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High incidence of *TERT* mutation in brain tumor cell lines

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Abstract

TERT promoter gene mutations are highly recurrent in malignant glioma. However, little information exists regarding their presence in experimental brain tumor models. To better characterize systems in which *TERT* mutation studies could be appropriately modeled experimentally, the *TERT* promoter was examined by conventional sequencing in primary brain tumor initiating cells (BTIC), two matched recurrent BTIC lines, a panel of established malignant glioma cell lines, and two meningioma cell lines. Telomerase gene expression was examined by quantitative PCR. We found that all glioblastoma BTIC lines harbored a *TERT* mutation, which was retained in two patient-matched recurrent BTIC. The *TERT*C228T or C250T mutation was found in 33/35 (94 %) of established malignant glioma cell lines and both meningioma cell lines examined. Brain tumor cell lines expressed variably high telomerase levels. Thus, a high percentage of glioma cell lines, as well as two meningioma cell lines, harbors *TERT* mutations. These data characterize tractable, accessible models with which to further explore telomerase biology in these tumor types.

Keywords

TERT; Telomerase; Glioma; Glioblastoma; Meningioma; Brain tumor initiating cells; Cancer

Introduction

Glioblastoma is the most aggressive primary brain tumor and carries a median survival of 12–14 months despite standard-of-care treatment [1]. To better understand its genomic landscape, extensive multi-platform genomic profiling approaches have been employed to characterize the somatic alterations that accumulate during the development of these tumors [2–4]. Collectively, these studies and others have revealed many of the recurrent genomic changes that underlie this cancer type. Of these, recurrent mutations in the promoter region of *TERT* are the most prevalent.

TERT encodes the catalytic subunit of the telomerase enzyme, which protects the telomeric ends of chromosomes and is considered necessary for human cellular transformation [5]. Additional extratelomeric roles for telomerase have also been described [6]. Telomerase is estimated to be overexpressed in 85–90 % of all human malignancies [7]. The recently discovered recurrent C→T mutations at positions 1,295,228 and 1,295,250 (C228T and C250T, respectively) in the *TERT* promoter [8] lead to increased telomerase expression, likely due to the creation of novel ETS family transcription factor binding sites [8, 9]. Together, these mutations have been identified in over 80 % of glioblastomas [10] as well as a significant percentage of other malignancies including urothelial cancer, melanoma, basal cell carcinoma, and myxoid liposarcoma [11]. In fact, primary glioblastoma represents the malignancy with the highest incidence of this recurrent mutation to date, suggesting that its presence is crucial to tumor development and/or maintenance. Moreover, the presence of *TERT* mutations in the absence of concomitant *IDH1* mutations portends a worse prognosis across all glioma grades [12, 13]. However, despite the critical importance of *TERT* mutations in glioblastoma, few in vitro glioblastoma models have been characterized to enable the study of this biology.

To identify in vitro experimental models of *TERT*-mutant brain tumors, we first annotated the *TERT* promoter mutation status in ten low-passage, patient-derived brain tumor initiating cells (BTIC) and provide the first evidence that these mutations are retained in patient-matched recurrent BTIC. We then characterized the *TERT* promoter mutation status in 35 well-studied glioblastoma cell lines as well as 2 meningioma cell lines and found that 35/37 (94.6 %) harbor either the C228T or C250T promoter mutation. *TERT*-mutated cell lines harbored high but variable levels of telomerase expression.

Materials and methods

Cell lines were obtained from 4 sources. Of 37 well-passaged cell lines, all but 6 were obtained from the Cancer Cell Line Encyclopedia [14]. IOMM-Lee, LN215, LN235, LN428, LN464, and SF767 were obtained from The RNAi Consortium, Broad Institute (Cambridge, MA). Normal human astrocytes were purchased from Lonza, Inc (Mapleton, IL). Brain tumor initiating cells (BTIC) were generated from patient specimens immediately ex vivo according to a previously described protocol [15]. Low-passage (5 passages from initial culture) BTICs were utilized for all experiments. Fluorescence in situ hybridization (FISH) and targeted cancer gene sequencing data were obtained as part of the clinical workflow at our institution.

The promoter region of the *TERT* gene was amplified by polymerase chain reaction using a modified previously described protocol [16]. Briefly, genomic DNA was isolated using the DNAeasy Blood and Tissue Kit (Qiagen). The region from 1,295,129 to 1,295,372 on chromosome 5 as annotated in the GRCh37 reference assembly was amplified from 200 to 300 ng of genomic DNA using the primers 5'-GCACAGACGCCAGGACCGCGCT-3' and 5'-TTCCCACGTGCGCAGCAGGACGCA-3' [16] for 40 cycles using Taq polymerase (New England Biolabs) and 29 PCRx Enhancer Solution (Invitrogen) in a total volume of 50 µl. The *TERT* promoter amplicon was gel purified using the Qiaquick Gel Extraction Kit (Qiagen). Amplicons were sequenced using the above primers via conventional Sanger technology.

