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Genomics of gallbladder cancer: the case for biomarker-driven clinical trial design

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Abstract

Background and aims—Gallbladder carcinoma is a rare, aggressive malignancy of the biliary tract associated with a poor prognosis. Despite the deployment of targeted therapies that have demonstrated marked survival benefits in many tumor types, traditional cytotoxic chemotherapy has remained the mainstay of treatment for unresectable and metastatic gall-bladder cancer.

Methods—Systematic review of ongoing and prior clinical studies shows a paucity of biomarker-driven therapeutic trials using targeted agents in gallbladder cancer. In fact, over the past 6 years, of the 38 therapeutic biliary tract protocols listed on clinicaltrials.gov, only 6 (21 %) utilized targeted therapies based upon tumor biomarkers or genomics. Now that we have entered the era of next-generation sequencing and precision medicine, we are beginning to identify common and specific genetic alterations in gallbladder carcinomas.

Results—A review of the literature reveals alterations in ARID1A, BRAF, CDKN2A/B, EGFR, ERBB2-4, HKN-RAS, PIK3CA, PBRM1, and *TP53*. Given the widespread use of tumor genomic profiling and the fact that most of the aforementioned alterations are pharmacologically tractable, these observations suggest the potential for new therapeutic strategies in this aggressive malignancy.

Conclusions—Taken together, further understanding of the genomic landscape of gallbladder cancer coupled with biomarker-driven clinical trials that match therapies to targets are urgently needed.

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Compliance with ethical standards

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Keywords

Biliary tract cancers; Cholangiocarcinoma; Gallbladder cancer; Targeted therapy

1 Introduction

Biliary tract tumors are aggressive malignancies that arise from biliary epithelium. These can be subclassified according to their location in the biliary tree or gallbladder. Thus, bile duct tumors may be further characterized as intrahepatic (intrahepatic cholangiocarcinoma, IHCC) or extrahepatic (extrahepatic cholangiocarcinoma, EHCC), which may be further subdivided into perihilar (Klatskin tumors) and distal cholangiocarcinomas. Together, these cancers constitute a rare set of malignancies with poor prognoses, because patients frequently present in later stages, and systemic chemotherapeutic regimens generally lack significant response rates. As a result, treatment goals are often palliative in nature [1, 2].

Due to the rarity of these malignancies, as well as their common cell of origin, the biology underlying the promotion and progression of these tumors has been studied as one disease entity (Fig. 1). In turn, treatment for all biliary tract cancers has been identical. But, with fairly recent developments in next-generation sequencing (NGS) and other molecular techniques, differentiation between these tumor entities has demonstrated that each tumor type (i.e., gallbladder cancer (GBCA), IHCC, and EHCC) has a unique somatic genomic landscape. As a result, examination of these molecular signatures may be important for identifying drugs targeting specific pathways that may be utilized in precision medicine approaches, as well as biomarker-driven clinical trial design [3]. In this review, we will focus upon the treatment of biliary tract tumors with traditional systemic chemotherapy and then provide an overview of the genomic alterations present in GBCA, as well as their implications for personalized targeted treatments.

2 Traditional therapy for biliary tract carcinomas

Due to the insidious nature of the disease, most biliary tract tumors are diagnosed at an advanced stage and are often unresectable or metastatic at presentation. Cytotoxic chemotherapy remains the mainstay of treatment for advanced disease (Table 1) [4–7]. The National Comprehensive Cancer Network (NCCN) guidelines [8] consider gemcitabine combined with cisplatin to be standard of care first-line chemotherapy for patients with biliary tract cancers based upon results of the largest randomized controlled phase III ABC-02 trial to date, which showed improved median overall survival with combination therapy versus gemcitabine alone (11.7 versus 9 months) [6]. Other active chemotherapy regimens include (1) gemcitabine with oxaliplatin or capecitabine, (2) capecitabine with cisplatin or oxaliplatin, (3) fluorouracil with cisplatin or oxaliplatin, (4) single-agent fluorouracil, (5) single-agent capecitabine, or (6) single-agent gemcitabine [9–11]. Across these various regimens containing these three drugs, response rates are generally in the range of 10 to 30 %, and prognosis remains poor with median overall survival times being generally less than 1 year. More recently, targeted therapies (e.g., panitumumab, cetuximab,

and erlotinib) have been studied in unselected metastatic and locally advanced patients with response activity statistically not significant (Table 2) [12–28].

As previously described, biliary tract tumors have often been grouped together in clinical trials and treatment algorithms, such as the NCCN guidelines [8]. However, they are distinct biologically and genomically. In line with this concept, differences in the epidemiology of GBCA and cholangiocarcinoma reflect the underlying differences in the factors driving the genesis of these cancers [29, 30].

3 Distinguishing the genomic landscape of biliary tract carcinomas

Gene sequencing has rapidly evolved over the last decade. As compared to older methodology, which relied upon sequencing one gene a time, more recent studies have utilized NGS to characterize tumors, detailing information on a multitude of genes known to be important in cancer signaling. Although frequently grouped together in biologic (Fig. 1), histologic, and clinical trial assignment, GBCA and cholangiocarcinomas have shared, but distinctive somatic genomic landscapes, suggesting that different treatment strategies are necessary for clinical trial design in each disease type.

