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Mechanisms of *TERT* Reactivation and Its Interaction with *BRAF*^{V600E}

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The telomerase reverse transcriptase (*TERT*) gene, which is repressed in most differentiated human cells, can be reactivated by somatic *TERT* alterations and epigenetic modulations. Moreover, the recruitment, accessibility, and binding of transcription factors also affect the regulation of *TERT* expression. Reactivated *TERT* contributes to the development and progression of cancer through telomere lengthening-dependent and independent ways. In particular, because of recent advances in high-throughput sequencing technologies, studies on genomic alterations in various cancers that cause increased *TERT* transcriptional activity have been actively conducted. *TERT* reactivation has been reported to be associated with poor prognosis in several cancers, and *TERT* promoter mutations are among the most potent prognostic markers in thyroid cancer. In particular, when a *TERT* promoter mutation coexists with the *BRAF*^{V600E} mutation, these mutations exert synergistic effects on a poor prognosis. Efforts have been made to uncover the mechanisms of these synergistic interactions. In this review, we discuss the role of *TERT* reactivation in tumorigenesis, the mechanisms of *TERT* reactivation across all human cancers and in thyroid cancer, and the mechanisms of interactions between *BRAF*^{V600E} and *TERT* promoter mutations.

Keywords: Telomerase; BRAF; Thyroid neoplasms; Genomics; Epigenomics

INTRODUCTION

Since the human telomerase reverse transcriptase (*TERT*) gene was first cloned in 1997 [1,2], extensive efforts have been made to reveal the mechanisms of *TERT* reactivation. With recent advances in high-throughput sequencing technologies, these mechanisms have been actively researched, with a particular focus on the genomic alterations involved in activating *TERT* in various cancers. Based on the recent pan-cancer analysis of The Cancer Genome Atlas (TCGA), the prevalence of *TERT* promoter mutations in cancers is reported to be 0% to 89%, with the highest rates in glioblastoma (89%), skin melanoma (72%), and bladder cancer (70%) [3]. In addition, *TERT* amplification is found in 0% to 22% of cancers, most frequently in ovarian cancer (22%) and adrenocortical carcinoma (15%). Among the subtypes of thyroid cancer, *TERT* promoter mutations are more common in poorly-differentiated thyroid cancer (PDTC; 21% to 47%) and anaplastic thyroid cancer (ATC; 55% to 73%) than in differentiated thyroid cancer (DTC), such as papillary thyroid cancer (PTC; 4% to 26%) and follicular thyroid cancer (FTC; 6% to 36%) [4,5]. Moreover, *TERT* promoter mutations often coexist with the *BRAF*^{V600E} mutation, both in PTC (odds ratio [OR], 2.4) and in PDTC/ATC (OR, 2.4) [6]. Thus, efforts are ongoing to decipher the mechanisms of its association with oth-

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er oncogenes and clinical outcomes. Reactivation of *TERT* and coexistence with $BRAF^{V600E}$ have been reported to be associated with poor prognosis in several cancers, particularly in thyroid cancer [5,7]. Therefore, understanding the mechanisms by which *TERT* affects tumor aggressiveness is important for improving the prognosis of these cancers. In this review, we present an overview of recent insights into the mechanisms of *TERT* reactivation, as well as its interactions with *BRAF* in human cancers, including thyroid cancer.

TERT REACTIVATION IN TUMORIGENESIS

Canonical functions of *TERT*: lengthening of telomeres

Key protection mechanisms against cancer are the repression of telomerase and the maintenance of short telomeres [8]. In most differentiated human cells, telomerase is silenced by transcriptional repression of the *TERT* gene, which encodes its catalytic component [9]. The lack of telomerase leads to progressive telomere erosion in dividing human cells. When telomeres have become critically short, they are detected by the DNA-damage repair machinery, and the cell dies or reaches a permanent growth arrest stage known as replicative senescence [10]. The senescence response is a potent tumor suppressive mechanism [11]. As infinite proliferation is a hallmark of malignant cells, up to 90% of human cancers overcome the senescence barrier by reactivating telomerase [8,12]. The telomere length is maintained by the reactivation of telomerase/*TERT* in most tumor cells, while a small proportion acquire immortality through the telomerase-independent alternative lengthening of telomeres (ALT) mechanism, a homologous recombination-based process (Fig. 1) [12,13].

