original reports abstract

Intrahepatic Cholangiocarcinoma: Genomic Heterogeneity Between Eastern and Western Patients

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PURPOSE Intrahepatic cholangiocarcinoma (IHCCA), a global health problem, is increasing in incidence and has differing etiologies worldwide. Next-generation sequencing (NGS) is rapidly being incorporated into the clinical management of biliary cancers. IHCCA is enriched with actionable mutations, and there are several promising targeted therapies under development. NGS data from Asia, where IHCCA is most prevalent, are limited.

METHODS Comprehensive genomic profiling of formalin-fixed paraffin-embedded tumor tissue from 164 Asian and 283 Western patients with IHCCA was performed using NGS. We measured the distribution of DNA repair genetic aberrations (GAs) in IHCCA, along with actionable mutations. Also, we evaluated the association between DNA repair GAs and tumor mutation burden (TMB). Based on the TMB status, patients were distinguished into 3 levels: low (< 6 mut/Mb), intermediate (6-10 mut/Mb), and high (TMB-H; ≥ 10 mut/Mb).

RESULTS Seventy-two percent of Asian patients had ≥ 1 actionable GA, with a significantly higher frequency in *KMT2C, BRCA1/2,* and *DDR2* compared with Western patients (P = .02, .003, and .003, respectively); 60.9% of Western patients had ≥ 1 actionable GA and higher frequency of *CDKN2A/B* and *IDH1/2* GAs (P = .0004 and < .001, respectively). GAs in nuclear factor kappa B pathway regulators and DNA repair genes occurred more frequently in Asian patients (P = .006 and .001, respectively). There was a higher frequency of TMB-H in Asian compared with the Western cohort (12.2% *v* 5.9%; P = .07).

CONCLUSION A higher burden of DNA repair mutations and frequency of patients with TMB-H in the Asian IHCCA cohort compared with the Western patients suggests a potential role for DNA repair and immune checkpoint inhibitors in the Asian population. Future clinical trials should account for this genetic heterogeneity.

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INTRODUCTION

Cholangiocarcinoma (CCA), a global health problem, constitutes about 10%-20% of all primary liver cancers worldwide. Geographic variation in CCA incidence has been attributed to differing risk factors in the affected population. In Western countries, including the United States, metabolic syndrome, inflammatory bowel disease, primary sclerosing cholangitis, hepatitis C, and alcohol abuse are the main CCA risk factors, and the age-standardized incidence rate is 1.6 per 100,000.[1] The incidence in Southeast Asia is extremely high, up to 71.3 per 100,000 in certain parts of Asia, and is associated with liver fluke and hepatitis B virus (HBV) infection.[2]

Notably, there is an increasing incidence of intrahepatic cholangiocarcinoma (IHCCA) worldwide, and its etiology is unclear.[3] Surgical resection is the only potentially curative treatment, but the majority of patients present with an advanced disease stage and have a limited therapeutic options. Recently, next-generation sequencing (NGS) has been rapidly incorporated into the clinical management of CCA, particularly in the United States. IHCCA, in particular, is enriched with a relatively high number of actionable mutations, and early reports indicate several promising leads with targeted therapies for fibroblast growth factor receptor 2 fusion (*FGFR*), isocitrate dehydrogenase-1 (*IDH1*), *BRAF* V600E mutations, and *HER2*/neu amplification.[2] Nevertheless, NGS data from Asia, where CCA is most prevalent, are limited. These data are critical toward development of targeted therapies for this disease.

CCA genetic profiling studies have identified genetic diversity between intrahepatic and extrahepatic subtypes. Similarly, there appears to be genetic diversity between liver fluke and nonliver fluke–associated CCA in Asia.[4] In this study, we explored the genomic heterogeneity of IHCCA between Asian and Western populations using a targeted NGS platform.

ASSOCIATED CONTENT

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

We compared the genomic landscape of intrahepatic cholangiocarcinoma in Asian and Western patients with this disease using next-generation sequencing. These differences may have therapeutic implications.

Knowledge Generated

Asian patients harbored a higher rate of DNA repair gene mutations compared with Western patients. Actionable driver gene alterations were more common in the Western patients. However, high tumor mutation burden (TMB) value (> 10 mut/Mb) was significantly more common in Asian patients than in their Western counterparts. Patients with high TMB had alterations in direct DNA repair genes and caretaker genes, especially in the Asian population.

Relevance

These results may reflect differences in disease etiology and are relevant for targeted therapy and immunotherapy trials for patients with intrahepatic cholangiocarcinoma.

METHODS

Comprehensive genomic profiling of formalin-fixed paraffin-embedded (FFPE) tumor tissue blocks from the primary tumor or metastatic lesions obtained from patients with IHCCA was performed in 283 patients from a single center in the United States and in 164 patients from China. In the United States, all genomic profiling analysis was performed using a Clinical Laboratory Improvement Amendments (CLIA)-validated platform (Foundation Medicine, Cambridge, MA). In China, genomic profiling was performed by using another CLIA-validated targeted sequencing platform (OrigiMed, Shanghai, China).[5] A bridging study comparing the 2 platforms indicated high concordance (96.3%; 26/27) for the same study samples (results indicated in Appendix Table A1). All study-enrolled patients signed a consent form allowing the release of their tissue blocks to be tested. All FFPE tissue blocks were sectioned and stained with hematoxylin and eosin and reviewed by an expert pathologist to confirm the diagnosis and presence of least 20% of the DNA derived from tumor cells. This research was approved by the institutional review boards at MD Anderson Cancer Center and Peking Union Medical College.

