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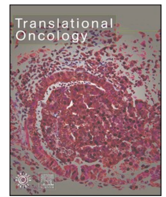
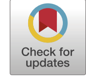


Original Research



Targeted genomic profiling revealed a unique clinical phenotype in intrahepatic cholangiocarcinoma with fibroblast growth factor receptor rearrangement

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A R T I C L E I N F O

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A B S T R A C T

Genomic aberrations (GAs) in fibroblast growth factor receptors (*FGFRs*) are involved in the pathogenesis of intrahepatic cholangiocarcinoma (ICC), and clinical trials have shown efficacy of FGFR inhibitors in treating ICC patients with *FGFR* GAs such as *FGFR2* rearrangement. To clarify the *FGFRs* GA profile and corresponding clinicopathological features in Chinese patients with ICC, a total of 257 cases were identified. Fourteen cases (5.45%) were positive for *FGFR2* rearrangement. Further analysis on the 110 *FGFR2* rearrangement negative cases showed that 13 patients present additional *FGFRs* GAs, including *FGFR3* rearrangement (2.73%), and *FGFRs* mutations. When compared with patients without *FGFRs* GAs, those with *FGFR2* or *FGFR3* rearrangement presented more under the age of 58 years, female sex, HBsAb positivity, CD10 expression, and PD-L1 expression. The clinical characteristics between patients with *FGFRs* mutation and those without *FGFRs* GAs were similar, with the exception that cases with *FGFRs* mutation have more hepatolithiasis. We concluded that *FGFR* rearrangement is associated with unique clinical phenotypes in ICC.

Introduction

Intrahepatic cholangiocarcinoma (ICC) is the second most diagnosed hepatobiliary tumor, which is characterized by late diagnosis, extraordinary heterogeneity, limited treatment option and dismal prognosis [1]. Epidemiological studies have shown that the incidence of the deadly tumor has significantly increased in recent years [1,2]. Risk factors attributed to the tumorigenesis of ICC include parasitic infection, viral hepatitis, hepatolithiasis, choledochal cysts, primary sclerosing cholangitis, diabetes, obesity, smoking, alcohol-related disorders, and genetic susceptibility [3,4]. Surgical resection remains the gold standard treatment, however a surgical approach with curative intention may not be feasible in majority of ICC cases as the disease is typically diagnosed at advanced stage. For locally advanced or metastatic disease, the chemotherapy combination of gemcitabine and cisplatin remains the only preferred systemic treatment, with a median survival of less than one year [5,6]. Therefore, there is an urgent need for more treatment modalities for this severe tumor.

Recently, advances in integrated sequencing technology have provided a compendium of ICC genomic aberrations, which creates unprecedented opportunities for precision targeted therapy to the tumor [7]. Genomic aberrations in fibroblast growth factor receptors (*FGFRs*) are among the most frequent events during ICC development [8]. The *FGFRs* are part of the larger receptor tyrosine kinases family and contain

Abbreviations: FGFR, Fibroblast growth factor receptor; FISH, fluorescent in situ hybridization; ICC, intrahepatic cholangiocarcinoma; TKI, tyrosine kinase inhibitor; GA, genomic aberration; FFPE, formalin-fixed paraffin-embedded; HBV, hepatitis B virus; HCV, hepatitis C virus; TNM, tumor-node-metastasis; CD10, cluster of differentiation 10; PD-L1, programmed cell death-ligand 1; IHC, immunohistochemistry; NGS, next-generation sequencing; BWA, Burrows-Wheeler Aligner; SNV, single nucleotide variant; STAR, spliced transcripts alignment to a reference; TKD, tyrosine kinase domain; HBsAg, hepatitis B surface antigen; HBsAb, anti-hepatitis B surface antibody; HBeAb, anti-hepatitis B virus e antibody; HBcAb, anti-hepatitis B virus core antibody.

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four members: *FGFR1, FGFR2, FGFR3*, and *FGFR4*. Upon binding of fibroblast growth factors, the FGFRs undergo receptor dimerization and initiate downstream signaling, which is essential for diverse physiologic processes [9]. The altered FGFRs because of different genetic aberrations (GAs), including chromosomal translocation and activating mutation, have been proved to play a key role in tumor onset and progression in several human malignancies [10]. In ICC, chromosomal translocations involving *FGFR2* have been frequently identified, resulting in the creation of oncogenic fusion proteins. The chimeric FGFR2 fusion proteins are assumed to undergo ligand-independent receptor dimerization resulting in a fully activated kinase, leading to activation of various oncogenic downstream pathways such as RAS/MAPK, PI3K/AKT, and JAK/STAT [11]. As the *FGFR2* rearrangement is particularly common in ICC as compared with other cancer types, it has been rapidly translated into a promising therapeutic target in this type of cancer [12, 13]. Several pre-clinical and clinical trials have shown the efficacy of FGFR inhibitors in treating ICC patients with *FGFR2* rearrangement, as well as with *FGFR3* rearrangement [14–22]. However, *FGFRs* GA profiling in ICC and corresponding clinico-pathological features remain unclear, which would hinder the optimal therapeutic application of the FGFR inhibitors.

In the present study from a Chinese ICC population, we sought to determine: 1) the *FGFR2* rearrangement status in a total of 257 cases; 2) the *FGFRs* GA profile in 110 *FGFR2* rearrangement-absent cases; and 3) the clinical and pathological features in cases with *FGFRs* GAs.

Materials and methods

Patients and specimens

A total of 257 ICC cases were enrolled in this study. We retrieved archived formalin-fixed, paraffin-embedded (FFPE) diagnostic material from surgical cases of ICC diagnosed at Eastern Hepatobiliary Surgery Hospital, Second Military Medical University, Shanghai, China, between September 2015 and August 2018. None of the enrolled patients received radiation therapy, chemotherapy, or other anticancer therapy before surgery. Glass slides were reviewed by pathologists (Drs. H. Dong and W. Cong) to confirm the pathological diagnosis of adenocarcinoma, determine the tumor grade, and select an appropriate paraffin block for ancillary studies. Cases with insufficient tumor for testing were excluded. Information including age, gender, cigarette smoking, alcohol drinking, hepatolithiasis, serum CA19–9, and radiological and pathologic reports, was obtained when available. An ever-smoker was defined as a smoker of at least 1 cigarette/day for 6 months or longer. An ever-drinker was defined as a person who reported drinking alcoholic beverages at least once a week for 6 months or longer. The serologic tests for hepatitis B virus (HBV) and hepatitis C virus (HCV) infection were performed using commercially available products (ELISA Processor III, Behring, Germany). Anti-programmed cell death-ligand 1 (PD-L1) (Clone 28–8; Abcam, Cambridge, MA, USA) and anti-cluster of differentiation 10 (CD10) (Clone 56C6; Maixin, Fuzhou, China) antibodies were selected. Immunohistochemistry (IHC) was performed using an automated staining system (Leica Bond III; Leica Microsystems). The antibody dilutions were optimized to 1:100 for both anti-PD-L1 and anti-CD10. PD-L1 positivity was defined as tumor cells that expressed PD-L1 on the cell membrane at any intensity. CD10 positivity was defined as any tumor cell that expressed CD10 on the cell membrane and/or cytoplasm at any intensity. Written informed consent for surgical resection and clinical research was obtained from each subject. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/ or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study was approval by the Medical Ethics Committee of the Eastern Hepatobiliary Surgery Hospital.

