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Cholangiocarcinoma: Molecular Pathways and Therapeutic Opportunities

Sumera Rizvi, MBBS[1], **Mitesh J. Borad, MD**[2], **Tushar Patel, MD**[3], and **Gregory J. Gores, MD**[1]

[1] Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, Minnesota

[2] Division of Hematology and Oncology, Mayo Clinic, Scottsdale, Arizona

[3] Departments of Internal Medicine, Transplantation and Cancer Biology, Mayo Clinic, Jacksonville, Florida

Abstract

Cholangiocarcinoma (CCA) is an aggressive biliary tract malignancy with limited treatment options and low survival rates. Currently, there are no curative medical therapies for CCA. Recent advances have enhanced our understanding of the genetic basis of this disease, and elucidated therapeutically relevant targets. Therapeutic efforts in development are directed at several key pathways due to genetic aberrations including receptor tyrosine kinase pathways, mutant IDH enzymes, the PI3K-AKT-mTOR pathway, and chromatin remodeling networks. A highly desmoplastic, hypovascular stroma is characteristic of CCAs and recent work has highlighted the importance of targeting this pathway via stromal myofibroblast depletion. Future efforts should concentrate on combination therapies with action against the cancer cell and the surrounding tumor stroma. As the mutational landscape of CCA is being illuminated, molecular profiling of patient tumors will enable identification of specific mutations and the opportunity to offer directed, personalized treatment options.

Keywords

cholangiocarcinoma; molecular pathogenesis; targeted therapy

Cholangiocarcinoma (CCA) is the most common primary biliary malignancy and accounts for 3% of all gastrointestinal malignances.[1] Cholangiocarcinomas arise from varying locations within the biliary tree, and are classified according to their anatomic origin into intrahepatic, perihilar, and distal cholangiocarcinoma. The second-order bile ducts are the anatomical boundary between intrahepatic CCA (iCCA) and perihilar CCA (pCCA), whereas the cystic duct is the point of distinction between pCCA and distal CCA (dCCA). Although these subtypes exhibit extensive desmoplasia and markers of cholangiocyte differentiation, each has a unique cancer biology and distinct therapeutic options.[1]

Address for correspondence Gregory J. Gores, MD, Department of Medicine and Physiology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905 (gores.gregory@mayo.edu)..

The desmoplastic response is marked by a prominent tumor microenvironment containing a striking aggregation of cancer-associated fibroblasts (CAF).[2] The desmoplastic stroma and the genetic heterogeneity of this malignancy pose therapeutic challenges.[3] The desmoplastic stroma may impede access of drugs to the tumor and promote potent survival signals. The genetic heterogeneity of the cancer precludes curative treatment options. Cholangiocarcinoma has an aggressive natural course with medial survival of less than 24 months after diagnosis.[4] Potentially curative surgery is an option for all three subtypes, albeit only for patients with early-stage disease. For a select group of patients with pCCA, neoadjuvant chemoradiation followed by liver transplantation is a consideration.[1] Currently, there are no curative medical therapies for CCA. Systemic chemotherapy with gemcitabine and cisplatin has become the practice standard for patients with advanced or metastatic disease. However, response to combination chemotherapy in CCA patients is typically limited, and the 5-year survival remains low.[5,6] An enhanced understanding of CCA carcinogenesis, tumor-stroma interactions, and key molecular pathways would herald targeted, individualized therapies with improvement in patient survival. We discuss the current knowledge regarding the genetic basis of this disease, essential molecular pathways involved in its carcinogenesis, and review potential targeted therapies that hold promise in CCA therapeutics, emphasizing a personalized medicine approach.

Inflammation and Carcinogenesis

A spectrum of malignancies including CCA arises in the background of chronic inflammation, which suggests that inflammation promotes carcinogenesis by imparting prosurvival signals and inducing genetic aberrations. Inflammatory pathways are not only key components in carcinogenesis, but also promote tumor invasion and migration. Primary sclerosing cholangitis (PSC), characterized by chronic biliary tract inflammation and liver injury, is the most common predis-posing condition for CCA in the Western world.[1] The incidence of CCA is particularly high in Southeast Asia, in part due to inflammation secondary to the occurrence of biliary infection by the hepatobiliary flukes *Opisthorchis viverrini* (*O. viverrini*) and *Clonorchis sinensis*. Another common CCA risk factor in Southeast Asia is hepatolithiasis, which is characterized by calculi occurring proximal to the hepatic duct confluence.[7] Prolonged biliary inflammation, cholestasis, and bacterial infections induced by these calculi also promote CCA development.[7]