The first study to begin delineating these differences was reported by Borger and colleagues in 2012 [31]. They studied 287 tumors from gastrointestinal cancer patients, including the biliary tract, colorectal, gastroesophageal, hepatic, pancreatic, and small intestine carcinomas. They evaluated 15 known cancer genes for 130 site-specific gene mutations. Mutations were identified within several of these genes, including KRAS (35 %), *TP53* (22 %), *PIK3CA* (10 %), *BRAF* (7 %), *APC* (6 %), *NRAS* (3 %), *IDH1* (2 %), *AKT1* (1 %), *CTNNB1* (1 %), and *PTEN* (1 %). While *IDH1* mutations were rare in other common gastrointestinal malignancies, they were identified in 3 tumors (25 %) of an initial series of 12 biliary tract carcinomas. In order to better define both *IDH1* and *IDH2* mutations, an additional 75 bile duct cancers making 87 total tumors (IHCC (N = 40), EHCC (N = 22) and GBCA (N = 25)) were examined (Table 3). On subset analysis, only IHCC (9 of 40, 23 %) had *IHD1* (20 %) or *IDH2* (3 %) mutations, whereas none were identified in EHCC or GBCA. In contrast, *KRAS* (23 %) and *TP53* (14 %) predominated in EHCC, while *PIK3CA* (12 %) mutations were the most common in GBCA. For this first time, this study began to molecularly distinguish the biliary tract cancers, as well as identified potentially new targets for therapy, representing a major paradigm shift in the field.

With the evolution of personalized medicine, many more patients are having molecular analyses performed on their tumors. Two large studies analyzing biliary tract carcinomas that utilized different molecular platforms were recently presented [32, 33]. Together, these studies provide further insight into the distinct molecular alterations identified in each sub-type. In the first study, 815 cases (IHCC (N = 434), EHCC (N = 126), GBCA (N = 244), and not otherwise specified (N = 11)) were evaluated using a commercial multiplatform profiling service (Caris Life Sciences) [32]. Testing included sequencing and protein expression analysis (IHC). In this analysis, 24 of 47 genes tested had mutations, with the highest rates in *TP53* (28 %), *KRAS* (18 %), *IDH1* (9 %), and *SMAD4* (6 %). In cholangiocarcinomas, *BRCA1* and *BRCA2* mutations were seen in 7.3 and 12.5 % of cases, respectively. But, they

were not observed in GBCA. On further analysis of GBCA (Table 3), TP53 (41 %) mutations were the most common. GBCA (15 %) also had high human epidermal growth factor receptor 2 (HER2) overexpression. Finally, GBCA showed a high frequency of PBRM1 underexpression (53 %). Therefore, multiplatform cancer profiling revealed additional distinct biomarker characteristics of biliary tract carcinomas that offer insights into disease biology and potential therapeutic interventions.

In the second study, 554 cases (IHCC (N = 412), EHCC (N = 57), and GBCA (N = 85)) were evaluated using a commercial comprehensive genomic profiling (CGP) service (Foundation Medicine) [33]. CGP was performed on hybridization-captured, adaptor ligation-based libraries to a mean coverage depth of >600× for 3230 exons of 182 cancer-related genes plus 37 introns from 14 genes frequently rearranged in cancer. All three biliary tract carcinoma subtypes shared genomic alterations in cell cycle regulation (e.g., *CDKN2A/B* loss, 17–19 %) and chromatin remodeling (*ARID1A*, 12–17 %). GBCA had high *ERBB2* (i.e., HER2 or HER2/neu) amplification rates (11 and 16 %, respectively). Moreover, *PIK3CA* (14 %) mutations were the most common in GBCA consistent with the Borger study [31]. Taken together, the NGS data demonstrate that GBCA have frequent *PIK3CA* and *TP53* mutations, EHCC have frequent *ARID1A*, *KRAS*, and *TP53* mutations, and IHCC have frequent *ARID1A, BAP1, IDH1, KRAS, PBRM1*, and *TP53* mutations. Therefore, there is a diverse somatic landscape of genomic alterations in biliary tract cancers that can serve to distinguish them, as well as may provide clinically rational targets for therapies. Herein, we focus upon the genomic aberrations that are most common and recurrently identified in GBCA, with particular attention to those that are clinically relevant as they are potentially actionable.

4 Genomic alterations in gallbladder cancer

4.1 APC

Adenomatous polyposis coli (*APC*) encodes a tumor suppressor protein that acts as an antagonist of the Wnt/β-catenin signaling pathway. It is also involved in cell migration, adhesion, transcriptional activation, and apoptosis. Genomic alterations in *APC* have been reported in 4 % of GBCA cases in one study (Table 3) [31]. Wnt pathway inhibitors are under development or in clinical trials for several tumor types. Celecoxib and sulindac are FDA-approved drugs that inhibit the Wnt/β-catenin pathway [36].

