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ORIGINAL ARTICLE

Comprehensive analysis of genomic alterations of Chinese hilar cholangiocarcinoma patients

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Abstract

Background Cholangiocarcinoma (CCA) is a rare malignant tumor of the biliary system. The heterogeneity of CCA leads to the lack of effective targeted treatment for CCA subtypes. The molecular characteristic of hilar CCA (hCCA) is still unclear.

Methods A total of 63 hCCA patients were enrolled from Shanghai Eastern Hepatobiliary Surgery Hospital. Formalin-fixed, paraffin-embedded tumor tissues, and matched blood were collected and deep sequencing targeting 450 cancer genes were performed. Tumor mutation burden (TMB) was measured by an algorithm developed in-house. Correlation analysis was performed by Fisher's exact test.

Results The most commonly mutated genes were *TP53* (51.7%), *NF1* and *KRAS* (20%, for both), *SMAD4* (16.7%), *FAT3* and *FRS2* (13.3%, for both), *NF1* (11.7%), and *KMT2C*, *MDM2*, and ATM (10%, for each) in hCCA. *ARID1A*, *GATA6*, and *PREX2* mutations commonly occurred in female and *KMT2C* mutations mainly occurred in patients under 60 years old. Statistical analysis showed the association between *ARID1A* mutation and tumor stage ($P=0.041$) and between *NF1* mutation and high TMB ($P=0.0095$). Furthermore, *ARID1B* mutation was identified to associate with the poor prognosis of Chinese hCCA patients ($P=0.004$).

Conclusion The mutational characterization of hCCA is different from both extrahepatic CCA and intrahepatic CCA. *ARID1B* is a potential biomarker for prognosis prediction of Chinese hCCA patients.

Keywords Cholangiocarcinoma · Genomic alteration · Tumor mutational burden · Disease-free survival · Biomarker

Introduction

Cholangiocarcinoma (CCA) is a rare malignant tumor of the biliary system, which seriously threatens the life of patients [1]. According to the location of the disease, CCA was classified into intrahepatic CCA which is located within the hepatic parenchyma and extrahepatic CCA which consisted of hilar CCA (hCCA) and distal CCA [2]. Surgery is still the effective treatment for early CCA, although only a small subset of patients could be diagnosis because of the unclear clinical symptoms of early CCA [3]. The insensitivity of CCA to radiotherapy and chemotherapy leads to poor prognosis [4, 5]. Targeted therapy and immunotherapy based on biomarkers are effective treatments for malignant tumors [6–8]. However, there are few effective biomarkers for CCA, which need to be developed and explored for early identification and diagnosis.

The location-based classification is helpful to determine the preoperative treatment in clinic. Anyway, the boundary

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between subtypes is still ambiguous [9]. The heterogeneity of CCA leads to the lack of effective targeted treatment for CCA subtypes differ in response to the treatment [10]. With the development of next-generation sequencing (NGS) technology, it is possible to discover the differences among subtypes at the molecular level. Recent studies have shown that there are different molecular characteristics between intrahepatic CCA and extrahepatic CCA [11, 12]. Comprehensive whole-exome and transcriptome sequencing in a large cohort of 260 patients also revealed potentially targetable genetic driver alterations [13]. For example, the specific common mutations in intrahepatic CCA were *IDH1*, *MCL1*, *PBRM1*, *FGFR2*, and *FGFR 3/4/19*, whereas *FBXW7*, *ERBB2*, and *RBM10* in extrahepatic CCA [11–13]. NGS studies revealed the genomic heterogeneity of CCA subtypes potentially affecting the future therapy trials [11]. Although extrahepatic CCA can be divided into hCCA and distal CCA, the prognosis of them were different. Waseem et al. reported that the mean survival of hCCA was lower than distal CCA, but similar to intrahepatic CCA [14]. Until now, few studies isolated hCCA and focused on its genomic characteristics.

In this study, we enrolled 63 Chinese hCCA patients to characterize their comprehensive genomic profiling, and aimed to identify the potential biomarkers for prognosis and provide evidence for further targeted therapy and immunotherapy.

Patients and methods

Patient enrollment and sample collection

From 2012 to 2019, 63 hCCA patients were enrolled from Shanghai Eastern Hepatobiliary Surgery Hospital according to the tumor locations. Informed consent was obtained from all patients and this study was approved by the Institutional Ethics Committee of Shanghai Eastern Hepatobiliary Surgery Hospital. According to the results of computed tomography or magnetic resonance imaging, the patients were given the necessary jaundice-reducing treatment. After the total bilirubin was less than 5 times of normal, surgical resection was performed. The tumor tissue samples were fixed in formalin, and then were embedded in paraffin within 24 h. Meanwhile, matched blood samples were collected as control. Formalin-fixed, paraffin-embedded (FFPE) tumor tissues containing at least 20% of tumor cells are considered to be composed of tumor tissue and can be used for further NGS detection.

