Telomere, Telomerase, and Cellular Senescence and Death

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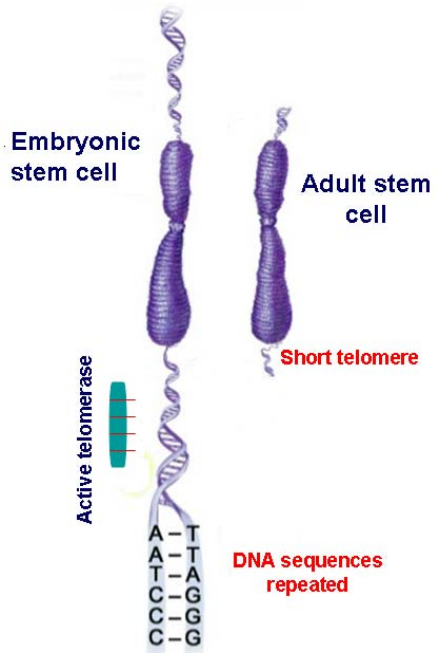
Telomerase is a specialized ribonucleoprotein composed of telomerase reverse transcriptase (TERT), an intrinsic RNA template (TR), and several associated proteins. Its main function is to stabilize telomeres, which protect chromosomes from recombination and end-to-end fusion. Telomeres also recognize DNA damage and control the replicative capability of cells. With each cell division some of the DNA is lost from the ends of chromosomes. This is due to the fact that conventional DNA polymerase can not fully replicate the 3' end of the lagging strands of the linear molecule. When telomeres reach a critical minimum length, cells can not divide and cellular senescence and apoptosis is induced, which coincides with the activation of p53, p21, and p16 in cells. Human somatic cells have the potential to undergo about 60 to 70 divisions after which they experience growth arrest and enter senescence. Telomerase preserves telomere length in stem cells, germ cells, and cancer cells by adding hexameric (TTAGGG) repeats to the ends of chromosomes, thereby circumventing the cumulative damage that occurs with each mitotic cell division. Telomerase recognizes the G-rich strand of an existing telomere repeat sequence and elongates it in the 5´-to-3´ direction.



Stem cells, germ cells, cancer cells, and certain somatic cells overcome telomere shortening by expressing telomerase, which allows them to maintain their telomere length and escape senescence. Embryonic stem cells with greater proliferative capacity have developed mechanisms that maintain telomere length through many cell divisions. Telomerase activity in embryonic stem cells guarantees that they are able to handle potentially large expansion demands, preserving their ability to maintain and repair the tissues. The level of telomerase activity is shown to be up-regulated in stem cells that undergo rapid expansion, such as committed hematopoietic progenitor cells, and in activated lymphocytes. On the contrary, adult stem cells exhibit low telomerase activity.

Dysregulation of telomerase expression has been linked to several human diseases. For example, dyskeratosis congenita, a progressive bone marrow failure syndrome, results from telomerase dysfunction due to mutations in either the RNA subunit or the telomerase RNA-associated protein dyskerin. These patients show visibly shorter telomeres than normal individuals. About 90% of cancer cells contain short telomeres, but exhibit high telomerase activity. For example, 75% of oral carcinomas, 80% of lung cancers, 93% of breast cancers, and 95% of colorectal cancers, have detectable telomerase activity. As cancer cells divide more often, they possess, on an average, shorter telomeres than normal cells. Hence, without an active telomerase to maintain telomere length, cancer cells could reach critically short telomere at a faster pace than normal cells.

The presence of telomerase activity is correlated with poor clinical outcome in cancer patients. Hence, telomerase inhibitors are considered as potential therapeutic agents for the management of tumor

progression. Promising approaches for telomerase inhibition include the use of mutant dominant/negative versions of human TERT (hTERT) and the use of antisense oligonucleotides directed against the template RNA component (hTR) of the telomerase holoenzyme. Although telomerase inhibitors can accelerate cancer cell senescence and apoptosis, they may also destroy normal proliferating cells and stem cells.

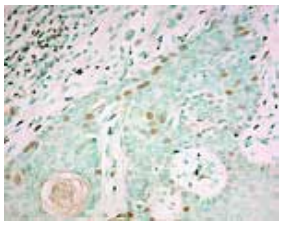
References:

Carroll, K.A., and Ly, H. 2009. *Int. J. Clin. Exp. Pathol.* **2,** 528
Cao, Y., et al. 2008. *Cancer Sci.* **99,** 1092.
Hiyama, E., and Hiyama, K. 2007. *Br. J. Cancer* **96,** 1020.
Li, S., and Blackburn E.H. 2006. *Cold Spring Harb. Symp. Quant. Biol.* **71,** 211
Wai, L.K. 2004. *MedGenMed.* **6,** 19.
Altshuler, M.L., et al. 2003. *Biochemistry (Mosc).* **68**, 1275.
Mokbel, K. 2003. *Curr. Med. Res. Opin.* **19**, 470.
Wu, X., et al. 2003. *J. Natl. Cancer Inst.* **95**, 1211 .
Cong Y.S., et al. 2002. *Micobiol. Mol. Biol. Rev.* **66,** 407.
Gomez, D., et al. 2002. *Cancer Res.* **62**, 3365.
Shay, J.W., et al. 2001. *Hum. Mol. Genet.* **10**, 677.
Rahat, M.A., et al. 1999. *Cancer* **85**, 919.
Roos, G., et al. 1998. *Int. J. Cancer* **79**, 343.
Wen, J., et al. 1998. *Mol. Diagn.* **3**, 29.
Kim, N.W. 1997. *Eur. J. Cancer* **33**, 781.
Shay, J.W. 1997. *J. Cell. Physiol.* **173**, 266.
Harrington, L., et al. 1997. *Science* **275**, 973.

