Genomic Profiling of Biliary Tract Cancer Cell Lines Reveals Molecular Subtypes and Actionable Drug Targets

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HIGHLIGHTS
BTC cell lines harbor similar genomic alterations to primary tumors
Transcriptomic profiling of BTC cell lines identified two molecular subtypes
MAPK signaling is activated in BTC via multiple mechanisms
BTC lines with deregulated ERBB2 or FGFRs respond to specific targeted therapies

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Genomic Profiling of Biliary Tract Cancer Cell Lines Reveals Molecular Subtypes and Actionable Drug Targets

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SUMMARY
Biliary tract cancers (BTCs) currently have no approved targeted therapies. Although genomic profiling of primary BTCs has identified multiple potential drug targets, accurate models are needed for their evaluation. Genomic profiling of 22 BTC cell lines revealed they harbor similar mutational signatures, recurrently mutated genes, and genomic alterations to primary tumors. Transcriptomic profiling identified two major subtypes, enriched for epithelial and mesenchymal genes, which were also evident in patient-derived organoids and primary tumors. Interrogating these models revealed multiple mechanisms of MAPK signaling activation in BTC, including co-occurrence of low-activity BRAF and MEK mutations with receptor tyrosine kinase overexpression. Finally, BTC cell lines with altered ERBB2 or FGFRs were exquisitely sensitive to specific targeted agents, whereas surprisingly, IDH1-mutant lines did not respond to IDH1 inhibitors 

INTRODUCTION
Biliary tract cancers (BTCs) include intra- and extrahepatic cholangiocarcinoma, gallbladder carcinomas, and ampullary carcinomas. The majority (80%-90%) of patients present with advanced disease, and each year 139,000 people die of BTC around the world, including 12,000 in the US (Charbel and Al-Kawas, 2011; Marciano-Bonilla et al., 2016). The incidence of the disease varies globally, with highest rates in northeastern Thailand and neighboring Laos and Cambodia where liver fluke infestations are endemic (Charbel and Al-Kawas, 2011). Furthermore, for reasons that are presently unknown, the incidence of intrahepatic cholangiocarcinoma is increasing in the western world (Shoda et al., 2012). Systemic chemotherapy has only modest activity in the metastatic setting, with gemcitabine plus cisplatin the standard of care, and there are currently no approved second-line or targeted therapies for BTC. Consequently, the median overall survival for these patients is approximately 12 months (Valle et al., 2010).

Initial sequencing studies aiming to characterize the genomic landscape of BTCs (Farshidfar et al., 2017; Jusakul et al., 2017; Li et al., 2014; Nakamura et al., 2015) identified a series of recurrently mutated genes, including loss-of-function mutations in the tumor suppressors TP53 and SMAD4 and the epigenetic modifiers ARID1A, ARID2, and BAP1, whereas activating mutations in KRAS, PIK3CA, and NRAS were the most common oncogenic events (Nakamura et al., 2015). More recent studies identified fusions involving PRKACA and PRKACB as other potential driver events (Nakamura et al., 2015), as well as mutations in ROBO2, RNF43 (Ong et al., 2012), RASA1, STK11, and MAP2K4 (Jusakul et al., 2017). These studies also identified potential therapeutically exploitable targets including mutations and amplifications of members of the ERBB family of receptor tyrosine kinases (Li et al., 2014), IDH1 mutations (Borger et al., 2012), and FGFR2 fusions (Arai et al., 2014); however, in many cases it remains to be determined whether these genomic alterations can be exploited for therapeutic benefit. To test this, reliable models harboring endogenous alterations in these potential targets are needed. In this regard, cell lines represent powerful models to study cancer biology and assess drug response.
Although a number of BTC cell lines have been established, their genomic profiles have not been extensively characterized and compared with that of primary BTCs. In this study, we comprehensively profiled 22 BTC cell lines by exome sequencing, copy number analysis, and RNA-seq analysis. We found that the most frequently observed genomic alterations in primary BTCs are preserved in cell lines validating their use as accurate model systems to study this disease. In addition, we identified two distinct molecular sub-sets of BTC cell lines that differ in expression of EMT genes and importantly demonstrate that these signatures are also evident in patient-derived organoid models and primary BTCs. We also demonstrate that the MAPK signaling pathway is deregulated by multiple mechanisms in BTC and identify a number of potential actionable drug targets for this disease.

