Distinct clinical and prognostic implication of IDH1/2 mutation and other most frequent mutations in large duct and small duct subtypes of intrahepatic cholangiocarcinoma

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Abstract

Background and Objective: IDH1/2, BAP1, ARID1A and PBRM1 have been reported as the most frequent mutant genes in ICCs, and their relationships with clinicopathological features and prognosis were researched in this study.

Methods: We collected clinical data of 130 ICC patients from January 2012 to December 2017. The IDH1/2 mutation and loss of BAP1, ARID1A and PBRM1 expressions were detected by DNA sequencing or IHC methods, and histological subtype of ICCs was determined by HE, Alcian blue and S100P staining.

Results: IDH1/2 mutation was related to decreased TBIL ($P=0.039$), ferritin ($P=0.000$) and higher histological differentiation ($P=0.024$), and was associated with prolonged DFS ($P=0.009$) and a trend toward increased OS ($P=0.126$) in small duct type of ICCs. IHC result of MsMab-1 was generally consistent with DNA sequencing for IDH1/2 mutant in ICCs ($\kappa=0.691$). Only BAP1 expression loss was correlated to prolonged DFS ($P=0.031$) and OS ($P=0.041$) in large duct type of ICCs.

Conclusions: IDH1/2 mutation may be a favorable predictor and be related to iron metabolism in small duct type of ICCs. Furthermore, we suggest that the detection of IDH1/2 mutation is indispensable to determine targeted therapy in small duct of ICCs, while it is not necessary in large duct of ICCs. MsMab-1 was a relatively effective multi-specific antibody against IDH1/2 mutant in ICCs. BAP1 expression loss was related to an improved prognosis in large duct type of ICCs.

1. Introduction

umors originated from the bile ducts can be subdivided anatomically into three subgroups: distal, perihilar, and intrahepatic cholangiocarcinoma (ICC). The ICCs represent approximately 20% of bile duct malignancies and account for 15-20% of primary liver cancers^[1]. ICC is related to a dismal prognosis with a postoperative 5-year overall survival (OS) rates of 17%^[2]. Meanwhile, the incidence of ICC is increasing in recent years^[3]. With the application of next-generation sequencing technology, many frequent genetic mutations have been discovered in ICCs, such as isocitrate dehydrogenase 1/2 (IDH1/2), KRAS, BAP1, ARID1A, PBRM1 and FGFR2-fusion^[4, 5]. Almost all literatures reported that the incidences of their mutations are more than 10% in cases with ICCs^[4-9].

In fact, the distinction between ICC and perihilar cholangiocarcinoma at second-order biliary branches is somehow artificial and seems to take in consideration of surgical implications rather than molecular, histological and anatomical aspects^[10]. As proposed by several investigators^[10-15], ICCs are much heterogeneous and can be histologically classified into two subtypes according to the size of displayed bile duct: large duct type and small duct type (also known as bile duct type and cholangiolar type). Moreover, this category individuates ICC subgroups with different clinical and molecular features^[12, 13]. For example, large duct type ICC representatively lack IDH1/2 mutation and FGFR2-fusion, characteristics

exclusively seen in small duct ICC ^[14]. Whereas, KRAS mutation was detected in 23% of large duct type and 1% of small duct type ICC respectively (P=0.00003) ^[15].

IDH1 and IDH2 genes encode the NADP⁺-dependent isocitrate dehydrogenase in the cytoplasm and mitochondria respectively, catalyzing the conversion from isocitrate to α -ketoglutarate (α -KG). IDH1/2 mutations have been detected in many human cancers, including glioma, chondrosarcoma, acute myeloid leukemia, and cholangiocarcinoma ^[16-19]. These mutations acquire neomorphic activity that transforms α -KG into oncometabolite 2-hydroxyglutarate (2-HG). 2-HG accumulation may inhibit specific α -KG dependent dioxygenases and participate in tumorigenesis involving cell signaling, extracellular matrix maturation, and epigenetic regulation ^[20, 21]. Although previous studies have researched the association of IDH mutation with prognosis and pathology in ICC patients, the data are conflicting ^[4, 6, 7].

