Cancer Treatment Reviews (2007) 33, 704–709

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ANTI-TUMOUR TREATMENT

Targeting ALT: The role of alternative lengthening of telomeres in pathogenesis and prevention of cancer

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Received 18 May 2007; received in revised form 22 June 2007; accepted 12 August 2007

KEYWORDS
ALT;
Telomere;
Cancer;
Senescence;
Crisis;
Recombination;
Tumor

Summary Telomere shortening in the course of cell divisions plays an important role in both suppression and pathogenesis of cancer. Telomere maintenance mechanisms such as telomerase and alternative lengthening of telomeres (ALT) are essential for long-term tumor growth. Consequently, interdiction of telomere lengthening has been proposed as an anti-cancer treatment but requires insight in the genes and pathways involved.
In this article, the molecular and functional details of ALT are reviewed, and proposed next steps towards a therapy aimed at preventing ALT in human cancers are described.

Introduction

Telomeres, the ends of chromosomes, are shortened with each cell division in most somatic cells, functioning as a ''mitotic clock'' which limits the number of divisions in cells (Hayflick limit).[1] When the telomere length is reduced below a critical value, cellular senescence takes place. Transformed cells whose checkpoint functions are damaged escape senescence and continue to divide with rapidly shortening telomeres. Eventually, telomere dysfunction results in massive chromosomal aberrations leading to cell death, a situation termed crisis.[2] Rare survivors show active lengthening of their telomeres, mostly by the enzyme telomerase. Telomerase is a reverse transcriptase, containing a catalytic subunit, hTERT, and an RNA, hTR, serving as a template for telomere elongation.

Telomerase has been detected in germ line and stem cells as well as in ~60% of immortal cells and ~85% of cancers.[3] Telomerase is therefore intimately involved in pathogenesis and progression of human cancers, which has led to the development of telomerase inhibitors as potential anticancer drugs.[4] However, with cancer being the result of considerable ongoing mutation and selection mechanisms, cancer cells will by their nature be able to potentially escape any drug treatment applied on them. Taking this into account, complete genetic deletion of telomerase in all somatic mitotic cells in combination with frequent stem cell reseeding has been proposed as an ultimate cancer treatment.[5] Using gene therapy, native stem cells would be replaced by engineered ones not possessing telomerase, and because these new stem cells would not be immortal, they would have to be reseeded regularly. After this treatment, cancers could arise, but they could not reach a life-threatening stage anymore because of lacking immortality.

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0305-7372/$ - see front matter
doi:10.1016/j.ctrv.2007.08.006

However, a major obstacle to this plan is the finding that some cancers develop despite showing no discernible telomerase activity. These cancers employ a different telomere maintenance mechanism, termed ''Alternative Lengthening of Telomeres'' (ALT).[6] ALT cells show a specific phenotype which is characterized by very heterogeneous telomere lengths, ALT-associated promyelocytic leukemia bodies (APBs) and rapid telomere deletions and elongations.[3] Elucidation of the details of ALT would be very helpful in targeting these cancer types.

The mechanism of ALT

ALT is a mechanism which is independent of telomerase. Experiments in which ALT cells were fused with normal somatic and telomerase-positive cancer cells showed that ALT can be suppressed in these cells, indicating the presence of repressors that normally prevent this pathway.[7] However, later studies demonstrated that ALT and telomerase can also coexist over a prolonged period in human cancer cells.[8–10]

The connection to recombination was found very quickly after the discovery of ALT. Cells of *Saccharomyces cerevisiae* can maintain their telomeres by recombination. Immortalized yeast cells surviving crisis can be separated into two categories[3]: Type 1 survivors show elongated telomeres with tandem repeats consisting of alternating telomere repeats and subtelomeric sequences. Type 2 survivors have only the telomeric repeat; most ALT cells resemble type 2 survivors which gave rise to the assumption that ALT is based on recombination.