Hybrid Selection and Sequencing

A custom hybridization capture panel including over 23,660 individually synthesized 5′-biotinylated DNA 120 bp oligonucleotides was used to target the exons of cancer-related genes, as well as selected introns of genes frequently rearranged in cancer. Hybridization capture followed the protocol of Hybridization capture of DNA libraries using xGen Lockdown Probes and Reagents (Version 3; Integrated DNA Technologies, San Diego, CA). Postcapture libraries were mixed together, denatured, diluted, and then sequenced. For the purpose of estimation of sequencing error rate, a PhiX spike-in was added as an external control to measure the percentage of reads with 0-4 mismatches, following the method described by Manley et al.[6]

Bioinformatics Pipeline

All types of genetic alterations, including single-nucleotide variant (SNV), short and long indels, copy number alterations (CNAs), and gene rearrangement, were called using a suite of bioinformatics pipelines.[5] Analysis of SNVs and indels began with the alignment of raw reads to the human genome reference sequence (hg19) with the Burrows-Wheeler Aligner (v0.62; BWA, Cambridge, MA), followed by polymerase chain reaction (PCR) duplicates removal using MarkDuplicates algorithm from Picard (version 1.47; Cambridge, MA). Local realignment and base quality recalibration for SNVs were performed using GATK (v3.1-1; Cambridge, MA) and subsequently called by MUTECT (v1.7; Cambridge, MA). The CNAs included: (1) amplification, defined as an increase in the number of gene segment copies by ≥ 8, and (2) homozygous deletion, defined as decrease of complete loss of gene segment copies in samples with > 20% purity. To identify these alterations, tumor cellularity was estimated by allele frequencies of sequenced single-nucleotide polymorphisms (SNPs). For detection of gene rearrangement, aligned reads with abnormal insert size of > 2,000 or zero bp were collected and used as discordant reads, that is, paired-end reads that could not be closely mapped to a genome reference, with each read of paired reads aligned to the same chromosomes or different chromosomes. Originally, the discordant reads with the distance less than 500 bp formed clusters were further assembled by fermi-lite to identify potential rearrangement breakpoints. The breakpoints were double confirmed by BLAT, and the resulting chimeric gene candidates were annotated.

Oncogenic Genetic Mutations

Statistical analysis was only based on oncogenic genetic variants and the variants of unknown significance; low-frequency (variant allele frequency < .01) variants were excluded. Oncogenic criteria included: (1) known pathogenic: oncogenic mutations supported by specific functional studies; (2) likely pathogenic: specific functional

TABLE 1. Demographics for Chinese and US Patients With Intrahepatic Cholangiocarcinoma

Variables	China (n = 164)	United States (n = 283)	*P*
Age, years			
Mean ± SD	59.6 ± 9.9	58.3 ± 12.5	.2419
Median	60	59	
Range	32 to approximately 88	22 to approximately 84	
Sex			
Male:female ratio	1.6:1	0.79:1	
Male	61.6	44.2	.0004
Female	38.4	55.8	
Race			
White	0	74.2	< .0001
Non-White	100	18.8	
Pathology differentiation			
Well	5.5	1.1	.002
Moderate	53	47	
Poor	41.5	45.6	
Stage at diagnosis			
I	4.9	13.4	< .0001
II	17.1	4.2	
III	37.8	39.6	
IV	40.2	37.5	
Smoking history, pack-years	14.6	40.3	
Alcohol	9.7	49	
Fhx			
Fhx of cancer	24.4	78	< .0001
Fhx of liver cancer	7.3	2.5	
Hx of cancer	9.1	14.8	
Hepatitis			
HBV	16.4	1	
HAV	1	0	
HCV	0	0.7	

NOTE. Data are % unless otherwise specified.

Abbreviations: Fhx, family history; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; Hx, history; SD, standard deviation.

studies exist on the mutations situated on the same genome loci and structural disrupting mutations on tumor suppressor genes, such as truncations, splicing sites, and frameshifts; (3) confirmed somatic: somatic mutations recorded on the public genomic database, such as COSMIC, and detected at least once in any tumor types.

Tumor Mutation Burden

Tumor mutation burden (TMB) was estimated for 157 US patients and 164 Chinese patients with IHCCA by counting its somatic mutations, including coding SNVs and indels per megabase of the sequence examined. Driver mutations and known germline alterations in dbSNP were not counted. We classified our patients according to the TMB scores into: (1) TMB low (TMB-L) if the number of mutations per megabase (mut/Mb) was < 6, (2) TMB intermediate (TMB-I) if the number of mutations per megabase was between 6 and 9, and (3) TMB high (TMB-H) if the number of mutations per megabase was ≥ 10. The TMB cutoff was defined per prior lung cancer trials.[7,8]

Microsatellite Instability

We determined the microsatellite instability (MSI) status in all patients with detectable TMB status. According to the

MSI score, we classified the samples as MSI high (MSI-H), defined as instability in ≥ 2 microsatellite loci; MSI low, defined as instability in only 1 loci; and microsatellite stable, defined as absence of any evidence of microsatellite loci instability. If the results for a sample were ambiguous, the analysis was performed a second time. PCR validation confirmed the diagnosis of MSI-H.

Statistical Analysis

Statistical analysis was performed with IBM SPSS version 24.0 (SPSS, Armonk, NY). *P* values < .05 were considered significant. We used χ^2 test or Fisher's exact test for categorical variables and the Kruskal-Wallis test for continuous variables. A box and whisker plot was performed to determine the distribution of TMB among Western and Asian patients with IHCCA.