Identification of genomic alterations and tumor mutation burden

DNAs of both FFPE tumor tissues and matched blood were obtained using QIAamp DNA FFPE Tissue Kit and QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany), respectively, and sequenced using the next-generation sequencing-based YuanSu450™ gene panel of OrigiMed (Shanghai, China), from where the laboratory was certified by College of American Pathologists (CAP) and Clinical Laboratory Improvement Amendments (CLIA). The genes were captured and sequenced with a mean depth of 800 × using Illumina Nova (Illumina, Inc., CA). Genomic alteration was identified as following [15]: single-nucleotide variants (SNVs) were identified by MuTect (v1.7). Insertion–deletions (Indels) were identified using PINDEL (V0.2.5). The functional impact of genomic alterations was annotated by SnpEff3.0. Copy-number variation (CNV) regions were identified by Control-FREEC (v9.7) with the following parameters: window = 50 000 and step = 10 000. Gene fusions were detected through an in-house developed pipeline. Gene rearrangements were assessed by Integrative Genomics Viewer (IGV). Tumor mutation burden (TMB) was calculated by counting the coding somatic mutations, including SNVs and Indels, per megabase of the sequence examined in each patient.

Statistical analysis

Statistical analyses were performed using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA). The Kaplan–Meier method and Cox regression were used to analyze survival. Fisher's exact test was used to analyze significant differences. $P < 0.05$ was considered statistically significant.

Results

Clinical characteristics of hCCA patients

A total of 63 hCCA patients with a median age of 59 years (range 38–85 years) were enrolled in this study. These samples consisted of 41 (65.1%) male and 22 (34.9%) female. According to the pathological examination records, the tumor of patients was classified into stage I (4/63, 6.4%), stage II (35/63, 55.6%), stage III (16/63, 25.4%), and stage IV (5/63, 7.9%). The tumor stage of 3 (4.8%) patients was unclear. Three of the 63 patients harbored hepatitis B virus and no one harbored hepatitis C virus. The 27% of patients were identified as lymph-node metastasis positive. 57 (90.5%) patients have had radical surgery, and 54 of them were followed up, including 33 patients received postoperative adjuvant chemotherapy, 15 patients did not receive postoperative adjuvant treatment, and 6 patients with unknown postoperative treatment. According to intraoperative exploration, 8 (12.7%) patients were diagnosed with vascular invasion, 54 (85.7%) had no vascular invasion, and 1 patient had unknown information. According to Bismuth–Corlette

classification [16], 8 patients were type I, 9 patients were type II, 17 patients were type IIIa, 25 patients were type IIIb, 2 patients were type IV, and 2 patients with unclear Bismuth–Corlette type. Patients' clinical or pathological information is summarized and shown in Table 1.

Genomic alterations in hCCA

Three of the 63 patients did not detect the effective alterations. A total of 545 clinically relevant genomic alterations in 263 genes were identified in 60 hCCA patients. All these alterations included 331 (60.7%) substitution/Indels, 102 (18.7%) truncations, 89 (16.3%) gene amplifications, 21 (3.85%) fusion/rearrangement, and 2 (0.37%) gene homozygous deletions (Table S1). The most commonly mutated genes were *TP53* (51.7%, 31/60), *NF1* and *KRAS* (20%, 12/60, for both), *SMAD4* (16.7%, 10/60), *FAT3* and *FRS2* (13.3%, 8/60, for both), *NF1* (11.7%, 7/60), and *KMT2C*, *MDM2*, and *ATM* (10%, 6/60, for each) (Fig. 1). The most common mutations of *ARID1A* and *SMAD4* were truncation mutant (10/12 and 7/10, respectively). The most common mutations of *FRS2* and *MDM2* were gene amplification (7/8 and 6/6). Notably, *FRS2* and *MDM2* amplifications were occurred simultaneously in 6 patients (Fig. 1).

Correlations between mutated genes and the clinical characteristics of Chinese hCCA patients

To explore the potential biomarker, we performed association analyses between mutated genes and clinical characteristics such as gender and age. The most frequent mutated genes were *TP53* (48.8%, 20/41), *KRAS* (17.07%, 7/41), *SMAD4* (14.6%, 6/41), *ATM* and *FAT3* (12.2%, 5/41, for both) in male, while *TP53* (50%, 11/22), *ARID1A* (36.4%, 8/22), *KRAS* (22.7%, 5/22), *FRS2*, *KMT2C*, *NF1*, and *SMAD4* (18.2%, 4/22, for each) in female. Statistical analysis showed that the mutational frequencies of *ARID1A* ($P=0.017$), *GATA6* ($P=0.039$), and *PREX2* ($P=0.039$) were significantly higher in female than in male patients (Fig. 2a).

Based on tumor stage, we classified stage I and II into a group, and stage III and IV into another group, and found that *ARID1A* mutations were mainly occurred in stage I/II group. Statistical analysis showed a significantly association between *ARID1A* mutations and tumor stage I/II ($P=0.041$) (Fig. 2b). In this study, there were 8 patients with vascular invasion. Statistical analysis showed that there was an association between *KRAS* mutation and vascular invasion ($P=0.043$) (Fig. 2c).