Inflammatory cells promote oxidative stress, which can promote genetic aberrations. Inducible nitric oxide synthase (iNOS) activation by inflammatory cytokines contributes to nitrosative stress by generation of excess nitric oxide.[8] DNA repair enzymes are susceptible to nitric oxide-mediated nitrosylation. Consequently, iNOS activation results in inhibition of DNA repair proteins, and single-stranded, double-stranded, and oxidative DNA lesions.[8] Enhanced iNOS expression occurs not only in PSC, but also in CCA indicating that it is involved in tumor formation and progression.[8,9]

Oxidative stress via generation of oxysterols from biliary cholesterol creates a milieu favorable for tumor development and progression. Oxysterols are cholesterol oxidation products present in human bile, and are increased in the bile of patients with biliary tract

inflammation.[10,11] Oxysterols are activators of the Hedgehog signaling pathway, a key developmental pathway implicated in CCA cell proliferation, migration, and invasion.[1,12,13]

Cyclooxygenase-2 is induced by various proinflammatory cytokines, and has been implicated in the carcinogenesis of CCA.[14] Oxysterols, bile acids, and iNOS all stimulate over-expression of cyclooxygenase-2.[14,15] Secondary bile acids in bile may also contribute to CCA biology. In addition to inducing cyclooxygenase-2, bile acids also activate receptor tyrosine kinases, such as epidermal growth factor receptor (EGFR), which mediate cell proliferation.[14]

Genetic Studies to Date

Recent advances in genome-wide technologies have provided deeper insight into the genetic basis of this challenging malignancy.[16] A recent whole-exome sequencing analysis of eight liver *O. viverrini*-related tumors and matched normal tissue identified 206 somatic mutations in 187 genes.[17] Of these, 15 genes were selected for validation in another 46 *O. viverrini*-related tumors. Somatic mutations were identified in genes with a known association with malignancy, such as tumor protein 53 (*TP53*) found to be mutated in 44.4% of cases, Kristen ras sarcoma viral oncogene homolog (*KRAS*) mutated in 16.7% of cases, and *SMAD4* that was mutated in 16.7% of cases. In addition, mutations were found in several newly implicated oncogenes including *MLL3* (mutated in 14.8% of cases), *ROBO2* (9.3%), *RNF43* (9.3%), *PEG3* (5.6%), and *GNAS* (9.3%). The biological functions of these genes broadly include deactivation of histone modifiers, G-protein activation, and loss of genomic stability.[17] KRAS mutations were associated with a poor survival in this cohort of patients.[17] In another recent whole-exome sequencing analysis, 108 cases of *O. viverrini*-related tumors and 101 cases of non-*O. viverrini*-related tumors from Asia and Europe were profiled.[18] Recurrent somatic mutations in *BAP1* and *ARID1A* were identified. *BAP1* and isocitrate dehydrogenase 1 and 2 (*IDH1*, *IDH2*) were noted to be mutated more frequently in nonliver–fluke-related CCAs, and *TP53* mutations occurred with increased frequency in liver–fluke-related tumors.[18] These findings imply that genetic alterations may vary according to etiological associations. Inactivating mutations of chromatin-remodeling genes such as *BAP1*, *ARID1A*, and *PBRM1* were also identified in exome sequencing of 32 iCCAs.[19] These findings postulate a connection between epigenetic regulation and CCA.

Mutations in genes encoding protein tyrosine phosphatases, particularly *PTPN3*, have been recently reported in iCCA.[20] Whole-exome sequencing of seven pairs of iCCA tumors and the surrounding nontumor tissue identified mutations in nine genes encoding protein tyrosine phosphatases. A prevalence screen of 124 paired samples was then conducted and identified somatic mutations in at least one of these nine protein tyrosine phosphatase genes in 51.6% of iCCAs, including *PTPN3* mutations in 41.1% of iCCAs. Higher recurrence rates were noted in patients with tumors containing activating mutations of *PTPN3*.[20]

A small number of studies have investigated chromosomal aberrations in CCA. In a recent study, gene-expression profile, high-density single-nucleotide polymorphism array, and mutation analyses were performed in 149 cases of iCCA.[21] This analysis identified two main biological classes of CCA: the inflammation class and the proliferation class.

Activation of inflammatory pathways, cytokine over-expression, and STAT3 activation were hallmarks of the inflammation class, which comprised 38% of the analyzed iCCAs. Features of the proliferation class, consisting of 62% of the analyzed iCCAs, included activation of oncogenic signaling pathways (such as RAS, mitogen-activated protein kinase, and MET), *KRAS* and *BRAF* mutations, DNA amplifications at 11q13.2, and deletions at 14q22.1.[21] In a recent integrated genomic analyses of 104 iCCAs and 59 matched controls, unique subclasses of patients were identified on the basis of *KRAS* mutations, early recurrence, and overall survival time.[22] Genes regulating inflammation and proteasome activities were associated with a poor prognosis in these patients.[22]

Activating mutations of *KRAS* and loss-of-function mutations of TP53 are a common occurrence in CCA.[23] *TP53* mutations were reported in 21% of CCAs in a review of studies with an aggregate of 229 CCA patients.[24] Direct DNA sequencing analysis of 69 CCAs identified *KRAS* and *BRAF* mutations in 22% and 45% of tumors.[25] Mutations have also been reported in other genes, albeit less frequently, including *EGFR*, *PIK3CA*, *NRAS*, and *APC*.[1]

Pathways and Targeted Therapies

Current standardized regimens available for medical therapy in cholangiocarcinoma have limited effectiveness and considerable side effects. There is a crucial need for development of targeted therapies that interfere with growth and progression of cancer cells while sparing normal cells (Fig. 1).