BAP1, ARID1A and PBRM1 represent the very frequently mutated histone modifying and chromatin remodeling genes in human cancer, such as renal cell carcinoma, ovarian carcinoma and ICC ^[4, 22, 23]. Due to the diversity of mutational hot-spots and the large scales of these genes, detection of these mutations is relatively difficult compared with IDH or KRAS. Alternatively, immunohistochemistry (IHC) staining is effective to detect the above mutations, because the presence of mutations correlates strongly with loss of the corresponding protein expressions ^[22, 24]. Some studies have estimated the association of these inactive mutations with clinical features and prognosis, but there are still many discrepancies ^[4, 8, 9, 25].

Here we investigated the mutational status of IDH1/2, BAP1, ARID1A and PBRM1, and for the first time, determined whether these mutations are correlated to clinicopathological characteristics and prognoses in each histological subtype of ICCs. Due to the heterogeneity of ICC, by analyzing their relationships in the subtypes, rather than the total cohort of ICCs, we could obtain more reasonable and accurate results to explain the inconsistencies reported previously ^[4, 6-9, 25].

2. Materials And Methods

2.1 Patients and Tissue Samples

For this study, we selected 130 consecutive patients with ICC who underwent surgical treatment at Tianjin Medical University Cancer Institute and Hospital from January 2012 to December 2017. Because of the challenge of determining exact anatomical origin of some tumors, the ICCs with similar features of perihilar carcinoma were excluded. Cases with combined HCC-CCA, composed of typical HCC and typical ICC, were excluded. All patients were reviewed to confirm the diagnosis of ICC and to restage according to the 8th edition 2017 AJCC staging system. After exclusion of 28 patients for prognostic analysis (including 11 cases lost to follow-up, 14 cases received non-R0 resection, 1 cases died of postoperative complications, and 2 cases died of some non-tumor-related causes), 130 and 102 patients were eventually used for comparisons of clinical characteristics and survival analyses respectively.

Formalin-fixed paraffin-embedded (FFPE) samples and hematoxylin-eosin (HE) staining slides of 130 surgical specimens were collected from Pathology Department of Tianjin Medical University Cancer Institute and Hospital. Tissue microarrays (TMAs) composed of 2-mm cores of FFPE tumor tissue were constructed for various staining by selecting a representative tumor areas and a typical peritumoral region from each case.

The Medical Ethics Committee of Tianjin Medical University Cancer Institute and Hospital approved this study (Approval No.: bc2019065), and informed consents were obtained from all patients or their legal guardian.

2.2 Clinical Data

Clinical data, including the patients' gender, age, surgical records, imaging examinations and laboratory tests were carefully reviewed and analyzed. All cases were regularly followed up every 3 months for tumor recurrence by screening of plasma tumor markers and imaging examinations. Disease-free survival (DFS) was measured from the date of surgery to the date of first recurrence or last follow-up, whereas OS was defined as the interval between the date of first diagnosis of ICC and the date of death or last follow-up.

2.3 Histopathology

HE staining was performed routinely on a set of TMAs. According to histological features, we subclassified ICCs into two types: large duct and small duct [11, 14, 15].

Type1 (large duct type) is made up of tall columnar tumor cells arranged in a large-sized glandular or tubular pattern and usually shows abundant desmoplastic stroma and low cellularity. Meanwhile, the tumor cells of type1 contain abundant eosinophilic, clear, or mucinous cytoplasm, and their nuclei are usually high grade. Extracellular mucin can be detected in glandular spaces. [Figure 1A]

Type2 (small duct type) has histological features of small-sized tubular or acinar component composed of low columnar to cuboidal tumor cells and usually shows scant stroma and high cellularity. This type can also be arranged in cribrate or solid patterns. The tumor cells of Type2 have a higher nuclear-to-cytoplasmic ratio compared with type1 tumors and usually contain scanty amphophilic or eosinophilic cytoplasm. [Figure 1D]

If there was any doubt in the process of classification, we can refer to whole mount sections for further observation. Ultimately, some cases, not clearly classified, were termed undetermined type.

Mucin production was stained by Alcian Blue Stain Kit (pH 2.5) (G1560; Solarbio Science & Technology Co., Ltd, Beijing, China) on TMAs to distinguish subtypes of ICCs [Figure 1B,E]. Alcian blue staining was scored semiquantitatively by the proportion of glandular lumens with mucin production on a scale from 0 to 2: score 0, <10%; score 1, 10% to 50%; and score 2, >50% or frequent intracytoplasmic mucin [10, 26].