RESULTS

Exome Sequencing of Biliary Cancer Cell Lines
A panel of 22 BTC cell lines derived from tumors from distinct anatomical locations within the biliary tree was assembled from international cell repositories and individual investigators (Table S1). The panel comprised the majority of BTC lines described in the literature (Homma et al., 1987, 1988; Knuth et al., 1985; Koyama et al., 1980; Ku et al., 2002; Kusaka et al., 1988; Miyagiwa et al., 1989; Yamada et al., 1997), including two cell lines, KKU-M055 and KKU-M213, derived from liver-fluke-associated intrahepatic cholangiocarcinoma (Obchoei et al., 2011; Tepsiri et al., 2005).

The total number of mutations (SNVs + InDels) ranged from 315 to 623 (mean 394) across the cell lines (Figures 1A–1C and Table S2). The average frequency of InDels in cell lines (0.27 InDels/Mb, range 0.16–0.42 InDels/Mb) was similar to that observed in primary cancers (0.32 InDels/Mb). Comparatively, the average mutation rate for cell lines (5.0 SNVs/Mb, range 3.9–7.9 SNVs/Mb) was higher than that observed in primary BTCs (mean 2.6 SNVs/Mb). No hypermutated cell lines (>25 mutations/Mb) were identified in the panel, consistent with the low frequency (5%) of hypermutated cases in primary BTCs (Nakamura et al., 2015).

The dominant somatic substitution pattern observed in primary BTCs are C > T/G > A transitions that are enriched at CpG dinucleotides, followed by T > C/A > G transitions and C > A/T > G transversions...
(Alexandrov et al., 2013; Nakamura et al., 2015). This pattern is similar in fluke- and non-fluke-derived tumours (Chan-On et al., 2013). A similar distribution of somatic substitution patterns was observed in BTC cell lines, with C > T (0.49 \pm 0.03) transitions as the dominant pattern observed, followed by T > C (0.15 \pm 0.02) transitions and C > A transversions (0.14 \pm 0.04) (Figure 1D).

In primary BTC, two predominant mutational signatures have been identified: (A/C/G)CG>(A/C/G)TG previously defined as Signature 1 by Alexandrov et al. (Alexandrov et al., 2013), which is the result of an endogenous mutational process initiated by spontaneous deamination of 5-methylcytosine, and TC(A/C/T) \_TG(A/C/T) and TCN > TTN, which is similar to the previously defined APOBEC-associated signature (Signature 2) (Alexandrov et al., 2013). Although the predominant signature in BTC cell lines was Signature 1 (Figure 1E), we identified one cell line (HuH28, intrahepatic) with the classical APOBEC signature (Figure 1F), which was also the cell line harboring the highest mutational load.

**Comparison of the Most Frequently Mutated Genes in Primary BTCs and BTC Cell Lines**

To determine if the genes most frequently mutated in primary BTCs are reflected in BTC cell lines, we compared the frequency of mutations with that reported by Nakamura et al. in which 13 significantly mutated genes were identified from sequencing 260 primary BTCs (Nakamura et al., 2015). Compared with primary disease, the mutational frequency of several known oncogenes and tumor suppressor genes
including TP53 (25.9% vs 63.6%), KRAS (18.0 vs 40.9%), and SMAD4 (8.8% vs 18.2%) were higher in cell lines (Figure 2A). Nevertheless, we observed a strong correlation between the most frequently mutated genes in primary cancers and cell lines (Pearson’s R = 0.962), demonstrating that although cell lines have a higher mutational frequency of major oncogenes and tumor suppressor genes, the relative proportion of these mutations is similar to that observed in primary disease (Figure 2B).