4.2 ARID1A

ARID1A encodes a member of the switch/sucrose nonfermentable (SWI/SNF) family. SWI/SNF is a chromatin remodeling complex that includes three putative DNA binding subunits (ARID1A, ARID1B, and PBRM1). *ARID1A* is aberrant (usually lost) in about 13 % of patients with GBCA [33]. The SWI/SNF genes have helicase and ATPase activities that regulate transcription through chromatin remodeling. Therefore, the SNF/SWI complex is required for transcriptional activation of genes normally repressed by chromatin. In addition, the C terminus of *ARID1A* can stimulate glucocorticoid receptor-dependent transcriptional activation. Moreover, when *ARID1A* expression is lost, activation of the PI3K/AKT/mTOR pathway is more frequent; increased microsatellite instability by

epigenetic silencing of the *MLH1* gene has also been reported [37]. The latter could be of interest because recent data suggest that immune checkpoint inhibitors (e.g., anti-PD-L1 therapies) are more effective in tumors with microsatellite instability, a condition that results from impaired DNA mismatch repair [38].

4.3 BRAF

BRAF is a member of the Raf family of serine-threonine kinases, as well as is a component of the MAPK (RAS-RAF-MEK-ERK) pathway involved in cellular growth signaling. Mutations in *BRAF* have been observed in about 1–6 % of gallbladder cancers [33, 34]. While the application of the *BRAF* inhibitors vemurafenib and dabrafenib for melanomas, which frequently harbor the *BRAF* V600E mutation, has resulted in high response rates [39–41], the use of *BRAF* inhibitors in many non-melanomatous cancers is a very active area of ongoing investigation.

4.4 CDKN2A/B

Cyclin-dependent kinase inhibitor 2A and cyclin-dependent kinase 4 inhibitor B (*CDKN2A/B*) losses were seen in about 6–19 % of gallbladder cancers [33, 34]. These genes lie in adjacent regions, leading to the co-occurrence of deletions. *CDKN2A* is a tumor suppressor gene that encodes both $p16^{INK4A}$, which modulates cyclin-dependent kinases CDK4 and CDK6, and $p14^{ARF}$, which prevents TP53 degradation. *CDKN2B* is a tumor suppressor gene that encodes both $p15^{INK4B}$, which modulates CDK4 and CDK6, and prevents cyclin D activation. Loss of *CDKN2A/B* results in the activation of the CDK4/6 complex with resultant cyclin D, Rb, and E2F activation (60), which regulates cell cycle progression. Inhibition of CDK4/6 with drugs like palbociclib may have activity in patients with loss of CDKN2A function [42]. This agent is currently FDA-approved for estrogen receptor-positive advanced breast cancer and currently being studied both alone and in combination with other agents in multiple other solid tumor types [43].

4.5 CTNNB1

Catenin (cadherin-associated protein) beta-1, also known as β-catenin, is a protein that is encoded by *CTNNB1*. Genomic alterations in this gene have been reported in 4 % of GBCAs in one study (Table 3) [31]. The protein encoded by *CTNNB1* is part of the adherens junction complex. These junctions are necessary for the creation and maintenance of epithelial cell layers. The encoded protein also anchors the actin cytoskeleton and may transmit contact inhibition signals that cause cells to stop dividing once the epithelial layer is complete. Finally, *CTNNB1* protein binds to the product of the *APC* gene and is an integral part of the canonical Wnt/β-catenin signaling pathway. Again, Wnt pathway inhibitors are under development or in clinical trials for several tumor types. At present, celecoxib and sulindac are only FDA-approved drugs that may inhibit the Wnt/β-catenin pathway [36].

4.6 *HER* family genes

HER family genes include *EGFR* (i.e., epidermal growth factor receptor or *HER1*), which is mutated altered in about 3.9 % of GBCA [34], but overexpressed in about 38.5 % of cases [44]; *ERBB2* and *ERBB3* are altered in about 10–16 and 12 % of GBCA, respectively [34,

35]. In fact, in one study of 57 patients, ERBB signaling (including *EGFR, ERBB2, ERBB3, ERBB4*, and their downstream targets, such as *NRG1*) was mutated in 36.8 % of tumors [34]. Moreover, these gene aberrations may confer a worse prognosis. Given the relatively infrequency of EGFR mutations/amplifications, clinical trials of EGFR inhibitor therapy in unselected patients have yielded low objective response rates [16–19], while no trials have been performed with *EGFR* genomic alterations as a biomarker for patient selection. High *ERBB2* amplifications rates are consistent with prior reports of *HER2* overexpression in biliary cancers [35, 44–47]. One study of *HER2* expression/amplification in 37,992 patients included 194 patients with GBCA. Of the subset, 9.8 % had HER2 overexpression (IHC) with a 92 % concordance with fluorescence *in situ* hybridization [35]. Preclinical experiments suggest that targeting HER2 induces apoptosis and inhibition of subcutaneous biliary tract tumors [48]. To date, small studies with lapatinib in unselected patients with biliary tract cancers have been unsuccessful [20]. However, in subset analysis, 5/8 (62.5 %) patients with *HER2* amplification/overexpression attained a complete or partial response with HER2-directed treatment [49]. Because members of the HER family function in tandem, ERBB3 must dimerize with ERBB2 in order to be active. Because ERBB3 is altered in about 12 % of GBCAs, pan-HER inhibitors (e.g., afatinib), as well as anti-Her antibodies (e.g., pertuzumab), may be active in these patients because they disrupt ERBB2-ERBB3 dimerization.

