2020

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Neuro-Oncology Advances
2(1), 1–11, 2020 | doi:10.1093/noajnl/vdaa025 | Advance Access date 28 February 2020

TERT, a promoter of CNS malignancies

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Abstract

As cells replicate their DNA during mitosis, telomeres are shortened due to the inherent limitations of the DNA replication process. Maintenance of telomere length is critical for cancer cells to overcome cellular senescence induced by telomere shortening. Telomerase reverse transcriptase (TERT) is the rate-limiting catalytic subunit of telomerase, an RNA-dependent DNA polymerase that lengthens telomeric DNA to maintain telomere homeostasis. *TERT* promoter mutations, which result in the upregulation of *TERT* transcription, have been identified in several central nervous system (CNS) tumors, including meningiomas, medulloblastomas, and primary glial neoplasms. Furthermore, *TERT* promoter hypermethylation, which also results in increased *TERT* transcription, has been observed in ependymomas and pediatric brain tumors. The high frequency of *TERT* dysregulation observed in a variety of high-grade cancers makes telomerase activity an attractive target for developing novel therapeutics. In this review, we briefly discuss normal telomere biology, as well as the structure, function, and regulation of TERT in normal human cells. We also highlight the role of TERT in cancer biology, focusing on primary CNS tumors. Finally, we summarize the clinical significance of *TERT* promoter mutations in cancer, the molecular mechanisms through which these mutations promote oncogenesis, and recent advances in cancer therapies targeting TERT.

Keywords

central nervous system tumors | telomerase promoter mutations | TERT

One hallmark of cancer is the ability of cancer cells to replicate indefinitely.[1] This replicative potential runs counter to Hayflick's rule, which imposes a limit on the replicative potential of a normal cell.[2] This limitation is a result of the directionality of DNA polymerase, which causes shortening of chromosome ends after each replicative cycle, leading to cellular senescence. The addition of telomeres to the ends of chromosomes allows for successive replicative cycles without chromosomal DNA shortening. Telomeres, which consist of noncoding TTAGGG nucleotide repeats, protect the ends of chromosomes from deterioration and end fusion to other chromosomes.[3,4] Telomeres are protected from DNA damage by binding to proteins that create a shelterin complex.[4] The telomerase enzyme is an RNA-dependent DNA polymerase that lengthens telomeric DNA to maintain telomere homeostasis.[5] Telomerase reverse transcriptase (TERT) is the rate-limiting catalytic subunit of telomerase.[6]

Telomerase activity is highly regulated. In most somatic cells with finite proliferative potential, telomerase activity is downregulated through epigenetic regulation of the *TERT* promoter.[7,8] Telomeres in these somatic cells shorten with successive mitoses, ultimately triggering cellular senescence.[9] In contrast, in stem cells and proliferative cells of self-renewing tissues, telomerase activity is not downregulated and counteracts the shortening of telomeres, allowing for increased replicative potential.

Cancer cells must maintain telomere length to circumvent cellular senescence. Telomerase upregulation can be found in 90% of malignancies, although the mechanism of activation is not always known.[9] One well-known mechanism, upregulation of *TERT* transcription, often occurs through *TERT* promoter mutations. Such mutations have been found in meningioma, glioblastoma, medulloblastoma, and

non-central nervous system (CNS) cancers.[9–12] Another well-described mechanism of *TERT* upregulation is *TERT* promoter methylation, which paradoxically results in increased *TERT* expression in glioblastoma, ependymoma, and medulloblastoma, along with several non-CNS cancers.[13–17]

In this review, we outline normal telomere biology and regulation of *TERT*. We discuss the mechanisms of *TERT* upregulation and its role in cancer, focusing on glioblastoma and other CNS tumors. We also describe the clinical and prognostic relevance of *TERT* mutations in CNS tumors. Finally, we discuss therapies that might target cancers with aberrant *TERT* upregulation.

Normal Telomere Biology

Telomeres span approximately 10–20 kb at the end of human chromosomes.[3,4,18] The noncoding repeats in telomeres bind proteins that form the shelterin complex.[3,4,18] Telomeres also consist of a 150–200 nucleotide, G-rich, single-stranded overhang, which ends with a 3′-OH group that is recognized by TERT.[3,4] This single-stranded overhang is protected from the DNA double-strand break repair machinery by folding back on itself to form the so-called T-loop and by recruitment of the shelterin complex.[3,18] The formation of this nucleoprotein structure protects chromosome ends from nonhomologous end joining and regulates the access of telomerase to telomeres.

Telomeres shorten with DNA replication due to an inability to fill in the gap with complementary DNA on the 5′-end of the DNA strand after the RNA primer is removed during replication, termed the "end replication problem."[19] The loss of telomeric repeats with successive replication cycles leads to an inability of the telomere to form the T-loop or recruit the shelterin complex. This causes a loss of chromosomal protection that leads to the formation of end-to-end chromosomal fusions and loss of cell viability.[3]

A human telomere contains enough repeats to withstand the loss of length for approximately 50–90 replication cycles in the absence of telomere elongation mechanisms.[4] Most cells undergoing continuous division, such as stem cells, overcome the end replication problem by expressing telomerase. The telomerase complex, which is comprised of TERT and an RNA molecule encoded by the *telomerase RNA component* (*TERC*) gene, binds to telomeres at the end of the single-telomere overhang.[3] Once TERT is bound, its enzymatic activity elongates the telomere, thus allowing the cell to undergo additional DNA replication cycles (Figure 1).