Non-canonical functions of *TERT*: interactions with oncogenic signaling pathways

TERT has also been shown to exhibit multiple biological activities, independently of its telomere lengthening function, regulating various genes and signal pathways involved in the hallmarks of cancer, such as cell proliferation, angiogenesis, resistance to apoptosis, inflammation, invasion, and metastasis (Fig. 1) [14,15]. Intriguingly, nuclear factor-κB (NF-κB) and β-catenin, master regulators of many oncogenic targets directly or indirectly driving various hallmarks of cancer, are known activators of *TERT* expression, and *TERT* regulates their transcriptional activities, forming a feed-forward loop in cancer cells [16]. *TERT* interacts with NF-κB p65, activating NF-κB target genes, including interleukin (IL)-6, tumor necrosis factor alpha, and IL-8, cytokines known to sustain inflammation and cancer progression, and upregulating matrix metalloproteinases, which are important for metastasis [16,17]. In addition, *TERT* directly interacts with the Wnt/β-catenin signaling pathway and amplifies its transcriptional output, stimulating the epithelial-mesenchymal transformation and stemness of cancer cells, which contribute to invasion and metastasis [18,19]. Therefore, *TERT* reactivation contributes to cancer development and progression by both telomere lengthening-dependent and independent mechanisms.

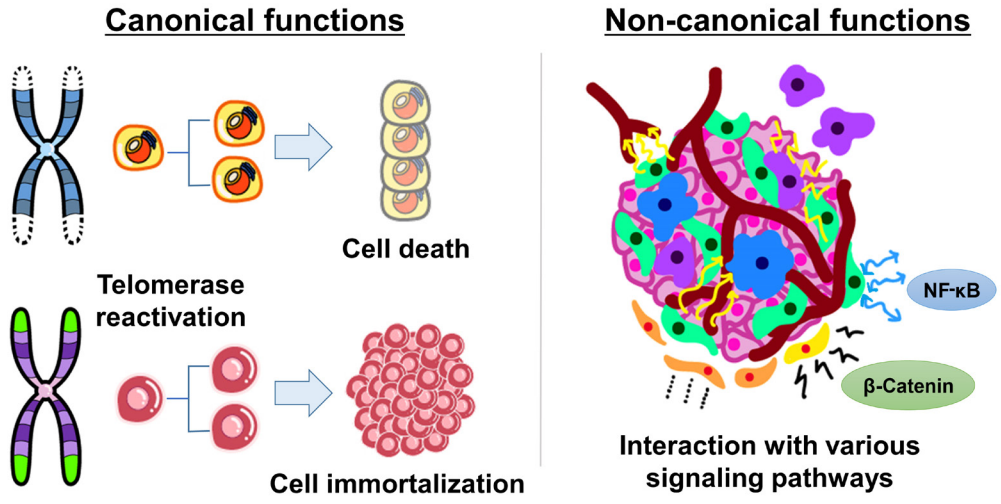

Fig. 1. Canonical and non-canonical functions of telomerase reverse transcriptase (*TERT*) in tumorigenesis. Telomerase/*TERT* reactivation causes cancer development and progression through telomere lengthening-dependent (canonical) and independent (non-canonical) mechanisms. NF-κB, nuclear factor-κB.

MECHANISMS OF *TERT* REACTIVATION IN CANCER

TERT reactivation can be caused by mechanisms including genetic (promoter mutations, amplifications, and rearrangements) and epigenetic (DNA methylation, histone acetylation/deacetylation, and non-coding RNAs) events (Fig. 2). Advances in high-throughput next-generation sequencing technologies have enabled researchers to unravel numerous genomic aberrations in various human malignancies. In an integrated analysis of 31 cancer types derived from the TCGA cohort, 95% of *TERT*-expressing samples had *TERT* aberrations, including *TERT* promoter mutations (31%), *TERT* amplification (3%), *TERT* structural variants (3%), *TERT* promoter structural variants (5%), or *TERT* promoter methylation (53%) [3].

