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TERT-TP53 mutations: a novel biomarker pair for hepatocellular carcinoma recurrence and prognosis

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Hepatocellular carcinoma (HCC) is the most prevalent form of liver cancer, and ranks among the most lethal malignancies globally, primarily due to its high rates of recurrence and metastasis. Despite the urgency, no reliable biomarkers currently exist for predicting tumor recurrence in HCC. Telomerase reverse transcriptase (*TERT*) promoter mutations (*TERTpm*) and cellular tumor antigen p53 mutations (*TP53m*) have been frequently documented in HCC, but their combined clinical significance remains undefined. In this study, we investigated the clinical implications of *TERTpm*, *TP53m*, and their co-occurrence in 50 HCC tissue samples using the next-generation sequencing (NGS) technology. We identified *TERTpm* (C228T) and *TP53m* in 16 (32%) and 24 (48%) samples, respectively. Our findings indicate that these mutations are more prevalent in male patients (100% for *TERTpm*, 83.33% for *TP53m*), in those with solitary tumors (87.5% for both), in individuals with G2-G3 hepatitis (100% / 83.3%), and in cases of moderately differentiated tumors (75.0% / 83.3%). Furthermore, patients with both *TERTpm* and *TP53m* exhibited a significantly higher risk of tumor relapse ($P < 0.05$) and shorter progression-free survival ($P < 0.05$). Collectively, our results suggest that presence of both *TERTpm* and *TP53m* may serve as a robust predictor of tumor recurrence and a marker of poor prognosis in HCC.

Keywords HCC, TERT promoter mutation, TP53 mutation, Recurrence, Prognosis

Liver cancer stands as a global health burden, ranking among the most prevalent and lethal malignancies worldwide. According to GLOBOCAN, it holds the third position in mortality and sixth in incidence, highlighting its devastating impact on public health[1,2], and hepatocellular carcinoma (HCC) constituting the majority of cases[3]. This grim reality is exacerbated in China, where HCC notably occupies the second and fourth positions in terms of mortality and incidence rates, respectively[1,2,4]. Despite advancements in therapeutic strategies, HCC, the primary form of liver cancer, continues to pose significant challenges to healthcare systems.

Hepatic resection, considered the gold standard for HCC treatment, is only applicable to approximately 20% of patients. Unfortunately, an astonishing 85% of patients experience intrahepatic recurrence or metastasis within five years post-surgery, contributing to the poor prognosis and high mortality rates among HCC patients[5,6]. Current methods for predicting and managing recurrence remain suboptimal, with regular imaging and serum alpha-fetoprotein (AFP) tests falling short in detecting early-stage recurrence, thereby underscoring the urgent need for novel predictive biomarkers[7–9].

It's well known that tumorigenesis is a complex process involving the accumulation of multiple genetic alterations[10,11]. However, the precise molecular mechanisms underlying the initiation and progression of HCC remain unclear. Recent years, remarkable improvements have been made in genomic studies of HCC, and a variety of mutated genes have been identified[12]. Among all, the tumor antigen p53 mutations (*TP53m*) and telomerase reverse transcriptase promoter mutations (*TERTpm*) are detected the most frequent genomic alterations, affecting 18.7%~48% and 14.9%~60% of all HCC patients, respectively[7,13,14].

The *TP53* gene is located in 17p13 and encode the cellular tumor antigen p53, a critical tumor suppressor protein containing transcriptional activation, DNA binding, and oligomerization domains[15]. p53 can prevent tumorigenesis by inducing cell-cycle arrest, apoptosis, cellular senescence, DNA repairing, and metabolism changes, etc[15,16]. Mutations in *TP53* are linked to over 50% of human cancers, including lung, liver, and breast

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cancers[17,18]. Conversely, the *TERT* gene is located in 5p15 and encode the telomerase reverse transcriptase, a rate-limiting and catalytic component of telomerase, which is an essential enzyme to extends telomeres, and plays an important role in the process of tumorigenesis, including cell growth, proliferation, invasion, metastasis, and angiogenesis[8,19]. *TERT* is typically suppressed in most normal somatic cells but is frequently activated in the majority of cancers, facilitating the immortal and indefinite growth of cancer cells[19]. *TERTpm* reported in many types of cancers are likely to introduce new binding sites for transcription factors such as ETS/TCF, which are associated with increased activity and expression of TERT[20–22].

Specific mutations in *TERT*, such as C228T (-124 bp) and C250T (-146 bp), are recognized as early genetic events in hepatocarcinogenesis, while TP53 mutations are associated with later stages of HCC progression[23,24]. Based on literature, these mutations not only play a pivotal role in HCC carcinogenesis and metastasis but also hold potential as prognostic indicators for HCC.

Despite the progress, the critical genes influencing HCC recurrence and progression are yet to be fully deciphered. Unraveling these genes could deepen our understanding of the molecular underpinnings of early recurrence in HCC and its associated poor outcomes, potentially leading to the discovery of new therapeutic targets.