RESULTS

Patient Characteristics

The mean age and standard deviation was 59.6 ± 9.9 and 58.3 ± 12.5 years for the Chinese and US cohorts, respectively. Among the Chinese cohort, 61.6% were male, with a significantly higher male-to-female ratio (1.6:1) compared with the US cohort. Although 74.2% of US patients were White, 53 (18.8%) of patients were non-White, including 20 (7.1%) Hispanics, 18 (6.4%) Asians, 14 (4.9%) Blacks, and 21 (7.5%) were other. The majority of the patients had stage III and IV disease, with no significant differences between the US (77%) and Chinese (78%) patients. However, there was a higher proportion of patients with stage I and II disease in the US and Chinese cohorts, respectively. The percentage of HBV-positive patients were higher in the Chinese cohort than in the US cohort. Furthermore, the Chinese patients had a significantly higher rate of well-differentiated adenocarcinoma compared with their US counterparts (*P* = .002; Table 1).

Western Versus Asian NGS Comprehensive Genomic Profiling

Comprehensive genomic profiling identified 1,007 genetic aberrations (GAs) in Chinese patients compared with 971 GAs in US patients (Appendix Table A2). Each patient harbored at least 1 GA, with an average of 6.1 (range, 1 to approximately 20) and 3.4 (range, 1 to approximately 16) GAs per tumor in Chinese and US patients, respectively. As indicated in Figure 1, the most commonly reported GAs in Chinese patients were tumor protein 53 (*TP53*; 41.5%), Kirsten rat sarcoma viral oncogene homolog (*KRAS*; 28.7%), AT-rich interactive domain-containing protein 1A (*ARID1A*; 18.3%),

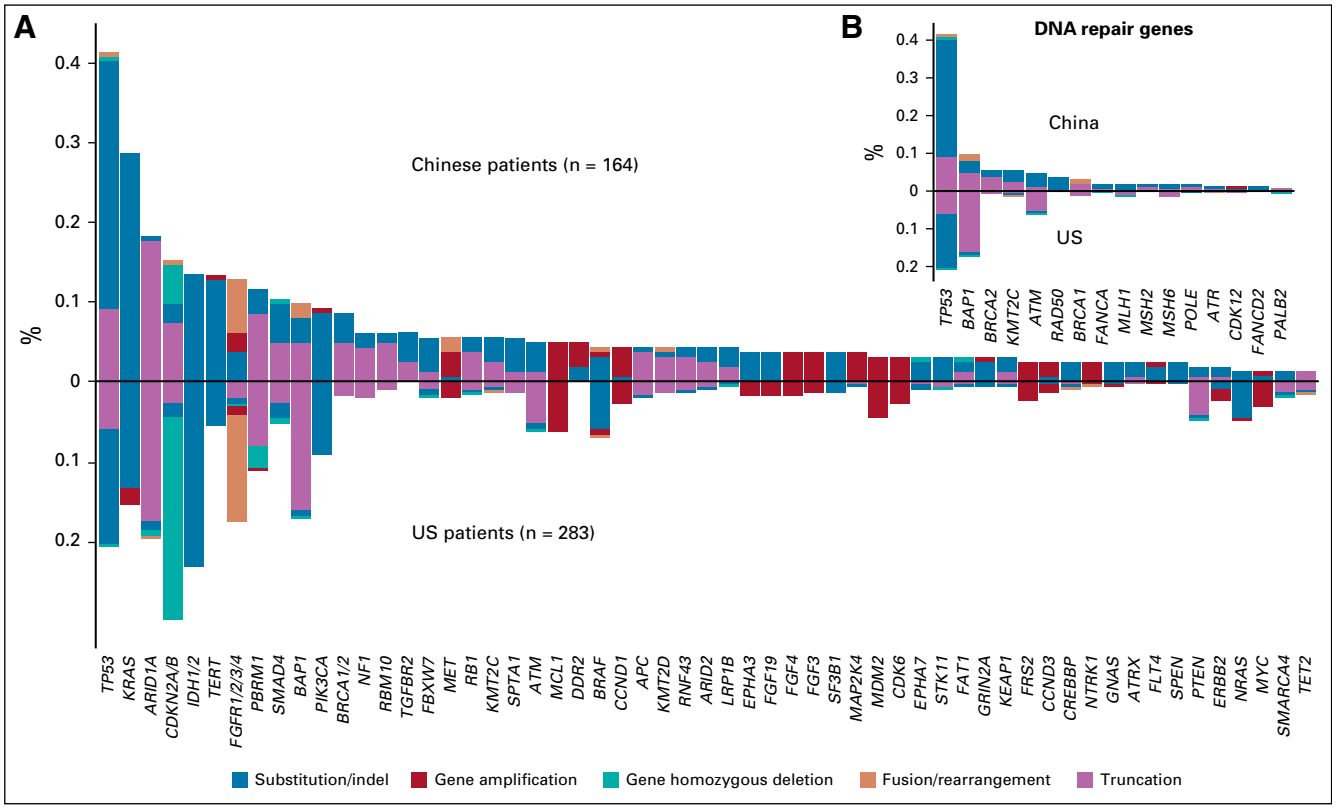

FIG 1. (A) The main graph shows the most common alterations of patients with intrahepatic cholangiocarcinoma (IHCCA). Each gene is separately stated in China (n = 164) and the United States (n = 283). (B) The affiliated chart indicates the alterations among direct and caretaker DNA repair genes. Mutations are colored according to mutation types of substitution/indel, gene amplification, gene homozygous deletion, truncation, and fusion/rearrangement. Alteration types are presented in the color key at the bottom.

cyclin-dependent kinase inhibitor 2A and 2B (*CDKN2A/B*; 15.2%), and isocitrate dehydrogenase 1 and 2 (*IDH1/2*; 13.4%). For the US patients, they were *CDKN2A/B* (30%), *IDH1/2* (23.3%), *TP53* (20.8%), *ARID1A* (19.8%), and fibroblast growth factor receptors *FGFR*1-4 (17.7%). Among the Chinese patients, 118 (72.0%) had at least 1 actionable GA, with a significantly higher frequency in *KMT2C, BRCA1/2,* and *DDR2* compared with US patients ($P = .02$, .003, and .003, respectively). In the US patients, 154 patients (60.9%) had at least 1 actionable GA, with significantly higher frequency of *CDKN2A/B* and *IDH1/2* GAs ($P = .0004$ and $< .001$, respectively; Table 2; Fig 1)

