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# Telomeres, Telomerase, and Tumorigenesis -- A Review

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Human telomeres function as a protective structure capping both ends of the chromosome. They are composed of long, repetitive sequences of TTAGGG, associated with a variety of telomere-binding proteins. Telomeres protect the chromosomes from end-to-end fusion, recombination, and degradation, all events that can lead to cell death. At cell replication, telomeres cannot be completely replicated. They are gradually shortened, and when the telomeres reach a critical threshold, cell replication is arrested in what is called "replicative senescence." Thus, telomeres act as an intrinsic "counting" mechanism of the cell's aging process. Telomerase is an enzymatic ribonucleoprotein complex that acts as a reverse transcriptase in the elongation of telomeres. Telomerase activity is almost absent in somatic cells, but it is detected in embryonic stem cells and in the vast majority of tumor cells. Tumor cells, in fact, may contain short and stable telomeres that confer immortality to the cancer cells, which are thus able to replicate indefinitely. The deregulation of telomeres thus plays an important role in the relationship between premature aging syndrome and cancer. This review describes the recent advances in the molecular characterization of telomeres, the regulation of telomerase activity in cancer pathogenesis, and the potential of targeting telomerase for cancer therapy.

In the early 1930s, Hermann J. Muller and Barbara McClintock described the telomere (from the Greek word "telos," meaning end, and "meros," meaning part) as a protective structure at the terminal end of the chromosome. When this structure is absent, end-to-end fusion of the chromosome may occur, with ensuing cell death. In the 1970s, James D. Watson described what he called "end-replication problems." During DNA replication, DNA-dependent DNA polymerase does not completely replicate the extreme 5' terminal end of the chromosome, leaving a small region of telomere uncopied. He noted that a compensatory mechanism was needed to fill this terminal gap in the chromosome, unless the telomere was shortened with each successive cell division.[1]

Meanwhile in the 1960s, Hayflick described a biological view of aging. He found that human diploid cells proliferate a limited number of times in a cell culture. The "Hayflick limit" is the maximal number of divisions that a cell can achieve in vitro. When cells reach this limit, they undergo morphologic and biochemical changes that eventually lead to arrest of cell proliferation, a process called "cell senescence.[2,3]"

Then in the 1970s, Olovnikov connected cell senescence with end-replication problems in his "Theory of Marginotomy," in which telomere shortening was proposed as an intrinsic clocklike mechanism of aging that tracks the number of cell divisions before the arrest of cell growth or replicative senescence sets in. Greider and colleagues,[1] in 1988, corroborated this theory when they observed a progressive loss in telomere length in dividing cells cultured in vitro.