Alternative lengthening of telomeres (ALT) is another mechanism of telomere elongation. While most immortal cell lines exhibit telomerase activity, some cell lines, including 10% of cancer lines, exhibit no telomerase activity but still have elongated telomeres as a result of ALT.[20] Although the details have not been elucidated, it is postulated that lengthening by ALT occurs by using recombination to copy telomeres from other chromosomes or from the same chromosome.[21,22] Whole exome sequencing of glioblastomas with wildtype *IDH* and *TERT* promoter identified an ALT positive subgroup of tumors with *ATRX* or *SMARCAL1* mutations. These mutations are mutually exclusive and confer a similar overall survival to *TERT* promoter mutations, suggesting that ALT plays an important role in glioblastoma.[23]

More recently, telomeres have been found to have functions other than DNA end protection, such as regulation of gene expression through transcriptional silencing of genes.[24] Although the mechanism is poorly understood, the conformation of telomeric DNA, including structures such as the T-loop, is also thought to contribute to telomere function. Additionally, RNA transcribed from telomeric DNA, so-called telomeric repeat-containing RNA, has been implicated in a variety of processes such as regulation of telomerase, heterochromatin organization at telomeres, and regulation of DNA expression.[4,24]

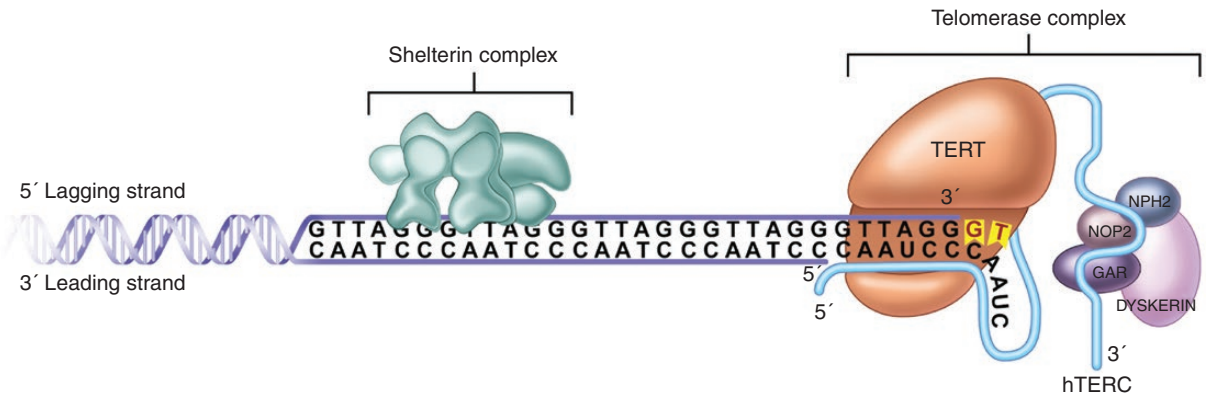

Fig. 1 Telomere elongation. After recruitment to the telomere by the shelterin complex, TERT catalyzes the addition of the telomere repeats using the template provided by TERC. This counteracts the shortening of chromosomal telomeres in the setting of DNA replication.

TERT Structure, Function, and Regulation

The TERT protein is comprised of 4 domains: the telomerase essential N-terminal domain (TEN), the reverse transcriptase domain (RT), the telomerase RNA-binding domain (TRBD), and the C-terminal extension domain.[4,25] The TEN domain binds RNA and telomeric DNA and contributes to catalysis.[26] The RT domain contains an insertion in the finger domain that distinguishes it from other reverse transcriptases.[25,26] TERT binds the *TERC*-encoded RNA in the TRBD to form the telomerase ribonucleoprotein.

TERT is located at chromosome 5p15.33 in humans, while *TERC* is located at chromosome 3q26.[24] TERC provides the template for synthesis of telomeric repeats, whereas TERT is the catalytic component of telomerase. The presence of TERT and TERC alone is sufficient for telomere elongation in vitro, but in vivo function also requires other components that serve additional roles, such as regulating attachment to telomeres and trafficking of telomerase components into the nucleus.[4,24,27]

The *TERT* gene is 40-kb long and consists of 15 introns and 16 exons with a 260-bp promoter core. The *TERT* promoter region contains GC boxes that bind the zinc finger transcription factor SP1, which increases *TERT* transcription, and E-boxes that bind both transcriptional enhancers and repressors.[4,7,24] The GC-rich areas around the transcription start site are also regions of epigenetic regulation through DNA methylation and chromatin remodeling.[4]

