex patterns, complete loss of polarity, marked nuclear atypia, and frequent mitosis. While IHC staining for p53 is usually negative in low-grade BilIN, it is often overexpressed in high-grade BilIN (71). The expression of p16 is relatively preserved in low-grade BilIN and decreased in high-grade BilIN (72). A notable mutation in BilIN lesions is alterations in *KRAS*, which is reported to be approximately 30% (73) (**Figures 4A,B**).

Differentiating BilIN from reactive epithelial atypia may be difficult, especially in biopsy samples. Reactive atypia shows overlapping attenuated basophilic cells with nuclei having fine and diffuse chromatin. The nucleoli are small or conspicuous. Mitotic activity may be prominent. Reactive epithelial atypia usually shows a gradual transition from uninvolved epithelium, in contrast to the abrupt change usually seen in BilIN. The IHC detection of S100P was shown to be useful, being mostly negative in reactive epithelial atypia. However, its expression increased sequentially from low-grade BilIN to high-grade BilIN and subsequently in iCCA (74).

Intraductal Papillary Neoplasm of the Bile Duct

Intraductal papillary neoplasm of the bile duct (IPNB) is defined as a grossly visible premalignant neoplasm showing intraductal papillary or villous growth of biliary-type epithelium (70). It is considered to be a counterpart of a similar tumor arising in the pancreas, the so-called intraductal papillary mucinous neoplasm (IPMN). IPNB is divided into low-grade and high-grade based on the highest degree of cytoarchitectural atypia.

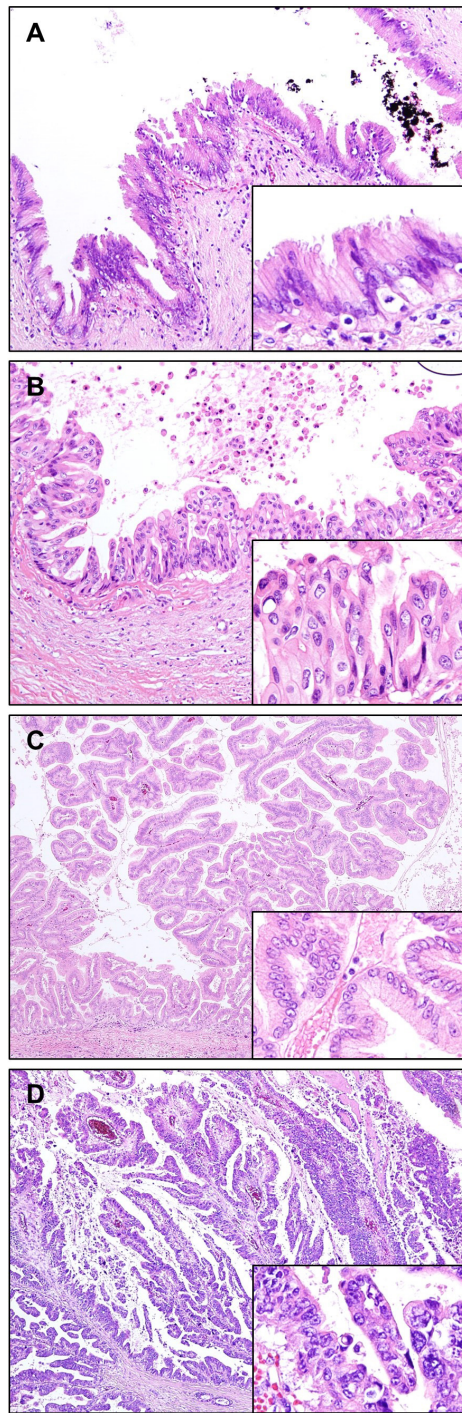


FIGURE 4 | Precursor lesions of intrahepatic cholangiocarcinoma. **(A,B)** Biliary intraepithelial neoplasia (BillIN). **(A)** Low-grade BillIN composed of columnar cells with intact nuclear polarity and minimal atypia. **(B)** High-grade BillIN showing stratification of cells with marked nuclear atypia and loss of polarity. **(C,D)** Intraductal papillary neoplasm of the bile ducts (IPNB). **(C)** Low-grade IPNB showing a papillary growth of columnar biliary type epithelial cells with mild pleomorphism and preserved nuclear polarity. **(D)** High-grade IPNB showing irregular papillary projections, composed of highly pleomorphic and stratified cells with increased nuclear-cytoplasmic ratio. Original magnification: 100× for **(A,B)**, 40× for **(C,D)**, 200× for inset images.

