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LRP1B mutation associates with increased tumor mutation burden and inferior prognosis in liver hepatocellular carcinoma

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Abstract

Background: Liver hepatocellular carcinoma (LIHC) is the most common primary liver cancer and the main cause of death in patients with cirrhosis. LRP1B is found to involve in a variety of cancers, but the association of LRP1B mutation with tumor mutation burden (TMB) and prognosis of LIHC is rarely studied.

Methods and Results: Herein, we analyzed the somatic mutation data of 364 LIHC patients from The Cancer Genome Atlas (TCGA) and found that LRP1B showed elevated mutation rate. Calculation of the TMB in LRP1B mutant and LRP1B wild-type groups showed that LRP1B mutant group had higher TMB compared with that in LRP1B wild-type group. Then survival analysis was performed and the survival curve showed that LRP1B mutation was associated with poor survival outcome, and this association remained to be significant after adjusting for multiple confounding factors including age, gender, tumor stage, mutations of BRCA1, BRCA2, and POLE.

Conclusion: Collectively, our results revealed that LRP1B mutation was related to high TMB value and poor prognosis in LIHC, indicating that LRP1B mutation is probably helpful for the selection of immunotherapy and prognosis prediction in LIHC.

Abbreviations: LIHC = liver hepatocellular carcinoma, LRP1B = lipoprotein receptor-related protein 1B, SMG = significantly mutated genes, SNP = single nucleotide polymorphism, SNV = single nucleotide variant, TMB = tumor mutation burden, TCGA = The Cancer Genome Atlas.

Keywords: liver hepatocellular carcinoma, LRP1B mutation, prognosis, tumor mutation burden

1. Introduction

As the most common primary liver cancer, liver hepatocellular carcinoma (LIHC) is the main cause of death in patients with cirrhosis.[1] Annually, approximately 1 million people are diagnosed with LIHC, resulting in more than 690,000 death worldwide.[2] The risk factors of LIHC include age, smoking, alcohol consumption, hepatitis B virus, hepatitis C virus, and genetic factors, etc.[3,4] Currently, the candidate therapies for LIHC mainly consist of chemotherapy, interventional radiology, and surgery, which have achieved important advances.[5,6] However, considering the fact that a large proportion of LIHC patients are diagnosed at advanced stage, it is believed that exploration of effective biomarkers for prediction of LIHC prognosis may confer an improved clinical outcome.[7]

As mentioned above, genetic factors are implicated in LIHC, which has been confirmed by previous studies. Compared with healthy controls, the LIHC patients exhibited elevated plasma microRNA-21 level, which was obviously decreased after surgery.[8] Lu et al identified 5 microRNAs, including hsa-mir-3677, hsa-mir-326, hsa-mir-511-2, hsa-mir-424, and hsa-mir-421, as important signatures for the diagnosis and prognosis of LIHC through analyzing the data of LIHC patients.[9] Xiao et al established a 4-gene-signature consisting of CBX2, PBK, CPEB3, and CLSPN, to effectively predict the survival outcome of LIHC patients.[10] Low density lipoprotein receptor-related protein 1B (LRP1B) is a member of low-density lipoprotein family, and frequently involves in a variety of cancers. However, to our knowledge, few studies have focused on the role of LRP1B mutation in LIHC.

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Availability of data and materials: The datasets analyzed during the current study are available in the TCGA repository [https://portal.gdc.cancer.gov/].

Ethics approval and consent to participate: Not applicable (this paper was provided based on researching in global databases and it did not involve any human or animal subjects).

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Highlight

1. LRP1B mutation rate was relatively high in liver hepatocellular carcinoma (LIHC) samples.
2. LRP1B mutation was associated with high tumor mutation burden (TMB) and inferior prognosis of LIHC patients.
3. LRP1B mutation may be helpful for immunotherapy selection and prognosis prediction in LIHC patients.