DNA and total RNA extraction

DNA and total RNA from FFPE tissue sections were extracted and purified using the MagMAX™ FFPE DNA/RNA Ultra Kit (ThermoFisher, A31881) and processed following the manufacturer's instructions. The quality of isolated DNA and total RNA was verified using QuantiFluor dsDNA System (Promega, E2760) and QuantiFluor RNA system (Promega, E3310) in Quantus™ Fluorometer E6150. RNA integrity was checked by running on the Agilent Bioanalyzer (RNA Nano chip).

Fluorescence in Situ Hybridization (FISH)

FGFR2 rearrangement was identified using a break-apart FISH probe kit (5′ flank and 3′ flank of *FGFR2* were labeled in green and orange, respectively) from AmoyDx (Amoy Diagnositcs, Xiamen, China), and the performance was done according to the manufacture's instruction. In briefly, the 4 μm-thick sections cut from FFPE tissue block were deparaffinized in xylene, rehydrated in gradient ethanol (100%, 85%, 70%) to deionized water. Sections were then boiled in the pretreatment solution (pH 7.0) for 20 min, and air-dried. Sections were digested in proteinase K working solution (final concentration was 0.05 mg/mL, pH 7.0) for 6 min, dehydrated in gradient ethanol (70%, 85%, 100%), and air-dried. 10 μL *FGFR2* break-apart probe described above was added on each slide. After sealing, slides were put on the hybridizer (Abbott Molecular, Des Plaines, IL), and codenaturation and hybridization were carried out at 85 °C for 5 min and 37 °C for overnight, respectively. After hybridization, slides were immersed in 2 × saline sodium citrate buffer (2 × SSC, pH 7.0) for 5 min, following washed in 0.1% NP40/2 × SSC at 46 °C for 7 min, then dehydrated and air-dried. Finally, DAPI solution was used as a counterstain, and slides were cover slipped.

The ZytoLight® SPEC FGFR2 Dual Color Break Apart Probe from ZytoVision (ZytoVision GmbH, Bremerhaven, Germany) was selected to confirm the result of the AmoyDx's probe. The probe was performed in a similar way described above according to the manufacture's instruction. In particular, the denaturation and hybridization condition were 75 °C for 10 min and 37 °C for overnight, respectively.

Analysis was performed using 100 ×, 1.4 NA oil objective under Olympus BX53 (Olympus, Tokyo, Japan) microscopy equipped with the appropriate filter sets including DAPI single bandpass, Green single bandpass and Orange single bandpass. For each specimen, 50–100 non-overlapping tumor cells were analyzed, positive was considered if separate green and orange signals or single green signal besides undivided signals had to be present in at least 15% of nuclei throughout the tumor. On the contrary, specimens with qualified FISH signals but not meeting the criteria were considered as negative. The representative images of each specimen were acquired with ProgRes cooled CCD camera (Analytik Jena AG, Jena, German) in monochromatic layers that were subsequently merged by the FISH 3.0 software (ImStar Therapeutics, Paris, France).

Library preparation and Next-Generation Sequencing (NGS) assay

The NGS assay was performed using a laboratory developed test kit from AmoyDx (Amoy Diagnositcs, Xiamen, China), which was designed to sequence the whole coding sequences of FGFR1, FGFR2, FGFR3, and FGFR4. Sequencing data was processed using a customized bioinformatics pipeline designed to detect several classes of genomic alterations, including nucleotide substitution, indel, and genomic rearrangement. The DNA (100 ng) was sheared using a Covaris M220 instrument. The input amount of RNA used for library preparation was 100 ng. The RNA fragmentation time was account for the degree of fragmentation determined by the RNA integrity check. After first and second strand synthesis, the dscDNA were mixed with fragmented DNA and purified using AMPure XP Beads (Beckman, A63880). The dscDNA and DNA mix were then repaired to make them blunt and phosphorylated, followed by dA-tailing and adaptor ligation. Sample indexes were added during the PCR

enrichment step. FGFR capture was conducted by hybridizing the pre-PCR libraries with biotinylated DNA baits at 65 °C for 16–24 h followed by extraction using Dynabeads MyOne™ Streptavidin T1 beads (Thermo Fisher, 65,601). The capture libraries were pooled and sequenced on Illumina Novaseq6000 with PE150 cycles. FASTQ files obtained from different samples were first processed by FormatFastq to complete basic QC and generate high-quality clean data. Valid sequencing data were then mapped to the human genome (UCSC hg19) by Burrows-Wheeler Aligner (BWA) to generate original alignment in BAM format. Then, a custom pipeline was used to do the variant calling and identify single nucleotide variant (SNV), insertion and deletion. For rearrangement detecting analysis, valid sequencing data were then mapped to the human genome (UCSC hg19) by STAR (Spliced Transcripts Alignment to a Reference) to generate original mapping results in BAM format, and Chimeric reads. Then, STAR-Fusion was used to call and filter candidate rearrangements with Chimeric reads. The most arguments in STAR and STAR-Fusion are default, but some arguments are optimized according our pre-experiments. The SNV or indel mutation was defined positive as ≥2% mutant allele fraction and ≥2 mutation supporting reads. The fusion was defined positive as ≥5 unique fusion reads. Germline mutation was filtered out using 1000genome database (allele frequency ≥1%) and ExAC and GnomAD database (allele count ≥2), and the remaining mutations except synonymous mutations were exported.

Statistical analysis

The associations between the occurrence of *FGFRs* GAs and clinicopathologic features were assessed utilizing χ^2 or Fisher exact tests, as appropriate. All reported *P* values were 2-sided, and $P < 0.05$ was considered significant. All statistical analyses were performed with Stata 16.0 (Stata, College Station, USA).

Results

In total, 257 ICC cases were subjected to FISH screening for *FGFR2* rearrangements based on the AmoyDx platform. Fourteen cases were observed positive for the presence of the rearrangement, which accounts for 5.45% of the cohort here analyzed. The *FGFR2* rearrangement status of 122 cases (114 negative and 8 positive patients) from our screening cohort was also explored by a second FISH platform (ZytoVision), and 100% concordance result between the two FISH platforms were obtained (Fig. 1).

The demographic and clinical characteristics of the cases harboring *FGFR2* rearrangement are summarized in Table 1. The median age of patients with *FGFR2* rearrangement was 49 years (range, 39 to 69 years). The number of women with *FGFR2* rearrangement ($n = 7$, 50.0%) was equal to the number of men ($n = 7$, 50.0%). Most of patients with *FGFR2* rearrangement were non-smoker ($n = 8$, 66.7%) and non-drinker ($n = 9$, 81.8%). All the *FGFR2* rearrangement positive patients had no hepatolithiasis. Eleven patients (78.6%) with *FGFR2* rearrangement presented a normal serum level of CA19–9 (< 39 U/mL). Patients with *FGFR2* rearrangement predominantly had positive HBsAb ($n = 9$, 69.2%), and smaller numbers of patients had positive HBsAg ($n = 3$, 23.1%) or negative HBsAb/HBsAg ($n = 1$, 7.7%). Thirteen patients (92.9%) presented with earlier disease (stage I or II) at the time of diagnosis. The tumor grade of the analyzed specimens was predominantly moderately differentiated ($n = 12$, 85.7%). By immunohistochemistry, 6 cases (46.1%) with *FGFR2*-rearrangement showed positive for CD10. Eight cases (57.1%) were positive for PD-L1 expression. Examples of CD10 and PD-L1 immunoreactivity are shown in Fig. 2.

