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Molecular diagnosis of intrahepatic cholangiocarcinoma

Hiroaki Haga and **Tushar Patel**

Department of Cancer Biology, Mayo Clinic, Jacksonville, Florida

Abstract

Intrahepatic cholangiocarcinomas (iCCA) are primary intrahepatic malignancies originating from biliary epithelia. While both hepatocellular cancer and iCCA can present as mass lesions within the liver, these cancers are distinct in their morphology, etiology, pathology, natural history and response to therapy. There is a need for accurate and sensitive molecular markers for the diagnosis of iCCA. Recent advances in elucidating molecular and genetic characteristics of iCCA offer the potential of molecular-based diagnosis of iCCA. Specific genetic mutations of IDH1/2, BAP1, p53, and KRAS, FGFR gene fusions and alterations in microRNA have all been described in iCCA. Although there are no accurate serum or biliary biomarkers currently available for diagnosis of iCCA, several potential candidates have been identified. Knowledge of specific genetic or molecular abnormalities offers potential for individualized approaches for the treatment of patients with iCCA in the future.

Keywords

Biliary cancers; Liver cancers; biomarkers; gene mutations; gene fusions

INTRODUCTION

Cholangiocarcinomas are rare malignant tumors arising from the biliary tract. These malignancies are aggressive and are associated with a very poor prognosis. Diagnosis of cholangiocarcinoma requires consideration of the clinical scenario, imaging studies, tumor markers and histologic evaluation. Based on anatomical locations, cholangiocarcinoma can be separated into three distinct tumor types namely intrahepatic cholangiocarcinoma (iCCA), perihilar cholangiocarcinoma (pCCA), and distal cholangiocarcinoma (dCCA). Despite their common association with the biliary tract, these types of cholangiocarcinomas differ in their clinical presentation.

The separation of these cancers into intrahepatic and extrahepatic cancers is best avoided because it has contributed to a lack of clarity in epidemiological, clinical and molecular studies as a result of inconsistent designation of pCCA as either intrahepatic or extrahepatic.

In this review, we will focus on emerging approaches for the molecular diagnosis of iCCA. iCCA are primary cancers of the liver that originate from the intrahepatic biliary ductal

Address for correspondence: Tushar Patel, MBChB, AGAF, Mayo Clinic, 4500 San Pablo Road, Jacksonville, Florida 32224, Tel: 904 956 3257, Fax: 904 956 3359, patel.tushar@mayo.edu.

system, and form an intrahepatic mass whereas pCCA are tumors located in the bifurcation of the right and left bile duct, and dCCA are tumors originating from the distal part of the extrahepatic bile duct beyond the attachment of the cystic duct. Whereas patients with dCCA or pCCA may present with unremitting jaundice and cholestasis with pruritus, pale stools and dark urine, obstructive symptoms or cholangitis are rare as a symptom of iCCA, except in advanced cases (1). In contrast, iCCA can be identified incidentally as mass lesions on imaging studies that are performed for investigation of other symptoms. In this context, they need to be distinguished from other benign and malignant hepatic lesions.

Populations at risk for developing iCCA

The peak incidence for iCCA is between ages 55 and 75 years. Unlike HCC, which is 5 to 6 times more prevalent in men, iCCA appears to have only a slight male predominance, with a male: female ratio of 2:3(2).

Chronic biliary tract inflammation is an established risk factor for iCCA. Specifically, primary sclerosing cholangitits (PSC), intrahepatic lithiasis, and parasite infections such as *Clonorchis sinensis* and *Opistorchis viverrini* are well-established risk factors for iCCA(3). Congenital abnormalities of the biliary system carry a risk of malignant transformation that can be as high as 15% after the second decade of life. Such abnormalities include fibrocystic liver disease, choledochal cysts, and Caroli's disease. Recently, chronic hepatitis infection has been recognized as a risk factor for iCCA(4). In addition to diabetes mellitus, IBD, and smoking, cirrhosis and hepatitis exposure are also risk factors for iCCA(5). In a recent meta-analysis of published case-control studies, we identified several risk factors for iCCA that included hepatitis B, hepatitis C, obesity, diabetes and alcohol(6). Exposure to certain toxins is also associated with iCCA. Several cases were identified amongst workers at a printing company in Osaka(7). All patients were exposed to 1,2-DCP for 7–17 years and diagnosed with cancer 7–20 years after their first exposure. An increased risk of iCCA has been associated with exposure to thorotrast (thorium dioxide) (3).

Genetic and molecular characteristics of iCCA

Most studies of genetic or molecular features of cholangiocarcnoma have not systematically defined these events in the three clinically defined types of cholangiocarcinomas even though these cancers differ in their presentation, biological behavior and management. Alterations in gene and protein expression that are specific for iCCA are now becoming recognized. Isocitrate dehydrogenase(IDH) mutations were more frequently observed in iCCA than in extrahepatic cancers(8, 9). The overexpression of p53 was not identified in BilIN lesions and was less frequent in iCCA (18.2%) compared with extrahepatic cancers (38.1%) or gall-bladder cancers (61.5%) (10). Chang et al showed that EGFR mutation was an independent prognostic marker in CCA in addition to tumor stage and differentiation. No simultaneous EGFR and KRAS mutations were found in extrahepatic cholangiocarcinoma and gallbladder carcinoma (11). Genetic mutations that have been reported in iCCA are listed in Table 1. Understanding specific genetic and molecular characteristics that are specific for iCCA when compared to other types of cholangiocarcinoma will be essential in order to develop new or more effective diagnostic approaches.

IDH1/2 mutations

Mutations in the genes encoding isocitrate dehydrogenase, IDH1 and IDH2, have been reported in 10–28% of cholangiocarcinomas(8, 9, 12, 13). They have also been reported in several other types of cancers such as gliomas, myeloid leukemias, chondrosarcomas and thyroid cancer(14). These mutations result in elevated levels of an oncometabolite, 2-hydroxyglutarate, which is associated with higher DNA CpG methylation and altered histone methylation that accompany a block in cellular differentiation(15). Mutations in IDH1 or IDH2 were associated with longer overall survival and were independently associated with a longer time to tumor recurrence after iCCA resection in multivariate analysis. IDH1 and IDH2 mutations were significantly associated with increased levels of p53 in iCCA, but no mutations in the p53 gene were found, suggesting that mutations in IDH1 and IDH2 may result in p53 activation(12).

BAP1 mutations

The BAP1 protein is a deubiquitinase belonging to the ubiquitin C-terminal hydrolase family and is involved in chromatin remodeling. In a complex with ASXL1, BAP1 deubiquitinates histone H2A(16). Somatic mutations in *BAP1* occurred in 8 of 32 iCCA (25%)(17). BAP1 was frequently mutated in CCA cases without O. viverrini infection(18). Most of the observed BAP1 mutations were predicted to result in loss of the HCF-1–binding domain and nuclear localization signal, both of which are necessary for the inhibition of cell proliferation and tumorigenesis(16).

