Telomerase Inhibition for Killing Cancer Cells

Mahnoor Patel

Department of Biotechnology, Veer Narmad South Gujarat University, Surat 395007, Gujarat, India.

Email: patelmahnoor786@gmail.com

Abstract

There is an upper limit to the number of times a cell can divide before it stops growing and enters a phase which is called senescence. For human embryonic diploid fibroblasts, the Hayflick limit is about 60 doublings. This limit is related to the end-replication problem faced by DNA polymerase as it moves along a template strand synthesizing a new strand. DNA polymerase must initiate synthesis from an RNA primer and it synthesizes a new strand beginning with the primer's 3' end. The need for the RNA primer means that the DNA polymerase cannot replicate the very 5' end of the lagging strand because the primer occupies the last few bases of the template strand. As a consequence, the DNA of a chromosome shortens by 50-100 nucleotides each time the chromosome replicates. Eukaryotic chromosomes have telomeres at their ends, which are long sections made up of a repeat sequences, TTAGGG in human cells. These sequences protect the ends of the chromosomes, thus preventing the chromosomes from interacting with each other. Telomeres are eroded with each division until they no longer function properly, with catastrophic results for the cell.

Principle

Telomeres are the terminal structure of linear chromosomes in eukaryotes. It consists of multiple repetitive sequences. They protect and confer stability to the ends of chromosomes. Telomeres also possess other functions like breakage prevention, end-to-end fusion and degeneration. During cell division, chromosome ends cannot be replicated by the polymerases responsible for the replication in eukaryotes, that's why the chromosomes continuously become shorter from the telomere ends. This type of shortening of chromosomes will eventually stop the cell division. In the case of cancer, the cancerous cells have a ribonucleoprotein enzyme known as telomerase which allows compensation of telomere shortening and continuation of the cell multiplication process. Most of the cancers need a high level of this enzyme to continue cell multiplication. This enzyme is absent in normal cells or present at very low level, the telomerase inhibitors cannot have a significant effect on normal cells. Telomerase is present in most of cancerous cells; its inhibition can be considered as a goal of cancer treatment in future.

Mechanism

Telomerase is a ribonucleoprotein enzyme that catalyzes the addition of telomere repeats at the ends of eukaryotic chromosomes. The RNA component of telomerase (hTer in human cells) anneals to the telomere repeat at the end of the chromosome and the protein component (hTERT) a reverse transcriptase, reverses transcribes the RNA component to add DNA repeats to the ends of chromosomes. Telomerase is silent in nearly all normal cells, except for germ cells, which must express telomerase so that eggs and sperm receive chromosomes ending in telomeres of right length. If hTERT is overexpressed in cells, these will continue to proliferate past the point when they could normally reach the Hayflick limit and senescence. Strikingly, more than 90% of human tumor overexpress telomerase, no other characteristic is common among so many different tumors. The vast majority of tumors depend on telomerase for continued cell division, which makes this enzyme an attractive therapeutic target. Perhaps inhibiting telomerase activity would stop cell growth. Overexpressing dominant-negative forms of hTERT in tumor cell lines eventually leads the cells into crisis.

Experiments

Scientists tried to use the mutant hTERT probably work by "Mopping up" associated proteins so that the endogenous hTERT cannot work efficiently. In an alternative strategy, investigators first tried using a short interfering RNA (siRNA) to knock down the endogenous telomerase RNA component, hTer. As expected, hTer depletion led to telomere loss and cell senescence, *in-vitro* and *in-vivo*. Second, they replaced hTer with a mutant-template hTer, which directed the addition of non-telomeric sequence at the telomeres. This mutant-template hTer also inhibited cell growth, and it did that more rapidly than the siRNA induced depletion of hTer. Third, and unexpectedly combining the siRNA with mutant-template hTer had a synergistic effect, which caused more rapid and greater inhibition of cell growth, accompanied by DNA damage. Interestingly, wild-type hTERT was needed, presumably so that mutant-template hTer/siRNA could interfere with it and direct the addition of the non-telomeric sequences. The quick-acting toxic effect of mutant-template hTer/siRNA is quite distinct from any effect of telomere shortening, which would require many cell division cycles for serious shortening to occur. The mutant telomerase RNA affected even cells that had lost p53 function, and this coupled with the near-universal telomerase activation in cancer cells, gives hope that this previously unsuspected pathway may provide a new method to target cancer cells.