**Functional Validation of Specific Mutations in BTC**

To validate the functionality of specific mutations, we examined corresponding mRNA expression of genes harboring truncating mutations by analysis of RNA-seq data generated for each cell line. As expected, cell lines harboring truncating TP53 mutations had significantly lower TP53 mRNA expression compared with TP53 wild-type cell lines or cell lines harboring TP53 point mutations (Figure 2C). Furthermore, immunohistochemical staining of the cell lines revealed high TP53 protein expression in mutant cell lines compared with wild-type lines or lines harboring truncating mutations (Figure 2D). Similarly, the single cell line harboring a homozygous inactivating mutation in BAP1 (TFK-1) had the lowest level of BAP1 mRNA expression among the cell lines (Figure S1A). We also identified three cell lines harboring mutations in the WNT pathway (KKU-M055, APC frameshift; TGBC18TKB, CTNNB1 T41A; and SNU-869, CTNNB1 S45P). KKU-M055 cells also harbored a deletion of APC (Chr5q22.2) consistent with loss of heterozygosity. As expected, these three lines had markedly higher Wnt reporter (TOPFLASH) activity, and expression of the Wnt target gene, AXIN2, compared with wild-type lines (Figures S2A and S2B). Notably, inactivating mutations in the E3 ubiquitin ligase RNF43 have also been reported to enhance canonical Wnt signaling due to failure to degrade FZD receptors on the cell surface (Koo et al., 2012), and we identified one cell line, Sk-ChA-1, harboring a biallelic inactivating RNF43 mutation, which also had low levels of RNF43 mRNA expression (Figure S1B). Surprisingly, however, TOPFLASH activity and Wnt target gene expression was not elevated in this line (Figures S2A and S2B), and Sk-ChA-1 cells were not preferentially sensitive to exogenous Wnt ligand (Figure S2C), collectively indicating that the inactivating RNF43 mutation in this line does not activate the Wnt pathway.

**DNA Copy Number Changes and Identification of Focal Regions of Amplification and Deletion in BTC Cell Lines**

To investigate changes in DNA copy-number, we utilized Illumina OmniExpress SNP arrays. The most commonly deleted genomic regions were chromosome 8p and chromosome 18, whereas the most commonly gained regions were 5p, 7p, 17q, and chromosome 20. We also identified focal regions significantly altered by DNA copy-number alterations in cell lines using GISTIC. Significantly amplified genes across the cell lines were KRAS (12p12.1), SLCO1B (12p12.2), ALG10 (12p11.1), hsa-mir-720/hsa-mir-1263/BCHE (3p26.1), and POU5F1B (8q24.21), and significantly deleted regions were CDKN2A/B (9p21.3), FHIT (3p14.2), WWOX (16q23.1), MACROD2 (20p12.1), and TPRG1 (3q28) (Figure 3A).

We also performed an analysis in which we determined the extent of overlap of the 33 homozygously deleted and 22 focally amplified genes identified in >2 primary BTCs by Nakamura et al. (Nakamura et al., 2015). Of the 33 homozygous deleted genes, 16 (47%) were also deleted in one or more cell line. All of these genes were located at chromosome 9p21.3 and included CDKN2A and CDKN2B. Confirming the deletion of CDKN2A and the adjacent region CDKN2B, mRNA expression of these genes was significantly lower in cell lines harboring homozygous deletions (Figure 3B). In comparison, 20 of the 22 amplified genes identified in primary disease were found to be also amplified in at least one BTC cell line (copy number ≥5), of which five genes (22%) had copy numbers ≥7 in one or more cell lines (MYC, YEATS4, CCND3, IKBKB, KRAS) (Tables S3 and S4). Notably, four of these five focally amplified genes displayed corresponding increases in mRNA expression (Figure 3C).