Overall, there were no significant differences between patients with and without *FGFR2* rearrangement regarding gender, cigarette smoking, alcohol drinking, hepatolithiasis, serum CA19–9, tumor grade, and tumor stage. However, some significant differences were noted. When compared with patients without *FGFR2* rearrangement, more *FGFR2* rearrangement patients presented before the age of 58 years ($P = 0.028$), HBsAb positivity ($P = 0.008$), CD10 expression ($P = 0.013$), and PD-L1 expression ($P = 0.024$) (Table 2).

Next, we performed NGS assay on 12 *FGFR2* rearrangement positive and 110 negative cases from the cohort, achiveing the same result on *FGFR2* rearrangement status between NGS and FISH platform. For *FGFR2* rearrangement positive cases, no further FGFR1–4 GA was observed. Multiple *FGFR2* rearrangement partners were discovered including *CUX1* ($n = 2$), *SORBS1, SHROOM3, WAC, TXNL4A, CBX5, COL16A1, ALAD, POF1B, FILIP1*, and *POC1B* (Fig. 3). For *FGFR2-POC1B* and *FGFR2-SORBS1*, respectively, 2 rearrangement transcripts were identified in a single case, because of the diversity of genomic breakpoints. To our knowledge, most of the *FGFR2* rearrangement partners have not previously been reported in ICC, except for *SHROOM3, SORBS1*, and *WAC* [22-24]. Among the 110 *FGFR2* rearrangement negative cases, 13 (11.8%) patients present *FGFRs* GAs, including *FGFR3* rearrangement ($n = 3$) (Fig. 3), and mutations at *FGFR1* ($n = 1$), *FGFR2* ($n = 3$), *FGFR3* ($n = 2$), and *FGFR4* ($n = 4$) (Table 3). The *FGFR3* rearrangements were *MYT1L-FGFR3, FGFR3-TACC3*, and *FGFR3-MSRB2* (Fig. 3), and only *FGFR3-TACC3* has been reported previously in ICC [24] and other cancers [25]. One 72-year-old male had coexisting 2 *FGFR2* mutations (Table 3). Rearrangement at *FGFR2–3* (*FGFRs*

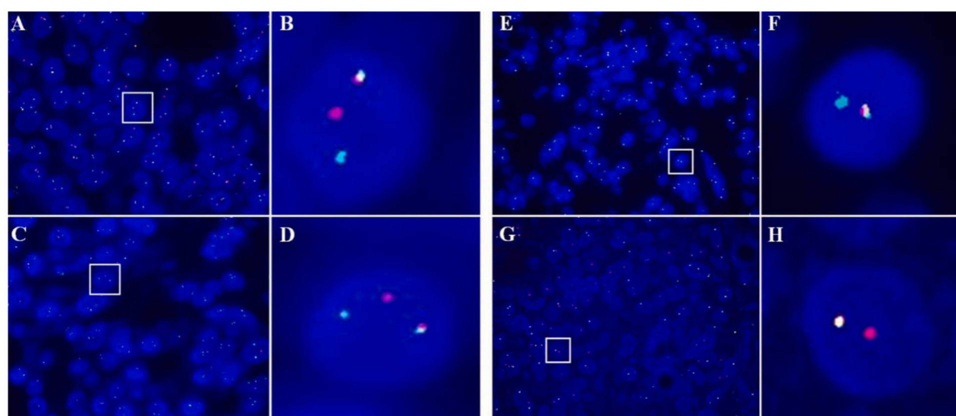

Fig. 1. Schematic representation of *FGFR2* rearrangement in intrahepatic cholangiocarcinoma, by *FGFR2* break-apart FISH probe. A Tumor positive for *FGFR2* rearrangement with AmoyDx FGFR2 Break-apart probe; the dominant positive signal pattern displays as separate green and orange signals. B Enlargement of boxed area in panel A. C Tumor positive for *FGFR2* rearrangement with ZytoVision FGFR2 Break-apart probe; the dominant positive signal pattern displays as separate green and orange signals. D Enlargement of boxed area in panel C. E Tumor positive for *FGFR2* rearrangement with AmoyDx FGFR2 Break-apart probe; the dominant positive signal pattern displays as single green signal besides undivided signals (5′ flank of *FGFR2* was labeled in green). F Enlargement of boxed area in panel E. G Tumor positive for *FGFR2* rearrangement with ZytoVision FGFR2 Break-apart probe; the dominant positive signal pattern displays as single orange signal besides undivided signals (5′ flank of *FGFR2* was labeled in orange). H, Enlargement of boxed area in panel G.

Table 1
Clinicopathological features of patients with intrahepatic cholangiocarcinoma with *FGFR2* rearrangement.

Sample *no.*	Gender	Age (years)	HBV status	CA19–9 (U/mL)	Tumor grade	TNM stage	CD10 expression	PD-L1 expression
1	Male	65	HBsAb+, HBeAb+, HBcAb+	33.6	Moderately differentiated	I	Negative	Negative
2	Female	56	HBeAb+, HBcAb+	106.3	Poorly differentiated	I	Negative	Positive
3	Female	54	HBsAb+, HBeAb+, HBcAb+	0.8	Moderately differentiated	I	n/a	Positive
4	Female	46	HBsAb+, HBeAb+, HBcAb+	66.8	Moderately differentiated	I	Negative	Positive
5	Male	56	HBsAb+, HBeAb+, HBcAb+	24.2	Moderately differentiated	II	Positive	Negative
6	Male	65	HBsAg+, HBeAb+, HBcAb+	21.0	Moderately differentiated	I	Positive	Positive
7	Female	45	HBsAb+, HBeAb+, HBcAb+	422.2	Moderately differentiated	I	Positive	Positive
8	Male	39	HBsAg+, HBeAb+, HBcAb+	7.6	Moderately differentiated	II	Positive	Negative
9	Female	47	HBsAb+, HBcAb+	0.6	Moderately differentiated	III	Negative	Negative
10	Male	48	n/a	25.2	Moderately differentiated	I	Negative	Negative
11	Female	45	HBsAb+, HBcAb+	11.7	Moderately differentiated	I	Negative	Positive
12	Male	69	HBsAg+, HBeAb+, HBcAb+	8.6	Moderately differentiated	I	Negative	Negative
13	Female	49	HBsAb+	16.1	Moderately differentiated	II	Positive	Positive
14	Male	58	HBsAb+, HBcAb+	8.3	Poorly differentiated	I	Positive	Positive

n/a, not available; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBsAb, anti-hepatitis B surface antibody; HBeAb, anti-hepatitis B virus e antibody; HBcAb, anti-hepatitis B virus core antibody; TNM, tumor-node-metastasis; CD10, cluster of differentiation 10; PD-L1, programmed cell death-ligand 1.

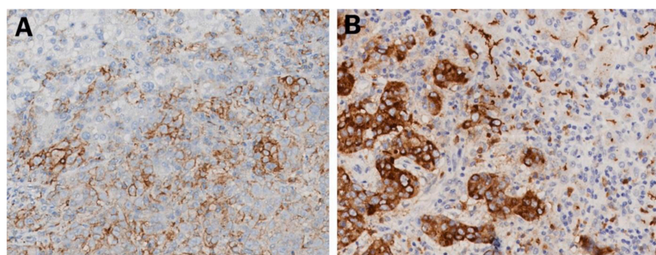

Fig. 2. Immunohistochemistry demonstrating PD-L1 and CD10 expression in intrahepatic cholangiocarcinoma. A Tumor stained with PD-L1 antibody, demonstrating cell membrane positivity. B Tumor stained with CD10 antibody, demonstrating cell membrane and cytoplasm positivity.

rearrangement) and mutation at *FGFR1–4* (*FGFRs* mutation) were mutually exclusive.