This study aims to explore the clinical relevance of TERT promoter mutations, TP53 mutations, and their co-occurrence in HCC. Utilizing the next-generation sequencing technology, we conducted a mutational analysis to assess the prevalence and frequency of these genetic alterations. Additionally, we characterized the clinical and laboratory features of the patients and discussed their prognostic implications. Among this study, we found that the co-existence of *TERTpm* and *TP53m* may serve as a potent indicator of tumor recurrence and a harbinger of poor prognosis in HCC.

Materials and methods

Patients, tissue samples, and clinicopathologic data

From June 2019 to October 2020, a total of 50 HCC patients whom were reported and diagnosed as HCC for the first time at the West China Hospital of Sichuan University were randomly selected. Fresh tumor tissues (≥ 60 mg) were obtained by surgical resection, and paired clinicopathologic information were collected. The laboratory and clinical parameters of these patients were described in Supplementary Table 1. Histopathological diagnoses were determined by at least two experienced pathologists. Informed consent was obtained from all subjects under protocols approved by Ethical Committee of the hospital. All patients provided written contents according to ethical regulations.

All patients after resection were followed up regularly from 2019 to January 28, 2024. The mean follow-up time was 49.2 ± 4.8 month (median, 51.0 months; range, 39.0–55.0 months). Laboratory, clinical, pathological, and radiographic parameters were evaluated to diagnose postoperative recurrence (including tumor marker levels, clinical symptoms, MRI, CT, PET-CT, MRI, and histopathological results). The internal between surgery and local recurrence, or distal metastasis, or death, was defined as progression-free survival (PFS).

DNA extraction

Genomic DNA was extracted from the fresh HCC tumor tissues of individual patients with the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the standard procedures. In brief, tissue samples (less than 10 mg) were digested overnight at 56 °C with protein K and Buffer ALT. After extraction with Buffer AL and reprecipitation in ethanol (97%), nucleic acids were then rinsed with Buffer AW1 and Buffer AW2. After that, DNA was solubilized in Buffer AE and stored at -20 °C. The concentration and purity of extracted DNA were determined using Qubit 3.0 Fluorometer (Life Invitrogen, Villebon, France). After that, DNA degradation and contamination were detected with agarose gel electrophoresis.

Next-generation sequencing (NGS)

As previously described, genomic DNA derived from tissues after fragmentation and purification was used to construct DNA library[25]. The human 1021 gene mutation detection kit (Gene+, Beijing, China) was used to capture the most common mutations in HCC, which including exons, introns, promoters, and fusion breakpoint regions of 1021 genes. Followed by hybridization and amplification, the Gene + Seq-2000 sequencer was applied to DNA sequencing.

After removing terminal adaptor sequences and low-quality data, remaining reads were mapped to the reference human genome (hg19) through the Burrows-Wheel Aligner (BWA) software. Then, duplicate reads were removed with Gene Analysis Toolkit. MuTect2 algorithm was utilized to call single-nucleotide variants (SNVs) and somatic insertions/deletions (InDel). GnomAD database was applied to filter germline mutations. Afterwards, mutated variant allele frequency (VAF) ≥ 0.01 was filtered.

Statistical analysis

Statistical analyses were performed by IBM SPSS Statistics 23.0 software and GraphPad Prism 9.1.1 software. Data were reported as frequencies (percentages), mean ± SD, or median (range).

Clinicopathological parameters of patients were compared using the Student's t-test, Pearson's chi-square (χ^2) test, or Fisher's exact test where appropriate. Recurrence odds ratios (ORs) and 95% confidence intervals (CIs) of patients were obtained via univariable and multivariable logistic regression. A two-tailed p value of < 0.05 was considered statistically significant.

Results

Patients' clinicopathological characteristics

Detailed characteristics of the enrolled 50 HCC patients are listed in (Supplement Table 1). The study cohort consisted of 41 women (82.0%) and 9 men (18.0%), with an average age at diagnosis of 55 (range, 24-87) years. All patients had chronic liver disease related to HBV infection, 27 patients had liver cirrhosis. Most of them (94.0%) had a single tumor, with a mean diameter of 5.80 ± 3.7 centimeters. Furthermore, 36 (72.0%) cases had moderately differentiated tumors, and 14 cases had poorly differentiated tumors. Besides, 17 (34.0%) cases were identified microvascular invasion (MVI) status from pathology reports.

Median duration of follow-up was 49 months (range, 39–55 months) after operation. Among these patients, 14 experienced local recurrence, 5 experienced distant metastasis, and 4 had both types of relapse during the follow-up period, with a median recurrence duration of 267 (range, 41-1131) days.

TERT promoter and *TP53* are frequently mutated in hepatocellular carcinoma

To better delineate the genomic landscape of HCC, 50 tumor tissues obtained from paired patients were sequenced with a 1021 gene panel, which covering 1021 genes frequently mutated in HCC and other solid tumors. Sequencing results revealed that the most recurrently mutated genes were *TP53* (48.0%, 24/50) and *TERT* (32.0%, 16/50), followed with *LRP1B* (26.0%, 13/50), *CTNNB1* (18.0%, 9/50) and *AXIN1* (14%, 7/50) (Fig. 1A), which were largely consistent with previous reports[24,26].