TERT Dysregulation in Cancer

TERT Promoter Mutations

Analysis of data from metastatic melanoma patients by Huang et al.[28] identified 2 recurrent C>T mutations in the *TERT* promoter region -124 (C228T) and -146 (C250T) base pairs from the transcriptional start site in 71% of patients. Subsequent screening for *TERT* promoter mutations found varying prevalence among different cancers (Table 1).[29] *TERT* promoter mutations are associated with increased *TERT* expression and telomerase activity.[10] Both commonly observed mutations (C228T and C250T) generate identical de novo binding sites for the E-twenty-six (ETS) family of transcription factors.[28] Even though there are 27 ETS transcription factors that all bind to similar sequences, only GA Binding Protein Transcription Factor Subunit Alpha (GABPA) selectively binds the mutant *TERT* promoter and not the wildtype version in several cancers.[9,30] GABPA functions as a homodimer or as a heterotetramer with GABPB. The *TERT* promoter mutant sites cooperate with native ETS sites to recruit GABPA/B as a heterotetramer and activate *TERT* transcription.[30] In contrast, a study using melanoma cell lines found only minimal binding of the GABPA homodimer to the mutant *TERT* promoter.[31]

Increased *TERT* transcription in the setting of *TERT* promoter mutation has also been found to occur in an NF-κβ-dependent manner. Unlike the C228T mutation, the C250T mutation creates a binding site for the NF-κβ subunit p52, allowing for the cooperative binding of NF-κβ and ETS1/2 to activate transcription.[32] Thus, despite having similar downstream effects, the 2 canonical *TERT* promoter mutations may distinctly alter *TERT* expression.

Table 1 Continued

TERT Promoter Mutations in CNS Neoplasms

TERT promoter mutations have been identified in CNS tumors, including primary glial neoplasms (up to 80%), medulloblastomas (20%), and meningiomas (6.4–11%).[29,33–40] As with other cancers, the 2 most common promoter mutations are C228T and C250T, which occur in a mutually exclusive fashion. The mutation frequency varies by subtype for each of these tumors.

In adult primary glial neoplasms, the frequency of *TERT* promoter mutations varies by WHO grade (Figure 2). Glioblastoma (WHO grade IV) has a high frequency of mutations (80%). WHO grade II and III lesions, such as oligodendrogliomas, have a relatively lower frequency of *TERT* mutations (60–70%), and WHO grade I tumors, such as astrocytomas, have the lowest frequency of *TERT* mutations (30–40%).[29,33,37,39] In patients with primary glial neoplasms, particularly glioblastoma, *TERT* promoter mutations were associated with co-mutations in epidermal growth factor receptor (*EGFR*), *CDKN2A/B*, and phosphatase and tensin homolog.[40] Data from multi-sector sequencing of glioblastoma tumors revealed *TERT* promoter mutations to be clonal in all tumor sectors, suggesting that these mutations represent an early oncogenic event in this cancer.[41,42]

In patients with meningiomas, *TERT* promoter mutations are associated with higher grade lesions (1.7% WHO grade I, 5.7% WHO grade II, and 20% WHO grade III) and with advancing tumor grade upon recurrence.[35,38] In patients with medulloblastoma, although the overall mutation frequency is reported as 20%, the frequency of *TERT* promoter mutations in the wingless (WNT) and sonic hedgehog (SHH) subtypes has been reported to be as high as 31% and 83%, respectively.[33,36]

TERT Promoter Hypermethylation

TERT promoter hypermethylation has more recently been identified as an additional contributor to *TERT* dysregulation in cancer cells.[13–17] Promoter hypermethylation may represent an alternative means of *TERT* upregulation or may work in conjunction with *TERT* promoter mutations to increase *TERT* expression in cancer cells. Hypermethylation of the promoter region would typically be expected to result in transcriptional silencing. However, in the case of the *TERT* promoter, a region termed the *TERT* hypermethylated oncological region (THOR) has been identified as a site of methylation that causes an increase in *TERT* expression when hypermethylated. THOR

Table 1 Summary of the Frequency of TERT Promoter Mutations in Central Nervous System Tumors

Cancer Type	Mutation Frequency (%)
CNS cancers	
Primary glioblastoma	70–80%[12]
Secondary glioblastoma	30–50%[12]
Astrocytoma	30–40%[12]
Oligoastrocytoma	35–55%[12]
Medulloblastoma	19.8%[11]
Meningioma	7.4%[11]
Ependymoma	3%[12]
Skin cancer—non-melanoma	
Atypical fibroxanthoma	93%[11]
Pleomorphic dermal sarcoma	76%[11]
Squamous cell carcinoma	60%[11]
Basal cell carcinoma	47.4%[11]
Merkel cell carcinoma	10.2%[11]
Melanomas	
Non-acral melanoma	35–80%[12]
Conjunctival melanoma	30–40%[12]
Mucosal melanoma	10–15%[12]
Acral melanoma	5–10%[12]
Uveal melanoma	0–1%[12]
Thyroid cancers	
Poorly differentiated thyroid carcinoma	42.9%[11]
Anaplastic thyroid carcinoma	39.2%[11]
Follicular thyroid carcinoma	17.1%[11]
Atypical follicular thyroid adenoma	16.7%[11]
Hürthle cell carcinoma	13.1%[11]
Differentiated thyroid carcinoma	12.1%[11]
Papillary thyroid carcinoma	11.0%[11]
Gynecological cancers	
Ovarian clear cell carcinoma	15.9%[11]
Squamous cell carcinoma of the cervix	9.9%[11]
Endometrial carcinoma	6.6%[11]
Ovarian low-grade serous carcinoma	4.9%[11]
Urological cancers	
Micropapillary urothelial carcinoma	100%[12]
Squamous cell carcinoma of the bladder	80%[12]
Infiltrating urothelial carcinoma with glandular differentiation	72%[12]
Urothelial carcinoma	60–70%[12]
Renal pelvic carcinoma	45%[12]
Ureter carcinoma	19%[11]
Urethral carcinoma	15–20%[12]
Inverted urothelial papilloma	15%[12]
Chromophobe renal cell carcinoma	13%[12]
Clear cell renal cell carcinoma	5–10%[12]
Renal cell carcinoma, unclassified	8%[12]
Digestive system cancers	
Combined hepatocellular cholangiocarcinoma	45%[12]
Hepatocellular carcinoma	41.2%[11]
Gallbladder cancer	4%[12]
Intrahepatic cholangiocarcinoma	0–5%[12]
Esophageal squamous cell carcinoma	0–2%[12]
Gastric cancer	0%[11]
Pulmonary cancers	
Malignant pleural mesothelioma	15.2%[11]
Lung and non-small cell lung carcinomas	0–2%[11]
Head and neck cancers	
Laryngeal carcinoma	27.2%[11]
Squamous cell carcinoma of head and neck	16.3%[11]
Soft tissue cancers	
Myxoid liposarcoma	69.4%[11]
Chondrosarcoma	50%†[11]
Fibrosarcoma	33.3%†[11]
Solitary fibrous tumor	24.1%[11]

