



When the Ends Are Really the Beginnings: Targeting Telomerase for Treatment of GBM

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Abstract

Purpose of Review High-throughput genomic sequencing has identified alterations in the gene encoding human telomerase reverse transcriptase (TERT) as points of interest for elucidating the oncogenic mechanism of multiple different cancer types, including gliomas. In gliomas, the TERT promoter mutation (TPM) and resultant overexpression of TERT are observed mainly in the most aggressive (primary glioblastoma/grade IV astrocytoma) and the least aggressive (grade II oligodendroglioma) cases. This article reviews recent research on (1) the mechanism of TERT activation in glioma, (2) downstream consequences of TERT overexpression on glioma pathogenesis, and (3) targeting TPMs as a therapeutic strategy.

Recent Findings New molecular classifications for gliomas include using TPMs, where the mutant group demonstrates the worst prognosis. Though a canonical function of TERT is established in regard to telomere maintenance, recent studies on non-canonical functions of TERT explore varied roles of telomerase in tumor progression and maintenance.

Summary Somatic alterations of the TERT promoter present a promising target for novel therapeutics development in primary glioma treatment.

Keywords Glioblastoma · GBM · Telomerase reverse transcriptase (TERT) · TERT promoter mutation (TPM) · Therapeutic targeting of TERT · Cellular functions of TERT

Introduction

Glioblastoma (GBM) is the most frequently reported malignant brain tumor histology (29.6%) [1]. Prognosis for patients who develop GBM is bleak, with average survival after diagnosis of 12–16 months [2]. Surgery, irradiation, and temozolomide delay tumor progression and extend patients' survival minimally, but these tumors universally recur and prove fatal [3]. The advent of genomic characterization in GBM [4] presented an avenue to develop effective targeted therapeutics, the full success of which is yet to be realized.

Next-generation sequencing analyses have identified telomerase alterations as points of interest for elucidating the oncogenic mechanism of multiple cancer types including gliomas [4–6]. An analysis of 18,430 samples across 31 cancer

subtypes reported that within the TERT-expressing tumors (95% of available samples), 29% harbored TERT promoter mutations and 50% were methylated at the TERT promoter [7]. In gliomas, the proportion of TERT-expressing tumors with TERT promoter mutations is much higher (80–90%), indicating this is the primary mechanism of telomerase activation. Identification of mutations in the TERT promoter in adult glioblastomas [4, 5] and ATRX mutations in adult low-grade and pediatric high-grade gliomas [8] has implicated dysregulation of telomere maintenance as a crucial step of glioma pathogenesis.

Diverse mechanisms for telomerase activation and telomere maintenance have been identified across species and cell types, though evidence for their roles in oncogenic mechanisms remains poorly understood. Given our current understanding of telomerase function in telomere lengthening and maintenance, the accepted hypothesis for the role of telomerase activation in cancer is in overcoming replicative senescence; however, more recently, a case for non-telomeric roles has been explored as well [9].

Initial reports of telomerase dynamics in glioblastoma show a stark expression difference between TERT promoter

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wild-type and TERT promoter mutant tumors along with an overwhelming cytoplasmic localization of telomerase [6]. Though non-canonical, non-telomere lengthening roles for telomerase are yet to be fully considered in glioblastoma, recent work has explored the importance of these functions. In this review, we present evidence for the role of TERT in glioma pathogenesis and its potential in developing new therapies.

Telomerase Activation in GBM

Approximately 83% of primary GBMs exhibit a mutation of C to T in the promoter of TERT [5] at either -124 or -146 bp upstream from the transcription start site. Large-scale genomic analyses have established the presence of the TERT promoter mutation across glioma subsets delineated by genomic events, such as chr7/chr10 alterations, previously considered to be initiating, but later suggested to be subsequent to the mutation acquisition [10•]. The mechanism of TERT overexpression due to the promoter mutation is not well understood, though many groups have suggested varying ideas.

Mutations in the TERT promoter region result in an ETS (E26 transformation-specific family transcription factor) binding site recognized by GABPA, a component of the multimeric transcription factor GABP, which facilitates reactivation of telomerase [11••]. Structurally, the GC-rich promoter sequence is prone to a G-quadruplex structure, which is misfolded in the presence of either C124T or C146T mutations. This tertiary structure functions as a transcriptional silencing element; in the presence of either mutation, the structure and function are lost, resulting in overexpression of TERT [12]. An additional theory of active histone marks opening chromatin around the TERT promoter is being explored in the context of GBM [13], validating a previous finding in another cancer type. A study in hepatocellular carcinoma cell lines, also known to have a high incidence of TPMs, presented evidence for RNA polymerase II preferentially being recruited to open chromatin identified with H3K4me2/3, rather than the H3K27me3 mark on the wild-type promoter allele [14•].