2.4 Immunohistochemistry

TMA sections were dewaxed and rehydrated in xylene and gradient ethanol, respectively. After antigen retrieval and endogenous peroxidase activity blocking, the slides were incubated with primary antibodies (4°C for 14h and 37°C for 1h) and HRP-conjugated secondary antibody (37°C for 1 h) in turn. Then the sections were visualized with 3,3'-diaminobenzidine (ZLI-9017; Zhongshan Goldbridge) for 5 min and counterstained with Hematoxylin. Appropriate internal or external positive controls and negative controls were designed and used for each round.

The primary antibodies used in the present study were as follows: S100P (clone EPR6142; dilution 1:300; Abcam, Cambridge, UK), IDH1/2 mutant (R132/172) (clone MsMab-1; dilution 1:200; EMD Millipore Corp., Billerica, MA, USA), BAP1 (ab199396; dilution 1:200; Abcam), ARID1A (clone EPR13501-73; dilution 1:500; Abcam), PBRM1 (HPA015629; dilution 1:200; Sigma-Aldrich).

The expression of S100P showed nuclear and cytoplasmic staining pattern and was evaluated semiquantitatively according to the percentage of positive tumor cells as follows: score 0, <1%; score 1, 1% to 25% ; score 2, 26% to 50%; score 3, 51% to 75%; and score 4, 76% to 100% ^[10]. [Figure 1C,F]

IDH1/2 mutant exhibited cytoplasmic and mild nuclear expression, while only nuclear staining was interpreted as immunopositive in the evaluation of BAP1, ARID1A, and PBRM1 expression. [Figure 2-3] A semiquantitative scoring system was used for the above 4 factors in terms of the proportion of positive neoplastic cells: score 0 (negative), < 1%; score 1 (focal or regional positive), 1% to 90%; score 2 (diffuse positive), 91% to 100% ^[27, 28].

2.5 Analysis of IDH1/2 mutation

Genomic DNA of ICCs was extracted from ten 10-um-thick sections micro-dissected from FFPE tissue blocks. Two pairs of primers were designed for hotspot mutations of IDH1/2 ^[10]. The first pair of primers for IDH1-R132: forward 5'- ACACGACGCTCTTCCGATCTACACATAACAAGTTGGAAATTTCTGG-3' and reverse 5'- GACGTGTGCTCTTCCGATCTAATCACCAAATGGCACCATAC-3'; the second pair of primers for IDH2-R140 and IDH2-R172: forward 5'-ACACGACGCTCTTCCGATCTCAGAGACAAGAGGATGGCTAGG-3' and reverse 5'- GACGTGTGCTCTTCCGATCTTGTCTCACAGAGTTCAAGCTG-3' (Tsingke Biological Technology CO., Ltd, Beijing, China). The target DNA sequences were amplified by polymerase chain reaction (PCR) using the primers above, and were further sequenced and analyzed for IDH1/2 mutation.

2.6 Statistical analysis

Categorical variables were presented as totals and frequencies, and evaluated by chi-square test or Fisher exact test, as appropriate; Continuous variables were described as means with standard deviations or medians with ranges, and compared with t test or Mann-Whitney U test, as appropriate. DFS and OS were calculated by Kaplan-Meier method, and assessed by log-rank test for univariate analysis. Cox proportional hazards model was conducted to adjust for the bias from tumor characteristics (e.g. TNM

stage, bilateral involvement) in analyzing prognostic value of gene mutations and other clinical factors. The results were recorded as hazard ratios (HR) and 95% confidence intervals (CIs). A two-tailed P value less than 0.05 was considered significant. SPSS statistical program (IBM SPSS Statistics 24, Chicago, IL, USA) was used for data analysis.