4.7 RAS

Mutations in *KRAS* are seen in 4–13 % of gallbladder tumors [31–34]. *KRAS* is part of the RAS family and mutations lead to constitutive activation of the RAF-MEK-ERK in the MAP kinase pathway. Other RAS family genes, including *NRAS* and *HRAS*, have also been identified in GBCAs, albeit less frequently in up to 2–4 and 2 % of cases, respectively [31, 34]. Within the MAPK pathway, MEK functions as a key downstream effector of RAS. Recently, trametinib, a small-molecule MEK inhibitor, has been FDA-approved in melanoma and is undergoing ongoing clinical research in other cancer types [50]. Another MEK1/2 inhibitor, selumetinib, has been studied in unselected patients with metastatic biliary tract cancers [23]. Objective responses were observed in 3/28 (12 %) patients; however, patients were not selected by mutation status. It is noteworthy that this is close to the fraction (2.5–37.5 %) of KRAS mutations in four large studies of biliary tract cancers [31–33, 51].

4.8 PI3K/AKT/mTOR pathway genes

Mutations in *PIK3CA* activate the AKT/mTOR pathway and have been described in many malignancies types, including colon, breast, gastric, and brain cancers. Somatic mutations are less common in biliary tract cancers; *PTEN* and *PIK3CA* mutations were observed in about 1 and 12–14 % of GBCA, respectively [31–33]. The presence of these mutations may render tumors sensitive to PI3K specific inhibitors currently under investigation, as well as mTOR inhibitors, such as everolimus, temsorolimus, and rapamycin. Consistent with this, there is a published phase II data demonstrating activity of everolimus and rapamycin in biliary tract cancers progressing after chemotherapy [52].

4.9 PBRM1

SWI/SNF is a chromatin-remodeling complex and includes three putative DNA binding subunits (ARID1A, ARID1B, and PBRM1). *PBRM1* (or *BAF180*) acts as a tumor suppressor gene. In clear cell renal carcinoma, somatic mutations lead to aberrant chromatin biology [53]. In GBCA, immunohisto-chemistry shows that underexpression of PBRM1 occurs in about 53 % [32]. It is unclear if this gene aberration can be targeted. However, in light of emerging data about the role of immunotherapy and checkpoint inhibition in patients with mutations in mismatch repair genes that increase the rate of somatic mutations [38], impaired chromatin remodeling has the potential to be another source of genetic instability and potential application of anti-PD-1 or anti-PD-L1 therapies.

4.10 SMAD4

SMAD4 is a transcription factor and tumor suppressor gene in the TGF-β signaling pathway that is mutated in several cancer types. Following pathway activation, TGF-β receptor complex phosphorylates receptor-regulated SMADs, which subsequently accumulate in the nucleus and act as transcription factors. Genomic alterations in *SMAD4* are seen in about 6 % of GBCAs [32]. Targeting SMAD4 is challenging, because TGF-β plays a dual role in tumorigenesis. During initiation and early progression of the tumor, TGF-β serves as a tumor suppressor, which is supported by the fact that loss or function mutations in members of the TGF-β signaling pathway cause unregulated cell growth and cancer. However, in late stages of tumor progression, elevated levels of TGF-β promote tumor growth. Currently, there are no studies with TGF-β modulators or anti-SMAD4 agents.

4.11 TP53

Between 4 and 41 % of GBCAs harbor a mutation in *TP53*, a member of the TP53-MDM2-MDMX axis [32, 33]. It functions as a tumor suppressor gene involved in cellular processes, including gene expression, DNA repair, and apoptosis. Mutations in *TP53* result in cancer cell growth and immortality. To date, targeting *TP53* mutations has proved to be a challenge. Several strategies have been suggested: (1) Wee-1 kinase inhibitors together with DNA damaging agents, (2) inhibitors of the p53-MDM2 interaction, which result in p53 stabilization, and (3) vascular endothelial growth factor receptor (VEGFR)/VEGF-targeting agents in patients with *TP53* mutations [54].

Wee-1 is a serine/threonine protein kinase that phosphorylates cyclin-dependent kinase 1 (Cdk1). It functions at the G2/M checkpoint of mitosis. Preclinical studies have demonstrated that cancer cell viability can be attenuated after Wee-1 inhibition. Moreover, this is augmented when cells are treated in combination with a conventional DNA-damaging therapy (e.g., radiation and/or cytotoxic chemotherapy). Mitotic catastrophe results from premature entry into mitosis with unrepaired DNA damage. As such, cancer cells become sensitized to conventional therapy by Wee-1 inhibition especially cells with insufficient G1-arrest due to deficient p53 signaling [55]. MK-1775 is a small-molecule Wee-1 inhibitor that induces apoptosis in *TP53*-deficient cells when used in combination with DNA-damaging chemotherapeutic agents (e.g., gemcitabine, carboplatin, cisplatin) [56]. But, in a phase I study in combination with these agents, no objective responses were observed [57]. However, Wee-1 inhibitors remain in clinical investigation [58].