Cytokines

Interleukin- (IL-) 6 is an inflammatory cytokine produced by cholangiocytes in the presence of inflammatory stimuli. It is also secreted by CCA cells and participates in survival and mitogenic signaling in these cells.[26,27] The phosphatidylinositol 3-kinase (PI3K)/AKT cell-signaling pathway also generates potent survival signals in several malignancies. AKT is strongly expressed and constitutively active in CCA. IL-6 upregulates the potent antiapoptotic Bcl-2 protein, myeloid cell leukemia sequence 1 (Mcl-1), via an AKT-dependent mechanism.[26] Mcl-1 is integral in tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) resistance in CCA.[28] Consequently, IL-6 inhibition reduces Mcl-1 expression and enhances TRAIL-mediated apoptosis.[26] IL-6 also acts via a signal transducer and activator of transcription- (STAT-) dependent mechanism to enhance Mcl-1 expression.[29] A suppressor of cytokine signaling 3 (SOCS) regulates the IL-6/STAT-3 signaling pathway in a feedback manner, and epigenetic silencing of SOCS-3 facilitates sustained IL-6/STAT-3 signaling with resultant enhanced Mcl-1 expression.[30] This information suggests several approaches to treating the inflammatory subtype of CCA. For example, neutralizing antibodies to IL-6 are in human clinical trials and may be therapeutic in CCA.[31,32] Likewise, inhibitors of the JAK kinases, which activate STAT3 downstream of IL-6 initiated signaling, are also being developed and warrant investigation in preclinical models of CCA (Fig. 2). Finally, analogous to the success of Bcl-2 antagonists in chronic lymphocytic leukemia, Mcl-1 antagonists could prove useful in treating CCA. Such antagonists are in development.[33,34] Sorafenib, albeit not a direct Mcl-1 inhibitor, induces apoptosis in cancer cells via downregulation and destabilization of Mcl-1.[35]

Notch Signaling Pathway and Inhibitors

The Notch signaling pathway has a central role in cell-fate determination during embryonic development in several organs including the biliary tree.[36] Notch dysregulation is implicated in inflammation and carcinogenesis. Notch expression can be induced by iNOS, and Notch-1 is upregulated in PSC and CCA.[37] Two preclinical studies utilizing cell fate-tracing techniques have demonstrated a role for Notch in conversion of mature adult hepatocytes into biliary precursors of iCCA.[38,39] Enhanced expression of the Notch intracellular domain (NICD) in hepatic cells is associated with iCCA occurrence.[40] Several inhibitors of the Notch pathway are in development. Notch signaling inhibition can occur by various mechanisms including inhibition of Notch receptor activation with γ-secretase inhibitors, inhibition of binding of the ligand with monoclonal antibodies, or inhibition of the transcriptional activity of NICD by blocking peptides (Fig. 3).[41]

Receptor Tyrosine Kinase Pathways and Inhibitors

Several growth factor tyrosine kinases are implicated in carcinogenesis and progression of CCAs. These include the ERBB family of receptor tyrosine kinases, fibroblast growth factor receptor (FGFR), and the hepatocyte growth factor (HGF) receptor, c-met.

The ERBB family of receptor tyrosine kinases is comprised of four different receptors, ERBB1 or EGFR, ERBB2 or HER-2/neu, and ERBB3 and 4.[42] The EGFR activation leads to downstream activation of mitogen-activated protein kinase (MAPK), a well-known oncogenic signaling pathway. Over-expression of ERBB2, an EGFR family member, has been linked to biliary epithelial tumor formation in mice.[43] Moreover, *ERBB2* amplification was recently described in a cohort of 100 CCA patients, with a reported prevalence of 3%.[44] Erlotinib, an EGFR inhibitor, has had limited success in human cholangiocarcinoma clinical trials.[45,46] This may partly be due to an insufficient understanding of EGFR signaling in the molecular pathogenesis of CCA, and failure to select a patient population overexpressing EGFR. Indeed, the reported prevalence of *EGFR* mutations and amplification is only 15% and 5%, respectively.[47] A recent molecular and genomic characterization of 104 surgically resected iCCAs demonstrated significant upregulation of HER2 signaling in tumors with the most malignant phenotype.[22] The HER2 upregulation was associated with poor prognosis and frequent coactivation of ERBB3 and EGFR.[22] Lapatinib, a dual inhibitor of EGFR and HER2, was significantly more effective in inhibition of CCA cell lines than trastuzumab, which selectively inhibits HER2.[22] Loss of function mutation in *ERRFI1*, an endogenous inhibitor of *EGFR*, *ERBB2*, *ERBB3*, and *ERBB4*, was detected in a patient with metastatic, treatment refractory iCCA.[48] Significant disease regression was observed in this patient after EGFR-targeted therapy with erlotinib.[48] Hence, the efficacy of ERBB inhibitors certainly merits further attention as we learn to select and stratify patients for targeted therapies (Fig. 1).