Telomerase expression levels were determined by quantitative RT-PCR. First-strand cDNA was synthesized from 1 µg of RNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Telomerase and GAPDH were amplified from 2 µl cDNA using TaqMan primers (assays Hs00972650 and Hs03929097, respectively, Applied Biosystems) for 40 cycles on an Eco Real-Time PCR System (Illumina).

Results

Recent work has shown that malignant glioma harbors a high incidence of *TERT* mutations [10, 17]. To identify tractable models to study telomerase biology in this disease type, we first determined the incidence of *TERT* mutations by conventional Sanger sequencing technology in a subset of low-passage (5 passages from initial culture), patient-derived brain tumor initiating cells (BTIC) established at our institution from patients with primary (i.e., de novo) glioblastoma [15]. Nine of the ten lines characterized by clinical fluorescence in situ hybridization or gene panel sequencing exhibited alterations typically identified in primary glioblastoma, including loss of chromosome 10q or *EGFR* amplification (Table 1). Of ten BTIC lines derived from patients with an average age of 52.7 (38–66), nine harbored the C228T *TERT* mutation and one harbored the C250T mutations (Table 1). We then assessed whether the *TERT* mutation was retained in two patient-matched BTIC lines established from the recurrent glioblastomas in patients from whom the original BTIC lines, B2 and B28, were derived. Both patients received adjuvant radiation treatment, temozolomide, and were enrolled in the ACT IV trial. In both recurrent BTIC, termed “B2-Rc” and “B28-Rc”, the same *TERT* mutations were retained. These data show that *TERT* mutations are found not only in primary BTIC but also in patient-matched recurrent BTIC.

Having demonstrated that BTIC lines harbor a high incidence of *TERT* mutations, we extended our analysis and determined the incidence of *TERT* promoter mutations in a large panel of well-characterized and frequently studied glioma cell lines. Strikingly, 33/35 (94 %) of the analyzed cell lines harbored either the C228T or C250T mutation (Table 2), with 12/35 (34 %) carrying the C250T mutation compared to 21/35 (60 %) that possessed the C228T mutation. This distribution of mutations is consistent with previous studies in primary gliomas showing a relative predominance of the C228T mutation compared to C250T [12]. In the 2/35 (6 %) cell lines that were wild type, no alternative mutations were identified in the examined region of the promoter.

Because additional studies show that meningiomas with *TERT* promoter mutations exhibit a significantly shorter time to recurrence [18] and a propensity to recur at a higher tumor grade [19], we also annotated the *TERT* promoter region in two well-studied meningioma cell lines. Both CH-157-MN and IOMM-Lee, which represent two of only a small panel of available meningioma cell lines, harbored the C228T *TERT* mutation (Table 2). Thus, most established, well-studied glioma and meningioma cell lines carry either the C228T or C250T *TERT* promoter mutations.

Because *TERT* promoter mutations are correlated with elevated telomerase expression, we examined the relative telomerase expression by quantitative RT-PCR in our panel of malignant glioma and meningioma cell lines as compared to normal human astrocytes (NHA), which harbor a wild-type *TERT* promoter sequence. *TERT*-mutant glioma cell lines expressed 2.2- to 286-fold higher telomerase expression relative to NHA (Fig. 1a). The 2 wild-type cell lines, CAS1 and SF172, exhibited telomerase expression that was 5.7-fold and 15.5-fold higher, respectively, than NHA as well. Meningioma cell lines expressed 145- to 400-fold higher telomerase expression relative to NHA (Fig. 1b). These results show that all *TERT*-mutant cell lines express a broad range of high levels of telomerase relative to untransformed astrocytes, and that well-passaged cell lines without the C228T/C250T mutations also express elevated telomerase levels (Fig. 1d).

Finally, we examined telomerase gene expression in the BTIC lines. BTIC lines derived from newly diagnosed glioblastomas expressed 3.2- to 12.9-fold higher telomerase levels relative to NHA (Fig. 1c). Moreover, the two recurrent BTIC lines, B2-Rc and B28-Rc, expressed nearly sevenfold more telomerase than NHA. These data show that *TERT*-mutant BTIC harbor telomerase expression that is retained in patient-matched recurrences.

