Tumorigenic effect of *TERT* and its potential therapeutic target in NSCLC (Review)

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Abstract. Non-small cell lung cancer (NSCLC), which accounts for ~85% of all lung cancer cases, is commonly diagnosed at an advanced stage and has a high patient mortality rate. Despite the increasing availability of treatment strategies, the prognosis of patients with NSCLC remains poor, with a low 5-year survival rate. This poor prognosis may be associated with the tumor heterogeneity of NSCLC, as well as its acquisition and intrinsic resistance to therapeutic drugs. It has been suggested that combination therapy with telomerase inhibition may be an effective strategy for the treatment of drug-sensitive and drug-resistant types of cancer. Telomerase is the key enzyme for cell survival, and ~90% of human cancers maintain telomeres by activating telomerase, which is driven by the upregulation of telomerase reverse transcriptase (*TERT*). Several mechanisms of telomerase reactivation have been described in a variety of cancer types, including *TERT* promoter mutation, epigenetic modifications via a *TERT* promoter, *TERT* amplification, and *TERT* rearrangement. The aim of the present study was to comprehensively review telomerase activity and its association with the clinical characteristics and prognosis of NSCLC, as well as analyze the potential mechanism via which *TERT* activates telomerase and determine its potential clinical application in NSCLC. More importantly, current treatment strategies targeting *TERT* in NSCLC have been summarized with the aim to promote discovery of novel strategies for the future treatment of NSCLC.

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Abbreviations: NSCLC, non-small cell lung cancer; TERT, telomerase reverse transcriptase; hTERT, human telomerase reverse transcriptase; SCLC, small cell lung cancer; HTERC, human telomerase RNA; Skp2, S-phase kinase-associated protein 2; TTF-1, thyroid transcription factor-1; ER, estrogen receptor; SP1, specificity protein 1; NF-κB, nuclear transcription factor κB; AP1, transcription factor activator protein 1; GABPA/B1, GA-binding protein transcription factor, α subunit/β subunit 1; H3K4me2/3, histone3 lysine 4 di/trimethyl; H3K27me3, histone H3 lysine 27 tri-methylation; EST, E-twenty-six binding site; SNP, single nucleotide polymorphism; EGFR, epidermal growth factor receptor; THOR, TERT hypermethylated tumor region; HDAC, histone deacetylase; HCC, hepatocellular carcinoma; TP53, tumor protein 53; ALK, anaplastic lymphoma kinase; 6-thio-dg, 6-thio-2'-deoxyguanosine; HIV1, human immunodeficiency virus 1; PFS, progression-free survival; OS, overall survival; ATM/CHK1, ataxia-telangiectasia-mutated/checkpoint kinase 1; HLA, human leukocyte antigen; MHC, major histocompatibility complex; APC, antigen-presenting cell; RFPL3, Ret finger protein like 3; CTL, cytotoxic lymphocyte; EGF, epidermal growth factor; CBP, CREB-binding protein

Key words: non-small cell lung cancer, telomerase reverse transcriptase, telomerase, telomerase inhibitors, prognosis

Contents

1. Introduction

Lung cancer seriously endangers human health, and has the highest morbidity and mortality rates among all malignant tumors worldwide (1). While the mechanisms underlying lung cancer pathogenesis have not been fully elucidated (2), previous studies have revealed that it may be associated with

tobacco consumption, living environment, genetic factors and the abnormal regulation of oncogenes and tumor-suppressor genes (3-5).

According to its histological characteristics, lung cancer is mainly divided into two major categories: Small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), which account for ~15 and ~85% cases, respectively (6). In the early stage of NSCLC, surgical treatment can achieve the best prognosis for patients (7). Unfortunately, the majority of patients diagnosed with NSCLC are at an advanced stage and therefore require comprehensive treatment. Over the past few decades, while the development of novel treatments, innovative surgical procedures and effective clinical management have improved the survival of patients with NSCLC to some extent, the 5-year survival rate remains poor at 10-15% (8-11). Therefore, improvements in therapeutic strategies are urgently required. Moreover, the discovery of molecular heterogeneity in NSCLC suggests the complexity of this cancer (12).

Basic biology suggests that telomere maintenance is an attractive candidate mechanism for potential cancer risk (13). The telomere is a special heterochromatic structure consisting of repetitive nucleotide sequences (TTAGGG) and a telomere-associated protein complex at the end of the linear chromosome, referred to as Shelterin. Moreover, it can protect the ends of chromosomes from end-to-end fusion and degradation, and then serves an important role in ensuring genomic stability and integrity (14). The length and stability of telomeres can determine the cell lifespan and are closely associated with cellular aging and tumor formation (15). In most normal cells, the telomere becomes progressively shorter after each cell division, and when it is shortened to a certain critical point, cells stop dividing and cellular senescence is triggered. This is considered as a strong tumor suppressor mechanism in humans (16-18). However, most immortal tumor cells can overcome their fate of senescence via telomere length maintenance, mainly by expressing or re-activating telomerase, which adds nucleotides to the ends of telomeres to extend the telomere length and cell proliferative potential (19).