Due to the immunogenicity of LIHC, immunotherapy has been emerging as a promising choice for LIHC treatment.[11] Tumor mutation burden (TMB) is related to neoantigen amount and plays a critical role in immunotherapy. It was reported that patients with high TMB were more sensitive to immune checkpoint inhibitor therapy, due to higher inherent immunogenicity.[12] In addition, accumulating evidence has suggested that TMB is associated with gene mutation in various cancers.[13] But to our knowledge, the relationship of LRP1B mutation with TMB in LIHC is rarely studied.

Herein, the somatic mutation data of LIHC were analyzed to explore the relationship between the mutation of LRP1B and TMB. In an attempt to elucidate the association between LRP1B mutation and LIHC prognosis, we conducted the survival and COX regression analyses. Our research may shed light on the effect analysis of LRP1B mutation on prognosis prediction, as well as selection of immunotherapy in LIHC.

2. Methods

2.1. Data source

We downloaded the somatic mutation data of this study (maf file) from The Cancer Genome Atlas (TCGA, www.cancergenome.nih.gov), which included 364 LIHC patients, with 358 patients having complete record of survival information. The clinical information of patients was included in Table 1.

Table 1

Clinicopathological characteristics of LIHC samples from TCGA database.

	OS status			
Parameters	**Alive (N = 233)**	**Dead (N = 131)**	**x^2**	***P*-value**
Age (mean ± SD)	58.02 ± 13.69	61.79 ± 13.68	0.11863	0.7305
Gender			3.2286	0.07236
Female	67	51		
Male	166	80		
Pathologic stage			23.022	3.996e-05
i	126	43		
ii	58	26		
iii	38	45		
iv	1	3		
Unknown	10	14		
Race			9.8764	0.05256
Asian	115	44		
White	101	77		
Black or African American	10	7		
American Indian or Alaska	2	0		
Unknown	5	3		

2.2. Mutation signature extraction

The SignatureAnalyzer software determines mutation signature based on Bayesian nonnegative matrix factorization method.[14] Here we used the SignatureAnalyzer software to extract the mutation signature from LIHC maf files.

2.3 TMB calculation

TMB represented the number of mutations per megabase in genome. The mutation number for each LIHC patient was calculated based on the maf files. The TMB was indicated as proportion of mutation number to exon length (30 M).

2.4. TMB distribution in LRP1B mutant and LRP1B wild-type samples

The LIHC samples were assigned into 2 groups, including LRP1B mutant and LRP1B wild-type groups. Two-sided *t* test was adopted to analyze the TMB difference between the 2 groups, and $P < 0.05$ was considered statistically significant. We investigated the effects of several confounding factors on TMB using Wilcoxon rank-sum test, with $P < 0.05$ as threshold.

2.5. Survival analysis

Survival analyses were performed by using survival and survminer packages in *R* software. The survival curve was plotted to investigate the influence of LRP1B mutation on survival outcome using survminer package. In order to further investigate the effects of several concurrent factors, including age, gender, and tumor stage on survival, the Cox regression model was established using survival package. Since the mutations of POLE, BRCA1, and BRCA2 could influence the damage and repair function of genome, thus affecting the mutations of other genes,[13] we included the mutations of POLE, BRCA1, and BRCA2 into the concurrent factors here.

2.6. Significantly mutated genes (SMG) analysis

The SMG was analyzed by using MutSigCV algorithm.[15] It is known that the mutations in tumors are classified into 2 types: driver mutation and passenger mutation. As driver mutation could confer a selective growth advantage to cells,[16] it is beneficial for the pathology research and treatment of tumors to screen driver mutation.

