

Neural Cancer Stem Cells: Focusing on Chromosome Ends

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Abstract

Telomeres are DNA tandem repeats associated with six proteins located at chromosome ends. Telomere shortening happens after each replication round in the majority of human cells, including adult stem cells, leading to telomere dysfunction and activation of senescence and/or apoptosis. In this commentary, key studies evidencing that telomerase overexpression (which is the most common telomere lengthening mechanism in cancer) confer stem-like properties to neural cells with tumorigenic potential, which is in accordance to previously proposed models of *TERT* overexpression as a mechanism for the emergence of neural cancer stem cells. In addition, the recent finding that telomerase inhibition is a promising anti-neural cancer stem cell therapy (at least in gliomas) is confronted with other studies, resulting in the identification of potential limitations: the presence of alternative lengthening of telomeres in neural cancer stem cells (already identified in glioma stem cells) and of the possibility that short telomeres is not an universal feature of neural cancer stem cells. Further investigation regarding telomere length measurement in neural cancer stem cells of different origins and development of clinically feasible and applicable methods to distinguish telomerase-positive from ALT-positive neural cancer stem cells are pointed as important perspectives to better understand the potential of the promising telomerase inhibition-based therapy to fight neural cancer stem cells.

Keywords: Telomere; Telomerase; Neural stem cell; Cancer stem cell; Telomerase inhibition

Abbreviations: ASC: Adult Stem Cell; CSC: Cancer Stem Cell; NSC: Neural Stem Cell; NCSC: Neural Cancer Stem Cell; ALT: Alternative Lengthening of Telomeres

Introduction

Telomeres and Telomerase, and Implications for Cancer and CSCs

Telomeres are 5' TTAGGG 3' DNA tandem repeats located at the ends of eukaryotic chromosomes, with a structure conformed by at least six associated proteins commonly referred to as telomere shelterin [1]. Due to the end replication problem (briefly, the incapacity of the DNA replication machinery to replicate the very ends of linear chromosomes) [2], the telomeres are shortened after each cell division. Considering that critical telomere shortening results in the recognition of chromosome ends as DNA damage sites (activating senescence and/or apoptosis responses), telomere length has been considered a key factor in cellular lifespan [3]. Telomere shortening occurs in cells that do not have activity of a ribonucleoprotein complex called telomerase, which promotes telomere lengthening by reverse transcription (catalyzed by the telomere reverse transcriptase subunit, encoded by the *TERT* gene) based on a RNA template (named telomerase RNA component, encoded by the *TERC* gene) [4,5]. Physiologically, telomerase is active in cells with a low degree of differentiation, including embryonic stem cells, germline stem cells and adult stem cells (ASCs). In the last, however, telomerase levels are sufficient only to delay telomere shortening, ultimately leading to ASC senescence or death [6,7]. Considering the importance of these cells in organism homeostasis, specifically in tissue self-renewal during life, telomere biology in ASCs is considered one of the fundamental aspects of physiological aging in models that consider the interplay among telomerase, tumor suppression and ASC viability [8].

These aspects of telomere biology have also been implicated in disease. There are well characterized syndromes (such as dyskeratosis congenita) caused by mutations in telomere-related genes, resulting in premature telomere shortening and, consequently, premature aging-

related phenotypes and impairments characteristic of ASC failure (especially in high-turnover tissues, such as the skin and the blood) [9-11]. In addition, telomere biology has major implications for cancer. Telomerase activity is one of the most prevalent cancer markers, present (at detectable levels) in approximately 85-90% of human cancers [12,13]. This makes telomerase an important target for anti-cancer therapies that aim to reduced or eliminate replicative immortality (which is considered a cancer hallmark) [14]. Another telomere-related cancer hallmark is genome instability: telomere shortening makes chromosome ends more prone to end-to-end fusions, which favors the accumulation of genomic abnormalities after each cell cycle, in a phenomenon known as breakage-fusion-bridge cycles. In this regard, critical telomere shortening is a common early event in carcinogenesis, which favors the accumulation of mutations and emergence of cells with proliferative advantages over the others, such as having telomerase active [14]. However, it is important to note that, since critical telomere shortening results in tumor suppression responses activation, telomere shortening can also be considered a tumor suppression mechanism that limits the lifespan of a given cell, thus reducing the chances of damage accumulation in a single cell. Whether telomere shortening act as a tumor suppression or a genome instability mechanism depend on the capacity of sensing critically shortened telomeres, which can have important consequences for the health outcomes of telomere shortening in a given individual (in an over-simplified dichotomy, tumor suppression responses may influence the likelihood of developing cancer or loss of tissue self-renewal capacity [15]).

