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

Copy Number

Gene expression

Exome

MAPK drivers

EMT subtypes

Actionable targets

- FGFR
- ERBB
- IDH1

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
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 in vitro. These findings establish BTC cell lines as robust models of primary disease, reveal specific molecular disease subsets, and highlight specific molecular vulnerabilities in these cancers.

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; Marcano-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.

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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 tums (Chan-On et al., 2013). A similar distribution of somatic substitution patterns was observed in BTC cell lines, with C > T (0.49 ± 0.03) transitions as the dominant pattern observed, followed by T > C (0.15 ± 0.02) transitions and C > A transversions (0.14 ± 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; TGBC18TK8, 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., 2013). 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 adjacentely located 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, IKBKβ, 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 hallmark 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 IDH1<sup>R132K</sup> mutant intrahepatic cholangiocarcinoma. As observed in SNU-1079 cells, AG-120 failed to inhibit growth of the IDH1<sup>R132K</sup> organoid, with instead a modest but significant 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 BTCs 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, TGBCl8TKB, which carried two hotspot mutations in ERBB2 (S310F and R678Q) (Figure S6A), which have been previously reported in primary BTC (Li et al., 2014). Notably, mRNA expression of ERBB2 was also highly elevated in TGBCl8TKB cells (Figure S6B). We also identified a second cell line, TKKK, with highly elevated levels of ERBB2 mRNA (Figure S6B). Copy number analysis of this cell line revealed an amplification in ERBB2 (Figure S6A), which was confirmed by qRT-PCR and in situ hybridization (Figures S6C and S6D). Importantly, both TGBCl8TKB 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 S6E and S6F).

**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 (TGBCl8TKB, BRAF<sup>S516L</sup> NSR2T, and Sk-Ch-A1, BRAF<sup>D594T</sup>) (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 TGBCl8TKB cells (Figure S6A) 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, MAP2K<sup>K57N</sup> 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 MEK<sup>WT</sup> G415 cells, MEK<sup>K57N</sup> 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–6D). Time course experiments also demonstrated that SCH772984 increased levels of active CRAF (pCRAF S338) in both MAP2K<sup>K57N</sup> mutant 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 MEK<sup>WT</sup> G415 cells after 6 h, they were strongly reactivated in MEK<sup>K57N</sup> mutant KKU-M055 cells, consistent with the MAP2K<sup>K57N</sup> acting to amplify BRAF/MAPK/ERK signaling (Figure 6F).

The role of MAP2K<sup>K57N</sup> 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 BGJ398 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 MEK1K57N Mutant Cell Line

(A) Mutation plot from cBioPortal showing location and frequency of occurrence of MEK1K57N mutation in human cancers. Lollipops designate mutation points.

(B and C) MTS assays of a MEK1K57N mutant (KKU-M055) and a MEKWT 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 MEK1K57N mutant and MEKWT 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 MEK1K57N mutant (KKU-M055) and MEKWT 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

Figure 7. Characterization of FGFR Overexpressing BTC Cell Lines

(A) Basal (Top) mRNA and (Bottom) protein expression of FGFR1 in the BTC cell line panel determined by qPCR and Western blot. M (molecular weight markers) in Kilo Daltons. Values shown in the top panel are mean ± SEM from a single experiment performed in technical triplicate. (B and C) Response of FGFR1 overexpressing KKU-M055 cells and non-overexpressing G415 cells to increasing concentrations of (B) BGJ398 and (C) erdafitinib. Cells were treated for 72 h and cell growth determined by MTS assays. Values shown are mean ± SEM from a single experiment performed in quadruplicate. Similar results were obtained in three independent experiments. (D and E) Response to KKU-M055 cells to erdafitinib in vivo. (D) Mice (n = 4 per group) were each injected with tumour cells in their right and left flanks and treated once daily via oral gavage with either erdafitinib (20 mg/kg) or vehicle (20% hydroxypropyl b cyclodextrin) for 18 consecutive days. Values shown in panel D are mean +/- SEM. (E) Representative images of resected tumours from mice treated with vehicle or erdafitinib. (F and G) mRNA expression of (F) FGFR3 and (G) FGFR4 in the BTC cell line panel determined by RNA-seq. FGFR3/4 overexpressing Mz-Ch-A2 cells are shown in blue. (H) FRS2 DNA copy number in BTC cell lines. (I and J) Response of FGFR3/4 overexpressing (blue) and non-overexpressing cell lines (black) to (I) BGJ398 and (J) erdafitinib in vitro. Cells were treated with drug for 72 h and cell growth determined by MTS assays. Values shown are mean +/- SEM from a representative experiment.
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-5198, inhibits colony formation of glioma cells transformed with mutant IDH1 (Rohle et al., 2013); however, despite lowering of 2-HG levels, AGI-5198 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 ERBB 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-existant activating ERBB2 mutation in BRAF mutant (IS80L, 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 MAP2K1\textsuperscript{K57N} 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 occur in 7%–14% of intrahepatic cholangiocarcinomas (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 misclassifying 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.

SUPPLEMENTAL INFORMATION
Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2019.10.044.

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AUTHOR CONTRIBUTIONS
D.K.L., D.M, W.W., I.Y.L., C.M.S., D.S.W., G.I., L.J.J., C.M.R., T.M., Y.S., and J.M.M: conducted, analyzed, and interpreted experiments.

D.K.L., D.M, D.S.W., Y.Y., T.L., G.I., F.C., M.N., D.C, A.J.W., N.C.T., O.M.S and J.M.M: conceived, designed, interpreted experiments, and/or supervised parts of the study.

D.K.L., D.M, G.I., A.S.D., O.M.S, and J.M.M: contributed to the writing of the paper.
REFERENCES
Abou-Alfa, G.K., Marocella, T.M., Javle, M., et al., (2019) and ClariDH: A global, phase 3, randomized, double-blind study of ivosidenib (IVO) vs placebo in patients with advanced cholangiocarcinoma (CC) with an isocitrate dehydrogenase 1 (IDH1) mutation. Presented at: 2019 ESMO Congress; September 27 to October 1, 2019; Barcelona, Spain. Abstract LBA10 PR, https://oncologypro.esmo.org/Meeting-Resources/ESMO-2019-Congress/ClariDH-A-global-phase-3-randomized-double-blind-study-of-ivosidenib-IVO-vs-placebo-in-patients-with-advanced-cholangiocarcinoma-CC-with-an-isocitrate-dehydrogenase-1-IDH1-mutation.