3. Results

3.1. Mutation spectrum of LIHC samples

An analysis of the maf files using maftools package in *R* software found that missense mutation was the main variant type in LIHC samples, single nucleotide polymorphism (SNP) accounted for a large proportion in the variant type and C > T was the major single nucleotide variant (SNV) type (Figure S1, Supplemental Digital Content, http://links.lww.com/MD/G807). The mutation rates of multiple genes were calculated, including the top 25 mutated genes (Fig. 1A), genes in LRP1B family (LRP1, LRP8, LRP6, LRP5L, LRP2, LRP4, LRP3, LRP10, LRP12, and LRP5) and genes, which probably influences the mutation rates of other genes (POLE, MLH3, BRCA1, and BRCA2) (Fig. 1B). As shown in Figure 1B, LRP1B presented high mutation rate among LRP1B family. Besides, the LIHC samples with LRP1B, BRCA1, BRCA2, MLH3, or POLE mutations showed high TMB values, suggesting that these genes were related to genomic stability.

3.2. Association of LRP1B mutation with TMB in LIHC

According to LRP1B mutation status, the LIHC samples were divided into LRP1B mutant and LRP1B wild-type groups, then their TMB was calculated. It was found that the TMB in LRP1B mutant group was higher than that in LRP1B wild-type group (P = .0045, Fig. 2A).

Then the effects of multiple confounding factors on TMB were investigated, including age, gender, tumor stage, and mutations of BRCA1, BRCA2, POLE, and LRP1B. As shown in Figure 2B, the TMB in LRP1B wild-type group was lower than that in LRP1B mutant group (odd ratio = 0.78).

3.3. Association of LRP1B mutation with prognosis of LIHC

We plotted the survival curves for LRP1B mutant and LRP1B wild-type samples. As shown in Figure 3A, compared with the LRP1B wild-type group, the LRP1B mutant group exhibited poor survival outcome (P = .00073). After controlling for the concurrent factors, including age, gender, tumor stage, mutations of POLE, BRCA1, and BRCA2, the LRP1B mutant group still showed worse survival outcome than the LRP1B wild-type group (P < 0.001, hazard ratio = 0.40, Fig. 3B).

3.4. Signatures 5 accounts for high percentage in LIHC samples

We extracted 4 mutation signatures from LIHC samples by using SignatureAnalyzer, which were named as W1, W2, W3, and W4 (Fig. 4A). After comparison of the 4 extracted mutation signatures with the mutation signatures in COSMIC database (https://cancer.sanger.ac.uk/cosmic/), we found that high similarities existed between W1 and Signature6, W2 and Signature5, W3 and Signature22, W4 and Signature25, respectively (Fig. 4B). Signature22 was observed in urothelial cancer and liver cancer, and was closely related to aristolochic acid. Signature6 was associated with DNA damage repair, which has been observed in 17 cancers. The detailed mechanism of Signature5 and Signature25 still remained unclear.

Then we calculated the proportion of each signature in LIHC samples and found that Signature5 showed high percentage in both individual and whole LIHC samples (Fig. 5).

