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Review

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Telomerase reverse transcriptase promoter mutations in hepatocellular carcinogenesis

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Abstract

Through several studies exploiting next-generation sequencing, we are obtaining a clearer picture of the complex genetic and molecular landscape of hepatocellular carcinoma (HCC). Consistent with the findings of other cancer types, telomerase reverse transcriptase (TERT) promoter mutations have been frequently reported in HCC. C228T and C250T are two major types of hot spot mutations in the TERT promoter region. Besides, in hepatitis B virus (HBV)-related HCC cases, the TERT promoter is recurrently interrupted by integration of HBV DNA. TERT promoter mutations are thought to be an early event in HCC carcinogenesis, and they are significantly associated with disease progression. In this review, we provide an updated overview of the somatic mutations in the TERT promoter region and discuss their possible roles in the development of HCC.

Keywords: Hepatocellular carcinoma, telomerase reverse transcriptase, mutation, hepatitis B virus

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common and deadliest cancers worldwide, ranking fifth and ninth in incidence, and second and sixth in mortality for males and females, respectively[1,2]. So far, only three molecular targeted agents, including sorafenib, lenvatinib and regorafenib, have been approved by the Food and Drug Administration for the treatment of HCC[3,4], and they only extend median survival by a few weeks to months[5]. Therefore, more research is needed to fill the gaps in knowledge of the genetic

and molecular landscape of HCC in order to develop target therapies. The genetic landscape of HCC is complicated and involves a number of pathways as well as a considerable amount of somatic mutations in a wide range of genes[6]. Among all these genetic alterations, telomerase reverse transcriptase (TERT) promoter mutations occur most frequently, affecting ~60% of all HCC patients[6-8]. In this mini-review, we mainly summarize the frequency, mechanisms and clinical prospect of TERT promoter mutations in HCC. To provide more background information, this review also briefly touches upon the TERT promoter mutations in various cancers, although HCC remains the main focus of our discussion throughout the whole paper.

THE STRUCTURE AND FUNCTION OF TERT

Human telomerase is a ribonucleoprotein polymerase that reverses the continuous telomere shortening in cell division by adding 5'-TTAGGG-3' repeats to the ends of chromosome[9]. It consists of two core subunits: the catalytic component TERT and the RNA component (TERC) that serves as a template for elongating telomeres[10,11].

The TERT component is encoded by the *TERT* gene, located on chromosome 5 in humans. It spans a length of about 40,000 base pairs (bp) with 16 exons[12]. Of note, the *TERT* gene is suppressed in most normal somatic cells (excluding germ cells and stem cells), ensuring that these cells only divide a finite number of times and do not surpass the Hayflick limit[13,14]. Normal somatic cells stop dividing when their telomeres become critically short, whereupon they enter a stage called senescence[15]. Cancer cells, however, overcome replicative senescence and achieve immortality by reactivating the *TERT* gene and upregulating TERT expression[14].

The regulation of TERT expression largely depends on the activity of the TERT promoter, especially the core functional fragment that consists of a 260 bp DNA sequence with several transcription factor binding sites, but distinctly lacking a TATA box or a similar sequence[16,17]. The binding motifs in the TERT promoter include two evolutionarily-conserved E-boxes (CACGTG), located at -242 bp and -34 bp to the translational start site, for c-Myc binding[18]. The binding of c-Myc to the E-box activates TERT transcription, suggesting a role of c-Myc in regulation of the expression of TERT[19,20]. GC-boxes (GGGCGG), the binding sites for zinc finger transcription factor Sp1, are the other characteristic sequences in the TERT promoter region[21]. There are at least five GC-boxes within the core promoter of TERT, and they function synergistically to maintain the promoter activity of TERT[22]. P53 has been shown to down-regulate TERT transcription in an SP1-dependent manner[23].