All *TERTpm* were found at the hotspot position of chr5, 1,295,228 G > A (C228T) (Fig. 1B; Table 1), while a total of 21 different *TP53m* were identified in 24 patients (Fig. 1C; Table 2). Among all *TP53m*, 17/24 were missense mutations, 3/24 were splice-site mutations, 2/24 were nonsense mutations, 1/24 was frameshift-deletion mutation, and 1/24 had coexistence of frameshift-deletion and missense mutations.

Association of *TERTpm* with *TP53m* in HCC

As summarized in Tables 3 and 16 cases harbored *TERTm*, and 24 cases harbored *TP53m*. Out of them, 16 cases harbored *TP53m* only, 8 cases harbored *TERTm* only, 8 cases harbored both *TP53m* and *TERTm*, and none of the two mutation types were detected in the other 18 cases.

In total, *TERTm* C228T was found in 33.3% (8 of 24) of *TP53m*-positive HCC vs. 30.8% of (8 of 26) *TP53m*-negative HCC, showing a higher trend of *TERTm* C228T in the *TP53m*-positive HCC, but the association was not statistically significant. Accordingly, these data indicate that there was an undeniable association of *TERTm* C228T with the *TP53m* in HCC.

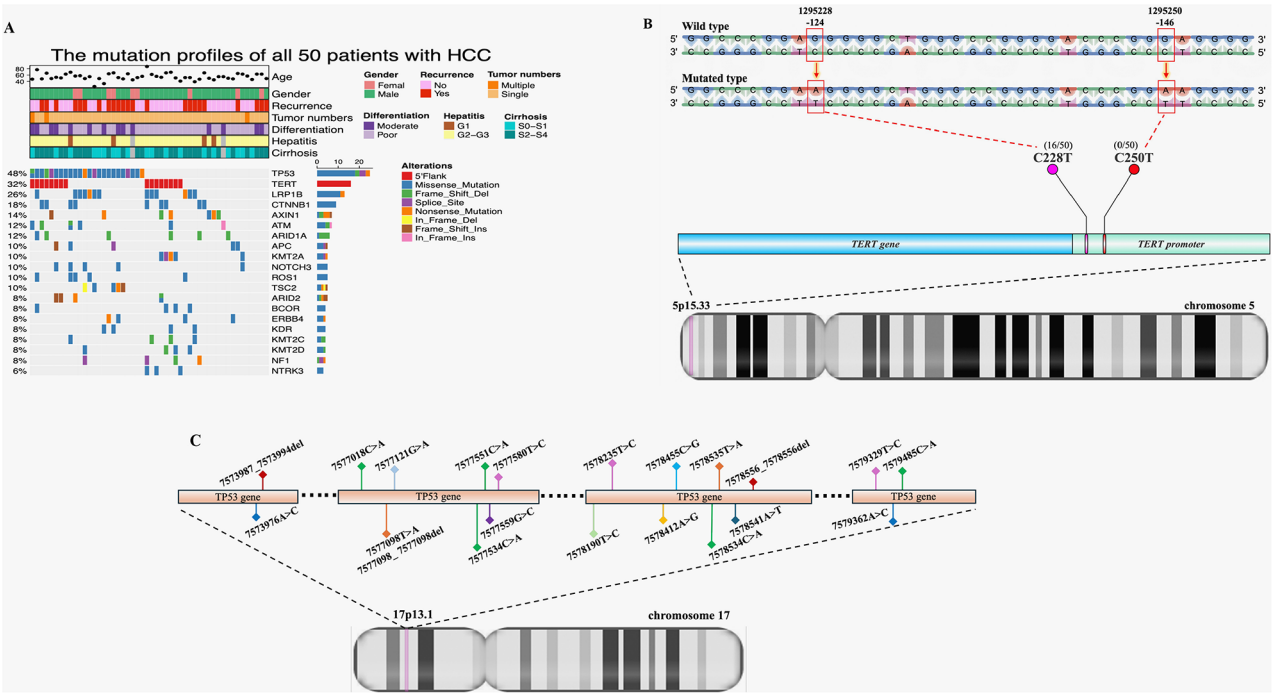

Fig. 1. Mutational profiles of studied cohort and the correlated mutation positions within *TERT* and *TP53*. (**A**) A heatmap illustrating the mutation frequencies of the top 20 genes, categorized by alteration type. Columns correspond to individual tumor samples, rows to specific genes, and color-coded boxes denote mutation types. Clinical data for each patient is displayed above the heatmap for reference. (**B**) Schematic presentation of TERT gene located on chromosome 5p, highlighting the positions of the C228T and C250T mutations within the promoter region. Figures in brackets indicate the number of mutated cases. (**C**) Schematic presentation of P53 gene located on chromosome 17p, highlighting the positions of the 21 distinct mutation types identified in this study.