When invasive carcinoma develops in this lesion, it is diagnosed as IPNB with associated invasive carcinoma. High-grade IPNBs are often associated with stromal invasive carcinoma, usually consisting of tubular adenocarcinoma and occasionally mucinous carcinoma (75).

Grossly, IPNBs appear as polypoid masses with dilatation of the bile ducts. These are usually isolated papillary lesions, whereas some IPNBs appear as multiple contiguous papillary or polypoid lesions. Some IPNBs are characterized by mucus hypersecretion, forming mucin-containing fusiform dilatation or cysts, similar to those observed in IPMNs (76). Microscopically, IPNBs form papillary structures with fine fibrovascular cores (**Figures 4C,D**). Four histological subtypes are generally accepted based on cytological appearance and immunophenotype, namely, – pancreatobiliary, intestinal, gastric, and oncocytic. Immunohistochemically, MUC1 is mostly expressed in the pancreatobiliary type. Gastric type usually express MUC5AC and MUC6, and the intestinal type frequently express MUC2. CK20 is positive in the intestinal type, but not in the gastric and oncocytic types (70, 77). The presence of two or more histopathological types is common in IPNB, therefore these tumors are diagnosed based on the most prevalent histopathological type. The pancreatobiliary type is most common, with higher prevalence in western countries than in Asia. In contrast, intestinal type is more common in Asian populations than in western populations, while oncocytic and gastric types are least frequent (78). Although the clinical implications of histopathologic subtypes are still controversial, the pancreatobiliary type is reported to be linked with a higher frequency of associated invasive carcinoma, frequent lymph node metastasis, and recurrence (77).

A recent consensus has proposed a different classification for IPNBs of type 1 and type 2 (79, 80). Type 1 IPNB shows more homogeneous appearance than type 2 IPNB, and is composed of regular villous, papillary, or tubular structures usually with low-grade dysplasia, but may present with high-grade dysplasia with foci of low-grade dysplasia. Mucin overproduction is frequently observed, whereas stromal invasion is uncommon. This is most commonly found in intrahepatic bile ducts. Histological similarity with IPMN of the pancreas is also characteristic. Type 2 IPNB exhibits heterogenous appearance composed of irregular and complicated villous, papillary or tubular structures. This is usually composed of high-grade dysplasia, and foci of low-grade dysplasia are absent or minimal. Invasive carcinoma is more frequently associated with type 2 IPNB than type 1 IPNB. Mucin overproduction is not common. Type 2 IPNB arises throughout the biliary tree, including intrahepatic and extrahepatic bile ducts.

The mutational profile studies on IPNB have reported that diverse cancer driver mutations are frequently observed, including *KRAS*, *TP53*, *STK11*, *CTNNA1*, *APC*, *SMAD4*, and *GNAS*. Type 1 IPNBs show higher mutation rates of *KRAS*, *GNAS*, and *RNF43*, whereas type 2 IPNBs have higher *TP53* and *SMAD4* mutation rates (81, 82).

Although IPNBs present papillary morphology, sometimes tubular growth pattern of epithelial components with less mucin production is predominantly observed, similar to intraductal tubulopapillary neoplasm (ITPN) of the pancreas (83). Such cases have been described as intraductal tubular neoplasms