†signifies sample size less than 5.

is a 433-bp region that contains 52 CpG sites located immediately upstream of the *TERT* core promoter.[17] Importantly, hypermethylation of THOR results in increased *TERT* expression regardless of *TERT* promoter mutation status. Furthermore, when THOR is unmethylated, the activating effects of *TERT* promoter mutations appear to be repressed compared to when THOR is hypermethylated.[17] These findings suggest that THOR hypermethylation represents an independent mechanism of increased *TERT* expression from *TERT* promoter mutations.

Remarkably, the frequency of THOR hypermethylation may be as high as the frequency of *TERT* promoter mutations in some cancers. Indeed, more than 70% of prostate, breast, hematological, and colon cancers are characterized by THOR hypermethylation.[17] In general, the frequency of THOR hypermethylation appears to be greater in cancers with a low frequency of *TERT* promoter mutations. Nonetheless, THOR hypermethylation has been observed with relatively high frequency even in cancers with a high frequency of promoter mutations and has been found to be approximately 63% in CNS tumors.[17]

Impact of TERT Dysregulation in Cancer Cells

All cancer cells acquire molecular aberrations that confer the properties of replicative immortality, sustained proliferative signaling, evasion of growth suppressors, resistance to cell death, angiogenesis, invasion, and

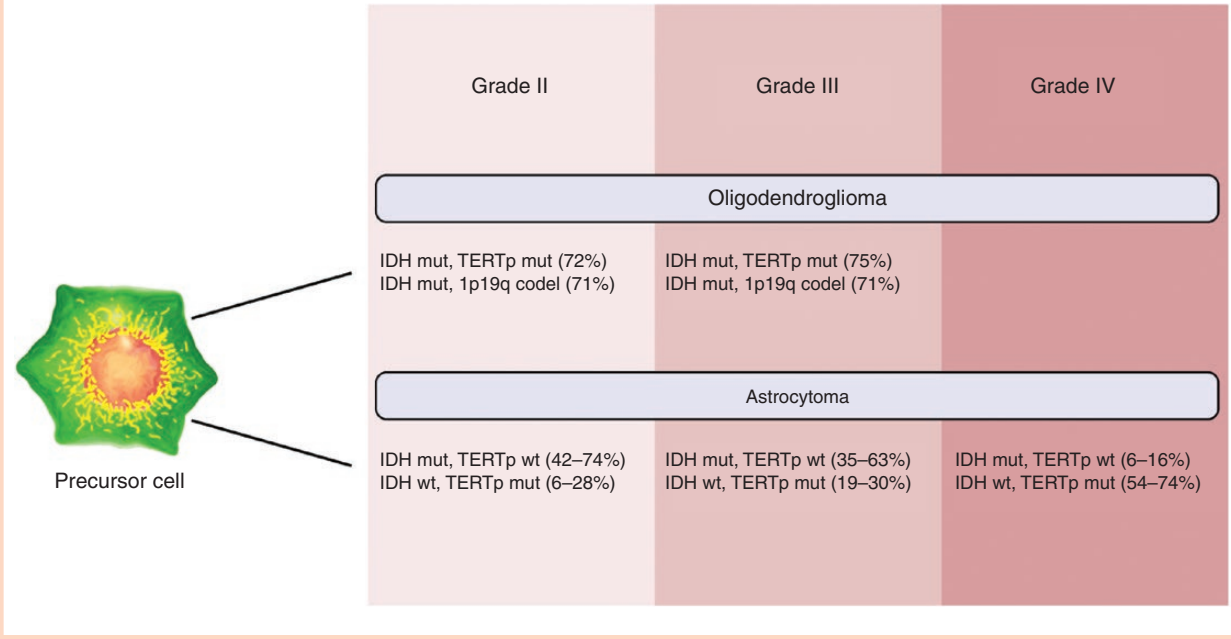

Fig. 2 Frequency of *TERT* promoter mutation, IDH mutation, and 1p19q co-deletion in glioma. The frequency of *TERT* promoter mutations (*TERTp* mut) increases with increasing grade of primary glial neoplasms. The presence of *TERT* promoter mutations in combination with other commonly observed genetic abnormalities in gliomas, such as *IDH* mutation (*IDH* mut) and chromosome 1p19q co-deletion (1p19q codel), has both diagnostic and prognostic implications. Frequency of selected clinically relevant genomic co-alterations are shown here.[33,43,44]

