**EDITORIAL**

# Promoting a new brain tumor mutation: *TERT* promoter mutations in CNS tumors

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Genomic research has recently linked two fundamental observations about the molecular biology of cancer. The first of these pivotal observations is that cancer is essentially a genetic disease, with tumor cells driven to grow inappropriately by acquired genetic alterations that deregulate cellular molecular machinery. The second observation is that ~85 % of tumors contain upregulated activity of the ribonucleoprotein complex telomerase, which is thought to help tumor cells avoid senescence and achieve immortality by replenishing the telomere cap on the tips of chromatin. For many cancers, it remained unclear how telomerase activity came to be upregulated in the first place, with no obvious genetic cause to explain why telomerase activity was inappropriately "switched on". Now, recent work has uncovered what may very well be the genetic basis for telomerase activation in many human cancers, particularly CNS tumors. This work, including discoveries reported in three articles and one letter in this issue of *Acta Neuropathologica*, centers around recurrent point mutations in the promoter of the telomerase reverse transcriptase gene (*TERT*).

*TERT* promoter mutations were first identified in a whole-genome sequencing screen of melanomas and reported early in 2013 [7]. At the same time, similar mutations were discovered using a hereditary linkage approach in patients with familial melanomas [5]. Strikingly, the mutations were situated within the *promoter* of the *TERT* gene, rather than the protein-coding sequence, representing the first identification of a highly recurrent cancer mutation outside the coding region of a gene. These discoveries in melanomas sparked a search for *TERT* promoter mutations in other cancer types. *TERT* promoter mutations were soon discovered in thyroid cancers, hepatocellular carcinomas, urinary epithelium tumors, and subtypes of sarcoma [9]. Such mutations turned out to be particularly prevalent in CNS tumors, occurring in a majority of glioblastomas and oligodendrogliomas, as well as in a portion of medulloblastomas and other CNS tumors [1, 9, 10, 13, 14].

In this issue, Nonoguchi and coworkers report *TERT* mutational status for 358 glioblastomas. They point out that *TERT* mutations occur in a majority of primary glioblastomas, i.e., tumors that arise de novo [13]. In contrast, the *TERT* mutations were rare among secondary glioblastomas, which develop slowly from lower-grade astrocytomas of WHO grade II–III, and which tend to contain *IDH1* mutations. While *TERT* mutations were associated with a poor prognosis among all glioblastoma patients, Nonoguchi and colleagues conclude this likely reflects their association with the poorer-performing primary glioblastomas.

Also in this issue, Koelsche and coworkers broaden the search for *TERT* promoter mutations to a wide variety of CNS tumor types by exploring a panel of 1,515 CNS tumors. They identify *TERT* mutations in rarer entities such as gliosarcomas (81 %) and solitary fibrous tumors (50 %) [10]. They also confirm that the *TERT* mutations are tightly correlated with 1p/19q codeletion in oligodendrogliomas [1, 9, 10] and mutually exclusive with *ATRX* mutations and *IDH1/IDH2* mutations in astrocytomas and glioblastomas.

In addition to gliomas, *TERT* mutations have been found to occur in 21 % of medulloblastomas [14]. In an article by Remke and coworkers [14] in this issue, the clinical implications of *TERT* mutations in patients with this tumor type were explored in a cohort of 466 medulloblastoma patients.

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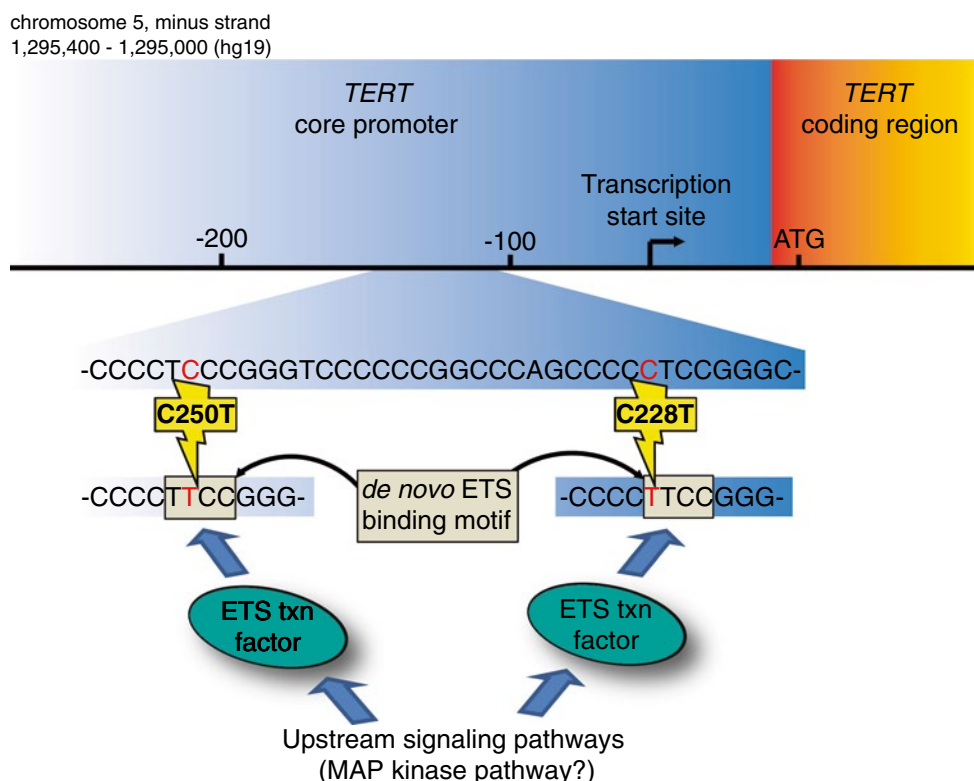e-mail: yan00002@mc.duke.edu; hai.yan@dm.duke.edu