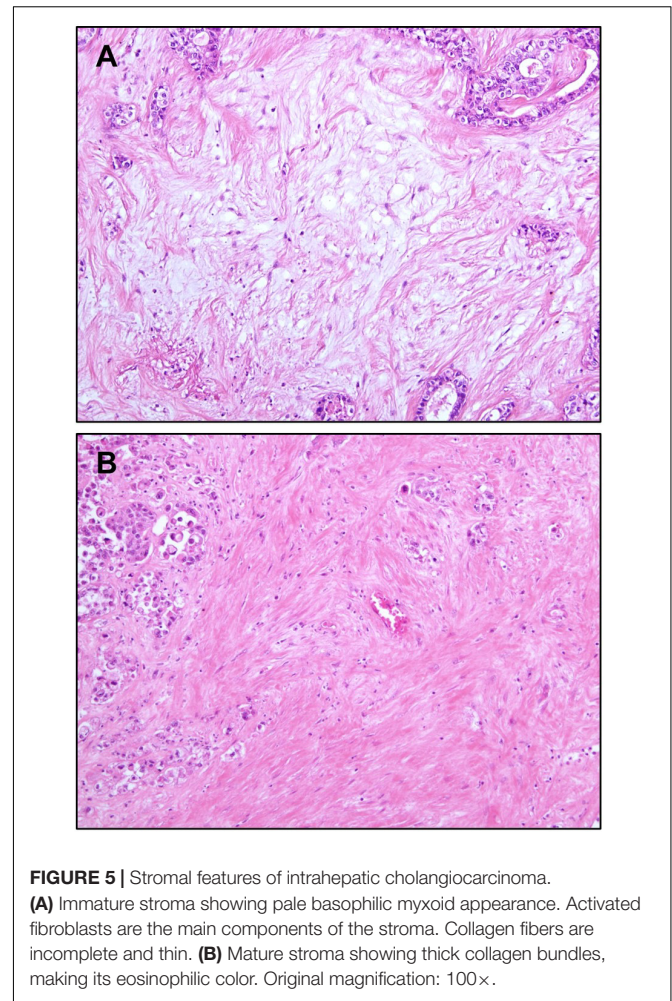
or ITPNs. Recently, ITPN has been reported to show more frequent intrahepatic occurrence in contrast to IPNB, which favors both intra- and extrahepatic locations. Furthermore, IPNB and ITPN differ in their genomic and epigenomic profiles. Recently, IPNB has been reported to share mutational profiles with extrahepatic CCA, including mutations in *TP53*, *SMAD4*, and *KRAS* and deletions on chromosomes 9q, 17p, and 18q. However, ITPN shows low overall mutational burden, and distinct DNA methylation pattern that clustered together with iCCA rather than extrahepatic CCA, suggesting that IPNB and ITPN are distinct entities (84).

TUMOR MICROENVIRONMENT OF INTRAHEPATIC CHOLANGIOCARCINOMA

One of the characteristics of iCCA is the abundance of fibrous stroma (85). The amount of fibrous stroma has been reported to be associated with poor prognosis (86). Furthermore, so-called “scirrhous type” iCCA, which is defined as iCCA having scirrhous area (where the amount of fibrous stromal component is at least equal to the area of epithelial component) more than 70% of the largest cut surface, has been reported to show worse prognosis than conventional iCCAs (87). More recently, the characteristics of immature and mature fibrous tumor stroma have been reported to be related to tumor behavior (88, 89). Immature stroma is composed of myxoid stroma with randomly oriented short keloid-like collagen bundles. In contrast, mature stroma shows multilayered mature collagen fibers (Figure 5). Accordingly, iCCAs with immature stroma have been reported to show poorer prognosis compared to iCCAs with mature stroma (88, 89).

Activated CAFs, one of the major components of the tumor microenvironment, have been demonstrated to facilitate tumor growth and progression, and promote immunosuppression in tumors (90). CAFs are thought to be recruited from hepatic stellate cells, portal fibroblasts, or circulating mesenchymal cells, but the exact source is currently unknown (91). Transforming growth factor β (TGF- β) and platelet-derived growth factor D (PDGF-D) secreted by tumor epithelial cells recruit CAFs. Recruited CAFs not only promote desmoplastic reaction by collagen and matrix metalloproteinases, but also cause tumor epithelial cells to proliferate, invade, and resist antitumor mechanisms by secreting growth factors such as PDGF-B and epidermal growth factors (92, 93). Patients with iCCA with a high proportion of activated CAFs were reported to have a shorter survival rate than patients with low CAF proportion (94).

Immune cells, including tumor-associated macrophages (TAMs) and tumor-infiltrating lymphocytes, are also main components of the tumor microenvironment. The hepatic macrophage population consists of activated macrophages derived from Kupffer cells or bone marrow-derived macrophages (95), and activated macrophages can be classified as M1 (classical) and M2 (alternative)-polarized types (96). The M2 phenotype forms the majority of TAM population in iCCA, having anti-inflammatory and pro-tumor functions mediated by



the secretion of anti-inflammatory cytokines, including IL-4, IL-10, IL-13, and TGF- β (97). These cells also promote intratumoral angiogenesis, which is vital for tumor survival and metastasis (85, 98). A high proportion of M2 TAMs in iCCA is correlated with increased invasiveness of tumor cells and poor disease-free survival (97, 99). In contrast, M1 TAMs have been reported to exert pro-inflammatory functions, including secretion of pro-inflammatory cytokines such as TNF- α , interleukin (IL)-6, and IL-1 β (99).