GAs in nuclear factor kappa B pathway regulators and DNA repair genes occurred more frequently in Chinese patients ($P = .006$ and .001, respectively; Fig 2). In our cohorts, we defined DNA repair genes to include the 20 most frequent previously reported DNA repair gene GAs, including direct DNA repair genes (*ATM, ATR, BRCA1, BRCA2, FANCA, FANCD2, MLH1, MSH2, MSH6, PALB2, POLD1, POLE, PRKDC, RAD50,* and *SLX4*) and caretaker genes (*BAP1, CDK12, KMT2C/MLL3, TP53,* and *BLM*).[9] Remarkably, Chinese patients harbored more DNA repair GAs compared with US patients with IHCCA, with predominant GAs in *TP53, BRCA1/2, KMT2C,* and *RAD50*. GAs in *BLM, POLD1, PRKDC*, and *SLX4* were not identified in either cohort. (Table 2; Fig 1)

Additionally, in the Chinese cohort, 10.4% of patients had TMB-H, 13.4% had TMB-I, and 76.2% had TMB-L compared with 5.7% with TMB-H, 15.3% with TMB-I, and 79% with TMB-L among US patients ($P = .18$, 0.45, and 0.65, respectively). Furthermore, there was no significant difference in the median values of TMB-H, TMB-I, and TMB-L groups among the Chinese (14, 8, and 2 mut/Mb, respectively) and US patients with IHCCA (19, 7, and 3 mut/Mb, respectively). Moreover, we identified that the rate of MSI-H in Chinese and US patients was 1.8% (n = 3) and 0.6% (n = 1), respectively, and all MSI-H patients were associated with high TMB values (Fig 2).

We explored the association between DNA repair gene GAs and TMB, and identified a significantly higher rate of TMB-I and TMB-H in patients who had combined direct and caretaker DNA repair GAs compared with patients without DNA repair GAs among the Chinese ($P < .001$) and US ($P = .05$) patients with IHCCA. This was particularly notable with alterations of both direct and caretaker DNA repair gene alterations (Table 3; Fig 3).

TABLE 2. Variations in the Genomic Aberrations Among Chinese and US Patients With Intrahepatic Cholangiocarcinoma

Gene	Chinese Patients (n = 164)	US Patients (n = 283)	*P*
Actionable GA			
CDKN2A/B	15.2	30.0	.0004
IDH1/2	13.4	23.3	< .0001
FGFR1/2/3/4	12.8	17.7	.2
PIK3CA	9.1	9.2	1
MET	5.5	2.1	.1
KMT2C	5.5	1.4	.02
FBXW7	5.5	2.1	.1
BRCA1/2	8.5	1.8	.003
DDR2	4.9	—	.0003
CCND1	4.3	2.8	.4
BRAF	4.3	7.1	.3
STK11	3.0	1.1	.1
RET	1.8	1.1	.7
PTEN	1.8	4.9	.1
PTCH1	1.8	1.4	.7
ERBB2	1.8	2.5	.8
CDK4	0.6	0.7	1
Other GA			
TP53	41.5	20.8	< .0001
ARID1A	18.3	19.8	.8
TERT	13.4	5.7	.008
PBRM1	11.6	11.3	1
SMAD4	10.4	5.3	.06
BAP1	9.8	17.3	.04
TGFBR2	6.1	—	< .0001
RBM10	6.1	1.1	.006
NF1	6.1	2.1	.04
SPTA1	5.5	1.4	.02
RB1	5.5	1.8	.05
MCL1	4.9	6.4	.7
ATM	4.9	6.4	.7
MDM2	3.0	4.6	.5
CDK6	3.0	2.8	1
NRAS	1.2	4.9	.06
MYC	1.2	3.2	.3

NOTE. Data are % unless otherwise specified.
Abbreviation: GA, genetic aberration.

DISCUSSION

IHCCA is an aggressive disease with limited treatment options. The advent of NGS offers promising breakthroughs with targeted therapy and immunologic interventions. However, it is important to identify genomic heterogeneity between populations because this will have a significant impact on precision medicine approaches for this cancer.

In our study, we performed comprehensive molecular profiling of 164 Chinese and 283 US patients with IHCCA to explore genomic heterogeneity between populations. We

FIG 2. (A) The distribution of tumor mutation burden (TMB) value and microsatellite instability (MSI) status in Chinese and US patients with intrahepatic cholangiocarcinoma. (B) Modulator genes of dysregulation pathways or gene subgroups with statistically significant levels between the 2 cohorts. Mutation types are presented in the color key at the bottom. MSS, microsatellite stable; mut/Mb, mutations per megabase.

noted important differences between Asian and Western patients with IHCCA. Through the results, Chinese patients had significantly higher frequency of *TP53* as well as *KMT2C, BRCA1/2, DDR, TERT, TGFBR2, RBM10, NF1, SPTA1*, and *RB1* GAs. In the Western cohort, GAs in *IDH1/2, BAP1*, and *CDKN2A/B* were more dominant. This

TABLE 3. Association Between DNA Repair GAs and TMB Among Chinese and US Patients With Intrahepatic Cholangiocarcinoma

TMB		% of Chinese Patients Evaluated for TMB (n = 164)			
Status	(n = 164)	Absent DNA repair GAs (n = 59)	Direct DNA GAs Only (n = 21)	Caretaker DNA GAs Only (n = 69)	Direct and Caretaker GAs (n = 15)
TMB-L	76.2	89.8	76.2	75.4	26.7
TMB-I	13.4	5.1	9.5	14.5	46.7
TMB-H	10.4	5.1	14.3	10.1	26.7
P value		Reference	.23	.06	< .001
TMB		**% of US Patients Evaluated for TMB (n = 157)**			
Status	**(n = 157)**	**Absent DNA repair GAs (n = 83)**	**Direct DNA GAs Only (n = 15)**	**Caretaker Genes Only (n = 53)**	**Direct and Caretaker GAs (n = 6)**
TMB-L	79	88.8	66.7	71.7	50.0
TMB-I	15.3	10.8	33.3	18.9	—
TMB-H	5.7	1.2	—	9.4	50.0
P value		Reference	.09	.03	.05

NOTE. Data are % unless otherwise specified.