TERT promoter mutations in human cancers

TERT promoter mutations are the most common non-coding mutations in human cancer. Since 2013, when two studies on melanoma were published [20,21], research exploring *TERT* promoter mutations in various cancers, including thyroid cancer, has robustly increased [22]. These mutations are recurrent C>T transitions occurring at chr5:1295228 (-124 or C228T) or chr5:1295250 (-146 or C250T) within the core promoter of *TERT*. *TERT* promoter mutations upregulate *TERT* transcription by creating a *de novo* binding site for E-twenty six (ETS) transcription factors [20,23] and recruiting these factors to the *TERT* promoter in a mutation-dependent manner [23,24].

The mutations can arise as in the context of malignant transformation (e.g., in the liver) [25], but overall, they represent a late event in most cancers. Tumor cells harboring *TERT* promoter mutations have short telomeres, suggesting that these mutations are a late event of tumorigenesis, after telomeres have become critically short [3,26,27]. Considering that the selective pressure for *TERT* promoter mutations should be strongest near replicative senescence after multiple cycles of replication, it is logical that these mutations are acquired late. In cancers caused by environmental exposure (e.g., skin and bladder), *TERT* promoter mutations can represent an early event [28]. Such alterations are not found in some cancer types, such as gastrointestinal or hematologic malignancies, which arise from tissues with high rates of self-renewal [3]. Because these tissues have intrinsic telomerase activity, allowing them to maintain telomere length over many cell divisions, selection for *TERT* promoter mutations may be less important for them.

In a pan-cancer study including 31 cancer types, *TERT* promoter mutations were detected in 27% (range, 0% to 89%) of the analyzed cases [3]. *TERT* promoter mutations have been demonstrated across a range of malignancies, namely in the central nervous system (89% of glioblastomas and 45% of low-

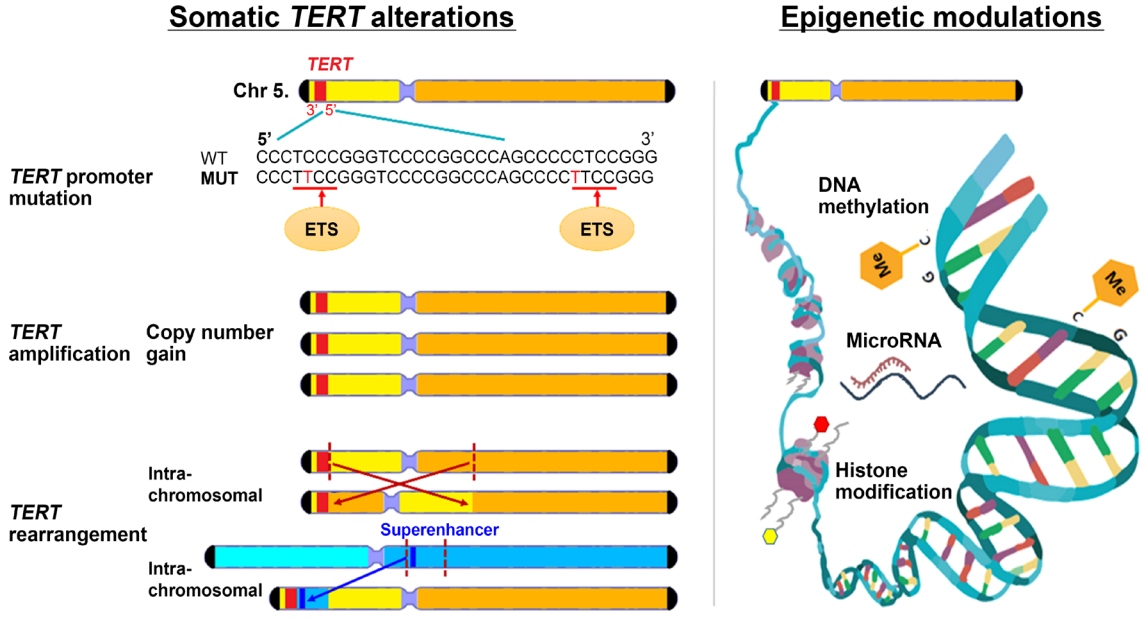

Fig. 2. Mechanisms of telomerase reverse transcriptase (*TERT*) reactivation in cancer. *TERT* reactivation can occur by somatic *TERT* alterations (promoter mutation, amplification, and rearrangement) and epigenetic modulation (DNA methylation, non-coding RNA, and histone modification). WT, wild-type; MUT, mutant; ETS, E-twenty six.