We next examined the differences in clinicopathological characteristics between patients with *FGFR2–3* rearrangement ($n = 17$), patients with *FGFR1–4* mutation ($n = 10$), and patients without *FGFRs* GAs ($n = 97$) (Table 4). Compared with patients without *FGFRs* GAs, those with *FGFR2–3* rearrangement presented more before the age of 58 years ($P = 0.017$), HBsAb positivity ($P = 0.003$), CD10 expression ($P = 0.021$), and PD-L1 expression ($P = 0.009$), which were consistent with that observed above for *FGFR2* rearrangement versus *FGFR2* non-rearrangement based on the cohort. In addition, more *FGFR2–3* rearrangements were identified in women ($P = 0.034$). The clinical characteristics between patients with *FGFR2–3* rearrangement and those with *FGFR1–4* mutation were significantly different, with cases with *FGFR2–3* rearrangement presenting more before the age of 58 years ($P = 0.013$), HBsAb positivity ($P = 0.041$), and CD10 expression ($P = 0.022$), but less hepatolithiasis ($P = 0.001$). There were no significant differences regarding gender and tumor stage. When compared with patients without *FGFRs* GAs, those with *FGFR2–3* rearrangement or *FGFR1–4* mutation presented a female predominance ($P = 0.020$) and an earlier tumor stage I/II ($P = 0.025$). Overall, the clinical characteristics between patients with *FGFR1–4* mutation and those without *FGFRs* GAs were similar, with the exception that cases with *FGFR1–4* mutation have more hepatolithiasis ($P = 0.005$).

Table 2
Clinicopathological characteristics of patients with intrahepatic cholangiocarcinoma with and without *FGFR2* rearrangement [*n* (%)].

Characteristics	No *FGFR2* rearrangement ($n = 243$)	*FGFR2* rearrangement ($n = 14$)	*p*-value[a]
Gender			0.265
Female	85 (34.0)	7 (50.0)	
Male	158 (65.0)	7 (50.0)	
Age (years)			
≤58	115 (47.3)	11 (78.6)	0.028
>58	128 (52.7)	3 (21.4)	
Hepatitis B surface antibody (HBsAb)			0.003
Positive	66 (27.9)	9 (69.2)	
Negative	171 (72.1)	4 (30.8)	
Serum CA19–9 (U/mL)			0.053
<39	120 (50.4)	11 (78.6)	
≧39	118 (49.6)	3 (21.4)	
Hepatolithiasis			0.079
Negative	193 (79.4)	14 (100.0)	
Positive	50 (20.6)	0 (0.0)	
Smoker			0.540
Non-smoker	148 (63.3)	8 (66.7)	
Current smoker	63 (26.9)	2 (16.7)	
Former smoker	23 (9.8)	2 (16.7)	
Drinker			1.000
Non-drinker	164 (73.2)	9 (81.8)	
Current drinker	46 (20.5)	2 (18.2)	
Former drinker	14 (6.3)	0 (0.0)	
Tumor grade			0.492
Well differentiated	4 (1.7)	0 (0.0)	
Moderately differentiated	167 (68.7)	12 (85.7)	
Poorly differentiated	72 (29.6)	2 (14.3)	
TNM stage			0.313
I/II	191 (78.6)	13 (92.9)	
III	52 (21.4)	1 (7.1)	
CD10 expression			0.013
Negative	189 (84.4)	7 (53.9)	
Positive	35 (15.6)	6 (46.1)	
PD-L1 expression			0.024
Negative	127 (71.8)	6 (42.9)	
Positive	50 (28.2)	8 (57.1)	

TNM, tumor-node-metastasis; HBsAb, anti-hepatitis B surface antibody; CD10, cluster of differentiation 10; PD-L1, programmed cell death-ligand 1.
[a] χ^2 or Fisher exact test, as appropriate.

Discussion

Our results demonstrated that patients with ICC with *FGFRs* rearrangement have distinct clinical phenotype compared with the general population of patients with ICC. Specifically, we observed significant enrichment for *FGFR2–3* rearrangement in patients age ≤ 58 years, of female, with positive serum HBsAb, and whose tumor expressed CD10 and PD-L1. Since *FGFRs* rearrangement-positive tumors can be sensitive to FGFR inhibitors [14-22], these observations suggest that molecular testing to detect *FGFRs* rearrangement in ICC should be prioritized for patients with these clinical and pathological features. In addition, our

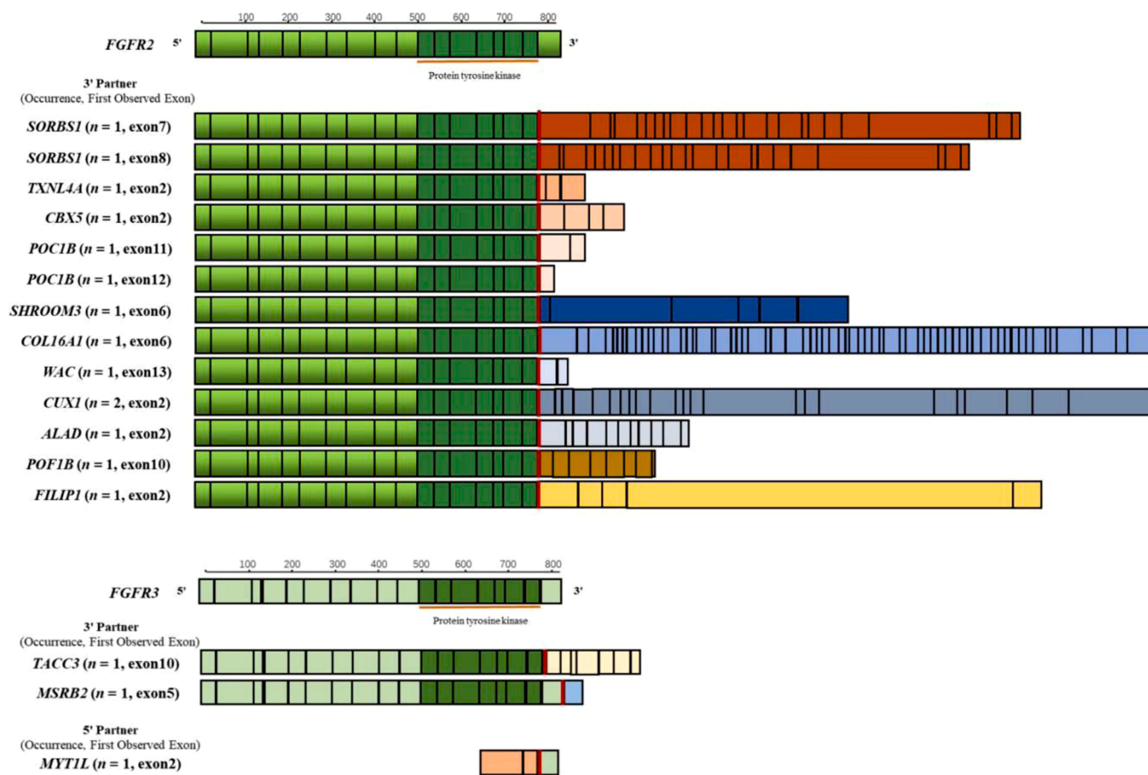

Fig. 3. *FGFR2* and *FGFR3* rearrangement partners in intrahepatic cholangiocarcinoma.