Abbreviations: GAs, genetic aberrations; TMB, tumor mutation burden; TMB-H, tumor mutation burden high; TMB-I, tumor mutation burden intermediate; TMB-L, tumor mutation burden low.

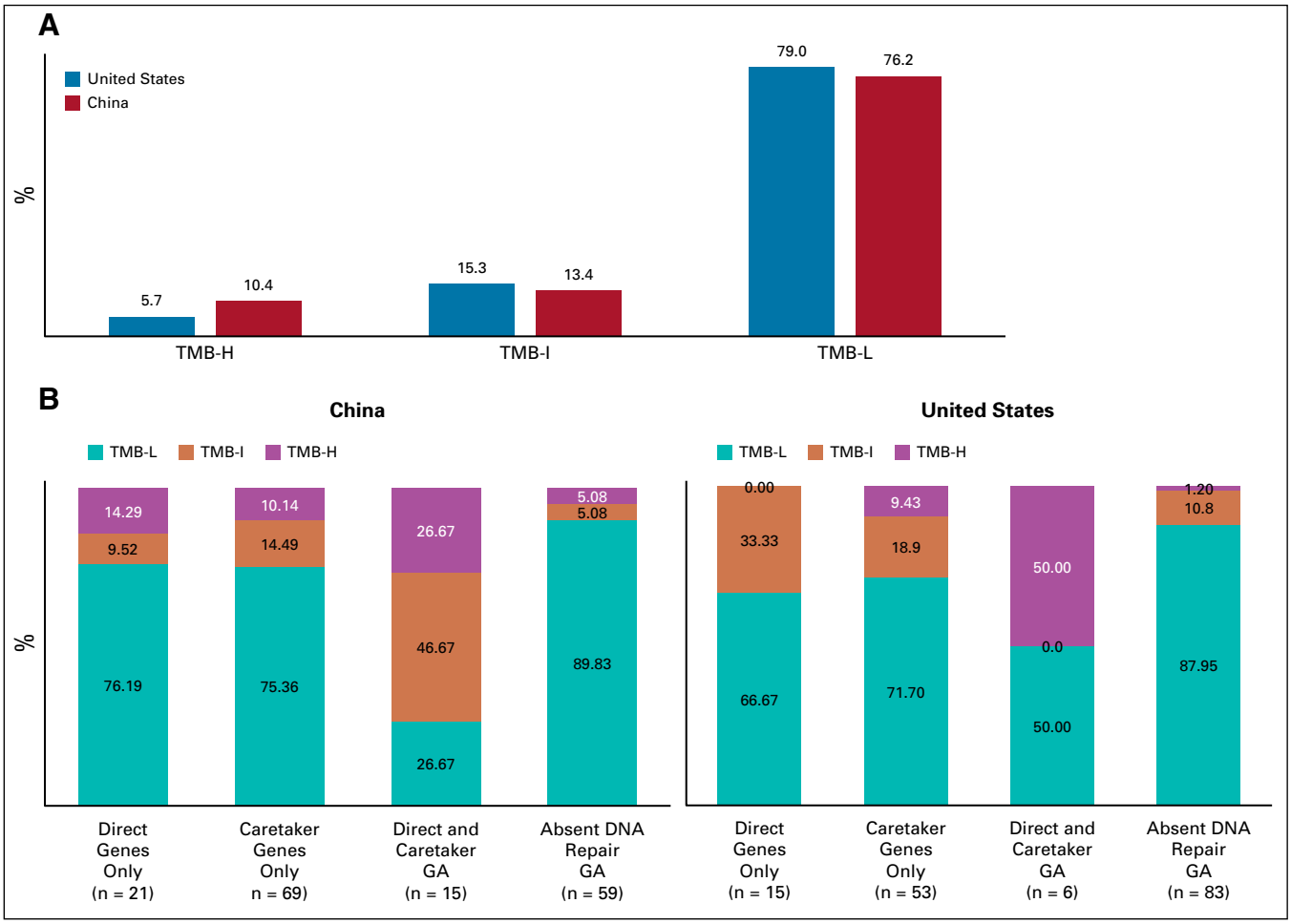

FIG 3. (A) The rate of levels through separating into tumor mutation burden high (TMB-H; (≥ 10 mut/Mb), TMB intermediate (TMB-I; 6-10 mut/Mb) and TMB low (TMB-L; ≤ 6 mut/Mb). (B) The correlation between TMB status and DNA repair genetic aberrations (GAs) by presenting the rates of TMB levels in different existence patterns of direct DNA repair GAs and caretaker GAs.

genetic diversity could be attributed to variations in the underlying disease risk factors (Table 2). In Western countries, CCA is associated with metabolic syndrome, inflammatory bowel disease, primary sclerosing cholangitis, and hepatolithiasis, whereas liver fluke and HBV are important risk factors in Asian countries. A previous study suggested that fluke-related CCAs are enriched with *ERBB2* amplification and *TP53* mutation, whereas fluke-negative CCAs have a higher rate of *IDH1/2*, *BAP1*, *FGFR/PRKA* GAs, and programmed death-1 (PD-1)/programmed death-ligand 1 (PD-L1) expression.[10,11] Furthermore, in a recent genetic profiling study of 103 Chinese patients with IHCCA, it was noted that *TP53* mutations were more commonly seen in HBV-related IHCCA, whereas *KRAS* mutation was more likely occur in HBV-negative IHCCA.[12]

