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REVIEW ARTICLE

THE ROLE OF TELOMERES AND TELOMERASE IN HUMAN CANCER

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Abstract : Human cancers/malignant transformation of normal cells occur from multiple independent genetic changes/mutations that can subvert the normal growth controls of cells, leading to distinct phenotypic changes and immortalization. Normal human somatic cells have a limited proliferative capacity both *in vitro* and *in vivo* and undergo senescence. Recent studies have implicated telomeres and telomerase in the regulation of lifespan of cells. Telomeres are the stretches of DNA consisting of tandem repeats of nucleotide sequences that cap chromosomes and prevent its degradation and play a role, both in normal control of cell proliferation and abnormal growth of cancers. They are highly conserved during evolution. Telomerase, the novel reverse transcriptase enzyme that synthesizes telomeric DNA is repressed in most human somatic cells, it results in telomere shortening with each cell division, leading to a process thought to contribute to senescence. Recent research proposes that activation of telomerase is important for cells to proliferate indefinitely and that all human cancer cells require activation of this enzyme to maintain telomeric DNA, to overcome cellular senescence and to attain immortality. Thus telomeres and telomerase offer potential for diagnostics, cancer therapy as well as for understanding the process of aging.

Key words : telomeres telomerase senescence cancer immortality

INTRODUCTION

Normal human somatic cells have limited proliferative capacity both in culture and *in vivo*. This phenomenon, termed replicative senescence has often been used as a model for cellular aging (1, 2). However, the progression of normal human cells to tumors involves the escape from limitations on proliferations imposed by cellular senescence. Transformation *in vitro* confers an extended lifespan to cells, but transformed cells eventually undergo a proliferative crisis accompanied by cell death, from which rare immortal clones emerge (3). Circumstantial evidence suggests that acquisition of extended proliferative capacity, and even of immortality, can also occur *in vivo* during the development of tumors (4). Immortalization of human cells may result in part from chromosomal or chromatin destabilization i.e. either spontaneously or induced by carcinogens, physical agents or DNA tumor viruses (5). Recent studies have implicated telomeres and telomerase in the regulation of cellular lifespan and have proposed that activation of the enzyme telomerase and

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stabilization of telomeres are necessary for human cells to become immortal, or capable of proliferating indefinitely (6-8). Hence, in this review, we emphasize the role of telomeres and telomerase in cancer and immortality.

TELOMERES

Eukaryotic chromosomal ends consist of specialized nucleoprotein structures called telomeres. Telomere is a Greek work (telos means end; meros means part) coined by the nobel laureate Hermann J. Muller (9) in 1930s. He was the first Scientist to recognize that chromosome ends, or telomeres, are essential to maintain chromosomal integrity. Thereafter, investigators (10) began to decipher its structure and confirmed that though the telomere carried no genes, it is vital for chromosomal survival. The telomeric DNA is highly conserved in all well-characterized eukaryotic nuclear chromosomes and is quite different from the termini of linear viral, extracellular plasmid, or mitochondrial DNA (11).

The telomeric DNA is formed of simple tandemly repeated nucleotide units with a G-rich strand oriented 5' to 3' toward the chromosomal end and sometimes protrudes as 3' overhangs. Although telomeric sequences can vary from species to species, a given organism has a characteristic repeat at all telomeres (12). In humans, these sequences are predominantly composed of TTAGGG repeats repeated 800-3000 times, making up a total of 5 to 15 kilobase pairs (13, 14). Proximal to the essential telomeric repeats, some chromosomal ends harbor additional common elements called subtelomeric repeats or telomere-associated sequences (15, 16). Unlike the telomeric repeats, these sequence are not conserved and their function remains unclear (17). In humans, the majority and most distal repeats are of the form TTAGGG, although variant forms such as TTGGGG and TGAGGG exist subterminally (13, 18). Initial work with ciliates and yeast suggested that telomeric DNA associates with specific proteins to form a telomeric nucleoprotein complex (19). Binding of the necessary protein may rely on the sequence of the repeat as the alteration of the telomeric sequence in both human and *Tetrahymena* cells causes formation of incomplete telomeres (20-22). Human and other telomeres have been demonstrated to associate with nuclear matrix protein fraction, which may include nuclear envelope and nuclear matrix (23).