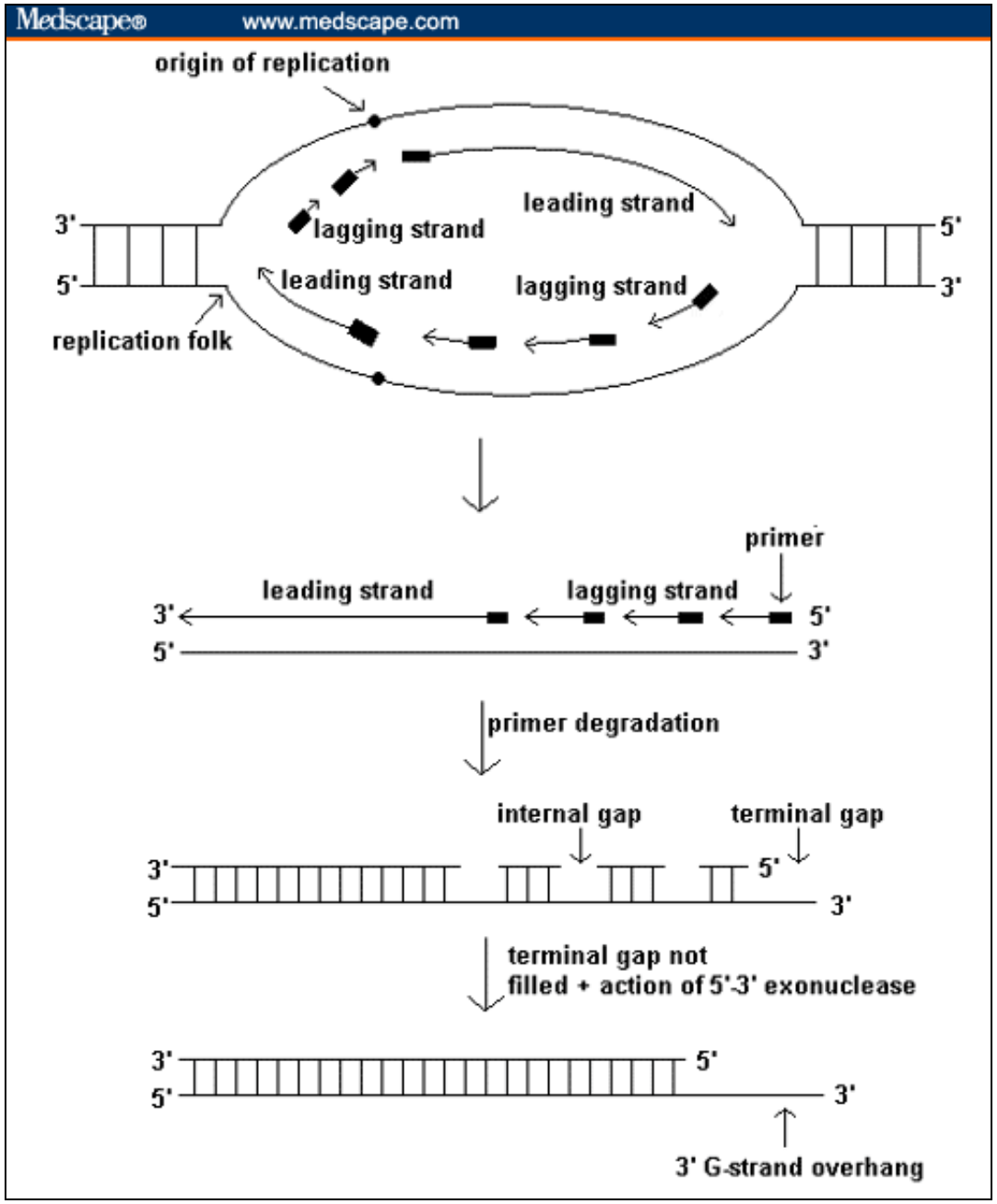
In 1978, Elizabeth Blackburn found that the molecular structure of telomeres in *Tetrahymena pyriformis* contains long repeating units rich in thymine (T) and guanine (G) residues. In 1984, she and her colleagues isolated telomerase, the enzyme responsible for the maintenance and elongation of telomere length. In 1989, Gregg reported the existence of telomerase activity in human cancer cell lines, which was thought to contribute to the immortality of tumor cells. At about the same time, Greider and associates found that telomerase was nearly always absent in normal somatic cells.[1]

In the 1990s, Shay and Harley detected telomerase in 90 of 101 human tumor cell samples (from 12 different tumor types), but found no activity in 50 normal somatic cell samples (from 4 different tissue types). Since then, more than 2600 human tumor samples have been examined and telomerase activity detected in about 90% of all tumor cells. The obvious implication is that telomerase may play a major role in the pathogenesis of cancer.[1]

Because of their role in physiologic aging, cancer pathogenesis, and premature aging syndromes (eg, progeria), telomeres and telomerase are currently under intensive investigation. This review focuses on the molecular structure of telomeres, telomerase and associating proteins, the role of telomere shortening, the activation of telomerase in cancer pathogenesis, and the potential of targeting telomerase for cancer therapy.