Moreover, our data demonstrated an overall high frequency of DNA repair GAs: in 62.6% of Chinese patients and 45.9% of US patients. Dysregulation of DNA repair pathway is often associated with accumulation of several GAs and higher TMB. Highly mutated tumors harbor neoantigens, which make them more responsive to immune checkpoint inhibitors. This has also been demonstrated in a previous study of lenvatinib with PD-1 inhibition for intrahepatic cholangiocarcinoma conducted by our group. In this study, high TMB was associated with longer progression-free survival.[13] Also, pembrolizumab has been approved by the Food and Drug Administration for the DNA mismatch repair deficient (MMR-d) cancers based on a phase II clinical trial that showed a 40% objective response rate and 78% progression-free survival rate in patients with colorectal cancer with MMR-d compared with 0% and 11%, respectively, for MMR-proficient patients.[14] Similar results have been achieved in noncolorectal cancers that included 4 patients (44%) with biliary tract cancer. Furthermore, it has been recognized that specific somatic mutations could lead to alteration in the immune biomarker expression. For instance, *BRCA1/2* mutated high-grade serous ovarian carcinoma had significantly higher CD3+ and CD8+ tumor infiltrating lymphocytes, as well as elevated PD-1 and PD-L1 expression, in tumor-associated immune cells compared with tumors without *BRCA1/2* mutations.[15,16] In our

study, Chinese patients had significantly higher *BRCA1/2* GAs (8.5%) compared with Western patients (1.8%).

We classified our patients based on the TMB score, and we considered TMB to be high if it was ≥ 10 mut/Mb based on results of the recently published CheckMate 568 trial that demonstrated 44% overall response rate in patients who had TMB ≥ 10 mut/Mb when treated with combined nivolumab and ipilimumab (irrespective of PD-L1 expression).[7] In our study, a minority of patients with IHCCA were TMB-H (12.2% and 5.9% in Asian and Western cohorts, respectively). Additionally, a significant association between DNA repair GAs and TMB-H and TMB-I has been determined. The role of combined poly (ADP-ribose) polymerase inhibitors and PD-1 inhibitors in advanced solid tumor is currently under investigation and may be applicable to this subgroup.

Our study has important limitations. We obtained the comprehensive genetic alteration data employing 2 targeted gene panels. In these panels: (1) the content genes had some dissimilarities; to address this issue, we filtered out differential genes and only included the 320 overlapped genes into the analysis, thereby ensuring the comparability; (2) sequencing platforms were Illumina HiSeq2500 (Illumina, San Diego, CA) for FoundationOne and NovaSeq (Illumina) for OrigiMed450, with effective depths of 500× and 700×, both reflecting the accuracy of sequencing results[17]; (3) the TMB algorithm of OrigiMed450 was based on the published algorithm of FoundationOne, and the genomic alterations were selected similarly[18]; (4) the OrigiMed450 platform uses blood samples from individual patients as their own control for eliminating the impact of germline polymorphisms, whereas FoundationOne uses the somatic-germline-zygosity algorithm and databases of dbSNP and ExAC for the same, and finally; and (5) we conducted a bridging study comparing the 2 platforms and showed a high degree of concordance. Despite these differences, we do not believe that the results are affected in a meaningful way by the differing platforms and are consistent with earlier reports. Furthermore, we have examined the clinical characteristics of the Chinese and US patients and demonstrated that demographic differences between these populations were minor, with most patients having an advanced disease stage. There was a higher proportion of hepatitis B exposure in the Asian cohort, and the pathophysiologic relationship between the viral infection and somatic mutations in cancer is unknown at this time. Future studies to investigate the genomic profiling in different populations, taking into consideration the disease risk factors, are warranted. Data regarding PD-1 and PD-L1 expression were not available; therefore, we could not identify variations in immune biomarker expression between the 2 cohorts in this study. To our knowledge, this is the first study to investigate genomic profiling variations between Asian and Western patients with IHCCA, and our data are likely to be informative toward future precision medicine trials for this cancer.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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APPENDIX

Methods

We identified 10 patients who had been sequenced using the FoundationOne platform; of these, 7 with adequate cellularity were chosen for analysis. The respected blocks were sectioned at 5 μm; the tumor content size and were confirmed by a trained pathologist, and a minimum of 5 unstained slides were submitted for confirmation using the OrigiMed platform. Institutional review board approval was obtained for this study. A high degree of concordance was noted between the platforms, as indicated in Table A1.

TABLE A1. Result of Actionable Alternation Between OrigiMed and FoundationOne Sequencing Platform