TERT PROMOTER MUTATIONS IN SEVERAL CANCERS

TERT promoter mutations are the most frequent somatic mutations in a variety of cancers. It has been widely reported that the two most common types of recurrent TERT promoter mutations are C228T and C250T, located at positions 1,295,228 and 1,295,250 on chromosome 5, or -124 bp and -146 bp of the ATG translational start site of the *TERT* gene[24-27]. In a systematic analysis involving 1,581 cancer cases of different types, 27.0% were found to have TERT promoter mutations[25]. Killela *et al*.[28] examined 1,230 tumor specimens of 60 different types and identified 231 TERT promoter mutations (18.8% of the total), among which C228T and C250T mutations accounted for 98%. Similarly, in a study where 1,515 tumors of the central nervous system were tested, 327 (21.6%) had TERT promoter mutations, and all except two contained either C228T or C250T[29]. Another study examined 150 cell lines of several cancer types from the Cancer Cell Line Encyclopedia and noted that 24 cell lines (16%) harbored either C228T or C250T mutations[26]. Statistics show that C228T is somewhat more prevalent than the C250T mutation [Table 1] in a wide range of cancer types, including various subtypes of CNS cancers, urogenital cancers, melanoma and thyroid cancer[25,26,28-37].

Table 1. Telomerase reverse transcriptase promoter mutations in multiple cancers

Cancer type	Number of cancer cases	Number of TERT mutations*	Number of different types of TERT promoter mutations**			Methods	Ref.
			C228T	C250T	C228T or C250T		
Cancer tissue							
Glioma, medulloblastoma, hepatocellular carcinoma, *etc*.	1230	231 (18.8)	179 (77.5)	48 (20.8)	227 (98.3)	PCR/Sanger sequencing	[28]
Bladder cancer, liver cancer, glioma, *etc*.	1581	426 (26.9)	/	/	/	Whole-genome/low-pass whole-genome sequencing	[25]
CNS cancers	1515	327 (21.6)	257 (78.6)	68 (20.8)	325 (99.4)	PCR/bidirectional sequencing	[29]
CNS, bladder, thyroid cancers, *etc*.	741	142 (19.2)	99 (69.6)	43 (30.3)	140 (98.6)	PCR/Sanger sequencing	[36]
Urogenital cancers	302	130 (43.0)	100 (76.9)	24 (18.5)	124 (96.4)	PCR/Sanger sequencing	[37]
Medulloblastoma	466	98 (21.0)	/	/	/	PCR/Sanger sequencing	[35]
Melanoma	287	109 (38.0)	51 (46.8)	40 (36.7)	91 (83.5)	PCR/Sanger sequencing	[32]
Bladder cancer	262	218 (83.2)	165 (75.7)	32 (14.7)	197 (90.4)	SNaPshot assay and Sanger sequencing	[34]
Melanoma	77	24 (31.2)	7 (29.2)	5 (20.8)	12 (50.0)	High-throughput sequencing/Sanger sequencing	[33]
Cancer cell line							
Melanoma	168	125 (74.4)	46 (36.8)	64 (51.2)	110 (88)	High-throughput sequencing/Sanger sequencing	[33]
Melanoma, liver, bladder cancers, *etc*.	150	24 (36.0)	/	/	24 (100)	Whole-genome sequencing, Sanger sequencing,	[26]
Urothelial bladder cancer	23	20 (87.0)	16 (80.0)	2 (10.0)	18 (90.0)	PCR/Sanger sequencing	[31]
Urothelial bladder cancer	32	28 (87.5)	25 (89.3)	3 (10.7)	28 (100)	PCR/Sanger sequencing	[30]

*Percentage in all cancer cases; **percentage in telomerase reverse transcriptase (TERT) mutation cases

Overall, it is widely accepted that glioma, melanoma, bladder cancer and HCC are among those commonly-affected by TERT promoter mutations[25,28,38].