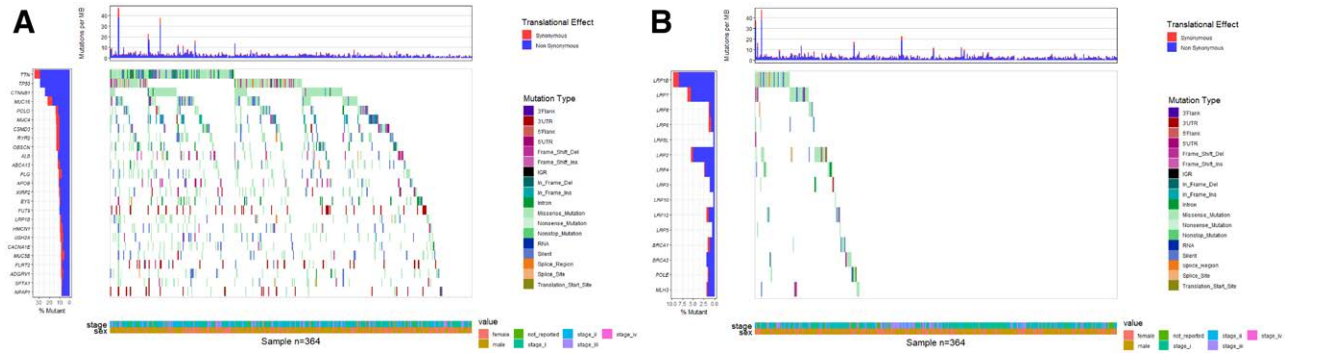

Figure 1. Mutation landscape of LIHC samples from TCGA. Mutations per megabase, mutation types, mutation rates across each LIHC sample and distribution of tumor stage by patient sex for the top 25 mutated genes (A), genes in LRP1B family (LRP1, LRP8, LRP6, LRP5L, LRP2, LRP4, LRP3, LRP10, LRP12, and LRP5) and genes that might influence the mutation rates of other genes (POLE, MLH3, BRCA1, and BRCA2) (B).

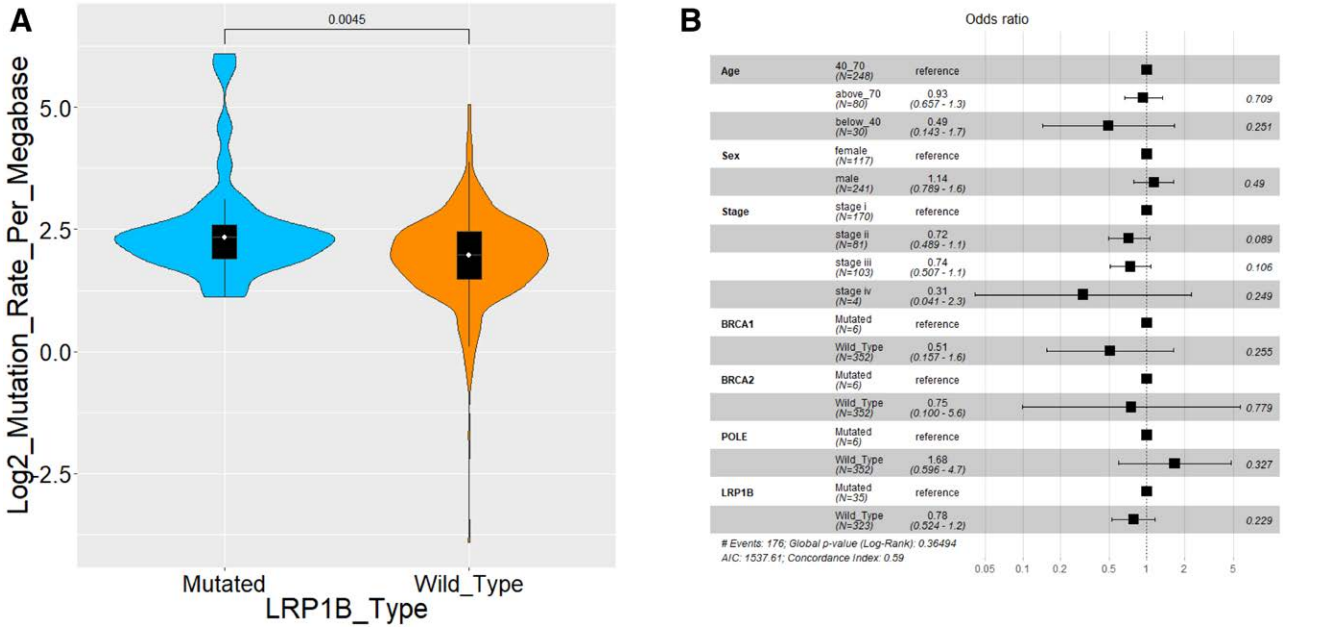

Figure 2. An association between LRP1B mutation and TMB is observed in LIHC patients. (A) TMB in LRP1B mutant and LRP1B wild-type groups. (B) The relationship of LRP1B mutation with TMB after adjustment of several factors, including age, gender, tumor stage, and mutations of POLE, BRCA1, and BRCA2.