Alexandrov, L.B., Nik-Zainal, S., Wedge, D.C., Aparicio, S.A., Behjati, S., Biankin, A.V., Bignell, G.R., Bolli, N., Borg, A., Borresen-Dale, A.L., et al. (2013). Signatures of mutational processes in human cancer. Nature 500, 415–421.

Arai, Y., Totoroki, Y., Hosoda, F., Shirota, T., Hama, N., Nakamura, H., Ojima, H., Furuta, K., Shimada, K., Okusaka, T., et al. (2014). Fibroblast growth factor receptor 2 tyrosine kinase fusions define a unique molecular subtype of cholangiocarcinoma. Hepatology 59, 1427–1434.

Borger, D.R., Tanabe, K.K., Fan, K.C., Lopez, H.U., Fantin, V.R., Straley, S.K., Schenken, D.P., Hezel, A.F., Ancukiewicz, M., Liebman, H.M., et al. (2012). Frequent mutation of isocitrate dehydrogenase (IDH1) and IDH2 in cholangiocarcinoma identified through broad-based tumor genotyping. Oncologist 17, 72–79.

Bunse, L., Pusch, S., Bunse, T., Sahm, F., Sanghvi, K., Friedrich, M., Alansary, D., Sonner, J.K., Green, R., Deumelandt, K., et al. (2018). Suppression of antitumor T cell immunity by the oncometabolite (R)-2-hydroxyglutarate. Nat. Med. 24, 1192–1203.

Charbel, H., and Al-Kawas, F.H. (2011). Cholangiocarcinoma: epidemiology, risk factors, pathogenesis, and diagnosis. Curr. Gastroenterol. Rep. 13, 182–187.

Dougherty, M.K., Muller, J., Ritt, D.A., Zhou, M., Zou, X.Z., Copeland, T.D., Connards, T.P., Veenstra, T.D., Lu, K.P., and Morrison, D.K. (2005). Regulation of Rap1 by direct feedback phosphorylation. Mol. Cell 17, 215–224.

Farshidfar, F., Zheng, S., Gingeras, M.C., Newton, Y., Shih, J., Robertson, A.G., Hinoue, T., Hoadley, K.A., Gibb, E.A., Roszk, J., et al. (2017). Integrative genomic analysis of cholangiocarcinoma identifies distinct IDH1-mutant molecular profiles. Cell Rep. 19, 2878–2880.

Gao, Y., Chang, M.T., McKay, D., Na, N., Zhou, B., Yanger, R., Torres, N.M., Muniz, K., Drosten, M., Babacan, M., et al. (2018). Allele-specific mechanisms of activation of MEK1 mutants determine their properties. Cancer Discov. 8, 648–661.

Helsten, T., Elkin, S., Arthur, E., Tomson, B.N., Carter, J., and Kurzrock, R. (2016). The FGFR landscape in cancer: analysis of 4,853 tumors by next-generation sequencing. Clin. Cancer Res. 22, 259–267.

Homma, S., Hasumura, S., Nagamori, S., and Kameda, H. (1988). Establishment and characterization of a human gall bladder carcinoma cell line NOZ. Cell Physiol. Jpn. 22, 474–479.

Homma, S., Nagamori, S., Fujise, K., Yamazaki, K., Hasumura, S., Sujino, H., Matsura, T., Shimizu, K., Kameda, H., and Takaki, K. (1987). Human biliary duct carcinoma cell line producing abundant mucin in vitro. Gastroenterol. Jpn. 22, 648–661.

Hyman, D.M., Piha-Paul, S.A., Won, H., Rodon, J., Saura, C., Shapiro, G.I., Juric, D., Quinn, D.I., Moreno, V., Doger, B., et al. (2018). HER kinase inhibition in patients with HER2- and HER3-mutant cancers. Nature 554, 189–194.

Iishi, Y., Sigel, C., Lowery, M.A., Goyal, L., Gliser, C., Jiang, L., Pandya, S., Wu, B., Choe, S., and Deshpande, V. (2018). Abstract A071: AG-120 (ivosidenib), a first-in-class mutant IDH1 inhibitor, promotes morphologic changes and upregulates liver-specific genes in IDH1 mutant cholangiocarcinoma. Mol. Cancer Ther. 17, A071.

Javle, M., Lowery, M., Shroff, R.T., Weiss, K.H., Springer, C., Borad, M.J., Ramanathan, R.K., Goyal, L., Sadeghi, S., Macarulla, T., et al. (2018). Phase II study of BGJ398 in patients with FGFR-altered advanced cholangiocarcinoma. J. Clin. Oncol. 36, 276–282.

Jusakul, A., Cutcutache, I., Yong, C.H., Lim, J.Q., Huang, M.N., Padmanabhan, N., Nellore, V., Kusaka, Y., Tokiwa, T., and Sato, J. (1988). Establishment of a cell line (G-415) from a human gallbladder carcinoma cell line producing abundant mucin in vitro. Gastroenterol. Jpn. 24, 188–192.

Kawakita, I., Yamagata, S., Fukutomi, H., Sakita, T., Kondo, I., and Kikuchi, M. (1980). Establishment and characterization of a cell line NOZ-1 from a human gallbladder carcinoma cell (HuCC-T1) producing abundant mucin in vitro. Gastroenterol. Jpn. 15, 95–97.

Koyama, S., Yoshioka, T., Mizushima, A., Kawakita, I., Yamagata, S., Fukutomi, H., Sakita, T., Kondo, I., and Kikuchi, M. (1980). Establishment of a cell line (G-415) from a human gallbladder carcinoma. Gan 71, 574–575.

Kusaka, Y., Totoroki, Y., and Sato, J. (1988). Establishment and characterization of a cell line from a human cholangiocellular carcinoma. Res. Exp. Med. (Berl) 188, 367–375.

Lapiniski, P.E., Bauler, T.J., Brown, E.J., Hughes, E.D., Saunders, T.L., and King, P.D. (2007). Generation of mice with a conditional allele of the p120 Ras GTPase-activating protein. Genesis 45, 762–767.