**Unsupervised Clustering of Cell Lines Based on Gene Expression Identifies Two Major Subgroups that Differ in Epithelial and Mesenchymal Characteristics**

We next performed RNA-seq analysis on the cell lines. Unsupervised clustering based on expression of all genes separated the cell lines into two major groups, comprising 7 and 13 cell lines (Figure 4A). Gene set enrichment analysis of the 411 genes differentially expressed between these groups identified the hallmarks epithelial-mesenchymal transition (EMT), mitotic spindle, and hypoxia with the most significant enrichment scores in the smaller cluster, whereas cholesterol homeostasis, IFN alpha and gamma response, and early and late estrogen response were significantly enriched in the larger cluster (Figure 4B). Consistent with enrichment of the EMT hallmark, expression of mesenchymal genes CTGF, FLNA, FN1, TGFβ1, and ZEB1 were higher in the smaller cluster (mesenchymal cluster), whereas expression of multiple
drivers (ELF3, KLF5, EHF) and markers of epithelial differentiation (CDH1, EPCAM, KRT19, KRT8, VILL) and tight junction components (CGN, CRB3, CLDN4, CLDN7, F11R, TJP3) were more highly expressed in the “epithelial cluster” (Figure 4A). Notably, four out of seven lines in the mesenchymal cluster were derived from metastatic gallbladder cases. To determine if these transcriptional differences translated to histological differences, representative cell lines from each cluster were grown as xenografts. All three lines from the mesenchymal cluster grew as poorly differentiated tumors with no glandular structure, whereas the three lines from the epithelial cluster grew as moderately differentiated tumors with clear evidence of glandular morphology (Figure 4C). Furthermore, the majority of cell lines from the mesenchymal cluster grew primarily as single cells or with spindle-like morphology in vitro, compared with cell lines from the epithelial cluster where many grew in patches of closely adhered cells (Figures S3 and S4).

To determine if these signatures were also evident in patient-derived organoid (PDO) models, we analyzed microarray data available from four recently generated PDOs, three of which were derived from well-to-moderately differentiated tumors (19T, 1T, 24T) and one that was derived from a moderately-to-poorly differentiated tumor (9T) (Saito et al., 2019). Expression of the epithelial genes CLDN4, EPCAM, TJP3, and KRT19 was highest in the well-to-moderately differentiated organoids, whereas expression of the mesenchymal genes TGFB1, ZEB1, FN1, and CALD1 was highest in the 9T organoid (Figure 4D), demonstrating the cell-line-derived gene expression signature of tumor histology is also evident in organoid models of the disease.

Finally, to determine if primary BTCs harboring these signatures could be identified, 35 primary BTCs profiled by the TCGA were clustered with the cell lines based on the EMT signature. This analysis identified two
primary BTCs that clustered with the mesenchymal lines and eight primary tumors that clustered with the epithelial lines (Figure S5). Notably, examination of the histopathology of the primary tumors in the mesenchymal cluster confirmed that case TCGA-ZU-A8S4 was a sarcomatoid carcinoma showing spindle cell (mesenchymal) morphology with no evidence of gland formation, whereas case TCGA-W5-AA2H showed some gland formation but also a high degree of tumor budding, forming small clusters of spindle shaped tumor cells (Figure S6). Comparatively, all eight primary cases that clustered with the epithelial lines showed clear evidence of gland formation and epithelioid cell morphology, without spindle cells or tumor budding (Figure S6).

Investigation of Actionable Genetic Alterations in BTC

**IDH1 R132C Mutation**

We identified one cell line (SNU-1079, intrahepatic) harboring an IDH1 R132C hotspot mutation, which was confirmed using Sanger sequencing (Figure S7A inset). Consistent with the neomorphic advantage conferred by this mutation (Ward et al., 2010), levels of the oncometabolite R-2-hydroxy-glutarate (2-HG) were markedly elevated in culture medium and cell pellets from this line (Figure S7A). Treatment of...
SNU-1079 cells with the mutant IDH1 inhibitor AGI-5198 significantly decreased 2-HG accumulation (Figure S7B), however, neither AGI-5198 nor the clinically used derivative AG-120 (ivosidenib) inhibited proliferation of this line (Figure S7C). To determine whether similar effects occurred in PDOs, we determined the effect of AG-120 in the 9T PDO, generated from a patient with an IDH1R132K mutant intrahepatic cholangiocarcinoma. As observed in SNU-1079 cells, AG-120 failed to inhibit growth of the IDH1R132K organoid, with instead a modest increase in cell proliferation observed (Figure S7D).

