

# Clinical Cancer Research



## Molecular Targeting of Neural Cancer Stem Cells: TTAGGG, You're It!

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## CCR Translations

Commentary on Castelo-Branco et al., p. 111

## Molecular Targeting of Neural Cancer Stem Cells: TTAGGG, You're It!

Anita B. Hjelmeland and Jeremy N. Rich

## Abstract

Telomerase is an important mechanism by which cancers escape replicative senescence. In neural tumors, cancer stem cells express telomerase, suggesting that this may explain their preferential tumorigenesis. Oligonucleotide telomerase targeting selectively disrupts cancer stem cell growth through the induction of differentiation, adding to the armamentarium of anticancer stem cell therapies. *Clin Cancer Res*; 17(1); 3–5. ©2011 AACR.

In this issue of *Clinical Cancer Research*, Castelo-Branco and colleagues (1) report that neural cancer stem cells activate telomerase and display preferential sensitivity to a telomerase inhibitor.

Cancers are complex organ systems with multiple constituent cell types and heterogeneity within the neoplastic cellular compartment. Tumorigenic cells within cancers are often rare, suggesting that not all neoplastic cells contribute to tumor maintenance. Clonal tumor cell variation derives from genetic diversity, presumably resulting from stochastic mutational events. Additionally, many cancers display cellular hierarchies, and epigenetically distinguished neoplastic cells (called cancer stem cells, tumor initiating cells, or tumor propagating cells) are functionally defined by their capacities to self-renew and propagate tumors that are phenotypically identical to the tumor from which they were derived (2). These models of cellular heterogeneity are not mutually exclusive, as many driver mutations in cancer regulate stem cell pathways. The significance of cancer stem cells is supported by their ability to resist conventional therapies and promote tumor angiogenesis and cellular dispersal (2). Core stem cell pathways (e.g., Notch, hedgehog, and wnt) are the focus of many drug development efforts and have shown some initial promise (3). However, because of cancer's rapid adaptability and the potential toxicity against normal stem cells, no single approach is likely to be universally effective (3).

Self-renewal and sustained proliferation require chromosomal replication. In mitosis, cells face the challenge of incomplete replication of chromosome ends due to the biochemistry of DNA polymerases (4). Chromosome ends are protected through repetitive sequences (TTAGGG in humans) that form complex four-stranded structures

(G-quadruplexes). During each round of cell division, the telomere progressively decreases in length unless it is replenished by a ribonucleoprotein enzyme complex called telomerase containing a template RNA [telomerase RNA component (TERC)] and reverse transcriptase [telomerase reverse transcriptase (TERT)]. Maintenance of long telomeres is critical for genomic integrity during self-renewal in embryonic stem cells and select progenitors. The degradation of telomeres with repeated mitoses suppresses transformation as cells hit a replicative limit and undergo senescence controlled by p53 and RB pathways. Rare cells overcome the p53 and RB checkpoints but enter crisis when the telomeres become critically short. To become fully transformed, a cancer cell must overcome these three barriers (p53, RB, and telomere maintenance) (5). Therefore, it is not surprising that mutational inactivation of p53 and RB is nearly universal in many cancers, and that abnormal telomere maintenance is a common feature as well. Two predominant mechanisms for telomere regulation in cancer have been described: aberrant expression of telomerase, and activation of the alternative lengthening of telomeres (ALT) pathway. The activation of stem cell programs and the importance of telomerase for immortalization led to the suggestion that cancer stem cells have elevated telomerase activity, which facilitates telomere maintenance (4).

In this issue of *Clinical Cancer Research*, Castelo-Branco and colleagues (1) examine telomerase activity and telomere maintenance of neural tumor (glioma and neuroblastoma) subpopulations in comparison with normal stem cells. These cancers are attractive models for studying tumor cell heterogeneity, as studies support cellular hierarchies in each tumor type and telomere biology has already been investigated in brain cancers (6–8). The authors display significant rigor in addressing some of the major deficits plaguing the cancer stem cell field. They avoid the use of cell lines that acquire artifactual self-renewal mechanisms due to cell culture conditions, and phenotypically validate the functional characteristics of cancer stem cells.

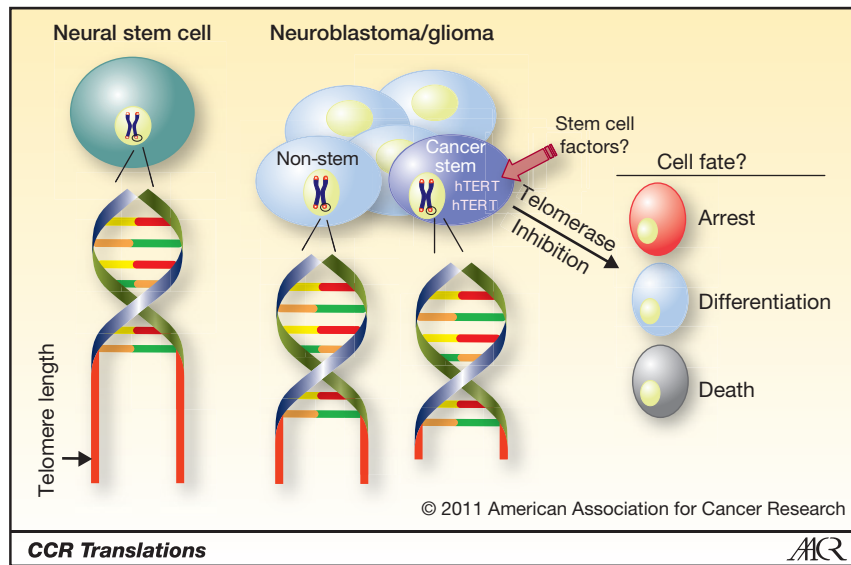
Telomerase expression in brain tumors may have prognostic significance, as elevated telomerase activity has been