metastasis.[1,45] Telomere length plays a complex role in oncogenesis. Critical shortening of telomeres results in increased rates of oncogenic chromosomal alterations such as deletions and chromosomal end fusions. At the same time, the shortening of telomeres activates the normal regulatory pathways that result in cellular senescence or even cell death. In 90% of cancers, telomerase upregulation is critical to achieve proliferative immortality.[4,20,24] The TERT subunit catalyzes the rate-limiting step of telomerase, making the *TERT* gene a logical target for upregulation in cancer cells. Indeed, Hahn et al.[46] demonstrated that ectopic expression of *TERT*, in addition to key oncogene expression and tumor suppressor dysfunction, is required for the transformation of human somatic cells into tumorigenic cells.

Analysis of 1230 tumors from 60 cancer types by Killela et al.[29] found 2 distinct groups of cancers—those with a high (≥15%) or a low (<15%) frequency of *TERT* promoter mutations. The 9 cancers in the high-frequency group all originated from tissues with low self-renewal and included tumors such as medulloblastoma and primary gliomas. This finding led to the hypothesis that *TERT* promoter mutations primarily benefit cancer cells originating from tissues with low self-renewal potential.[8] In cancers originating from the hematopoietic system or gastrointestinal tract, for example, *TERT* promoter mutations would be predicted to have less of an impact on *TERT* expression since these tissues already express *TERT*. Accordingly, the frequency of *TERT* promoter mutations in these cancers is low.[8,10,29] In contrast, cancers arising from cells with low replicative potential that do not express *TERT*, such as astrocytes or melanocytes, would gain a replicative advantage over other cells via aberrant overexpression of *TERT* resulting from promoter mutations.[10,28,29,40,47] Although this explanation is corroborated by the relative frequency of these mutations observed in several cancers, there are some notable exceptions, including non-melanoma skin cancers, which have a high frequency, of *TERT* promoter mutations.[48] These exceptions suggest the possibility that an oncologic advantage may be conferred through alternative functions of the TERT protein or of telomeres themselves.

Indeed, while *TERT* promoter mutations are associated with increased telomerase expression in some cancers, there is growing evidence that *TERT* promoter mutations are not always associated with longer telomeres.[39] In human fibroblast cell lines that maintain long telomeres through ALT, the expression of oncogenic *H-RAS* led to inefficient tumorigenicity. However, overexpression of *TERT* in addition to H-RAS resulted in robust malignant transformation of ALT fibroblasts, suggesting that TERT may have telomere-lengthening-independent roles in tumor initiation and/or maintenance.[49] Additionally, Chiba et al.[50] found that during malignant transformation in melanoma, *TERT* promoter mutation-driven telomerase expression does not prevent telomere shortening. Findings such as these have led to the hypothesis that increased telomerase expression may allow for the persistence of genetically unstable clones by maintaining telomeres just above a critically short length, thus avoiding cellular senescence while also promoting rapid disease evolution.

TERT may also have functions entirely independent of telomere biology. For example, TERT is capable of

performing RNA-dependent RNA polymerase (RdRP) functions via its association with BRG1, an SWI/SNF-related chromatin remodeling protein, and nucleostemin, a GTP-binding protein, forming the TERT–BRG1–NS complex (TBN complex). The TBN complex synthesizes double-stranded RNAs that are processed into short-interfering RNAs that regulate heterochromatin assembly and mitotic progression.[51] Inhibitors targeting these non-canonical functions of TERT serve as promising anticancer agents. One such inhibitor, Eribulin, that targets the RdRP activity ofTERT is discussed in a later section.

Additionally, in an in vitro model, TERT knockout somatic cells were reprogrammed into pluripotent stem cells by the introduction of an enzymatically inactive TERT mutant, suggesting that TERT may maintain a stem-like cell state independently of telomere lengthening.[52] Potential mechanisms of this function include direct modulation of the Wnt/β-catenin signaling pathway and increased *EGFR* expression.[53,54] Finally, TERT may promote invasion and metastasis of cancer cells by altering the expression of extracellular matrix remodeling and epithelial-to-mesenchymal transition related genes.[55–57]

Clinical Significance of Aberrant *TERT* Expression

TERT promoter mutations occur in specific clinical and phenotypic subtypes of various cancers. They are found mainly in adult rather than pediatric tumors and are sometimes associated with more aggressively behaving subtypes of cancers including melanomas, gliomas, meningiomas, and hepatocellular carcinomas.[4,33,38,40] Given the normal age-dependent shortening of telomeres, it is unsurprising that *TERT* promoter mutations are associated with increased age at diagnosis for these cancers. The clinical impact of these mutations has been most extensively studied in melanomas, where *TERT* promoter mutations were found to be associated with markers of poor prognosis, such as increased Breslow thickness and metastasis, and with poor overall survival and disease-free progression.[4,29,47] The clinical impact of these mutations has similarly been studied in primary CNS tumors, including gliomas and meningiomas.