TERT PROMOTER MUTATIONS IN HCC

The genomic landscape of HCC involves a number of pathways as well as somatic mutations in a wide range of genes, including *TP53*, *CTNNB1*, *AXIN1*, *CDKN2A*, *ARID2*, *ARID1A*, *TSC1/TSC2*, *RPS6KA3*, *KEAP1*, *MLL2*, and several epigenetic modifications[6]. Despite the complexity of the genomic landscape of HCC, the single most significant factor is genomic changes on TERT promoter, which include point mutations, hepatitis B virus (HBV) DNA integrations, amplifications and epigenetic modifications. TERT promoter point mutations contribute more frequently (54%-60%) to the reactivation of telomerase in HCC than the exclusively-present HBV insertions in the TERT promoter (10%-15%) and TERT amplification (5%-6%)[6-8]. Therefore, we are going to thoroughly discuss TERT promoter mutations while briefly touching upon other genomic and epigenomic alterations on TERT promoter in HCC.

TERT promoter point mutations

A few prominent studies on HCC demonstrated that TERT promoter mutations were found in about 30%-60% of the total cases[8,39-49]. Consistent with the findings in other cancer types, the two most common mutations were *C228T* and *C250T*, and the former was more prevalent than the latter in HCC [Table 2][8,39-47]. As shown in Table 2, there are no cases with both *C228T* and *C250T* mutations, which implies that these two hot spot

Table 2. Telomerase reverse transcriptase promoter mutations in hepatocellular carcinoma

Number of HCC cases	Number of TERT mutations (%)	Number of different types of TERT promoter mutations (%*)			Methods	Ref.
		C228T	C250T	C250T or C228T		
469	254 (54.2)	236 (92.9)	11 (4.3)	247 (97.2)	PCR/bidirectional sequencing	[8]
316	103 (32.6)	96 (93.2)	5 (4.9)	101 (98.1)	PCR/Sanger sequencing	[43]
305	179 (58.7)	166 (92.7)	11 (6.1)	177 (98.9)	PCR/Sanger sequencing	[42]
276	85 (30.8)	84 (98.8)	1 (1.2)	85 (100)	PCR/Sanger sequencing	[44]
196	87 (44.4)	/	/	/	Whole-genome sequencing	[48]
195	57 (29.5)	54 (94.7)	3 (5.3)	57 (100)	PCR/Sanger sequencing	[45]
160	46 (28.8)	32 (69.6)	14 (30.4)	46 (100)	PCR/Sanger sequencing	[39]
44	15 (34.1)	10 (66)	5 (34)	15 (100)	PCR/Sanger Sequencing	[40]
190	57 (30.0)	50 (87.7)	7 (12.3)	57 (100)	PCR/bidirectional sequencing	[46]
127	64 (50.4)	62 (96.9)	2 (3.2)	64 (100)	PCR/Sanger sequencing	[47]
123	45 (36.6)	43 (95.6)	2 (4.4)	45 (100)	PCR/Sanger sequencing	[41]
125	85 (68.0)	/	/	/	PCR/Sanger sequencing	[49]

*Percentage in telomerase reverse transcriptase (TERT) mutation cases. HCC: hepatocellular carcinoma

mutations are mutually exclusive. Furthermore, a comprehensive review evaluating the distribution of TERT promoter mutations in 1,939 primary HCC from four continents also showed that TERT promoter mutations had almost the same level of prevalence in all continents, with slightly higher mutation rates in Europe (56.6%) and Africa (53.3%) than in America (40%) and Asia (42.5%), and that C228T mutation was universally more frequent than C250T[1].

Apart from the high frequency of TERT promoter mutations in HCC, another piece of useful information indicated by several lines of evidence is that TERT promoter mutations are associated with a few factors, including virus status, gender, age and tumor size of the patients. TERT promoter mutations were more frequent in HCC patients infected with hepatitis C virus[7,8,39,41,42,47,48,50] than in those infected by HBV. One study suggested that this phenomenon could be explained by the high rate of HBV DNA insertions in the TERT promoter[42]. Furthermore, several studies reported higher TERT promoter mutations rate in men[7,39,42], in older patients[7,50], in patients with smoking[51], in patients with smaller tumors[42], in patients with low serum levels of alpha-fetoprotein[42], and in patients with *CTNNB1* mutations[8,42,47], while other papers either disagreed with or did not find these associations.