Table 3
FGFRs mutations in *FGFR2* rearrangement-negative patients with intrahepatic cholangiocarcinoma.

Sample no.	Gene	Transcript	Coding sequence change	Clinical significance
1	*FGFR1*	NM_023110	Exon6, c.742G > *A*: p. V248M	Likely benign
2	*FGFR2*	NM_000141	Exon 7, c.870G > *T*: p. W290C	Pathogenic
			Exon 3, c.185G > *A*: p. C62Y	Uncertain significance
3	*FGFR2*	NM_000141	Exon 14, c.1976A > *T*: p. K659M	Likely pathogenic
4	*FGFR2*	NM_000141	Exon 14, c.1880T > *A*: p. L627[a]	Uncertain significance
5	*FGFR3*	NM_000142	Exon 6, c.713G > *A*: p. R238Q	Benign
6	*FGFR3*	NM_000142	Exon 7, c.796G > *A*: p. V266M	Uncertain significance
7	*FGFR4*	NM_213,647	Exon 9, c.1183C > *T*: p. L395F	Uncertain significance
8	*FGFR4*	NM_213,647	Exon 10, c.1310G > *A*: p. R437H	Uncertain significance
9	*FGFR4*	NM_213,647	Exon 3, c.187G > *T*: p. G63C	Uncertain significance
10	*FGFR4*	NM_213,647	Exon 10, c.1276G > *A*: p. G426S	Uncertain significance

[a] Nonsense mutation.

data presented the first *FGFRs* GA profiling in *FGFR2* rearrangement-absent ICC, which suggest that patients that are negative for *FGFR2* rearrangement may harbor other *FGFR* alterations that may be amenable to targeted therapies similar to *FGFR2* rearrangements through clinical trials.

FGFR2 rearrangement, the most promising therapeutic target biomarker in ICC, were identified in 5.45% of patients in the present study. Previous smaller studies have reported *FGFR2* rearrangement in 5.5% to 50% of ICC patients [21,23,26–35]. It is obvious that the frequency of *FGFR2* rearrangement were lower in Asian ICC patients than that in Caucasian patients, possibly reflecting the differences in ethnicity, causative etiology, and compositions of various clinical characteristics. Importantly, in ICC lacking *FGFR2* rearrangement, we revealed *FGFR3* rearrangement and *FGFR1–4* mutations, with frequencies of 2.7% and 9.1%, respectively. Several pre-clinical and clinical trials have shown the efficacy of FGFR inhibitors in treating ICC patients with *FGFR3* rearrangement or certain *FGFRs* mutations, as well as with *FGFR2* rearrangement [14–17]. Thus, the findings of biomarker profile in our data may expand the proportion of potential FGFR-targetable cases in ICC. Several studies have already identified secondary resistance mutations to FGFR-targeted therapies, most of which occurred in the Tyrosine Kinase Domain (TKD) of the *FGFR* genes [35–37]. However, in our dataset, all the *FGFRs* mutations were detected in non-TKD and none of the ICC patients received FGFR inhibitors, representing primary mutations. The associations of these primary mutations with the sensitivity and resistance to FGFR inhibitors merit further studies.

FGFR pathway GAs have been examined in relation to clinical and pathological characteristics in biliary tract cancers, with several studies on Caucasian patients reporting an association. Javle et al. [21] have reported that *FGFR2* rearrangements or mutations in ICC were associated with younger age at onset and female sex. Graham et al. [27] have reported that *FGFR2* rearrangement in mixed intrahepatic, perihilar and extrahepatic cancer was associated with a female predominance. Jain et al. [22] have reported in mixed intrahepatic, extrahepatic and gallbladder cancer, *FGFR* and *FGF19* GAs occurred more frequently in younger patients and presented at an earlier tumor stage. In the present study, we provided further valuable information that *FGFR2–3* rearrangement in ICC was associated with younger age (≤ 58 years), female sex, serum HBsAb positivity, and tumoral CD10 and PD-L1 expression, while *FGFRs* GAs were associated with an earlier tumor stage. HBV infection has been proved to be associated with an increased risk of ICC incidence [38]. It has been reported that *FGFR2* rearrangement positive cases had a propensity for hepatitis virus infection (HCV or HBV) in

Table 4
Clinicopathological characteristics of patients with *FGFR2–3* rearrangement versus those with *FGFR1–4* mutation or those without rearrangement and mutation.

	No *FGFRs* rearrangement or mutation (n = 97)	*FGFR2–3* rearrangement (n = 17)	*FGFR1–4* mutation (n = 10)	p-value*	p-value#
Gender				0.034	0.598
Female	26 (26.8)	9 (52.9)	5 (50.0)		
Male	71 (73.2)	8 (47.1)	5 (50.0)		
Age (years)				0.017	0.013
≤58	49 (50.5)	14 (82.4)	3 (30.0)		
>58	48 (49.5)	3 (17.6)	7 (70.0)		
Hepatolithiasis				0.124	0.001
Negative	81 (83.5)	17 (100.0)	4 (40.0)		
Positive	16 (16.5)	0 (0.0)	6 (60.0)		
HBsAb status				0.003	0.041
Positive	25 (26.6)	11 (68.8)	2 (22.2)		
Negative	69 (73.4)	5 (31.2)	7 (77.8)		
CD10 expression				0.021	0.022
Negative	76 (80.9)	8 (50.0)	9 (100.0)		
Positive	18 (19.1)	8 (50.0)	0 (0.0)		
PD-L1 expression				0.009	0.069
Negative	58 (71.6)	6 (37.5)	6 (85.7)		
Positive	23 (28.4)	10 (62.5)	1 (14.3)		

* *FGFR2–3* rearrangement vs. *FGFRs* negative.
# FGFR2–*3* rearrangement vs. *FGFR1–4* mutation.

mixed intrahepatic and extrahepatic cancer from a Japanese population [29]. However, we observed significant enrichment for *FGFR* rearrangement in patients with positive HBsAb, rather than those with positive HBsAg (P = 0.381), consistent with the findings from another study in China reporting no significant association between *FGFR2* rearrangement in ICC and HBV infection [34]. Although PD-L1 expression, as a potential predictor for anti-PD-1/PD-L1 immune checkpoint inhibitors (ICIs) treatment, was enriched in *FGFR* rearrangement-positive ICCs, it is still unknown whether implications for combining FGFR inhibitor and anti-PD-1/PD-L1 agent could enhance treatment response for these patients. It has been reported that, in patients with advanced urothelial cancer with *FGFR* alterations, sequential application of FGFR inhibitor and PD-1/PD-L1 inhibitor enhanced the ICI response rate to approximately 30% in contrast to only 3.6% for patients receiving initial PD-1/PD-L1 inhibitors [39]. CD10, a cell surface ectoenzyme, is widely expressed on different types of cancers, and has been associated with tumor progression, therapeutic resistance, and molecular dysregulation in the tumor microenvironment [40]. Further studies are needed to explore whether the CD10 and/or PD-L1 expression impact the response of targeted therapy and immunotherapy in ICCs.