Telomerase is a ribonucleoprotein enzyme composed of multi-subunits, and its core holoenzyme includes a catalytic protein subunit referred to as human telomerase reverse transcriptase (hTERT encoded by the *TERT* gene on chromosome 5p15.33) and an RNA component known as human telomerase RNA (hTERC encoded by *TERC* gene on chromosomal region 3q26) (20,21). The *TERT* gene is stringently repressed, which consequently leads to telomerase silencing. Therefore, *TERT* regulation is the rate-limiting factor for telomerase activity (22). Previous studies have demonstrated that, compared with TERC, the correlation between *hTERT* expression and telomerase has a consistency of 88.9% (23). Telomerase activity is hardly detectable in most somatic cells, apart from those in the spinal cord and peripheral blood or bone marrow (24). However, *TERT* expression and telomerase activity can be detectable in up to 90% of tumor tissues, which may cause immortal cells to sustain tumor growth (19). Thus, targeting telomerase is more universal compared with most other cancer targets, and it represents a critical target specific to cancer cells.

Accumulating evidence has shown that genetic factors serve an important role in regulating *TERT* expression, which is associated with *TERT* point mutations, DNA amplification, rearrangement and transcription, and predicted telomerase activity (25-28).

The aim of the present review was to summarized the clinical significance of telomerase activity in NSCLC and discuss the mechanism underlying telomerase activity regulation by *TERT*, as well as its effect on NSCLC. The mechanism of action and the current research progress of targeted telomerase drugs in NSCLC were also evaluated.

2. Telomerase activity and *hTERT* mRNA in NSCLC

As aforementioned, increased telomerase activity has been observed in various types of human malignancies, including NSCLC tissues. Several studies have reported that *hTERT* mRNA and telomerase activity were significantly higher in cancerous tissue compared with those in the lung parenchyma free from neoplastic infiltration (29-32), which confirms that telomerase activity may serve an important role in the tumorigenesis of lung cancer. A large number of early studies examined the possibility of using telomerase activity as a tumor marker in NSCLC, and the expression rates of telomerase in each study are presented in Table I. Moreover, the correlation between telomerase activity and clinical characteristics of NSCLC has been extensively investigated (Table I), indicating that telomerase activity is significantly associated with the prognosis of patients with NSCLC and may be used as a diagnostic or prognostic indicator for these patients (31,33-39). In general, it has been suggested that telomerase activity is independent of age, sex, smoking history, and the histological characteristics of the tumor, but it was found to be significantly associated with tumor stage (34,36-38,40,41), the grade of differentiation (32,38-40) and lymph node metastasis (31,36,41). However, in other studies, it was indicated that the stage of tumor status was not associated with telomerase activity (33,42).

NSCLC, non-small cell lung cancer. The expression of *hTERT* mRNA in lung cancer is frequently detected (29,34,42,43). Thus, the correlation between *hTERT* mRNA and the clinical characteristics, diagnosis and prognosis of patients with NSCLC has also been widely reported. For example, a previous study identified a positive correlation between telomerase activity and *hTERT* expression level (34). Moreover, Metzger *et al* (42) found that telomerase activity is significantly higher than *hTERT* mRNA expression in patients with NSCLC. It has also been reported that *hTERT* is upregulated in the peripheral blood of patients with NSCLC, and shows high specificity and sensitivity in the diagnosis of NSCLC. A combination of s-phase kinase-associated protein 2 (Skp2) and thyroid transcription factor-1 (TTF-1), was found to significantly improve the sensitivity and accuracy of diagnosis, and can be used as a diagnostic indicator of NSCLC (36,44). Unlike telomerase activity which is unrelated to clinical characteristics, it was found that *hTERT* was a significantly associated with histological classification rather than tumor grade (42). *hTERT* mRNA expression differs across the pathological types of NSCLC (45), and a low *hTERT* mRNA expression was found to be significantly correlated with lung squamous cell carcinoma (42). However, the prognostic significance of

Table I. Detection rate of telomerase activity and its relationship with the clinical characteristics and prognosis in NSCLC.

Rate, % (no./total)	Clinical characteristics	Prognosis in NSCLC	(Refs.)
60.3 (47/78)	The activity was mostly associated with late stage		(40)
95.7 (45/47)	The telomerase activity was higher in the low differentiation group	There was no association with 2-year survival, and it was not possible to determine whether telomerase activity could be a prognostic factor	(30)
75 (36/48)	The telomerase activity was associated with lymph node metastasis	Markers that can be used to evaluate the occurrence, development and diagnosis of tumors	(31)
86.7 (13/15)		May serve as a tumor-specific marker	(32)
75 (27/36)	The telomerase activity was independent of tumor stage and differentiation	May serve as a diagnostic marker	(33)
77.6 (45/58)	The telomerase activity was associated with tumor stage	Is associated with apoptosis disorder and has prognostic significance	(34)
86.6 (123/142)		May be used as a prognostic marker	(35)
75.8 (42/62)	The telomerase activity was associated with lymph node metastasis, tumor differentiation and stage	May be used as a diagnostic marker of malignant diseases, and the prognostic marker was not described	(36)
66 (40/60)	The telomerase activity was mostly associated with late-stage disease and poor differentiation	May be used as a prognostic indicator	(37)
82.5 (54/103)	The activity was mostly associated with late stage and poorly differentiated groups	Telomerase activity was an independent prognostic factor	(38)
85.3 (58/68)	Telomerase activity increased in patients with lymph node metastasis and advanced stage	Telomerase activity was positively correlated with the risk of lung cancer recurrence and mortality and may be used as a prognostic indicator	(41)

NSCLC, non-small cell lung cancer.

hTERT expression in NSCLC remains controversial among researchers. For example, Metzger *et al* (42) revealed that high *hTERT* mRNA expression was associated with 5-year survival rates. Furthermore, Wang *et al* (34,46) reported that the mRNA expression level of *hTERT* and apoptotic index were associated with clinical outcome, suggesting that *hTERT* mRNA has prognostic significance in patients with NSCLC. However, another study suggested that *hTERT* has diagnostic significance, but cannot be used as a prognostic indicator (45).