Tumor-infiltrating lymphocytes include B cells (CD20⁺), helper T cells (CD4⁺), cytotoxic T cells (CD8⁺), and regulatory T cells (Tregs, FOXP3⁺). The major proportion of tumor-infiltrating lymphocytes comprises T cells rather than B cells (100). The distribution and proportion of CD4⁺ and CD8⁺ T cells varies among iCCAs, and increased population of these cells is correlated with better prognosis (8, 85). In addition, iCCAs with B cell infiltration have been reported to be associated with better survival than iCCAs without B cells (101). Treg cells are a subset of CD4⁺ T cells that suppress innate and adaptive immune responses mainly by secreting IL-10 and TGF- β , which are known to promote tumor progression by inhibiting antitumor immune response. Regarding iCCA, while there are a few studies on

the clinical aspects of the presence of Treg cells, there is no sufficient evidence to draw a conclusion. Therefore, additional investigation is required (101–103).

With the advent of immune checkpoint blockade therapeutics, the expression status of cell surface proteins with immune escape mechanisms is currently under active investigation. Cytotoxic T-lymphocyte antigen-4 (CTLA-4), expressed on the surface of Treg cells, suppresses cytotoxic T cell activity by binding to CD80 of antigen-presenting cells (85). High CTLA-4 expression has been reported to be related to worse relapse-free survival of patients with CCA, raising the possibility of effective immunotherapy targeting CTLA-4 (104). Programmed death 1 (PD-1), expressed on T cells, and its ligand programmed death ligand 1 (PD-L1), on tumor epithelial cells, are other major immune checkpoints of interest. Binding of PD-L1 to PD-1 diminishes the immunological function of cytotoxic T cells. Approximately 9–30% of iCCA has been reported to be PD-L1 positive as observed by IHC staining (105–107). Recently, our group reported that *KRAS* alteration and chromosomal instability were associated with resistance to PD-1/PD-L1 blockade immunotherapy, whereas high intratumoral tumor-infiltrating lymphocyte density was associated with a favorable immunotherapy response in patients with CCA (35). Many clinical trials for iCCA using immune checkpoint inhibitors are ongoing based on the expression status of markers, including PD-1, PD-L1, and CTLA-4, with expectations of promising results in the near future (108).

MULTI-OMICS FEATURES OF INTRAHEPATIC CHOLANGIOCARCINOMA

Germline and Somatic Mutational Profile of Intrahepatic Cholangiocarcinoma

Germline Predisposition

There are a few germline predispositions for cancers, including proto-oncogenes and tumor suppressor genes, either by inheritance from parents or *de novo* mutation at the zygote level. Approximately 8–12% of iCCAs have been reported to have known pathogenic or possibly deleterious germline mutations, and the most commonly found germline-mutated genes are *BRCA1* and *BRCA2*, which are associated with DNA repair mechanism and hereditary cancer syndromes (109–112) (Table 1). Other germline variants linked with iCCA include *APC*, an antagonist of the Wnt signaling pathway, *BAP1*, which mediates deubiquitination, and mismatch repair mechanism-related genes, namely *MLH1* and *MSH2* (109, 111, 113). However, evidence regarding the association between known hereditary cancer syndromes and iCCA is currently not fully established and requires further investigation.

Somatic Short Mutations, Structural Variations, and Copy Number Aberrations

Among somatic mutations identified in iCCA, the most well-known and frequent variants are at exons 5–8 of *TP53* and

TABLE 1 | Summary of germline mutations reported in intrahepatic cholangiocarcinoma.