MET tyrosine kinase plays an integral role in carcinogenesis by promoting tumor invasion, protection from apoptosis, and angiogenesis.[49] Binding to HGF or scatter factor activates MET. HGF and MET expression is enhanced in CCAs, and is associated with activation of ERBB family members, especially ERBB2.[1] An integrative genomic analysis of iCCA identified a proliferation class of iCCA that is characterized by activation of oncogenic

signaling pathways such as MET.[21] *MET* amplification has been described in malignancies including gastric, esophageal, ovarian, and nonsmall cell lung cancer, and is associated with a poor clinical outcome.[50,51] *MET* amplification is also linked to resistance to EGFR and ERBB2 inhibitors and may predict sensitivity to MET inhibitors.[50,51] A recent study utilizing DNA sequencing of 28 iCCAs detected MET amplification in two cases (7%).[51] Crizotinib is a small molecule inhibitor of MET and ROS receptor tyrosine kinases. The U.S. Food and Drug Administration has approved crizotinib for the treatment of metastatic lung cancer in patients with anaplastic lymphoma kinase-positive tumors. Crizotinib, cabozantinib, and tivozanib have therapeutic potential in targeted cancer chemotherapy of CCA tumors with MET amplification (Fig. 1). Tivozanib is currently being evaluated in a phase II trial in adult patients with advanced hepatocellular carcinoma (ClinicalTrials.gov identifier: NCT01807156).

ROS1 Fusion Kinases and Inhibitors

ROS1 fusions proteins have a significant oncogenic role in several malignancies and have been recently identified in CCA.[52] Subsequent preclinical studies have validated fused-in-glioblastoma-c-ros-oncogene 1 (*FIG-ROS*) as a potent oncogene in a mouse model of CCA.[53] In this model, FIG-ROS mediated iCCA development was Kras dependent, and led to development of aggressive and metastatic tumors in the presence of Kras.[53] In tumors harboring *Kras* and *p53* mutations, FIG-ROS inactivation led to the inhibition of tumor growth. Accordingly, ROS1 inhibitors have potential as targeted therapy in a subset of patients harboring ROS1 fusion proteins. Crizotinib, a ROS1 inhibitor, is approved for use in select patients with non-small cell lung cancer. Recent reports have indicated that a specific ROS1 mutation, ROS1(G2032R), confers resistance to crizotinib. Foretinib, another ROS1 inhibitor, maintains clinical effectiveness in the setting of ROS1 domain mutations including ROS1(G2032R) (Fig. 1).[54]

FGFR Fusions and Inhibitors

FGFR is a receptor tyrosine kinase involved in a myriad of biological processes including cell transformation, angiogenesis, and tissue repair.[55] Fusions of the *FGFR* gene have been reported in solid cancers. Gene fusions are a class of driver mutations with an essential role in certain cancers, such as the BCR-ABL gene in chronic myeloid leukemia.[56] Chronic myeloid leukemia patients with the BCR-ABL fusion gene respond well to imatinib, a small-molecule kinase inhibitor, signifying the value of targeted therapy. Recently, *FGFR2-BICC1* gene fusion was described in two cases of CCA by Wu et al.[56] A high prevalence (13.6%) of *FGFR2* gene fusions was reported in a cohort of 102 CCA cases.[55] In addition to detecting several cases with *FGFR2-BICC1* fusions, a single case of a novel FGFR gene fusion, *FGFR2-AHCYL1*, was also identified in this cohort.[55] More recently, single cases of *FGFR2-MGEA5* and *FGFR2-TACC3* gene fusions have been described in iCCA.[48]