Further, TERT promoter mutations are early somatic genetic alterations in hepatocarcinogenesis, playing important roles in malignant transformation of preneoplastic cirrhotic lesions[42,52]. Nault *et al.*[52] found that the frequency of TERT promoter mutations increased as premalignant lesions transformed into HCC, from 6% in low-grade dysplastic nodules and 19% in high-grade dysplastic nodules to 61% in early HCC and 42% in small and progressed HCC; mutations in 10 other recurrent genes only emerged in small and progressed HCC. Similarly, Huang *et al.*[43] demonstrated that the mutation rates also increased in a stepwise manner during advanced HCC progression and reached a maximum of 45% in patients with stage C. Calderaro *et al.*[53] found that there were 64.6% (208/322) cases with TERT promotor mutations; HCC phenotypes were tightly associated with gene mutations, including TERT promoter mutations, and transcriptomic classification.

As the proportion of nonalcoholic fatty liver disease (NAFLD)-related HCC patients is increasing due to increased prevalence of metabolic syndrome, especially in Western countries[54-56], there have been studies investigating TERT promoter mutations in NAFLD-related HCC. One research analyzed the genetic aberrations of 11 tumor samples from 10 NAFLD-HCC patients and found that TERT promoter mutation *C228T* occurred in 9/11 (82%) cases[56]. On the contrary, in another study, the prevalence of TERT promoter mutations *C228T* and *C250T* was very low (3.2%) in patients with NAFLD[57]. Obviously, the TERT promoter mutation state in NAFLD-related HCC is far from conclusive.

TERT promoter insertional mutations by HBV DNA integration

HBV infection has been shown to be a causative factor of HCC, especially in Asians where chronic hepatitis B infection is prevalent. Integration of HBV DNA into the human genome of HCC cells is evident in HBV-related HCC[8,40,48,58-64]. Several lines of evidence demonstrate that the integration sites of HBV are not random. Integration of certain genomic sites, including near or within the genes of TERT[8,48,59-65], MLL4[48,59,61-63,65] and CCNE1[48,61-63,65] are more frequently identified in HCC[48].

To date, 13 independent studies have identified a total of 262 integrations of HBV DNA in the *TERT* gene, meaning that in more than 20% HBV-related HCC cases, *TERT* gene is interrupted by HBV integration[7,58,65-75]. *TERT* is the most susceptible gene for HBV integration, followed by MLL4 (79 integrations), CCNE1 (22 integrations) and CCNA2 (19 integrations)[76]. According to our pool analysis of the results from these articles[7,58,65-75], among the 262 HBV integrations in TERT, 73.28% (192/262) occur in the TERT promoter region, including 26% in the core functional fragment (-223 bp to -14 bp from the ATG translational start site). As the regulation of TERT expression largely depends on the activity of the TERT promoter region, especially the core functional fragment, HBV integration in the TERT promoter may have an important functional role in HCC development.

A few studies suggested that HBV tended to integrate in common chromosomal fragile sites, where DNA replication was delayed and DNA sequences were more susceptible to breakage[63,64]. Nevertheless, the findings that TERT was a recurrent integration site but not a fragile site demand new explanation[64]. More recent studies have therefore presented new possibilities. One study proposed that HBV preferentially integrates into *TERT* gene because disruption at these loci lowers the threshold for malignant transformation and thus grants a selective advantage to carcinogenesis[59]. Another two studies, using a similar line of reasoning, suggested that the recurrence of HBV integrations into TERT promoter region in HCC could be due to the potential growth advantage that augmented TERT expression provides for the clonal expansion and carcinogenesis of hepatocytes[60,62]. In TCGA database, the HCC with HBV DNA insertion into the TERT promoter displays the highest level of TERT RNA expression among all HCCs, suggesting an HBV cis-activating event did exist[48].