Another approach to targeting p53 is to use an MDM2 inhibitor, which would be active in the presence of wild-type p53. Interestingly, MDM2 overexpression is associated with poor prognoses in GBCA [59]. There are several small-molecule MDM2 inhibitors, such as analogs of MI-219 and Nutlin-3, which are in development.

Finally, a third approach to targeting mutant *TP53* has also been suggested based upon a retrospective study, which showed that *TP53*-mutant patients with advanced cancers had longer progression-free survival times when treated with bevacizumab-containing regimens, as compared to patients with wild-type *TP53* [54]. Furthermore, mutations in *TP53* have been correlated with increased *VEGF-A* mRNA expression, which is the target of bevacizumab [60].

5 Overview of clinical trials with targeted agents

The paradigms for treating advanced malignancies have changed over the past 60 years. Historically, chemotherapeutic agents have been used to treat numerous types of cancers. Scientists are now attempting to use the growing knowledge of cancer biology, genomics, and immune regulation to create targeted therapies that are less harmful to benign cells and more deadly to cancer cells. To date, molecular matching approaches performed in late-stage diseases have yielded critical insights into personalized cancer therapy [61–64]. Therefore, there is precedent for this methodology.

The concept that the identification of somatic alterations in GBCA (Table 3) can be paired with targeted agents against cognate genomic alterations has already been demonstrated to lead to higher response rates in multiple cancer types [3, 65]. For instance, the use of vemurafenib and trametinib in *BRAF V600E*-mutated melanoma patients has led to substantial survival improvements [39]. Similarly, targeting *EGFR* mutations and the *EML4-ALK* fusion product in lung cancer with erlotinib and crizotinib, respectively, has led to remarkably improved outcomes [66]. Finally, targeting the PI3K/AKT/mTOR pathway with cognate inhibitors used in combination (but not as single agents) resulted in stable disease for greater than 6 months and partial response rates of up to 45 % in individuals with *PIK3CA* mutations [63].

Despite these aforementioned studies in other diseases, a review of PubMed shows a limited number of published clinical trials using targeted agents in biliary tract cancers (Table 2). With the exception of one report, treatment was administered to an unselected population. The only trial that reported results in a selected population utilized the anti-EGFR antibody, panitumumab, in a *KRAS* wild-type population [22]. Because response rates in these studies were quite variable, it is difficult to assess the contribution of the targeted agent. Moreover, in several of the trials using targeted agents alone, response rates were quite low, suggesting the importance of biomarker selection. Indeed, recent large meta-analyses (~70,000 patients) suggest that matched targeted therapy results in improved outcomes, but that targeted therapy without matching often has low or negligible salutary effects [67, 68]. Further, the PFS in phase I trials of mainly matched targeted agents or local therapies in patients with advanced, refractory GBCA and cholangiocarcinoma were similar to that of the first, second, and last-line therapy with FDA-approved agents [3]. Furthermore, treatment with hepatic

arterial infused oxaliplatin, and inhibitors of angiogenesis, HER-2, or MEK resulted in a stable disease for more than 6 months with partial response in 28 % of heavily pretreated patients with biliary tract malignancies.

6 Ongoing trials

A search of clinicaltrials.gov for new biliary tract and related protocols registered during the past 6 years identified 38 protocols (search criteria: trials registered in database during the period 1/1/2009 to 10/1/2015; gallbladder cancer or cholangiocarcinoma or biliary tract cancer). Review of these protocols determined that only six of them utilized targeted agents against EGFR and MEK that were aimed at a population that was biomarker selected (Table 2). To our knowledge, there are no trials of immunotherapy with PD-1 or PD-L1 blockade in patients with GBCA.

7 Conclusion

Gallbladder cancers are rare and aggressive tumors, with a paucity of clinical trials using biomarker-guided targeted treatment. Standard chemotherapy treatments result in median survival of about 1 year. Genomically matched or immunotherapeutic options are not presently FDA-approved. Complicating therapeutic decisions, GBCA is grouped with other biliary tract malignancies, such as intrahepatic and extrahepatic cholangiocarcinomas, yet GBCA biology is distinct. In fact, most biologic studies lump the signaling pathways of these cancers together, despite their emerging differences (Fig. 1). In lung cancer and melanoma, using matched targeted therapies based on specific molecular or biologic alterations that point to genomically targeted treatment and/or exploitation of immunotherapy strategies have resulted in significant improvements in survival and serve as the paradigm shift in the cognitive approach to thoughtful clinical trial design. As such, pathological documentation of the primary and/or the meta-static disease is crucial for genomic analysis. Multiple potentially actionable genomic alterations have been identified in GBCA (Table 3). Moreover, several of the genes share common downstream signaling pathways including MAPK ($N = 7$), PI3K/AKT/mTOR ($N = 5$), phospholipase Cγ ($N = 2$), Wnt/β-catenin ($N = 2$), and the chromatin remodeling complex ($N = 2$) (Table 4). Additional pathways (include TP53/MDM2/MDMX, TGF-β, Src, JNK, JAK/STAT, PKC, FGF, cytoplasmic NADPH production, transcription regulation, and cell cycle regulation) are associated with only one gene each. Despite the number of alterations or pathways with available cognate-targeted agents (Table 4), very few have been studied in clinical trials and a search of clinicaltrials.gov reveals a continued paucity of such protocols. Developing a biomarker-driven approach for clinical trial design of a rare malignancy such as GBCA is urgently needed.