3. Results

3.1 Large duct and small duct type of ICCs

3.1.1 Subclassification of ICCs

We classified 130 cases with ICC into 3 subtypes on the basis of histological features, S100P expression and Alcian blue staining in the first round. 12 cases (9.2%) were recognize as typical large duct type, which met three standards—HE, type1; S100P, score 3-4; and Alcian blue, score 1-2. Meanwhile, 73 cases (56.2%) were typical small duct type, which satisfied the following conditions: HE, type2; S100P, score 0-1; and Alcian blue, score 0-1. The remaining 45 cases (34.6%) were intermediate type that exhibited mixtures of two subtypes or indeterminate features. [Supplementary Table S1]

In the second round, according to the above criteria, we set classification efficiency of three factors as follows: HE > S100P > Alcian blue. Then 45 cases of intermediate type were divided into 2 subcategories. [Supplementary Table S2] Finally, we identified 27 (20.8%) and 103 (79.2%) cases as large duct and small duct type ICCs, respectively. [Table 1] The concordant rate and κ value of interpretations between two observer were 96.9% and 0.904, respectively.

3.1.2 Clinical characteristics and prognosis of ICC subtypes

Our study cohort included 71 males (54.6%) and 59 females (45.4%), with a mean age of 57.8 ± 9.7 years (range from 28 to 77). 44 cases (33.8%) underwent lymphadenectomy.

The large duct type was more likely to have higher level of CA19-9 ($P=0.002$), higher frequencies of lymphadenectomy ($P=0.002$), nerve invasion ($P=0.025$), satellite lesions ($P=0.009$), smaller size of tumor ($P=0.021$) and more aggressive tumor stage of pT ($P=0.041$), pM ($P=0.019$), and TNM classification ($P=0.04$) than small duct type. [Supplementary Table S3]

The median follow-up period of 102 ICC cases was 25.2 months (range from 4.9 months to 100.0months). Although patients with large duct type had worse prognosis than those with small duct type in univariate analysis (DFS, $P=0.063$; OS, $P=0.031$) [Figure S1], the histological subtype was not an independent prognostic factors of ICCs in multivariate analysis ($P>0.10$).

3.2 IDH1/2 mutations in ICCs

3.2.1 IDH1/2 mutations by DNA sequencing and IHC

DNA sequencing showed 21 patients (16.1%) harbored IDH1/2 mutation, including IDH1-R132C, IDH1-R132G, IDH1-R132H, IDH1-R132L, IDH2-R172K, and IDH2-R172W. But no mutation of IDH2-R140 was detected. Meanwhile, IDH1/2 mutant was detected in 14 cases (10.8%) by MsMab-1, which is specific for IDH1-R132G, IDH1-R132H, and IDH2-R172W according to instructions. [Table 2] IHC analysis verified the specificity of MsMab-1 according to results detected by DNA sequencing, and showed that sensitivity and specificity of MsMab-1 to detect specific types of IDH1/2 mutation were 81.8% (9 of 11) and 95.8% (114 of 119), respectively. Accordingly, MsMab-1 was a relatively effective multi-specific antibody against IDH1/2 mutant in ICCs ($\kappa = 0.691$).

3.2.2 Clinical implications of IDH1/2 mutation in small duct type of ICCs.

IDH1/2 mutation was detected in 3.7% (1/27) of cases with large duct type and 19.4% (20/103) of patients with small duct type, respectively. Patients with IDH1/2 mutation had decreased TBIL (total bilirubin) ($P=0.039$), Fe (ferritin) ($P=0.000$) and higher histological differentiation ($P=0.024$) in small duct type. [Table 3]

Univariate and multivariate analyses exhibited that cases with IDH1/2 mutation had significantly improved prognosis than those without IDH1/2 mutation in terms of DFS ($P=0.006$) and OS ($P=0.031$) in total cohort of ICCs. [Figure 4 A-B, Supplementary Table S4] And more notably, Kaplan-Meier analysis showed IDH1/2 mutation was associated with prolonged DFS ($P=0.009$) and a trend toward increased OS ($P=0.126$) in small duct type of ICCs. Further multivariate analysis confirmed IDH1/2 mutation was a favorable independent predictor of DFS ($P=0.022$, HR=3.452, 95% CI=1.191-10.004), and was not significantly related to OS ($P=0.114$, HR=2.686, 95% CI=0.789-9.144) in patients with small duct type. [Figure 4C-D, Table 4]