Of the two hotspot mutations, C124T is more common than C146T in GBM. While some groups posit that both mutations are responsive to similar mechanisms, in a GBM cell line carrying the C146T mutation, evidence suggests ETS is insufficient for telomerase expression and requires non-canonical NF- κ B signaling [15]. This observation that the mechanism for reactivation at the C124T mutation could be distinct from the mechanism of reactivation at the C146T mutation is an important consideration for determining therapeutic potential, as it may provide an avenue for cells to escape dependency on one or another mutation.

Telomere lengths in GBM are seen to be shorter than matched normal tissue, further supporting the role of telomere maintenance mechanisms in glioma pathogenesis. Paradoxically, these shorter telomere lengths in tumors are observed even though the tumor overexpresses telomerase. Telomerase activity is implicated in telomere maintenance [16]; chromosomal instability from too short and too long telomeres is avoided through mechanisms for telomere maintenance [17]. The observation of short telomeres maintained in primary GBM is contrasted with the observation of longer telomeres maintained in secondary GBM and low-grade gliomas, suggesting differing strategies for addressing chromosomal instability [10•].

In melanoma cells, a two-step mechanism for tumorigenesis in response to the TERT promoter mutation provides an explanation for the observation that the presence of TERT promoter mutations coincides with shorter tumor telomeres, common to cutaneous melanoma and glioblastoma [18••]. In a melanoma cell line, it was demonstrated that unduly short telomeres led to genomic instability, contributing to malignant transformation; telomerase activation extends, and thereby protects, these short, unprotected telomeres. This is consistent with observations in yeast, where telomere lengths are heterogeneous across chromosome arms and where telomere length-dependent mechanisms in the cell respond to the length of the shortest telomere [19]. Recent work has also established the identity of a telomere trimming protein called TZAP (telomeric zinc finger-associated protein) which recognizes unprotected long telomeres and activates a telomere trimming process which limits individual telomere length [20].

Epigenetic considerations related to gliomas and TPMs involve histone 3.3 (H3FA). A common mark for silent chromatin, H3K27me3, is seen concurrently with normal levels of telomerase expression and wild-type TERT promoter; a common mark for active chromatin, H3K4me2/3, is seen concurrently with TPMs [14•]. Interestingly, the alternate telomere maintenance mechanism driven by ATRX mutations is observed in low-grade and secondary gliomas and seen concurrently with H3K27M mutations, thus removing the silencing mark. As a methyltransferase specific for lysine 27, EZH2 is an important point of study when understanding the mechanism of aberrant TERT expression. In glioma cell lines, TERT expression has a positive effect on EZH2 expression and diminished EZH2 expression has a negative effect on TERT expression [21].

The same study provided evidence towards a non-canonical role for TERT in metabolic reprogramming and DNA damage responses in GBM. However, care must be taken in interpreting data elucidating metabolic mechanisms in GBM, as currently used model systems for studying GBM pathogenesis significantly misrepresent the natural metabolic state [22].

Mechanistic Consequences of Telomerase Overexpression in GBM

The necessity to escape from replicative senescence and the almost ubiquitous presence of the mutant TERT promoter in primary gliomas suggests activation of telomerase expression being a necessary component of gliomagenesis [23]. The current understanding of telomerase function is in telomere lengthening and maintenance, though recent literature suggests it may play other roles as well. Canonical and selected non-canonical functions of telomerase are summarized in Fig. 1. Through characterization of a mitochondrial localization sequence, a nuclear localization sequence, and phosphorylation sites relevant to cellular localization, it has become apparent that elucidation of the mechanistic consequences of the TERT promoter mutation in glioma pathogenesis has not yet been fully considered beyond canonical telomere lengthening functions.