Recent studies have suggested that mutant IDH may promote cholangiocarcinoma development by suppressing HNF4A expression and blocking hepatocyte differentiation (Saha et al., 2014), and initial data from clinical trials of AG-120 in IDH1 mutant cholangiocarcinoma have reported an upregulation of liver-specific genes in serial biopsy samples (Ishii et al., 2018). However, treatment of SNU-1079 cells with AGI-5198 or AG-120 failed to increase expression of HNF4A or other hepatocyte markers (MGST1, CYP27A1, ALB) or markers of epithelial (EPCAM) or mesenchymal (VIM) transition (Figure S7E). Finally, as increased benefit of IDH1 mutant BT Cs to chemotherapy was recently reported (Molenaar et al., 2018), we assessed the sensitivity of this line to gemcitabine. Although SNU-1079 cells were not exquisitely sensitive to gemcitabine, it ranked among the more sensitive lines (Figure S7F). However, pre-treatment of SNU-1079 cells with AGI-5198 did not further enhance sensitivity, suggesting the sensitivity of IDH-mutant tumors to chemotherapy may not be directly related to elevated 2-HG levels (Figure S7G).

**ERBB2 Mutation and Amplification**

Mutations in the ERBB family of receptor tyrosine kinases, particularly ERBB2 and ERBB3, occur in ~10% of BTCs and we identified one cell line, TGBCB18TKB, which carried two hotspot mutations in ERBB2 (S310F and R678Q) (Figure 5A), which have been previously reported in primary BTC (Li et al., 2014). Notably, mRNA expression of ERBB2 was also highly elevated in TGBCB18TKB cells (Figure 5B). We also identified a second cell line, TKKK, with highly elevated levels of ERBB2 mRNA (Figure 5B). Copy number analysis of this cell line revealed an amplification in ERBB2 (Figure 3A), which was confirmed by qRT-PCR and in situ hybridization (Figures 5C and 5D). Importantly, both TGBCB18TKB and TKKK cells were markedly more sensitive to the ERBB2 inhibitors lapatinib and AZD8931 compared with WT lines, establishing these mutations as potential drug targets in BTC (Figures 5E and 5F).

**ERK-MAPK Signaling Is Activated by Multiple Mechanisms in BTC Cell Lines**

Integration of the exome sequencing data and DNA copy number analysis revealed multiple mechanisms of ERK/MAPK pathway activation in BTC cell lines. Specifically, KRAS mutations were identified in 8/22 cell lines, whereas amplification of KRAS was observed in three cell lines of which one line (NOZ) also harbored a KRAS mutation (Table S5). In addition, we identified two cell lines harboring low-activity BRAF mutations (TGBCB18TKB, BRAF516L, NS32T, and Sk-Ch-A1, BRAF619YAT) (Table S5). Unlike activating BRAF mutations (V600E), these mutants act as amplifiers of RAS signaling and often coexist with other forms of RAS activation (Yao et al., 2017). The identification of ERBB2 hotspot mutations in TGBCB18TKB cells (Figure 5A) is consistent with this mechanism. Similarly, we identified a truncating mutation in RASA1 in Sk-Ch-A1 cells (Table S2 and Figure S1), which encodes the Ras GTPase-activating protein p120-RasGAP, which suppresses RAS signaling by converting RAS to the inactive GDP-bound form (Lapinski et al., 2007).