HBV integrations promote the development of HCC by inducing global genomic instability, elevating expression of adjacent genes, viral-host fusion transcripts and secondary mutations of host or viral genes, as well as by DNA copy number variations and proteins with oncogenic activity (*X* and *preS* gene products)[58,61,64,65]. Recently, based on the discovery that both HBV integration and somatic mutations in the TERT promoter were more frequent in male patients with HCC, Li *et al.*[69] proposed a novel mechanism in which sex hormones, along with GABPA play a role in regulating TERT expression. They analyzed 101 HBV-related HCC cases using a capture-next-generation sequencing platform and concluded with convincing evidence that the integration of HBV DNA, whose sequence contains both androgen- and estrogen-responsive elements, into the TERT promoter permits the androgen-receptor to up-regulate and the estrogen-receptor to down-regulate TERT transcription in a HNF4α-dependent manner[62].

OTHER GENOMIC AND EPIGENOMIC ALTERATIONS ON TERT PROMOTER IN HCC

TERT amplification in HCC

Totoki *et al.*[8] showed that TERT focal amplification was detected in 6.7% of the total 608 cases. Schulze *et al.*[77] observed less than 5% of TERT focal amplification in the 243 liver tumors. However, while both studies described the occurrence of TERT focal amplification in HCC, none of them investigated its effect on TERT expression level. Thus, more research is needed to confirm the role of TERT amplification in liver carcinogenesis.

Epigenetic modification of TERT promoter in HCC

As for epigenetic regulation of TERT promoter in HCC, Iliopoulos *et al.*[78] observed a strong negative correlation between TERT promoter methylation and TERT expression in all liver tissues they studied,

proposing for the first time that the hypermethylation of TERT promoter and the methylation of histone H3-K9 resulted in the inhibition of c-Myc binding in E-box 1, which in turn inactivated TERT expression. However, this result contrasts with previous studies, which showed that TERT promoter epigenetic modification had either a positive correlation or no correlation with TERT expression and telomerase activity in other cancer types[79-83]. A more recent study examining 125 HCC cases in the Han Chinese population found that the promoter of the *TERT* gene is significantly hypermethylated, and it further showed that the hypermethylation is associated with higher expression of TERT, suggesting that TERT promoter hypermethylation contributes to the progression of liver carcinogenesis via elevating TERT expression level[84]. Overall, there is no definite conclusion regarding whether hypermethylation of TERT promoter has a positive or negative correlation with TERT expression and telomerase activity.

MECHANISMS OF TERT PROMOTER MUTATIONS CONTRIBUTING TO THE DEVELOPMENT OF HCC AND OTHER CANCERS

Although TERT promoter mutations are strongly associated with several cancers, the mechanism by which TERT promoter mutations lead to cancer development is not fully understood. How TERT promoter mutations increase TERT expression and whether the up-regulation of TERT directly translates into active telomerase activity that eventually contributes to tumorigenesis are two important questions requiring answers.

Mechanisms of TERT promoter in other cancers

It is currently accepted that C228T and C250T, the two most common mutation types in TERT promoter region, both create an 11-bp binding motif (5'-CCCCTTCCGGG-3') for E-twenty-six (ETS) transcription factors[26,85,86]. In glioblastoma, a total of five ETS transcription factors were found (ELF1, ETS1, ETV3, ETV4 and GABPA) that modulate TERT expression. GABPA complexes with GABPB to form a fully functional heterodimer GABP transactivator, it was the only factor that reproducibly regulated TERT expression in a mutation-specific manner[86]. Akincilar *et al*.[24], using cell lines from several cancer types, including melanoma, glioblastoma, colon, and prostate cancers, *etc*., reported that TERT promoter mutations enhanced the binding of GABPA, mediating long-range chromatin interaction (at chr5: 1,556,087-1,558,758, a region 300 kb upstream of promoter), enrichment of active histone markers H3K4Me3 and H3K9Ac and subsequent POL2 recruitment, thus driving TERT transcription. Another study suggested a slightly different mechanism. According to work by Li *et al*.[85], the TERT promoter with C250T mutation was driven by NF-κB signaling. On activation of this signaling pathway, p52 (NF-κB2) is recruited to the C250T region, but not the C228T region, and cooperates with ETS factors ETS1/2 to drive efficient TERT transcription[85]. TERT promoter mutations are widely found together with BRAF V600E alteration in human cancers, particularly in thyroid cancer and melanoma[87-92]. A recent study found that that TERT promoter mutations and BRAF V600E cooperatively upregulated TERT expression and promoted the oncogenic behaviors in the papillary thyroid cancer cells[93].