FGFR2 fusions

Recent studies have reported the presence of FGFR fusions in cholangiocarcinoma. Wu et al. detected a novel kinase fusion, FGFR2-BICC1, in two cholangiocarcinoma patients(19). Moreover Arai et al. detected FGFR2 fusion in nine of 66 patients (13.6%) with iCCA, of which seven patients had FGFR2-AHCYL1 fusion and two patients had FGFR2-BICC1 (20). Ross et al. reported three gene fusion, FGFR2-BICC1, FGFR2-KIAA 1598 and FGFR2-TACC3, in primary iCCA (21). FGFR gene fusion positive cancers have been shown to have enhanced susceptibility to FGFR inhibitors over activating point mutations of FGFR(19, 20). Borad et al., reported three patients possessing an FGFR2 gene fusions (FGFR2-MGEA5, FGFR2-TACC3 and FGFR2-BICC1), of which two patients received targeted therapy for FGFR2 (22). A patient with a FGFR2-MGEA5 fusion was treated with ponatinib monotherapy with anti-tumor activity documented. In another patient with a FGFR2-TACC3 fusion with progression on pazopanib, stable disease was noted with treatment using a pan-FGFR inhibitor ponatinib (22). Of note, tyrosine kinase inhibitors that target anaplastic lymphoma kinase (ALK) are particularly effective in the treatment of a distinct subset of lung adenocarcinoma carrying ALK fusions(23). These emerging results regarding FGFR2 fusions suggest that oncogenic activation of FGFR2 may represent a therapeutically actionable event and that identification of FGFR2 fusions may have therapeutic implications.

Other genetic changes

Gain-of-function mutations in KRAS downstream of EGFR represent one of the most frequent mutations found in iCCA (8–54%) (24–27). Moreover, mutations in KRAS were detected in 30% of bile from patients with primary sclerosing cholangitis (PSC), suggesting that it is an early event contributing to the malignant transformation of cholangiocytes(28). KRAS were associated with poor overall survival in all patients with iCCA (29).

The p53 tumor suppressor gene is localized on chromosome 17p13 and encodes a 53-kD nuclear phosphoprotein. It plays a central role in DNA repair and apoptosis, thus regulating epithelial cell homoeostasis(30). More than 90 different mutations have been described in TP53. A review of 10 studies which included 229 cases found a total of 21% (49 patients) with mutations in TP53, ranging from 23% in Asia, 14% in Europe, and 26% in the USA(31).

Analysis of mutations was reported in a large set of iCCA patients by Robertson et al. Of 54 cases, KRAS mutations were present in 7.4%, and BRAF mutations in 7.4%; these were mutually exclusive. Mutant cases were associated with a higher tumor stage at time of resection and a greater likelihood of lymph node involvement. These cases were also associated with a worse long-term overall survival(32).

Exome sequencing of eight CCA with *Opisthorchis viverrini* (*Ov*) infection and 15 CCA without *Ov* infection identified a total of 245 somatic mutations in 224 genes. These analyses identified the mutation of TP53, KRAS2, SMAD4, and CDKN2A as frequently mutated genes similar to other prior reports(33).

microRNAs

Alterations in microRNA are associated with modulation of tumor cell proliferation, alteration in sensitivity to chemotherapy, or alteration in epithelial–mesenchymal transition (EMT). Upregulation of microRNA such as miR-26A, miR-31, miR-21, and miR-421 as well as downregulation of microRNAs such as miR-494, miR-370, and miR-138 has been reported in CCA tissues and can modulate cell proliferation through several different mechanisms (34–37). Similary, miRNA associated with chemotherapy resistance include miR-21 which is up-regulated or mir-320 which is down-regulated in CCA. miR-21 can modulate gemcitabine-induces apoptosis by phosphatase and tensin homolog deleted on chromosome 10(PTEN)-dependent activation of PI 3-kinase signaling(34). During EMT, epithelial cells lose their cell polarity and cell–cell adhesion and can gain migratory and invasive properties to become mesenchymal cells(38). The activation of miR-200c can lead to a reduction of EMT with reduced cell migration and invasion in iCCA cells(39). Likewise, miR-214 may be important in regulating metastasis of iCCA because downregulation of this miRNA can promote EMT by directly targeting the Twist gene (40).

Proteins

Many proteins have been associated with iCCA (Table 4). Biological processes that have been implicated in tumorigenesis include EMT, which contributes to invasion and metastasis (41). An EMT phenotype resulting from expression of E-cadherin, β-catenin, vimentin and

fibronectin has been reported in iCCA, and MET associated proteins such as CD151, GATA6, Gli1 and S100A4 have been postulated as biomarkers. Korita et al reported a positive correlation of vimentin expression with differentiation and poor survival in iCCA(42). Fibronectin expression occurs in many carcinomas and has been correlated with poor survival and metastasis(43). A study by Gu et al. reported that the combination of β-catenin negativity with positive expression of vimentin or fibronectin showed worse prognosis whereas β-catenin positivity with loss of vimentin or fibronectin expression showed the best prognosis (44).

Recent studies have demonstrated that CD151 forms structural and functional associations with the proto-oncogene that encodes c-Met protein and is involved in the regulation of downstream pathways of the c-Met/hepatocyte growth factor (HGF) system(45). Huang et al. examined the expression of CD151/c-Met by immunohistochemistry in a tissue microarray. Overexpression of CD151 was implicated in metastasis and invasion of iCCA. Thus, either CD151 or c-Met overexpression may be potential molecular therapeutic targets for iCCA. Tian et al. reported that 67LR was regulated by GATA6 through binding to its promoter in CCA cells, indicating that aberrant expression of GATA6 correlates with poor prognosis and promotes tumor cell invasion and metastasis, possibly through promoter binding mediated regulation of 67LR (46). In iCCA tissues, Gli1 nuclear immune-intensity is associated with intrahepatic metastasis and venous invasion(47). Gli1 is a transcriptional target of the hedgehog pathway, and Gli1 expression serves as an indicator of activated hedgehog signaling. Elevated Gli1 expression was linked with cancer development and progression(48). Furthermore, blocking hedgehog signaling by cyclopamine or siRNA-targeting Gli1 results in apoptosis and growth inhibition in iCCA cells. Thus, detection of hedgehog pathway activation may have therapeutic value(47). S100A4 may also represent a potential therapeutic target. Fabris et al. reported that nuclear expression of S100A4 by neoplastic ducts in CCA was a strong predictor of metastasis and reduced survival after resection. S100A4-silenced EGI-1 cells (human CCA cell line) demonstrated reduced motility, invasiveness, and MMP-9 secretion in vitro, without changes in cell proliferation(49).

Molecular diagnosis of iCCA

Serum biomarkers

At this time there are no available serum biomarkers that are useful for early or accurate diagnosis of iCCA. Both CA 19-9 and carcinoembryonic antigen (CEA) can be elevated in these cancers but are not specific and may also be elevated in other cancers or in the setting of cholestasis in the absence of malignancy, and following liver injury (50). Thus, their accuracy for the diagnosis of cholangiocarcinoma is limited. Other potentially useful markers include DU-PAN-2, CA 125 and interleukin-6(51). CYFRA 21-1 concentrations again were shown to be significantly higher in CCA than in HCC or in patients with benign liver diseases. Moreover, sensitivity to detect CCA was superior for CYFRA 21-1 as compared with alpha fetoprotein, CEA, and CA 19-9 levels (87% versus 17, 35, and 61%, respectively)(52). Combinations of these markers might improve the sensitivity and specificity for the diagnosis of cholangiocarcinoma.