Genomic instability in a cancer cell can arise from a number of different mechanisms, and telomere maintenance is an obvious contributor to the oncogenic process. Unprotected

telomere ends produced by rapid lengthening, disrupted expression of shelterin complex proteins, or lack of histone wrapping can all contribute to free double-stranded ends available for homologous recombination. While primary gliomas can employ telomerase activation to protect against telomere damage-induced genomic instability, low-grade and secondary gliomas employ ATRX and the metabolic product of mutant IDH, found to be mutually exclusive with TPMs in adult GBM. The product of IDH R132H is 2-hydroxyglutarate (2HG) which can suppress homologous recombination, while ATRX mutations override this suppression [27]. The capacity to overcome genomic instability is paramount to a glioma's survival in the face of rapid proliferation and the genomic events which contribute to progression.

Reports of telomerase activity within non-nuclear regions in the cell suggest there may be non-telomere lengthening roles for this enzyme [6]. In the last decade, the volume of research covering extra-telomeric roles for telomerase and TERT has significantly increased. Though these studies have not been done in the context of gliomas, they have been conducted across various cancers [28] and provide a starting point

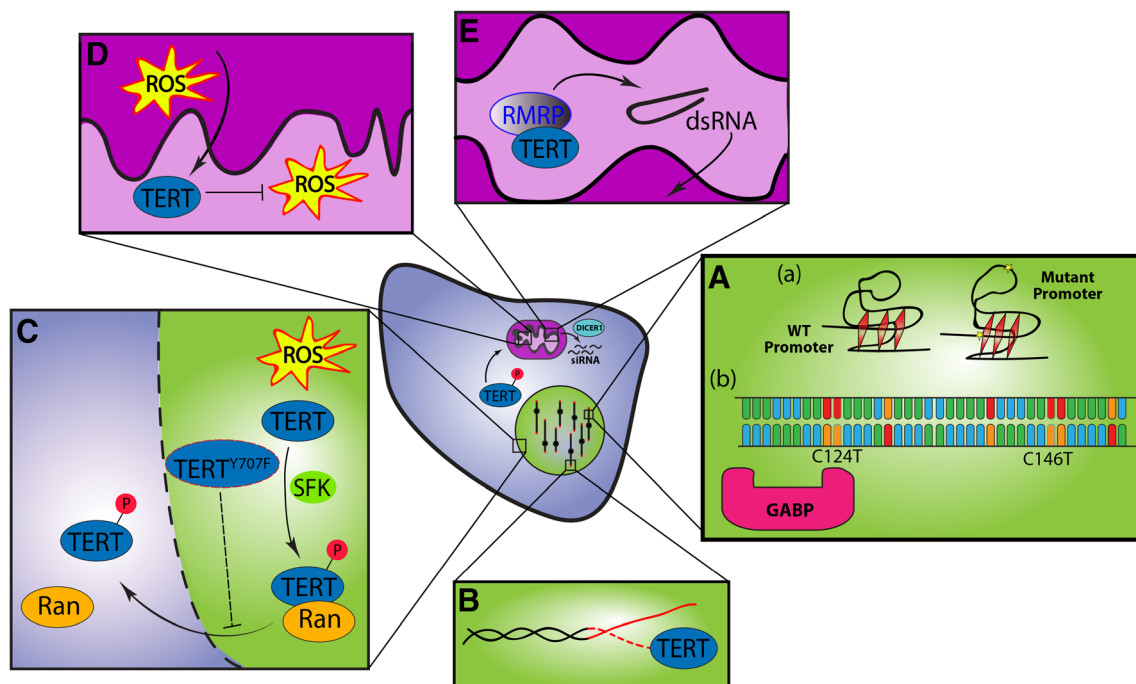


Fig. 1 Summary of canonical and non-canonical functions of TERT. (A) Human telomerase reverse transcriptase (TERT) is found on chromosome 5. Mutations in the promoter region of TERT are at hg19 positions 1,295,228 and 1,295,250, or -124 and -146 bp from the start site, respectively. Hypothesized mechanisms of TERT overexpression related to promoter mutations include (a) loss of tertiary G-quadruplex structure in promoter region and (b) a de novo binding sequence for GABP within the mutated promoter. (B) Canonical function of active telomerase is to elongate telomeres using telomerase RNA component (TERC) as a template. (C, D) Under oxidative stress, phosphorylated TERT can be exported from the nucleus and shuttled into mitochondria, to abrogate production of mitochondrial reactive oxygen species [24]. Src family