Discussion

Herein, we annotated the region of the *TERT* promoter that harbors the highest percentage of recurrent mutations in a large panel of glioma cell lines, commonly studied meningioma cell lines, and a subset of BTIC. Gliomas have been found to exhibit the highest incidence of *TERT* promoter mutations across many cancer types, suggesting a major role of telomerase in tumor development and, likely, in tumor maintenance as well. In the short time since its discovery, *TERT* mutation status in glioma has also become a critical prognostic factor. Specifically, *TERT* mutations in both low-grade and high-grade *IDH1* wild-type glioma portend a more aggressive natural history [13, 16, 20]. Conversely, *TERT* mutations concomitant with *IDH1* mutations define a low-grade oligodendroglioma phenotype that presages the best prognosis for patients with these tumors. Thus, there is tremendous utility to the identification of accessible cell line models with *TERT* mutations to further study telomerase biology in glioma.

It is particularly important to characterize tractable in vitro models to study telomerase biology in brain tumors because its functions are complex. Although the effects of telomerase on telomere maintenance are well acknowledged, the recent observation that telomere length may be shorter in *TERT*-mutant gliomas compared to wild-type counterparts suggests that telomere lengthening functions may not be the only cancer-relevant function of

this enzyme [12]. Indeed, prior work showed that immortalized cells could be transformed with a mutant telomerase unable to lengthen telomeres in vivo, providing experimental evidence that additional extratelomeric functions of this enzyme may be operative in the cancer setting [21]. Specifically, there appear to be possible telomere-independent roles for telomerase in regulating downstream Wnt signaling, in producing siRNAs through an RNA-dependent RNA polymerase function, and regulating apoptosis [6]. Further work is needed to clarify the physiologic relevance of these putative telomerase-dependent functions to the process of glioma formation and/or maintenance.

We found that nearly all of the cell lines we studied harbored either the C228T or C250T *TERT* promoter mutation, which correlated with variable levels of increased telomerase expression. While the high total percentage of *TERT* mutations in the 35 glioma cell lines reported herein (94 %) is consistent with the high incidence of *TERT* mutation seen in this disease type, there may also be a selection bias for *TERT*-mutant lines that may adapt well to cell culture over time. Our report thus provides the most comprehensive annotation of the *TERT* mutation status in a large panel of the most commonly studied glioma cell lines employed in the study of malignant glioma. A small subset of the malignant glioma cell lines we characterized have recently been shown to carry *TERT* promoter mutations [9, 22–24], and our results are concordant with these findings.

It is notable that there appears to be a broad distribution of telomerase overexpression in our cell lines, as has been observed in other studies [12]. The higher levels of expression observed in some of the established glioma and meningioma cell lines may either mirror outliers observed in primary tumor samples or perhaps reflect a selection process for longstanding in vitro passage adaptation. Additional work will thus be necessary to determine both the etiologies and the consequences of the differences in the magnitudes of telomerase expression between tumors. Moreover, it will be important to consider the alternative mechanisms of telomerase overexpression in the absence of promoter mutations. In the case of the wild-type CAS-1 cell line, *TERT* is amplified and thus may be a possible mechanism that leads to increased telomerase expression relative to NHA. In other *TERT* wild-type settings, it is possible that elevated activation of some of the known transcriptional regulators of telomerase expression, such as c-Myc and b-catenin, may drive telomerase expression [25]. Additionally, epigenetic silencing of transcriptional repressors of telomerase may contribute to its regulation [11]. These mechanisms may also contribute to the high telomerase levels observed in the *TERT*-mutant context as well. It may also be important to consider the post-transcriptional regulation of the telomerase–holoenzyme complex. For instance, the assembly of the multi-component shelterin complex with the appropriate stoichiometry as well as its finely regulated trafficking within the nucleus is critical for telomerase function and provides additional points of cancer-relevant dysregulation that could augment telomerase activity in the absence of *TERT* promoter mutations or elevated telomerase transcription. Ongoing work is directed at exploring telomerase regulation in the glioma setting.

In addition to the well-established malignant glioma lines that we studied, we also characterized the *TERT* promoter mutations in primary patient-derived BTIC. Nine of ten BTIC harbored the C228T *TERT* promoter mutation, whereas one BTIC line carried the

C250T mutation. Interestingly, in the two cases in which we also established BTIC from the patients' glioblastoma recurrences, we also detected the same *TERT* mutations. While the preservation of *TERT* mutation was recently described [12], we provide the first evidence of *TERT*-mutant recurrent BTIC, suggesting a feasible method with which to study *TERT* mutation in primary and patient-matched recurrent settings. Although both recurrent BTIC exhibited decreased telomerase expression compared to their matched cell lines derived from newly diagnosed glioblastoma, additional work in larger paired samples will be needed to validate this trend and explore its biological relevance. It is unlikely that *TERT* mutation itself confers an advantage to establishing low-passage, patient-derived cultures because we have successfully established BTIC in over 90 % of our attempts.