3.3 Expression status of BAP1, ARID1A and PBRM1 in ICCs

3.3.1 Loss of BAP1, ARID1A and PBRM1 expressions

BAP1 expression was retained in 72 cases (55.4%), while 47 cases (36.2%) and 11 cases (8.5%) showed complete and focal loss of BAP1 expression respectively. Complete or regional loss of ARID1A and PBRM1 staining was observed in 28 cases (21.5%) and 45 cases (34.6%), respectively. [Supplementary Table S5] There was obviously positive correlation among their expression loss ($P<0.05$), especially between BAP1 and PBRM1 ($P=0.000$). [Supplementary Table S6]

3.3.2 Clinical implications of BAP1, ARID1A and PBRM1 expression loss in subtypes of ICCs.

The loss of BAP1 expression was associated with decreased ALT (alanine aminotransferase), AST (aspartate aminotransferase), TBIL and higher histological differentiation in large type of ICCs, while it was correlated to larger size of tumor in small duct type. [Supplementary Table S7] All clinical factors significantly related to the loss of ARID1A expression, were within small duct type of ICCs, such as decreased ALT, AST, lower incidence of liver cirrhosis and earlier TNM stage. [Supplementary Table S8] PBRM1 loss correlated decreased AST, TBIL, IBIL (indirect bilirubin), and CA19-9 in the large duct type of

ICCs. [Supplementary Table S9] In addition, the loss of BAP1, ARID1A and PBRM1 expressions had similar distribution in large duct and small duct type of ICC patients ($P>0.05$). [Supplementary Table S7-9]

Kaplan-Meier curves showed loss of BAP1, ARID1A and PBRM1 expressions were not significantly associated DFS and OS in patients with ICCs ($P>0.05$). [Supplementary Table S4] While univariate and multivariate analyses exhibited BAP1 expression loss was correlated to prolonged DFS ($P=0.031$) and OS ($P=0.041$) in large duct type of ICCs. [Figure 5] Besides this, none of inactive mutations was statistically associated with DFS or OS in either subtype of ICCs ($P>0.05$). [Table 4]

3.4 A typical case of ICC patient with IDH1 mutation

A 61-year-old Chinese man was diagnosed with liver cancer and diffuse intrahepatic and abdominal lymph node metastasis by abdominal magnetic resonance (MR) in May 2017, and subsequently confirmed as ICC (stage IV) with IDH mutation (IDH1-R132C) by biopsy and gene sequencing. He received dasatinib (100mg, once a day) combined with chemotherapy (gemcitabine, once every three weeks, twenty-five cycles) since June 2017. Abdominal MR in July 2017 showed stable disease (SD). Abdominal MR in January 2019 revealed progressive disease (PD). Therefore, in the same month, this patient was administered with lenvatinib (12mg, once a day) instead of dasatinib and chemotherapy. Encouragingly, this case still survives with ECOG performance status 1 at the end of this study (September 2019). Figure 6 summarized the patient's serial images of abdominal MR and diameter curves of representative tumors during follow-up period.

4. Discussion

In the present study, we identified 27 (20.8%) and 103 (79.2%) cases as large duct and small duct type ICCs respectively, according to accepted classification criteria^[10, 14], but the proportion of large duct type in ICCs was lower than previously reported^[10, 15]. To ensure the accuracy of this research, those cases with similar features of perihilar carcinoma that have been excluded should be divided into large duct type. Meanwhile, ICC patients with cholelithiasis, often recognized as large duct type^[15], were rarely admitted to our hospital.

Consistent with previous reports^[11, 13, 15], we found that the small duct type ICCs had improved prognosis than large duct type ICCs in univariate analysis. However, in our research, histopathological subtype was not an independent predictor of postoperative survival in multivariate analysis; Furthermore, the small duct type of ICCs was closely associated with tumor TNM stage. So the bias of tumor stage from both subtypes may account for the inconsistency, and the histological subtype was not an effective prognostic factor in patients with ICCs.