Several other several candidate serum marker for iCCA have been reported, including KL-6 mucin(53), hTERT mRNA(54), A1BG/AFM ratio(55) and MUC5AC(56). Candidate markers in serum are listed in Table 2. Matsuda et al. reported that Wisteria floribunda agglutinin (WFA) can differentiate iCCA lesions from normal bile duct epithelia (57). Using serum as well as bile from patients with CCA or benign bile duct diseases, they reported the use of WFA-positive L1CAM, enriched from serum by the WFA-assisted affinity capturing, to distinguish malignant from benign epithelia. Diagnostic accuracy for CCA was increased by combining the assay with a high sensitive assay detecting WFA-positive sialylated mucin 1 (overall accuracy = 0.84, AUC = 0.93)(58). Shen et al. reported that SSP411 (also known as spermatogenesis-associated protein 20), was a potential serum diagnostic biomarker for CCA, with a sensitivity of 90.0% and specificity of 83.3% at a cutoff value of 0.63(59). SSP411, a thioredoxin family member, is a novel spermatid-expressed gene which is thought to play a role in sperm maturation, fertilization and/or embryo development(60). In another report, serum DKK1 levels were significantly higher in iCCA patients than in healthy volunteers. On ROC analyses, a serum DKK1 level of 2.49 ng/mL had an area under the curve = 0.872, with 75.7% sensitivity and 100% specificity for diagnosis of iCCA. High DKK1 expression in iCCA tissues was associated with elevated matrix metalloproteinase 9 (MMP9), vascular endothelial growth factor C (VEGF-C) expression, and high lymph node metastasis(61).

Bile biomarkers

Candidate markers in bile are reported in Table 3. The frequency of CA 19-9 detection in bile from patients with both benign and neoplastic diseases of the pancreaticobiliary tract ranges from 46 to 61% and specificity of 60 to 70%(51). However, a higher frequency of elevated CA 19-9 in bile has been reported in patients with acute cholangitis as compared with CCA(62). Similarly, the results regarding the diagnostic value of CEA levels in bile are contradictory. The sampling time as measured before and after biliary drainage procedures has been shown to influence the CA 19-9 and CEA levels, and is a major variable that influences the value of bile biomarkers.

S100 protein has been reported as a candidate bile biomaker(63). The bile levels of S100P were increased significantly in patients with cholangiocarcinoma compared with those in patients with lithiasis(63). Moreover, Baraniskin et al. reported that measurement of RNU2-1f levels in bile fluids enabled the differentiation of patients with CCA from controls in all cases. Furthermore, RNU2-1f levels in bile fluids of patients with CCA were significantly higher than in patients with PSC(64).

Li et al. (65) reported that analysis of microRNA within extracellular vesicles obtained from bile enabled diagnosis of CCA. A panel of 5 microRNAs (miR-191, miR-486-3p, miR-1274b, miR-16 and miR-484) demonstrated a sensitivity of 67% and specificity of 96%. Importantly, their control group contained 13 PSC patients, 16 patients with biliary obstruction of varying etiologies (including benign biliary stricture, papillary stenosis, choledocholithiasis, extrinsic compression from pancreatic cysts, and cholangitis), and 3 patients with bile leak syndromes. Their findings establish the importance of using extracellular vesicles, rather than whole bile, for developing disease biomarkers (66).

Tissue diagnosis

Distinguishing hepatic metastases from distant adenocarcinoma and intrahepatic cholangiocarcinoma is difficult because of the similarities in morphological appearances. Current approaches to diagnosis require the exclusion of other primary malignancies. Tissue markers with high specificity for iCCA may be helpful for the diagnosis in these difficult cases. In addition, tissue markers that correlate with disease progression, or response to therapy would be of particular clinical value in defining prognosis or future care. Selected candidate tissue markers along with their clinical or pathological correlations are shown in Table 4. Finally, specific therapies may also be suggested by demonstration of drug-relevant targets within tissues.

Therapeutic options for iCCA

For patients with localized disease, surgical resection should be considered as this is the only strategy with the potential for cure. A critical factor is the extent of hepatic resection that is necessary and also compatible with a functionally adequate remnant liver. Advances in techniques to predict residual tumor volume and function, and portal embolization enable more extensive resections than were possible previously(67). For resections with either positive margins or residual tumor or positive lymph nodes, re-resection or ablation should be considered if feasible(68). Locoregional approaches, such as ablation, transarterial chemoembolization (TACE) and transarterial radioembolization have been used in patients with cholangiocarcinoma(69). However, the experience has been limited and these modalities have not been systematically evaluated. Although the response to external beam radiation therapy in patients with intrahepatic cholangiocarcinoma has been poor, anecdotal evidence indicates that a reduction in tumor burden can be achieved with stereotactic body radiotherapy treatment (SBRT)(70). For patients with advanced cancers that are unresectable or those with metastatic disease, systemic therapy with gemcitabine plus cisplatin is a first-line approach(71). Alternative choices, particularly in individuals who might not tolerate the combination of gemcitabine and cisplatin would include gemcitabine monotherapy, 5FU-based regimens, or supportive care (72). Future directions in therapy of iCCA are likely to be based on the emerging knowledge of the molecular pathogenesis of these cancers. Drugs that can target many of the oncogenic pathways that have been identified such as FGFR2 fusions, IDH mutations, EGFR and others are available, and may form the basis for targeted individualized therapy in the future.

Conclusions

There is a need for accurate and sensitive molecular markers for iCCA. Similarly, a knowledge of specific genetic or molecular abnormalities offers the potential for more refined and individualized approaches to the treatment of patients with iCCA through the use of appropriate targeted therapies. Understanding genetic and molecular changes associated with these cancers therefore holds the promise for future assays that may be helpful for disease diagnosis, prognosis, or treatment.