The human telomeres consist of long, repetitive TTAGGG subunits, which are associated with a variety of telomere-binding proteins. These repeating sequences comprise a portion of the double-stranded telomeric DNA, which has an overhanging, single-stranded, G-rich 3' end. The human somatic cells will enter into replicative senescence after a limited number of cell replications. This phenomenon is attributed to the end-replication problem.

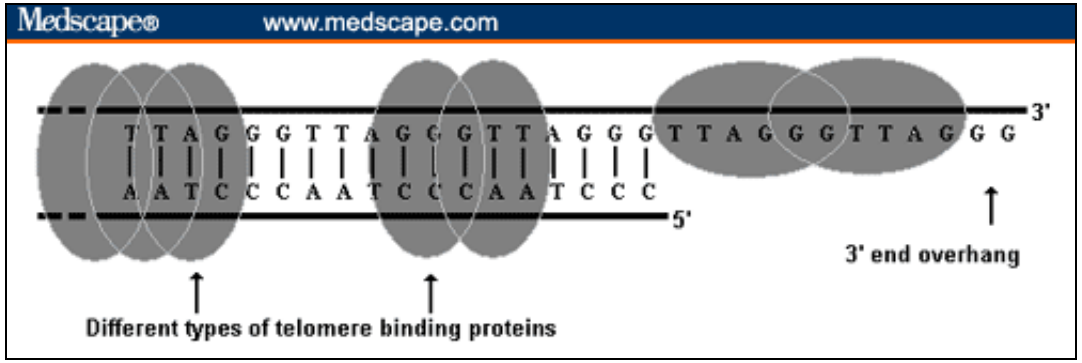
At 1 or more concurrent sites within the replicating chromosome, DNA polymerase starts with a primer at the 3' end and runs toward the 5' end of the template, forming a 5' to 3' leading strand and a lagging daughter strand.[3,4] The leading strand runs toward the replication fork, whereas the synthesis of the lagging strand (consisting of Okazaki fragments) begins at the replication fork and runs in the opposite direction (Figure 1). When the synthesis is complete, the primers are degraded and internal gaps or spaces are formed at each site of replication.