er-grade gliomas), skin (72% of melanomas), urothelial bladder (70%), liver (45%), head and neck (24%), and thyroid (10% of PTCs) [7]. The prognostic impacts of *TERT* promoter mutations and their potential use as clinical biomarkers in various cancers have been evaluated. *TERT* promoter mutations are associated with decreased overall survival in bladder cancer [29], brain tumors [30,31], melanoma [32], laryngeal cancer [33], as well as in thyroid cancer [5,34-37], with hazard ratios of 1.3 to 21.1.

TERT amplification in human cancers

The amplification of oncogenes is a frequent event in cancer and typically causes gene overexpression. Since *TERT* amplification in human cancer was first identified in 2000 [38,39], many studies have shown this genomic event in many different types of malignancies, and in recent years, copy number variations have been more successfully detected by next-generation sequencing. In the TCGA study, *TERT* focal amplifications were found in 4% (range, 0% to 22%) of all examined tumors, with high frequencies in ovarian cancer (22%), adrenocortical carcinoma (15%), esophageal cancer (14%), and non-small cell lung cancer (13%) [3]. *TERT* amplifications play an important role in the diagnosis of various solid tumors, including breast phyllodes tumors, non-small cell lung cancer, and bladder cancer. *TERT* amplifications also predict a poor prognosis in breast cancer [40,41], bladder cancer [42], non-small cell lung cancer [43], and acral-lentiginous melanoma [44].

TERT rearrangements in human cancers

TERT rearrangements involving the gene promoter or the gene body are another *TERT* reactivation mechanism that occurs in human cancers. Of particular note, in the majority of *TERT* promoter rearrangements, super-enhancers directly overlap with the juxtaposed *TERT* coding sequence. This repositioning of enhancer elements through rearrangement leads to massive transcriptional upregulation of *TERT* and strong chromatin remodeling of the affected region [45]. In support of this hypothesis, *TERT* promoter rearrangements were reported to result in the highest *TERT* expression level among somatic *TERT* aberrations [3,46]. *TERT* rearrangement with telomerase activation was first reported in an immortal fibroblast cell line in 2009 [47]. Recently, using high-throughput sequencing, structural variations involving rearrangements of the *TERT* gene have been identified in many cancer types, including neuroblastoma [45,48,49], hepatocellular carcinoma [50], kidney chromophobe cancer [51], sarcoma, and prostate cancer [3]. *TERT* rearrangements have been most comprehensively analyzed in neuroblastoma, and recurrent rearrangements proximal to the *TERT* gene have been reported in high-risk tumors with a poorer outcome [48,52].

Epigenetic mechanisms regulating *TERT* in human cancers

Epigenetic mechanisms, which consist of alterations other than direct DNA sequence changes, can modulate *TERT* transcription. *TERT* gene transcription is regulated not only by the assembly of transcription factors at promoter or enhancer regions, but also the modification of their accessibility to DNA, a process controlled by epigenetic mechanisms including DNA methylation, histone methylation, and histone acetylation [53]. The *TERT* promoter region is rich with binding motifs for multiple transcript factors including MYC proto-oncogene, specificity protein 1 (SP1), ETS, activator protein 1 (AP1), signal transducer and activator of transcription 3 (STAT3), and tumor protein p53 [54,55], and also contains binding sites for repressors such as CCCTC-binding factor (CTCF), SIN3 transcription regulator family member A (SIN3A), and MYC-associated zinc finger protein (MAZ) [56,57]. In general, hypermethylation of CpG islands of gene promoters is associated with gene silencing, while hypomethylation of CpG islands is associated with gene overexpression. However, *TERT* promoter methylation has been reported to be paradoxically correlated with *TERT* overexpression in most *TERT*-positive tumor cells, while the absence of *TERT* methylation has been found in some *TERT*-negative tumors and *TERT*-negative normal cells [58]. An explanation for this is that the unmethylated promoter sequence favors repressor binding; therefore, the hypermethylated state interferes with the binding of transcriptional repressors, resulting in upregulated *TERT* expression and telomerase activity [59]. In addition, associations between *TERT* promoter hypermethylation and poor prognoses have been reported in brain tumors [60], pancreatic [61] and prostate cancers [62], and paragangliomas [63]. Other epigenetic mechanisms including histone modifications [64-66] and non-coding RNA interactions with *TERT* [58] may also be involved in *TERT* regulation.