Patient ID	Gene	Type	Chr	Position	AA Change	Freq/CNs OrigiMed	Freq/CNs FoundationOne
1	*ARID1A*	Substitution	Chr 1	27094441	p.D1050G	0.57	0.45
1	*BAP1*	Substitution	Chr 3	52443586	p.Q36*	0.52	0.46
1	*CDKN2A*	Indel	Chr 9	21994233	p.E33Gfs*30	0.34	0.53
1	*MCL1*	CNV	—	—	—	Amp	Amp
2	*APC*	Substitution	Chr 5	112162934	p.V513A	0.5	0.46
2	*ARID1A*	CNV	—	—	Exon 3_20 del	Del	Del
2	*IDH1*	Substitution	Chr 2	209113113	p.R132S	0.3	0.27
2	*PBRM1*	CNV	—	—	Exon 15_23 del	Del	Del
3	*ARID1A*	Indel	Chr 1	27105936	p.D1850fs*33	0.28	0.09
3	*ATM*	Substitution	Chr 11	108183194	p.K1992T	0.51	0.55
3	*CDKN2B*	Substitution	Chr 9	22006147	p.D86N	0.47	0.50
3	*IDH1*	Substitution	Chr 2	209113113	p.R132C	0.26	0.15
3	*IDH1*	Indel	Chr 2	209110091	p.P158Tfs*3	0.13	0.08
3	*MCL1*	Substitution	Chr 1	150551669	p.A113V	0.39	0.44
3	*NRAS*	Substitution	Chr 1	115256529	p.Q61R	0.3	0.17
4	*ARID1A*	Indel	Chr 1	27106727	p.P2114Rfs*21	0.35	0.13
4	*BAP1*	Indel	Chr 3	52437288	p.I586Hfs*57	0.38	0.11
4	*FGFR2*	Rearrangement	—	—	FGFR2-NOL4 fusion	Fusion	Fusion
4	*MLH1*	Substitution	Chr 3	37056021	p.L259*	0.70	0.11
4	*PBRM1*	Indel	Chr 3	52678768	p.K284Rfs*16	0.37	Negative
5	*ATM*	Indel	Chr 11	108122620	p.M557Nfs*9	0.24	0.20
5	*IDH1*	Substitution	Chr 2	209113113	p.R132C	0.22	0.21
6	*ARID1A*	Indel	Chr 1	27089591	p.Y850Wfs*21	0.3	0.34
6	*IDH1*	Substitution	Chr 2	209113113	p.R132C	0.3	0.26
6	*KRAS*	Substitution	Chr 12	25398284	p.G12D	0.32	0.25
7	*BAP1*	Substitution	Chr 3	52443574	p.Q40*	0.18	Positive
7	*FGFR2*	Rearrangement	—	—	FGFR2-SLMAP fusion	Fusion	Fusion

Abbreviations: AA, amino acids; chr, chromosome; CNs, copy numbers; CNV, copy number variation; del, deletion; freq, frequency; ID, identification.

TABLE A2. Comprehensive Genomic Profiling Identified 1,007 GAs in Chinese Patients Compared With US Patients

Gene	US Patients (n = 283) %	Chinese Patients (n = 164) %
TAF1	0.4	1.2
SOX9	0.4	1.2
SLIT2	0.4	1.2
RUNX1T1	0.4	1.2
RAF1	0.4	1.2
PRKCI	0.4	1.2
MAP2K2	0.4	1.2
LZTR1	0.4	1.2
JAK1	0.4	1.2
FGF23	0.4	1.2
EPHB1	0.4	1.2
EMSY	0.4	1.2
DOT1L	0.4	1.2
CDK12	0.4	1.2
BCORL1	0.4	1.2
ATR	0.4	1.2
VHL	0.4	0.6
PRDM1	0.4	0.6
MYCN	0.4	0.6
JAK2	0.4	0.6
IGF1R	0.4	0.6
FLT1	0.4	0.6
ERBB4	0.4	0.6
CRKL	0.4	0.6
CIC	0.4	0.6
CHEK2	0.4	0.6
CD36	0.4	0.6
CBL	0.4	0.6
BCOR	0.4	0.6
B2M	0.4	0.6
AXIN1	0.4	0.6
ACVR1B	0.4	0.6
TERC	0.4	0.0
STAT4	0.4	0.0
STAG2	0.4	0.0
PDCD1LG2 (PD-L2)	0.4	0.0
PC	0.4	0.0
PASK	0.4	0.0
HRAS	0.4	0.0
FH	0.4	0.0
FGF10	0.4	0.0
FANCE	0.4	0.0

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TABLE A2. Comprehensive Genomic Profiling Identified 1,007 GAs in Chinese Patients Compared With US Patients (Continued)

Gene	US Patients (n = 283) %	Chinese Patients (n = 164) %
CYLD	0.4	0.0
CSF1R	0.4	0.0
CKS1B	0.4	0.0
CDKN1B	0.4	0.0
CD274 (PD-L1)	0.4	0.0
BCL2L1	0.4	0.0
AXL	0.4	0.0
AURKA	0.4	0.0
ALK	0.0	1.2
BRIP1	0.0	1.2
DICER1	0.0	1.2
ERG	0.0	1.2
ETV6	0.0	1.2
FANCD2	0.0	1.2
FGF14	0.0	1.2
GRM3	0.0	1.2
PARK2	0.0	1.2
PDGFRA	0.0	1.2
SDHA	0.0	1.2
TBX3	0.0	1.2
TSHR	0.0	1.2
XPO1	0.0	1.2
ABL1	0.0	0.6
ABL2	0.0	0.6
ARAF	0.0	0.6
BCL6	0.0	0.6
BRD4	0.0	0.6
CARD11	0.0	0.6
CD79A	0.0	0.6
CDC73	0.0	0.6
CDKN1A	0.0	0.6
CEBPA	0.0	0.6
CHEK1	0.0	0.6
CTNNA1	0.0	0.6
EPHA5	0.0	0.6
ETV5	0.0	0.6
FANCG	0.0	0.6
FOXL2	0.0	0.6
GATA3	0.0	0.6
GNAQ	0.0	0.6
H3F3A	0.0	0.6
HNF1A	0.0	0.6

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TABLE A2. Comprehensive Genomic Profiling Identified 1,007 GAs in Chinese Patients Compared With US Patients (Continued)