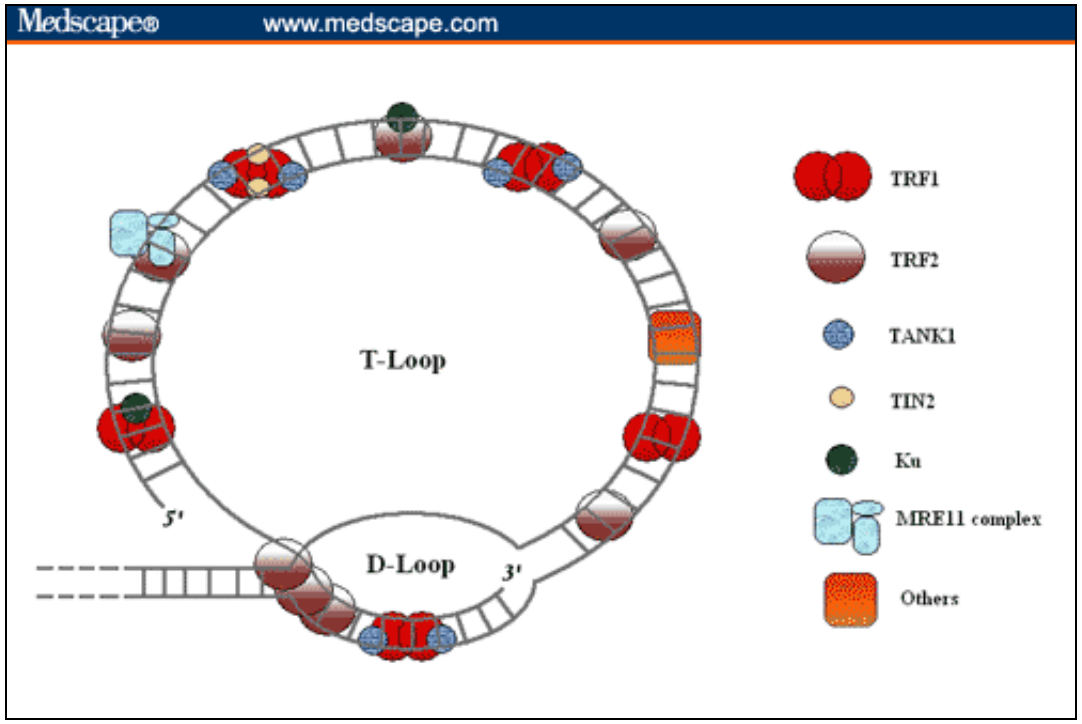

Telomere shortening during DNA replication. The degradation of the primer on the lagging strand and the action of a putative 5' to 3' exonuclease lead to shortening of the 5' end of the telomere and the formation of a 3'-end overhang structure.

The gaps between the newly replicated fragments of the lagging strand are filled by the action of DNA ligase; the terminal gap, the space left by the primer at the end of both strands, is not filled. The terminal gap is further enlarged by the action of putative 5' to 3' exonuclease, which degrades 130-210 nucleotides. Thus, the 5' end of the telomere is shortened with each replication.[3,5,6] After a finite number of replications, the telomere reaches a critical length, the senescence checkpoint, and either cell-proliferation arrest or cell death occurs (physiologic aging). If, however, the cell escapes or avoids this checkpoint, chromosomal instability or end-to-end fusion of the chromosome occurs, which may contribute to cell death or carcinogenesis.

In the classic view, the mammalian telomere was conceived as a linear structure (Figure 2). Recent electron microscopic studies revealed that the telomere end is actually a large duplex structure consisting of 2 loops.[7] This model suggests that the telomere folds back on itself to form such a large telomere loop (T-loop). The 3' G-strand overhang binds to the double-stranded telomere repeat sequence of the 5'-end strand, forming a displacement loop (D-loop) (Figure 3). In this way, the T-loop-D-loop mask the overhang structure, and some suggest that this plays a protective role by sequestering the overhang terminal inside the double strand.




Classic view of telomere structure -- linear structure of telomeres associated with different types of telomere-binding proteins.




New view of telomere structure -- duplex structure of telomeres consisting of a telomere (T)-loop and a displacement (D)-loop, associated with telomere-binding proteins.

Other studies have shown that telomere integrity and function require at least 3 factors: a minimal length in the TTAGGG repeating sequence, integrity of the overhang, and regulation of telomere-binding proteins.[8] There are 2 major telomere-binding proteins, TRF1 (telomeric repeat binding factor 1) and TRF2 (telomeric repeat binding factor 2). Both may function individually or through interaction with other proteins. TRF1 is found in the double-strand TTAGGG sequence of the T-loop. It is a homodimer, and it functions by inducing the shallow bend in telomeric DNA, thus aiding in loop formation and stabilization.

TRF1 is also a negative regulator of telomere length, and length regulation is dependent on a feedback mechanism involving telomerase. Experimental evidence indicates that the amount of TRF1 within a cell affects telomere length. Overexpression of wild-type TRF1 will decrease telomeric length, whereas overexpression of mutant TRF1 will lead to telomere elongation by activation of telomerase.[8-10]

A variety of proteins and enzymes have been identified interacting with TRF1 in regulating telomeric length. Poly-(ADP-ribose)-polymerase-TANK 1 (tankyrase 1) or poly-(ADP-ribose)-polymerase-TANK 2 (tankyrase 2) binds to TRF1. These enzymes are responsible for the ribosylation of TRF1 and thus inhibition of TRF1 binding to telomere DNA. As a consequence, the telomerase can access the telomere and allow telomere elongation. Alternatively, tankyrases act as positive regulators of telomere length.[9,10]

Conversely, binding of TRF1 to the protein TIN2 functions as a negative regulator of telomere length, because it leads to compaction of telomeric DNA, stabilization of

the T-loop, and limitation of accessibility to telomerase.[11] The interaction of TRF1 with the protein Pot1 in the overhang is thought to be a way to convey information from the double-strand region to the single-strand region.[8]