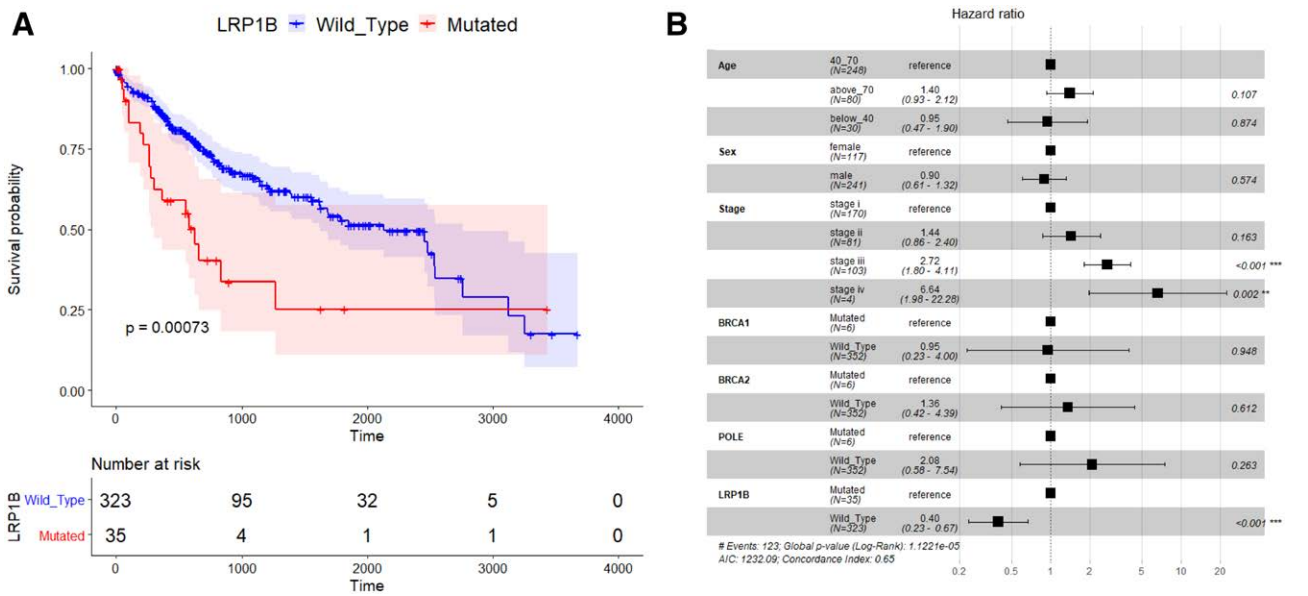

Figure 3. The mutation of LRP1B is related to poor survival outcome of LIHC. (A) Survival curve of LRP1B mutant samples and LRP1B wild-type samples. (B) The relationship of LRP1B mutation with survival outcome after controlling for multiple factors, including gender, age, tumor stage, and POLE, BRCA1, BRCA2 mutations.