Gene	Frequency of occurrence (%)	References
<i>BRCA1</i>	1–3	(109–111)
<i>BRCA2</i>	1–3	(109–111)
<i>MLH1</i>	2	(109)
<i>MSH2</i>	2	(109)
<i>MUTYH</i>	2	(111)
<i>BAP1</i>	1	(111)
<i>PMS2</i>	1	(111)
<i>APC</i>	1	(111)

hotspots at codons 12/13 of *KRAS*, which are involved in cell cycle arrest/DNA repair and mitogen-activated protein kinase (MAPK) signaling pathway, respectively (114–116). Other MAPK pathway genes such as *NRAS* and *BRAF* are also frequently mutated in iCCA (117). Owing to the advancement and wide use of massive parallel sequencing techniques, many other driver gene mutations have been discovered in the last decade. Single-nucleotide variants of chromatin remodeling-related genes such as *ARID1A*, *BAP1*, and *PBRM1* have been reported with frequencies ranging from 6 to over 30% (115, 118–120). Moreover, mutations in *IDH1/2*, which acts as an epigenetic regulator, are most frequently observed, with an average incidence of approximately 15% (115, 121–124). Other somatic short mutations include Akt signaling pathway-associated genes such as *PTEN*, *PIK3CA*, and *PIK3C2A*, and *SMAD4*, a TGF- β signaling pathway gene (115, 118, 125, 126).

The most frequently found structural variation in iCCA is *FGFR* gene fusion, notably *FGFR2*, which has been reported in 6–14% of iCCAs (127, 128). The most common fusion partner is *BICC1*; however, several other genes were also found, including *AHCYL1* and *PPHLN1* (128–130). Driver gene amplification was found in *ERBB2* (2–12%), *MDM2* (0–13%), *EGFR* (1–16%), and *CCND1* (10–13%). Deletion of 9p21.3, or the locus including genes *CDKN2A* and *CDKN2B* is found in 10–20% of iCCA (13, 126, 127, 131).

Microsatellite instability-high cases are usually determined by three methods namely, observing the size change in more than three out of five marker loci by polymerase chain reaction, IHC for mismatch repair proteins including, *MLH1*, *MSH2*, *MSH6*, and *PMS2*, or estimation of tumor mutation burden by NGS. Such cases are known to be rare (around 1%) in iCCA (126, 132–134). The mutational characteristics of iCCA are summarized in Table 2.

Genetic alterations are also correlated with pathological features. Hotspot mutations in *KRAS* have been reported in periductal infiltrating type, but not in mass-forming type (135). Histopathologically, small duct type has been reported to have more frequent *BAP1* and *IDH1/2* hotspot mutations and *FGFR2* fusion, and lower incidence of *KRAS* mutation than large duct type (12, 26, 51, 122, 136, 137). On the contrary, large duct type is known to have frequent mutations in *TP53*, *KRAS* and some

TABLE 2 | Major somatic variants and reported incidence in intrahepatic cholangiocarcinoma.

	Groups	Gene or locus	Frequency of occurrence (range, %)	References
Small nucleotide variants	DNA repair	<i>TP53</i>	2.5–39.3	(13, 35, 115, 120, 123, 125, 126, 131)
		Chromatin remodeling	<i>ARID1A</i>	7–36
	MAPK signaling pathway	<i>BAP1</i>	6–16	(13, 35, 116, 121, 123, 126, 131)
		<i>PBRM1</i>	9–14.3	(13, 35, 116, 119, 121, 123, 126, 131)
		<i>KRAS</i>	2–30.3	(13, 35, 115, 121, 123, 125, 126, 131)
		<i>NRAS</i>	3–9.3	(13, 115, 117, 119, 125, 126, 131)
		<i>BRAF</i>	3–5	(13, 35, 117, 125, 126, 131)
	Epigenetic regulator	<i>IDH1</i>	5–36	(13, 35, 115, 126, 131)
		<i>IDH2</i>	3.7–36	(13, 115, 117, 119, 121, 126, 131)
	TGF- β signaling pathway	<i>SMAD4</i>	0–9	(13, 35, 115, 126, 131)
	Akt signaling Pathway	<i>PTEN</i>	0.6–11	(13, 115, 117, 125, 126, 131)
		<i>PIK3CA</i>	3–7	(13, 35, 115, 117, 119, 120, 125, 126, 131)
		<i>PIK3C2A</i>	0–7.1	(117)
Structural variation	Translocation	<i>FGFR2</i>	6–14	(13, 127, 128, 131)
		Amplification	<i>CCND1</i>	10–13
	<i>EGFR</i>	1–16	(126, 131)	
	<i>ERBB2</i>	2–12	(35, 126, 131)	
	<i>MDM2</i>	0–13	(131)	
Deletion	9p21.3 (CDKN2A/B)	10–20	(126, 131)	
Microsatellite instability			~1	(126, 131, 133)

TGF- β pathway genes, including *SMAD4*, *TGFBR2*, *FBXW7*, and *MYC* (35).