Mechanisms of TERT promoter mutations in HCC

TERT promoter mutation was a later oncogenic event. Pilati *et al*.[94] have screened TERT promoter in a large series of liver cancers including adenomas, borderline lesions hepatocellular adenomas (HCA)/HCC, HCC derived from adenomas and classical HCC, and found TERT promoter mutations did not exist in classical adenomas, but in borderline lesions HCA/HCC (17%) and HCC cases derived from adenomas (56%) which frequency was similar to that in classical HCC (54%).

There are only a few studies focusing on the mechanism of how TERT promoter mutations influence TERT expression and lead to malignant transformation of liver cells [Figure 1]. Telomerase activation is important to maintaining telomere length that confers cancer cells infinite ability to overcome the proliferation barrier. One study demonstrated that *TERT* mRNA expression and telomerase activity were higher in patients with

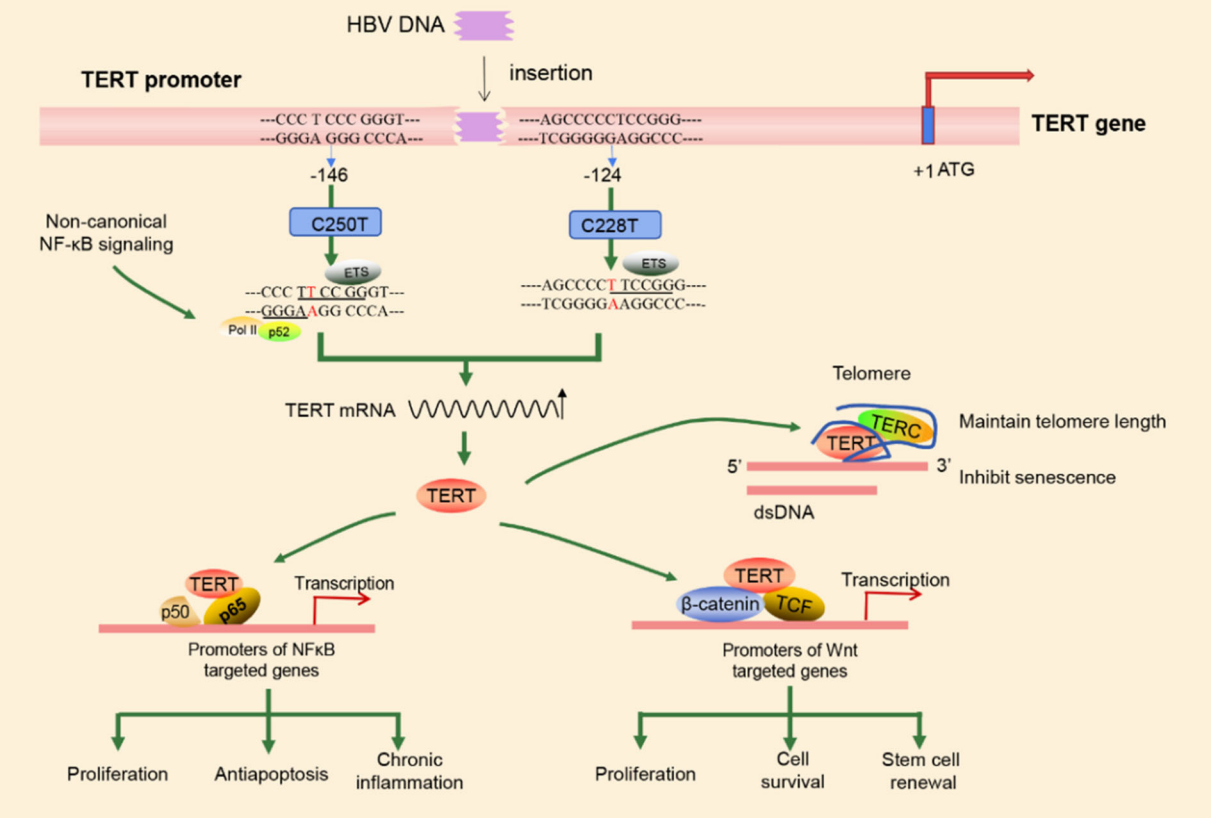

Figure 1. Proposed model for telomerase reactivation by telomerase reverse transcriptase (TERT) promoter mutations. The C228T and C250T TERT promoter mutation both create an E-twenty-six (ETS) binding motif (the mutational hotspots are in red) to modulate TERT mRNA expression. P52 (NF-κB2) is recruited to the C250T region, but not the C228T region, and cooperates with ETS factors to drive efficient TERT transcription. The elevated TERT expression enhances cell malignant behavior through a telomere lengthening-dependent manner (maintaining telomere length or inhibiting senescence), and/or a telomere lengthening-independent manner (TERT acting as a transcriptional modulator regulating genes related to Wnt and NF-κB signaling pathways thereby promoting cell proliferation, antiapoptosis, and stem cell renewal). Hepatitis B virus (HBV) DNA insertion into TERT promoter is another possible mechanism of hepatocarcinogenesis, which may cause HBV promoter/enhancer-driven transcription of TERT

HCC who had both single nucleotide polymorphism (SNP) rs2853669 and promoter mutations of *TERT* gene[95]. The rs2853669 variant and the TERT promoter mutation C228T combined to induce TERT promoter methylation and increase TERT expression, resulting in a longer telomere length compared to the wild-type rs2853669 and TERT promoter[95].