MECHANISMS OF *TERT* REACTIVATION IN THYROID CANCER

TERT promoter mutations in thyroid cancer

An association between *TERT* promoter mutation and increased *TERT* expression has been demonstrated in thyroid cancer [3,67,68]. The mechanism through which *TERT* expression is enhanced by *TERT* promoter mutations in thyroid cancer is that

ETS transcription factors selectively bind and activate the mutant *TERT* promoter [68,69], which is consistent with the mechanisms proven in other cancers. The prevalence of *TERT* promoter mutations is significantly higher in ATC and PDTC than in DTC [4,5]. Moreover, the *TERT* expression levels induced by promoter mutations have also been reported to be higher in ATC than in DTC, which may be due to the expansion of subclones with *TERT* promoter mutations in ATC [46,70]. In the pan-cancer study of the TCGA cohort, the proportion of samples without *TERT* expression was higher in PTC than in cancers of other organs; a total of 22% of analyzed tumors had neither detectable *TERT* expression nor ALT-related abnormalities, and 79% of PTC cases did not have them [3]. *TERT* promoter mutations are well-known to be associated with high-risk clinicopathologic characteristics and poor outcomes of DTC [5,34-37]. *TERT* expression has also been reported to be correlated with aggressive tumor behavior and a poorer prognosis in DTC, even independent of *TERT* promoter mutation status [67,71-73].

TERT amplification in thyroid cancer

Few studies have investigated *TERT* aberrations other than *TERT* promoter mutations in thyroid cancer. In a recent study of follicular thyroid tumors, *TERT* copy number gain was observed in six of 77 (8%) FTCs, four of 19 (21%) follicular tumors of uncertain malignant potential (FT-UMPs), and two of 43 (5%) follicular adenomas (FAs), and these proportions were not significantly different between groups [71]. Moreover, *TERT* copy number gain was associated with an increased risk for recurrence in FTC after adjusting for covariates. Another recent study investigated mechanisms of *TERT* activation in various types of thyroid cancer including PTC, FTC, Hürthle cell cancer (HCC), medullary thyroid cancer (MTC), and PDTC/ATCs [74]. Increased *TERT* copy numbers were found in one of 107 (0.9%) PTCs, two of 22 (9.1%) FTCs, four of 29 (13.8%) HCCs, 0 of 22 (0%) MTCs, and two of four (50.0%) PDTC/ATCs. Thus, the prevalence of *TERT* amplification was higher in aggressive cancer types, such as HCC and PDTC/ATC, although the sample size was limited. Interestingly, among thyroid cancer cases with *TERT* copy number gain, co-occurrence with *TERT* promoter mutations (C228T) was found in two of four HCC and one of two PDTC/ATC samples.

TERT rearrangements in thyroid cancer

TERT fusion genes were reported in one PTC case in the TCGA study (myotubularin-related protein 12 [*MTMR12*]-*TERT*) and in one widely-invasive FTC (wiFTC) case of our previous study (phosphodiesterase 8B [*PDE8B*]-*TERT*), which were detected using RNA-sequencing [46]. These intra-chromosomal rearrangements showed the typical characteristic of fusion genes that *TERT* expression after the breakpoint was markedly increased. Moreover, an inter-chromosomal translocation at an upstream region of *TERT* (t[2;5][2q:5p]) was demonstrated in a wiFTC case, using RNA-sequencing and whole-genome sequencing. This rearrangement remarkably enhanced *TERT* expression by super-enhancer hijacking. Like other types of cancer, *TERT* expression was notably upregulated in thyroid cancer samples with *TERT* structural rearrangements in comparison to those with promoter mutations [46].