From an etiological point of view, liver fluke *O. viverrini* infection-related iCCA had a higher *TP53* mutation rate, while *BAP1* and *IDH1/2* mutations were more frequently found in non-fluke-related cases (131, 138). *TP53* mutation was also found to be significantly correlated with hepatitis B virus (HBV) infection (127, 139). Regarding patient outcome, worse overall survival of patients with mutated *TP53*, *KRAS*, and *TERT* or deleted *CDKN2A* has been reported (126).

Genomic-Transcriptomic Profiles: Molecular Classification of Intrahepatic Cholangiocarcinoma

Several multi-omics approaches have been reported in the past decade, and several molecular classifications of iCCA have been presented (Table 3).

Inflammation/Proliferation Class

Integrated gene expression and mutational analyses performed by Sia et al. revealed two classes (inflammation and proliferation) of iCCA (53). The inflammation class accounted for approximately 40% of iCCA, and it was characterized by activation of immune response-related pathways, including dendritic cell signature and cytokines such as IL-4 and IL-10. The proliferation class showed activation of several oncogenic pathways including receptor tyrosine kinase pathway genes, such as *EGF*, *RAS*, *AKT*, *MET*, and other growth factor genes. Patient outcomes were worse in the proliferation class than in the inflammation class.

Prognosis-Based Classes

Transcriptomic profiling of iCCA and perihilar CCA by Andersen et al. revealed two prognostic groups (C1 and C2) with 5-year survival rate. The group with poor prognosis (C2) indicated increased activation of *VEGF/ERBB*, *CTNNB1/MYC*, and *TNF* signaling network and *KRAS* mutation, whereas these characteristics were not seen in the group with good prognosis (C1) (140). Recently, the gene expression pattern of iCCAs with cholangiolocellular differentiation trait, having favorable prognosis, was reported to be similar to that of C1, and has a signature including upregulated expression of inflammation-related genes and downregulated expression of proliferation-related genes based on Gene Ontology terms (52, 141).

Tumor Microenvironment-Based Classes

A recent study on the classification of iCCA according to its tumor microenvironment presented four subtypes based on gene signature analysis: “immunogenic,” which shows high innate and adaptive immune cell infiltration, “myeloid-rich,” which has strong macrophage and myeloid signatures, “mesenchymal,” with strong activated fibroblast signature, and “immune-desert,” which is characterized by lowest expression of all signatures (142). The immunogenic subtype had the best outcome, whereas the mesenchymal subtype had the worst outcome, in agreement with the prognostic features of the tumor microenvironment of iCCA discussed previously.

Other Classifications

Multi-omics data from TCGA project revealed that *IDH1/2*- and *PBRM1*-mutant subgroups showed upregulation of

TABLE 3 | Notable classification of intrahepatic cholangiocarcinoma from multi-omics studies.