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References

1. Patel T. Cholangiocarcinoma. Nat Clin Pract Gastroenterol Hepatol. 2006 Jan; 3(1):33–42. [PubMed: 16397610]
2. Shaib YH, Davila JA, McGlynn K, El-Serag HB. Rising incidence of intrahepatic cholangiocarcinoma in the United States: a true increase? J Hepatol. 2004 Mar; 40(3):472–7. [PubMed: 15123362]
3. Dodson RM, Weiss MJ, Cosgrove D, Herman JM, Kamel I, Anders R, et al. Intrahepatic cholangiocarcinoma: management options and emerging therapies. J Am Coll Surg. 2013 Oct; 217(4):736–50. e4. [PubMed: 23890842]
4. Welzel TM, Graubard BI, El-Serag HB, Shaib YH, Hsing AW, Davila JA, et al. Risk factors for intrahepatic and extrahepatic cholangiocarcinoma in the United States: a population-based case-control study. Clin Gastroenterol Hepatol. 2007 Oct; 5(10):1221–8. [PubMed: 17689296]
5. Shaib YH, El-Serag HB, Davila JA, Morgan R, McGlynn KA. Risk factors of intrahepatic cholangiocarcinoma in the United States: a case-control study. Gastroenterology. 2005 Mar; 128(3): 620–6. [PubMed: 15765398]
6. Palmer WC, Patel T. Are common factors involved in the pathogenesis of primary liver cancers? A meta-analysis of risk factors for intrahepatic cholangiocarcinoma. J Hepatol. 2012 Jul; 57(1):69–76. [PubMed: 22420979]
7. Kumagai S, Kurumatani N, Arimoto A, Ichihara G. Cholangiocarcinoma among offset colour proof-printing workers exposed to 1,2-dichloropropane and/or dichloromethane. Occup Environ Med. 2013 Jul; 70(7):508–10. [PubMed: 23493378]
8. Kipp BR, Voss JS, Kerr SE, Barr Fritcher EG, Graham RP, Zhang L, et al. Isocitrate dehydrogenase 1 and 2 mutations in cholangiocarcinoma. Hum Pathol. 2012 Oct; 43(10):1552–8. [PubMed: 22503487]
9. Borger DR, Tanabe KK, Fan KC, Lopez HU, Fantin VR, Straley KS, et al. Frequent mutation of isocitrate dehydrogenase (IDH)1 and IDH2 in cholangiocarcinoma identified through broad-based tumor genotyping. Oncologist. 2012; 17(1):72–9. [PubMed: 22180306]
10. Hsu M, Sasaki M, Igarashi S, Sato Y, Nakanuma Y. KRAS and GNAS mutations and p53 overexpression in biliary intraepithelial neoplasia and intrahepatic cholangiocarcinomas. Cancer. 2013 May 1; 119(9):1669–74. [PubMed: 23335286]
11. Chang YT, Chang MC, Huang KW, Tung CC, Hsu C, Wong JM. Clinicopathological and Prognostic Significances of EGFR, KRAS and BRAF Mutations in Biliary Tract Carcinomas in Taiwan. J Gastroenterol Hepatol. 2013 Dec 27.
12. Wang P, Dong Q, Zhang C, Kuan PF, Liu Y, Jeck WR, et al. Mutations in isocitrate dehydrogenase 1 and 2 occur frequently in intrahepatic cholangiocarcinomas and share hypermethylation targets with glioblastomas. Oncogene. 2013 Jun 20; 32(25):3091–100. [PubMed: 22824796]
13. Sia D, Tovar V, Moeini A, Llovet JM. Intrahepatic cholangiocarcinoma: pathogenesis and rationale for molecular therapies. Oncogene. 2013 Oct 10; 32(41):4861–70. [PubMed: 23318457]
14. Amary MF, Bacsi K, Maggiani F, Damato S, Halai D, Berisha F, et al. IDH1 and IDH2 mutations are frequent events in central chondrosarcoma and central and periosteal chondromas but not in other mesenchymal tumours. J Pathol. 2011 Jul; 224(3):334–43. [PubMed: 21598255]
15. Grassian AR, Pagliarini R, Chiang DY. Mutations of isocitrate dehydrogenase 1 and 2 in intrahepatic cholangiocarcinoma. Curr Opin Gastroenterol. 2014 Feb 24.
16. Pena-Llopis S, Vega-Rubin-de-Celis S, Liao A, Leng N, Pavia-Jimenez A, Wang S, et al. BAP1 loss defines a new class of renal cell carcinoma. Nat Genet. 2012 Jul; 44(7):751–9. [PubMed: 22683710]

17. Jiao Y, Pawlik TM, Anders RA, Selaru FM, Streppel MM, Lucas DJ, et al. Exome sequencing identifies frequent inactivating mutations in BAP1, ARID1A and PBRM1 in intrahepatic cholangiocarcinomas. Nat Genet. 2013 Dec; 45(12):1470–3. [PubMed: 24185509]
18. Chan-On W, Nairismagi ML, Ong CK, Lim WK, Dima S, Pairojkul C, et al. Exome sequencing identifies distinct mutational patterns in liver fluke-related and non-infection-related bile duct cancers. Nat Genet. 2013 Dec; 45(12):1474–8. [PubMed: 24185513]
19. Wu YM, Su F, Kalyana-Sundaram S, Khazanov N, Ateeq B, Cao X, et al. Identification of targetable FGFR gene fusions in diverse cancers. Cancer Discov. 2013 Jun; 3(6):636–47. [PubMed: 23558953]
20. Arai Y, Totoki Y, Hosoda F, Shirota T, Hama N, Nakamura H, et al. Fibroblast growth factor receptor 2 tyrosine kinase fusions define a unique molecular subtype of cholangiocarcinoma. Hepatology. 2013 Oct 9.
21. Ross JS, Wang K, Gay L, Al-Rohil R, Rand JV, Jones DM, et al. New Routes to Targeted Therapy of Intrahepatic Cholangiocarcinomas Revealed by Next-Generation Sequencing. Oncologist. 2014 Feb 21.
22. Borad MJ, Champion MD, Egan JB, Liang WS, Fonseca R, Bryce AH, et al. Integrated Genomic Characterization Reveals Novel, Therapeutically Relevant Drug Targets in FGFR and EGFR Pathways in Sporadic Intrahepatic Cholangiocarcinoma. PLoS Genet. 2014 Feb.10(2):e1004135. [PubMed: 24550739]
23. Gerber DE, Minna JD. ALK inhibition for non-small cell lung cancer: from discovery to therapy in record time. Cancer Cell. 2010 Dec 14; 18(6):548–51. [PubMed: 21156280]
24. Isa T, Tomita S, Nakachi A, Miyazato H, Shimoji H, Kusano T, et al. Analysis of microsatellite instability, K-ras gene mutation and p53 protein overexpression in intrahepatic cholangiocarcinoma. Hepatogastroenterology. 2002 May-Jun;49(45):604–8. [PubMed: 12063950]
25. Kiba T, Tsuda H, Pairojkul C, Inoue S, Sugimura T, Hirohashi S. Mutations of the p53 tumor suppressor gene and the ras gene family in intrahepatic cholangiocellular carcinomas in Japan and Thailand. Mol Carcinog. 1993; 8(4):312–8. [PubMed: 8280380]
26. Ohashi K, Nakajima Y, Kanehiro H, Tsutsumi M, Taki J, Aomatsu Y, et al. Ki-ras mutations and p53 protein expressions in intrahepatic cholangiocarcinomas: relation to gross tumor morphology. Gastroenterology. 1995 Nov; 109(5):1612–7. [PubMed: 7557145]
27. Tannapfel A, Benicke M, Katalinic A, Uhlmann D, Kockerling F, Hauss J, et al. Frequency of p16(INK4A) alterations and K-ras mutations in intrahepatic cholangiocarcinoma of the liver. Gut. 2000 Nov; 47(5):721–7. [PubMed: 11034592]
28. Kubicka S, Kuhnel F, Flemming P, Hain B, Kezmic N, Rudolph KL, et al. K-ras mutations in the bile of patients with primary sclerosing cholangitis. Gut. 2001 Mar; 48(3):403–8. [PubMed: 11171833]
29. Jang S, Chun SM, Hong SM, Sung CO, Park H, Kang HJ, et al. High throughput molecular profiling reveals differential mutation patterns in intrahepatic cholangiocarcinomas arising in chronic advanced liver diseases. Mod Pathol. 2013 Nov 1.
30. Chang F, Syrjanen S, Syrjanen K. Implications of the p53 tumor-suppressor gene in clinical oncology. J Clin Oncol. 1995 Apr; 13(4):1009–22. [PubMed: 7707100]
31. Khan SA, Thomas HC, Toledano MB, Cox IJ, Taylor-Robinson SD. p53 Mutations in human cholangiocarcinoma: a review. Liver Int. 2005 Aug; 25(4):704–16. [PubMed: 15998419]
32. Robertson S, Hyder O, Dodson R, Nayar SK, Poling J, Beierl K, et al. The frequency of KRAS and BRAF mutations in intrahepatic cholangiocarcinomas and their correlation with clinical outcome. Hum Pathol. 2013 Dec; 44(12):2768–73. [PubMed: 24139215]
33. Ito T, Sakurai-Yageta M, Goto A, Pairojkul C, Yongvanit P, Murakami Y. Genomic and transcriptional alterations of cholangiocarcinoma. J Hepatobiliary Pancreat Sci. 2014 Feb 13.
34. Meng F, Henson R, Lang M, Wehbe H, Maheshwari S, Mendell JT, et al. Involvement of human micro-RNA in growth and response to chemotherapy in human cholangiocarcinoma cell lines. Gastroenterology. 2006 Jun; 130(7):2113–29. [PubMed: 16762633]
35. Hummel R, Hussey DJ, Haier J. MicroRNAs: predictors and modifiers of chemo- and radiotherapy in different tumour types. Eur J Cancer. 2010 Jan; 46(2):298–311. [PubMed: 19948396]