kinases (SFKs) (Src, Fyn, and Yes) phosphorylate tyrosine 707 (Y707) of TERT under oxidative stress to facilitate its transport from the nucleus [25]. A mutation in Y707 has been shown to prevent nuclear transport of TERT to mitochondria. (E) In the mitochondria, TERT associates with the RNA component of mitochondrial RNA processing endoribonuclease RMRP, and this complex, an RNA-dependent RNA polymerase (RdRP), produces RMRP-derived double-stranded RNAs (dsRNAs) that are further processed into small interfering RNAs (siRNAs) in a Dicer-dependent manner that controls the endogenous levels of RMRP [26••]. TERT has also been shown to play a role as a transcriptional modulator in the WNT/ β -catenin signaling pathway (not shown in schematic)

for probing novel mechanisms in telomerase-overexpressing gliomas and elucidating biologically significant mechanisms in glioma pathogenesis. In the summary to follow, TERT refers to the catalytic subunit of telomerase, rather than the holoenzyme.

One non-canonical function of TERT relates to MYC, which is elevated in 60–80% of GBM [29]. In an *in vitro* model of lymphoma, it was demonstrated that TERT can regulate the expression of MYC target genes and is required for both stabilizing MYC and regulating its ubiquitination [30]. Another non-canonical function relates to Wnt/ β -catenin signaling, though there have been conflicting reports regarding the role of TERT, based on cell type. In the context of mouse embryonic stem cells, TERT was demonstrated to act as a cofactor for Wnt-regulated transcriptional responses, which affect proliferation or differentiation [31]. In an *in vitro* model of breast cancer, either TERT overexpression or Wnt activation recapitulates tumorigenesis; however, no association between TERT and WNT target genes was detected in a subsequent *in vivo* study [32]. Thus, conflicting accounts between differing model systems should be considered. A biological role of WNT signaling is well established in maintenance of glioma stem cell (GSC) and glioma cell invasion [33, 34]. Treatments that inhibit TERT expression, thereby diminishing a cofactor necessary for Wnt-regulated transcriptional responses, could, therefore, diminish maintenance of the GSC phenotype as well as the invasive potential of glioma cells.

Non-canonical functions of TERT in mitochondria are also well established [24, 26•, 35], although not in context of gliomas, and could play an important role in tumorigenesis. When localized to the mitochondrial matrix, TERT binds to mitochondrial DNA, thereby protecting it from oxidative stress [36]. This observation of TERT localization to the mitochondrial matrix is associated to increased oxidative stress, suggesting a dedicated signaling mechanism for TERT-dependent protection from mitochondrial DNA damage. Another documented mitochondrial function relates to production of siRNAs, through an RNA-dependent RNA polymerase (RdRP) formed by TERT in complex with the RNA component of mitochondrial RNA processing endoribonuclease (RMRP) [26••]. Such observations suggest the relevance of TERT overexpression in context of DNA damage response mechanisms, rather than primarily immortalization.

An *in vitro* study across multiple cancers, including GBM, demonstrated that TERT associates with the RNA polymerase III subunit RPC32 to enhance tRNA expression and increases protein production for increased cancer cell proliferation [37]. While this non-canonical role of TERT occurs in the nucleus, it further supports that the widely held assumption of increased telomerase activity being primarily dedicated to telomeric extension is biologically inappropriately constrained.

Evidence from an *in vitro* study of melanoma suggests biological involvement of the RAS-ERK pathway in

activating telomerase and suggests a competitive benefit in BRAF mutations and TERT promoter mutations occurring together, as they contribute to maintaining an open chromatin state of the promoter [38, 39]. In primary GBM, the prevalence of this BRAF mutation is relatively low (around 6–7%); however, pediatric CNS tumors display higher prevalence as do lower-grade adult gliomas. In glioma, only one case has ever been recorded carrying both BRAF and TERT promoter mutations, in a rare tumor, which was of partial epithelioid glioblastoma and partial low-grade astrocytoma pathology [40].

High telomerase expression is predictive of more aggressive gliomas, which has also been attributed to persistence of glioma stem cells [41]. It was demonstrated that persistent enzyme-deficient TERT expression was sufficient in glioma stem cells to maintain stemness through inducing EGFR expression [42]. The persistence of glioma stem cells presents a major barrier to effective treatment, and if telomerase is truly a required component of the EGFR axis, then inhibition of telomerase activity will be even more crucial to achieve in order to produce controlling therapies for GBM.