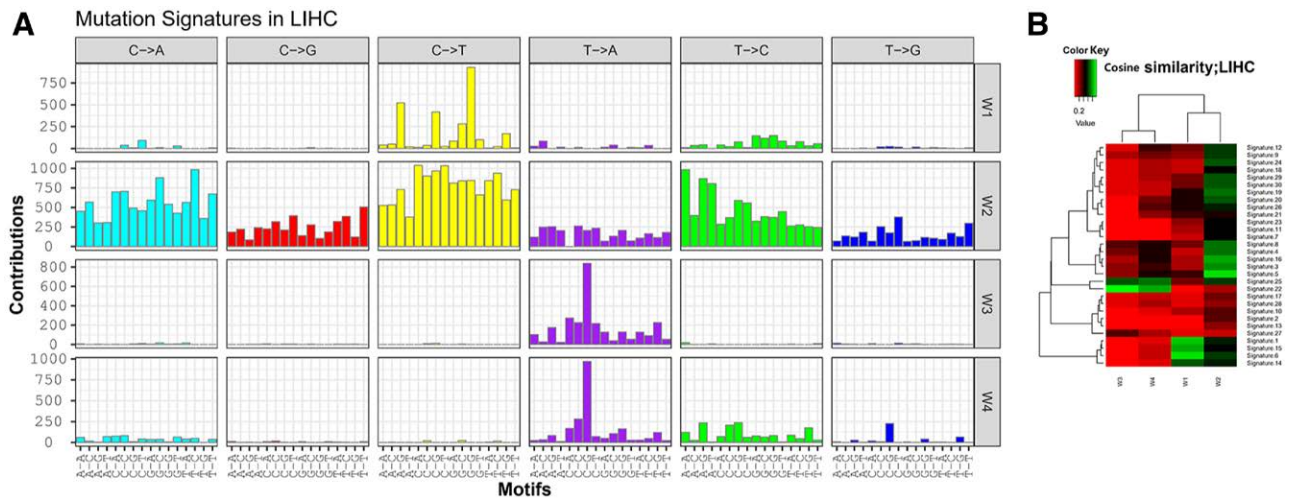

Figure 4. There are high similarities between W1–W4 that are extracted from LIHC samples with Signature6, Signature5, Signature22, Signature25 derived from COSMIC database. (A) 4 mutation signatures, including W1–W4 that were extracted from LIHC samples. (B) The similarities between extracted signatures from LIHC samples and signatures were derived from COSMIC database.

3.5. TP53, RB1, and CTNNB1 are potential mutation-driver genes in LIHC

The LIHC samples were assigned into LRP1B mutant and LRP1B wild-type groups, then MutSigCV algorithm was used for the analysis of SMGs in the 2 groups. We selected the top 10 of SMGs for investigation of their mutations in LRP1B mutant (Fig. 6A) and LRP1B wild-type groups (Fig. 6B) (detailed results were shown in Table S1, Supplemental Digital Content, http://links.lww.com/MD/G808). It was found that TP53, RB1, and CTNNB1 appeared in these 2 groups and showed high mutation rates in LRP1B mutant samples, indicating that they were potential mutation-driver genes in LIHC (Fig. 7).

4. Discussion

LIHC is one of the common malignancies and the third leading cause for cancer death worldwide.[17] Due to the lack of early diagnosis and treatment, LIHC has become a main health burden.[18] In our study, after analyzing the somatic mutation data of 364 LIHC patients from TCGA database, we found that LRP1B had a relatively high mutation rate in LIHC samples. Moreover, LRP1B mutation was obviously associated with high TMB and poor prognosis of LIHC patients, and the association remained significant after adjusting for several confounding factors such as age, gender, tumor stage, mutations of BRCA1, BRCA2, and POLE.

LRP1B belongs to low density lipoprotein receptor gene family, and encodes a 600 kDa-protein, which is expressed in multiple tissues such as thyroid, brain and salivary gland.[19] Previous studies have proved that LRP1B was frequently involved in various cancers. Inactivation of LRP1B has been found in several cancers such as esophageal carcinoma, glioblastoma, gastric cancer, melanoma, oral squamous cell carcinoma, and urothelial cancer, etc[20] Except for the inactivation of LRP1B, LRP1B mutation was also reported to be associated with cancers. For