We also identified one cell line (KKU-M055) with a K57N mutation in MAP2K1 (MEK1), which has been previously observed in lung adenocarcinoma and melanoma (Figure 6A). As with low-activity BRAF mutations, MAP2K1K57N was recently classified as a class II MEK mutant, which is partially dependent on upstream RAF to drive ERK signaling and likely acts as an amplifier of RAS signaling (Gao et al., 2018). Notably, compared with MEK1WT G415 cells, MEK1K57N mutant KKU-M055 cells were highly resistant to growth inhibition or signaling inhibition induced by the allosteric MEK inhibitor trametinib or the ERK inhibitor SCH772984 (Figures 6B–6E). Time course experiments also demonstrated that SCH772984 increased levels of active CRAF (pCRAF S338) in both MAP2K1WT and WT cell lines (Figure 6F), which is an expected effect of this drug due to relief of ERK-mediated inhibitory phosphorylation of CRAF (Dougherty et al., 2005). However, although pERK levels remained suppressed in MEK1WT G415 cells after 6 h, they were strongly reactivated in MEK1K57N mutant KKU-M055 cells, consistent with the MAP2K1K57N acting to amplify BRAF/MAPK/ERK signaling (Figure 6F).

The role of MAP2K1K57N as an amplifier of RAS signaling suggested KKU-M055 cells may also harbor alterations in upstream components of the RAS/MAPK pathway. As no mutations in RAS, BRAF, NF1, and RASA1...
were present, we investigated mRNA expression of the major receptor tyrosine kinases (RTKs), which revealed marked overexpression of FGFR1 in this cell line (Figure 7A). Furthermore, KKU-M055 cells were highly sensitive to the FGFR inhibitors BGJ398 and erdafitinib both in vitro and in vivo (Figures 7B–7E), suggesting proliferation of KKU-M055 cells is driven by FGFR1, with the MEKK57N mutation likely acting to amplify FGFR-driven MAPK signaling.

FGFR3 and FGFR4

Finally, we utilized the RNA-seq data to perform an outlier analysis in order to identify other cell lines that expressed exceptionally high levels of targetable receptor tyrosine kinases. This approach identified high levels of FGFR3 and FGFR4 mRNA in Mz-ChA-2 cells (Figures 7F and 7G). Interrogation of the signaling components downstream of FGFR in this cell line also revealed a focal low-level amplification of the FGFR docking protein FRS2 (Figure 7H) (Turner and Grose, 2010). Treatment of Mz-ChA-2 cells with the FGFR inhibitors BGIJ398 and erdafitinib induced exquisite sensitivity to both inhibitors compared with non-overexpressing lines (Figures 7I and 7J).

DISCUSSION

BTCs are a genomically heterogeneous group of cancers featuring a substantial number of low prevalence mutations. In this study, we profiled the genomic landscape of 22 BTC cell lines derived from various anatomical locations in the biliary tract and demonstrate that the most commonly mutated driver genes,
Figure 6. Characterization of MEK<sup>K57N</sup> Mutant Cell Line

(A) Mutation plot from cBioPortal showing location and frequency of occurrence of MEK<sup>K57N</sup> mutation in human cancers. Lollipops designate mutation points.

(B and C) MTS assays of a MEK<sup>K57N</sup> mutant (KKU-M055) and a MEK<sup>WT</sup> cell line (G415) treated with (B) the MEK inhibitor trametinib or (C) the ERK inhibitor SCH772984 for 72 h. Values shown are mean ± SEM from a single experiment performed in quadruplicate. Similar results were obtained in two independent experiments.

(D and E) Effect of (D) trametinib or (E) SCH772984 on pERK protein levels in MEK<sup>K57N</sup> mutant and MEK<sup>WT</sup> cell lines. Cells were treated with drug for 24 h and pERK levels determined by Western blot. MW (molecular weight markers) in Kilo Daltons.

(F) Time course Western blot analysis of the effect of ERK inhibitor (SCH772984, 500 nM) treatment on pERK and pCRAF protein levels in MEK<sup>K57N</sup> mutant (KKU-M055) and MEK<sup>WT</sup> G415 cells.
Mutational signatures, and deletions and amplifications observed in primary tumors were also present in cell lines. Cell lines exhibited a higher mutational burden to that reported in primary BTCs. This may be partly related to the lack of available normal genomic DNA for comparison. As a result, our somatic variant calling was dependent on comparisons to databases of known SNPs and germline mutations that may have overcalled the number of somatic mutations. Furthermore, cancer cell lines have undergone additional passages since the time of resection and hence have had the time to acquire additional mutations in vitro. Finally, it is possible that studies of primary BTC may under-call somatic variants, particularly tumors that have a high content of normal cells.