Patient	Age	Gender	Start_Position	End_Position	DNA change of TERT	Variant classification	Variant type
1	M	62	1,295,228	1,295,228	G228A	5'Flank	SNP
3	M	87	1,295,228	1,295,228	G228A	5'Flank	SNP
4	M	63	1,295,228	1,295,228	G228A	5'Flank	SNP
8	M	56	1,295,228	1,295,228	G228A	5'Flank	SNP
12	M	66	1,295,228	1,295,228	G228A	5'Flank	SNP
15	M	47	1,295,228	1,295,228	G228A	5'Flank	SNP
17	M	53	1,295,228	1,295,228	G228A	5'Flank	SNP
20	M	67	1,295,228	1,295,228	G228A	5'Flank	SNP
21	M	53	1,295,228	1,295,228	G228A	5'Flank	SNP
26	M	51	1,295,228	1,295,228	G228A	5'Flank	SNP
32	M	51	1,295,228	1,295,228	G228A	5'Flank	SNP
37	M	77	1,295,228	1,295,228	G228A	5'Flank	SNP
38	M	69	1,295,228	1,295,228	G228A	5'Flank	SNP
40	M	49	1,295,228	1,295,228	G228A	5'Flank	SNP
45	M	41	1,295,228	1,295,228	G228A	5'Flank	SNP
49	M	48	1,295,228	1,295,228	G228A	5'Flank	SNP

Table 1. Mutation details of patients with *TERTpm*.

Patient	Age	Gender	Start_Position	End_Position	Variant classification	Variant type	Reference allele	Seq allele	dbSNP_RS
2	M	64	7,577,018	7,577,018	Splice_Site	SNP	C	A	
4	M	63	7,577,098	7,577,098	Missense_Mutation	SNP	T	A	
5	M	49	7,578,534	7,578,534	Missense_Mutation	SNP	C	A	rs866775781
7	M	24	7,577,018	7,577,018	Splice_Site	SNP	C	A	
9	F	64	7,577,121	7,577,121	Missense_Mutation	SNP	G	A	rs121913343
10	M	49	7,579,485	7,579,485	Nonsense_Mutation	SNP	C	A	rs869312782
11	M	52	7,579,329	7,579,329	Missense_Mutation	SNP	T	C	rs121912658
12	M	66	7,573,987	7,573,994	Frame_Shift_Del	DEL	GCCTCATT	-	novel
14	F	45	7,577,534	7,577,534	Missense_Mutation	SNP	C	A	rs28934571
15	M	47	7,577,098	7,577,098	Frame_Shift_Del	DEL	T	-	
			7,577,580	7,577,580	Missense_Mutation	SNP	T	C	rs587780073
			7,578,535	7,578,535	Missense_Mutation	SNP	T	A	rs1057519996
21	M	53	7,578,412	7,578,412	Missense_Mutation	SNP	A	G	
25	F	57	7,577,534	7,577,534	Missense_Mutation	SNP	C	A	rs28934571
29	F	58	7,578,455	7,578,455	Missense_Mutation	SNP	C	G	rs730882000
32	M	51	7,577,551	7,577,551	Missense_Mutation	SNP	C	A	
33	M	55	7,573,976	7,573,976	Missense_Mutation	SNP	T	C	rs141402957
34	F	67	7,577,534	7,577,534	Missense_Mutation	SNP	C	A	rs28934571
35	M	69	7,578,235	7,578,235	Missense_Mutation	SNP	T	C	
36	M	33	7,577,559	7,577,559	Missense_Mutation	SNP	G	C	rs28934573
37	M	77	7,579,362	7,579,362	Missense_Mutation	SNP	A	C	rs1057523496
39	F	52	7,578,190	7,578,190	Missense_Mutation	SNP	T	C	rs121912666
40	M	49	7,578,556	7,578,556	Splice_Site	DEL	T	-	
48	M	56	7,579,485	7,579,485	Nonsense_Mutation	SNP	C	A	rs869312782
49	M	48	7,578,541	7,578,541	Missense_Mutation	SNP	A	T	
50	M	48	7,577,534	7,577,534	Missense_Mutation	SNP	C	A	rs28934571

Table 2. Mutation details of patients with *TP53m*.

Association of *TERTpm* and *TP53m* with clinical-pathological parameters in HCC

We next evaluated the relationship of *TERTpm* and *TP53m* with the classical clinicopathological features of HCC, respectively. As shown in Table 4, *TERTpm* C228T and *TP53m* were more frequent in male patients (100% / 83.33%), in patients with single tumor (87.5% / 87.5%), in patients with G2-G3 hepatitis disease (100% / 83.3%), and in patients with well differentiated tumors (75.0% / 83.3%). Although, patients with *TERTpm* had significantly lower AFP level (≤ 20 ng/mL, $P < 0.05$) than negative ones, we did not find any association with other laboratory tests, which including total bilirubin (TBIL), LDH, AST, etc.

TERTpm+ ($n=16$)		TERTpm- ($n=34$)	
TP53m-	TP53m+	TP53m-	TP53m+
8/26 (30.8%)	8/24 (33.3%)	18/26 (69.2%)	16/24 (66.7%)
$P>0.999$		$P>0.999$	

Table 3. Association of *TERTpm* with *TP53m* in HCC~. +: positive, − negative.