Overexpression of *FGFR2-BICC1* is associated with enhanced cell proliferation and altered cell morphology. Based on mechanistic studies, Wu et al proposed that FGFR fusion partners mediate oligomerizations, which initiates activation of the respective FGFR kinase in tumors harboring these mutations.[56] Only cells harboring the FGFR gene fusions were sensitive to FGFR inhibitors,[55,56] indicating a role for targeted FGFR kinase inhibition in

patients with tumors containing these gene fusions (Fig. 1). Indeed, FGFR genetic alterations have been reported to be the most significant predictor for sensitivity to the pan-FGFR inhibitor BGJ398.[57] BGJ398 and another small molecule FGFR inhibitor, PD173072, suppressed downstream MAPK signaling and oncogenic activity of FGFR fusion kinases.[55] Clinical efficacy of BGJ398 is currently being investigated in a phase I study in adult patients with advanced solid tumors harboring FGFR1/FGFR2 amplification or FGFR3 mutation (ClinicalTrials.gov identifier: NCT01004224). Targeted therapy with ponatinib, another pan-FGFR inhibitor, in an iCCA patient with the *FGFR2-MGEA5* fusion resulted in a decrease in tumor necrosis and levels of the tumor marker, CA 19-9.[48] Ponatinib also demonstrated preliminary antitumor activity with tumor-size reduction in an iCCA patient harboring the *FGFR2-TACC3* fusion.[48] Dovitinib, another small molecule FGFR inhibitor, was shown to be an effective and tolerable therapy in a phase II trial in patients with metastatic renal cell cancer.[58] Median progression-free survival and overall survival were 3.7 and 11.8 months, respectively.[58] Dovitinib is currently in a phase I study in combination with gemcitabine and capecitabine in patients with advanced solid tumors including pancreatic and biliary cancers (ClinicalTrials.gov identifier: NCT01497392).

IDH/IDH2 Mutations and Inhibitors

IDH1 and *IDH2* mutations have been reported in 10% to 28% of cholangiocarcinomas, occurring primarily in iCCA.[59] In a cohort of 94 CCA cases, the prevalence of *IDH1* and *IDH2* mutations was 15% and 7%, respectively.[60] *IDH* mutations were associated with clear cell change, poorly differentiated histology, and longer overall survival.[60] Similarly, in a separate cohort of 326 iCCA patients, *IDH* mutations had a prevalence of 10% and were associated with longer overall survival and longer time to recurrence.[61] Increased levels of p53 and DNA hypermethylation are observed in the setting of IDH-mutant tumors.[61] *IDH2* mutations are observed less frequently in iCCA with a prevalence of 27% compared with 73% prevalence of *IDH1* mutations.[59] A range of *IDH1/2* mutations exists and varies according to the IDH amino acid residues mutated. Arginine 132 (R132), *IDH1 R132C*, is mutated in 44% of iCCA cases whereas *IDH1 R132G* alteration is seen in 14% iCCAs containing mutated samples.[59] The mutated IDH enzymes convert α-ketoglutarate to 2-hydroxyglutarate (2-HG) rather than isocitrate, resulting in an approximate100-fold increase in levels of this oncometabolite in IDH-mutant tumors.[59] As 2-HG is present in negligible amounts in normal cells, it has utility as a biomarker in patients with IDH mutations who may be candidates for therapy with IDH inhibitors. This oncometabolite likely promotes carcinogenesis by affecting methylation changes of the genome.[59] Small molecule inhibitors of IDH1 and IDH2 are being developed.[62,63] AG1–5198, a selective IDH1 inhibitor, blocked the production of 2-HG in a dose-dependent manner and stunted the growth of IDH1-mutant gliomas cells, but not IDH1 wild-type cells.[62] The effects of IDH1 inhibition included increased expression of genes associated with gliogenic differentiation and demethylation of histone.[62] Similarly, treatment with AGI-6780, a selective IDH2 inhibitor, induced differentiation in IDH2-mutant hematological cell lines.[63]

Having demonstrated promise in preclinical studies, IDH inhibitors are now being investigated in clinical trials (Fig. 1). Preliminary results from an ongoing phase I trial of AG-221, a first in class inhibitor of mutated IDH2, have indicated promising clinical activity

without dose-limiting toxic effects in patients with IDH-mutant driven hematological malignancies (ClinicalTrials.gov identifier: NCT01915498). Clinical benefit has been noted in 7 out of 10 patients in this study thus far. AG-120, a specific IDH1 inhibitor, is the subject of a phase I study in patients with IDH1-mutant associated advanced solid tumors including cholangiocarcinoma (ClinicalTrials.gov identifier: NCT02073994).