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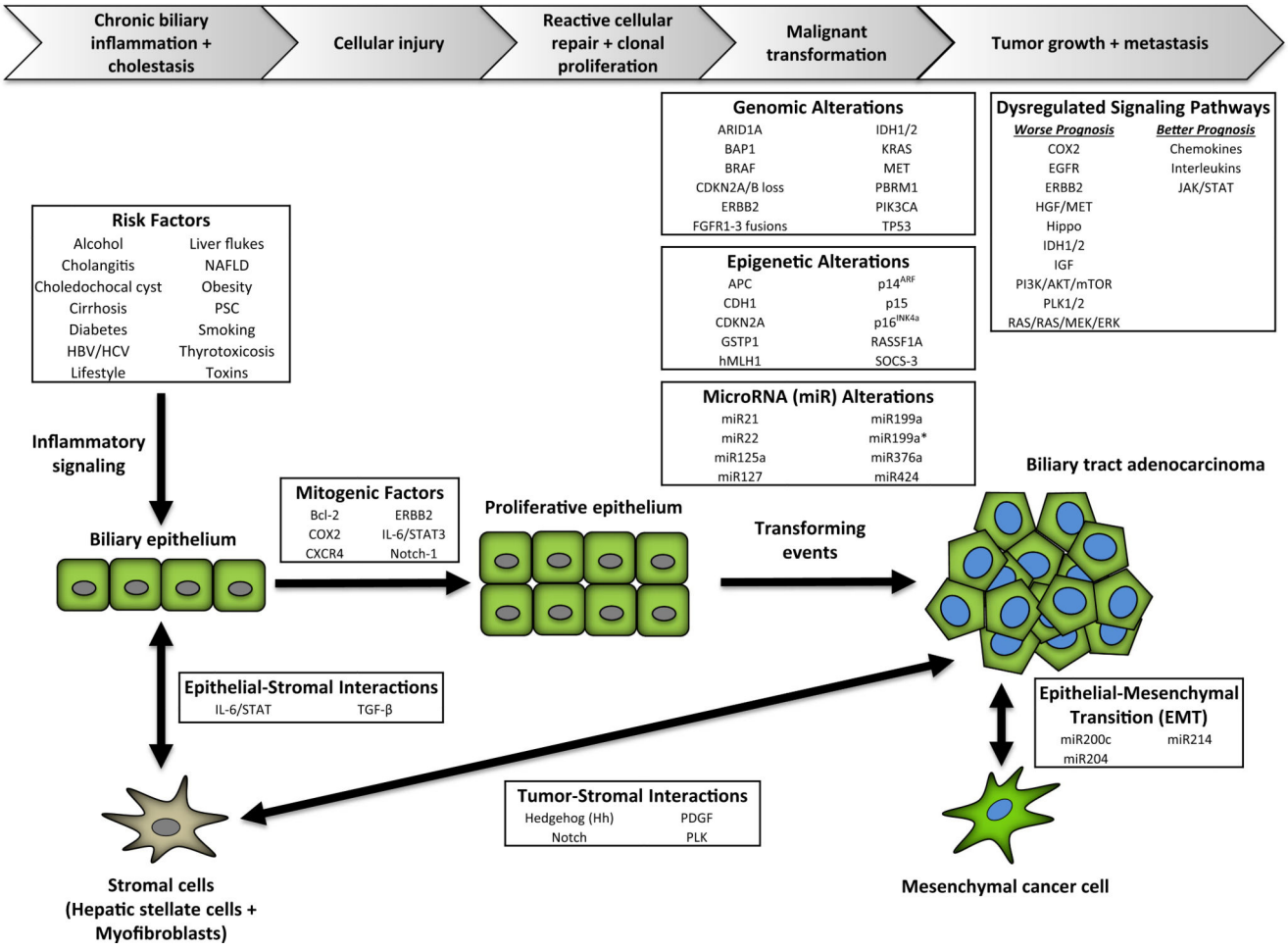

Fig. 1.

Vogelgram of biliary carcinogenesis. The progression from benign biliary epithelium to biliary tract adenocarcinoma has been assumed to be identical between intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, and gallbladder cancer. All three are thought to occur thru series of stages including chronic biliary inflammation and cholestasis caused by several risk factors followed by cellular injury, reactive cellular repair, clonal proliferation, malignant transformation, tumor growth, and metastasis. Each one of these steps is regulated by my factors including epithelial-stromal interactions, mitogens, genomic alterations, epigenetic alterations, microRNAs, dysregulated signaling pathways, epithelial-to-mesenchymal transitions, and tumor-stromal interactions. Reproduced from Sicklick and Fanta, Chapter 8B: Molecular pathogenesis of biliary tract cancer in Blumgart's Surgery of the Liver, Biliary Tract, and Pancreas, 6th Edition, Editors: Jarnagin, et al., 2016 with permission of Elsevier Inc.

