Telomerase and Anticancer Treatment

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Current chemotherapy uses compounds of organometallic nature that act with different mechanisms of action. Many pharmacological studies are directed toward the creation of compounds with more specific and selective activity toward tumor targets, including telomerase. The design and synthesis of such compounds with specific antitelomerase activity must consider the mechanism of action of the enzyme and its structure. The discovery of a close correlation between telomerase activation, cell immortalization and oncogenesis has suggested that telomerase inhibitors could be potent therapeutic agents, capable of selectively killing cancer cells. Inhibition of telomerase is expected to lead toward shortening of telomeres to a critical length, such that replicative senescence and cell death due to irreparable chromosomal damage can result. It has been observed that cancer cells generally have shorter telomeres than the normal replicative cell population, probably because the malignant cells have undergone more divisions. Therefore, the inhibition telomeres of cancer cells after a few cycles of cell division, without the normal cells suffering harmful consequences during therapy. Telomerase is certainly an interesting target on which to continue to study molecules that inhibit its function to obtain a specificity of therapeutic intervention and a reduction of the nonspecific cytotoxicity of chemotherapy.

Keywords: Cancer; hTR; Telomerase; TERT.

In normal human somatic cells, the telomeres, the ends of chromosomes, consist of approximately 15,000 base pairs with a characteristic hexanucleotide repeat sequence, TTAGGG12. Telomere sequences and their associated proteins form an important structure that prevents chromosome terminal fusion, translocation, and degradation by exonucleases3-5. Thus, telomeres preserve the integrity of the chromosome by allowing its complete replication and ensure the proper replication of essential genes6-8. Telomeres are progressively shortened at each cell division (loss of about 200 base pairs at each cycle), due to the so-called “terminal replication problem”, until they reach a minimum length, called “crisis”, which produces a strong apoptotic stimulus or the manifestation of a senescent phenotype9-13. This phenomenon, termed “replicative-type senescence”, induces in normal somatic cells the programmed cessation of cell division and limitation of proliferative potential. Therefore, this process has been considered as a mechanism of intrinsic control of tumor suppression: it is hypothesized that it may act as a molecular regulation (“clock”) of the amount of cell replication. In cancer cells, telomerase, an RNA-dependent DNA polymerase (retrotranscriptase), adds repetitive DNA sequences
(TTAGGG) to the telomeres, thus preserving the length of chromosomal terminals14-17. Thus, telomerase confers unlimited proliferative capacity on the cell, thus playing a central role in the neoplastic process16-20. The unlimited proliferative potential that results from the preservation of the telomere length has been defined as one of the six fundamental characteristics that underlie tumor development21.

**Telomerase: Function and Structure**

Telomeres are specialised structures of heterochromatin that act as a protective cap for the ends of chromosomes. In most organisms, telomeres consist of repeats of short guanine-rich sequences, complexed with proteins. In humans and other vertebrates, the repeat sequence of telomeres is 5’-TTAGGG-3’.

Each termination of each human chromosome has between 1,000 and 2,000 repeats, with an approximate total of 6-12 kb. The number of repeats and base composition vary between species, but the functions of telomeres are conserved and can be summarized as follows 1) maintenance of chromosomal stability, in terms of protection from the phenomena of degradation by exonucleases, fusion with the ends of other chromosomes by ligases, rearrangement, and recombination; 2) spatial organisation of the cell nucleus and anchoring of the chromosomes themselves during DNA replication; 3) influence on the transcription of genes located in the vicinity of chromosome ends23,24. Telomeric DNA has a double-stranded conformation; at the end of the chromosome, however, the guanine-rich 5’ 3’ strand protrudes 50-150 nucleotides more than the complementary strand rich in cytosine. Telomeric DNA-binding proteins form an intricate multi-protein complex and contribute to maintaining telomere stability and regulating its length. In human cells, the TRF1 and TRF2 proteins specifically recognise the TTAGGG repeat sequence. The function of regulating telomere length, inhibiting its elongation once the critical size has been reached, appears to be assigned to the TRF1 protein. TRF2, on the other hand, hinders the fusion of endings between chromosomes, resulting in remarkable telomere stability. Telomere replication is a late event in the cell cycle and, due to the previously discussed mechanism of DNA replication, incomplete at 5. ends of each newly synthesised strand. At each cell division, human telomeres undergo a loss of approximately 100 base pairs (16 TTAGGG repeats), thus undergoing progressive shortening. After a certain number of mitoses, the telomere reaches a critical length, which triggers a signal to stop cell division and the beginning of a period termed cellular senescence25.

Telomerase is a ribonucleoprotein belonging to the large family of reverse transcriptases, a polymerase that synthesises DNA from an RNA template; its peculiarity lies in the fact that it has its own RNA template as an integral part of the enzyme. Telomerase binds to the 3’ end of eukaryotic chromosomes and adds single-stranded TTAGGG repeat units. Therefore, active telomerase requires two essential components: 1) an RNA subunit, containing a nucleotide sequence that acts directly as a mold for the addition of telomeric repeats; 2) a protein subunit, which is assigned the function of catalyzing telomere synthesis.