Gene	US Patients (n = 283) %	Chinese Patients (n = 164) %
INPP4B	0.0	0.6
IRF2	0.0	0.6
IRF4	0.0	0.6
IRS2	0.0	0.6
JAK3	0.0	0.6
KDM5C	0.0	0.6
MAGI2	0.0	0.6
MITF	0.0	0.6
MYB	0.0	0.6
NOTCH1	0.0	0.6
PDK1	0.0	0.6
PMS2	0.0	0.6
PPP2R1A	0.0	0.6
PRKAR1A	0.0	0.6
RAD51	0.0	0.6
RANBP2	0.0	0.6
RARA	0.0	0.6
RUNX1	0.0	0.6
SDHC	0.0	0.6
SMAD2	0.0	0.6
SMAD3	0.0	0.6
SMO	0.0	0.6
SNCAIP	0.0	0.6
SOX10	0.0	0.6
SPOP	0.0	0.6
SUFU	0.0	0.6
SYK	0.0	0.6
ZNF217	0.7	0.6
U2AF1	0.7	0.6
PTPN11	0.7	0.6
PALB2	0.7	0.6
FGF6	0.7	0.6
CDK4	0.7	0.6
TSC2	0.7	0.0
STAT3	0.7	0.0
PAX5	0.7	0.0
KEL	0.7	0.0
KDM6A	0.7	0.0
CUX1	0.7	0.0
SETD2	0.7	1.2
MAP3K1	0.7	1.2
HGF	0.7	1.2

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TABLE A2. Comprehensive Genomic Profiling Identified 1,007 GAs in Chinese Patients Compared With US Patients (Continued)

Gene	US Patients (n = 283) %	Chinese Patients (n = 164) %
GLI1	0.7	1.2
GATA1	0.7	1.2
FOXP1	0.7	1.2
CUL3	0.7	1.2
CCND2	0.7	1.2
CDKN2A/B	30.0	15.2
TP53	20.8	41.5
IDH1/2	23.3	13.4
ARID1A	19.8	18.3
BAP1	17.3	9.8
FGFR1/2/3/4	17.7	12.8
KRAS	15.5	28.7
PBRM1	11.3	11.6
PIK3CA	9.2	9.1
BRAF	7.1	4.3
MCL1	6.4	4.9
ATM	6.4	4.9
TERT	5.7	13.4
SMAD4	5.3	10.4
PTEN	4.9	1.8
NRAS	4.9	1.2
MDM2	4.6	3.0
MYC	3.2	1.2
CCND1	2.8	4.3
CDK6	2.8	3.0
FRS2	2.5	2.4
ERBB2	2.5	1.8
EGFR	2.5	1.8
NF1	2.1	6.1
MET	2.1	5.5
FBXW7	2.1	5.5
APC	2.1	4.3
MYST3	2.1	1.8
SMARCA4	2.1	1.2
CCNE1	2.1	0.6
MDM4	2.1	0.0
RB1	1.8	5.5
FGF4	1.8	3.7
FGF19	1.8	3.7
EPHA3	1.8	3.7
TET2	1.8	1.2
AKT2	1.8	0.6

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TABLE A2. Comprehensive Genomic Profiling Identified 1,007 GAs in Chinese Patients Compared With US Patients (Continued)

Gene	US Patients (n = 283) %	Chinese Patients (n = 164) %
NFKBIA	1.8	0.0
SPTA1	1.4	5.5
KMT2C	1.4	5.5
RNF43	1.4	4.3
SF3B1	1.4	3.7
FGF3	1.4	3.7
CCND3	1.4	2.4
TSC1	1.4	1.8
PTCH1	1.4	1.8
PREX2	1.4	1.8
MSH6	1.4	1.8
MLH1	1.4	1.8
MAP2K1	1.4	1.8
PIK3R1	1.4	1.2
PIK3C2B	1.4	0.6
NF2	1.4	0.6
MUTYH	1.4	0.6
MLL2	1.4	0.0
RBM10	1.1	6.1
ARID2	1.1	4.3
STK11	1.1	3.0
EPHA7	1.1	3.0
BRCA1	1.1	3.0
CREBBP	1.1	2.4
RET	1.1	1.8
ERBB3	1.1	1.8
CDH1	1.1	1.8
ASXL1	1.1	1.8
ZNF703	1.1	1.2
KDM5A	1.1	1.2
CTNNB1	1.1	1.2
IKBKE	1.1	0.6
DNMT3A	1.1	0.6
WT1	1.1	0.0
RICTOR	1.1	0.0
ARFRP1	1.1	0.0
AKT3	1.1	0.0
BRCA2	0.7	5.5
LRP1B	0.7	4.3
MAP2K4	0.7	3.7
KEAP1	0.7	3.0
GRIN2A	0.7	3.0
FAT1	0.7	3.0
NTRK1	0.7	2.4
GNAS	0.7	2.4
ERRFI1	0.7	1.8
ARID1B	0.7	1.8
EP300	0.4	4.3
SPEN	0.4	2.4
FLT4	0.4	2.4
ATRX	0.4	2.4
POLE	0.4	1.8
NOTCH3	0.4	1.8
NFE2L2	0.4	1.8
KDR	0.4	1.8
GATA6	0.4	1.8
FANCA	0.4	1.8
BARD1	0.4	1.8
TGFBR2	0.0	6.1
DDR2	0.0	4.9
KMT2D	0.0	4.3
RAD50	0.0	3.7
AMER1	0.0	3.0
CDKN2B	0.0	3.0
INHBA	0.0	2.4
TOP2A	0.0	2.4
AR	0.0	1.8
CHD4	0.0	1.8
FANCL	0.0	1.8
FLT3	0.0	1.8
KIT	0.0	1.8
KMT2A	0.0	1.8
MSH2	0.0	1.8
NOTCH2	0.0	1.8
PDGFRB	0.0	1.8
PIK3CG	0.0	1.8
ROS1	0.0	1.8
VEGFA	0.0	1.8

Abbreviations: GAs, genetic aberrations; PD-L1, programmed death-ligand 1; PD-L2, programmed death-ligand 2.