TRF2 is found in the double-stranded TTAGGG of the T-loop and in the loop-tail junction. TRF2 plays an important role in chromosome stabilization. Recent studies have found that overexpression of mutant TRF2 leads to the loss of TRF2 bound at the telomere, the loss of overhang, induction of the p53-dependent damage pathway, end fusion, and growth arrest as in replication senescence.[8-10] Its regulatory function is independent of telomerase. The localization of TRF2 in the loop-tail junction implies a role in stabilizing the overhang structure at the T-loop by facilitating strand invasion. This prevents a single-strand sequence from being recognized as a DNA break with ensuing activation of the DNA damage response.[9]

TRF2 may interact with protein hRAP1 in regulating telomeric length. The overexpression of hRAP1 may cause telomere elongation.[8] TRF2 may also recruit the MRE11 complex, which is composed of the proteins RAD50, MRE11, and NBS1. This complex is involved in double-strand breakage repair.[8] The DNA-dependent protein kinase (DNA-PK) complex, which is composed of Ku70, Ku86, and DNA-PK catalytic subunits, also interacts with TRF2, and it functions by preventing telomeric fusion through the repair of double-strand breaks.[12,13]

The interactions between the loop structures of the telomere and its associating proteins are important because they allow us to establish molecular models of the telomere complex. However, further investigations of their functions, and identification of new elements, are required to better understand the correlation between their regulatory functions and protection of chromosome ends.

The telomerase enzyme, a ribonucleoprotein that functions as a reverse transcriptase, is the main positive regulator of telomere length. This enzyme consists of 2 main components, a telomere RNA component (TERC) and a telomere reverse transcriptase (TERT).[8,12,14] A number of studies have revealed that telomerase activity is almost absent in normal somatic cells. However, a low level of telomerase activity has been discovered in mitotically active cells, including skin, lymphocytes, and endometrium. Telomerase activity in these proliferating cells is regulated by growth.

Stem cells express telomerase to maintain telomere length throughout their life cycle.[15,16] About 90% of cancer cells contains short telomeres and high levels of telomerase activity. For example, 75% of oral carcinomas, 80% of lung cancers, 84% of prostate cancers, 85% of liver cancers, 93% of breast cancers, 94% of neuroblastomas, 95% of colorectal cancers, and 98% of bladder cancers have detectable telomerase activity.[17] It remains to be determined whether telomerase may represent a sensitive biomarker for diagnosis, early detection, and prognosis of cancer.