Prognostic Importance and Therapeutic Opportunities of Telomerase in GBM

Prognostically, genomic alterations to TERT in various glioma subtypes can indicate better or worse outcomes (Table 1). The observation of TERT promoter mutations in adult primary glioblastoma was an important contribution to defining clinically relevant subtypes for diagnosis [52]. As the current understanding stands, telomere length is used as a surrogate measure for telomerase activity due to telomere mRNA expression being a poor measure for telomerase activity. Multiple tools have been developed to measure telomere length [53–55], including bioinformatics tools using whole-genome sequencing data [56, 57]; however, the accuracy of these tools is yet to be confirmed in comparison to a widely accepted “gold-standard.” Nevertheless, in glioma patients, tumor telomere length compared to leukocyte telomere length served as an independent prognostic marker of overall survival and progression-free survival [58].

One group has followed the detection of telomerase mRNA in serum from human cancer patients as well as exosomes from cancer cell lines. Although serum levels of telomerase mRNA are very low and unstable, an exosome-based method was able to detect telomerase mRNA in patient serum across cancer types [59•]. This preliminary study demonstrated that exosomal TERT mRNA was positively associated to disease stage and showed use in determining disease progression. Although glioma patients were not included in this patient set, increasing progress on understanding tumor RNA

Table 1 Multivariate prognostic implications with TERT promoter mutation (TPM) in glioblastoma

References	TPM presence in GBM cohort (IDHwt) (%)	Conclusions
Batista et al. 2016 [41]	66.9	TPM confers poor prognosis when also carrier of rs2853669 SNP
Spiegel-Kreinecker et al. 2015 [43]	73	TPM associated to shorter overall survival and shortest OS in GBMs with TPM and rs2853669 SNP
Simon et al. 2015 [44]	80.3	The worst overall survival associated to GBMs with TPM and rs2853669 SNP
Mosrati et al. 2015 [45]	86	The worst overall survival associated to GBMs with TPM and rs2853669 SNP
Gao et al. 2016 [46]	32.7	TPM with long relative telomere length predicted radiotherapy resistance
Fan et al. 2016 [47]	46.3	GBMs with TPM occur in right temporal lobe
Batista et al. 2016 [41]	66.9	GBMs with TPM more frequently located in frontal lobe
Ersoy et al. 2017 [48]	76	Imaging biomarkers from VASARI protocol are weak for predicting TPM
Arita et al. 2016 [49]	60	GBMs with TPM and unmethylated MGMT have the worst PFS after radiation and TMZ
Nguyen et al. 2017 [50]	75	GBMs with TPM and unmethylated MGMT have the worst PFS after radiation and TMZ
Labussiere et al. 2014 [51]	75.7	EGFR amplification confers slight survival advantage with TPM

traversing the blood-brain barrier (BBB) into plasma may allow for this method to be used in the context of brain tumors.

Though our understanding of the glioma-genic mechanisms of telomerase is incomplete as of yet, with the current understanding of activating mechanisms of telomerase, there has been development on molecular therapies for telomerase inhibition (Table 2). In the general sphere of cancer therapy, multiple methods have been reported such as small-molecule

inhibitors, telomerase biogenesis inhibitors, gene therapy using telomerase promoter-driven expression of a suicide gene, and nucleoside analogues incorporated in telomere synthesis to cause telomere dysfunction [71]. Only a select number of these therapies have shown activity in solid tumors, and even fewer have been applied in the context of gliomas.

Imetelstat (GRN163L) is a competitive telomerase inhibitor which originally had success against myeloproliferative

Table 2 Telomerase-related therapies currently studied in glioblastoma

Therapy	Study conclusion(s)	Mechanism of action	Clinical stage	BBB penetration	Refs.
Telomestatin	Effective against patient-derived GSCs and GSC-derived mouse intracranial tumors	Telomeric G-quadruplex ligand	Pre-clinical	NA	[60, 61]
6OTD (Y2H2-6M(4)-oxazole telomestatin derivative)	Medicinally optimized telomestatin derivative suppresses growth of GSC-derived mouse intracranial tumors	G-quadruplex stabilization	Pre-clinical	Yes	[62]
GRN163L	Effective against patient-derived GBM tumor-initiating cells and orthotopic GBM xenograft tumors	Oligonucleotide complementary to RNA template of TERT	Pre-clinical	Yes	[63]
Eribulin mesylate	Suppresses growth of intracerebrally injected GBM cell lines	Specific inhibitor of RNA-dependent RNA polymerase (RdRP) activity of TERT	Phase II	Yes	[64]
BIBR1532	Nonspecific growth inhibition across GSCs and NSGCs	Competitive inhibition at enzyme active site	Pre-clinical	NA	[65]
Braco19	Suppresses cell proliferation and reduces telomerase activity in GBM cell lines	Telomeric G-quadruplex ligand	Pre-clinical	NA	[66]
GTC365/GTC260	Reduces telomerase activity and selectively suppresses viability of GBM cell lines	TERT promoter G-quadruplex-misfolding redirection	Pre-clinical	NA	[67, 68]
RHPS4	Suppresses cell proliferation and activates DNA damage in GBM cell lines	G-quadruplex stabilization, particularly of relatively short telomeres	Pre-clinical	NA	[69]
Telomelysin	Demonstration of activity and safety, though limited clinically relevant response across study groups	TERT promoter-driven oncolytic adenovirus	Phase I	NA	[70]