As reported in past studies, IDH1/2 mutation is detected in 10.2%-28.8% of ICCs^[4, 6, 7, 29], and occurs exclusively in small duct type of ICCs^[10, 15]. In this study, we founded that 16.1% of patients with ICC harbored IDH1/2 mutation. Meanwhile, the incidence of IDH1/2 mutation is 3.7% and 19.4% in large duct

and small duct type of ICCs, respectively. Consequently, the clinical features and prognostic value of IDH1/2 mutation should be investigated in small duct type, rather than large duct type. If studied in the total cohort of ICCs, the relationship between IDH1/2 mutation and ICCs may be inaccurate due to confounding from patients with large duct type. Yuchen Jiao, et al. [4] identified that ICC patients with IDH1/2 mutation had 3-year overall survival of 33% compared to 81% for patients with IDH1/2 wild-type ($P=0.0034$), but Pu Wang, et al. [7] reported IDH1/2 mutation was significant associated with improved prognosis in ICCs (4-years tumor recurrence rate was 45.3% vs. 71.5%; $P=0.046$). At the same time, one study on advanced stage ICCs from Massachusetts General Hospital Cancer Center [29] found that there were no significant differences in DFS and OS between IDH mutation and IDH wild-type populations. In the present study, univariate and multivariate analyses showed patients with IDH1/2 mutation had significant prolonged DFS and a trend toward increased OS in small duct type of ICCs. In addition, IDH1/2 mutation was not associated with tumor stage and was related to higher histological differentiation. Consequently, we considered IDH1/2 mutation is a favorable predictor for small duct type ICCs. As for total cohort of ICCs, the predict value of IDH1/2 mutation would be affected by bias from large duct type ICCs. Due to worse prognosis of large duct type ICCs, cases with IDH1/2 mutation had significantly improved DFS and OS than those with IDH1/2 wild-type in total cohort of ICCs. Our results may be account for the conflicting predictive values of IDH1/2 mutation in ICCs described previously. In small duct type of ICCs, IDH1/2 mutation was correlated to a trend toward better OS and the differences were not reach statistical significance. How to explain the inconsistent prognosis of IDH mutation between DFS and OS? As the development of therapies, active and appropriate treatment can obviously prolong survival after tumor recurrence. We further analyzed the treatment of cases with small bile duct type after recurrence and found that most of them were treated with chemotherapy or palliative care. Only 9 patients underwent reoperation or radiofrequency ablation (RFA), and coincidentally, all of them were classified as IDH wild-type. Therefore, this discrepancy can be explained by the bias of different treatments after tumor recurrence in two groups. Furthermore, the typical case described earlier also proved that ICC patient with IDH1/2 mutation had a better prognosis, even if in advanced stage, because he reached long-term (20 months) SD after treated with dasatinib. In general, IDH1/2 mutation is a favorable predictor for small duct type ICCs, and has higher prognostic value in DFS than in OS.

When analyzing the clinical characteristics of ICC patients with IDH1/2 mutation, some investigators found that it related to lower level of CA19-9, lower lymph node metastasis, and smaller size of tumors [6, 29]. We also identified the small duct type of ICCs had similar features described above. Since IDH mutations were typically detected in small duct type of ICCs, we considered these factors (CA19-9, lower lymph node metastasis and size of tumor) might be associated with small duct type, rather than IDH1/2 mutation. We firstly found that IDH1/2 mutation was strongly associated with decreased Fe and TBIL in small duct type of ICCs. The association of IDH1/2 mutation with iron and bilirubin metabolism has not been reported previously, but in tricarboxylic acid cycle, iron homeostasis is regulated by haem and citric acid, which is closely related to the activity of IDH [30, 31]. Accordingly, the mechanism of IDH mutation down-regulating Fe in ICCs needs further study. Because ICCs with IDH1/2 mutation was categorized as small duct type and localized at the periphery of bile duct, biliary obstruction was rare in contralateral bile

duct and hepatic duct. Therefore, most of patients with IDH1/2 mutation do not show elevated TBIL, that is, lower TBIL can be considered as clinical characteristic of small bile duct ICCs.

In gliomas, IDH1/2 mutation is the most frequent type and can be detected by DNA sequencing or IHC methods. Although DNA sequencing is regarded as gold standard, IHC seems to be slightly more accurate, easier to perform, and less costly for detecting IDH1/2 mutation in glioma patients^[32]. We firstly used MsMab-1 for detecting IDH1/2 mutation in ICCs, and found that the results were generally consistent with DNA sequencing. However, IDH1-R132C and IDH1-R132L, two common mutant types in ICCs, cannot be recognized by IHC so far, because their specific antibodies have not been commercially established yet. So multi-specific antibodies against hotspot mutations of IDH1/2 in ICCs should be constructed and validated effectively in future.