Prognostic Impact of TERT Promoter Mutations on Gliomas

Several genetic aberrations that modify prognosis have been discovered in gliomas. Mutations in *isocitrate dehydrogenase 1* (*IDH1*) are associated with lower histological grade, prolonged progression-free survival, and increased response to chemoradiotherapy.[58] These mutations tend to also be associated with secondary gliomas, which progress from an antecedent lower grade to higher grade disease.[37,59] Meanwhile, 1p and 19q co-deletions are associated with better survival in oligodendrogliomas and also correlate with *IDH1* mutations.[60] *Methylguanine methyltransferase* (*MGMT*) promoter methylation is yet another critical determinant of prognosis and treatment response. Glioblastomas with *MGMT* promoter methylation have been shown to have a more favorable treatment response to alkylating agents with a resultant survival benefit.[61] The various combinations of these genetic abnormalities have identified subtypes of gliomas with clinically distinct behavior. Factoring the presence of *TERT* promoter mutational status into these combinations may allow for better characterization of gliomas, with implications for precision medicine.

TERT promoter mutation frequency increases with increasing WHO grade in gliomas (Figure 2).[29,37] Across all subtypes of glioma, *TERT* promoter mutations are independently associated with worse overall survival.[37,62,63] The frequency of mutations is inversely correlated with *IDH1* mutation in all gliomas but positively correlated with 1p and 19q co-deletions in oligodendrogliomas.

The presence of *TERT* promoter mutations in combination with *IDH1* mutations changes overall survival in glioma patients. Gliomas with *TERT* promoter mutations without *IDH1* mutation have worse overall survival than those with wildtype *TERT* promoter and *IDH1* mutation. Meanwhile, gliomas with wildtype *TERT* promoter and wildtype *IDH1* have an intermediate overall survival. Paradoxically, gliomas with both *TERT* promoter mutation and *IDH1* mutation have improved overall survival compared to patients with wildtype *TERT* promoter and *IDH1* mutation.[37,62,63] Interestingly, concomitant mutations in *TERT* and *IDH1* occur at nearly the same frequency as concomitant mutations in *IDH1* and 1p and 19q co-deletions, providing further insight into the molecular etiology of oligodendrogliomas.[64] Therefore, the improved survival in this group of patients is not a result of biological interaction between these mutations but rather identification of a lower grade glioma.[37,63] This is corroborated by the fact that the small group of gliomas with co-mutations in *TERT* promoter and *IDH1* without 1p and 19q co-deletions correspond to gliomas with aggressive behavior and survival similar to those with *TERT* promoter mutations and *IDH1* wildtype.[37,62,63]

The important role of *TERT* promoter mutations on prognosis and clinical behavior is highlighted by the recently published cIMPACT-NOW update 3, which recommends that IDH wildtype grade II and III infiltrating astrocytomas with *TERT* promoter mutations, *EGFR* amplification, and/or chromosome 7 gain and 10 loss be graded as WHO grade IV.[65] Although not currently graded as WHO grade IV tumors, these tumors have clinical, histopathological, and radiographic behavior similar to WHO grade IV tumors.[63,66] Appropriate stratification of patients based on the genetic profile of their gliomas is thus critically important in informing how aggressively they should be treated. To this end, Shankar et al.[67] developed a rapid intraoperative genotyping assay to detect *TERT* promoter and *IDH1* mutational status in WHO grade II and III gliomas. This intraoperative molecular information has the potential to aid in real-time surgical decision making and may allow for precision therapy at the time of surgery.

Prognostic Impact of TERT Promoter Mutations on Other Primary CNS Tumors

TERT promoter mutations are associated with higher grade meningiomas.[38] Across all grades of meningioma,

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the mutation has been associated with a more aggressive natural history. Grade I tumors with the mutation were associated with a higher rate of recurrence with increased risk of progression to a higher grade in recurrent tumors. Analysis of recurrent meningiomas without malignant progression revealed a lack of the *TERT* mutation compared to recurrent meningiomas with malignant progression.[35] Similarly, grade II and III tumors with *TERT* mutations were associated with higher recurrence rates as well as shorter time to progression and/or recurrence.[38,42] In high-grade meningiomas, the *TERT* promoter mutation was associated with worse overall survival (2.7 years vs 10.8 years).[42]

The *TERT* promoter mutation is the most common recurrent somatic point mutation observed in medulloblastomas and occurs primarily in adults with the SHH and WNT subtypes.[34,36] The distribution of promoter mutation frequency among the various subtypes of medulloblastoma is not surprising given that the SHH subtype is the most common subtype in adult medulloblastoma patients and, as observed with other cancers, the mutation has a higher frequency among older patients. Interestingly, unlike with other cancers, *TERT* promoter mutations in SHH medulloblastomas are associated with a more favorable overall survival and with a lower incidence of metastatic disease on presentation. Group 4 medulloblastomas with the *TERT* promoter mutation follow the pattern observed in gliomas and meningiomas, with worse overall survival than those with wildtype *TERT* promoter.[34] In the WNT and group 3 medulloblastoma subtypes, the *TERT* promoter mutation does not appear to make a difference in overall survival. While the underlying biology explaining these survival differences is poorly understood, stratification of patients based on mutation status can inform discussions of prognosis and targeted therapeutics as they become available in the future.