The RNA subunit, called TER in general (TR, in mammals; hTR, in humans; TLC1, in yeasts), has a highly conserved secondary structure found in ciliates and vertebrates (Romero and Blackburn, 1991). The human RNA subunit, hTR (human Telomerase RNA) consists of 451 nucleotides, of which eleven represent the CUAACCUAAC template sequence encoding telomeric repeats (TTAGGG). The coding gene is in the distal part of the long arm of chromosome 326-29.

The TERT protein, encoded by the gene of the same name located on chromosome 5 (hTERT in humans, that is, human Telomerase Reverse Transcriptase), is a 127 kDa polypeptide that belongs to the family of reverse transcriptases. Among the characteristic conserved motifs, an important one is a triad of aspartates, which contributes to the formation of binding sites in the polymerase active site; any mutation in one of the aspartates results in altered catalytic functionality. The catalytic subunit of telomerase is conserved in yeast, protozoa and mammals30. Furthermore, telomerase-associated proteins appear to play a key role in allowing the enzyme to act in vivo. In yeast, there are at least two proteins associated with the enzyme complex, Est1p and Est3p. However, yeast extracts that have undergone deletion of Est1 or Est3, are capable
of supporting the addition of telomeric repeats in vitro. In the ciliated protozoan Tetrahymena, the p80 protein shows significant homology to a larger protein present in mammalian telomerase, TP1/TEP1. The biological role of these proteins is still being studied. In general, the telomerase complex may consist of many components, each of which is a potential target for the regulation of the enzyme.

Most human somatic cells, except for the hematopoietic system and germ cells, lack telomerase activity and show progressive telomere shortening, resulting in senescence. Telomerase activity is tightly regulated during normal growth and development to avoid unrestricted proliferation directed toward malignancy. Telomerase activity has been observed in several human foetal tissues, such as muscle, lung, and skin, suggesting that the enzyme is active during development and is repressed in adult tissues. The first normal human cells to be telomerase positive were lymphocytes subjected to proliferation stimulation. Other types of normal human cells that exhibit telomerase activity include the intestinal epithelium, the oesophageal epithelium, the endometrium, the basal keratinocytes, the cervix epithelium and stem cells of the hematopoietic system31-35.

Recent data show that, within each tissue, a small subpopulation still maintains low levels of telomerase activity, albeit to an extent insufficient to prevent telomere erosion. Studies on the expression of the catalytic subunit of human telomerase (hTERT) in normal tissues agreed with the data obtained from the measurement of telomerase activity by the TRAP assay. Analysis by in situ hybridization revealed the presence of hTERT in a variety of normal cells and tissues with high proliferative capacity, such as different types of epithelial cells, hematopoietic precursors, and spermatogonia. Finally, immunohistochemical studies using anti-hTERT antibodies showed the expression of hTERT in normal colon proliferative epithelial cells36-39.

In most normal cells, where telomerase activity is lacking, the expression of hTERT cannot even be detected, while hTR and some of its associated proteins are still detectable. These observations suggest that hTERT is an essential factor in telomerase activity and that its regulation plays a key role in the process of cellular immortalisation. The mechanism by which telomerase is repressed in normal somatic cells and is instead activated in tumour cells appears to involve regulation of hTERT expression but is still far from being elucidated. Analysis of hTERT expression has suggested the presence of at least two mechanisms of regulation of telomerase activity: 1) transcriptional control of the hTERT gene; 2) alternative splicing of hTERT transcripts. Recent studies have shown that the hTERT promoter is inactive in normal human somatic cells, while it is activated during immortalisation; detailed analysis of the promoter sequence has revealed binding sites for several transcription factors. The existence of hTERT transcripts, which result in the formation of a truncated or inactive form of the protein, indicates that, in addition to transcriptional activation/repression of the hTERT gene, telomerase activity may be regulated through the alternative splicing mechanism35,40.

**Table 1. Features of normal, cancerous, and hTERT-expressing fibroblasts**

<table>
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<th>Characteristics</th>
<th>Normal</th>
<th>Cancer</th>
<th>hTERT</th>
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<td>Contact inhibition of</td>
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<td>absent</td>
<td>present</td>
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<td>growth</td>
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<td>Anchorage-dependence</td>
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<td>present</td>
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<td>Cell cycle checkpoints</td>
<td>present</td>
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<td>Proliferative life-span</td>
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biomolecular events of the carcinogenesis process and the cytohistological evidence so far considered as the “gold standard” for the diagnosis of neoplasia is desirable. Consistent data indicate that a series of molecular events, leading to the activation of the telomerase enzyme, are the basis for a process of cellular carcinogenesis. Therefore, this enzyme is a tumor marker whose determination can ensure high diagnostic sensitivity and specificity (Table I).