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**Figure 1.** Antitelomerase-based neural stem cell therapies. Normal neural stem cells display longer telomeres than cancer stem cells, which also display elevated levels of hTERT. Cancer stem cells display preferential sensitivity to the telomerase inhibitor imetelstat (GRN163L), an oligonucleotide that directly inhibits telomerase enzymatic activity. Glioma and neuroblastoma stem cells treated with imetelstat have attenuated cell growth associated with decreased neurosphere formation capacity, an indicator of self-renewal. Decreased self-renewal with telomerase inhibition may also be due to increased differentiation or cell death.

linked to poor patient survival. The percentage of telomerase-positive brain tumors varies significantly between studies, ranging from 30% to 100% in the most malignant cancers, but consistent increases in telomerase activity have been noted with brain tumor grade (9). Because telomerase activity has not been detected in normal brain, telomerase has been identified as an attractive therapeutic target for brain tumors (9–12). However, the significant variability in telomere length among brain tumor specimens suggests that targeting telomerase may only be beneficial against a subset of brain tumors. Indeed, targeting telomerase in glioma cells with longer telomeres does not have a significant effect until >30 population doublings have occurred. It should be noted, however, that telomerase may regulate cellular phenotypes independently of its telomere maintenance activity, and subsets of cancers may depend on ALT pathways (8).

In the current report, Castelo-Branco and colleagues (1) prospectively segregate tumor propagating populations using the CD15 [stage specific embryonic antigen-1 (SSEA-1), Lewis X antigen] cell surface marker and find that telomerase activity is specific to the CD15+ subpopulation in glioma specimens with no detectable activity in nonstem CD15– cells or normal neural stem cells. Their data, as well as those obtained by Marian and colleagues (10) in CD133+ glioma cells, show that cancer stem cells have shortened telomeres compared with normal brain or nonstem glioma cells. Because decreased telomere length characterizes glioma stem cells regardless of the marker used for isolation, cancer stem cells do not share the long telomeres that are characteristic of normal stem cells. These data suggest that cancer stem cells may be the rare cells that escape senescence to become immortalized (4).

The study by Castelo-Branco et al (1) further shows that the elevated telomerase activity in glioma stem cells is due to increased *hTERT* mRNA expression, although the mole-

cular mechanisms responsible for this transcriptional dysregulation remain unknown. Histone acetylation and methylation of CpG islands regulate *TERT* expression, suggesting a role for epigenetic modification that is likely different in cancer stem cells. The *TERT* promoter also contains binding sites for multiple transcription factors, including those known to be involved in the biology of cancer stem cells. C-myc and hypoxia-inducible factor (HIF) binding elements are present in the promoter of *TERT*, and both are critical regulators of glioma stem cells. Ets-mediated *TERT* transcription is activated by epidermal growth factor, a core component of cancer stem cell maintenance. Thus, further studies are needed to delineate the stem cell pathways that mediate transcriptional elevation of *TERT* in cancer stem cells.

Antitelomerase pharmaceuticals have been developed on the basis of these findings. One of these pharmaceuticals, imetelstat (GRN163L, an oligonucleotide that directly inhibits telomerase enzymatic activity), has shown efficacy against glioma cell lines and xenografts (10–12). The shortened telomeres in cancer cells and elevated telomerase activity of neural stem cells suggest a high potential therapeutic index. Glioma and neuroblastoma stem cells treated with imetelstat display attenuated cell proliferation and neurosphere formation capacity, an indicator of self-renewal (1). Telomerase targeting may critically shorten the limited telomeres of cancer stem cells, triggering DNA damage responses. Subsequent cell cycle arrest may be followed by cell death (10) or differentiation (1, 11) (Fig. 1). However, the increased DNA repair capacity and apoptotic resistance of cancer stem cells may permit long-term resistance. Ultimately, investigators will need to conduct lineage tracing experiments to fully examine the fate of individual cells with telomerase inhibition strategies.

In the study by Castelo-Branco et al (1), imetelstat treatment immediately after implantation of neuroblastoma

stem cells improved the survival of xenograft-bearing mice, but treatment of established tumors did not significantly improve survival, which is a common outcome for monotherapies in many cancers. Marian et al (10) found that the viability of glioblastoma stem cells treated with temozolomide was further decreased by treatment with imetelstat, with minimal additional benefit from irradiation. They suggest that imetelstat may increase DNA damage induced by either temozolomide or irradiation to promote growth arrest. In conclusion, these studies strongly support the potential benefits of targeting telomerase as an anticancer stem cell therapy in combination with conventional ther-

apy (or other anticancer stem cell therapies) in advanced cancers. Imetelstat is in early clinical development, and future studies may identify patients with increased sensitivity to telomerase targeting or resistance (e.g., as a result of having tumors with active ALT mechanisms).

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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