Epigenetic mechanisms regulating *TERT* in thyroid cancer

Epigenetic mechanisms can regulate *TERT* transcriptional activity in thyroid cancer. Lee et al. [59] identified the 52 CpG-containing *TERT* hypermethylated oncological region (THOR) as a cancer-associated epigenetic mechanism of *TERT* upregulation in various human tumor types and found frequent (>45%) cancer-associated DNA hypermethylation in most cancer types. However, exceptionally, in thyroid tumors (including 31 PTCs, two FTCs, and five FAs), only one case of PTC (3%) showed high THOR methylation, while the other 97% of thyroid tumors had hypomethylated THOR. In the aforementioned study of follicular thyroid tumors, *TERT* promoter hypermethylation (methylation index >18%) was found in 25 of 77 (32%) FTCs, three of 25 (12%) FT-UMPs, and 0 of 42 (0%) FAs, and was associated with recurrence of FTC after adjusting for covariates [71].

MECHANISMS OF SYNERGISTIC INTERACTION BETWEEN *BRAF*^{V600E} AND *TERT* PROMOTER MUTATIONS IN CANCER PROGRESSION

TERT promoter mutations in thyroid cancer have been shown to be associated with the *BRAF*^{V600E} mutation, and the coexistence of these two mutations exerts a synergistic negative effect on clinical outcomes [5,35-37,75]. The co-occurrence of *BRAF*^{V600E} and *TERT* promoter mutations has been identified in thyroid cancer, melanoma, and glioma, and appears to be associated with tumor aggressiveness [76,77]. Therefore, the mechanisms underlying the interaction between these mutations have been explored (Table 1).

In a melanoma study, the ETS1 transcription factor, upregulated as a downstream target of the activated mitogen-activated protein kinase (MAPK) pathway due to *BRAF* or *NRAS* muta-

Table 1. Studies of Mechanisms Underlying the Synergistic Interactions between *BRAF*^{V600E} and *TERT* Promoter Mutations in Cancer

Study	Study subjects (*n*)	Suggested mechanism
Melanoma		
Vallarelli et al. (2016) [78]	Cell lines of melanoma (8) and melanocytes (1)	*TERT* promoter mutations form a direct link between *TERT* expression and MAPK pathway activation due to *BRAF* or *NRAS* mutations via the transcription factor ETS1 in melanoma.
Li et al. (2016) [79]	Cell lines of melanoma (7)	RAS-ERK signaling activation by *BRAF*^{V600E} or *NRAS* mutations maintains an active chromatin state at the mutant *TERT* promoters in melanoma, facilitating the recruitment of RNA polymerase II and thereby leading transcriptional activation of *TERT*.
Glioma		
Gabler et al. (2019) [80]	Cell lines of glioma (12)	*BRAF*^{V600E}-induced expression and phosphorylation of ETS1 enhance *TERT* expression and *TERT* promoter activity in gliomas with both mutations of *BRAF*^{V600E} and *TERT* promoter.
Thyroid cancer		
Liu et al. (2018) [69]	Cell lines of thyroid cancer (8), melanoma (7), colon cancer (1), embryonic kidney (1), thyrocytes (1)	*BRAF*^{V600E}/MAPK pathway promotes phosphorylation and binding of the FOS transcription factor to the GABP promoter, increasing GABPB expression and formation of the GABPA-GABPB complex. This complex selectively binds and activates the mutant *TERT* promoter, upregulating *TERT* expression in human cancer.
Song et al. (2019) [68]	Tissue samples of papillary thyroid cancer (331; 266 from TCGA and 65 from their own cohort); cell lines of thyroid cancer (8; 2 papillary and 6 anaplastic thyroid cancers) and thyrocytes (2)	ETS transcription factors, such as ETV1, ETV4, and ETV5, which are upregulated by *BRAF*^{V600E}/ MAPK pathway activation, selectively bind to the mutant *TERT* promoter in thyroid cancer. *TERT* expression is increased by the coexistence of *BRAF*^{V600E} and *TERT* promoter mutations, and the pathways related to immune response or adhesion molecules are activated by *TERT* expression.
Bullock et al. (2019) [81]	Tissue samples of normal thyroid (59) and papillary thyroid cancer (498; all from the TCGA cohort); cell lines of thyroid cancer (3; 1 papillary and 2 anaplastic thyroid cancers) and thyrocytes (1)	ETV5 is the most transcriptionally upregulated ETS gene in papillary thyroid cancer and is strongly correlated with *BRAF* and *RAS* mutational status. ETV5 preferentially binds the mutant *TERT* promoter allele and enhances *TERT* transcription, cooperating with FOXE1 to further increase *TERT* promoter activity.