In this cohort, most of the patients were over 40 years old, including 9 patients under 50 years old (1 of them was 38 years old), 14 patients between 50 and 59 years old, 29 patients between 60 and 69 years old, and 11 patients over 70 years old. Based on genomic alterations, we found the mutation of *KMT2C* mainly occurred in the patients under 60 years old. Statistical analysis also showed a significant association between age and the mutation of *KMT2C* ($P=0.002$) (Fig. 2d). We also analyzed the clinical characteristic of lymph-node metastasis and no significantly associated gene mutations were detected.

We identified the TMB value of 60 patients with clinically relevant genomic alterations. The median TMB value was 3.8 mutations/Mb, ranged from 0 to 49.5 mutations/Mb. To explore TMB-related mutations, we divided patients into mutant and wild-type groups for each mutated gene.

Table 1 Clinicopathologic features of 63 hilar cholangiocarcinoma patients

Total	63
Age	
Median (range)	59 (38–85)
TMB	
Median (range)	3.8 (0–49.5)
Gender	
Male	41 (65.1%)
Female	22 (34.9%)
Tumor stage	
I	4 (6.4%)
II	35 (55.6%)
III	16 (25.4%)
IV	5 (7.9%)
Not available	3 (4.8%)
Hepatitis B virus	
Positive	3 (4.8%)
Negative	60 (95.2%)
Hepatitis C virus	
Positive	0 (0%)
Negative	63 (100%)
Metastatic lymph nodes	
Yes	17 (27.0%)
No	43 (68.3%)
Not available	3 (4.7%)
Radical surgery	
Yes	57 (90.5%)
No	4 (6.4%)
Not available	2 (3.1%)
Postoperative chemotherapy	
Yes	37 (58.7%)
No	17 (27.0%)
Not available	9 (14.3%)
Vascular invasion	
Yes	8 (12.7%)
No	54 (85.7%)
Unknown	1 (1.6%)