Variables		Total ($n=50$)	TERTpm+ ($n=16$, 32%)	TERTpm- ($n=34$, 68%)	*P*-value	TP53m+ ($n=24$, 48%)	TP53m- ($n=26$,52%)	*P*-value
Age (years)		55 (24–87)	59 (41–87)	53 (24–69)	0.090	56 (24–87)	54 (33–77)	0.553
Gender: n (percentage)	F	9 (18.0%)	0	9 (26.5%)	**0.043**	4 (16.7%)	5 (19.2%)	>0.999
	M	41 (82.0%)	16 (100%)	25 (73.5%)		20 (83.3%)	21 (80.8%)	
Height (cm): mean (range)		165 (149–180)	164 (150–172)	165 (149–180)	0.815	166 (149–180)	163 (150–178)	0.089
Weight (kg)		64 (33–103)	67 (50–84)	63 (33–103)	0.235	66 (33–103)	63 (41–82)	0.881
Clinical-pathological data								
Tumor numbers	Single	47 (94.0%)	14 (87.5%)	33 (97.1%)	0.237	21 (87.5%)	26 (100%)	0.103
	Multiple	3 (6.0%)	2 (12.5%)	1 (2.9%)		3 (12.5%)	0	
Tumor diameter (cm): mean ± SD		5.80 ± 3.7	5.2 ± 2.1	6.1 ± 4.2	0.221	5.1 ± 3.3	6.4 ± 3.9	0.509
Differentiation	Poor	14 (28.0%)	4 (25.0%)	10 (29.4%)	0.746	4 (16.7%)	10 (41.7%)	0.119
	Moderate	36 (72.0%)	14 (75.0%)	24 (70.6%)		20 (83.3%)	16 (61.5%)	
WHO grade	II	38 (76%)	14 (87.5%)	24 (70.6%)	0.292	16 (66.7%)	22 (84.6%)	0.190
	III	12 (24%)	2 (22.5%)	10 (29.4%)		8 (33.3%)	4 (15.4%)	
Hepatitis	G1	7 (14.0%)	0	7 (20.6%)	0.081	4 (16.7%)	3 (11.5%)	0.697
	G2-G3	43 (86.0%)	16 (100%)	27 (79.4%)		20 (83.3%)	23 (88.5%)	
Cirrhosis	Yes	27 (54.0%)	8 (55.0%)	19 (55.9%)	0.767	14 (58.3%)	13 (50.0%)	0.584
	No	23 (46.0%)	8 (55.0%)	15 (44.1%)		10 (41.7%)	13 (50.0%)	
Macrovascular invasion	Yes	17 (34.0%)	5 (31.2%)	12 (35.3%)	>0.999	10 (41.7%)	7 (26.9%)	0.373
	No	33 (66.0%)	11 (68.8%)	22 (64.7%)		14 (58.3%)	19 (73.1%)	
Recurrence	Yes	23 (46.0%)	9 (56.3%)	14 (41.2%)	0.373	15 (62.5%)	8 (30.8%)	**0.046**
	No	27 (54.0%)	7 (43.7%)	20 (58.8%)		9 (37.5%)	18 (69.2%)	
PFS (days)		462 ± 395	479 ± 396	463 ± 395	0.172	462 ± 395	511 ± 402	0.806
Early recurrence (≤ 12 month)	Yes	15 (30%)	7 (43.8%)	8 (23.5%)	0.191	11 (45.8%)	4 (15.4%)	**0.030**
	No	35 (70%)	9 (56.2%)	26 (76.5%)		13 (54.2%)	22 (84.6%)	
Lab data								
AFP (ng/mL)	>20	28 (56.0%)	5 (31.2%)	23 (67.6%)	**0.031**	11 (45.8%)	17 (65.4%)	0.2542
	≤20	22 (44.0%)	11 (68.8%)	11 (32.4%)		13 (54.2%)	9 (34.6%)	
HBV-DNA	Positive	29 (58.0%)	7 (43.7%)	22 (64.7%)	0.2224	13 (54.2%)	16 (61.5%)	0.7749
	Negative	21 (42.0%)	9 (56.3%)	12 (35.3%)		11 (45.8%)	10 (41.7%)	
TBIL (μmol/L)		13.67 ± 6.9	13.70 ± 6.3	13.65 ± 7.2	0.497	13.22 ± 8.0	14.08 ± 5.8	0.557
ALB (g/L)		41.8 ± 4.5	42.7 ± 4.5	41.4 ± 4.5	0.715	41.2 ± 5.5	42.4 ± 3.3	0.457
AST (IU/L)		63 (16–477)	36 (18 − 16)	76 (16–477)	0.675	58 (16–477)	68 (19–385)	0.245
ALT (IU/L)		64 (12–663)	44 (12–26)	73 (13–663)	0.520	58 (12–369)	70 (13–663)	0.710
GLB (g/L)		27.4 ± 6.5	28.7 ± 5.8	26.8 ± 6.8	0.467	28.3 ± 5.4	26.7 ± 7.4	0.603
ALP (IU/L)		118 (49–451)	107 (55–76)	123 (49–451)	0.582	113 (49–451)	123 (58–405)	0.457
GGT (IU/L)		84 (14–440)	95 (18–329)	79 (14–440)	0.537	88 (15–440)	81 (14–329)	0.561
TP		68.9 ± 6.6	71.5 ± 6.8	67.7 ± 6.3	0.516	68.8 ± 7.6	69.0 ± 5.7	0.429
LDH		198 (101–828)	172 (136–240)	210 (101–828)	0.435	178 (117–444)	217 (101–828)	0.501
UREA		5.1 ± 1.3	5.6 ± 1.2	4.9 ± 1.3	0.312	5.3 ± 1.2	5.0 ± 1.4	0.298
CREA		77 ± 14	80 ± 10	75 ± 15	0.525	74 ± 13	80 ± 14	0.111
EGFR		92.89 ± 13.88	89.66 ± 13.38	94.41 ± 14.04	0.485	95.12 ± 13.15	90.83 ± 14.46	0.473
CYSC		3.0 (0.6-102.6)	1.0 (0.7–1.4)	3.9 (0.6-102.6)	0.605	5.2 (0.6-102.6)	1.0 (0.6–1.2)	0.597
GLU		5.62 ± 2.20	6.37 ± 3.07	5.27 ± 1.59	0.394	5.74 ± 2.75	5.51 ± 1.60	0.473