In recent years, TERT has been considered to have some other direct effects on carcinogenesis in addition to its function on maintaining telomere length[96]. Studies revealed that TERT acts as a transcriptional activator that activates the transcription of genes targeted by Wnt and NF-κB signaling to play a role in cell proliferation, antiapoptosis, and stem cell renewal[96,97]. In HCC, TERT expression level was higher in almost all cases with TERT promoter mutations than that in those without the mutations, and elevated TERT expression is closely related to the development of HCC[42,94]. Based on the significant association between TERT promoter and CTNNB1 mutations as well as previous studies showing the interaction between TERT and Wnt/β-catenin pathway, it was proposed that TERT promoter mutations and activation of the Wnt/β-catenin pathway together lead to malignant transformation[42,97]. By contrast, another research revealed that, while TERT expression did increase in the HCC cohort overall, it was not significantly correlated with TERT promoter mutations[48]. They suggested that TERT promoter mutations might cooperate with CDKN2A silencing to promote *TERT* mRNA expression. CDKN2A gene encodes the tumor suppressor gene $p16^{INK4A}$, whose down-regulation together with up-regulated TERT expression is critical for epithelia cell

immortalization[98]. Anyhow, there are only a few studies focusing on the mechanisms of TERT promoter mutations in HCC. Whether it shares the same mechanisms with other cancers requires further research in the future.

TERT PROMOTER MUTATIONS IN DIAGNOSIS, PROGNOSIS AND THERAPY OF HCC

A study detected the TERT promoter mutations in plasma cell-free DNA (cfDNA) in 218 patients with HCC, and the prevalence of TERT mutations was 47.7%, which was similar to the prevalence (44.4%) of 196 HCCs derived from the TCGA database[57]. Meanwhile, they also measured the prevalence of TERT promoter mutations in cfDNA of 81 patients with cirrhosis, and the frequency was 8.6%[57]. Since the frequency of TERT promoter mutations gradually increases during the process of cirrhosis and liver cancer, the TERT promoter mutations in the cfDNA in the serum can be detected as an important index for evaluating the development of HCC. However, there still remains a problem with specificity since the TERT promoter mutation is very common in various tumors so that the mutations in cfDNA cannot accurately reveal the source of the lesion.