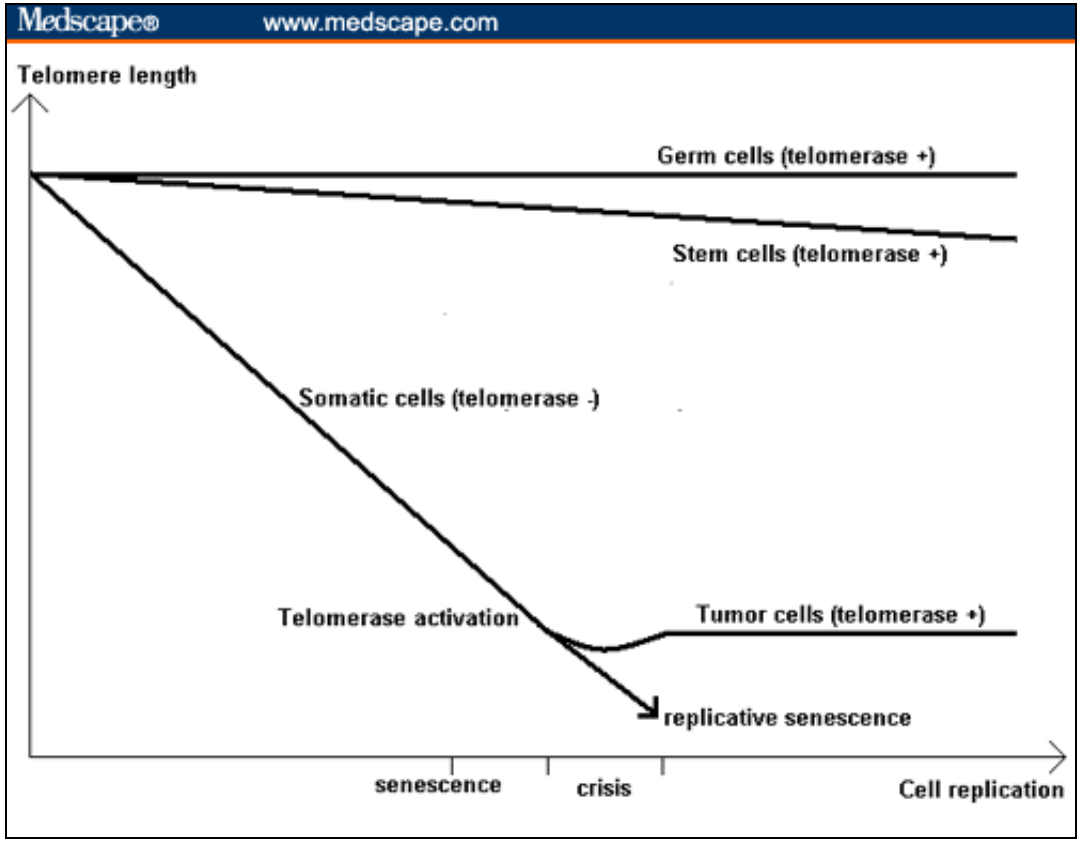
Telomerase-negative normal human cells, such as retinal pigment epithelial cells and foreskin fibroblasts, transfected with vectors encoding human (h)TERT, exhibit telomere elongation, whereas telomere-negative control cells exhibit telomere shortening and senescence.[18] Similarly, telomerase-deficient mice showed telomere-exhausted chromosome ends, end-to-end fusion, and the loss of viability. The introduction of telomerase prevented all these events, indicating that telomerase may enhance survival, promote proliferation, and extend life span.[8]

The expression of telomerase activity is regulated at different levels, including transcription, mRNA splicing, and maturation and modification of hTERT and hTERC. hTERT seems to be the most important determinant in the regulation of telomerase expression. Positive regulators of hTERT transcription include the oncogene *cMyc*, the transcription factor Sp1, the human papillomavirus 16 protein E6, and steroid hormones. Negative regulators of hTERT transcription include the transcription factor Mad1, the tumor suppressor protein p53, pRB, E2F, the Wilms' tumor 1 tumor suppressor protein, the myeloid cell-specific zinc finger protein 2, and a number of antiproliferation and differentiation agents, such as interferon-gamma and transforming growth factor-beta[19]

Telomere length serves as intrinsic biological clocks regulating the life span of the cell, in which the numbers of cell cycles are counted before the cell undergoes replicative senescence. Thus, telomeres limit the capacity of a cell to replicate, by inducing senescence as a sort of tumor-suppressing mechanism. As discussed, with successive cell divisions, telomeres are gradually shortened. When they reach a critical length, the induction of senescence is associated with the activation of a number of pathways, including p53, p21, and the Rb/$p16^{INK4A}$ cell-cycle checkpoint pathway.[3] As a consequence, cell growth is arrested and apoptosis occurs.