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The roles of telomere biology in cancer have been receiving an extra degree of attention due to the emergence of the cancer stem cell (CSC) theory. According to this model, the capacity of tumor cells to acquire new characteristics (due to genomic instability) may favor the emergence of stem-like cancer cells. These cells are currently suggested to be a key aspect in therapy resistance (because CSCs lie in a protective microenvironment and are quiescent in well-established tumors) and, consequently, tumor recurrence (since CSCs may, when needed to maintain “tumor homeostasis”, proliferate at elevated rates) [16]. These characteristics are typical of ASCs, which depended (among other factors) on telomerase activity/telomere integrity to maintain tissue homeostasis. The importance of telomerase for ASCs and the common features between ASCs and CSCs suggest that telomerase activity is a potentially promising target for anti-cancer therapies also in the context of CSCs, highlighting the importance of understanding the roles of telomere biology in CSCs of different origins.

Roles of telomerase in NCSCs

Considering that telomere dysfunction is a consequence of the end-replication problem, it is a logical notion that telomere-related aging phenotypes are more pronounced in high-turnover tissues. In dyskeratosis congenita, for example, the classical clinical manifestation involves skin-related phenotypes, and the main cause of death is bone marrow failure [17,18]. Interestingly, telomere syndromes can also cause lung disease (where the cell turnover is very slow), mainly when combined with other factors such as cigarette smoking, indicating that telomere dysfunction also affects slow-turnover tissues as a component risk factor for disease [19]. Importantly, the importance of telomere dysfunction has also been observed in other slow-turnover tissues [9]. Indeed, there are key pieces of experimental evidence that points to a role of telomere biology in several organs. Firstly, it is important to consider that ASCs have been identified in both high and slow-turnover tissues in humans and murines [20], indicating that tissue self-renewal is an important homeostasis mechanism. Secondly, both telomere length and *TERT* expression can be used to identify primitive cells in a given lineage [8,21]. Combining these evidences, one could hypothesize that telomere dysfunction causes aging-related phenotypes throughout the body by impairing ASCs. This has been investigated in an elegant *in vivo* strategy, which demonstrated that telomerase reactivation in genetically engineered *TERT*-deficient mice capable of somatic *TERT* reactivation eliminates degenerative phenotypes in several organs and tissues. Importantly, telomerase reactivation has also reversed neurodegeneration by restoring neural progenitor cell populations, resulting in alleviation of hyposmia and recovery of innate olfactory avoidance responses [22], thus showing the importance of telomere biology for neural stem cells (NSCs) function.

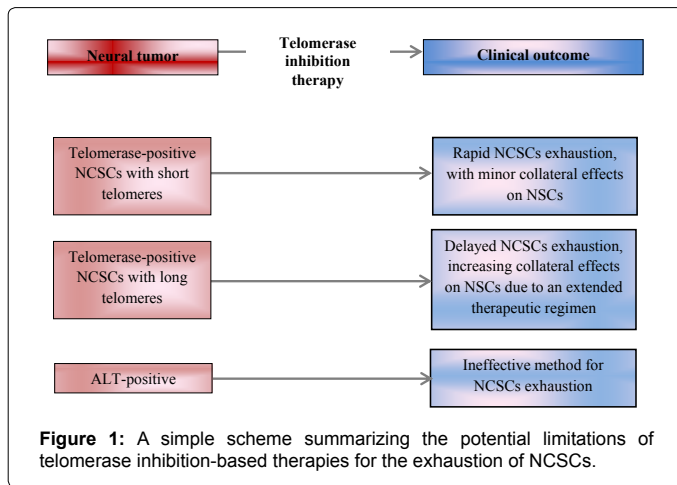
In fact, the importance of telomere integrity and telomerase activity for NSCs has been investigated in different studies, as previously reviewed [23]. Evidence also supports the notion that telomere biology is an important mechanism of neural cancer stem cells (NCSCs). It has been shown that *TERT* transduction in fetal neural progenitor cells results in loss of both diploid karyotype and contact inhibition, as well as anchorage independence and capacity to form neuroblastoma-like tumors *in vivo*, indicating that telomerase activation may favor the accumulation of oncogenic properties in primitive neural cells [24]. *TERT* transduction has also been shown to reduce the differentiation potential of Ntera-2 (NT2) human teratocarcinoma cells following retinoic acid treatment *in vitro* [25], which is in accordance to the reduction of telomerase activity of NT2 and SK-N-SH neuroblastoma cells upon their differentiation *in vitro* [26] and loss of telomerase

activity in NSCs upon differentiation into astrocytes when cultured with TGF-beta [27]. Moreover, *TERT* overexpression in P19 embryonal carcinoma cells retain markers of neuroepithelial precursors, do not complete neuronal differentiation and present an extended proliferation capacity [28], which are classical stem-like properties. However, there is evidence that supports that *TERT* overexpression in neural progenitor cell does not result in cancer [29]. It has been shown that immortalized lineage-restricted neural progenitors from human fetal spinal cord by retrovirus-mediated *TERT* overexpression cultured with basic fibroblast growth factor have been shown to give rise to functional phenotypically-restricted neural subpopulations, with no evident tumorigenesis [30]. In addition, there are defined protocols for generating lines of immortalized neural progenitor cells, whose neural progeny are phenotypically restricted, post-mitotic and functionally competent [31]. These results indicate that telomerase activity in neural progenitor cells may favor tumorigenesis (including the emergence of stem-like phenotypes, such as reduced differentiation) in cells that have a tumorigenic potential [25], which can be avoided or prevented by the use of well-defined protocols that induce differentiation (this has been previously discussed in other contexts [32]).