PI3K-AKT-mTOR Pathway

Binding of growth factors, such as HGF and FGF, to their respective receptor tyrosine kinases leads to activation of the PI3K cell signaling pathway. Subsequent AKT activation leads to phosphorylation and activation of the mammalian target of rapamycin (mTOR) pathway.[47] Deregulation of the PI3K-AKT-mTOR pathway fosters tumor development, cell proliferation and survival, tumor invasion, and angiogenesis. PIK3CA mutations were identified in 5 of 94 resected CCA specimens using MassARRAY technology.[47] In a cohort of Chinese CCA patients, *PIK3CA* mutations were identified in 34.2% of cases.[64] Exome sequencing of 32 iCCAs detected somatic mutations in several members of the PI3K pathway including *PIK3CA*, *PIK3C2A*, *PIK3C2G*, and phosphatase and tensin homolog (*PTEN*).[19] Loss of the lipid phosphatase PTEN results in constitutive activation of this pathway. In addition to mutations of *PTEN*, the oncomiR miR-21 inhibits expression of PTEN in CCA cells causing enhanced AKT/mTOR signaling.[65] Various PI3K pathway inhibitors are under investigation in multiple clinical trials of human cancer (Fig. 1).[66] GDC-0980, an orally bioavailable potent inhibitor of class I PI3K and mTOR kinase, and BAY 80–6946, a highly selective and potent pan-class PI3K inhibitor, are entering clinical studies.[67,68] A phase I trial of everolimus, an mTOR inhibitor, in combination with gemcitabine and cisplatin demonstrated promising clinical activity in patients with treatment refractory solid tumors including cholangiocarcinoma.[69]

KRAS Mutations

Mutations in *KRAS* have been frequently described in CCA, along with other mutations in *NRAS*, *BRAF*, and downstream MAPK effector pathways.[23,25] The proliferation subclass reported by Sia et al was notable for MAPK pathway activation.[21] Strategies to therapeutically target tumors with *KRAS* mutations have focused on targeting downstream effector pathways of KRAS such as Raf/MEK/ERK and PI3K/AKT. Phase II studies of a MEK inhibitor in a mixed population of biliary tract cancers that included CCA have shown some promise (Fig. 1), and studies of sorafenib are underway (SWOG0514). Combination therapy targeting both Raf/MEK/ERK and PI3K/Akt effector pathways similar to that reported for other *KRAS* dependent tumors is rational, but this approach may be limited by toxicities. Direct inhibitors of KRAS may offer promise if they become available in the future.

BAP1 Mutation and HDAC Inhibitors

Somatic mutations in *BAP1*, a chromatin-remodeling gene, have previously been reported in renal cell carcinoma, but not in CCA until recently.[19] In a cohort of 32 iCCA cases, the prevalence of inactivating mutations in *BAP1* was 25%.[19] Patients with these mutations had a trend, albeit not statistically significant, toward shorter survival times.[19] In a separate

cohort of 209 CCAs, *BAP1* mutations were detected in 10.5% of non-liver–fluke-related CCAs, compared with 2.8% of liver– fluke-related CCAs.[18] Functional studies demonstrated a tumor suppressor role for this gene.[18] The discovery of this mutation expands the mutation spectrum of CCA and heralds a role for drugs targeting chromatin remodeling, such as the histone deacetylase (HDAC) inhibitors vorinostat and panobinostat (Fig. 1).[19,70]

Targeting CAFs in the Tumor Stroma

Cholangiocarcinomas have a characteristic hypovascular, desmoplastic stroma containing α-smooth muscle actin (α-SMA) positive cancer-associated fibroblasts (CAFs).[71] Cancer-associated fibroblasts promote tumor progression and migration by production of factors including matricellular proteins, growth factors, chemokines, and matrix metalloproteinases. These factors enhance proliferation, tumor invasion and migration, and induce a profibrogenic response.[1] A fibrogenic matrix precedes CCA development and fosters malignant growth.[72] It has been postulated that the desmoplasia characteristic of CCA may serve as a niche fostering tumor development rather than as a response to the tumor.[2,72] This notion underscores the importance of fibrosis in CCA carcinogenesis, and supports the potential of antifibrotic therapies in CCA chemoprevention. Preclinical studies have demonstrated a reduction in fibrosis and carcinogenesis in CCA with 1D11, a transforming growth factor β (TGF-β) antagonist, as well as curcumin, a nutraceutical agent.[73,74] Further preclinical and clinical studies are needed to explore the role of antifibrotic therapies in CCA chemoprevention.

Recent work has highlighted the strong potential of selective targeting of CAF in CCA chemotherapeutic efforts (Fig. 4).[75] The BH3 mimetic, Navitoclax, significantly enhanced selective CAF apoptosis, decreased expression of α-SMA, and reduced tumor burden and metastasis while improving survival in an orthotopic model of CCA.[75]

Paracrine sonic Hedgehog signaling, a developmental pathway, promotes the desmoplastic response in CCA.[2] Such signaling has a well-established role in pancreatic adenocarcinoma. Reduction of the desmoplastic response via Hedgehog inhibition increased intratumor vascular density, and the intratumoral concentration of gemcitabine, contributing to transient stabilization of the disease.[76] Clinical studies using Hedgehog antagonists as combination therapy are underway in pancreatic cancer.[2,77] Although results to date have been disappointing in pancreatic cancer, one may still envision a similar role for Hedgehog inhibition in combination chemotherapy in CCA.