An important hallmark of ALT, also linking it to recombination and DNA repair, are APBs which are similar to promyelocytic leukemia bodies (PNBs) that are involved in DNA damage response and apoptosis.[11] APBs contain the promyelocytic leukemia protein (PML), extrachromosomal telomeric DNA repeats (ECTR) and numerous telomere-associated proteins, among them TRF1, TRF2, Mre11, NBS1 and RAD50.[12] APBs are found in ~5% of all ALT cells, particularly during late S/G_2 phase.[13] The function of the APBs is unclear. It was hypothesized that they could be both storage places and functional platforms where ALT takes place[11]; indeed, DNA synthesis has been shown to occur in APBs.[12] Both native telomere repeats and ECTR are present in these bodies,[12] however, APBs do not seem to be essential for ALT.[14]

Several mechanisms involving recombination are possible for ALT. Recombination leading to elongation of telomeres could occur with the telomere using itself, other telomeres or ECTR as a template. Furthermore, telomeric exchange between sister chromatids (T-SCE) or between different chromosomes (T-ICE) could account for the ALT phenotype.

Elevated levels of telomeric recombination have been observed in several studies.[15–17] However, this increase in recombination is not genome-wide, but restricted to the telomeric region.[18] It has also been demonstrated that sister chromatid exchange is much higher in telomeres than in the rest of the genome in ALT.[19] Telomerase inhibits T-SCE,[20] which could explain why telomerase-positive cells do not show this effect.

The recombination events in ALT are closely linked to DNA repair systems. Many of the proteins that are found in APBs are involved in double-strand break repair; it has been proposed that critically short telomeres are detected and considered as DNA damage, followed by attempts to repair the dysfunctional telomeres that lead to their elongation.[11] In line with this is that the Mre11/RAD50/NBS1 (MRN) complex appears to be essential for both DSB repair and ALT.[21]

Also the WRN and BLM helicases, the enzymes lacking in Werner's syndrome and Bloom's syndrome, are associated with ALT and APBs, probably because of their unwinding activity at the telomeres. WRN and BLM are able to unwind telomeric structures and intermediates of recombination events, and might thus play a crucial role in recombination-mediated elongation of telomeres.[22] Both enzymes have been shown to be regulated by telomere-repeat binding factors TRF1 and TRF2.[23,24] TRF2 stabilizes telomeres by protecting the t-loop, a structure at the end of the telomere where the single-stranded 3′ end is inserted into the antecedent double-stranded sequence. In this way, TRF2 inhibits recombination.[25] Its absence leads to rapid telomeric dysfunction and senescence. Interestingly, TRF2 not only stabilizes telomeres, but is also involved in an ATM-dependent DNA damage repair pathway after phosphorylation.[26] The MRN complex and the telomere-associated proteins TRF1 and TRF2 are required for APB formation and probably play a significant role in ALT.[21]

It has been hypothesized that unequal sister chromatid exchange could produce lengthened telomeres in the absence of telomerase.[16] In this scenario, sister chromatids exchange parts which are unequally large whereby, after cell division, one sister cell inherits a lengthened telomere at the expense of the other one. If T-SCE events are abundant enough, this could allow cells to maintain their telomere lengths and also explain the rapid elongations seen in ALT. Shorter telomeres are more prone to T-SCEs since they are poorly ''capped'' and thus more recombinogenic. The longest telomeres, on the other side, provide the largest targets for strand invasion, resulting in a stochastic transfer from long to short telomeres. However, if more than one such T-SCE per cell division occurs, one sister cell would have to inherit all the short telomeres and the other one all the long lengthened ones.[27]

The existence of telomeric interchromosomal exchange (T-ICE) in normal cells has been deduced from the observation that cells with short telomeres accumulated in pre-senescent cultures.[28] This could help to explain why the beginning of senescence mostly does not seem to depend on the shortest telomeres, but on average telomere length.[28] Thus, T-SCE and T-ICE in ALT cells could be dysregulated forms of mechanism also rarely occurring in normal cells.

A telomere could use itself or other telomeres as templates for elongation. Additionally, the presence of large amounts of ECTR suggests that they also could be involved in recombination. In the yeast species *Kluyveromyces lactis*, telomeres can be maintained by a roll-and-spread mechanism where small ECTR circles are used as templates for rolling circle replication.[29] The produced long tracts of telomeric DNA can be inserted into telomeres by homologous recombination and spread by intertelomeric recombination. Rolling circle replication might also take place directly at the telomere.