Several limitations should be noted in the present study. Firstly, the number of samples remains relatively small, especially for the *FGFRs* GA subsets, which reflects the relatively rare molecular subsets of ICC. Despite these limitations, this study is important, as it is, to our knowledge, the first profiling of *FGFR* GAs in a cohort of patients with ICC. Secondly, the cohort investigated is made up of surgery patients who are mostly in earlier tumor stage (stage I/II: 79.4%). Since the incidence of *FGFRs* GAs in ICC may be higher in surgically resectable disease stages [31], future studies should address the rate of these GAs in patients with later tumor stages, as well as in different tumor locations (primary tumor vs. metastases). Thirdly, the *FGFRs* GAs detected by NGS array was not validated by other methods such as PCR-based first--generation sequencing. Finally, all the patients in the present study did not received the FGFR inhibitors, which hindered our further analysis on the association between *FGFRs* GAs and clinical response to FGFR inhibitors. We recognize that the detection of candidate *FGFRs* GAs does not necessarily indicate its relevance as a potential therapeutic target. Thus, the functional consequences of these *FGFRs* GAs, especially mutations, await further investigation.

In conclusion, our data showed that *FGFR2* and *FGFR3* rearrangement in ICC is associated with unique clinical phenotypes, with features of younger age at onset, female sex, serum HBsAb positivity, and tumoral CD10 and PD-L1 expression.

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Authors' contribution

Conceptualization, Z.Z., H.D., J.W., W.C. and Q.X.; methodology, X. C. and H.L.; investigation, W.D., X.G., H.Y., J.F., S.G., X.C. and H.L.; resources, W.D. and H.Y.; writing—original draft preparation, Z.Z., H. D., X.C. and H.L.; writing—review and editing, W.D., W.C. and Q.X.; supervision, Z.Z.; funding acquisition, Z.Z.. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

Two authors, X.C. and H.L, work at the Amoy Diagnostics Co., Ltd. whose product, the break-apart FISH probe kit, was used to detect *FGFR2* rearrangement in the manuscript. Other authors listed in the authorship declared they had no competing financial interests.

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References

[1] A.E. Sirica, G.J. Gores, J.D. Groopman, F.M. Selaru, M. Strazzabosco, X.Wei Wang, A.X. Zhu, Intrahepatic Cholangiocarcinoma: continuing challenges and translational advances, Hepatology 69 (2019) 1803–1815.
[2] P. Bertuccio, M. Malvezzi, G. Carioli, D. Hashim, P. Boffetta, H.B. El-Serag, C. La Vecchia, E. Negri, Global trends in mortality from intrahepatic and extrahepatic cholangiocarcinoma, J. Hepatol. 71 (2019) 104–114.
[3] O. Clements, J. Eliahoo, J.U. Kim, S.D. Taylor-Robinson, S.A. Khan, Risk factors for intrahepatic and extrahepatic cholangiocarcinoma: a systematic review and meta-analysis, J. Hepatol. 72 (2020) 95–103.
[4] J.L. Petrick, B. Yang, S.F. Altekruse, A.L. Van Dyke, J. Koshiol, B.I. Graubard, K. A. McGlynn, Risk factors for intrahepatic and extrahepatic cholangiocarcinoma in the United States: a population-based study in SEER-Medicare, PLoS ONE 12 (2017), e0186643.
[5] R.K. Kelley, J. Bridgewater, G.J. Gores, A.X. Zhu, Systemic therapies for intrahepatic cholangiocarcinoma, J. Hepatol. 72 (2020) 353–363.
[6] J. Valle, H. Wasan, D.H. Palmer, D. Cunningham, A. Anthoney, A. Maraveyas, S. Madhusudan, T. Iveson, S. Hughes, S.P. Pereira, M. Roughton, J. Bridgewater, A.