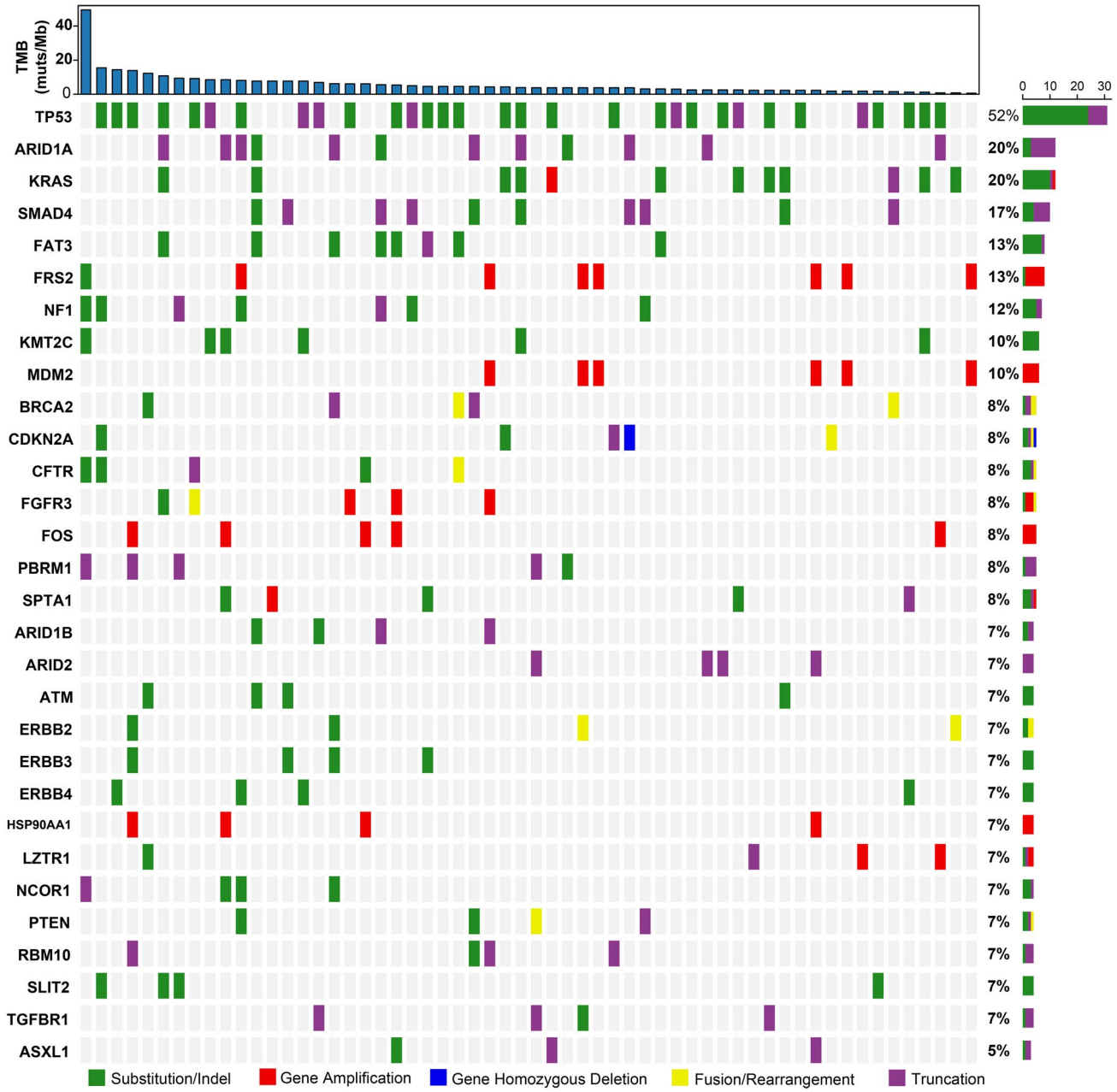

Fig. 1 Mutational landscape of 60 Chinese hCCA patients. The X-axis shows each case sample and the Y-axis shows each mutated gene. The bar graph upside shows the TMB value of the patients. The bar graph on the right shows the numbers of each mutated gene. Green represents substitution/indel mutations, red represents gene amplification mutations, blue represents gene homozygous deletion mutations, yellow represents fusion/rearrangement mutations, and purple represents truncation mutations

Our results showed that patients with *NF1* mutation had a significant higher TMB than those without *NF1* mutations ($P = 0.0095$) (Fig. 2e).

Bismuth–Corlette IIIa tumors located at the confluence of left and right hepatic ducts and invaded right hepatic ducts and Bismuth–Corlette Type IIIb tumors located at confluence of left and right hepatic ducts and invaded left hepatic ducts. We also analyze the association between mutated genes and Bismuth–Corlette subtype IIIa and IIIb. However, there were not any mutated genes associated with the invasion direction of the third subtype tumor was detected. Interestingly, a significant association between gender and invasion directions of Bismuth–Corlette subtype III tumor were identified (Fig. 2f).

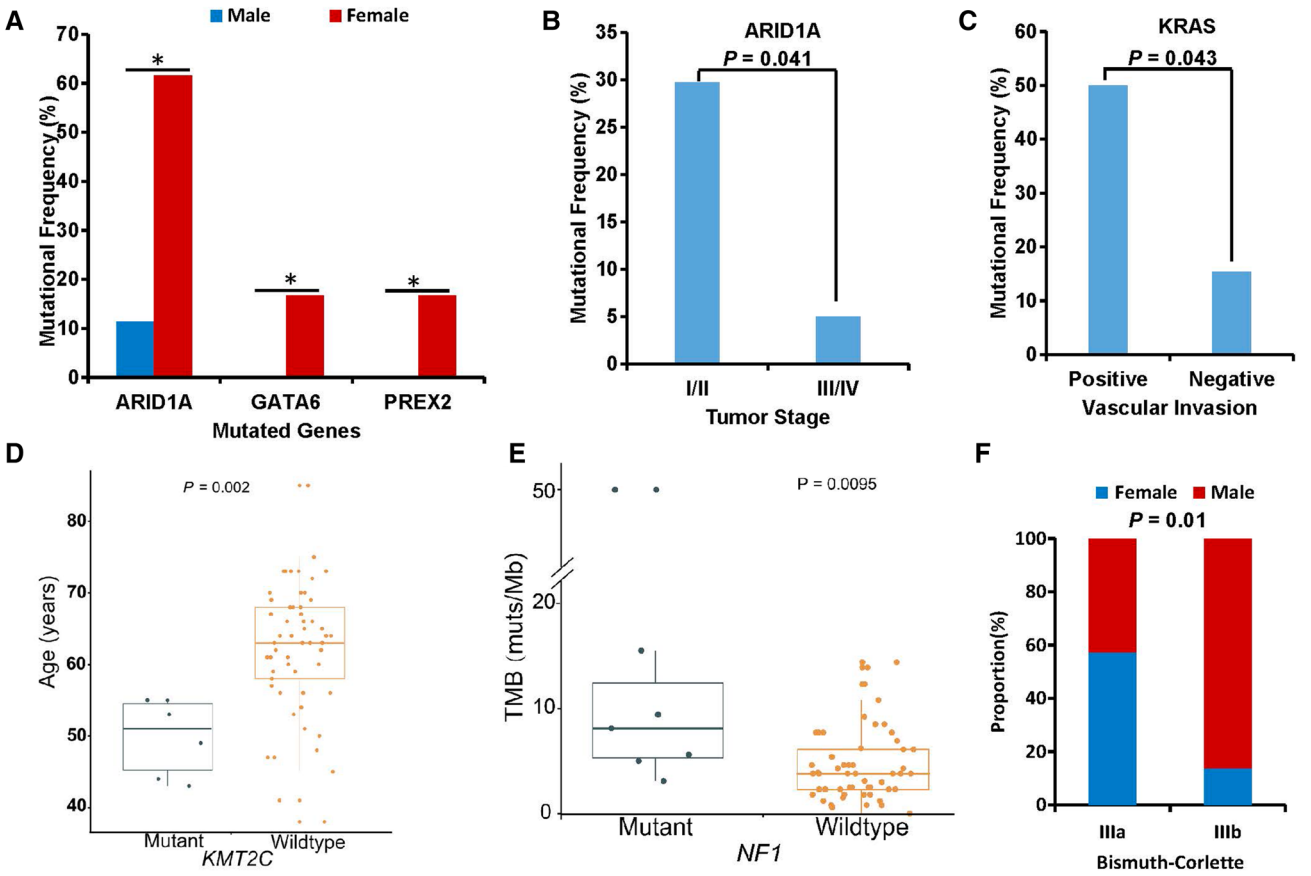

Fig. 2 The correlation between mutated genes and gender (**a**), tumor stage (**b**), vascular invasion (**c**) age (**d**), and TMB (**e**). The significant differences were marked with * for $P < 0.05$