Antibodies for Telomerase and Related Proteins

Anti-hTERT (348-358) Rabbit pAb

Purified. Immunogen used was a synthetic peptide corresponding to amino acids 348-358 of hTERT. Recognizes the ~128 kDa hTERT protein. Suitable for immunohistochemistry (paraffin sections).



Detection of hTERT by staining paraffin sections. Sample: Human breast carcinoma. Primary antibody: Anti-hTERT (348-358) Rabbit pAb (Cat. No. PC563) (2.5 µg/ml). Detection: DAB with methyl green counterstain

Cat. No. PC563 100 µg

Anti-Telomerase (Ab-1) Rabbit pAb

Undiluted serum. Immunogen used was a synthetic peptide corresponding to amino acids from an internal region of the human telomerase reverse transcriptase (hTERT). Recognizes the ~125 kDa telomerase (TERT) protein. Reacts with human and mouse samples. Suitable for immunoblotting, immunoprecipitation, and immunohistochemistry (paraffin sections).

Cat. No. 582000 100 µl

Anti-Telomerase (Ab-2) Rabbit pAb

Undiluted serum. Immunogen used was a synthetic peptide corresponding to an internal region of hTERT. Reacts with human and mouse samples. Suitable for immunoblotting, immunoprecipitation, and immunohistochemistry (frozen and paraffin sections).

Cat. No. 582005 100 µl

Anti-Telomeric Repeat Binding Factor-1 Rabbit pAb

Purified. Immunogen used was a synthetic peptide corresponding to amino acids near the C-terminus of human TRF-1, conjugated to KLH. Recognizes the ~43 kDa human TRF-1 protein. Suitable for ELISA and immunoblotting.

Cat. No. 581420 50 µg

Telomerase Inhibitors

Product	Cat. No.	Comments	Size	Price
3´-Azido-3´-Deoxythymidine (AZT)	194348	Inhibitor of HIV-1 reverse transcriptase that blocks the incorporation of nucleotides into newly synthesized DNA. Causes irreversible telomere shortening.	10 mg	
InSolution™ AZT, Triphosphate, Tetralithium Salt	194950	A reverse transcriptase inhibitor that acts by docking to the active site of HIV reverse transcriptase. Also reported to inhibit telomerase activity *in vitro* (IC_{50} = 30 μM).	1 μmol	
3´-Deoxy-2´,3´-didehydrothymidine	257920	A reverse transcriptase inhibitor that causes a consistent and rapid telomere shortening in vegetatively growing *Tetrahymena*.	25 mg	
Ellipticine, 9-Hydroxy-, Hydrochloride	324680	An antitumor alkaloid that is reported to inhibit telomerase activity in cultured pancreatic cancer cells, in a time- and concentration-dependent manner, possibly through inhibition of protein kinases. It also acts as a potent inhibitor of topoisomerase II (IC_{50} = 3.3 μM).	10 mg	
(–)-Epigallocatechin Gallate	324880	A polyphenolic constituent of green tea with potent antitumor, anti-inflammatory, and antioxidant properties. Strongly and directly inhibits telomerase activity in cell-free systems and in cancer cell lines.	10 mg	
PIPER (N,N′-*bis*[2-(1-Piperidino)ethyl]-3,4,9,10-perylenetetra-carboxylic Diimide)	528120	A perylene-based ligand that potently inhibits human telomerase activity by binding to G-quadruplex DNA. The strongest binding site for PIPER appears to be the 3'-boundary of the G-quadruplex. Can also bind non-specifically to nucleic acids.	10 mg	
Telomerase Inhibitor III, Sodium Salt (5´-d(TTAGGG)-3´; TAG-6)	581004	A short hexameric phosphorothioate oligonucleotide (PS-ODN) telomere mimic that inhibits telomerase activity in cell lysates and lengthens cell doubling time *in vitro* and *in vivo* at concentrations less than 2.5 μM.	150 nmol	
Telomerase Inhibitor VI, Sodium Salt (5´-CAGUUAGGGUUAG-3´; 2´-*O*-MeRNA)	581006	A 13-nucleotide 2'-O-MeRNA possessing terminal phosphorothioate linkages. Potently inhibits telomerase activity (IC_{50} = 2 nM at 23˚C and 3 nM at 37˚C).	100 nmol	
Telomerase Inhibitor IX (N,N´-bis(2,3-Dihydroxybenzoyl)-1,3-phenylenediamine)	581011	A cell-permeable, potent, and reversible inhibitor of telomerase activity (IC_{50} = 670 nM, TRAP lysate prepared from U937 cells). Prolonged treatment with MST-312 has been reported to result in telomere shortening and growth arrest in U937 cells. Does not inhibit the activity of *Taq* DNA polymerase (IC_{50} > 3 μM).	10 mg	
TMPyP4	613560	A potent inhibitor of human telomerase (IC_{50} = 6.5 μM). TMPyP4 binds strongly to DNA quadruplexes by stacking on the G-tetrads at the core of the quadruplex, resulting in telomerase inhibition. Fluoresces highly in the presence of quadruplex DNA.	25 mg	