B.C.T. Investigators, Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer, N. Engl. J. Med. 362 (2010) 1273–1281.
[7] A. Lamarca, J. Barriuso, M.G. McNamara, J.W. Valle, Molecular targeted therapies: ready for "prime time" in biliary tract cancer, J. Hepatol. 73 (2020) 170–185.
[8] A. Saborowski, U. Lehmann, A. Vogel, FGFR inhibitors in cholangiocarcinoma: what's now and what's next? Ther. Adv. Med. Oncol. 12 (2020), 1758835920953293.
[9] J. Wang, X. Xing, Q. Li, G. Zhang, T. Wang, H. Pan, D. Li, Targeting the FGFR signaling pathway in cholangiocarcinoma: promise or delusion? Ther. Adv. Med. Oncol. 12 (2020), 1758835920940948.
[10] N. Porebska, M. Latko, M. Kucinska, M. Zakrzewska, J. Otlewski, L. Opalinski, Targeting cellular trafficking of fibroblast growth factor receptors as a strategy for selective cancer treatment, J. Clin. Med. 8 (2018) 7.
[11] A. Mahipal, S.H. Tella, A. Kommalapati, D. Anaya, R. Kim, FGFR2 genomic aberrations: achilles heel in the management of advanced cholangiocarcinoma, Cancer Treat. Rev. 78 (2019) 1–7.
[12] F. Li, M.N. Peiris, D.J. Donoghue, Functions of FGFR2 corrupted by translocations in intrahepatic cholangiocarcinoma, Cytokine Growth Factor Rev. 52 (2020) 56–67.
[13] T. Yang, L. Liang, M.D. Wang, F. Shen, FGFR inhibitors for advanced cholangiocarcinoma, Lancet Oncol. 21 (2020) 610–612.
[14] M. Javle, M. Lowery, R.T. Shroff, K.H. Weiss, C. Springfeld, M.J. Borad, R. K. Ramanathan, L. Goyal, S. Sadeghi, T. Macarulla, A. El-Khoueiry, R.K. Kelley, I. Borbath, S.P. Choo, D.Y. Oh, P.A. Philip, L.T. Chen, T. Reungwetwattana, E. Van Cutsem, K.H. Yeh, K. Ciombor, R.S. Finn, A. Patel, S. Sen, D. Porter, R. Isaacs, A. X. Zhu, G.K. Abou-Alfa, T. Bekaii-Saab, Phase II Study of BGJ398 in patients with FGFR-Altered Advanced Cholangiocarcinoma, J. Clin. Oncol. 36 (2018) 276–282.
[15] J.B. Egan, D.L. Marks, T.L. Hogenson, A.M. Vrabel, A.N. Sigafoos, E.J. Tolosa, R. M. Carr, S.L. Safgren, E.E. Hesles, L.L. Almada, P.A. Romecin-Duran, E. Iguchi, A. Ala'Aldeen, J.A. Kocher, G.R. Oliver, N. Prodduturi, D.W. Mead, A. Hossain, N. E. Huneke, C.M. Tagtow, S. Ailawadhi, S.M. Ansell, M.S. Banck, A.H. Bryce, E. M. Carballido, A.A. Chanan-Khan, K.K. Curtis, E. Resnik, C.D. Gawryletz, R.S. Go, T.R. Halfdanarson, T.H. Ho, R.W. Joseph, P. Kapoor, A.S. Mansfield, N. Meurice, A. A. Nageswara Rao, G.S. Nowakowski, A. Pardanani, S.A. Parikh, J.C. Cheville, A. L. Feldman, R.K. Ramanathan, S.I. Robinson, R. Tibes, H.D. Finnes, J. B. McCormick, R.R. McWilliams, A. Jatoi, M.M. Patnaik, A.C. Silva, E.D. Wieben, T. M. McAllister, K.M. Rumilla, S.E. Kerr, K.N. Lazaridis, G. Farrugia, A.K. Stewart, K. J. Clark, E.J. Kennedy, E.W. Klee, M.J. Borad, M.E. Fernandez-Zapico, Molecular modeling and functional analysis of Exome Sequencing-derived variants of unknown significance identify a Novel, Constitutively Active FGFR2 Mutant in Cholangiocarcinoma, JCO Precis. Oncol. 2017 (2017). PO.17.00018.
[16] R. Bahleda, A. Italiano, C. Hierro, A. Mita, A. Cervantes, N. Chan, M. Awad, E. Calvo, V. Moreno, R. Govindan, A. Spira, M. Gonzalez, B. Zhong, A. Santiago-Walker, I. Poggesi, T. Parekh, H. Xie, J. Infante, J. Tabernero, Multicenter Phase I Study of Erdafitinib (JNJ-42756493), Oral Pan-Fibroblast growth factor receptor inhibitor, in patients with advanced or refractory solid tumors, Clin. Cancer Res. 25 (2019) 4888–4897.
[17] J.O. Park, Y.-.H. Feng, Y.-.Y. Chen, W.-.C. Su, D.-.Y. Oh, L. Shen, K.-.P. Kim, X. Liu, Y. Bai, H. Liao, J. Nie, M. Qing, Q. Ji, J. Li, M. Zhao, P.D. Porre, M. Monga, Updated results of a phase IIa study to evaluate the clinical efficacy and safety of erdafitinib in Asian advanced cholangiocarcinoma (CCA) patients with FGFR alterations, J. Clin. Oncol. 37 (2019), 4117-4117.
[18] G.K. Abou-Alfa, V. Sahai, A. Hollebecque, G. Vaccaro, D. Melisi, R. Al-Rajabi, A. S. Paulson, M.J. Borad, D. Gallinson, A.G. Murphy, D.Y. Oh, E. Dotan, D. V. Catenacci, E. Van Cutsem, T. Ji, C.F. Lihou, H. Zhen, L. Feliz, A. Vogel, Pemigatinib for previously treated, locally advanced or metastatic cholangiocarcinoma: a multicentre, open-label, phase 2 study, Lancet Oncol. 21 (2020) 671–684.
[19] R. Bahleda, F. Meric-Bernstam, L. Goyal, B. Tran, Y. He, I. Yamamiya, K. A. Benhadji, I. Matos, H.T. Arkenau, Phase I, first-in-human study of futibatinib, a highly selective, irreversible FGFR1-4 inhibitor in patients with advanced solid tumors, Ann. Oncol. 31 (2020) 1405–1412.
[20] V. Mazzaferro, B.F. El-Rayes, M. Droz Dit Busset, C. Cotsoglou, W.P. Harris, N. Damjanov, G. Masi, L. Rimassa, N. Personeni, F. Braiteh, V. Zagonel, K. P. Papadopoulos, T. Hall, Y. Wang, B. Schwartz, J. Kazakin, S. Bhoori, F. de Braud, W.L. Shaib, Derazantinib (ARQ 087) in advanced or inoperable FGFR2 gene fusion-positive intrahepatic cholangiocarcinoma, Br. J. Cancer 120 (2019) 165–171.
[21] M. Javle, T. Bekaii-Saab, A. Jain, Y. Wang, R.K. Kelley, K. Wang, H.C. Kang, D. Catenacci, S. Ali, S. Krishnan, D. Ahn, A.G. Bocobo, M. Zuo, A. Kaseb, V. Miller, P.J. Stephens, F. Meric-Bernstam, R. Shroff, J. Ross, Biliary cancer: utility of next-generation sequencing for clinical management, Cancer 122 (2016) 3838–3847.
[22] A. Jain, M.J. Borad, R.K. Kelley, Y. Wang, R. Abdel-Wahab, F. Meric-Bernstam, K. A. Baggerly, A.O. Kaseb, H.O. Al-shamsi, D.H. Ahn, T. DeLeon, A.G. Bocobo, T. Bekaii-Saab, R.T. Shroff, M. Javle, Cholangiocarcinoma with FGFR Genetic aberrations: a unique clinical phenotype, JCO Precis. Oncol. 2018 (2018). PO.17.00080.
[23] M.J. Borad, M.D. Champion, J.B. Egan, W.S. Liang, R. Fonseca, A.H. Bryce, Integrated genomic characterization reveals novel, therapeutically relevant drug targets in FGFR and EGFR pathways in sporadic intrahepatic cholangiocarcinoma, PLoS Genet. 10 (2014), e1004135.
[24] A. Jusakul, I. Cutcutache, C.H. Yong, J.Q. Lim, M.N. Huang, N. Padmanabhan, V. Nellore, S. Kongpetch, A.W.T. Ng, L.M. Ng, S.P. Choo, S.S. Myint, R. Thanan, S. Nagarajan, W.K. Lim, C.C.Y. Ng, A. Boot, M. Liu, C.K. Ong, V. Rajasegaran, S. Lie, A.S.T. Lim, T.H. Lim, J. Tan, J.L. Loh, J.R. McPherson, N. Khuntikeo, V. Bhudhisawasdi, P. Yongvanit, S. Wongkham, Y. Totoki, H. Nakamura, Y. Arai, S. Yamasaki, P.K. Chow, A.Y.F. Chung, L. Ooi, K.H. Lim, S. Dima, D.G. Duda, I. Popescu, P. Broet, S.Y. Hsieh, M.C. Yu, A. Scarpa, J. Lai, D.X. Luo, A.L. Carvalho, A.L. Vettore, H. Rhee, Y.N. Park, L.B. Alexandrov, R. Gordan, S.G. Rozen, T. Shibata, C. Pairojkul, B.T. Teh, P. Tan, Whole-genome and epigenomic landscapes of etiologically distinct subtypes of Cholangiocarcinoma, Cancer Discov. 7 (2017) 1116–1135.