ARID1B and *RBM10* mutations were associated with the disease-free survival

Fifty-four patients with radical surgery were followed up and 51 of them were detected the effect alterations. The median disease-free survival (DFS) was 16 months (ranged from 1 to 54 months). Taking DFS as a continuous variable, we found the association between *ARID1B* and *RBM10* mutations and DFS. Survival curve analysis showed that patients harboring *ARID1B* and *RBM10* mutations had a shorter DFS time than those without mutations (Fig. 3a). To further confirm this result, gender, age, TMB, and other clinical types of patients were considered, and a multivariate cox regression analysis was performed. The results showed that *ARID1B* ($P = 0.004$) mutations were still significantly associated with shorter DFS, while *RBM10* did not associate with DFS any more (Fig. 3b). This result indicated that the association between *RBM10* and DFS was easily neutralized by other clinical characters. Meanwhile, multivariate cox regression analysis also showed that gender ($P = 0.023$) might be a potential factor in response to the correlation between these mutated genes and DFS.

Actionable target mutations of hCCA

Actionable alterations in various types of cancers were collected and summarized by the OncoKB team, and 17 clinically relevant genes with 26 potential therapies for CCA [17], such as cobimetinib/binimetinib/trametinib were potential target drug for *KRAS* mutations, debio1347/BGJ398/erdafitinib/AZD4547 were potential target drug for *FGFR* mutations, and trametinib/cobimetinib was potential target drug for *NF1* mutations. In this cohort, there were 12 actionable mutated genes in 34 (54%) hCCA patients. The most common drug target mutations were *KRAS* (19.05%), *FGFR* (15.87%), and *NF1* (11.11%) (Table 2). Interestingly, cobimetinib is the potential target drug for both *KRAS* mutation and *NF1* mutation, and our data showed that nearly 30.16% (19/63) of hCCA patients harboring *KRAS* mutations or *NF1* mutations may potentially benefit from it.