Li, M., Zhang, Z., Li, X., Ye, J., Wu, X., Tan, Z., Liu, C., Shen, B., Wang, X.A., Wu, W., et al. (2014). Whole-exome and targeted gene sequencing of gallbladder carcinoma identifies recurrent mutations in the ErBβ pathway. Nat. Genet. 46, 872–876.

Lowery, M.A., Abou-Alfa, G.K., Burris, H.A., Janku, F., Shroff, R.T., Cleary, J.M., Saba Azad, N., Goyal, L., Maher, E.A., Gore, L., et al. (2017). Phase I study of AG-120, an IDH1 mutant enzyme inhibitor: results from the cholangiocarcinoma dose escalation and expansion cohorts. J. Clin. Oncol. 35, 4015.

Marcano-Bonilla, L., Mohamed, E.A., Mounajed, T., and Roberts, L.R. (2016). Biliary tract cancers: epidemiology, molecular pathogenesis and genetic risk associations. Chin. Clin. Oncol. 5, 61.

Miyaizawa, M., Ichida, T., Tokiwa, T., Sato, J., and Sasaki, H. (1989). A new human cholangiocellular carcinoma cell line (HuCC-T1) producing carbohydrate antigen 19-9 in serum-free medium. In Vitro Cell Dev. Biol. 25, 503–510.

Molenaar, R.J., Maciejewski, J.P., Wilmink, J.W., and van Noorden, C.J.F. (2018). Wild-type and mutated IDH1/2 enzymes and therapy responses. Chin. Clin. Oncol. 7, 1116–1135.

Mohammed, S., Heck, A.J., Maurice, M.M., et al. (2012). Tumour suppressor RNF43 is a stem-cell E3 ligase that induces endocytosis of Wnt receptors. Nature 488, 665–669.

Muhammad, A., Heid, A., Mirza, M., et al. (2012). Mutational analysis of the FGFR3 gene in patients with advanced cholangiocarcinoma. J. Hepatol. 56, 1949–1960.
Oncotarget

HER2 in advanced biliary tract cancer.
S.W., et al. (2016). Therapeutic implication of
Bang, J.H., Jin, M.H., Lee, K.H., Kim, T.Y., Han,
S.W., et al. (2016). Therapeutic implication of

Obshoei, S., Weakley, S.M., Wongkhams, S.,
Wongkhams, C., Sawanyawisuth, K., Yao, Q., and
Chen, C. (2011). Cyclophilin A enhances cell
proliferation and tumor growth of liver fluke-
associated cholangiocarcinoma. Mol. Cancer

Ong, C.K., Subimereb, C., Painojtul, C.,
Wongkhams, S., Cuticushe, I., Yu, W.,
McPherson, J.R., Allen, G.E., Ng, C.C., Wong,
B.H., et al. (2012). Exome sequencing of liver
fluke-associated cholangiocarcinoma. Nat.
Genet. 44, 690-693.

Rohle, D., Popovici-Muller, J., Palaskas, N.,
Turcan, S., Grommes, C., Campos, C., Tsoi, J.,
Clark, O., Oldrim, B., Komisopoulou, E., et al.
(2013). An inhibitor of mutant IDH1 delays growth
and promotes differentiation of glioma cells.
Science 340, 626-630.

Ross, J.S., Wang, K., Gay, L., Al-Rohil, R., Rand,
J.V., Jones, D.M., Lee, H.J., Sheehan, C.E.,
Otto, G.A., Palmer, G., et al. (2014). New routes to targeted therapy of
intrahepatic cholangiocarcinomas revealed by
next-generation sequencing. Oncologist 19,
235-242.

Saha, S.K., Jordan, D.D., Kleinstiver, B.P., Vu, P.,
Najm, M.S., Yeo, J.C., Shi, L., Kato, Y., Levin, R.S.,
Webber, J.T., et al. (2016). Isocitrate
dehydrogenase mutations confer dasatinib
hypersensitivity and SRC dependence in
intrahepatic cholangiocarcinoma. Cancer Discov.
6, 727-739.

Saha, S.K., Parachoniak, C.A., and Bardeesy, N.
(2014). IDH mutations in liver cell plasticity and
biliary cancer. Cell Cycle 13, 3176–3182.

Saito, Y., Muramatsu, T., Kanai, Y., Ojima, H.,
Sukeda, A., Hiroaka, N., Arai, E., Sugiyama, Y.,
Matsuzaki, J., Uchida, R., et al. (2019). Establishment of patient-derived organoids and
drug screening for biliary tract carcinoma. Cell
Rep. 27, 1265–1276.e64.

Seok, J., Yoon, S.H., Lee, S.H., Jung, J.H., and
Lee, Y.M. (2019). The oncometabolite
dihydroxyglutarate induces angiogenic activity
through the vascular endothelial growth factor
receptor 2 signaling pathway. Int. J. Oncol. 54,
753–763.

Shoda, J., Ishige, K., Sugiyama, H., and
Kawamoto, T. (2012). Biliary tract carcinoma:
clinical perspectives on molecular targeting
strategies for therapeutic options.
J. Hepatobiliary Pancreat. Sci. 19, 342–353.

Tepsiri, N., Chaturat, L., Sripa, B., Namwat, W.,
Wongkhams, S., Bhudhisawasdi, V., and
Tassaneeyakul, W. (2005). Drug sensitivity and
drug resistance profiles of human intrahepatic
cholangiocarcinoma cell lines. World J.
Gastroenterol. 11, 2748–2753.

Turner, N. and Grose, R. (2010). Fibroblast
growth factor signalling: from development to
cancer. Nat. Rev. Cancer 10, 116–129.

Valle, J., Wasan, H., Palmer, D.H., Cunningham,
D., Anhoney, A., Maraveyas, A., Madhusudan, S.,
Iveson, T., Hughes, S., Pereira, S.P., et al. (2010). Cisplatin plus gemcitabine versus gemcitabine
for biliary tract cancer. N. Engl. J. Med. 362, 1273–
1281.