TERT as a Therapeutic Target

The high frequency of *TERT* promoter mutations in cancers makes telomerase activity an attractive target for the development of therapeutic interventions. Additionally, normal cells typically have lower telomerase activity and maintain longer telomeres than cancer cells, allowing cancer cells to be preferentially targeted by telomerase-inhibiting therapies. Despite this, there are challenges to targeting *TERT* in the treatment of cancers. Telomere shortening to a length that induces cell death requires multiple cell cycles after TERT inhibition is initiated. This results in a lag to the response of these therapies during which time tumor burden increases. Furthermore, since *TERT* is normally highly expressed in rapidly dividing cells, targeting TERT can have side effects, particularly on the hematopoietic system.[24] One strategy to overcome these challenges may involve the use of other, more immediately acting treatments in conjunction with TERT-based therapies.

To date, no FDA-approved treatments targeting telomerase are available, but approaches including use of small molecule inhibitors, antisense oligonucleotides, immunotherapy, and G-quadruplex stabilizers are being investigated, with some therapies in various clinical trial phases.[24] Promising approaches to TERT targeting include use of the small molecular inhibitor imetelstat (GRN163L), immunotherapy, GABPβ1L inhibition, as well as targeting RdRP activity of TERT using eribulin (Figure 3).

Imetelstat

Imetelstat is the only small molecule inhibitor of TERT to be evaluated in clinical trials.[24] Imetelstat is a 13-bp RNA oligonucleotide with a thio-phosphramidate backbone that allows for the formation of RNA duplexes.[68] The molecule also contains a lipid moiety that increases cellular uptake and retention.[69] Unlike traditional antisense oligonucleotides, imetelstat was not designed to bind to mRNA but rather to bind to TERT.[24] In vitro studies demonstrated telomerase inhibition with concomitant telomere shortening in several cell lines including tumors of the bladder, breast, lung, liver, prostate, and pancreas. In vivo studies using mouse xenograft lung cancer models showed similar results, in addition to reduced tumor growth and metastasis and increased sensitivity to chemotherapy.[24,70]

In vitro studies of a glioblastoma cell line by Marian et al.[71] found that imetelstat produced a dose-dependent inhibition of telomerase, with long-term treatment leading to progressive telomere shortening, decreased proliferation, and cell death in glioblastoma tumor-initiating cells. Furthermore, combining treatment with

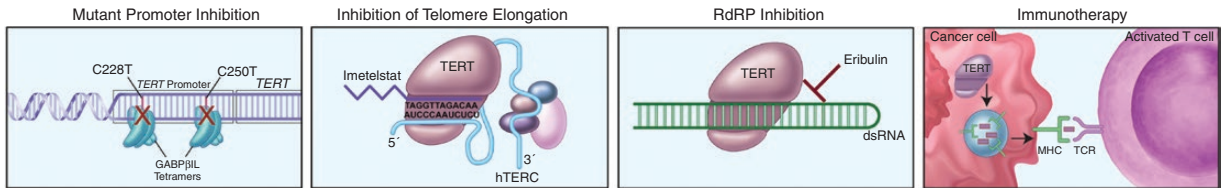

Fig. 3 Treatment strategies for *TERT* promoter mutant tumors. Various treatment strategies taking advantage of the presence of *TERT* promoter mutations have been proposed, including targeting the GABPβ1L tetramers that bind to the mutant promoter sites as transcription factors, blocking the *TERT* active site using small molecule inhibitors such as Imetelstat that bind to TERC, inhibition of RNA-dependent RNA polymerase (RdRP) activity with small molecule inhibitors such as Eribulin, and vaccine therapy targeting cells with increased *TERT* expression.

radiation and temozolomide reduced cancer cell survival and enhanced activation of the DNA damage response pathway. In vivo studies using mouse xenograft glioblastoma models revealed that imetelstat is able to cross the blood-brain barrier with 70% inhibition of telomerase activity and reduced growth of subcutaneously implanted tumors. While some imetelstat clinical trials have been completed, some have been suspended due to hematological toxicity.[24] A phase 2 clinical trial investigating imetelstat use for recurrent pediatric primary glial neoplasms showed 95% inhibition of intratumoral telomerase activity, but also showed significant hematological toxicity and was halted due to 2 patient deaths related to intratumoral intracranial hemorrhage.[72]

Immunotherapy

Cancer cells express protein fragments of telomerase as cell surface antigens via the human leukocyte antigen (HLA) class I pathway.[73,74] These epitopes can be targeted by cytotoxic T cells to kill cancer cells. The goal of anti-telomerase immunotherapy is to sensitize the immune system to tumor cells expressing TERT epitopes, resulting in the activation of T cells.