3. Regulatory mechanism of *TERT* regarding telomerase activity in NSCLC

TERT promoter mutation in NSCLC. The *TERT* promoter is a 260 bp region (-1,800 to +2,300 relative to ATG) and its core sequence is located at 330 bp upstream and 37 bp downstream from ATG. The promoter region is rich in GC, and lacks TATA and TCCA boxes; however, it contains multiple binding sites that regulate gene transcription and are responsible for the transcriptional activity of *TERT* and the activation of telomerase (47,48). Somatic mutations in the promoter of the *TERT* gene are the most common non-coding mutations in cancer (49). In a comprehensive analysis of The Cancer Genome Atlas (TCGA) dataset for 31 types of cancer, 27% of the analyzed samples contained one of these promoter mutations (25). It has also been suggested that Myc, estrogen receptor (ER), specificity protein 1 (SP1), nuclear transcription factor κB (NF-κB), p53, transcription factor activator protein 1 (AP1) and E2F have binding sites in this region and regulate the expression of *TERT* (47,50). Furthermore, the core promoter mutation of the *TERT* gene creates new binding sites for E-twenty-six (ETS) transcription factors, which provides a mechanism for telomerase with regards to cancer-specific telomerase reactivation (51-54). *TERT* promoter mutations occur predominantly at two hot spots, C228T and C250T, based on their genomic coordinates (Fig. 1) (55) Several reports have shown that tumors with either mutation tend to express higher levels of *TERT* and increased telomerase activity, thus suggesting a stimulatory effect on *TERT* expression (25). A study by Stern *et al* (49) revealed that *TERT* promoter mutations recruit GABPA/B (GA-binding protein transcription factor, α subunit/β subunit 1 and exhibit histone3 lysine4 di/trimethyl markers of chromatin activity, whereas wild-type cell lines retain epigenetic silencing markers-histone H3 lysine 27 tri-methylation, which confirms that only the mutant promoters are transcriptionally active. Activating mutations in the *TERT* promoter maintain telomere length and genomic stability by activating telomerase expression, thereby allowing

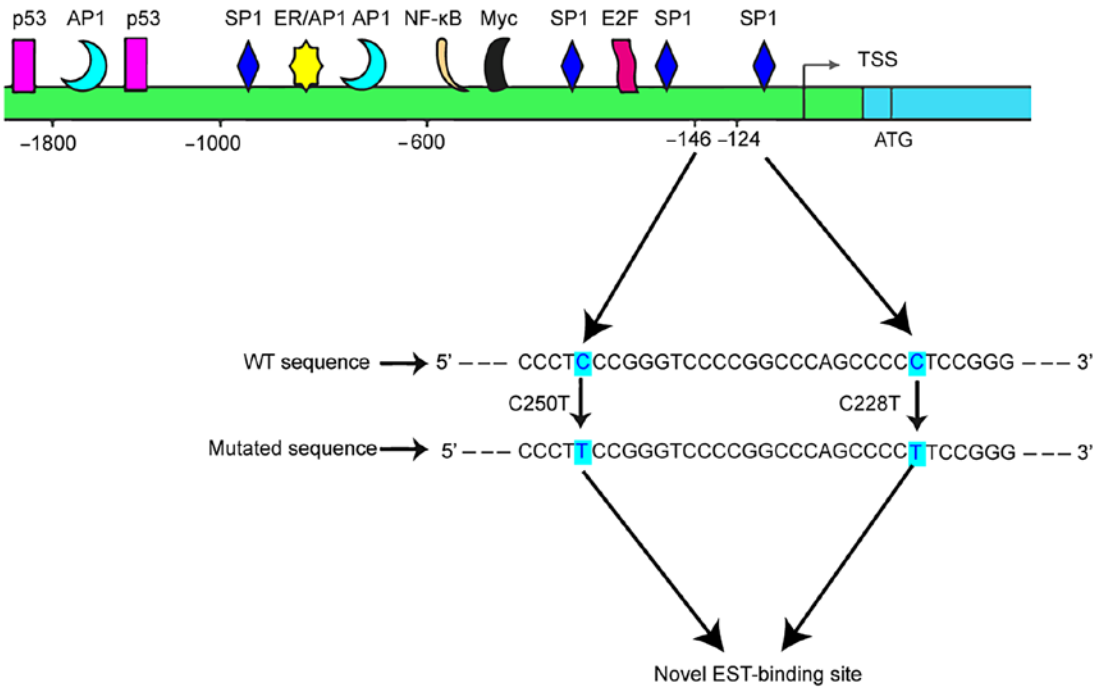

Figure 1. *TERT* gene promoter binding sites and common promoter mutation points. Myc, ER, SP1, NF-κB, p53, AP1 and E2F regulate *TERT* expression at the binding sites in this region. ATG is the first codon, and the blue region is the *TERT* gene coding region. The green region is the *TERT* gene promoter region. TSS: Transcription start site. C228T and C250T are *TERT* promoter mutations that occur primarily in two hot spots to create new E-twenty-six binding site (EST) transcription factors. ER, estrogen receptor; SP1, specificity protein 1; AP1, transcription factor activator protein 1; TSS, transcription start site; EST, E-twenty-six binding site; TERT, telomerase reverse transcriptase.

malignant cells to continue to divide preventing senescence or apoptosis (56). However, some cancer types lack *TERT* promoter mutations, but telomerase activity is detected, suggesting that other mechanisms of telomerase activation may be involved in *hTERT*-dependent tumors lacking *TERT* promoter mutations.