**Fig. 1** *TERT* promoter mutations generate de novo ETS binding sites. The C228T mutation within the *TERT* promoter occurs 146 bp upstream of the ATG start codon of *TERT*, at chr5 1,295,228 (hg19). The C250T mutation occurs 126 bp upstream of the ATG start codon of *TERT*, at chr5 1,295,250. Both mutations generate a de novo sequence, 5′-CCCCTTCCGGG-3′, which contains the ETS transcription factor binding motif 5′-TTCC-3′. The new ETS motif may recruit transcription factors from the ETS family, some of which are downstream targets of MAP kinase signaling

As with gliomas, *TERT* mutations occurred in distinct subsets of medulloblastomas. The *TERT* mutations tended to occur in medulloblastomas from older patients, and were particularly frequent among the older patients with medulloblastomas from the SHH (83 %) and WNT (31 %) gene expression-based subgroups. In contrast, the *TERT* mutations were rare among the Group 3 and 4 tumors (<5 %).

What is the function of the *TERT* mutations? Two mutations account for the vast majority of the somatic *TERT* mutations in CNS and other tumor types. Both alterations are cytosine to thymine (C > T) transitions and have been termed C228T and C250T based on their genomic coordinates on chromosome 5. These events occur 124 and 146 base pairs upstream of the ATG start codon of *TERT*, respectively, as shown in Fig. 1. Both of those alterations generate an identical 11-bp nucleotide motif (5′-CCC-CTTCCGGG-3′) containing a consensus binding site for E-twenty-six (ETS) transcription factors. Generation of this motif suggests that the mutated promoter gains the ability to recruit transcription factors that might upregulate *TERT* protein expression. In line with this possibility, the mutated *TERT* promoter drives higher expression of an experimental reporter gene in cell lines compared to the wild-type promoter [7]. Also, tumors containing *TERT* promoter mutations are correlated with higher *TERT* mRNA expression than wild-type tumors [1]. Additional rarer mutations have also been discovered in the *TERT* promoter, including C249T and C228A, which do not result in generation of an ETS box per se [9, 10], adding some complexity to the situation. Also, patients with hereditary melanomas harbor *TERT* promoter mutations at a different position than the mutations that arise in sporadic tumors [5], indicating that germline *TERT* mutations might have a slightly different functional impact than the somatic *TERT* promoter mutations.

The *TERT* mutations allowed delineation of two genetic mechanisms for telomere maintenance among many tumors. The gliomas, including oligodendrogliomas, astrocytomas, and glioblastomas, are a particularly interesting example. While oligodendrogliomas (WHO grade II and III) and primary glioblastomas (WHO grade IV) have been known for years to have activated telomerase, astrocytomas of grades II and III and secondary glioblastomas WHO grade IV seldom have activated telomerase. Instead, these astrocytomas have been found to employ an alternative lengthening of telomeres (ALT) phenotype [4, 11].

ALT is a homologous recombination-mediated mechanism of telomere length maintenance that is independent of telomerase activity. Thus, telomerase activation and ALT are two different mechanisms employed by different tumors to maintain their telomeres. Intriguingly, the astrocytomas WHO grade II–III and secondary glioblastomas WHO grade IV with ALT were recently found to harbor mutations in *ATRX* that are thought to cause or contribute to the ALT phenotype [8]. In contrast, new data indicate that primary glioblastomas and oligodendrogliomas frequently harbor *TERT* mutations [9, 13]. These results together provide a genetic basis for telomere deregulation in most higher-grade gliomas, with *ATRX* mutations accounting for ALT in grade II–III astrocytomas and secondary glioblastomas, and *TERT* promoter mutations accounting for the telomerase activation seen in oligodendrogliomas and primary glioblastomas.