Both meningioma cell lines we studied harbored *TERT*C228T mutations and exhibited significant levels of telomerase expression. Because primary, patient-derived meningioma cells are notoriously challenging to grow reliably and study in vitro, our data show that the CH-157-MN and IOMM-Lee cell lines—2 of only a small subset of cell lines commonly studied in meningioma biology—represent tractable models with which to study telomerase biology in this disease context. This line of study is particularly important because *TERT* mutation has been shown to correlate with a significantly shorter time to recurrence [18] as well transformation from lower grade to higher grade disease at time of recurrence [19].

In summary, we provide a comprehensive annotation of the *TERT* mutation status in a large panel of heavily studied malignant glioma cell lines, several well-characterized meningioma cell lines, and a panel of BTIC lines. Given the clear importance of recurrent *TERT* promoter mutations in these tumor types, further work using these cell line models will be important to better understand the contribution of *TERT* mutations to the cancer phenotype.

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Fig. 1.

Telomerase gene expression in brain tumor cell lines. Telomerase gene expression was determined by quantitative RT-PCR in established malignant glioma cell lines (a), meningioma cell lines (b), and low-passage, patient-derived BTIC as well as 2 patient-matched recurrent BTIC (c). The telomerase expression of all cell lines examined by $TERT$ mutation is shown in (d)

Table 1*TERT* status in primary and recurrent glioblastoma BTIC

Histology	BTIC line	<i>TERT</i> mutation	Age at presentation or recurrence	Other clinical genomic alterations
Primary glioblastoma	B2	C228T	56	Clinical trial evaluation: EGFRvIII ⁺
Recurrent glioblastoma	B2-Rc	C228T	57	Panel sequencing: PTEN pY315*
Primary glioblastoma	B28	C228T	38	Clinical trial evaluation: EGFRvIII ⁺ FISH: <i>EGFR</i> amplification
Recurrent glioblastoma	B28-Rc	C228T	39	Panel sequencing: PIK3CA pH1047R, <i>EGFR</i> amplification
Primary glioblastoma	B36	C228T	55	FISH: 1p36/19q13 loss, Chr 10q loss
Primary glioblastoma	B49	C228T	60	FISH: Chr 10q loss Panel sequencing: PTEN pL320S, multiple TP53 mutations
Primary glioblastoma	B51	C228T	43	FISH: <i>EGFR</i> amplification, Chr 10q loss
Primary glioblastoma	B58	C228T	66	FISH: Chr 10q loss
Primary glioblastoma	B65	C228T	53	Clinical trial evaluation: EGFRvIII ⁺ FISH: <i>EGFR</i> amplification, Chr 10q loss Panel sequencing: EGFR pC636Y, PTEN pE7*
Primary glioblastoma	B66	C228T	36	FISH: Chr 7 polysomy, Chr 10q loss, Chr 19q13 loss Panel sequencing: EGFR F254I, TP53 P169Qfs*42, PTEN pR378Ifs
Primary glioblastoma	B67	C228T	60	FISH: Chr 10q loss
Primary glioblastoma	B71	C250T	60	None identified by FISH or panel sequencing

Table 2*TERT* promoter status in malignant glioma and meningioma cell lines

Cell line	<i>TERT</i> mutation	Cell line	<i>TERT</i> mutation	Cell line	<i>TERT</i> mutation
<i>Established malignant glioma cell lines</i>					
A1207	C250T	LNZ308	C228T	SF295	C228T
A172	C228T	LN215	C228T	SF767	C250T
AM38	C250T	LN229	C228T	SNU1105	C228T
CAS1	WT	LN235	C250T	SNU201	C250T
DBTRG	C250T	LN319	C250T	SW1783	C228T
DKMG	C250T	LN340	C228T	T98G	C250T
GB1	C228T	LN382	C228T	U178	C228T
GMS10	C228T	LN428	C228T	U251	C228T
HS683	C250T	LN443	C250T	U343	C228T
KALS1	C228T	LN464	C228T	U87	C228T
KNS60	C250T	SF126	C228T	YKG1	C228T
KNS81	C228T	SF172	WT		
<i>Established meningioma cell lines</i>					
CH-157 MN	C228T	IOMM-Lee	C228T		