Table 4. Clinicopathological parameters of the HCC cohort.

Interestingly, *TP53m*-positive patients revealed a significantly higher recurrence risk than *TP53m*-negative (62.5% / 30.8%, $P<0.05$) ones. Though it was not statistically significant, there do have a higher trend of recurrence rate in *TERTpm*-positive patients (56.3% / 41.2%). Further, we found that patients with *TERTpm* (43.8% / 23.5%) or *TP53m* (45.8% / 15.4%, $P<0.05$) have a tumor relapse earlier (< 12 month) than those negative ones.

Taken together, the analyses above suggest potential prognostic value of *TERTpm* and *TP53m* in HCC.

TERTpm coexistence with *TP53m* was associated with worse prognostic in HCC

As presented in Table 5, to further explore the potential prognostic value of *TERTpm* and *TP53m*, we also examined the individual effects of *TERTpm* alone, *TP53m* alone, and coexistence of *TERTpm* and *TP53m* on clinicopathological outcomes of HCC. Surprisingly, whereas the effects of *TP53m* alone and *TERTpm* alone were lost, coexistence of *TERTpm* and *TP53m* was more commonly and more significantly associated with the aggressive clinicopathological characteristics of HCC, including higher risk of recurrence ($P<0.05$), and earlier relapse period (≤ 12 month, $P<0.05$).

In survival analysis, we found that coexistence of *TERTpm* and *TP53m* had the shortest PFS ($P<0.05$) (Fig. 2). Given that there was no patients died in the follow-up duration, another 207 HCC patients with complete outcome information was obtained from The Cancer Genome Atlas (TCGA) liver hepatocellular carcinoma database for survival analysis (Supplementary Table 2). Among these, 8 patients had *TP53m* alone, 52 had *TERTpm* C228T alone, 19 had both, and 128 had neither. The median follow-up time overall was 953 days (range, 3-1826 days), and 115 patients died during this period. The all-cause mortality rates of patients with both negative, *TP53m* alone, *TERTpm* alone, and both positive were 52.34% (67/128), 87.5% (7/8), 50% (26/52), and 78.95% (15/19), respectively (Supplementary Table 3).

Based on these data, it was clear that the coexistence of *TERTpm* and *TP53m* had considerably worse survival outcome.

Discussion

Hepatocellular carcinoma (HCC) is a formidable adversary in the landscape of global health, with its high recurrence rate being a major contributor to the poor prognosis and elevated mortality rates among patients. Despite the utility of laboratory markers like alpha-fetoprotein (AFP) and imaging techniques in diagnosing recurrence, the early detection of this phenomenon remains elusive[7–9]. This study, therefore, aimed to identify predictive biomarkers that could foresee recurrences in HCC, a pursuit that is both timely and crucial.

The genesis of tumors is a complex tapestry woven by multiple genetic alterations, and while the molecular underpinnings of HCC's initiation and progression are not fully elucidated, certain mutations stand out. *TP53* mutations and *TERT* promoter mutations are reported to be the most common somatic alterations in hepatocellular carcinoma[7,13,24]. Notably, *TERTpm* have been linked to enhanced telomerase activity and TERT