36. Karakatsanis A, Papaconstantinou I, Gazouli M, Lyberopoulou A, Polymeneas G, Voros D. Expression of microRNAs, miR-21, miR-31, miR-122, miR-145, miR-146a, miR-200c, miR-221, miR-222, and miR-223 in patients with hepatocellular carcinoma or intrahepatic cholangiocarcinoma and its prognostic significance. Mol Carcinog. 2013 Apr; 52(4):297–303. [PubMed: 22213236]
37. Chusorn P, Namwat N, Loilome W, Techasen A, Pairojkul C, Khuntikeo N, et al. Overexpression of microRNA-21 regulating PDCD4 during tumorigenesis of liver fluke-associated cholangiocarcinoma contributes to tumor growth and metastasis. Tumour Biol. 2013 Jun; 34(3): 1579–88. [PubMed: 23417858]
38. Thiery JP. Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer. 2002 Jun; 2(6):442–54. [PubMed: 12189386]
39. Oishi N, Kumar MR, Roessler S, Ji J, Forgues M, Budhu A, et al. Transcriptomic profiling reveals hepatic stem-like gene signatures and interplay of miR-200c and epithelial-mesenchymal transition in intrahepatic cholangiocarcinoma. Hepatology. 2012 Nov; 56(5):1792–803. [PubMed: 22707408]
40. Li B, Han QQ, Zhu Y, Yu Y, Wang JH, Jiang XQ. Down-regulation of miR-214 contributes to intrahepatic cholangiocarcinoma metastasis by targeting Twist. Febs J. 2012 Jul; 279(13):2393–8. [PubMed: 22540680]
41. Huber MA, Kraut N, Beug H. Molecular requirements for epithelial-mesenchymal transition during tumor progression. Curr Opin Cell Biol. 2005 Oct; 17(5):548–58. [PubMed: 16098727]
42. Korita PV, Wakai T, Ajioka Y, Inoue M, Takamura M, Shirai Y, et al. Aberrant expression of vimentin correlates with dedifferentiation and poor prognosis in patients with intrahepatic cholangiocarcinoma. Anticancer Res. 2010 Jun; 30(6):2279–85. [PubMed: 20651380]
43. Sudo T, Iwaya T, Nishida N, Sawada G, Takahashi Y, Ishibashi M, et al. Expression of mesenchymal markers vimentin and fibronectin: the clinical significance in esophageal squamous cell carcinoma. Ann Surg Oncol. 2013 Dec; 20(Suppl 3):S324–35. [PubMed: 22644514]
44. Gu MJ, Choi JH. Epithelial-mesenchymal transition phenotypes are associated with patient survival in intrahepatic cholangiocarcinoma. J Clin Pathol. 2014 Mar; 67(3):229–34. [PubMed: 24062361]
45. Ke AW, Shi GM, Zhou J, Wu FZ, Ding ZB, Hu MY, et al. Role of overexpression of CD151 and/or c-Met in predicting prognosis of hepatocellular carcinoma. Hepatology. 2009 Feb; 49(2): 491–503. [PubMed: 19065669]
46. Tian F, Li D, Chen J, Liu W, Cai L, Li J, et al. Aberrant expression of GATA binding protein 6 correlates with poor prognosis and promotes metastasis in cholangiocarcinoma. Eur J Cancer. 2013 May; 49(7):1771–80. [PubMed: 23313142]
47. Tang L, Tan YX, Jiang BG, Pan YF, Li SX, Yang GZ, et al. The prognostic significance and therapeutic potential of hedgehog signaling in intrahepatic cholangiocellular carcinoma. Clin Cancer Res. 2013 Apr 15; 19(8):2014–24. [PubMed: 23493353]
48. Sanchez P, Hernandez AM, Stecca B, Kahler AJ, DeGueme AM, Barrett A, et al. Inhibition of prostate cancer proliferation by interference with SONIC HEDGEHOG-GLI1 signaling. Proc Natl Acad Sci U S A. 2004 Aug 24; 101(34):12561–6. [PubMed: 15314219]
49. Fabris L, Cadamuro M, Moserle L, Dziura J, Cong X, Sambado L, et al. Nuclear expression of S100A4 calcium-binding protein increases cholangiocarcinoma invasiveness and metastasization. Hepatology. 2011 Sep 2; 54(3):890–9. [PubMed: 21618579]
50. Nakeeb A, Lipsett PA, Lillemoe KD, Fox-Talbot MK, Coleman J, Cameron JL, et al. Biliary carcinoembryonic antigen levels are a marker for cholangiocarcinoma. Am J Surg. 1996 Jan; 171(1):147–52. discussion 52–3. [PubMed: 8554130]
51. Nehls O, Gregor M, Klump B. Serum and bile markers for cholangiocarcinoma. Semin Liver Dis. 2004 May; 24(2):139–54. [PubMed: 15192787]
52. Kashihara T, Ohki A, Kobayashi T, Sato T, Nishizawa H, Ogawa K, et al. Intrahepatic cholangiocarcinoma with increased serum CYFRA 21–1 level. J Gastroenterol. 1998 Jun; 33(3): 447–53. [PubMed: 9658330]