Most of the most common cancers, such as breast, prostate, lung, liver, pancreatic, and colon cancer, exhibit telomerase activity, highlighting the key role of this enzyme in tumor pathogenesis. Telomerase may already be active at a pre-neoplastic stage or, in other cases, its activity gradually increases in parallel with tumor progression. In many cases, telomerase activity is directly proportional to the aggressiveness of the tumor and its ability to metastasize.

The telomerase enzyme is now considered a new marker for tumor cell detection and represents a potential target for selective chemotherapy. Inhibitors of this enzyme, in fact, could cause a progressive reduction of telomeres in tumor cells alone, until a minimum length of the crisis is reached, inducing a strong apoptotic stimulus.

Understanding the regulatory mechanisms underlying the expression and activity of telomerase could also accelerate the development of new therapeutic modalities for cancer therapy. A telomerase inhibitory strategy would have several advantages, such as directly limiting tumor growth, acting synergistically with existing inhibitors and amplifying their efficiency, it could be used, after initial chemotherapy or surgery, as an adjuvant to block neoplastic cell recovery by increasing susceptibility to immune system activity or killing with traditional chemotherapeutic agents. Moreover, this antitelomerase strategy would be particularly interesting in situations of developing cell turnover, resulting from the use of angiogenesis inhibitors. Systemic administration of telomerase inhibitors could affect the activity present in stem and germ cells. This effect would be minimal because the telomere length of tumor cells, which is relatively shorter, would reach a critical erosion value before irreversible damage is established in other cell types.

Criteria that should be met by a compound with anti-telomerase activity include reducing telomerase activity without initially affecting cell growth capacity; shortening the telomere with each replicative cycle; leading to cell death or tumor growth arrest; initial telomere length influences the time it takes for a reduction in cell proliferation to be observed; reduction in proliferative capacity, or shortening of telomeres should not be due to the action of chemically similar molecules. Many experimental approaches aimed at creating an antitelomerase strategy have been reported in recent years and the results presented so far seem to encourage research in this direction.

Some strategies that were shown to be antitelomerase were not selective, such as: induction of oxidative damage with anticancer drugs such as bleomycin and doxorubicin, directed at telomeres to make them unrecognizable and therefore no longer elongable by telomerase.

Another nonselective strategy was based on experimental evidence of the formation of paired bases between guanines of the same telomeric chain, which by associating with each other generate cyclic complexes, known as G-quadruplexes. Such a G-quadruplex structure could make the telomeric portion inaccessible to telomerase; in fact, the hTERC template RNA region requires an unwounded DNA primer for telomere extension to be possible. Therefore, molecules [1,4-2,6-bis(â-aminoalkanamido) anthrancene-9,10-diene and a cationic porphyrin derivative, tetra (N-methyl-4-pyridyl) porphine (TMPyP4)], stabilizing these structures of G-quadruplex, were produced. Selective strategies of inhibition of telomerase have involved the use of base analogues [dideoxyguanine (ddG), 3'-azido-3'deoxythymidine (AZT) and carbovir] that would interfere with the neosynthesis of the telomere at the catalytic site of the enzyme. Other selective strategies have involved the use of antisense oligonucleotides of TP1 mRNA and DNA antisense oligonucleotides in vitro. Recently, a modified oligonucleotide, sequence-specific peptide nucleic acid (PNA), which hybridizes RNA more efficiently than antisense and is more resistant to proteases, has been used in vitro and in vivo in pancreatic cancer cells. Finally, another selective telomerase inhibition approach consisted
of the design, based on the hTERT RNA sequence, of hammerhead ribozymes (Rz), RNA chains endowed with autocatalytic activity, consisting of a conserved portion, called the catalytic site, and two tails that have a sequence complementary to that of the target RNA. These molecules bind very specifically to their ribonucleic targets, causing them to be catalyzed at particular sites, usually consisting of the GUC, GUU, or CUC triplets.

CONCLUSIONS

In normal adult tissues, telomerase activity and TERT mRNA expression are greatly reduced. As a result, normal cells in culture are short-lived and undergo growth arrest, called senescence. However, in many cancers, TERT gene expression and telomerase activity are prominent. This has led to proposals for the use of telomerase inhibitors for cancer therapy. Telomerase, since it is therefore active in most malignant tumors and only in a low percentage of normal tissues, could be used as a tumor marker and/or prognostic indicator and provides an excellent target for antitumor therapy.

Evidence of a close correlation between telomerase activation, cell immortalization, and oncogenesis suggests that telomerase inhibitors could be potent therapeutic agents, capable of selectively killing cancer cells. Inhibition of telomerase leads to shortening of telomeres to a critical length, such that it results in replicative senescence and cell death due to irreparable chromosomal damage.

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Authors’ contributions

All authors participated in the research design, data analysis, and the writing of the manuscript. All authors approved the final version of the manuscript.

Conflict of interest

The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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