Variables		TERTpm- / TP53m- (*n* = 18, 36%)	TERTpm+ / TP53m- (*n* = 8, 16%)	*P* value	TERTpm- / TP53m+ (*n* = 16, 32%)	Pvalue	TERTpm+ / TP53m+ (*n* = 8, 16%)	Pvalue
Age (years)		54 (39–67)	61 (41–87)	0.110	56 (24–69)	0.763	54 (47–66)	0.420
Gender	F	3 (16.7%)	0	0.529	6 (37.5%)	0.250	0	0.529
	M	15 (83.3%)	8 (100%)		10 (62.5%)		8 (100%)	
Height (cm)		162 (153–180)	167 (162–170)	0.947	160 (149–170)	0.279	164 (150–172)	0.267
Weight (kg)		64 (49–83)	63 (50–78)	0.679	60 (33–103)	0.413	65 (61–84)	0.114
Tumor numbers	Single	17 (94.4%)	8 (100%)	> 0.999	16 (100%)	> 0.999	6 (75.0%)	0.215
	Multiple	1 (5.6%)	0		0		2 (25.0%)	
Tumor diameter (cm)		5.6 ± 3.6	5.45 ± 1.92	0.749	6.75 ± 4.31	0.859	5.6 ± 3.5	0.476
Differentiation	Poor	4 (22.2%)	0	0.277	6 (37.5%)	0.457	4 (50.0%)	0.197
	Moderate	14 (77.8%)	8 (100%)		10 (62.5%)		4 (50.0%)	
WHO grade	II	14 (77.8%)	8 (100%)	0.277	10 (62.5%)	0.457	6 (75.0%)	> 0.999
	III	4 (22.2%)	0		6 (37.5%)		2 (25.0%)	
Hepatitis	G1	3 (16.7%)	0	0.529	4 (25%)	0.682	0	0.529
	G2-G3	15 (83.3%)	8 (100%)		12 (57%)		8 (100%)	
Cirrhosis	Yes	10 (55.6%)	3 (37.5%)	0.673	9 (56.3%)	> 0.999	5 (62.5%)	> 0.999
	No	8 (44.4%)	5 (62.5%)		7 (43.7%)		3 (27.5%)	
Macrovascular invasion	Yes	7 (38.9%)	0	0.062	5 (31.3%)	0.729	5 (62.5%)	0.401
	No	11 (61.1%)	8 (100%)		11 (68.8%)		3 (27.5%)	
Recurrence	Yes	5 (27.8%)	3 (37.5%)	0.667	9 (56.3%)	0.163	6 (75.0%)	**0.038**
	No	13 (72.2%)	5 (62.5%)		7 (43.7%)		2 (25.0%)	
Recurrence (≤ 12 month)	Yes	2 (11.1%)	2 (25%)	0.563	6 (37.5%)	0.110	5 (62.5%)	**0.014**
	No	16 (88.9%)	6 (75%)		10 (62.5%)		3 (27.5%)	

Table 5. Association of *TERsTpm*, *TP53m*, and their co-existence with tumor recurrence.

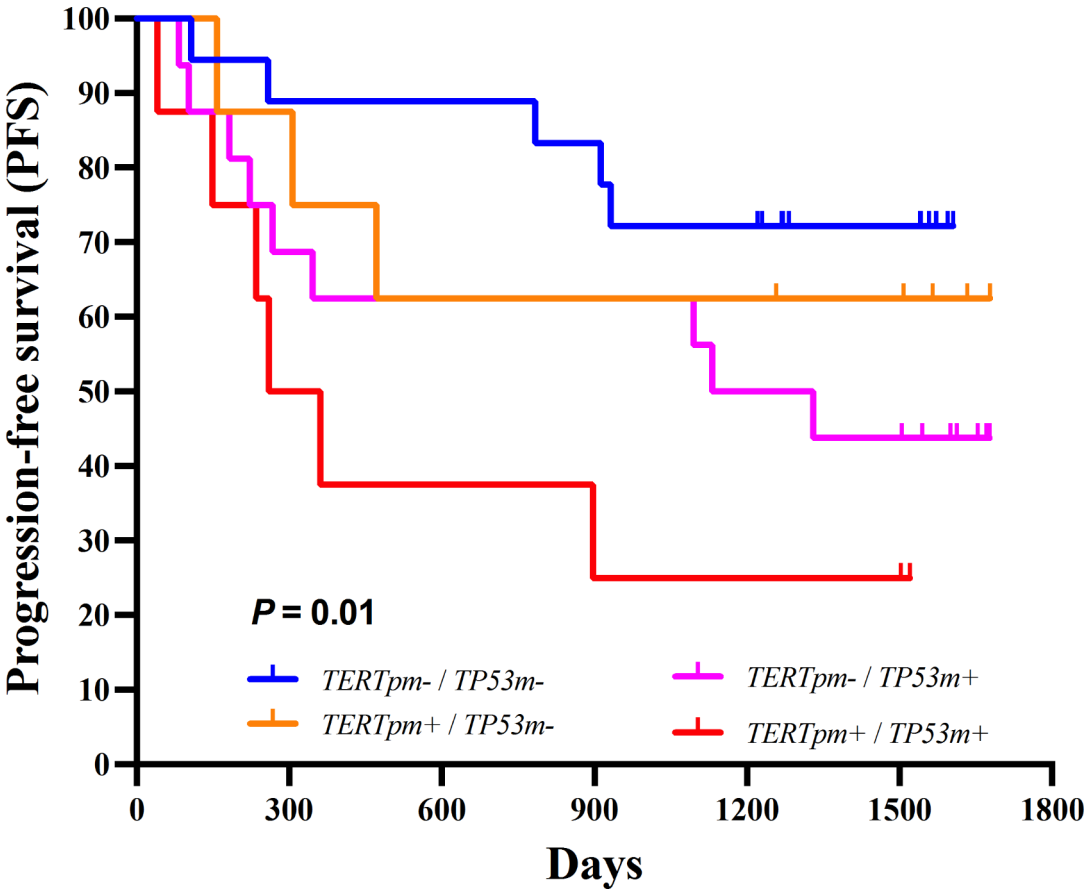

Fig. 2. The correlation of TERT promoter status and TP53 status with progression-free survival. +: positive, – negative.

mRNA expression across various cancers, including meningioma, urothelial carcinoma, glioblastoma, and HCC. These alterations are associated with cellular immortalization and a grim prognosis[20,27–29]. TP53 mutations, in turn, often result in the inactivation of TP53 and p53 protein, leading to chromosomal instability and unchecked proliferation, hallmarks of many cancers[30,31]. However, the interplay between TERTpm and TP53 mutations and their collective impact on HCC recurrence is not well understood, a gap this study sought to bridge.