53. Xu H, Inagaki Y, Tang W, Guo Q, Wang F, Seyama Y, et al. Elevation of serum KL-6 mucin levels in patients with cholangiocarcinoma. Hepatogastroenterology. 2008 Nov-Dec;55(88):2000–4. [PubMed: 19260467]
54. Leelawat K, Leelawat S, Ratanachu-Ek T, Trubwongchareon S, Wannaprasert J, Tripongkaruna S, et al. Circulating hTERT mRNA as a tumor marker in cholangiocarcinoma patients. World J Gastroenterol. 2006 Jul 14; 12(26):4195–8. [PubMed: 16830373]
55. Tolek A, Wongkham C, Proungvitaya S, Silsirivanit A, Roytrakul S, Khuntikeo N, et al. Serum alpha1beta-glycoprotein and afamin ratio as potential diagnostic and prognostic markers in cholangiocarcinoma. Exp Biol Med (Maywood). 2012 Oct; 237(10):1142–9. [PubMed: 23104505]
56. Bamrungphon W, Prempracha N, Bunchu N, Rangdaeng S, Sandhu T, Srisukho S, et al. A new mucin antibody/enzyme-linked lectin-sandwich assay of serum MUC5AC mucin for the diagnosis of cholangiocarcinoma. Cancer Lett. 2007 Mar 18; 247(2):301–8. [PubMed: 16793202]
57. Matsuda A, Kuno A, Kawamoto T, Matsuzaki H, Irimura T, Ikehara Y, et al. Wisteria floribunda agglutinin-positive mucin 1 is a sensitive biliary marker for human cholangiocarcinoma. Hepatology. 2010 Jul; 52(1):174–82. [PubMed: 20578261]
58. Matsuda A, Kuno A, Matsuzaki H, Kawamoto T, Shikanai T, Nakanuma Y, et al. Glycoproteomics-based cancer marker discovery adopting dual enrichment with Wisteria floribunda agglutinin for high specific glyco-diagnosis of cholangiocarcinoma. J Proteomics. 2013 Jun 24.85:1–11. [PubMed: 23612463]
59. Shen J, Wang W, Wu J, Feng B, Chen W, Wang M, et al. Comparative proteomic profiling of human bile reveals SSP411 as a novel biomarker of cholangiocarcinoma. PLoS One. 2012; 7(10):e47476. [PubMed: 23118872]
60. Shi HJ, Wu AZ, Santos M, Feng ZM, Huang L, Chen YM, et al. Cloning and characterization of rat spermatid protein SSP411: a thioredoxin-like protein. J Androl. 2004 Jul-Aug;25(4):479–93. [PubMed: 15223837]
61. Shi RY, Yang XR, Shen QJ, Yang LX, Xu Y, Qiu SJ, et al. High expression of Dickkopf-related protein 1 is related to lymphatic metastasis and indicates poor prognosis in intrahepatic cholangiocarcinoma patients after surgery. Cancer. 2013 Mar 1; 119(5):993–1003. [PubMed: 23132676]
62. Ker CG, Chen JS, Lee KT, Sheen PC, Wu CC. Assessment of serum and bile levels of CA19–9 and CA125 in cholangitis and bile duct carcinoma. J Gastroenterol Hepatol. 1991 Sep-Oct;6(5): 505–8. [PubMed: 1657243]
63. Sato Y, Harada K, Sasaki M, Nakanuma Y. Clinicopathological significance of S100 protein expression in cholangiocarcinoma. J Gastroenterol Hepatol. 2013 Aug; 28(8):1422–9. [PubMed: 23621473]
64. Baraniskin A, Nopel-Dunnebacke S, Schumacher B, Gerges C, Bracht T, Sitek B, et al. Analysis of U2 Small Nuclear RNA Fragments in the Bile Differentiates Cholangiocarcinoma from Primary Sclerosing Cholangitis and Other Benign Biliary Disorders. Dig Dis Sci. 2014 Jan 31.
65. Li L, Masica D, Ishida M, Tomuleasa C, Umegaki S, Kalloo AN, et al. Human bile contains microRNA-laden extracellular vesicles that can be used for cholangiocarcinoma diagnosis. Hepatology. 2014 Feb 4.
66. Patel T. Extracellular vesicle non-coding RNA - new players in the diagnosis and pathogenesis of cholangiocarcinoma. Hepatology. 2014
67. Yokoyama Y, Nishio H, Ebata T, Igami T, Sugawara G, Nagino M. Value of indocyanine green clearance of the future liver remnant in predicting outcome after resection for biliary cancer. Br J Surg. 2010 Aug; 97(8):1260–8. [PubMed: 20602507]
68. Patel T. Cholangiocarcinoma--controversies and challenges. Nat Rev Gastroenterol Hepatol. 2011 Apr; 8(4):189–200. [PubMed: 21460876]
69. Ahmadzadehfar H, Biersack HJ, Ezziddin S. Radioembolization of liver tumors with yttrium-90 microspheres. Semin Nucl Med. 2010 Mar; 40(2):105–21. [PubMed: 20113679]
70. Kopek N, Holt MI, Hansen AT, Hoyer M. Stereotactic body radiotherapy for unresectable cholangiocarcinoma. Radiother Oncol. 2010 Jan; 94(1):47–52. [PubMed: 19963295]

71. Valle J, Wasan H, Palmer DH, Cunningham D, Anthoney A, Maraveyas A, et al. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. N Engl J Med. 2010 Apr 8; 362(14):1273–81. [PubMed: 20375404]
72. Hezel AF, Zhu AX. Systemic therapy for biliary tract cancers. Oncologist. 2008 Apr; 13(4):415–23. [PubMed: 18448556]
73. Endo K, Ashida K, Miyake N, Terada T. E-cadherin gene mutations in human intrahepatic cholangiocarcinoma. J Pathol. 2001 Mar; 193(3):310–7. [PubMed: 11241409]
74. Andersen JB, Spee B, Blechacz BR, Avital I, Komuta M, Barbour A, et al. Genomic and genetic characterization of cholangiocarcinoma identifies therapeutic targets for tyrosine kinase inhibitors. Gastroenterology. 2012 Apr; 142(4):1021–31. e15. [PubMed: 22178589]
75. Sia D, Hoshida Y, Villanueva A, Roayaie S, Ferrer J, Tabak B, et al. Integrative molecular analysis of intrahepatic cholangiocarcinoma reveals 2 classes that have different outcomes. Gastroenterology. 2013 Apr; 144(4):829–40. [PubMed: 23295441]
76. Leone F, Cavalloni G, Pignochino Y, Sarotto I, Ferraris R, Piacibello W, et al. Somatic mutations of epidermal growth factor receptor in bile duct and gallbladder carcinoma. Clin Cancer Res. 2006 Mar 15; 12(6):1680–5. [PubMed: 16551849]
77. Tannapfel A, Sommerer F, Benicke M, Katalinic A, Uhlmann D, Witzigmann H, et al. Mutations of the BRAF gene in cholangiocarcinoma but not in hepatocellular carcinoma. Gut [Research Support, Non-US Gov't]. 2003 May; 52(5):706–12.
78. Tannapfel A, Weinans L, Geissler F, Schutz A, Katalinic A, Kockerling F, et al. Mutations of p53 tumor suppressor gene, apoptosis, and proliferation in intrahepatic cholangiocellular carcinoma of the liver. Dig Dis Sci. 2000 Feb; 45(2):317–24. [PubMed: 10711445]
79. Leelawat K, Narong S, Wannaprasert J, Ratanashu-ek T. Prospective study of MMP7 serum levels in the diagnosis of cholangiocarcinoma. World J Gastroenterol. 2010 Oct 7; 16(37):4697–703. [PubMed: 20872971]
80. Uenishi T, Yamazaki O, Tanaka H, Takemura S, Yamamoto T, Tanaka S, et al. Serum cytokeratin 19 fragment (CYFRA21–1) as a prognostic factor in intrahepatic cholangiocarcinoma. Ann Surg Oncol. 2008 Feb; 15(2):583–9. [PubMed: 17955299]
81. Enjoji M, Yamaguchi K, Nakamuta M, Nakashima M, Kotoh K, Tanaka M, et al. Movement of a novel serum tumour marker, RCAS1, in patients with biliary diseases. Dig Liver Dis. 2004 Sep; 36(9):622–7. [PubMed: 15460847]
82. Koopmann J, Thuluvath PJ, Zahurak ML, Kristiansen TZ, Pandey A, Schulick R, et al. Mac-2-binding protein is a diagnostic marker for biliary tract carcinoma. Cancer. 2004 Oct 1; 101(7): 1609–15. [PubMed: 15378479]
83. Mao X, Chen D, Wu J, Li J, Zhou H, Wu Y, et al. Differential expression of fascin, E-cadherin and vimentin: Proteins associated with survival of cholangiocarcinoma patients. Am J Med Sci. 2013 Oct; 346(4):261–8. [PubMed: 23221510]
84. Yao X, Wang X, Wang Z, Dai L, Zhang G, Yan Q, et al. Clinicopathological and prognostic significance of epithelial mesenchymal transition-related protein expression in intrahepatic cholangiocarcinoma. Onco Targets Ther. 2012; 5:255–61. [PubMed: 23091390]
85. Huang Q, Liu L, Liu CH, Shao F, Xie F, Zhang CH, et al. Expression of Smad7 in cholangiocarcinoma: prognostic significance and implications for tumor metastasis. Asian Pac J Cancer Prev. 2012; 13(10):5161–5. [PubMed: 23244128]
86. Zhai B, Yan HX, Liu SQ, Chen L, Wu MC, Wang HY. Reduced expression of P120 catenin in cholangiocarcinoma correlated with tumor clinicopathologic parameters. World J Gastroenterol. 2008 Jun 21; 14(23):3739–44. [PubMed: 18595142]
87. Zhang C, Bai DS, Huang XY, Shi GM, Ke AW, Yang LX, et al. Prognostic significance of Capn4 overexpression in intrahepatic cholangiocarcinoma. PLoS One. 2013; 8(1):e54619. [PubMed: 23349941]
88. Iguchi T, Aishima S, Taketomi A, Nishihara Y, Fujita N, Sanefuji K, et al. Fascin overexpression is involved in carcinogenesis and prognosis of human intrahepatic cholangiocarcinoma: immunohistochemical and molecular analysis. Hum Pathol. 2009 Feb; 40(2):174–80. [PubMed: 18835624]