Vaccaero, J., Guedj, N., Claperon, A., Nguyen Ho-
Boulcbois, T.H., Paradis, V., and Fouassier, L.
(2017). Epithelial-mesenchymal transition in cholangiocarcinoma: from clinical evidence to
regulatory networks. J. Hepatol. 66, 424–441.

Wang, P., Dong, Q., Zhang, C., Kuan, P.F., Liu, Y.,
Jeck, W.R., Andersen, J.B., Jiang, W., Savich, G.L.,
Tan, T.X., et al. (2013). Mutations in isocitrate
dehydrogenase 1 and 2 occur frequently in
intrahepatic cholangiocarcinomas and share
hypermethylation targets with glioblastomas.
Oncogene 32, 3901–3100.

Ward, P.S., Patel, J., Wise, D.R., Abdel-Wahab,
O., Bennett, B.D., Collier, H.A., Cross, J.R., Fantin,
V.R., Hedvat, C.V., Perl, A.E., et al. (2010). The
common feature of leukemia-associated IDH1
and IDH2 mutations is a neomorphic enzyme
activity converting alpha-ketoglutarate to 2-
hydroxyglutarate. Cancer Cell 17, 225–234.

Xu, S., Zhan, M., and Wang, J. (2017). Epithelial-
to-mesenchymal transition in gallbladder cancer: from clinical evidence to cellular regulatory
networks. Cell Death Discov. 3, 17069.

Yamada, N., Chung, Y., Ohtani, H., Ikeda, T.,
Onoda, N., Sawada, T., Nishiguchi, Y., Hasuma,
T., and Sowa, M. (1997). Establishment and
characterization of a new human gallbladder
carcinoma cell line (OCUG-1) producing TA-4. Int.
J. Oncol. 10, 1251–1255.

Yao, Z., Yaeger, R., Rodrik-Outmezguine, V.S.,
Tao, A., Torres, N.M., Chang, M.T., Drosten, M.,
Zhao, H., Cecchi, F., Hembrough, T., et al. (2017).
Tumours with class 3 BRAF mutants are sensitive
to the inhibition of activated RAS. Nature 548,
234–238.

Zhang, K., Chu, K., Wu, X., Gao, H., Wang, J.,
Yuan, Y.C., Loera, S., Ho, K., Wang, Y., Chow, W.,
et al. (2013). Amplification of FRS2 and activation
of FGFR/FRS2 signaling pathway in high-grade
liposarcoma. Cancer Res. 73, 1298–1307.
Supplemental Information

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

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Figure S1. mRNA expression of genes harboring inactivating mutations. Related to Figure 2. mRNA expression of (A) BAP1 (B) RNF43 and (C) RASA1 in BTC lines determined by RNA-seq.
Figure S2. Validation of altered Wnt signaling in BTC cell lines, Related to Figure 2. (A) Wnt reporter activity in BTC cell lines which are mutant or wild type for APC, CTNNB1 or RNF43 determined by the ration of TOPFLASH/FOPFLASH (TOP/FOP) luciferase reporter assay. Values shown are mean + SEM from a representative experiment performed in quadruplicate. (B) Basal mRNA expression of the Wnt target gene AXIN2 in BTC cell lines determined by qPCR. Values shown are mean + SEM from a representative experiment performed in technical triplicate. (C) Effect of Wnt3a conditioned medium of mRNA expression of AXIN2 in BTC cell lines. Cells were treated with increasing concentrations of Wnt3a conditioned medium for 24h and AXIN2 mRNA expression determined by qPCR. Values shown are mean + SEM from a representative experiment performed in technical quadruplicate.
Figure S3. Morphology of BTC cell lines from the mesenchymal and epithelial clusters when grown in 2D cell culture, Related to Figure 4. Cell lines in the exponentially growing phase were imaged by light microscopy.
Figure S4. Summary of anatomical location and molecular subtype of BTC cell lines, Related to Figure 4. Cell lines were classified as epithelial or mesenchymal based on their basal gene expression profile which was assessed by RNA-seq analysis.
Figure S5. Identification or primary BTCs harboring the mesenchymal (blue text) and epithelial (orange text) signatures identified in BTC cell lines. Related to Figure 4. 35 primary BTCs for which RNA-seq data was available were clustered along with the 20 BTC cell lines based on the expression of the 411 genes differentially expressed between the epithelial and mesenchymal BTC cell line clusters. Two primary BTCs (TCGA-W5-AA2H and TCGA-ZU-A824) clustered with the cell lines harboring the mesenchymal signature, and 8 primary BTCs (TCGA-W5-AA36, TCGA-4G-AAZT, TCGA-W5-AA38, TCGA-W5-AA2O, TCGA-W5-AA2I, TCGA-W5-AA2X and TCGA-3X-AACV) clustered with the BTC cell lines harboring the epithelial signature. Data obtained in part from the TCGA Research Network: https://www.cancer.gov/tcga.
Figure S6