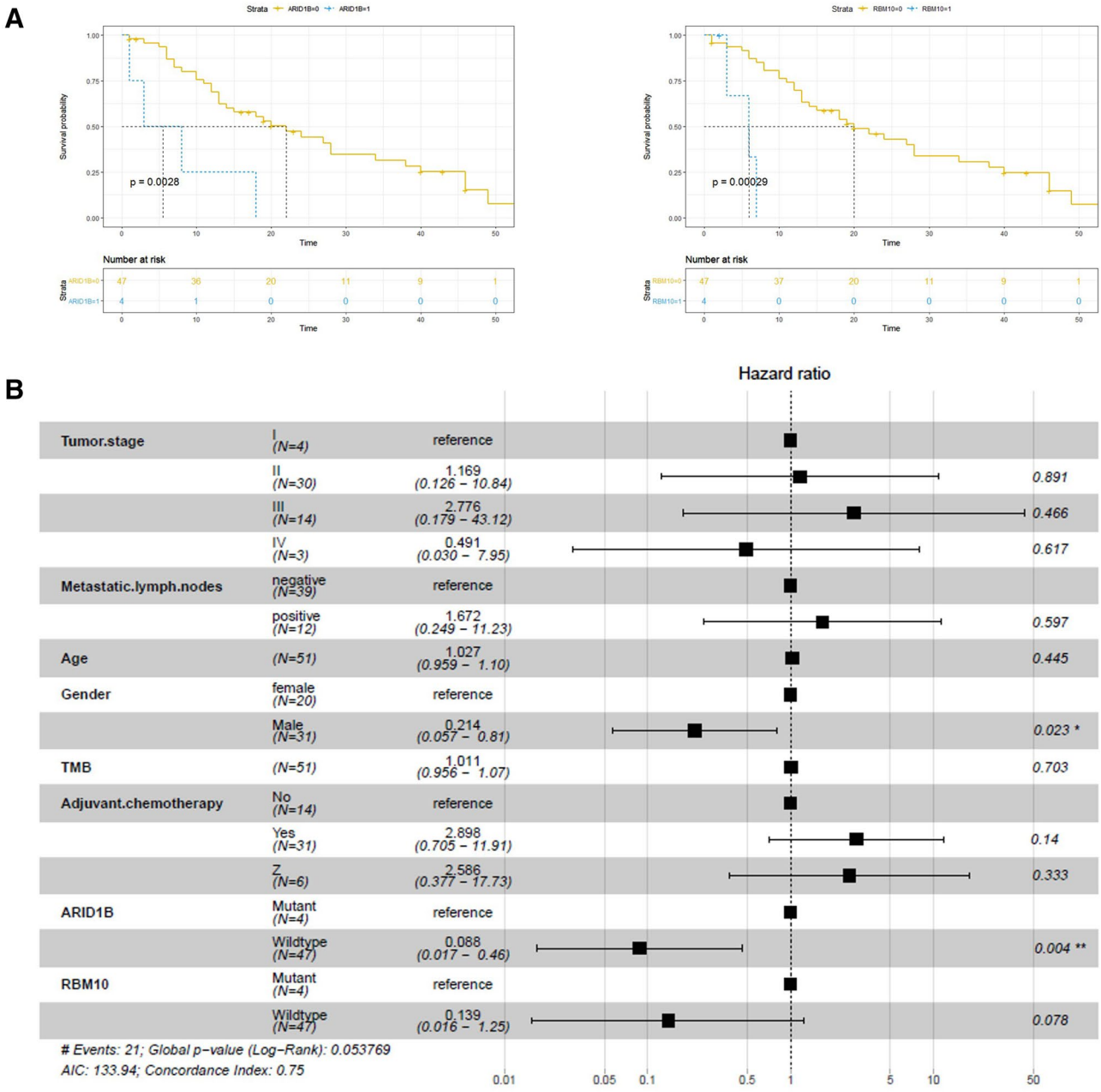

Fig. 3 Correlation analysis between mutated genes and disease-free survival (DFS). **a** Kaplan–Meier curves of the DFS in patients with (red)/without (blue) *ARID1B* and *RBM10* mutations. **b** Multivariate cox regression analysis to confirm the correlation between DFS and *ARID1B* and *RBM10* mutations. Forest plot showed the risk of DFS in various subgroups of patients such as gender, age, and tumor grade

Discussion

CCA is a tumor with high heterogeneity, which occurred in the locations of intrahepatic, hilar, and distal common bile duct. Previous studies have suggested that hCCA and distal CCA are included in extrahepatic CCA, thus distinguishing them from intrahepatic CCA [18]. However, some intrahepatic CCA is the invasion of hCCA [18]. Akita et al. divided the intrahepatic CCA into perihilar CCA and peripheral CCA based on histologic [19]. Particularly, hCCA have been variably and inconsistently coded as either intrahepatic CCA or distal CCA. Although these three types of CCA are distinct in their presentation and natural history, as well as the approach to diagnosis and management [20, 21], few hCCA molecular characteristics have been reported. Here, we enrolled 63 hCCA patients and identified the mutational profile. In addition to the most common mutations of *TP53*, *KRAS*, *SMAD4*, *ARID1A,* and *CDKN2A/B* in CCA [20–22],

Table 2 Comparative analysis of druggable genes in hCCA, intrahepatic CCA, and extrahepatic CCA

GENES	Drugs	hCCA (%)	Intrahepatic CCA (%)	Extra-hepatic CCA
KRAS	Cobimetinib, Binimetinib, Trametinib	19.05	28.70	46.00
CDKN2A	Abemaciclib, Palbociclib, Ribociclib	7.94	15.20	20.00
BRAF	PLX8394	3.17	4.30	8.00
IDH1	Ivosidenib	0.00	23.30	2.50
ATM	Olaparib	7.94	4.90	3.75
PTEN	AZD8186, GSK2636771	6.35	1.80	1.25
MET	Crizotinib	4.76	5.50	1.25
NF1	Trametinib, Cobimetinib	11.11	6.10	NA
FGFR1/2/3	Debio1347, BGJ398, Erdafitinib, AZD4547	15.87	12.80	NA
NTRK3	Larotrectinib, Entrectinib	1.59	NA	NA
CDK12	Pembrolizumab, Nivolumab, Cemiplimab	4.76	NA	NA

the high mutation frequencies of *MDM2* and *FRS2* were detected in hCCA.

MDM2 and *FRS2* are located at 12q13-15 chromosomal band and they are close to each other. This may be the main reason for the co-amplification of *MDM2* and *FRS2* in this cohort. Amplification of 12q13-15 region often occurred in liposarcoma tumors and low-grade osteosarcoma [23]. Previous studies showed that *FRS2* and *MDM2* amplification associated with the differentiation of liposarcoma [24]. *FRS2* is a downstream binding protein of tyrosine kinase receptor and involved in the process of cell differentiation, proliferation, and tumorigenesis [25]. *FRS2* can be phosphorylated by *FGFRs* to activate downstream pathways, such as MAPK and PI3K/Akt/mTOR pathways, so as to make tumor progress [25, 26]. In breast cancer, *FRS2* is a biomarker with high risk of tamoxifen adjuvant therapy [27]. The high frequency of *MDM2* and *FRS2* amplification in hCCA supported the specific molecular mutational feature of hCCA, which may provide evidence for further precision medicine of hCCA.

KMT2C is a tumor suppressor due to its frequent mutations in multiple types of tumors [28–30]. *KMT2C* is associated with the poor prognosis in acute myeloid leukemia [31]. While in breast cancer, the association between *KMT2C* mutation and prognosis is controversial [32, 33]. Wang et al. reported that *KMT2C* mutations were more frequently occurred in patients over 50 years [33]. While in this study, the patients with *KMT2C* mutation were all under 60 years old, which indicated the association between *KMT2C* and age in hCCA. However, the function of *KMT2C* mutations is limited [33, 34].