89. Gu FM, Gao Q, Shi GM, Zhang X, Wang J, Jiang JH, et al. Intratumoral IL-17(+) cells and neutrophils show strong prognostic significance in intrahepatic cholangiocarcinoma. Ann Surg Oncol. 2012 Aug; 19(8):2506–14. [PubMed: 22411204]

90. Higashi M, Yamada N, Yokoyama S, Kitamoto S, Tabata K, Koriyama C, et al. Pathobiological implications of MUC16/CA125 expression in intrahepatic cholangiocarcinoma-mass forming type. Pathobiology. 2012; 79(2):101–6. [PubMed: 22286058]

91. Wang Z, Zheng T, Wu Q, Wang J, Wu C. Immunohistochemical analysis of the mTOR pathway in intrahepatic cholangiocarcinoma. Neoplasma. 2012; 59(2):137–41. [PubMed: 22248270]

92. Lee D, Do IG, Choi K, Sung CO, Jang KT, Choi D, et al. The expression of phospho-AKT1 and phospho-MTOR is associated with a favorable prognosis independent of PTEN expression in intrahepatic cholangiocarcinomas. Mod Pathol. 2012 Jan; 25(1):131–9. [PubMed: 21874010]

93. Boonjaraspinyo S, Boonmars T, Wu ZL, Loilome W, Sithithaworn P, Nagano I, et al. Platelet-derived growth factor may be a potential diagnostic and prognostic marker for cholangiocarcinoma. Tumor Biol. 2012 Oct; 33(5):1785–802.

94. Huang XY, Ke AW, Shi GM, Ding ZB, Devbhandari RP, Gu FM, et al. Overexpression of CD151 as an Adverse Marker for Intrahepatic Cholangiocarcinoma Patients. Cancer. 2010 Dec 1; 116(23): 5440–51. [PubMed: 20715158]

95. Dong LW, Hou YJ, Tan YX, Tang L, Pan YF, Wang M, et al. Prognostic significance of Beclin 1 in intrahepatic cholangiocellular carcinoma. Autophagy. 2011 Oct; 7(10):1222–9. [PubMed: 21654208]

96. Zhou JX, Li Y, Chen SX, Deng AM. Expression and prognostic significance of cancer-testis antigens (CTA) in intrahepatic cholagiocarcinoma. J Exp Clin Cancer Res. 2011; 30:2. [PubMed: 21211023]

97. Shafizadeh N, Grenert JP, Sahai V, Kakar S. Epidermal growth factor receptor and HER-2/neu status by immunohistochemistry and fluorescence in situ hybridization in adenocarcinomas of the biliary tree and gallbladder. Hum Pathol. 2010 Apr; 41(4):485–92. [PubMed: 20040392]

98. Yoshikawa D, Ojima H, Iwasaki M, Hiraoka N, Kosuge T, Kasai S, et al. Clinicopathological and prognostic significance of EGFR, VEGF, and HER2 expression in cholangiocarcinoma. Br J Cancer. 2008 Jan 29; 98(2):418–25. [PubMed: 18087285]

99. Utispan K, Thuwajit P, Abiko Y, Charngkaew K, Paupairoj A, Chau-in S, et al. Gene expression profiling of cholangiocarcinoma-derived fibroblast reveals alterations related to tumor progression and indicates periostin as a poor prognostic marker. Mol Cancer. 2010; 9:13. [PubMed: 20096135]

100. Xu Y, Zhu M, Zhang S, Liu H, Li T, Qin C. Expression and prognostic value of PRL-3 in human intrahepatic cholangiocarcinoma. Pathol Oncol Res. 2010 Jun; 16(2):169–75. [PubMed: 19757198]

101. Sato Y, Harada K, Itatsu K, Ikeda H, Kakuda Y, Shimomura S, et al. Epithelial-mesenchymal transition induced by transforming growth factor-{beta}1/Snail activation aggravates invasive growth of cholangiocarcinoma. Am J Pathol. 2010 Jul; 177(1):141–52. [PubMed: 20489148]

102. Riener MO, Fritzsche FR, Clavien PA, Pestalozzi BC, Probst-Hensch N, Jochum W, et al. IMP3 expression in lesions of the biliary tract: a marker for high-grade dysplasia and an independent prognostic factor in bile duct carcinomas. Hum Pathol. 2009 Oct; 40(10):1377–83. [PubMed: 19467694]

103. Hashimoto N, Yachida S, Okano K, Wakabayashi H, Imaida K, Kurokohchi K, et al. Immunohistochemically detected expression of p27(Kip1) and Skp2 predicts survival in patients with intrahepatic cholangiocarcinomas. Ann Surg Oncol. 2009 Feb; 16(2):395–403. [PubMed: 19034576]

104. Lee SM, Lee KE, Chang HJ, Choi MY, Cho MS, Min SK, et al. Prognostic significance of CD44s expression in biliary tract cancers. Ann Surg Oncol. 2008 Apr; 15(4):1155–60. [PubMed: 18214619]

105. Karamitopoulou E, Tornillo L, Zlobec I, Cioccari L, Carafa V, Borner M, et al. Clinical significance of cell cycle- and apoptosis-related markers in biliary tract cancer: a tissue microarray-based approach revealing a distinctive immunophenotype for intrahepatic and extrahepatic cholangiocarcinomas. Am J Clin Pathol. 2008 Nov; 130(5):780–6. [PubMed: 18854271]

106. Aishima S, Nishihara Y, Iguchi T, Taguchi K, Taketomi A, Maehara Y, et al. Lymphatic spread is related to VEGF-C expression and D2–40-positive myofibroblasts in intrahepatic cholangiocarcinoma. Modern Pathol. 2008 Mar; 21(3):256–64.