**Mesenchymal cluster**

TCG-W5-AA2H

TCG-ZU-A8S4

**Epithelial cluster**

TCG-W5-AA39

TCG-W5-AA36

TCG-4G-AAZT

TCG-W5-AA38

TCG-W5-AA2O

TCG-W5-AA2I

TCG-W5-AA2X

TCG-3X-AAVC

Figure S6. Cellular morphology of primary tumours clustered among the cell lines harbouring the mesenchymal or epithelial signature, Related to Figure 4. Images obtained from the TCGA Research Network: https://www.cancer.gov/tcga.
Figure S7. Characterization of IDH<sup>R132C</sup> mutant SNU-1079 cells, Related to Figure 2. (A) Levels of 2-HG in the culture medium or cell lysates in IDH<sup>R132C</sup> (SNU-1079) or IDH<sup>WT</sup> (SNU-1196, TKKK, Sk-Ch-A1, OZ) cell lines. Values shown are mean + SD from a representive experiment performed in duplicate. Inset: Sanger sequencing chromatogram of IDH1 R132C mutation in SNU-1079 cells. (B) SNU-1079 cells were treated with increasing concentrations of the mutant IDH1 inhibitor AGI-5198 for up to 72h and 2-HG levels determined in the culture medium. Values shown are mean +/- SEM of a representive experiment performed in triplicate. (C) Effect of AGI-5198 and AG-120 on proliferation of SNU-1079 cells. Cells were treated with AGI-5198 or AG-120 for 72h and cell proliferation determined using the MTS assay. Values shown are mean +/- SEM of n=3 independent experiments. (D) Effect of AG-120 on proliferation of IDH<sup>R132L</sup> or IDH<sup>WT</sup> biliary cancer organoids. Values shown are mean +/- SD of a representive experiment performed in quadruplicate. Similar results were obtained in an independent experiment. (E) Effect of AGI-5198 and AG-120 on expression of markers of hepatocyte differentiation and EMT. SNU-1079 cells were treated with AGI-5198 or AG-120 for 72h and changes in gene expression determined by qPCR. Values shown are mean +/- SEM of a representive experiment performed in triplicate. (F) Relative sensitivity of BTC cell lines to gemcitabine. Cells were treated with DMSO or gemcitabine (20 uM) for 72h and cell viability determined by MTS assay. Values shown are mean +/- SD of n=2 independent experiments. (G) Effect of combination treatment with AGI-5198 and gemcitabine on proliferation of IDH mutant SNU-1079 cells. Cells were treated with drug combinations for 72h and cell proliferation determined by MTS assay. Values shown are mean +/- SD from a representative experiment performed in technical quadruplicate.
Table S1. Biliary tract cancer cell lines, source and origin, Related to Figure 1. HSRRB-Japan Health Sciences Foundation; Zurich University (Prof. A Knuth); KCLB-Korean Cell Line Bank; RIKEN Bioresources Centre, Japan.

| Cell line      | Gender, Age | Differentiation grade of primary | Anatomical location of primary tumour | Primary/Metastatic | Liver Fluke | Source          | Ref  |
|----------------|-------------|----------------------------------|--------------------------------------|-------------------|------------|-----------------|------|
| HuH28          | Female, 37Y | Undiff                           | Intrahepatic                         | Primary           | No         | HSRRB (1)       |      |
| SNU-1079       | Male, Unk   | Mod Diff                         | Intrahepatic                         | Primary           | No         | KCLB (2)        |      |
| TKKK           | Male, Unk   | Mod Diff                         | Intrahepatic                         | Primary           | Yes        | RIKEN N/A       |      |
| KU-M055        | Male, 56Y   | Poorly Diff                      | Intrahepatic                         | Primary           | Yes        | KU (3)          |      |
| KU-M213        | Male, 58Y   | Adenosquamous                    | Intrahepatic                         | Primary           | Yes        | KU (4)          |      |
| HuCCT1         | Male, 56Y   | Mod Diff                         | Intrahepatic                         | Metastatic (Ascites) | No     | RIKEN (5)       |      |
| OZ             | Male, 71Y   | Well Diff and Poorly Diff        | Extra/Intrahepatic                   | Metastatic (Ascites) | No     | HSRRB (6)       |      |
| SNU-1196       | Unk, Unk    | Mod Diff                         | Extrahepatic                         | Primary           | No         | KCLB (2)        |      |
| Sk-ChA-1       | Female, 47Y | Undiff                           | Extrahepatic                         | Metastatic (Ascites) | No     | Zurich Uni. (7) |      |
| TFK-1          | Male, 63Y   | Mod Diff                         | Intrahepatic                         | Primary           | No         | DSMZ (8)        |      |
| SNU-245        | Unk, Unk    | Well Diff                        | Intrahepatic                         | Primary           | No         | KCLB (2)        |      |
| EGI-1          | Male, 52Y   | Poorly Diff                      | Extrahepatic                         | Primary           | No         | DSMZ (9)        |      |
| SNU-308        | Unk, Unk    | Well-Moder Diff                  | Gallbladder                          | Primary           | No         | KCLB (2)        |      |
| TGBCT14TKB     | Female, Unk | Undiff (Anaplastic)             | Gallbladder                          | Primary           | No         | RIKEN (10)      |      |
| TGBCT2TKB      | Female, Unk | Well/Poorly Diff                | Gallbladder                          | Primary           | No         | RIKEN (10)      |      |
| MZ-ChA-2       | Female, 63Y | Mod Diff                         | Gallbladder                          | Metastatic (Liver) | No     | Zurich Uni. (7) |      |
| G415           | Male, 68Y   | Undiff                           | Gallbladder                          | Metastatic (Ascites) | No     | RIKEN (11)      |      |
| NOZ            | Female, 48Y | Mod Diff                         | Gallbladder                          | Metastatic (Ascites) | No     | HSRRB (12)      |      |
| OCGU-1         | Male, 43Y   | Poorly Diff                      | Gallbladder                          | Metastatic (Ascites) | No     | HSRRB (13)      |      |
| SNU-478        | Unk, Unk    | Poorly Diff                      | Ampullary                            | Primary           | No         | KCLB (2)        |      |
| TGBCT18TKB     | Female, 79Y | Unk                              | Ampullary                            | Primary           | No         | RIKEN N/A       |      |
| SNU-869        | Unk, Unk    | Well Diff                        | Ampullary                            | Primary           | No         | KCLB (2)        |      |