NF1 encodes a GTPase activating protein and functions as a tumor suppressor gene in immature myeloid [35, 36]. Mutations of *NF1* may lead to increased proliferation and tumorigenesis [37]. In CCA, low frequency of *NF1* mutation was detected [12, 20–22]. A similar mutation frequency of *NF1* in hCCA to intrahepatic CCA was detected in this study. Also, we first identified the association between *NF1* mutation and high TMB in CCA. High TMB means to have more potential opportunity to benefit from immunotherapies [38, 39]. PD-L1 expression is also a biomarker for immunotherapy prediction [40]. Mou et al. reported intrahepatic CCA patients with high TMB and PD-L1-positive which exhibited a successful response to the combination of immunotherapy and chemotherapy [41]. Wang et al. also showed increased expression of PD-L1 on *NF*-associated tumors [42]. Together, our result implied that patients with *NF1* mutation may have potential opportunity to be benefit from Immunotherapy.

ARID1A mutation is a frequent event in endometriosis-related ovarian carcinomas [43]. Low expression of *ARID1A* correlates with poor prognosis in intrahepatic CCA [44]. Although we did not detect the correlation between *ARID1A* mutation and DFS in this study, we found the significant association between *ARID1A* mutation and early tumor. *ARID1A* mutation and *GATA 6* mutation were associated with gender in this study. These results are similar with the previous study in kinds of cancers [45, 46]. The previous study showed that *GATA6* was a new predictor for poor prognosis of ovarian cancer [47]. Interestingly, our results also showed that gender may be a potential factor associated with DFS. These results implied the possible association between *ARID1A* and *GATA6* mutations and the prognosis of female hCCA patients. However, further confirmation is still needed.

Vascular invasion is associated with high tumor grade [48]. There were 8 patients with vascular invasion in this study and all of them were of high tumor stage (III/IV). Interestingly, the association between *KRAS* mutation and vascular invasion were identified. *KRAS* mutation is a predictor for poor prognosis in many cancers [49, 50]. Similarly, although patients with KRAS mutation may benefit from

cobimetinib/binimetinib/trametinib, our results also support that patients with *KRAS* mutations may have a higher risk of vascular invasion and poor prognosis.

Mutations in the *ARID1B* gene, which shares approximately 60% similarities in amino acid sequence with *ARID1A*, are a component of *SWI/SNF* chromatin remodeling complex and may play a role in cell cycle activation [51]. It is reported that the low expression of *ARID1B* is associated with the poor prognosis in bladder urothelial carcinoma and ovarian clear cell carcinoma [52, 53]. *RBM10* is involved in the tissue damage repair and plays an important role in tumor progression in many cancer types [54–57]. The mutation of *RBM10* was associated with the poor prognosis in lung adenocarcinoma [58]. In this study, our results showed the significant association between the mutations of *ARID1B* and *RBM10* and short DFS. Further multivariate cox regression analysis confirms the associations between *ARID1B* and DFS, but not support the association between *RBM10* and DFS. This may be due to the small cohort in this study. However, our results supported that Chinese hCCA patients with *ARID1B* mutation may have a poor prognosis. In total, we first reported the association between the mutation of *ARID1B* and short DFS, and suggested that *ARID1B* may be a potential prognosis biomarker for hCCA.

So far, there have been many studies on the mutation characteristics of CCA [21]. Previous studies have shown that the molecular characteristics of patients from different regions are different [12, 20]. All cases come from a single case center is a deficiency of this study. It is possible that the mutation characteristics of hCCA patients in this study may be different from those in other parts. To avoid the differences caused by regions, we compared the mutational characteristics of Chinese hCCA, intrahepatic CCA [22], and extrahepatic CCA [20]. The most common mutations of intrahepatic CCA were *TP53*, *ARID1A*, *CDKN2A*/B, *TERT*, *IDH1*/2, *FGFR1/2/3/4*, *PBRM1*, and *SMAD4* [22]. While the most common mutations of extrahepatic CCA were *TP53*, *KRAS*, *SMAD4*, *ARID1A*, *CDKN2A*/B, *TERT*, and *RBM10* [20]. In this study, the high-frequency mutations were *TP53*, *KRAS*, *ARID1A*, *SMAD4*, *FGFR1/2/3/4*, *FRS2*, *CDKN2A/B,* and *MDM2*. Compared with the most common mutated genes from intrahepatic CCA and extrahepatic CCA, we described the molecular characteristic of hCCA as follows: (I) Gene mutations similar to those in intrahepatic CCA, including *TP53*, *KRAS*, *FGFR*, *PBRM1*, and *NF1*. The mutational frequencies of *TP53* and *KRAS* were significantly lower in hCCA and intrahepatic CCA than in extrahepatic CCA. Mutation information of *FGFR*, *PBRM1*, and *NF1* from extrahepatic CCA was not available. (II) The mutational frequency of *IDH1* was significantly lower in hCCA than in intrahepatic CCA, but similar to that of extrahepatic CCA. (III) Genes with higher mutational frequency in hCCA than in intrahepatic CCA and extrahepatic CCA, such as *MDM2* and *FRS2*. (IV) Mutations in hCCA are similar to those in intrahepatic CCA and extrahepatic CCA, such as *SMAD4*, *ARID1A*, *CDKN2A/B*, *TERT*, and *RBM10*. Interestingly, there was a significant difference in *SMAD4* mutation frequency between intrahepatic CCA and extrahepatic CCA, but no significant difference between hCCA and both intrahepatic CCA and extrahepatic CCA (Fig. 4). Meanwhile, lower frequency of *KRAS*, *CDKN2A*, *BRAF*, and *IDH1*, and higher frequency of *ATM* and *PTEN* were in hCCA. These results indicated less opportunity to benefit from the therapy of cobimetinib/binimetinib/trametinib (*KRAS*), abemaciclib/palbociclib/ribociclib (*CDKN2A*), PLX8394 (*BRAF*), and ivosidenib (*IDH1*), and more opportunity to benefit from the therapy of olaparib (*ATM*) and AZD8186/GSK2636771 (*PTEN*) in