Base of classification	Number of cases	Molecular classification and characteristics	References
Inflammation versus proliferation signature	149	<ul style="list-style-type: none"> ● Inflammation class <ul style="list-style-type: none"> - Enriched in immune response-related pathways - Overexpression of <i>IL-4</i> and <i>IL-10</i> (Th2 marker) - Favorable prognosis ● Proliferation class <ul style="list-style-type: none"> - Enriched in oncogenic pathways including RTK and angiogenic pathways, increased expression of <i>EGF</i>, <i>RAS</i>, <i>AKT</i>, <i>MET</i>, and growth factors - Worse outcome compared to inflammation class 	Sia et al. (53)
Prognosis	104*	<ul style="list-style-type: none"> ● Cluster 1 (group with good prognosis) <ul style="list-style-type: none"> - No <i>KRAS</i> mutation - Absence or weak expression of <i>HER2</i> and <i>MET</i> ● Cluster 2 (group with poor prognosis) <ul style="list-style-type: none"> - Enriched <i>VEGF/ERBB</i>, <i>CTNNB1/MYC</i>, and TNF pathway and <i>KRAS</i> mutation 	Andersen et al. (140)
Tumor microenvironment	78	<ul style="list-style-type: none"> ● Immune desert subtype <ul style="list-style-type: none"> - Minimal expression of all TME signatures ● Immunogenic subtype <ul style="list-style-type: none"> - High innate and adaptive immune cell presence - Strong activation of fibroblasts and inflammatory and immune checkpoint pathways - Best outcome ● Myeloid-rich subtype <ul style="list-style-type: none"> - Strong monocyte-derived myeloid cell signatures - Low lymphoid signatures ● Mesenchymal subtype <ul style="list-style-type: none"> - Strong active fibroblast signatures - Worst outcome 	Job et al. (142)
TCGA project	32	<ul style="list-style-type: none"> ● <i>IDH</i>-mutant cluster <ul style="list-style-type: none"> - <i>IDH1/2</i> mutation - Enriched mitochondrial gene expression - Loss of function of <i>ARID1A</i> and <i>PBRM1</i> ● <i>CCND1</i> amplification cluster <ul style="list-style-type: none"> - Highly methylated - <i>BAP1/FGFR</i> cluster ● <i>BAP1</i> mutation or <i>FGFR2</i> fusion ● Survival difference is not significant between clusters 	Farshidfar et al. (13)
Etiologic factor-associated	69	<ul style="list-style-type: none"> ● Cluster 1 <ul style="list-style-type: none"> - Liver fluke-related - <i>ARID1A</i>, <i>BRCA1/2</i>, and <i>TP53</i> mutations - <i>ERBB2</i> amplification - CpG island hypermethylation ● Cluster 2 <ul style="list-style-type: none"> - Partly liver-fluke-related - <i>TP53</i> mutation - High expression of <i>CTNNB1</i>, <i>WNT5B</i> and <i>AKT1</i> ● Cluster 3 <ul style="list-style-type: none"> - High CNA burden - Enriched immune-related pathways ● Cluster 4 <ul style="list-style-type: none"> - Associated with viral hepatitis - <i>BAP1</i> or <i>IDH1/2</i> mutation - High expression of <i>FGFR</i> family proteins - CpG shore hypermethylation - Favorable prognosis 	Jusakul et al. (131)

*Whether only intrahepatic cholangiocarcinoma was included is not certain.

CNA, copy number aberration; HCC, hepatocellular carcinoma; IL, interleukin; RTK, receptor tyrosine kinase; TCGA-CHOL, The Cancer Genome Atlas-Cholangiocarcinoma Consortium; TME, tumor microenvironment.

mitochondrial genes and downregulation of chromatin-modifying genes such as *ARID1A* and *ARID1B* due to hypermethylation of the promoter CpG region, while cases with

FGFR2 fusion showed downregulation of mitochondrial genes (13). Furthermore, another study has proposed a classification based on the correlation of multi-omics features with etiologic

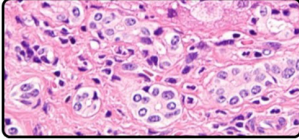
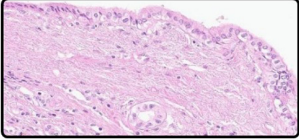
Intrahepatic Cholangiocarcinoma		
Classification	Small Duct Type	Large Duct Type
Gross Type	Mass-forming	Mixed Periductal Infiltrating
Cell of Origin	 Canal of Hering Bile ductule	 Columnar cholangiocytes Peribiliary glands
Main Etiology	Chronic hepatitis HBV / HCV Alcoholic / Metabolic	Hepatoolithiasis Liver fluke PSC
Immuno-histochemistry & Mucin stain	NCAM N-cadherin CRP	S100P Mucin
Frequent Mutations	<i>BAP1</i> <i>IDH1/2</i> <i>FGFR2 fusion</i>	<i>KRAS</i> <i>TP53</i> <i>SMAD4</i>
Suggested Molecular Classification*	Inflammation Class	Proliferation Class
Patient Outcome	Favorable	Poor