1. Kusaka Y, Tokiwa T, Sato J. Establishment and characterization of a cell line from a human cholangiocellular carcinoma. Res Exp Med (Berl) 1988;188:367-75
2. Ku JL, Yoon KA, Kim IJ, Kim WH, Jang JY, Suh KS, et al. Establishment and characterisation of six human biliary tract cancer cell lines. Br J Cancer 2002;87:187-93
3. Tepsiri N, Chaturat L, Sripa B, Namwat W, Wongkham S, Bhudhisawasdi V, et al. Drug sensitivity and drug resistance profiles of human intrahepatic cholangiocarcinoma cell lines. World J Gastroenterol 2005;11:2748-53
4. Obchoei S, Weakley SM, Wongkham S, Wongkham C, Sawanyawisuth K, Yao Q, et al. Cyclophilin A enhances cell proliferation and tumor growth of liver fluke-associated cholangiocarcinoma. Molecular cancer 2011;10:102
5. Miyagiwa M, Ichida T, Tokiwa T, Sato J, Sasaki H. A new human cholangiocellular carcinoma cell line (HuCC-T1) producing carbohydrate antigen 19/9 in serum-free medium. In Vitro Cell Dev Biol 1989;25:503-10
6. Homma S, Nagamori S, Fujise K, Yamazaki K, Hasumura S, Sujino H, et al. Human bile duct carcinoma cell line producing abundant mucin in vitro. Gastroenterol Jpn 1987;22:474-9
7. Knuth A, Gabbert H, Dippold W, Klein O, Sachsse W, Bitter-Suermann D, et al. Biliary adenocarcinoma. Characterisation of three new human tumor cell lines. Journal of hepatology 1985;1:579-96
8. Saijyo S, Kudo T, Suzuki M, Katayose Y, Shinoda M, Muto T, et al. Establishment of a new extrahepatic bile duct carcinoma cell line, TFK-1. Tohoku J Exp Med 1995;177:61-71
9. Zach S, Birgin E, Rückert F. Primary Cholangiocellular Carcinoma Cell Lines. J Stem Cell Res Transplant 2015;2:1013
10. Ghosh M, Koike N, Yanagimoto G, Tsunoda S, Kaul S, Hirano T, et al. Establishment and characterization of unique human gallbladder cancer cell lines. Int J Oncol 2004;24:1189-96
11. Koyama S, Yoshioka T, Mizushima A, Kawakita I, Yamagata S, Fukutomi H, et al. Establishment of a cell line (G-415) from a human gallbladder carcinoma. Gan 1980;71:574-5
12. Homma S, Hasumura S, Nagamori S, Kameda H. [Establishment and characterization of a human gall bladder carcinoma cell line NOZ]. Hum Cell 1988;1:95-7
13. Yamada N, Chung Y, Ohtani H, Ikeda T, Onoda N, Sawada T, et al. Establishment and characterization of a new human gallbladder carcinoma cell line (OCUG-1) producing TA-4. Int J Oncol 1997;10:1251-5
Table S2. Mutated genes in 22 BTC cell lines, Related to Figure 1. Table is provided as a separate excel file.
**Table S3.** Significantly Amplified and Deleted loci in biliary tract cancer cell lines, Related to Figure 3. Significantly amplified and deleted loci across the 22 BTC cell lines determined using Illumina OmniExpress SNP arrays.

| Amplified loci | Cytoband | q value | residual q value | wide peak boundaries       |
|----------------|----------|---------|------------------|-----------------------------|
|                | 12p12.1  | 2.74E-11| 2.83E-10         | chr12:24970589-25581258     |
|                | 12p12.2  | 0.00012196 | 0.0022211       | chr12:20202918-21312923     |
|                | 12p11.1  | 0.0049466 | 0.034311        | chr12:33940680-37927113     |
|                | 3q26.1   | 0.046253  | 0.046253        | chr3:161570646-166861677    |
|                | 8q24.21  | 0.065588  | 0.065588        | chr8:128129043-128408115    |

| Deleted loci  | Cytoband | q value | residual q value | wide peak boundaries       |
|--------------|----------|---------|------------------|-----------------------------|
|              | 9p21.3   | 2.34E-21| 2.34E-21         | chr9:21865843-22451030      |
|              | 3p14.2   | 9.50E-11| 9.50E-11         | chr3:59029190-61549294      |
|              | 16q23.1  | 0.00018762| 0.00018762   | chr16:78129907-79628180     |
|              | 20p12.1  | 0.00039386| 0.00039386   | chr20:14295646-16034338     |
|              | 3q28     | 0.093074  | 0.093074        | chr3:188605427-189356940    |
**Table S4.** Copy number variations in 22 BTC cell lines, Related to Figure 3. DNA copy-number was assessed using Illumina OmniExpress SNP arrays. Table is provided as a separate excel file.
**Table S5.** Mechanism of MAPK pathway deregulation in BTC cell lines, Related to Figures 5 and 6. MAPK pathway alterations were assessed using exome sequencing, copy number analysis or RNA-seq analysis.

| Cell line       | MAPK pathway alteration                  |
|-----------------|------------------------------------------|
| OZ              | **KRAS Q61L**                             |
| SNU869          | **KRAS G12D**                             |
| TGBCl4TKB       | **KRAS G13C**                             |
| EGI1            | **KRAS G12D**                             |
| G415            | **KRAS G13D**                             |
| HUCCT1          | **KRAS G12D**                             |
| KKUM213         | **KRASG13C**                              |
| NOZ             | **KRAS G12V, KRAS Amp**                   |
| SNU1196         | **KRAS Amp**                              |
| SNU245          | **KRAS Amp**                              |
| TGBC18TKB       | **BRAF I581L, N582T**                     |
| Sk-Ch-A1        | **BRAF D594T, RASA1 R726***               |
| KKU-MO55        | **MAP2K1 K57, FGFR1 overexpression**      |
| TKKK            | **WT**                                    |
| SNU478          | **WT**                                    |
| TFK-1           | **WT**                                    |
| SNU1079         | **WT**                                    |
| TGBCl2TKB       | **WT**                                    |
| SNU308          | **WT**                                    |
| HUH28           | **WT**                                    |
| MzChA2          | **WT**                                    |
| OCUG1           | **WT**                                    |
Table S6. Short tandem repeat (STR) profiles of BTC cell lines, Related to Figure 1. STR profiling was performed on DNA isolated from each cell lines using the GenePrint 10 System.