The *TERT* promoter mutation was first identified in melanoma and, subsequently, in common types of cancer such as central nervous system, bladder and thyroid cancers (50). However, the frequency of the *TERT* promoter mutation varies significantly among different cancer types. An early study on *TERT* promoter mutations in 60 different cancer types revealed that tumors can be categorized either into low (<15%) or high (≥15%) mutation types, depending on the frequencies (57). The mutation of the promoter usually occurs in cancers with low self-renewal rates, such as liver cancer, melanoma, brain tumor and low-grade bladder cancer, which may trigger telomerase activation (57). Current studies have reported differences in the frequency of *TERT* promoter mutations by type of cancer and histology. Several studies have analyzed the role of the *TERT* promoter mutation in the etiology of various types of cancer, and have reported inconsistent results. A meta-analysis was conducted to examine the relationship between *TERT* promoter mutation and patient age, sex, tumor stage, metastatic status and cancer prognosis [5-year overall survival (OS) rate]. The results demonstrated that the *TERT* promoter mutation was associated with sex, age and distant metastasis and, thus, it was suggested that the *TERT* promoter mutation may be a poor prognostic factor for patients with cancer (58).

Currently, the majority of studies report the prevalence and the prognostic characteristics of the *TERT* promoter mutation in NSCLC and its potential as a clinical biomarker. It has been demonstrated that *TERT* promoter mutations have a low frequency in a very small proportion of patients with NSCLC, whereas some studies did not identify mutations in the *TERT* promoter (55,58-61) and suggested that it may be a prognostic marker for NSCLC patients. For example, Ma *et al* (55) revealed that *TERT* promoter mutations occurred repeatedly in 2.57% of patients with NSCLC, and most mutations were observed in elderly patients. Yuan *et al* (58) reported similar findings, suggesting that the *TERT* promoter mutation was clinically age related and that the incidence of the *TERT* promoter mutation was very low, at ~5.8%. Moreover, Jung *et al* (26) studied the regional mutations and clinical characteristics of the *TERT* promoter. The authors found that the rate of the *TERT* promoter mutation was 2.2% (4/188 NSCLC cases), which was closely associated with regional lymph node infiltration (P<0.01), and further survival analysis suggested a poor prognosis.

Single-nucleotide polymorphisms (SNPs) are DNA sequence polymorphisms caused by variations in a single nucleotide at the genomic level (62). The rs2853669 SNP is located in the promoter region of the *TERT* gene, and it is significantly associated with telomere and survival in NSCLC cases with an *EGFR* mutation (63). Moreover, the rs2853669 T/C allele is significantly associated with a shorter relative telomere length (as opposed to C/C and T/T; P=0.039 and P=0.023, respectively, in patients without *EGFR* mutations) and lower *TERT* mRNA expression (compared with C/C and *T/T*; both P<0.001) (63).

Epigenetic regulation of the TERT promoter in NSCLC. *TERT* promoter epigenetic regulation is another mechanism

associated with *TERT* expression dysregulation (25), and basic epigenetic regulations of *TERT* expression include histone methylation, DNA methylation and histone acetylation (64). The promoter region of the *TERT* gene has a dense CG-rich CpG island, suggesting a role for methylation in the regulation of *hTERT* expression. DNA methylation of the *TERT* gene promoter is emerging as a powerful epigenetic biomarker in cancer diagnosis (65). Earlier studies analyzed the methylation status of 27 CpG sites within the *TERT* promoter core region. The results revealed that *TERT* promoter hypermethylation was positively correlated with *hTERT* mRNA expression and telomerase activity, indicating that methylation may be involved in the regulation of *hTERT* expression (66). In contrast to normal DNA methylation, the *TERT* promoter region has a non-methylated/hypomethylated CpG island in normal somatic cells, while telomerase-positive cells have a partially or totally methylated promoter region (64,67).

In a CpG island methylation-dependent and non-methylation mode, *hTERT* expression can be regulated at multiple levels (67). Devereux *et al* (67) analyzed the regulation of the *TERT* expression by detecting the CpG site methylation status of 37 different cell lines from normal and cancerous tissues. It was found that the promoters of most *hTERT*-negative normal cells and a few *hTERT*-expressing cell lines were unmethylated/hypomethylated, while most *hTERT*-expressing cell lines were partially or completely methylated. Moreover, a methylation inhibitor was added to the promoter methylation status of the *TERT*-negative cell line, and *TERT* expression was observed. These findings suggest that methylation can negatively regulate *hTERT* expression (67).

Epigenetic therapy has recently become a popular and promising treatment for a variety of cancer types. Among these therapies, histone deacetylase (HDAC) inhibition has been attracting considerable attention (28,68,69). Several studies have shown that HDAC inhibitors, such as chidamide and vorinostat, may inhibit telomerase activity via epigenetic alteration in NSCLC cells (70,71).