Table 1

Genetic mutations in intrahepatic cholangiocarcinoma

Mutation	location	Type of alteration	Association	Freguency	reference
E-cadherin	iCCA	Activating mutations	DH	12%	(73)
EGFR	iCCA	Activating mutations	OS	0–15%	(74–76)
GNAS	iCCA	Activating mutations	OS	7.4%	(29)
IDH1/2	iCCA	Inactivating mutations	OS (after surgery)	10–28%	(8, 9, 12)
K-ras	iCCA	Activating mutations	OS	8–54%	(24–27)
BRAF	iCCA	Activating mutations	OS, LNM, ATS	1–22%	(74, 75, 77)
p53	iCCA	Inactivating mutations	OS, LNM, ATS	21–37%	(10, 31, 78)

DH, Differentiation histology; OS, Overall survival; LNM, Lymph node metastasis; ATS, Advanced tumor stage

Table 2

Candidate Markers in serum

Gene/protein	location	material	method	Association	reference
DKK1	iCCA	serum	ELISA	OS	(61)
WFA-L1CAM	iCCA	serum, bile	ELISA	Diagnosis	(57, 58)
A1BG/AFM ratio	CCA	serum	Western blot	Diagnosis, OS (after surgery)	(55)
SSP411	CCA	serum, bile	ELISA, Western blot	Diagnosis	(59)
MMP7	CCA	serum	ELISA	Diagnosis	(79)
CYFRA21-1	iCCA	serum	ECLIA	LNM, ATS, VI, IM	(80)
KL-6 mucin	iCCA	serum	ELISA	Diagnosis,	(53)
MUC5AC mucin	CCA	serum	sandwich ELISA	Diagnosis	(56)
hTERT mRNA	CCA	serum	Real time RT-PCR	Diagnosis	(54)
RCAS1	CCA	serum	ELISA	Diagnosis	(81)

OS, Overall survival; LNM, Lymph node metastasis; ATS, Advanced tumor stage; VI, Vascular invasion; IM, Intrahepatic metastases; ELISA, Enzyme-Linked ImmunoSorbent Assay

Table 3

Candidate Markers in bile

Gene/protein	location	material	method	Association	reference
WFA-L1CAM	iCCA	serum, bile	ELISA	Diagnosis	(57, 58)
SSP411	CCA	serum, bile	ELISA, Western blot	Diagnosis	(59)
Mac-2BP	CCA	bile	ELISA	Diagnosis	(82)

ELISA, Enzyme-Linked ImmunoSorbent Assay

Table 4

Candidate Markers in tissues

gene/protein	location	poor prognosis	compartment	method	Association	reference
Fibronectin	iCCA	positive	membranous	IHC	LNM, ATS	(44)
vimentin	iCCA	positive	membranous	IHC	ATS, DH	(44, 83, 84)
E-cadherin	iCCA	negative	membranous	IHC	LNM, ATS, DH, OS, NI	(44, 83–86)
β catenin	iCCA	negative	membranous	IHC	TS, DH, LNM	(44)
Fibronectin-β catenin	iCCA	positive/negative	membranous	IHC	LNM, OS	(44)
vimentin-β catenin	iCCA	positive/negative	membranous	IHC	DH, NI, LNM, ATS, OS	(44)
vimentin-E cadherin	iCCA	positive/negative	membranous	IHC	DH, NI, RMI, ATS	(44)
GATA6	CCA	positive	Nuclear	IHC	OS, OS (after surgery), LNM	(46)
Gli 1	iCCA	positive	Nuclear	IHC	OS (after surgical)	(47)
Capn4	iCCA	positive	cytoplasmic	IHC(Tissue microarray)	OS (after surgery), LNM, ATS	(87)
DKK1	iCCA	positive	cytoplasmic	IHC	OS (after surgery), ATS	(61)
Fascin	iCCA	positive	cytoplasmic, membranous	IHC	DH, VI, LNM, DM, OS	(83, 88)
IL-17	iCCA	positive	cytoplasmic	IHC(Tissue microarray)	OS (after surgery)	(89)
MUC16	iCCA	positive	cytoplasmic	IHC	OS (after surgery)	(90)
N-cadherin	iCCA	positive	membranous	IHC	VI	(84)
p-4EBP1	iCCA	positive	cytoplasmic	IHC	OS	(91)
p-AKT1	iCCA	negative	cytoplasmic	IHC	OS	(92)
PDGF	CCA	negative	cytoplasmic	IHC	OS, ATS, DM	(93)
p-mTOR	iCCA	negative	cytoplasmic, membranous	IHC	OS	(91)
PTEN	iCCA	negative	cytoplasmic	IHC	OS	(92)
Smad7	CCA	positive	cytoplasmic	IHC	LNM, NI, OS, OS (after surgery)	(85)
CD151	iCCA	positive	membranous	IHC(Tissue microarray)	OS, OS (after surgery), DH, VI, LNM	(94)
S100A4	CCA	positive	Nuclear	IHC	OS, OS (after surgery)	(49)
Beclin1	iCCA	negative	cytoplasmic	IHC	LNM, OS, OS (after surgery)	(95)
MAGE-A3/4	iCCA	positive	cytoplasmic	IHC	OS	(96)
c-Met	iCCA	positive	cytoplasmic	IHC	LNM, DH, OS, OS (after surgery)	(94)
EGFR	CCA	positive	membranous	immunohistochemistry FISH	OS, OS (after surgery)	(97, 98)
Periostin	iCCA	positive	stromal compartment	IHC	OS	(99)

gene/protein	location	poor prognosis	compartment	method	Association	reference
PRL-3	iCCA	positive	cytoplasmic	IHC	ATS, VI, LNM, OS	(100)
snail	CCA	positive	membranous	IHC	OS (after surgery), LNM	(101)
IMP3	CCA	positive	cytoplasmic	IHC(Tissue microarray)	OS	(102)
p27	iCCA	negative	Nuclear	IHC	OS (after surgery), LNM	(103)
Skp2	iCCA	positive	Nuclear	IHC	OS (after surgery)	(103)
CD44	CCA	positive	membranous	IHC	OS, ATS	(104)
P120-catenin	iCCA	negative	membranous	IHC	DH, ATS, OS	(86)
p16	CCA	negative	Nuclear	IHC(Tissue microarray)	OS	(105)
VEGF-C	iCCA	positive	cytoplasmic	IHC	OS (after surgical), LNM	(106)

LNM, Lymph node metastasis; DH, Differentiation histology; OS, Overall survival; ATS, Advanced tumor stage; VI, Vascular invasion; NI, Neural invasion; TS, Tumor size; DM, Distant metastasis; IM, Intrahepatic metastases; RMI, Resection margin involve; IHC, Immunohistochemistry; FISH, fluorescence in situ hybridization

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