Table 1

Current treatment strategies in biliary tract cancers

Clinical situation	Treatment	Median survival
Resectable disease (adjuvant)	Surgical resection; followed by:	33 months
	Fluoropyrimidine-based chemoradiation, or fluoropyrimidine or gemcitabine chemotherapy, or observation	
Unresectable disease	Gemcitabine + Cisplatin	11.7 months
	Fluoropyrimidine-based chemoradiation	9.8 months
Metastatic disease	Gemcitabine + Cisplatin	11.7 months
	Fluoropyrimidine- or gemcitabine-based chemotherapy	5.1 to 15.4 months

Table 2

Targeted therapy clinical trials for biliary tract cancers: completed phase II/III trials and all phase I/II/III trials listed in clinicaltrials.gov (2009–2015)

Completed trials				
Targeted therapy	Selected population	No. of patients	Drug target(s)	Overall response rate
Cetuximab + gemcitabine + capecitabine [12]	No	34	EGFR	17.6 %
Cetuximab + gemcitabine [13][a]	No	44	EGFR	20.4 %
Cetuximab + GEMOX [14][b]	No	133	EGFR	30 %
Cetuximab + GEMOX [15][b]	No	30	EGFR	63 %
Erlotinib + bevacizumab [16]	No	53	EGFR, VEGF-A	12%
Erlotinib + docetaxel [17]	No	25	EGFR	0%
Erlotinib + Sorafenib [18]	No	34	EGFR + BRAF, VEGFR	6%
Erlotinib [19]	No	42	EGFR	8%
Lapatinib [20]	No	57 (17 biliary)	EGFR, ERBB2	0%
Panitumumab + gemcitabine + irinotecan [21][a]	No	35	EGFR	31 %
Panitumumab + GEMOX [22][b]	Yes (*KRAS* wild-type)	46	EGFR	33 %
Selumetinib [23]	No	28	MEK1/2	12%
Sorafenib + gemcitabine + cisplatin [24]	No	39	BRAF, VEGFR	12%
Sorafenib [25]	No	36	BRAF,VEGFR	0%
Sorafenib [26]	No	46	BRAF,VEGFR	2%
Sunitinib [27]	No	56	PDGFR, KIT, VEGFR, RET, CSF-1R, FLT3	8.9 %
Ongoing trials				
Targeted therapy	Selected population	Study phase	Drug target(s)	NCI identifier
Afatinib	No	I	EGFR and Her2	NCT01679405
Afatinib	No	I	EGFR and Her2	NCT02451553
Bevacizumab	No	II	VEGF-A	NCT00881504
Bevacizumab	No	II	VEGF-A	NCT01007552
Binimetinib	KRAS- or BRAF-mutant	I	MEK	NCT00959127
Binimetinib	No	I	MEK	NCT02105350
Binimetinib	No	II	MEK	NCT01828034
Cabozantinib	No	II	MET and VEGFR2	NCT01954745
Cediranib	No	II	VEGFR	NCT01229111
Cediranib	No	II/III	VEGFR	NCT00939848
Cetuximab	No	I	EGFR	NCT01216345
Cetuximab	No	II	EGFR	NCT01267344

Dabrafenib + trametinib	BRAF V600E mutant	II	BRAF + MEK	NCT02034110
DKN-01	Dkk-1 expressing tumor		Dickkopf-1 (Dkk-1; inhibits canonical Wnt/β-catenin)	NCT02375880
Dovotinib	No	I	FGFR3	NCT01497392
Erlotinib	No	I	EGFR	NCT00987766
Erlotinib + sorafenib	No	II	EGFR + BRAF, VEGFR	NCT01093222
Everolimus	No	I	PI3K/AKT/mTOR	NCT00949949
Imatinib	No	II	KIT, PDGFRA	NCT01153750
MK-2206	No	II	AKT	NCT01425879
Panitumumab	No	II	EGFR	NCT00948935
Panitumumab	KRAS wild-type	II	EGFR	NCT01320254
Pazopanib	No	II	VEGFR, KIT, PDGFR	NCT01855724
Ponatinib	FGFR2 fusion	II	FGFR2 fusion	NCT02265341
Ramucirumab	No	II	VEGFR2	NCT02520141
Refametinib	No	II	MEK	NCT02346032
Regorafenib	No	II	VEGFR2, TIE2	NCT02053376
Regorafenib	No	II	VEGFR2, TIE2	NCT02115542
RRx-001	No	II	ROS-mediated pan-epigenetic agent	NCT02452970
Selumetinib	No	I	MEK1/2	NCT01949870
Selumetinib + MK-2206	No	II	MEK1/2 + AKT	NCT01859182
Silmitasertib	No	I/II	Casein kinase 2 (CK2)	NCT02128282
Sorafenib	No	II	BRAF. VEGFR	NCT00919061
SPI-1620	No	II	Endothelin B receptor	NCT01773785
Sunitinib	No	II	PDGFR, KIT, VEGFR, RET, CSF-1R FLT3	NCT01718327
Trametinib	No	II	MEK	NCT02042443
Trametinib	No	IIa	MEK	NCT01943864
Veliparib	Known or suspected BRCA1/BRCA2 germline mutation	I	PARP	NCT01282333