The *TERT* gene has been published as an epigenetic marker. In a previous study, the methylation patterns of formalin-fixed paraffin-embedded specimens from 144 NSCLC cases and 7 healthy controls were analyzed. The results demonstrated that the *TERT* methylation levels were significantly altered in NSCLC tissues, whereas 12 NSCLC cell lines were identified as having promoter methylation (72). Therefore, it was suggested that the *TERT* gene has potential as an epigenetic marker, which can be used as a diagnostic or prognostic marker. However, further research is required to elucidate its regulatory pathways.

TERT amplification in NSCLC. The gene amplification function is one of the most common mechanisms of oncogene activation (73). *TERT* gene amplification has been observed in some tumor cells including lung cancer, cervical tumors, breast cancers, and neuroblastomas, suggesting that the *TERT* site may be a common amplification target during oncogenesis (74). *TERT* amplification may be one factor causing the *hTERT* upregulation and telomerase activation in cancer (73,74). For example, Takuma *et al* (75) reported that *TERT* gene amplification appeared to serve a critical role in inducing *hTERT* mRNA in hepatocellular carcinoma, but no significant correlation was identified between h*TERT* gene amplification and its mRNA expression. Some studies have suggested that low-level amplification may be the reason for the lack of correlation with its expression (76,77).

TERT DNA copy number gain may be detected in the early stage of NSCLC (78). *TERT* amplification is prevalent in lung squamous cell carcinoma and lung adenocarcinoma, plays an important role in the diagnosis of NSCLC and may be considered as a poor prognostic marker (73,78). Zhu *et al* (73) reported that *hTERT* upregulation is significantly associated with *TERT* amplification in NSCLC (P=0.03), and that the *TERT* gene amplification is a prognostic factor for short-term recurrent-free survival (hazard ratio=2.16; P=0.03). Moreover, Alidousty *et al* (79) revealed that co-mutation of tumor protein 53 (*TP53*) and anaplastic lymphoma kinase *(ALK*) in NSCLC can lead to chromosome instability and 5% of patients with the *TP53* mutation have *TERT* gene amplification. To understand the prevalence and biological role of *TERT* amplification in adenocarcinoma with *ALK* translocation, the study found that *TERT* gene amplification was present in 5 (4.6%) of the 109 ALK^+ cases, and these patients had genomic instability (79).

TERT rearrangements. The mechanism of gene structure rearrangement involves a DNA double-strand break repair process, in which complex conversational transfer of repeating units occurs within or between alleles. Genomic rearrangements can result in inverted orientations, tandem duplications, interchromosomal changes, deletions, and amplification. Furthermore, genomic rearrangement can affect the chromosomal region of the 5p15.33 proximal *TERT* gene (80). Rearrangements involving *TERT* caused by hundreds of tandem duplications and templated insertions activate the *TERT* promoter and induce a strong upregulation of *TERT* (81). The *TERT* coding sequence can be juxtaposed with a strong enhancer element via the rearrangement of *TERT*, resulting in extensive chromatin remodeling and DNA methylation in affected regions (80). Moreover, Peifer *et al* (80) observed that the remodeling of the genomic environment eliminated transcriptional silencing of *TERT* and placed telomerase activation at the center of this tumor transformation. *TERT* rearrangement in neuroblastoma suggests a poor prognosis and is associated with other telomere maintenance mechanisms, including alternative lengthening of telomeres and *MYCN* amplification (82). However, further studies are required to understand whether *TERT* rearrangement occurs in NSCLC and its potential clinical implications.

4. Current telomerase-targeted therapies in NSCLC

Standard and targeted cancer therapies are almost universally failing in patients with advanced cancer due to plasticity/heterogeneity of the tumor and acquired or intrinsic drug resistance (83). Previous studies have reported that combination therapy with drug telomerase inhibition may be an effective strategy for the treatment of drug-sensitive and drug-resistant cancer types (83-86). Telomerase is silenced in normal cells, but is reactivated in 90% of tumor cells (19); thus, it is considered an attractive target for cancer therapies. Moreover, targeting telomerase is more pervasive, specific, and critical to cancer cells compared with most other targets.

Table II. Telomerase inhibitors in NSCLC.

Agent	Type	Target	Mechanism	Therapeutic mechanism in NSCLC	(Refs.)
6-thio-dg	Nucleoside analogue (telomerase substrate precursor)	Telomere	Incorporated into telomeres by telomerase and causes telomere dysfunction	Prolongs the time of disease control in patients with drug-resistant NSCLC	(88)
BIBR1532	Non-competitive inhibitor of small-molecule telomerase	Telomerase (*TERT* and *TERC*)	Interferes with telomerase processing	Increases the sensitivity of *KRAS*-mutant Calu-3 cells to chemotherapy drugs; Increased radio-sensitivity of NSCLC cells	(89-91)
Imetelstat	Competitive inhibitor of telomerase activity telomerase	Template region or the active site of the	Causes telomere shortening	Short telomeres were more sensitive compared with long telomeres	(92-94)
GV1001/ Vx-001	Telomerase vaccines	Immune system	Induces a specific immune response	Immunogenic and safe for patients with NSCLC	(100)

NSCLC, non-small cell lung cancer; 6-thio-dg, 6-thio-2'-deoxyguanosine; *TERT*, telomerase reverse transcriptase.