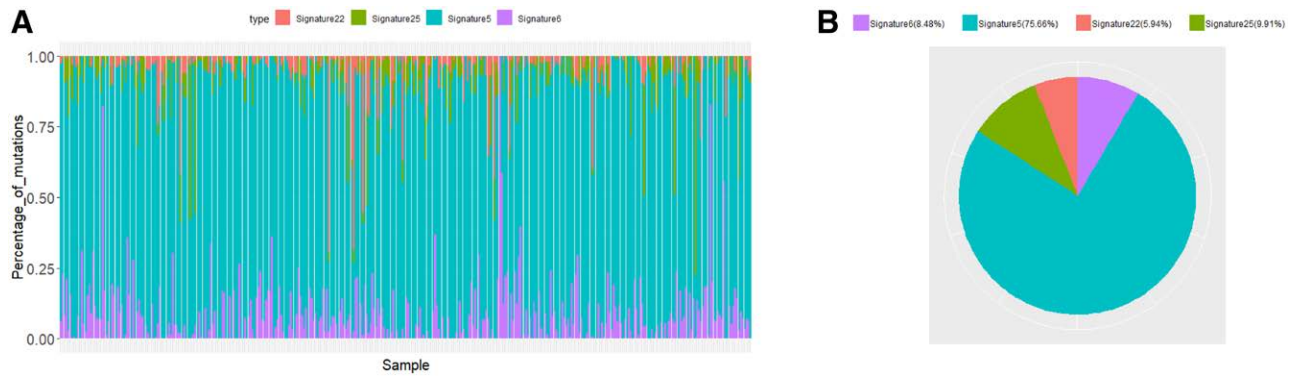

Figure 5. Signature 5 accounts for a high proportion in LIHC samples. (A) The proportions of Signature5, Signature6, Signature22, and Signature25 in individual LIHC sample. (B) The proportions of Signature5, Signature6, Signature22, and Signature25 in overall LIHC samples.

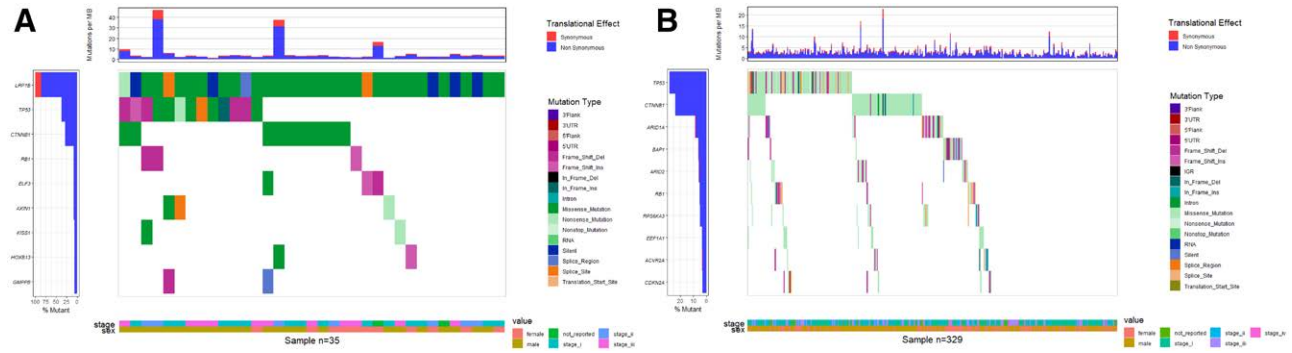

Figure 6. SMGs TP53, RB1, and CTNNB1 are observed in both LRP1B mutant and LRP1B wild-type groups. (A) Top 10 SMGs in LRP1B mutant group. (B) Top 10 SMGs in LRP1B wild-type group.