TERT, telomerase reverse transcriptase; MAPK, mitogen-activated protein kinase; ETS, E-twenty six; RAS-ERK, RAS-extracellular signal-regulated kinase; FOS, fos proto-oncogene; GABP, GA-binding protein; TCGA, The Cancer Genome Atlas; ETV, ETS variant transcription factor; FOXE1, forkhead box E1.

tions, increased *TERT* transcription in melanoma cells carrying *TERT* promoter mutations [78]. Moreover, another study of melanoma showed that aberrant activation of RAS-extracellular signal-regulated kinase (ERK) signaling by *BRAF*^{V600E} or *NRAS* mutations maintained an active chromatin state and facilitated the recruitment of RNA polymerase II, leading to transcriptional activation of *TERT* at the mutant *TERT* promoter [79]. A recent study of glioma showed that, in *BRAF*^{V600E}/*TERT* promoter double-mutant gliomas, the expression and phosphorylation of ETS1 selectively binding to the mutant *TERT* promoter was downregulated by *BRAF* inhibition, and *TERT* expression was reduced by an ETS factor inhibitor [80]. Thus, this study suggested that the ETS transcription factor may be a promising therapeutic target in double-mutant gliomas.

Mechanisms of interaction between *BRAF*^{V600E} and *TERT* promoter mutations in thyroid cancer

Liu et al. [69] investigated the molecular mechanisms underlying the interactions between *BRAF*^{V600E} and *TERT* promoter mutations using cancer cell lines, primarily including thyroid cancer and melanoma cells. They reported that *BRAF*^{V600E} activated the mutant *TERT* promoter by fos proto-oncogene (FOS), which is a downstream effector of MAPK signaling, and the GA-binding protein (GABP) complex. The *BRAF*^{V600E}/MAPK pathway promoted phosphorylation and binding of the FOS transcription factor to the *GABPB* promoter, increasing GABPB expression and formation of the GABPA-GABPB complex. This complex selectively bound to the mutated *TERT* promoter and strongly upregulated *TERT* expression.

In our recent study of PTC, we confirmed that *TERT* mRNA

expression was increased by the coexistence of *BRAF*^{V600E} and *TERT* promoter mutations using RNA sequencing data from a relatively large number of PTC tumor samples (266 PTCs from TCGA and 65 PTCs from Seoul National University Hospital) [68]. The changes in the intracellular signaling pathways by *BRAF*^{V600E} were further augmented by adding the *TERT* promoter mutation, and the pathways related to inflammation or adhesion molecules were upregulated by *TERT* expression, which is consistent with one of the non-canonical roles of *TERT*—namely, activating the NF-κB signaling pathway. Moreover, among the transcription factors that can regulate *TERT* promoter activity, the expression of ETS, especially ETS variant transcription factor 1 (ETV1), ETV4, and ETV5, showed the most significant difference in *BRAF*^{V600E}-mutant PTCs. The mechanism was validated using thyroid cancer cell lines, as ETV1, ETV4, and ETV5 were upregulated by activation of the *BRAF*^{V600E}/MAPK pathway and selectively bound to the mutant *TERT* promoter.