Paracrine EGF signaling has also been implicated in tumor-stroma crosstalk. Myofibroblast-derived EGF induced EGFR activation with enhanced migratory and invasive properties in CCA cells.[78] An EGF receptor inhibitor, gefitinib, abolished these effects, underscoring the potential of EGFR pathway targeting in CCA. Several studies have demonstrated paracrine platelet-derived growth factor (PDGF) signaling in CCA. Myofibroblast-derived PDGF-BB protects CCA cells from TRAIL-induced apoptosis via a Hedgehog-dependent mechanism.[79] Inhibition of the PDGF receptor, PDGFR-β, with imatinib induced CCA cell apoptosis.[79] Further preclinical studies demonstrated a reduction in tumor size with imatinib treatment in a rodent model of CCA.[80] Despite these promising preclinical studies, erlotinib

and imatinib have had been relatively disappointing in limited clinical studies in human CCA.[81]

Summary

Advances in the molecular and genetic basis of cancer and the ongoing development of tumor-specific therapeutics has heralded the era of personalized or precision medicine. Significant progress has been made in understanding the mutational landscape of cholangiocarcinoma, a notoriously difficult-to-treat malignancy. Continued dissection of the key pathways and genetic drivers of CCA progression will direct efforts at individualized therapy for patients based on the driver mutation for their particular tumor. Limited mutation-directed therapeutic efforts in CCA have been encouraging. Tumor immunotherapy is another frontier in cancer treatment. Inhibition of immune receptors such as programmed cell death 1 has shown promise in certain malignancies.[82] Although cholangiocarcinoma is a genetically heterogeneous cancer, its stroma is more genetically uniform. Preclinical studies have demonstrated tumor regression and enhanced survival with selective deletion of CAF from the CCA tumor microenvironment.[75] Therefore, the stroma represent an additional potential target in the treatment of CCA. Further work is needed to direct development of combination therapies, which will target not only the cancer cell, but also the cancer stroma in CCA.

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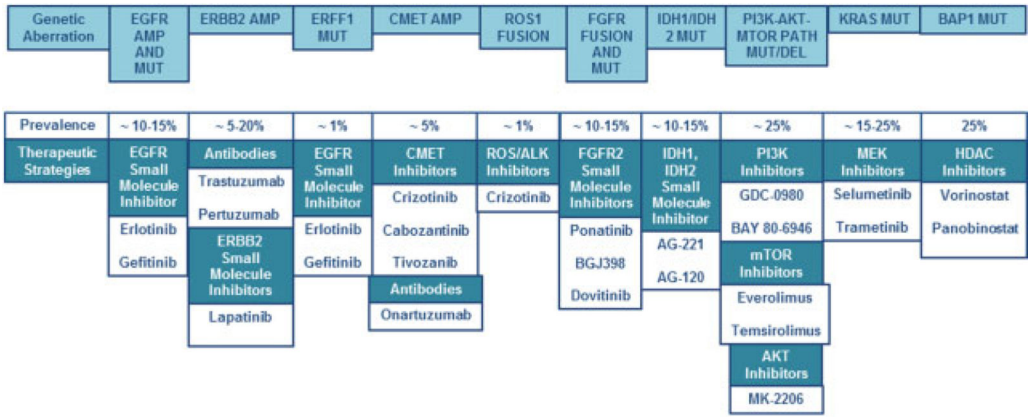

Fig. 1.

Molecular pathways in cholangiocarcinoma (CCA) and inhibitors. Several molecular pathways are dysregulated in CCA. Inhibitors targeting these pathways are entering human clinical trials. The prevalence of each genetic aberration and potential therapeutic strategies are listed.

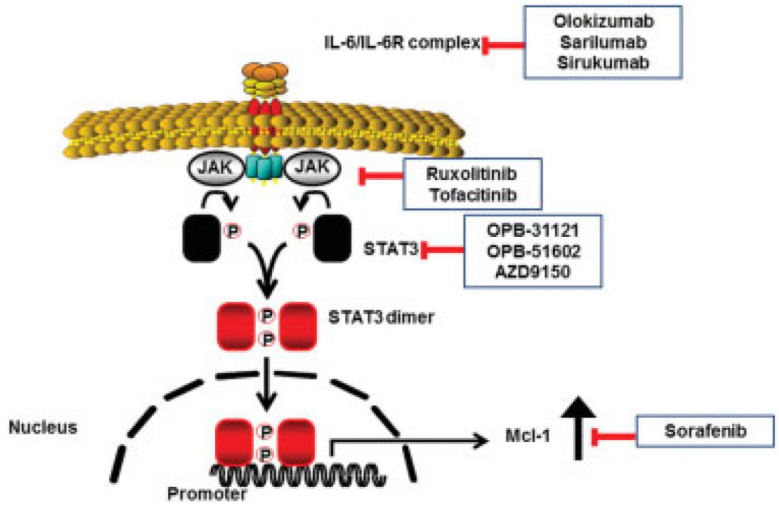

Fig. 2.