Surgery remains the mainstream of potentially curative treatment for ICC so far, at the same time, chemotherapy, radiotherapy and targeted therapy can be used as adjunctive treatments^[33]. Because of the low incidence of IDH1/2 mutations (3.7%), ICC patients with large duct type are suggested to select postoperative chemotherapy without the need for genetic sequencing. In contrast, due to the considerable incidence of IDH1/2 mutations (19.4%) in small bile duct type of ICCs, DNA sequencing should be recommend to detect mutant IDH1/2 and choose appropriate adjuvant therapies: if IDH1/2 mutation are detected, the corresponding target drug (dasatinib, ivosidenib or enasidenib) could be a promising treatment strategy^[34-36]. As the establishment of specific antibodies against mutant IDH1/2, IHC will be an easy and cheap way to detect IDH1/2 mutation.

Besides, the implications of other most frequent mutations were investigated in each subtype of ICCs. Frustratingly, these inactive mutations were not significantly associated DFS and OS in small duct type of ICCs. Nonetheless, only BAP1 expression loss was correlated to prolonged DFS and OS in the large duct type of ICCs. Most previous studies^[4, 25, 28] showed that these factors did not have any prognostic meaning in ICCs, but Sarcognato S et al.^[8] found that the retained expression of BAP1 were related to a poor prognosis, and two studies^[8, 9] drew conflicting conclusions about predictive value of ARID1A in ICCs. Anyhow, our study can explain the inconsistency about the prognostic value of BAP1: loss of BAP1 expression was related to improved survival in large duct type ICCs, and was not associated with prognosis in small duct type ICCs; After combining two subtypes into one group, BAP1 would be considered as a favorable or irrelevant predictor in ICCs according to the ratio of large duct and small duct type ICCs.

There were limitations in this study. Firstly, on account to the small sample of patients included in large duct type, 27 and 21 cases enrolling in comparison of clinical characteristics and survival analysis respectively, some factors with potential value may not reach statistical significance. This was also the reason why KRAS mutation, frequent in large duct type ICC, was not included in this study. Secondly, FGFR2-fusion, another frequent mutation followed by IDH1/2, BAP1, ARID1A and PBRM1 in ICCs, were not investigated in this article.

Declarations

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Author Contributions

BM: study concept and design, data collection and analysis, experiment performing, manuscript writing, statistical analysis; HM: study concept and design, data analysis, manuscript writing; YT: data interpretation, technical and material support; YW, TS, TZ, QW, YC and HL: data collection; QL and WZ: study concept, data collection, data interpretation, and critical revision of the manuscript.

Abbreviations

ICC, intrahepatic cholangiocarcinoma; OS, overall survival; IDH, isocitrate dehydrogenase; α -KG, α -ketoglutarate; 2HG, 2-hydroxyglutarate; IHC, immunohistochemistry; FFPE, formalin-fixed paraffin-embedded; HE, hematoxylin-eosin staining; TMA, tissue microarray; DFS, disease-free survival; PCR, polymerase chain reaction; HR, hazard ratios; CIs, confidence intervals; TBIL, total bilirubin; Fe, ferritin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; IBIL, indirect bilirubin; RFA, radiofrequency ablation; SD, stable disease; MR, magnetic resonance; PD, progressive disease; DBIL, direct bilirubin; CEA, carcinoembryonic antigen.

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Tables

Table 1. Integrated results of the ICC subclassification.

Subtype	HE			AB			S100P				
	Large	Small	underdetermined	0	1	2	0	1	2	3	4
Large duct	12	0	15	3	6	18	0	2	0	10	15
Small duct	0	86	17	92	8	3	68	20	6	9	0

Table 2. Comparisons of IDH1/2 mutations in ICCs between DNA sequencing and IHC methods.