FIGURE 6 | Clinico-pathologic and molecular summary of intrahepatic cholangiocarcinoma (iCCA). Macro and microscopic, immunohistochemical, mutational, and clinical overview of iCCA. HBV, hepatitis B virus; HCV, hepatitis C virus; PSC, primary sclerosing cholangitis; S100P, S100 calcium-binding protein P; NCAM, neural cell adhesion molecule; CRP, c-reactive protein. *Based on the classification by Sia et al. (53).

background, showing that liver fluke-associated clusters 1 and 2, which harbor *TP53* mutation and *ERBB2* amplification in common, can be differentiated based on hypermethylated CpG island (for cluster 1). Furthermore, liver fluke-negative clusters 3 and 4 can be subdivided based on immune-related pathway enrichment (for cluster 3), and *IDH1/2* mutations, *FGFR2* fusion, and hypermethylated CpG promoter shores associated with viral hepatitis (for cluster 4) (131).

Moleculo-Pathological Correlation

The two major pathological types, small duct type and large duct type iCCA differ in their molecular characteristics. Small duct type iCCAs frequently have *BAP1*, *IDH1/2*, and *FGFR* mutations, while large duct type iCCAs more commonly show *KRAS*, *TP53*, and *SMAD4* mutations. Interestingly, iCCA with cholangiolocellular differentiation trait, which belongs to the small duct type, has been reported to be correlated with inflammation class and group with good prognosis (C1) (52, 53, 140). The pathological, clinical, and molecular characteristics of iCCA based on currently available evidence are summarized in **Figure 6**.

Perspectives on Targeted Therapies

Large-scale genomic analyses have identified target molecules for chemotherapy of patients with iCCA. Thus far, use of fibroblast growth factor receptor (FGFR) inhibitors and isocitrate dehydrogenase (IDH) 1 and 2 inhibitors are promising strategies against iCCA (130, 143).

Fibroblast growth factor receptor family proteins are localized on the cell membrane and transfer extracellular growth signals through intracellular tyrosine kinase domains. Pemigatinib, an oral inhibitor of FGFR1-3, has been approved by the United States Food and Drug Administration (FDA) for the treatment of patients with refractory advanced CCA with *FGFR2* fusion (130).

Isocitrate dehydrogenase 1 and 2 proteins are essential components of the tricarboxylic acid cycle, that normally generate NADPH *via* conversion of isocitrate into α -ketoglutarate. Mutant *IDH1/2* proteins accelerate this process, resulting in excess production of the byproduct, 2-hydroxyglutarate, which acts as an oncometabolite by interfering with histone and DNA methylation regulation (143). A recent phase 3 clinical trial of the *IDH1* inhibitor ivosidenib in patients

with advanced iCCA has shown promising results in increasing overall survival, and the FDA has approved its use in previously treated *IDH1*-mutated iCCA patients (143, 144).

Even though there is hope for these approved target agents, application is limited to those who harbor specific mutations, and antitumor efficacy is limited by intratumoral heterogeneity and drug resistance. Other targeted protein inhibitor molecules are in active clinical trials, including other inhibitors of FGFR family; multi-kinase inhibitors that act on epidermal growth factor receptor, vascular endothelial growth factor receptor and platelet-derived growth factor receptor; specific tyrosine kinase inhibitors targeting HER2, BRAF inhibitors, and immune checkpoint inhibitors (145, 146). To detect a variety of potential actionable mutations, the European Society for Medical Oncology has recommended routine use of NGS in patients with advanced cholangiocarcinoma (147).

CONCLUSION

Intrahepatic cholangiocarcinoma is a very heterogeneous malignancy with respect to histomorphology and molecular perspectives. The tumor microenvironment of iCCA also varies significantly depending on the type of immune cell infiltration and tumor stromal characteristics. Histopathological classification of small duct and large duct types shows differences

in etiology, molecular features, and clinical outcomes. Analyses of NGS and multi-omics studies have suggested molecular classifications of iCCA and identified *FGFR2* fusion and *IDH1/2* mutations as indications for targeted drugs. Further studies are needed for better pathological-molecular correlation and marker development for targeted therapy as well as immunotherapy to improve the treatment efficacy of patients with iCCA.

AUTHOR CONTRIBUTIONS

TC and YP contributed to the conception and design of this manuscript. TC performed the literature search, data analysis, and wrote the first draft of the manuscript. YP critically revised the manuscript. Both authors approved the submitted version.

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