A major finding of the current study was the identification of sub classes of cell lines that differed primarily in the expression of genes involved in EMT, cell adhesion, differentiation, migration and developmental processes. Consistent with these transcriptional differences, the mesenchymal cluster was enriched for cell lines derived from metastatic gallbladder cancers, and morphological and histological analyses of the cell lines in this cluster revealed they were enriched for cell lines that had lost characteristics of epithelial differentiation. Importantly, we also observed similar differential expression of these epithelial/mesenchymal genes in tumor organoids derived from moderately and poorly differentiated tumors. The
advancement of 3D culture technology is now enabling the generation of increasing numbers of PDO model systems for use in translational research, and comparison of cell line and organoid platforms is of increasing interest. In this regard, it is noteworthy that our initial comparisons of these models, albeit using small numbers, suggest reasonable overlap. Finally, intersection of the epithelial and mesenchymal signatures identified in cell lines with RNA-seq data available through the TCGA revealed the existence of primary BTCs harboring both signatures, indicating these signatures are applicable to primary tumors. Indeed, primary BTCs that have lost expression of epithelial markers or that have gained expression of mesenchymal markers have been previously reported and associated with poorer outcome (Vaquero et al., 2017; Xu et al., 2017).

In comparison to our cell line analysis, prior gene expression profiling of primary BTCs identified four major groups of BTCs (Jusakul et al., 2017; Nakamura et al., 2015), including a group characterized by high expression of cytokines and immune checkpoint molecules. Gene expression profiling of intrahepatic cholangiocarcinomas also identified two major subclasses characterized by expression of proliferative and inflammatory genes, respectively. The contribution of stromal and inflammatory cells to the transcriptional signature of primary cancers likely contributes to these subtypes not being observed in cell lines and highlights an advantage of analyzing cell line models to reveal insights into biological differences among samples that may otherwise be masked by strong stromal signatures.

The genomic analysis of the BTC cell lines also revealed a number of potential actionable targets. In this regard, we tested a series of therapeutic targets established in other cancers for which we identified the corresponding endogenous genetic change in a BTC cell line. We identified the R132C hotspot mutation in IDH1 in the SNU-1079 line, which had corresponding high levels of the onco-metabolite 2-HG. Notably, this cell line had the lowest mutational load among the cell lines and interestingly did not harbor mutations in any other established tumor suppressor genes or oncogenes, consistent with a potential epigenetic mechanism of tumor promotion in IDH mutant cancers (Farshidfar et al., 2017; Wang et al., 2013). The inhibitor of mutant IDH1, AGI-S198, inhibits colony formation of glioma cells transformed with mutant IDH1 (Rohle et al., 2013); however, despite lowering of 2-HG levels, AGI-S198 or its clinically used derivative AG-120 had no effect on proliferation of SNU-1079 cells. Similarly, we observed that a PDO harboring an IDH1R132L mutation was also refractory to AG-120. These effects are consistent with pre-clinical studies in IDH1 mutant chondrosarcoma (Saha et al., 2016), as well as recent clinical evidence in cholangiocarcinoma where objective responses were only observed in 6% of IDH1 mutant patients treated with AG-120 (Lowery et al., 2017). Notably, the outcomes of the ClarIDHy phase III trial were recently reported in which IDH1 mutant cholangiocarcinoma patients treated with AG-120 (ivosidenib) experienced a significant improvement in progression-free survival (2.7 months) compared with patients treated with placebo (1.4 months) (Abou-Alfa et al., 2019). Consistent with our findings in pre-clinical models, objective response were rare (2.4%), raising the possibility that inhibition of mutant IDH1 may elicit anti-tumour activity through non-cell autonomous mechanisms. Indeed, the oncometabolite 2-HG has been previously reported to promote angiogenesis (Seok et al., 2019) and suppress anti-tumour T cell immunity (Bunse et al., 2018).