Mutant gene	DNA sequencing			MsMab-1 staining		
	Nucleotide change	Amino acid change	Number	Positive	Negative	Reactivity by manual
IDH1	CGT>TGT	R132C	6	1	5	No
IDH1	CGT>GGT	R132G	5	4	1	Yes
IDH1	CGT>CAT	R132H	4	4	0	Yes
IDH1	CGT>CTT	R132L	2	1	1	No
IDH2	AGG>TGG	R172W	2	1	1	Yes
IDH2	AGG>AAG	R172K	2	0	2	No
Wild type			109	3	106	No

Table 3. Comparisons of clinicopathological characteristics between IDH1/2 mutant and wild type of ICCs

Clinical variables	Total cohort			Small duct type		
	IDH1/2 mutant type	IDH1/2 wild type	P-value	IDH1/2 mutant type	IDH1/2 wild type	P-value
TBIL (umol/L)			0.122			0.039
<21	20(95.2%)	85(78.7%)		20(100.0%)	65(79.3%)	
≥21	1(4.8%)	23(21.3%)		0(0.0%)	17(20.7%)	
INR			0.025			0.054
≤1.0	17(81.0%)	59(54.6%)		16(80.0%)	47(56.6%)	
≥1.0	4(19.0%)	49(45.4%)		4(20%)	36(43.4%)	
CEA (ug/L)			0.162			0.067
<5	19(90.5%)	82(75.9%)		19(100.0%)	68(81.9%)	
≥5	2(9.5%)	26(24.1%)		0(0.0%)	15(18.1%)	
Fe (ug/L)			0.000			0.000
<200	17(85.0%)	42(41.2%)		17(89.5%)	34(44.2%)	
≥200	3(15.0%)	60(58.8%)		2(10.5%)	43(55.8%)	
Histological differentiation			0.028			0.024
Low	5(23.8%)	53(50.0%)		5(25.0%)	44(53.0%)	
Moderately to high	16(76.2%)	53(50.0%)		15(75.0%)	39(47.0%)	
Histological subtype			0.074			
Large duct	1(4.8%)	26(23.9%)				
Small duct	20(95.2%)	83(76.1%)				

Table 4. Univariate and multivariate analyses for prognostic value of frequent mutations in the subtypes of ICCs

Clinical variables	DFS in large duct type		OS in large duct type		DFS in small duct type		OS in small duct type	
	Univariate P-value	Multivariate P-value/ HR (95% CI)	Univariate P-value	Multivariate P-value/ HR (95% CI)	Univariate P-value	Multivariate P-value/ HR (95% CI)	Univariate P-value	Multivariate P-value/ HR (95% CI)
IDH1/2					0.009	0.022	0.126	0.114
Mutant vs. wild						3.452(1.191-10.004)		2.686(0.789-9.144)
BAP1	0.031	0.059	0.041	0.039	0.952		0.454	
Loss vs. retained		1.711(0.979-2.990)		2.985(1.038-4.186)				
ARID1A	0.432		0.580		0.357		0.626	
Loss vs. retained								
PBRM1	0.672		0.159		0.284		0.614	
Loss vs. retained								

Figures

Figure 1

The representative histology, mucin production and S100P expression of large duct type (A-C) and small duct type (D-F) of ICCs. These images were stained by HE (A, D), Alcian blue (B, E), and S100P (C, F), respectively. Scale bar represents 200 μ m.

Figure 2

The representative HE and IHC images of IDH1/2 mutant negative (A, B) and positive (C, D) staining in cases with ICC. These corresponding images of HE (A, C) were presented as control of each IHC staining (B, D), respectively. Scale bar represents 200 μm .

Figure 3

The representative HE and IHC images of BAP1 (A-D), ARID1A (E-H) and PBRM1 (I-L) expression in cases with ICC. The nuclear staining was detected in cases with the normal expression of BAP1 (B), ARID1A (F) and PBRM1 (J). Similarly, the loss of BAP1 (D), ARID1A (H) and PBRM1 (L) expression were interpreted as inactive mutations of these genes. These corresponding images of HE (A, C, E, G, I, K) were presented as control of each IHC staining (B, D, F, H, J, L), respectively. Scale bar represents 200 μ m.

Figure 4

Kaplan-Meier curves showing patients with IDH1/2 mutation had prolonged DFS (A) and OS (B) in total cohort of ICCs; and significant prolonged DFS (C) and a trend toward increased OS (D) were associated with IDH1/2 mutation in small duct type of ICCs.

Figure 5

Kaplan-Meier curves showing patients with loss of BAP1 had improved DFS (A) and OS (B) in large duct type of ICCs.

Figure 6

The serial images of abdominal MR (A) and diameter curves of 3 representative tumors (B) from a typical case during follow-up period. Three representative tumors were marked with corresponding colored arrows in the first MR image.

Supplementary Files

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