Telomerase complexes provide multiple potential sites for inhibitor development (87). In NSCLC, different telomerase inhibitors including 6-thio-2'-deoxyguanosine (6-thio-dg), BIBR1532, imetelstat, and telomerase-derived peptide, destroy or block different components and action pathways of telomerase, thereby blocking telomerase activity and ultimately limiting the growth and development of tumors (Table II). In addition, several telomerase inhibitors have been used in preclinical models and clinical trials (Table III).

6-thio-dg. 6-thio-dg is a nucleoside analogue and telomerase substrate recognized by telomerase, and it is incorporated into newly synthesized telomeres. 6-thio-dg causes telomere dysfunction and rapid cell death in tumor cells that are telomerase-positive and in fibroblasts expressing hTERT, but not in telomerase-negative cells (88). Moreover, 6-thio-dg may be a novel telomerase-dependent anticancer therapy (83,86). In a mouse xenograft study based on A549 lung cancer cells, 6-thio-dg reduced tumor growth and this effect was more pronounced compared with that induced by 6-thioguanine treatment (88). Mender *et al* (83) used human xenograft, syngeneic and genetically engineered mouse lung cancer models to detect the effects of 6-thio-dg on targeted therapy of chemotherapy-resistant human lung cancer cells and mouse models. The results revealed that erlotinib-, gemcitabine/cisplatin- and paclitaxel/carboplatin-resistant cells were highly sensitive to 6-thio-dg, which indicated that 6-thio-dg could prolong disease control with minimal toxicity in patients with drug-resistant lung cancer (83).

BIBR1532. BIBR1532 is a potent, selective, and non-competitive small-molecule inhibitor of telomerase that induces senescence in human cancer cells. Its mechanism is similar to that of non-nuclear human immunodeficiency virus 1 (HIV1) reserve transcriptase inhibitors (84,89). BIBR1532 inhibits natural and recombinant human telomerase, including the human telomerase RNA components and TERT, by interfering with the enzyme's processing ability (89). A study of the effects of BIBR1532 combined with chemotherapy on drug-resistant leukemia and breast cancer cells and their parental cells revealed that cells treated with BIBR1532 exhibited gradually shortened telomeres, had a reduced proliferative ability and were sensitive to chemotherapy (84). It has been shown that a *KRAS* mutation can increase telomerase activity, *hTERT* expression and telomere length in lung adenocarcinoma cells (Calu-3 cell line) (90). However, BIBR1532 reduced telomere length and inhibited the proliferation, colony formation and migration of *Kras*-mutant Calu-3 cells. Specifically, BIBR1532 increased the sensitivity of *Kras*-mutant Calu-3 cells to chemotherapy drugs (90).

BIBR1532 has the potential to be used as a radiosensitizer in the clinical setting (91). Ding *et al* (91) studied the effect of BIBR1532 on the NSCLC cellular response to radio-sensitization and observed increased IR-induced telomere dysfunction, disruption of chromosomal stability, inhibition of the ataxia-telangiectasia-mutated/checkpoint kinase 1 (ATM/CHK1) pathway, and reduced DNA damage repair. This study also demonstrated that BIBR1532 at non-toxic dose level, interfered with telomerase function and effectively enhanced the radio-sensitivity of NSCLC cells.

Imetelstat. Imetelstat is a competitive inhibitor of telomerase activity, which leads to the shortening of telomeres in most cancer cells (92-94), thereby reducing tumor growth. Imetelstat considers the template region or the active site of the telomerase as the target, and directly binds to the RNA component of the telomerase at the active site of the enzyme (92,95,96). Imetelstat has been shown to be effective *in vivo* in the treatment of lung metastasis in xenograft animal models (80). Moreover, Frink *et al* (94) studied the efficacy of imetelstat in NSCLC

Table III. Telomerase therapeutics currently undergoing clinical trials.

Agent	NCT identifier	Trial	Results	(Refs.)
Imetelstat	NCT01137968	Phase II	Imetelstat maintenance therapy did not improve PFS in patients with advanced NSCLC after first-line treatment	(97)
GV1001	NCT00509457	Phase I/II	Well-tolerated, minor side effects, no bone marrow toxicities; Immune response in 13/24 patients	(99)
GV1001	NCT01579188	Phase II	Well-tolerated; Immune response developed in 16/20 patients	(99)
Vx-001	NCT01935154	Phase II: NSCLC	Failed to meet primary endpoint (median OS: 11.3 and 14.3 months for the placebo and the Vx-001, respectively)	(106)

NSCLC, non-small cell lung cancer; OS, overall survival; PFS, progression-free survival.

cell lines, and found that the short telomeres were more sensitive compared with the long telomeres, and that continuous application led to inhibition of continuous telomere shortening and eventual growth inhibition. A clinical trial evaluated imetelstat as a transformation maintenance therapy in patients with advanced NSCLC. The results demonstrated that patients with short telomeres administered imetelstat treatment tended to have longer median progression-free survival (PFS) and overall survival (OS); however, patients with long telomeres given imetelstat treatment had no improvement in median PFS or OS. Furthermore, maintenance therapy with imetelstat did not improve PFS in patients with advanced NSCLC after first-line treatment (97). Therefore, the clinical development of imetelstat in NSCLC requires further investigation.