The human fibroblasts from young persons have long telomeres, whereas older individuals have shorter telomeres. Similarly, patients with premature aging syndrome (eg, Werner's syndrome and Hutchinson-Gilford's syndrome) have shorter telomeres as compared with healthy individuals of the same age.[20] The introduction of the catalytic subunit of hTERT into normal human cells leads to the indefinite and unchecked proliferation. Thus, it seems that telomere shortening culminates in cellular senescence or M1 (mortality stage 1).[12, 21-23]

When the cell-cycle arrest checkpoint pathway is bypassed, telomeres will undergo further shortening (Figure 4). An extremely short telomere will be recognized as a double-stranded break, with activation of DNA-damage response proteins, including ATM, Ku, and Mrt-2/Rad17. These proteins, in turn, lead to homologous recombination or nonhomologous end joining of the chromosome. Cells with unstable and aberrant chromosomes are in the crisis phase or M2 (mortality stage 2) and readily undergo apoptosis.[21,23,24]



The telomere-telomerase hypothesis -- changes in telomere length are detected in germ cells, stem cells, somatic cells, and tumor cells.

However, in tumor cells, bypassing of the cell-cycle checkpoint and of the crisis phase will lead to further shortening of telomeres and the activation of telomerase.[25,26] Thus, the telomeres are stabilized at a constant length. Some human tumor cells lack telomerase activity and are able to maintain their telomere length by an alternative mechanism, alternative lengthening of telomeres.[27,28] Telomere length in these cancer cells is extremely short, about 2-4 kb vs approximately 20 kb in stem cells and embryonic cells.[29]

The stabilized telomeres confer cell immortalization,[30] and immortalized tumor cells thus overcome the barrier of senescence/M1 and the crisis phase/M2, and have the ability to proliferate indefinitely. Of note, however, immortalization is not sufficient for malignant transformation.

Malignant transformation requires a multiple-step process, including the activation of oncogenes, the inactivation of tumor suppressor genes (eg, p53 and pRB), and uncontrolled mitogenic stimulation.[31] Conversely, human fibroblasts transduced with the simian virus 40 antigen do not undergo malignant transformation when the *ras* oncogene is activated, if they have not been immortalized.

These findings indicate that malignant transformation requires various oncogenic factors. Telomerase activation confers immortality but not neoplastic properties to cancer cells.[32]

Much evidence indicates that telomere maintenance confers an immortal phenotype to cancer cells. The loss of telomere sequences, on the other hand, may prevent it by inducing apoptosis.[33] Telomerase and telomeres thus offer a variety of potential targets for the development of anticancer therapies. Three main experimental approaches have been followed in recent investigations.

First, the enzymatic activity of telomerase may be inhibited by various telomerase inhibitors, with the induction of accelerated senescence and cell death. This approach may, however, affect the growth of telomerase-positive normal proliferating cells and stem cells (eg, hemopoietic stem cells, intestinal stem cells, and basal cells of the skin). It remains to be determined whether normal cells do indeed have differential sensitivity to such inhibitors and can withstand toxicity in vivo, thanks to longer telomeres and lower division rates.

Second, there is the elimination of telomerase-expressing cells by immune effectors. TERT-positive cells express TERT in association with major histocompatibility complex class 1 on their cell surfaces. Cells can then be recognized and eliminated by CD8+ cytotoxic T cells. This would provide the rapid elimination of tumor cells with no delay effect. However, TERT-expressing normal cells also would be affected.

Third, there is the expression of a mutant telomerase RNA template, with disruption of telomere function and the induction of cell death. Once more, this approach may act on telomerase-dependent tumor cells and on telomerase-dependent normal cells.[34,35] In addition, antitelomerase approaches would not be effective in telomerase-independent tumor cells (approximately 15% of cancer cells).[35]

The detection of telomerase activity, by the telomeric repeat amplification protocol (TRAP) assay, may be of diagnostic value. This polymerase chain reaction (PCR)-based assay is able to detect 1-10 TRAP-positive cells in a mixed population. For example, nearly 95% of test samples are TRAP-positive in breast cancer. Real-time quantitative reverse transcriptase-PCR is being used to detect the expression of hTERT mRNA. Nonetheless, these diagnostic tests should be used in association with other well-established methods of cancer diagnosis.[36]

Because of its potential usefulness for the development of novel anticancer therapies, telomere biology has been under intense investigation. Further research and a better understanding of the structure, function, expression, and regulation of telomeres and telomerase are essential before manipulation of this system approaches clinical applicability.

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