The situation in medulloblastomas, in which the *TERT* promoter mutations occur mostly in the adult subset of patients even though it is a predominantly pediatric tumor, highlights an important age-based distribution of the mutations. A similar situation is observed among glioblastomas. While *TERT* mutations are overall frequent among glioblastomas, they almost never occur in pediatric glioblastomas [9, 10]. Why are the *TERT* mutations virtually absent from tumors in children? Since telomerase is thought to be active in stem and progenitor cells early in life, this conspicuous age distribution has raised the possibility that childhood tumors may arise from cells that already have activated telomerase and, therefore, do not need to acquire a *TERT* mutation to aberrantly upregulate telomerase.

The high frequency of *TERT* mutations, and their association with distinct patient subgroups, raises the possibility that *TERT* mutations could aid in classification of gliomas and other CNS tumors. This possibility is especially tantalizing because the *TERT* mutations are relatively unambiguous and could be assayed by determining the sequence of only a few base pairs within the genome. Already, Remke and colleagues [14] have demonstrated a straightforward Taqman-based assay assessing the presence of the two most common *TERT* mutations. Determining *TERT* mutation status may be useful for stratification of patients for clinical trials to determine whether specific therapies are effective in different genetic subtypes of tumors. In addition, the mutations may be helpful for prognostication: for instance, those patients harboring tumoral *TERT* mutations survive longer than other patients among the group of SHH medulloblastomas [14].

Major questions remain in regards to the *TERT* mutations. Preliminary studies suggest differences in survival between patients with and without *TERT* mutations for several tumor types [9, 13]. For instance, patients with *TERT*-mutated glioblastomas have a poorer survival than *TERT* wild-type glioblastomas. However, this effect may reflect the improved survival of patients with *IDH1*-mutated glioblastomas, who almost always fall into the *TERT* wild-type group [13]. Thus, it remains to be seen whether *TERT* status is an independent prognostic indicator among glioblastomas [13].

It is important to note that a subset of adult primary glioblastomas exhibiting telomerase activation seem to lack *TERT* mutations. What genetic or epigenetic alteration is responsible for telomerase activation in these tumors? In a letter in this issue, Arita and colleagues [2] tested whether adult glioblastomas with upregulated *TERT* mRNA expression, but no *TERT* promoter mutation, might have a hypermethylated *TERT* promoter that could account for increased *TERT* mRNA expression. In fact, methylation of the *TERT* promoter had been found to be strongly associated with increased *TERT* expression in *pediatric* brain tumors, indicating that methylation, and not mutation, of the *TERT* promoter can account for *TERT* upregulation in certain tumors [3]. This finding stood in contrast to the typical role for promoter methylation, in which methylation usually silences expression of the associated gene. In contrast to the situation in pediatric glioblastomas, Arita and coworkers found that *adult* glioblastomas with high *TERT* expression, but with a wild-type *TERT* promoter, did not have increased *TERT* promoter methylation. This work leaves an open question as to the mechanism behind telomerase activation in the tumors that do not harbor either *TERT* promoter mutations or *TERT* promoter hypermethylation.

Further study of the *TERT* mutations may reveal new therapeutic hypotheses for several deadly types of CNS tumors. Years of study of telomerase as a therapeutic target, long before the discovery of the promoter mutations, has already resulted in several inhibitors of telomerase. These include imetelstat, which has elicited telomere shortening and tumor cell death in animal glioma models [12]. Might *TERT* mutation status help identify patients who best respond to telomerase-based therapies? Another consideration is that tumors treated with telomerase inhibitors may evolve resistance by developing ALT [6]. If so, combination therapies that also disrupt ALT may ultimately be necessary to target the telomere maintenance pathway in cancer. Finally, generation of ETS transcription factor binding sites by the *TERT* mutations points towards recruitment of one or more transcription factors to the *TERT* promoter as a critical step for tumor development. In this way, the discovery of the *TERT* promoter mutations may point the way towards innovative therapeutic strategies aimed at the molecular pathway *upstream* of that critical transcription factor or factors. Further research promises to pinpoint the role of *TERT* promoter mutations in CNS tumor pathogenesis and to lead the way towards improved tumor classification and treatment based on these unique mutations.

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