For this reason, using the next-generation sequencing technology, we identified the genetic landscape and mutation signatures of HCC in liver tumor tissues from 50 HCC patients. The association of *TP53m*, *TERTpm*, and their coexistence with clinicopathological features and outcome was explored to further clarify their potential value in HCC.

We identified that all tumor tissues involved in this study harbored genetic alterations, among which *TP53m* and *TERTpm* (C228T) were the most frequent, with a prevalence of 48% and 32%, respectively. Previous published studies showed that the incidence of *TP53m* and *TERTpm* (C228T) in HCC were 18.7%~48% and 14.9%~60%, respectively[7,13]. Overall, our results were consistent with those of most previous findings, indicating the importance of *TP53m* and *TERTpm* (C228T) in HCC. After that, other mutated genes such as *LRP1B* (26%), *CTNNB1* (18%) and *AXIN1* (14%) were also identified in the present study, which were similar to the previous researches[24,25,32,33]. Our results showed that patients with *TP53m* were significantly associated with moderately differentiated tumors and early recurrence. Meanwhile, *TERTpm* were considerably associated with male gender and lower serum AFP levels. However, neither *TP53m* nor *TERTpm* was linked to age, body measurements, tumor characteristics, or other clinical features and laboratory features, suggesting a more nuanced relationship between these genetic alterations and clinical manifestations.

Among the 50 HCC patients, 23 cases experienced intrahepatic tumor recurrence or distant metastases, 9 harbored *TP53m* alone, 3 harbored *TERT*pm alone, 6 harbored both, and 5 harbored neither. According to these data, we can infer that patients with *TP53* mutations were more likely to experience tumor recurrence than negative ones. While the recurrence rate of HCC in patients with *TERTpm* was not statistically different from those *TERTpm*-negative ones, the presence of *TERTpm* did elevate the probability of tumor recurrence in patients from 41.2 to 56.3%, especially the early recurrence rate was improved from 23.5 to 43.8%. However, there was no significant difference of recurrence rate in patients with neither *TP53m* alone nor *TERTpm* alone, compared to those with negative *TP53m* and negative *TERT*pm. By contrast, coexistence of *TERTpm* and *TP53m* indicated a higher likelihood of tumor recurrence, and poor progression-free survival. This finding is intriguing,

as it points to a potential synergistic effect between these mutations, a hypothesis supported by the known interaction between p53 and TERT.

Previous studies have found a strong interaction between p53 and TERT. On the one hand, p53 as a transcription factor have been shown to repress *TERT* mRNA level[34,35]. On the other hand, suppression of TERT could induce DNA damage and apoptosis in a p53-dependent way[36,37]. Further, both *TERTpm* and *TP53m* are associated with tumorigenesis. Thus, we speculated that genomic mutations of both *TERT* and *TP53* have strengthened the interaction between the two, which promote tumor progression and lead to poorer prognosis in HCC patients.

To address this hypothesis, we consulted the current literature on p53-TERT and found that p53 was a powerful inhibitor of the *TERT* promoter activity. *TP53m* were responsible for upregulation of *TERT* mRNA level, by physically interacting with other transcription factors such as Sp1 (activate transcription of *TERT*) to nucleated onto the *TERT* promoter[38,39]. Since *TERTpm* could introduce new binding sites for transcription factors such as ETS/TCF, and *TERT* promoter also contain Sp1 binding motifs. Taken together, it is thus overwhelmingly probable that *TERTpm* C228T may introduce a new binding site for Sp1, facilitating increased p53 binding and TERT activation. However, further work is required to investigate the exact mechanisms and their functional effects, since there is a lack of evidence at this point to support our hypothesis.

In conclusion, this study provides a comprehensive genomic portrait of HCC, identifying *TP53m* and *TERTpm* as common somatic alterations. The coexistence of these mutations appears to be a promising marker for tumor recurrence and poor prognosis, offering valuable insights for postoperative surveillance and personalized treatment strategies. While our findings are promising, they also underscore the need for larger-scale studies to validate these observations and explore the underlying mechanisms, potentially paving the way for novel therapeutic interventions in HCC.

Data availability

Raw data for the sequencing analysis included in this study have been deposited in Gene Expression Omnibus (GEO), accession number GSE273254.

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Declarations

Competing interests

The authors declare no competing interests.

Ethical approval

The studies involving human participants were reviewed and approved by the Biomedical Ethics Committee of West China Hospital of Sichuan University [Reference No. 2019 (203)]. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Additional information

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