Search terms on clinicaltrials.gov included gallbladder cancer, cholangiocarcinoma, or biliary tract cancer; 1/1/2009 to 10/1/2015

[a] Most studies included a variety of biliary tract tumors; however, studies marked with an asterisk only included cholangiocarcinomas

[b] GEMOX, gemcitabine + oxaliplatin

Table 3

Most common somatic genomic alterations and immunohistochemical changes in gallbladder carcinoma

Genomic alteration[a]	**GBCA (*N*=25) [31]**	**GBCA (*N*=64) [32]**	**GBCA (*N*=85) [33]**	**GBCA (*N*=57) [54]**
APC	4%			
AR1D1A			15 %	
BRAF			1%	6%
CDKN2A/B loss			19%	6%
CTNNB1	4%			
HRAS				2%
IDH1		1.5 %		
EGFR (*ERBB1*)				4%
ERBB2 amplification			16%	
ERBB2				10%
ERBB3				12%
ERBB4				4%
FGFR1-3 fusions amplifications			5%	
KRAS	4%	13%	11%	8%
MAP2K4				4%
MAPK10				6%
MYC				4%
NRAS	4%			2%
NRG1				3%
PIK3CA	12%		14%	6%
SRC				2%
TP53	4%	41 %		47%
Immunohistochemistry (IHC)	GBCA (*N*=194) [35]	GBCA (*N*=244) [32]		
ERBB2 overexpression	9.8 %	15%		
PBRM1 underexpression		53 %		

All percentages are rounded to the nearest whole number

[a]Mutation unless otherwise specified

Table 4

Matching genomic alterations with targeted therapies in gallbladder cancer: theoretical actionability meriting investigation

Altered gene target	Downstream pathway(s) affected	FDA-approved targeted therapeutics[a]	Alternative agent; developmental therapeutics
APC	Wnt/β-catenin	Celecoxib and sulindac (act as WNT inhibitors) [36]	
AR1D1A	Chromatin remodeling complex; PI3K/AKT/mTOR	Everolimus, temsirolimus (mTOR inhibitors) [37]	loss may cause microsatellite instability and hence PD-1 blockade may also be applicable [37, 38]
BRAF	MAPK	Vemurafenib and dabrafenib (BRAF inhibitors); trametinib (MEK inhibitor) [39-41]	
CDKN2A/B	Cell cycle	Palbociclib (CDK4/6 inhibitor) [42, 43]	
CTNNB1	Wnt/β -catenin	Celecoxib and sulindac (act as WNT inhibitors) [36]	Wnt inhibitors in clinical trials (e.g., PRI-724)
EGFR (*ERBB-1*)	MAPK, PI3K/AKT/mTOR, JNK	Afatinib, erlotinib, gefitinib, panitunumab, ibrutinib (weak), lapatinib, cetuximab (EGFR inhibitors and antibodies) [69, 70]	
HER2/c-neu (ERBB-2)	MAPK, PI3K/AKT/mTOR, JAK/STAT, PKC, phospholipase Cγ	Afatinib, lapatinib, pertuzumab, (ado-) trastuzumab emtansine (Her2/3 inhibitors and antibodies) [47, 49, 70]	
HER 3 (*ERBB-3*)	MAPK, PI3K/AKT/mTOR, phospholipase Cγ	Afatinib, pertuzumab (Her2/3 inhibitor and antibody) [70]	
HRAS	MAPK	Trametinib (MEK inhibitor; unclear efficacy) [50]	Selumetinib (MEK-1/2 inhibitor) [23]
FGFR1-3	FGF	Pazopanib, regorafenib, ponatinib, lenvatinib (FGFR inhibitors) [71]	
1DH1	Cytoplasmic NADPH production	5-Azacytidine, decitabine (DNA methyltransferase inhibitors) [72]	Glutaminase inhibitors in clinical trials
KRAS	MAPK	Trametinib (MEK inhibitor) [50]	Selumetinib (MEK-1/2 inhibitor) [23]
MYC	Transcription		Bromodomain inhibitors (e.g., JQ1) [73]
NRAS	MAPK	Trametinib (MEK inhibitor; unclear efficacy) [50]	Selumetinib (MEK-1/2 inhibitor) [23]
PBRM1	Chromatin remodeling complex		
PIK3CA	PI3K/AKT/mTOR	Everolimus, temsirolimus [52] (mTOR inhibitors; may be used in combination since single agent activity in some matched setting has been low [63])	
SMAD4	TGF-β		
SRC	Src	Bosutinib, dasatinib, ponatinib, vandetanib (Src inhibitors) [74]	
TP53	TP53/MDM2/MDMX	VEGF inhibitors (retrospective study shows longer PFS with bevacizumab in TP53 mutant versus wild-type tumors [54]; mutations in TP53 result in increased VEGF-A transcripts [60]	Wee-1 inhibitor (e.g., MK-1775) in clinical trials [58, 75]

[a] Lists include FDA-approved agents, but off-label indications