NA not available

neoplasms and recently demonstrated potent telomerase inhibition in CNS tumors [72••]. This report of intratumoral telomerase inhibition in brain malignancies is promising due to the demonstration that imetelstat is found in tumor tissue from pediatric CNS tumor patients. Another class of therapeutics being developed in the specific context of gliomas is G-quadruplex stabilizers [12], which stabilize the tertiary structure misfolding which results from the TERT promoter mutation. In vitro results of these molecules have shown promise in the context of melanoma and they are currently being investigated in context of gliomas [67•].

Recent advances in telomerase inhibitory therapies have shown relative successes in myeloid tumors, but have been disappointing overall in solid tumors. Whether such therapies can be effective for glioblastoma, specifically, will be dependent upon potency, specificity, and the molecule's ability to cross the BBB. Penetration of the BBB presents a major obstacle in drug development for brain tumors. Innovations in drug delivery have presented unique and promising solutions to this issue.

Aside from the limitation of effectiveness caused by inefficient drug delivery, a better understanding of the molecular pathways impacted by telomerase overexpression is needed. Current methods of measuring drug efficacy are dependent on a clear understanding of these mechanisms. If overexpressed TERT plays canonical telomere lengthening roles in gliomas, then the effectiveness of telomerase inhibition is dependent on how quickly a critical mass of telomeres shortens (dependent on rate of cell division) below a critical telomere length [18••, 71]. This would imply a greater efficacy of telomerase inhibitory drugs in gliomas with shorter telomeres. However, if overexpressed telomerase plays non-canonical roles in glioma, then different pharmacodynamic measures will be necessary to identify phenotypic subtypes of gliomas which will be vulnerable or resistant to telomerase inhibition. Importantly, there is evidence (under the appropriate genomic conditions) showing cancer cells can switch to the ALT mechanism for telomere lengthening when telomerase activity is inhibited [73]. However, since an extremely low percentage of cases with a TERT promoter mutation concurrently present with ATRX mutations, the mechanistic chronology of mutation acquisition may shed light upon how consequential this finding is.

Conclusion

Much of the available literature primarily addresses how telomerase is overexpressed as a result of the two hotspot promoter mutations. These studies are performed in embryonic stem cells, which naturally express telomerase (unlike all somatic cells), or established melanoma or glioblastoma cell lines which naturally harbor TPMs. Furthermore, large-scale

genomic analyses provide insight towards patterns in common mutations, but fail to offer a biological explanation of the specific mechanism of oncogenic transformation driven by telomerase. As the most prevalent non-coding mutation in cancer, the TERT promoter mutation provides a curious platform for understanding therapeutic potential. Much effort has resulted in acquiring an understanding of the mechanism of activation that allows these specific mutations to confer telomerase overexpression in those cells. However, the diversity of mechanisms consequent to telomerase overexpression indicates caution is necessary in ascribing one therapy across multiple cancers which display telomerase overexpression, or even the same promoter mutation.

Ultimately, the therapeutic potential of the TERT promoter mutant cannot be fully appreciated without a better understanding of the function of telomerase within glioblastoma cells. Admittedly, the enzyme is known to have a conserved function across species; however, assuming a singular function for this enzyme within the cancer cell is inappropriate and misguided. More research is warranted in glioblastoma, specifically, to elucidate the complete function of telomerase and whether it plays a truncal role in dysregulating foundational cellular processes such as replicative potential, genomic instability, and metabolic functions.

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Compliance with Ethical Standards

Conflict of Interest Saumya R. Bollam, Michael E. Berens, and Harshil D. Dhruv declare no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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