Telomerase-derived peptide (GV1001 and Vx-001). The first step in cancer immunotherapy is to identify the target tumor-associated antigens. The telomerase-derived peptide is one of the tumor-associated antigens that bind to human leukocyte antigen class I and class II molecules, effectively activating $CD8^+$ and $CD4^+$ T-cell subsets, and telomerase is widely expressed in tumors (Fig. 2) (98). Therefore, telomerase is an attractive target antigen for cancer immunotherapy. The safety, immune response and antitumor effects of several vaccines based on telomerase-derived peptides have been evaluated in numerous types of cancer (99). In total, three hTERT vaccines, GV1001, Vx-001 and GRNVAC1, have been successfully used to induce anti-telomerase immune responses in cancer patients (100), and GV1001 and Vx-001 have also been studied in NSCLC.

An early phase I/II study examined the safety, tolerability and clinical response of a combination of telomerase peptide GV1001 (hTERT: 611-626) and HR2822 (hTERT: 540-548) in patients with NSCLC, and observed that these were both immunogenic and safe for patients with NSCLC. In addition, the induction of a GV1001-specific immune response may lead to an objective tumor response (101). A phase III trial in patients vaccinated following radiation and chemotherapy, and an 8-year update on a previous I/II trial in patients with NSCLC after inoculation of telomerase peptide GV1001, revealed that the patients' immune tolerance was good, and there was immunity in most patients with NSCLC and the establishment of a lasting memory T cells. The high immune response rate and low toxicity support the concept of combining chemoradiotherapy with immunization (99). Other studies reported that T-regulated cells and myeloid-derived suppressor cells are associated with the impaired clinical efficacy of the vaccine response and the environment of toggle-induced cytokines (102).

Vx-001 is a restricted telomerase-specific antitumor vaccine, which is composed of a 9-mer Cryptic TERT (572) peptide and its optimized variant TERT (572Y). Early studies have shown that the Vx-001 vaccine is well-tolerated and can induce T-cell-specific immune response, which is closely associated with an improvement in clinical prognosis (103-105).

A randomized, double-blind, phase II clinical trial examined the clinical activity of the Vx-001 cancer vaccine as maintenance immunotherapy following chemotherapy in patients with stage IV NSCLC. The primary endpoint of the study was OS. The results did not reach the primary endpoint, and it was found that Vx-001 significantly prolonged the survival of patients with NSCLC. The median OS was 14.3 months in the vaccine Vx-001 patients vs. 11.3 months in patients in the placebo groups. The 6-month disease control rates were 33.7 vs. 25.7% in the vaccine Vx-001 group vs. the placebo groups, respectively. OS was significantly prolonged in 29.2% of the vaccinated patients compared with those who did not respond. These authors suggested that Vx-001 induced a specific $CD8^+$ T-cell immune response (106).

5. Discussion

A powerful antitumor strategy in humans involves inhibiting telomerase and maintaining shorter telomeres over longer evolutionary periods. Moreover, abnormal *TERT* gene disinhibition/telomerase reactivation is key to the malignant transformation of human cells (107).

Recent studies have reported that telomerase expression and patterns are unique among histopathological types of lung cancer and can predict the prognosis of patients (108). Telomerase can be used as a predictor of disease recurrence and cancer-related death in patients with early NSCLC after surgery (109). As aforementioned, numerous studies have revealed that telomerase and *hTERT* can be used as diagnostic markers of NSCLC. Although most studies suggest that these factors can also be used as prognostic indicators,

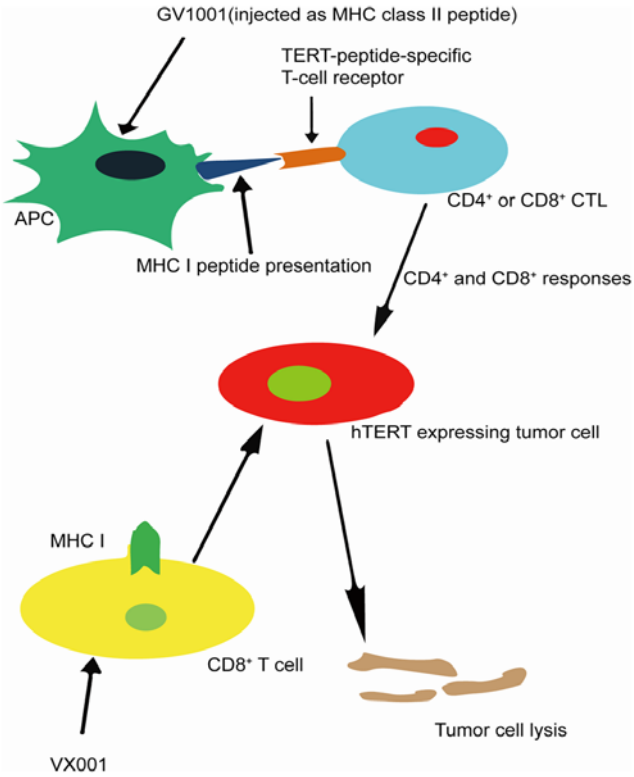

Figure 2. Telomerase immunotherapy. Telomerase-derived peptide binds to human leukocyte antigen class I and class II molecules, effectively activating $CD8^+$ or $CD4^+$ T-cell subsets to target and kill telomerase-positive tumor cells. GV1001 is an MHC class II-restricted peptide, which is further processed into an MHC class I peptide by APC triggering the $CD4^+$ or $CD8^+$ CTL response in hTERT-expressing tumor cells. VX001 has an enhanced affinity for MHC class I molecules and is mediated by $CD8^+$ T cells. MHC, major histocompatibility complex; APC, antigen-presenting cell; CTL, cytotoxic lymphocyte; hTERT, human telomerase reverse transcriptase.