The evidence discussed above suggests a role for telomerase in the immortalization of neural progenitor cells and in acquiring stem-like characteristics in neural cells with potential tumorigenic capacity, indicating that telomerase activation in neural cancer cells may be an important step for the emergence of NCSCs from a tumor mass. In this regard, it has been shown that *TERT* and *NOTCH1* upregulation associates with *in vivo* (engrafted in the central nervous system of immunosuppressed mice) tumorigenicity of clonally derived neural stem/progenitor cell cultures from the olfactory bulb that presented maintenance of growth factor dependence and multipotentiality at late passages *in vitro*, although tumor formation happened only in a subset of mice that received cells with these characteristics [33], indicating that telomerase activation may occur during neural tumorigenesis and that such cells are selected over telomerase-negative tumor cell populations. Other studies relating telomerase activity and NCSCs have been reviewed elsewhere [23], resulting in the proposal of a model in that *TERT* overexpression is required for NCSCs establishment. In addition, this review also suggested telomere biology as a promising target for anti-NCSCs therapies. However, studies more recent than this publication are of great relevance in this regard. For instance, telomerase inhibition has been shown to specifically target glioma NCSCs (which suffered proliferation arrest, cell maturation, DNA damage, loss of self-renewal and stem cell capabilities), since no telomerase activity was found in NSCs, which also had long telomeres when compared to glioma NCSCs, indicating that the exhaustion of these cells by telomerase inhibition may be an efficient and specific (given the telomerase independency of NSCs) therapeutic approach for neural tumors [34,35].

Telomerase inhibition to exhaust NCSCs: potential limitations and perspectives

Although inhibiting telomerase as a therapeutic approach anti-NCSCs is promising, there are some factors that must be considered, especially given the concern of the implications for cognitive function caused by collateral effects of cancer therapy on neural stem cells expressed in the modern literature [36]. First, it is important to recognize that telomerase activity levels in human adult stem/progenitor cells are sufficient to only delay rather than fully prevent telomere shortening in different stem cells compartments, such as hematopoietic, skin, intestinal crypt and pancreas [37-40], and neural [41]. This may



seem unexpected since tissues such as the hematopoietic and the skin are known to be highly dependent on telomerase activity for their long-term homeostasis, which indicates that low levels of telomerase activity may still be of functional importance. However, given the longer telomeres of NSCs [34], it is plausible to assume that a short-term telomerase inhibition might not have significantly detrimental consequences for NSCs. Additionally, telomerase inhibition seems to be among the best alternatives to target telomere biology in glioma NCSCs, since telomestatin treatment has been reported to have mild negative effects on neural progenitor cells [42]. The two possibly most important limitations of targeting telomerase to fight NCSCs are summarized in two key studies. First, the telomerase-independent recombination-based telomere lengthening mechanism termed alternative lengthening of telomeres (ALT) has been shown to confer to glioma NCSCs the capacity to sustain long-term proliferation and, importantly, ALT-positive glioma NCSCs are more resistant (both *in vitro* and *in vivo*) to ionizing radiation than their telomerase-positive counterparts [43]. Another study showed that a specific subpopulation of adamantinomatous craniopharyngioma cells presents overexpression of the Wnt/ β -catenin pathway, and that such cells share properties with normal pituitary progenitor/stem cells, including relatively long telomeres [44], indicating that stem-like adamantinomatous craniopharyngioma cells (plausibly CSCs) have long telomeres and, then, this would be an intracranial (although not literally from neuronal origin) tumor resistant to telomerase inhibition.

Since telomerase activity favors the acquisition of stem-like properties in neural cells with tumorigenic potential and is restricted to NCSCs (at least in gliomas) when compared to their normal stem cells counterparts, targeting telomerase as an anti-NCSC therapeutic approach seems to be promising. However (as illustrated in Figure 1), such therapy is likely to be less effective in ALT-positive NCSCs (since they - which have been evidenced to occur - do not depend on telomerase to lengthen their telomeres) and in the possibility that some telomerase-positive NCSCs might have long telomeres (consequently, NCSC telomere exhaustion would require more extended therapies, increasing the risk of side effects in NSCs telomere homeostasis). The notion that efficiency of telomerase inhibition depends on CSC telomere length indicates the importance of determining whether short telomeres is an universal feature of glioma NCSCs, and such investigation is required for other neural tumors, such as medulloblastomas (especially considering that each of these tumors can have different cells of origin, as well as some cells of origin in common [45]). In conclusion, further studies to establish which neural tumors are more likely to have their

NCSCs exhausted by telomerase inhibition (i.e., telomerase-positive NCSCs with short telomeres) and develop methods clinically feasible and applicable to distinguish telomerase-positive from ALT-positive NCSCs are needed to accurately assess the potential and range of application of telomerase inhibition-based therapies to fight NCSCs and reduce risk of neural tumor recurrence.

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