IL-6/STAT3 signaling and inhibitors. Binding of IL-6 to its receptor activates Janus kinase (JAK) with subsequent phosphorylation, activation, dimerization, and nuclear translocation of STAT3. STAT3 upregulates Mcl-1 transcription. Inhibitors of IL-6, JAK, STAT3, and Mcl-1 are currently in development.

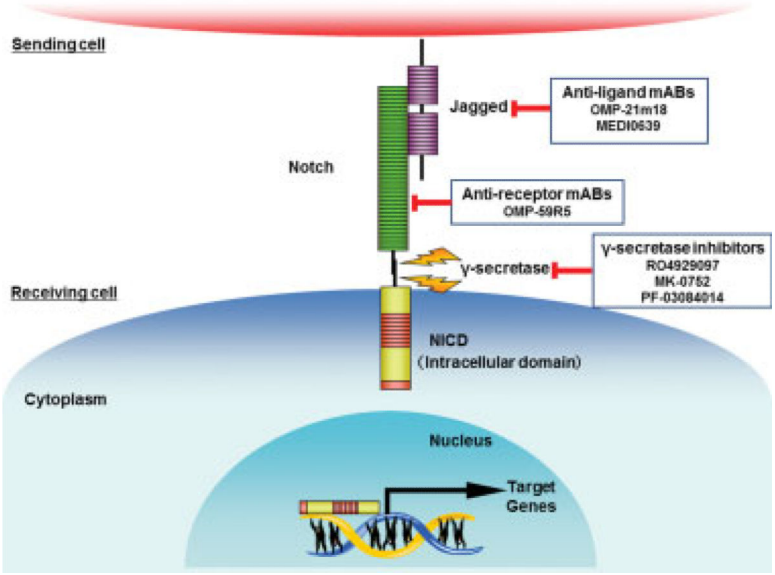

Fig. 3.

Notch signaling pathway and inhibitors. The Notch signaling pathway has been implicated in cholangiocarcinoma (CCA) carcino-genesis. Enhanced expression of NICD occurs in CCA. Several inhibitors of the Notch pathway are currently in development. These include inhibitors of γ-secretase, a key enzyme in the Notch pathway, as well as monoclonal antibodies (mABs) targeting the Notch receptors and ligands.

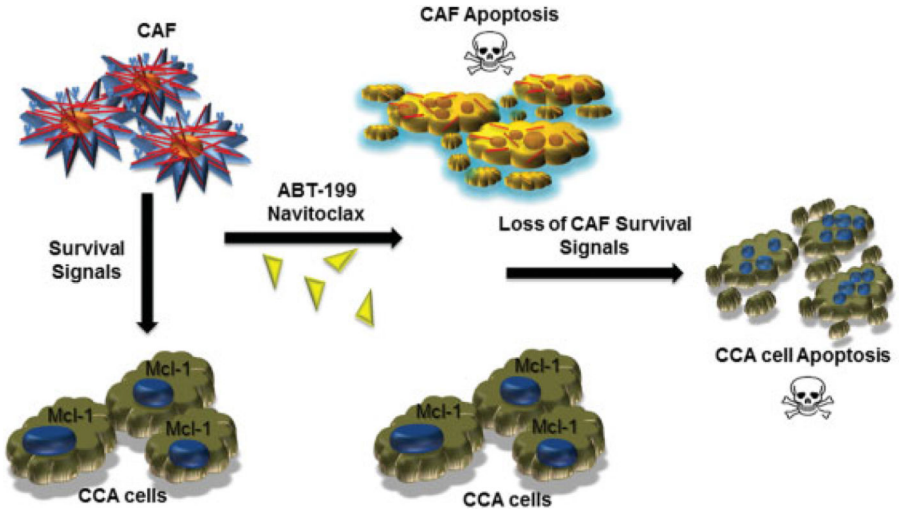

Fig. 4.

Selective deletion of cancer-associated fibroblasts (CAFs) from cholangiocarcinoma (CCA) tumor microenvironment. Cancer-associated fibroblasts produce factors that promote survival of tumor cells. Cancer-associated fibroblasts are primed or sensitized for apoptosis and hence can be selectively targeted by BH3 mimetics such as navitoclax and ABT-199. Cholangiocarcinoma cells overexpress Mcl-1, which does not bind navitoclax, and hence are not a direct target of navitoclax. However, CAF deletion from the tumor microenvironment leads to secondary cancer cell death due to loss of CAF-mediated prosurvival signals.[75]