On the other hand, we detected two cell lines harboring mutations and amplification of members of the ERBB receptor family, which demonstrated sensitivity to ERBB2-targeting agents. These findings are consistent with case reports and small clinical studies reporting clinical responses of BTCs to ERBB2 targeted agents (Hyman et al., 2018; Nam et al., 2016) and collectively support the fact that ERBB2 amplification/mutations represent a promising therapeutic target in BTC.

An important finding of the current study is the identification of multiple mechanisms of ERK-MAPK pathway deregulation in BTC, whereby in addition to identifying mutations in KRAS in 36% of the cell lines, we identified KRAS amplifications in three lines, inactivating mutations in BRAF in two lines and a MAP2K1K57T mutation in one cell line. Notably, both inactivating BRAF mutations and the MAP2K1K57T mutation have been suggested to act as amplifiers of ERK-MAPK signaling and frequently co-exist with mutations in upstream components of the ERK-MAPK pathway (Gao et al., 2018; Yao et al., 2017). Indeed, detailed investigation of the cell lines harboring these mutations identified a co-existent activating ERBB2 mutation in BRAF mutant (S831L, N582T) TGBC18TK8 cells, an inactivating RASA1 mutation in BRAF-mutant Sk-ChA-1 cells, and high levels of FGFR1 expression in MAP2K1K57T mutant KKU-M055 cells. An important implication of these findings is that tumors found to harbor low-activity “amplifier mutations” in the MAPK pathway in commonly used cancer gene panel sequencing tests should be further
interrogated for amplifications or overexpression of RTKs, as this may yield robust therapeutic targets. Proof of concept of this approach was our finding that MAP2K1K57N mutant KKU-M055 cells express high levels of FGFR1 and are exquisitely sensitive to FGFR inhibition.

We also identified high levels of FGFR3 and FGFR4 expression in Mz-ChA-2 cells. Further interrogation of the FGFR signaling pathway in this line also revealed an amplification of FRS2, and Mz-Ch-A2 cells were highly sensitive to FGFR inhibition in vitro. This finding is consistent with observations in liposarcoma, where cell lines harboring FRS2 amplifications have also been reported to be sensitive to FGFR inhibitors (Zhang et al., 2013). Importantly, gene fusions involving FGFR2
carcinomas (Helsten et al., 2016; Ross et al., 2014), and these tumors have been reported to be clinically responsive to FGFR inhibitors (Javle et al., 2018). Although we did not identify any cell lines harboring FGFR2 fusions, our findings suggest that the subset of BTCs driven by aberrant FGFR signaling and amenable to FGFR inhibition extend beyond those harboring FGFR2 fusions alone.

In summary, we characterized the exome, copy number, and transcriptome of a large panel of BTC cell lines and demonstrated that at the genomic level these cell line represent accurate models of primary disease. We also demonstrated that BTC cell lines can be separated into two major groups based on their transcriptional profiles, which is primarily driven by differential expression of genes involved in epithelial differentiation and EMT and which are also observed in both PDOs and primary tumors. We also identify a number of potential actionable drug targets for this disease (ERBB2, FGFR1, and FGFR3/4) and others that require additional investigation (IDH1) and provide a resource to facilitate the ongoing discovery and validation of potential therapeutic targets in BTC.

**Limitations of the Study**

An inherent limitation of the genomic analysis of historically established cell lines is the lack of normal genomic DNA for comparison, which may have resulted in miscalling of some genetic alterations. Cell lines also potentially acquire additional (epi-)genetic changes during passage, which may cause them to differ from primary tumors. Finally, the relatively small number of BTC cell lines available for genomic characterization is insufficient to capture all of genomic changes that drive BTC.

**METHODS**

All methods can be found in the accompanying Transparent Methods supplemental file.
REFERENCES


HER2 in advanced biliary tract cancer.