certain studies have not confirmed their effect on prognosis. Therefore, further studies are required to determine whether telomerase can be used as a prognostic factor of NSCLC. Early diagnosis is important for the prognosis of patients with NSCLC, particularly in the diagnosis of advanced NSCLC, and upregulation of *TERT* may be considered as an early molecular event in lung cancer (108). Therefore, the combination use of *TERT* with other lung cancer-related factors can be considered in the early stage to improve the diagnostic rate of patients with NSCLC.

TERT is a key gene that controls telomerase expression (40). Abnormalities in the *TERT* gene, such as *TERT* gene mutation, amplification and rearrangement, can regulate the expression level of *hTERT*, activate telomerase, maintain telomere length and promote tumor growth (27). In NSCLC, telomerase activity is higher compared with hTERT expression, indicating that telomerase activity is not only associated with the regulation of *hTERT*, but may also be expressed by tumor cells via other mechanisms. Studies have shown that telomerase activity is associated with TERC and telomere-related proteins (40,110). Accumulating evidence has shown that *TERC* serves an important role in regulating the maintenance of telomerase and other functions important in human cancer (111). However, the mechanism of telomerase activation requires further investigation.

Tumor formation is a multifactorial, staged process that requires multiple gene transformations, including mutations in several oncogenes and inactivation of ≥2 tumor-suppressor genes, as well as alterations in apoptotic regulation and DNA repair genes (112). *TERT* is both an effector and a regulator in cancer, and it can interact with other target genes to regulate the proliferation of tumor cells (113). Ret finger protein like 3 (RFPL3) is a tumor-specific *hTERT* promoter-binding protein that can promote the growth of lung cancer by activating *hTERT* expression (114). Moreover, *EGF* upregulates the expression levels of RFPL3 and hTERT proteins in NSCLC cells via the MEK pathway, promotes cell proliferation and inhibits apoptosis. RFPL3 overexpression increases the expression level of *hTERT* and is associated with MEK signaling proteins (115). The transcription co-activator CREB-binding protein (CBP) is a novel *hTERT*-binding protein that contributes to the upregulation of *hTERT* expression and tumor growth. Furthermore, upregulation of CBP may predict poor prognosis in human lung cancer (116). Interleukin (IL)-6 is an important cytokine in the development of lung cancer. *TERT* regulates the expression of numerous genes, including IL-6, and may serve a unique role in lung adenocarcinoma (117). Early studies have reported that the *TP53* gene mutation and high telomerase activity co-induce the occurrence and low differentiation of NSCLC (117). Moreover, the co-occurrence of *TP53* gene mutation and telomerase activity may be associated with the malignancy of NSCLC (118,119). Although it has been shown that *TERT* can interact with its target genes in NSCLC, the mechanism of interaction is not fully understood and needs to be further examined.

The close relationship between telomerase and tumors makes telomerase inhibition a novel and promising therapeutic approach to cancer treatment. However, there are still numerous challenges to be addressed with regards to research on the mechanism of telomerase inhibition. First, the specific mechanism underlying the role of the *TERT* gene in tumor development is incompletely understood. For example, whether *TERT* and other genes jointly influence the occurrence and development of tumors remains unknown and, if the specific association between the co-mutation of *TERT* gene and other genes is identified, it may prove helpful in the selection of suitable telomerase inhibitors. Second, the mechanism of telomerase reactivation before carcinogenesis and the molecular mechanism of inhibition of telomerase function after carcinogenesis are yet to be elucidated, and require further clarification. Moreover, although high telomerase activity is present in tumor tissues, telomerase is also highly active in embryonic cells, bone marrow stem cells, germ cells and activated immune cells (118). Therefore, telomerase inhibition may also prove harmful against normal cells. The biological characteristics of telomerase prolong the time lag between drug administration and clinical response, which may lead to drug toxicity in patients. Therefore, if an inhibitor that only targets telomerase-positive tumor cells is developed, it may help improve drug toxicity in patients.

6. Conclusions

The *TERT* gene is associated with the activation of telomerase and is closely related to the proliferation and regulation of

tumor cells. *hTERT* can be used as a diagnostic marker of NSCLC. At present, some clinical studies have confirmed the feasibility of targeting telomerase in various tumors, including NSCLC. The development of telomerase inhibitor drugs targeting the *hTERT* gene may provide a novel approach to gene therapy for NSCLC and improve the efficacy of NSCLC treatment. In the future, with the further research on telomerase, the understanding of its related mechanism and function will become clearer. Moreover, the relationship between telomerase and human aging and tumor development will be further elucidated, and the application of telomerase in the treatment of NSCLC may prove to be a promising therapeutic method.

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Availability of data and materials

All information provided in this review is documented by relevant references.

Authors' contributions

XCP and JC contributed to this review with the design. LY, MW, YHZ, LDY and NL reviewed the references. LY, JC and XCP wrote the manuscript. LY, WZ, ZQY and NL designed and produced the tables and figures. XCP and JC acquired the funding. All authors read and approved the manuscript for publication.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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