Bullock et al. [81] also identified that ETV5 was significantly upregulated in PTCs, especially in those harboring *BRAF*^{V600E} or *RAS* mutations, and preferentially bound to the mutant *TERT* promoter with allele-specific affinity, although they investigated only a subset of ETS genes among all ETS transcription factors and did not check ETV1 and ETV4. Two subsequent studies [68,81] did not find an association between *BRAF*^{V600E} and expression of GABPA, GABPB1, or GABPB2 in PTC samples. Moreover, a recent study found that GABPA was negatively associated with *TERT* expression and promoter mutations, and acted as a tumor suppressor in thyroid cancer [82]. This discrepancy between *in vitro* cell lines and tumors from PTC patients suggests a more complicated relationship of *TERT* promoter mutations with the ETS transcription factor family, which requires further investigation. However, it is at least recognized that the mechanism of synergistic effects between *TERT* promoter and *BRAF*^{V600E} mutations on the aggressiveness of thyroid cancer may be explained by the activation of *TERT* transcription and telomerase activity, which results from binding of *BRAF*^{V600E}-induced ETS transcription factors to the mutant *TERT* promoter.

CONCLUSIONS

Since telomerase repression is a powerful mechanism of tumor suppression, *TERT* reactivation is found in most human cancers. *TERT* reactivation occurs by somatic *TERT* alterations and epigenetic modulations. Moreover, the recruitment, accessibility, and binding of transcription factors as activators or repressors also affect the regulation of *TERT* expression (Fig. 3).

TERT promoter mutations are the most frequent non-coding alterations in human cancer, and have been detected in various types of cancer, including thyroid cancer. Other *TERT* aberrations (*TERT* amplification, rearrangement, and *TERT* promoter

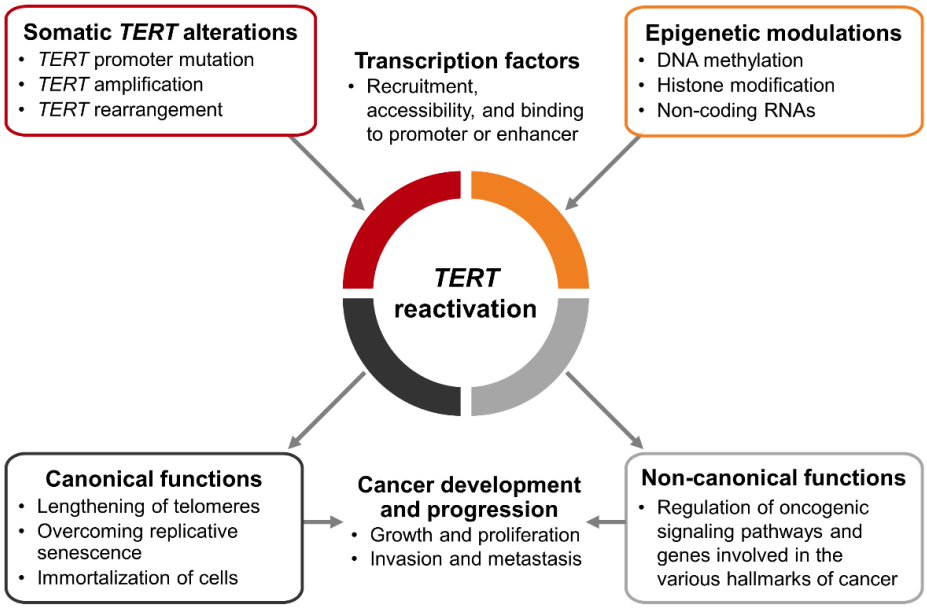

Fig. 3. Schematic representation of the mechanisms related to the causes and effects of how telomerase reverse transcriptase (*TERT*) reactivation leads to cancer development and progression.

methylation) involved in telomerase activation are relatively less studied than *TERT* promoter mutations in thyroid cancer, and further research is therefore needed.

TERT reactivation causes cancer development and progression through telomere lengthening-dependent and independent ways, and has been reported to be associated with poor prognoses in various cancers. In melanoma, glioma, and thyroid cancer, in which the coexistence of $BRAF^{V600E}$ and *TERT* promoter mutations has been documented and investigated, these two mutations show synergistic interactions that contribute to poor outcomes. As the mechanism underlying this synergism, it has been demonstrated that activation of the MAPK signaling pathway by the $BRAF^{V600E}$ mutation creates an active chromatic state or enhances binding of ETS transcription factors at the mutant *TERT* promoter, thereby increasing *TERT* expression and telomerase activity. Understanding the mechanisms of *TERT* reactivation is important, as these insights could be used to prevent and treat refractory cancers with increased *TERT* expression.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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