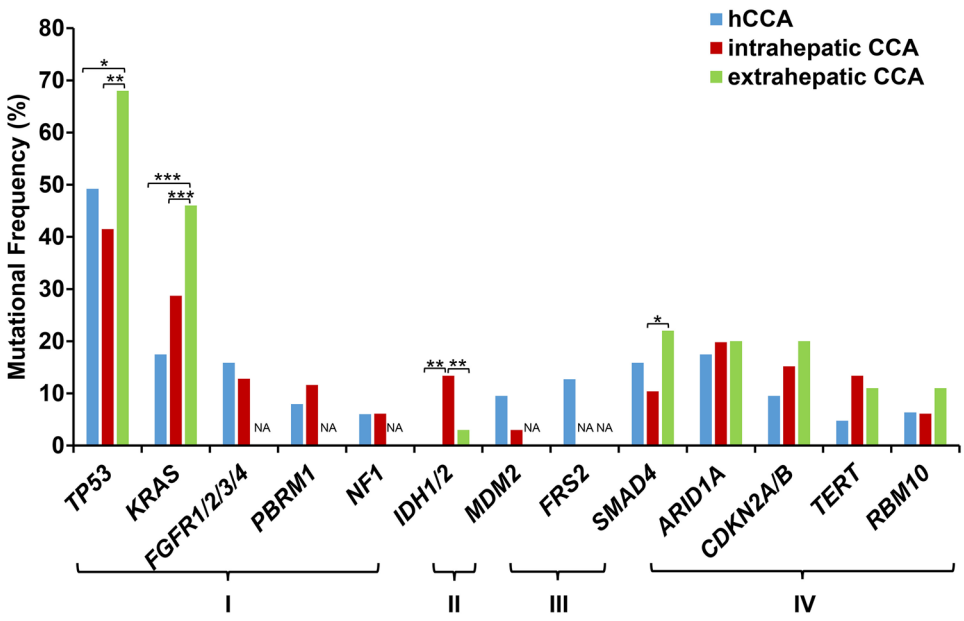

Fig. 4 Comparative analysis of high frequently mutated genes in hCCA (blue), intrahepatic CCA (red), and extrahepatic CCA (green). The X-axis represents the most mutated genes and the Y-axis represents the mutation frequency of each gene in different CCA subtypes. The significant differences were marked with * for $P<0.05$, ** for $P<0.01$, and *** for $P<0.001$, *NA* not available

hCCA. Although no available alteration mutations in extrahepatic CCA, the frequency of *NF1* was higher in hCCA than in intrahepatic CCA. This indicated the more opportunity to benefit from trametinib and cobimetinib in hCCA. In general, the opportunity to benefit from target drug of hCCA patients were different from those of intrahepatic CCA and extrahepatic CCA. Few studies reported the mutation characteristic of distal CCA. Although we failed to compare the molecular characteristics between hCCA and distal CCA, our results supported that hCCA is different from the previously reported intrahepatic CCA and extrahepatic CCA. The specific molecular feature of hCCA is of great significance in guiding the target drug treatment and further precision therapy of CCA.

In conclusion, we firstly identified mutational landscape of hCCA and detected the correlation between mutated gene and clinical characteristics. Our results suggested potential biomarkers such as *ARID1B*, for potential therapy and prognosis of Chinese hCCA. Objectively, the single sampling and the small number of samples are shortcomings of this study. Further study with the expanded number of samples is still needed to confirm and supplement our results here. However, our research provided the molecular evidence that hCCA differs from intrahepatic CCA and extrahepatic CCA, and provided the evidence for guiding precise therapeutic strategies of Chinese hCCA.

Author contributions FF, XW, QG, YW, YY, QC, BL, BY, and CL collected patients' consents and samples, and analyzed data; XS, QH, LZ, CG, and XJ wrote the manuscript; XJ and CG designed and supervised the study.

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Data availability The datasets used and analyzed in this study are available from the corresponding author upon reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval The project was approved by the Ethics Committee of Shanghai Eastern Hepatobiliary Surgery Hospital.

Informed consent Informed consent for participation was obtained from all subjects.

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