| Cell Line | D5S818 | D13S317 | D7S820 | D16S539 | vWA | TH01 | Amelogenin | TPOX | CSF1PO | D21S11 | REFERENCE | STR match / result |
|-----------|--------|---------|--------|---------|-----|------|-----------|------|--------|--------|------------|---------------------|
| EGI-1     | 13     | 11      | 9,13   | 13      | 17,19 | 6,9  | X,Y      | 8,11 | 12,13  | 28, 33,2 | Equivalent Ref #DSMZ ACC-385 | EV=0.97               |
| G415      | 12     | 8       | 11,12  | 12      | 16   | 7    | X        | 11   | 12     | 32,2   | Equivalent Ref RIKEN #RCB2640 | EV=0.92               |
| HuCCT1    | 12,13  | 11,13   | 10,11  | 11,12   | 18   | 7,10 | X,Y      | 8    | 11,12  | 31     | Equivalent Ref JCRB NIBIO #JCRB0426 | EV=1.0                |
| HuH28     | 9,12   | 9       | 10,11  | 9       | 17   | 9    | X        | 8    | 9,12   | 30,33,2 | Equivalent Ref JCRB NIBIO #JCRB0426 | EV=0.96               |
| KKU-M055  | 11     | 11      | 7,8    | 10,13   | 17,19 | 9    | X        | 8    | 10,11  | 29,31  | Equivalent Ref JCRB #JCRB1551    | EV=1.0                |
| KKU-M213  | 9      | 8,12    | 11     | 9,11    | 18   | 7    | X        | 8    | 9,13   | 29     | Equivalent Ref JCRB #JCRB1557    | EV=1.0                |
| Sk-Ch-A1  | 11,13  | 11,12   | 10     | 9,13    | 16,18 | 6    | X        | 8    | 12,13  | 28     | Not available                            | EV=0.91               |
| MzChA2    | 10     | 12      | 11,12  | 11      | 15,17 | 8    | X        | 11   | 12     | 28,30  | Not available                            | EV=0.98               |
| NOZ       | 13     | 8,12    | 10,11  | 9,11    | 19   | 7,9  | X        | 8    | 11     | 30,31  | Equivalent Ref JCRB NIBIO #JCRB1033 | EV=0.91               |
| OCUG1     | 10     | 8,11    | 10,11  | 9,13    | 16   | 7,9,3 | X        | 11   | 10,12  | 28,29  | Equivalent Ref JCRB NIBIO #JCRB0191 | EV=0.88               |
| OZ        | 13,14  | 8,12    | 10     | 10      | 14,16 | 6,9  | X,Y      | 9,11 | 11,12  | 29     | Equivalent Ref JCRB NIBIO #JCRB1032 | EV=1.0                |
| SNU245    | 7      | 9,13    | 8,10   | 9,13    | 14,18 | 9    | X        | 8    | 11     | 27,30  | Equivalent Ref KCLB #00245          | EV=0.96               |
| SNU308    | 9      | 8       | 7,12   | 10,13   | 16   | 8    | X        | 8    | 12     | 30,31  | Equivalent Ref KCLB #00308          | EV=1.0                |
| SNU478    | 9,12   | 9,11    | 8,11   | 12      | 15,16 | 7    | X        | 10,11| 10,12  | 30     | Equivalent Ref KCLB #00478          | EV=1.0                |
| SNU869    | 12     | 12      | 8,11   | 9       | 15    | 7,9  | X        | 8,10 | 10     | 29,30  | Equivalent Ref KCLB #00869           | EV=1.0                |
| SNU1079   | 10,13  | 8       | 8,12   | 10,11   | 16,18 | 8    | X,Y      | 8    | 12     | 30     | Equivalent Ref KCLB #01079           | EV=1.0                |
| SNU1196   | 11     | 9       | 8,11   | 11,12   | 14   | 7    | X        | 10,11| 10     | 30     | Equivalent Ref KCLB #01196           | EV=1.0                |
| TFK-1     | 9,12   | 14      | 10     | 9       | 14,17 | 6    | X,Y      | 8    | 10     | 30     | Equivalent Ref DSMZ #ACC-344         | EV=1.0                |
| TGBC2TKB  | 11     | 8,11    | 9,13   | 9,11    | 14,17 | 9    | X        | 8    | 14     | -      | Equivalent Ref RIKEN #RCB1130       | EV=1.0                |
| TGBC14TKB | 12     | 12      | 10     | 9       | 14    | 6,7  | X        | 8    | 12     | 28,30  | Equivalent Ref RIKEN #RCB1186        | EV=0.95               |
| TGBC18TKB | 14     | 8       | 11,12  | 9       | 18    | 9,9,3 | X        | 8    | 14     | 31,32,2 | Equivalent Ref RIKEN #RCB1169       | EV=0.92               |
| TKKK      | 13     | 8,12    | 10,11  | 11      | 16    | 6    | X,Y      | 8    | 11     | 29     | Equivalent Ref RIKEN #RCB1807       | EV=0.96               |
**Transparent Methods**

**Biliary tract cancer cell lines**
The source and original publications describing the 22 BTC cell lines investigated are listed in Supplemental Table 1. All cell lines were maintained in Dulbecco's Modified Eagle Medium supplemented with 10% FBS, 1 mM HEPES buffer and penicillin/streptomycin (100 U/mL) at 37°C and 5% CO$_2$. Cell line authentication was performed using the GenePrint® 10 System (Promega, USA) (Supplemental Table 6). Cell lines were routinely tested for mycoplasma status by the MycoAlert Mycoplasma Detection Kit (Lonza) and confirmed to be negative.

**Exome-capture sequencing:** Exome-capture was performed using the Agilent SureSelect XT Human All Exon v5 and 100 bp paired-end read sequencing performed on an Illumina HiSeq 2000 System. Raw FASTQ paired-end reads were aligned against the human hg19 reference sequence using the Burrows-Wheeler Alignment (BWA) tool (Li and Durbin, 2009). Variants were annotated against databases of known human germline variations (dbSNP, build 135, SAO = 1), 1000 Genomes Project database (build 20110521), Mills et al dataset of small insertions and deletions (Mills et al., 2011) and germline variants detected in 114 normal colorectal tissues analyzed in our laboratories. Somatic mutation signatures were generated using the “SomaticSignatures” package. Significantly mutated genes determined in MutSig.

**RNA-seq analysis:** Total RNA was extracted from cell pellets using the High Pure RNA Isolation Kit (Roche, Basel, Switzerland) as per manufacturer’s protocol, which included a DNase I step. cDNA synthesis, library preparation of six indexed samples and RNA-Seq analysis was performed on an Illumina HiSeq2000 to a minimum depth of >20 million 100bp single end reads. Raw reads were assessed for good quality using the FASTQC software. Alignment of transcript sequences to the human reference genome (build hg19) was performed using the TopHat (2.1.0) software with default parameters (Trapnell et al., 2012). Gene regions were identified based on alignments of the RefSeq human database by the UCSC genome browser (hg19). Normalized gene expression values were calculated by counting aligning reads per kilobase per million reads mapped (RPKM) and counts per million (CPM). Absence of gene expression was defined as a RPKM value of <1. *FGFR2* fusion transcripts were investigated by visualisation of aligned transcripts using the Integrative Genomics Viewer v2.4 (Broad Institute, Cambridge MA, USA) (Robinson et al., 2011).