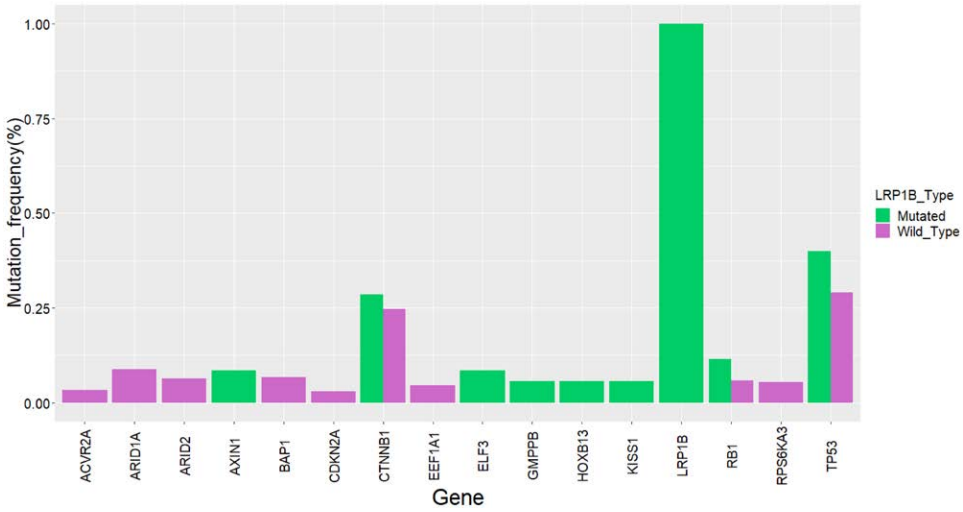

Figure 7. SMGs TP53, RB1, and CTNNB1 show high mutation rates in LRP1B mutant group.

example, Langbein et al found that hemi- and homozygous deletion of LRP1B was observed in 27% of the urothelial cancer cases, and 49% of the G3 urothelial cancer cases presented allelic loss within LRP1B gene region, which demonstrated that the LRP1B mutation was related to high grade of urothelial cancer.[21] Xiao et al reported high prevalence of LRP1B mutation in lung adenocarcinoma patients accompanied by chronic Obstruction Pulmonary Disease.[22] But to the best of our knowledge, LRP1B mutation has seldom been studied in LIHC yet. Cheng et al have recently demonstrated that the potential correlation between LRP1B mutation and hepatocellular carcinoma (HCC) patients' poor clinical response to immune checkpoint inhibitor treatment,[23] whereas our study have firstly revealed the correlation between LRP1B mutation and the prognosis of LIHC patients.

The LIHC samples were divided into mutant and wild-type LRP1B groups, and survival analysis revealed that the LRP1B mutant group had inferior survival outcome compared with

the LRP1B wild-type group, which was independent of multiple confounding factors, including age, gender, tumor stage, and mutations of BRCA1, BRCA2, and POLE. The dysfunction of LRP1B was previously reported to affect the clinical outcome of glioblastoma patients.[24] Moreover, LRP1B was a putative tumor suppressor and regulated tumor cell growth.[25] Down-regulation of LRP1B was observed in colon cancer, promoting the growth, migration and metastasis of colon cancer cells.[26] LRP1B expression level was decreased in renal cell cancer (RCC), and LRP1B silence enhanced the growth, migration and invasion of RCC cells.[25] Therefore, we speculated that LRP1B mutation might exert negative impacts the prognosis of LIHC patients by regulating the growth of tumor cells.

In addition, we found that compared with the LRP1B wild-type group, the TMB value in the LRP1B mutant group was significantly increased. TMB plays a key role in the prediction of response to immunotherapy,[27] and cancer patients with high TMB have superior efficacy of immunotherapy and clinical outcome.[28] Nevertheless, more details between TMB and the prognosis of LIHC patients still remain unclear. In this work, LRP1B mutation was observed to relate to both TMB and poor prognosis of LIHC, while the interactions between the 3 deserve more investigation. Besides, TP53, RB1, and CTNNB1 exhibited higher mutation rates in LRP1B mutant LIHC samples, whether they partly played driver roles on LRP1B mutant and high TMB in LIHC patients might not be concluded basing on our present results. However, the association between LRP1B mutation and TMB we identified might give more reference information for the immunotherapy selection and clinical management of LIHC. Finally, there are still several limitations in this work. For instance, our present sample size was limited by the public data we used; expanded clinical samples are conducive to improve the reliability of our results.

5. Conclusions

In summary, our research showed that LRP1B had a relatively high mutation rate in LIHC samples, which was related to high TMB and inferior prognosis in LIHC patients. Our study may be significant for the immunotherapy guidance and prognosis prediction of LIHC.

Author contributions

GY and YLC put forward the ideas of this article, wrote this article, and analyzed the data. All authors involved in acquisition of data and analysis and interpretation of data. HM, FF, HY Z, HK L, QW, and QQX revised the manuscript. All authors read and approved the final manuscript.

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