[25] Y.M. Wu, F. Su, S. Kalyana-Sundaram, N. Khazanov, B. Ateeq, X. Cao, R.J. Lonigro, P. Vats, R. Wang, S.F. Lin, A.J. Cheng, L.P. Kunju, J. Siddiqui, S.A. Tomlins, P. Wyngaard, S. Sadis, S. Roychowdhury, M.H. Hussain, F.Y. Feng, M.M. Zalupski, M. Talpaz, K.J. Pienta, D.R. Rhodes, D.R. Robinson, A.M. Chinnaiyan, Identification of targetable FGFR gene fusions in diverse cancers, Cancer Discov. 3 (2013) 636–647.
[26] J.S. Ross, K. Wang, L. Gay, R. Al-Rohil, J.V. Rand, D.M. Jones, H.J. Lee, C. E. Sheehan, G.A. Otto, G. Palmer, R. Yelensky, D. Lipson, D. Morosini, M. Hawryluk, D.V. Catenacci, V.A. Miller, C. Churi, S. Ali, P.J. Stephens, New routes to targeted therapy of intrahepatic cholangiocarcinomas revealed by next-generation sequencing, Oncologist 19 (2014) 235–242.
[27] R.P. Graham, E.G. Barr Fritcher, E. Pestova, J. Schulz, L.A. Sitailo, G. Vasmatzis, S. J. Murphy, R.R. McWilliams, S.N. Hart, K.C. Halling, L.R. Roberts, G.J. Gores, F. J. Couch, L. Zhang, M.J. Borad, B.R. Kipp, Fibroblast growth factor receptor 2 translocations in intrahepatic cholangiocarcinoma, Hum. Pathol. 45 (2014) 1630–1638.
[28] C.R. Churi, R. Shroff, Y. Wang, A. Rashid, H.C. Kang, J. Weatherly, M. Zuo, R. Zinner, D. Hong, F. Meric-Bernstam, F. Janku, C.H. Crane, L. Mishra, J. N. Vauthey, R.A. Wolff, G. Mills, M. Javle, Mutation profiling in cholangiocarcinoma: prognostic and therapeutic implications, PLoS ONE 9 (2014), e115383.
[29] Y. Arai, Y. Totoki, F. Hosoda, T. Shirota, N. Hama, H. Nakamura, H. Ojima, K. Furuta, K. Shimada, T. Okusaka, T. Kosuge, T. Shibata, Fibroblast growth factor receptor 2 tyrosine kinase fusions define a unique molecular subtype of cholangiocarcinoma, Hepatology 59 (2014) 1427–1434.
[30] H. Nakamura, Y. Arai, Y. Totoki, T. Shirota, A. Elzawahry, M. Kato, N. Hama, F. Hosoda, T. Urushidate, S. Ohashi, N. Hiraoka, H. Ojima, K. Shimada, T. Okusaka, T. Kosuge, S. Miyagawa, T. Shibata, Genomic spectra of biliary tract cancer, Nat. Genet. 47 (2015) 1003–1010.
[31] D. Sia, B. Losic, A. Moeini, L. Cabellos, K. Hao, K. Revill, D. Bonal, O. Miltiadous, Z. Zhang, Y. Hoshida, H. Cornella, M. Castillo-Martin, R. Pinyol, Y. Kasai, S. Roayaie, S.N. Thung, J. Fuster, M.E. Schwartz, S. Waxman, C. Cordon-Cardo, E. Schadt, V. Mazzaferro, J.M. Llovet, Massive parallel sequencing uncovers actionable FGFR2-PPHLN1 fusion and ARAF mutations in intrahepatic cholangiocarcinoma, Nat. Commun. 6 (2015) 6087.
[32] F. Farshidfar, S. Zheng, M.C. Gingras, Y. Newton, J. Shih, A.G. Robertson, T. Hinoue, K.A. Hoadley, E.A. Gibb, J. Roszik, K.R. Covington, C.C. Wu, E. Shinbrot, N. Stransky, A. Hegde, J.D. Yang, E. Reznik, S. Sadeghi, C. S. Pedamallu, A.I. Ojesina, J.M. Hess, J.T. Auman, S.K. Rhie, R. Bowlby, M. J. Borad, N. Cancer Genome Atlas, A.X. Zhu, J.M. Stuart, C. Sander, R. Akbani, A. D. Cherniack, V. Deshpande, T. Mounajjed, W.C. Foo, M.S. Torbenson, D.E. Kleiner, P.W. Laird, D.A. Wheeler, A.J. McRee, O.F. Bathe, J.B. Andersen, N. Bardeesy, L. R. Roberts, L.N. Kwong, Integrative genomic analysis of cholangiocarcinoma identifies distinct IDH-Mutant Molecular Profiles, Cell. Rep. 18 (2017) 2780–2794.
[33] M.A. Lowery, R. Ptashkin, E. Jordan, M.F. Berger, A. Zehir, M. Capanu, N. E. Kemeny, E.M. O'Reilly, I. El-Dika, W.R. Jarnagin, J.J. Harding, M.I. D'Angelica, A. Cercek, J.F. Hechtman, D.B. Solit, N. Schultz, D.M. Hyman, D.S. Klimstra, L. B. Saltz, G.K. Abou-Alfa, Comprehensive molecular profiling of intrahepatic and extrahepatic cholangiocarcinomas: potential targets for intervention, Clin. Cancer Res 24 (2018) 4154–4161.
[34] X.H. Pu, Q. Ye, J. Yang, H.Y. Wu, X.W. Ding, J. Shi, L. Mao, X.S. Fan, J. Chen, Y. D. Qiu, Q. Huang, Low-level clonal FGFR2 amplification defines a unique molecular subtype of intrahepatic cholangiocarcinoma in a Chinese population, Hum. Pathol. 76 (2018) 100–109.
[35] L. Goyal, S.K. Saha, L.Y. Liu, G. Siravegna, I. Leshchiner, L.G. Ahronian, J. K. Lennerz, P. Vu, V. Deshpande, A. Kambadakone, B. Mussolin, S. Reyes, L. Henderson, J.E. Sun, E.E. Van Seventer, J.M. Gurski Jr., S. Baltschukat, B. Schacher-Engstler, L. Barys, C. Stamm, P. Furet, D.P. Ryan, J.R. Stone, A. J. Iafrate, G. Getz, D.G. Porta, R. Tiedt, A. Bardelli, D. Juric, R.B. Corcoran, N. Bardeesy, A.X. Zhu, Polyclonal Secondary FGFR2 mutations drive acquired resistance to FGFR inhibition in patients with FGFR2 Fusion-Positive Cholangiocarcinoma, Cancer Discov. 7 (2017) 252–263.
[36] M.A. Krook, A. Lenyo, M. Wilberding, H. Barker, M. Dantuono, K.M. Bailey, H. Z. Chen, J.W. Reeser, M.R. Wing, J. Miya, E. Samorodnitsky, A.M. Smith, T. Dao, D. M. Martin, K.K. Ciombor, J. Hays, A.G. Freud, S. Roychowdhury, Efficacy of FGFR inhibitors and combination therapies for acquired resistance in FGFR2-Fusion Cholangiocarcinoma, Mol. Cancer Ther. 19 (2020) 847–857.
[37] M.A. Hatlen, O. Schmidt-Kittler, C.A. Sherwin, E. Rozsahegyi, N. Rubin, M. P. Sheets, J.L. Kim, C. Miduturu, N. Bifulco, N. Brooijmans, H. Shi, T. Guzi, A. Boral, C. Lengauer, M. Dorsch, R.D. Kim, Y.K. Kang, B.B. Wolf, K.P. Hoeflich, Acquired on-target clinical resistance validates FGFR4 as a driver of hepatocellular carcinoma, Cancer Discov. 9 (2019) 1686–1695.
[38] J.H. Tan, W.Y. Zhou, L. Zhou, R.C. Cao, G.W. Zhang, Viral hepatitis B and C infections increase the risks of intrahepatic and extrahepatic cholangiocarcinoma:

evidence from a systematic review and meta-analysis, Turk. J. Gastroenterol. 31 (2020) 246–256.

[39] A.O. Siefker-Radtke, A. Necchi, E. Rosenbaum, S. Culine, E.F. Burgess, P. H. O'Donnell, S.T. Tagawa, Y. Zakharia, A. OHagan, A.N. Avadhani, B. Zhong, A. E. Santiago-Walker, T. Roccia, Y. Loriot, Efficacy of programmed death 1 (PD-1) and programmed death 1 ligand (PD-L1) inhibitors in patients with FGFR mutations and gene fusions: results from a data analysis of an ongoing phase 2 study of erdafitinib (JNJ-42756493) in patients (pts) with advanced urothelial cancer (UC), J. Clin. Oncol. 36 (2018) 450.

[40] D. Mishra, S. Singh, G. Narayan, Role of B cell development marker CD10 in cancer progression and prognosis, Mol. Biol. Int. 2016 (2016), 4328697.