**DNA copy number analysis:** DNA copy number was assessed using Illumina HumanOmniExpress-24 BeadChip and analysed using GenomeStudio (Illumina). SNPs showing germline alterations, based on 637 normal samples, were excluded. DNA copy-number segmentation and absolute copy number estimates was performed using OncoSNPv2.18 (Yau et al., 2010), and significantly altered regions identified using GISTIC2.0 (van Dyk et al., 2013).

**Cell block generation and Immunohistochemistry (IHC):** Cultured cell lines (minimum 1x10$^7$) were pelleted and coagulated in human plasma (150 µL) and bovine thrombin (1units/µL) for 5 mins (Pfizer, New York, NY, USA), fixed in 10% formalin, and paraffin embedded. p53 was detected using an anti-p53 antibody (DO-7 clone, Novocastra, 1:100), followed by signal detection using an enzyme-conjugated multimer secondary antibody, UltraView Universal DAB detection kit (Ventana), using standard protocols.

**Luciferase reporter assays:** Cells lines seeded in 96 well plates were transfected with 0.1 µg/well of the β-catenin-TCF reporter plasmid (TOPFLASH,) or control plasmid (FOPFLASH) (van de Wetering et al., 1997) using Lipofectamine 2000 (0.2µL/well, Thermo Fisher Scientific). Renilla-TK (0.04 µg/well) was used to control for transfection efficiency. After 48h, cells were harvested in passive lysis buffer (Promega), and luciferase activity measured using the Dual Luciferase Reporter Assay System (Promega) on a Spectromax L Microplate reader (Molecular Devices).

**MTS assays**
Cell proliferation was determined by MTS (3-(4,5-dimethyl-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) assay using the CellTiter 96® AQueous One Solution Assay kit (Promega) as per manufacturer’s instructions.
Organoid proliferation
Cells from cholangiocarcinoma organoids 1T, 9T and 24T were plated (1.2 x 10^3 / well) were cultured for 4 days as described previously (Saito et al., 2019). Organoids were treated with AG-120 (Cayman chemical) for 6 days and cell viability determined by WST assay using the Cell Counting Kit-8 (Dojindo).

Western blotting
20-40 µg of protein was resolved under denaturing conditions (SDS-PAGE) and transferred to PVDF membrane. Membranes were blocked in Odyssey blocking buffer (Li-Cor Bioscience) and probed with anti-pERK1/2 (Thr202/Tyr 204, cat no 4370, Cell Signaling Technology, Danvers MA), anti-ERK1/2 (Cat no: 9107, Cell Signaling), anti-pCRAF (Ser338, Cat no: 9427, Cell Signaling), anti-FGFR1 (Cat no. 9740 Cell Signaling), anti-β-actin (Cat no. A5316, Sigma-Aldrich, MO, USA) or anti-β-tubulin (ab6046, Abcam, Cambridge UK) at room temperature for 1h. Anti-rabbit or mouse secondary antibodies were incubated at room temperature 1h. The infrared fluorescence image was obtained using Odyssey infrared imaging system (Li-Cor Bioscience).

Xenograft studies
Animal experiments were approved by the Austin Health Animal Ethics Committee. 1-2 x 10^6 cells comprising 100 µL of cells (in PBS) and 100 µl Matrigel (Corning, NY, USA) were subcutaneously injected into the right and left flanks of male Balb/c nu/nu mice. Tumours were grown for a maximum of 4 weeks, or until the combined tumour size reached 1 cm^3. Resected tumours were fixed in 10% formalin, and stained with haematoxylin and eosin. For erdafitinib experiments, mice were treated daily for 18 days with 20 mg/kg erdafitinib (MedChem Express, NJ, USA) or vehicle (20% hydroxypropyl b cyclodextrin, Sigma Aldrich) by oral gavage. Tumour growth was measured every second day by caliper.

Data and software availability
RNA-seq data is accessible via the Gene Expression Omnibus, with accession number GSE138772 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE138772).
Supplemental References

Li, H., and Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25, 1754-1760.

Mills, R.E., Pittard, W.S., Mullaney, J.M., Farooq, U., Creasy, T.H., Mahurkar, A.A., Kemeza, D.M., Strassler, D.S., Ponting, C.P., Webber, C., et al. (2011). Natural genetic variation caused by small insertions and deletions in the human genome. Genome Res 21, 830-839.

Robinson, J.T., Thorvaldsdottir, H., Winckler, W., Guttman, M., Lander, E.S., Getz, G., and Mesirov, J.P. (2011). Integrative genomics viewer. Nat Biotechnol 29, 24-26.

Saito, Y., Muramatsu, T., Kanai, Y., Ojima, H., Sukeda, A., Hiraoka, N., Arai, E., Sugiyama, Y., Matsuzaki, J., Uchida, R., et al. (2019). Establishment of Patient-Derived Organoids and Drug Screening for Biliary Tract Carcinoma. Cell reports 27, 1265-1276 e1264.

Trapnell, C., Roberts, A., Goff, L., Pertea, G., Kim, D., Kelley, D.R., Pimentel, H., Salzberg, S.L., Rinn, J.L., and Pachter, L. (2012). Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nature protocols 7, 562-578.

van de Wetering, M., Cavallo, R., Dooijes, D., van Beest, M., van Es, J., Loureiro, J., Ypma, A., Hursh, D., Jones, T., Bejsovec, A., et al. (1997). Armadillo coactivates transcription driven by the product of the Drosophila segment polarity gene dTCF. Cell 88, 789-799.

van Dyk, E., Reinders, M.J., and Wessels, L.F. (2013). A scale-space method for detecting recurrent DNA copy number changes with analytical false discovery rate control. Nucleic Acids Res 41, e100.

Yau, C., Mouradov, D., Jorissen, R.N., Colella, S., Mirza, G., Steers, G., Harris, A., Ragoussis, J., Sieber, O., and Holmes, C.C. (2010). A statistical approach for detecting genomic aberrations in heterogeneous tumor samples from single nucleotide polymorphism genotyping data. Genome Biol 11, R92.
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