The prognostic value of TERT promoter mutations remains controversial. Kawai-Kitahata *et al*.[7] and Huang *et al*.[43] performed survival analyses and demonstrated that TERT promoter mutations were associated with poor overall survival and could be prognostic markers for HCC[7,43]. However, Ko *et al*.[95] found that the presence of TERT promoter mutations alone did not translate into poor prognosis, but that the SNP rs2853669 and the -124C>T mutation combined were associated with poor survival rates. Further, Lee *et al*.[39] reported that longer telomere length, but not TERT promoter mutations, was independently associated with poor overall survival. Besides showing TERT promoter mutations' correlation with poorer overall survival in HCC, Li *et al*.[99] also demonstrated that TERT amplifications were associated with shortened overall survival independent of other clinicopathological parameters such as age, gender and TNM staging. Thus, while we are sure that genetic changes at *TERT* gene have prognostic value, we are uncertain about exactly which factor(s) - TERT promoter mutations alone, the combination of the SNP rs2853669 and the -124C>T mutation, longer telomere length or TERT amplifications - directly indicate(s) poor prognosis.

It is believed that TERT is a promising but also challenging driver gene to target. There are no drugs specifically targeting *TERT* gene yet, although a few inhibitors have been used to target amplified genes in HCC: epidermal growth factor receptor inhibitors like Gefitinib targeting amplified EGFR, MET, MAPK1, MAPK3 and CRKL, Crizontinib and vemurafenib targeting BRAF and ERBB2, and alisertib targeting amplified AURKA[99]. According to Dhanasekaran *et al*.[100], the somatic mutations associated with liver tumor development lie in genes whose products are not easily or safely targeted, and that mutant TERT, TP53, CTNNB1, and MYC are even believed to be undruggable. Nevertheless, the study also reveals that a synthetic TERT DNA vaccine, INO-1400, is being tested in a phase 1 trial of patients with solid tumors (NCT02960594) and that some trials are using TERT promoter mutation as a biomarker for study enrollment (NCT02766270)[100]. Since a traditional strategy to target TERT is challenging, it is suggested that new strategies, such as microRNA-based therapeutics, should be developed to target driver genes like *TERT* or their pathways[100]. In fact, one study explored the potential of a novel immunotherapy using TERT-derived peptide (TERT461) as a vaccine by investigating its safety and immunogenicity and characterizing the TERT-specific T cell responses induced[101]. Their results showed that the vaccination induced TERT-specific immunity in 10/14 (71.4%) of the patients, and that 57.1% of patients treated with TERT461 peptide-specific T cells could prevent HCC recurrence after vaccination[101]. Another study also concluded that CypB, SART2, SART3, p53, MRP3, AFP, and TERT are promising tumor-associated antigens (TAAs) in HCC immunotherapy[102]. Besides, not only do they suggest that the administration of the TAAs or peptides containing their epitopes as vaccines after HCC treatment is likely to be effective, but they also demonstrated that the concurrent use of anti-CTLA-4 antibodies may further improve antitumor immunity[102]. Therefore,

while it remains challenging to target *TERT* gene, new strategies are emerging to achieve this goal and make more effective therapy possible.

CONCLUSION

Our knowledge regarding the role of TERT promoter mutations in HCC is expanding; nevertheless, there remain many puzzles to be solved. Although the pattern of TERT promoter mutations in HCC is well-established, little is known about the mechanism through which TERT promoter mutations reactivate telomerase and promote tumor development. We are not yet sure how either somatic mutations or HBV integrations in the TERT promoter lead to malignant transformation and whether they can be prognostic biomarkers in HCC; nevertheless, we are confident that untangling the mechanisms relevant to TERT promoter can be a key for developing target therapy for HCC.

DECLARATIONS

Authors' contributions

Writing the initial manuscript: Ma ZX, Yang CM
Revision of the manuscript: Yang CM, Ma ZX, Li MG
Drafting the outline of the manuscript, critical revision of the manuscript for intellectual content, finalizing the manuscript, and obtaining the funding: Tu H

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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