One strategy used to achieve this goal is the development of TERT vaccines. Two vaccines have been used to generate an anti-telomerase response in cancer cells: GV1001 and Vx001. GV1001 is a 16-amino acid, HLA class II-restricted peptide that contains a part of the amino acid sequence of the TERT active site. Once injected, the vaccine is processed endogenously to yield an HLA class I peptide as well. This vaccine therefore activates a $CD4^+$ and a $CD8^+$ cellular response. Phase II trials investigating GV1001 alone or in combination with other therapies have been completed for malignant melanoma and hepatocellular carcinoma. Phase III GV1001 trials are ongoing for non-small cell lung carcinoma and metastatic pancreatic cancer. Vx001 is a cryptic peptide vaccine containing a TERT amino acid sequence hidden within a protein structure. Endogenous processing of the peptide results in the HLA class I presentation with a $CD8^+$ T-cell response. A phase 2 trial investigating Vx001 in non-small cell lung cancer is currently ongoing.

Another strategy is to prime antigen-presenting dendritic cells ex vivo and then inject the primed cells back into the patient. GRNVAC1 is a dendritic cell vaccine consisting of autologous dendritic cells transduced with mRNA-encoding TERT and LAMP1, which guides TERT proteins into lysosomes for breakdown and presentation. This results in a polyclonal immune response when the primed dendritic cells are injected back into the patient. One phase 2 trial for GRNVAC1 in acute myelogenous leukemia has been completed. All 3 TERT vaccines have been shown to be well tolerated in cancer patients, with little effect on normal cells and no autoimmunity.[24]

GABPβ1L Inhibition

Recently, Mancini et al.[75] demonstrated that disruption of the β1L isoform of GABP reverses the replicative immortality of glioblastoma cells in a *TERT* promoter mutation-dependent manner. As previously discussed, the C228T and C250T mutations allow specifically for the binding of the heterotetramer form of the ETS transcription factor GABP.[30] GABPβ1L is a tetramer forming isoform of GABP that is not necessary for normal development but binds to the mutant *TERT* promoter site. The authors knocked down GABPβ1L in *TERT* promoter mutant glioblastoma cells in vitro, resulting in decreased GABP binding, *TERT* expression, and cell viability. These effects of GABPβ1L knockdown were not seen in wildtype *TERT* promoter glioblastoma cells. Concordantly, knocking down GABPβ1L in a mouse xenograft model of glioblastoma impaired tumor growth and increased mouse survival.[75] Targeting GABPβ1L rather than TERT itself may represent a means of specifically targeting tumor cells bearing *TERT* promoter mutations while sparing normal cells, thus avoiding the hematopoietic side effects observed with drugs such as imetelstat.

Eribulin

Eribulin mesylate is a synthetic analog of halichondrin B, a natural isolate from marine sponges and was originally developed as an inhibitor of microtubule dynamics. In the United States, it is approved for the treatment of refractory metastatic breast cancer in patients who have previously received 2 or more chemotherapeutic agents.[76] Yamaguchi et al.[77] demonstrated that eribulin inhibited the growth of platinum-resistant ovarian cancer cells via inhibition of the RdRP activity, a non-telomere lengthening role of TERT. In a xenotransplantation glioblastoma mouse model, intraperitoneal injection of eribulin led to a significant reduction in intracranial tumor growth demonstrating a promising role for this drug in glioblastoma treatment. A phase II trial in patients with recurrent glioblastoma treated postoperatively with temozolomide, radiation, and bevacizumab is currently ongoing in Japan.[78]

Conclusions

Telomeres protect the ends of chromosomes from deterioration and end fusion to other chromosomes but shorten with successive mitotic cycles in normal cells. In highly replicative cells, the *TERT* expression maintains telomere length. *TERT* expression is otherwise very tightly regulated in normal somatic cells, with most cells silencing expression and entering a senescent state as their telomeres shorten. Two recurrent *TERT* promoter mutations have been detected in many cancers. In glioblastoma, meningioma, and medulloblastoma, *TERT* promoter mutation frequency appears to increase with a worsening grade of tumor. In glioblastoma, the mutational frequency is particularly high and the prognostic implications of the *TERT* mutation vary depending on the presence of other mutations. The underlying biology explaining these survival differences is poorly understood and warrants further investigation. Nevertheless, the clinical significance of *TERT* mutation highlights the importance of annotating its status in every CNS tumor. Presently, therapies targeting TERT or telomere

maintenance are not currently part of standard-of-care regimens, but several candidates are undergoing clinical trials. While promising, targeting TERT is not without challenges. The treatment effect is dependent on telomere shortening that only occurs after several mitotic cycles. Furthermore, rapidly dividing cells, such as hematopoietic cells, depend on TERT activity and are sensitive to its inhibition. Ultimately, many aspects of TERT function and regulation remain unknown. More recently, there is mounting evidence that TERT has functions other than telomere elongation and may play other roles such as regulating gene expression, mitochondrial activity, epithelial–mesenchymal transitions, and DNA damage repair. As our understanding of the role of telomeres and *TERT* in cancer biology improves, more therapeutic opportunities will likely be identified.

Funding

National Institutes of Health (R25NS090978 to B.P.; K08NS092912 and R01NS112712 to G.P.D.). The Damon Runyon Cancer Research Foundation (to G.P.D.)

Conflict of interest statement. None declared.

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