Telomeres appear to carry out at least three functions: (i) They protect the ends of double stranded DNA from degradation, fusion and recombination (10), (ii) Since the telomeres are located at the nuclear periphery, they may play a role in attaching the ends of chromosomes to nuclear membrane (24), (iii) Telomeres may provide a solution to the end replication problem (25); since all known polymerases require primer and synthesize DNA in the 5' to 3' direction, the 3' ends of linear DNA pose a problem to the replication machinery. Telomeric repeats may temporarily nullify this trends by providing a cushion of extendable non coding sequence at the chromosomal ends.

Telomere length plays an important role in cellular aging (6, 26), and immortalization of cells has been suggested by a number of investigators (27, 28). Somatic cells (lymphocytes) telomeres appear to be significantly shorter than spermline (sperm) telomeres from the same person (13, 14). It is now known that in most tissues, chromosomes loose their telomeric (TTAGGG) repeats with each cell division. The rate of telomere loss may vary from cell type to cell type. For example, skin and lymphocytes loose 15-40 bp per year of their telomeric DNA, whereas the telomeres of the fibroblasts, embryonic kidney cells, mammary epithelium and cervial cells in culture loose 50-200 bp of their DNA per population doubling and eventually stop dividing at a senescent stage (28-32). Based on this and other evidence, it has been proposed that decay of telomeric DNA represents a molecular clock that counts cell division and limits the replication of primary cells (28).

Sperm telomeres on the other hand increase in length with age, indicating that telomeres are actively maintained and even increased in length in germ cells (6). The correct sequence of telomeric repeat is required for its function, since addition of telomeric DNA harboring a mutant telomeric sequence to the ends of the endogenous *Tetrahymena* telomeres led to telomere instability and death (20).

Pathak et al (33, 34) have observed that only certain chromosomes (Nos. 1, 2, 3, 4, 6, 7, 10, 12, 14) are involved in their telomeric DNA associations, indicating that their telomeric DNA is lost more often than that of other chromosomes that remain intact. Harley et al (29) reported that a loss of 2 kb from mean telomeric length may imply a large increase in the proportion of cells missing TTAGGG from at least one telomere, since each cell contains 92 telomeres and the distribution of telomere length is wide. The loss of even a single telomere could render the chromosome unstable. This could aid in the end-to-end fusion between sister chromatids or with another chromosome, in the form of a dicentric or ring chromosome in anaphase abnormality, and in other types of instability included in the breakage-fusion bridge-cycle (10). Thus, the decreased length of telomere can lead to chromosomal instability and genetic changes of possible significance for tumor development (26, 29, 30). Furthermore, reductions in telomere length have been observed in different human cancers such as colorectal carcinoma (30), childhood leukemia (35), endometrial adenocarcinoma (36), neuroblastoma (37), ovarian carcinoma (38) and renal carcinoma (39) and this may represent a common pathway in cancer development. The shortening of telomeres could lead to a succession of events : chromosomal instability, additional genetic changes, increased proliferation, reactivation of telomerase, and ultimately cancer development (7, 40-42).

The telomere hypothesis suggests that loss of telomeres could act as a mitotic clock, reflecting the replicative history of normal somatic cells, and as a genetic time bomb, contributing to chromosomal abnormalities in cell transformation. Further, immortalization of a cell involves activation of telomerase, probably at or near crisis. Chromosomal abberations initiated by critical shortening of telomeres contributes to mutation and immortalization (6, 26).

TELOMERASE

Telomerase is a ribonucleoprotein enzyme capable of extending ends of chromosomes with a specific telomeric sequence by using a portion of its internal RNA component as the template (43). Greider and Blackburn (44) first identified and characterized the activity of telomerase in the ciliate *Tetrahymena*. A similar biochemical activity was characterized in the immortal human HeLa cell line by Morin (45). The cloning of the 159 nucleotide RNA component of *Tetrahymena* telomerase (46) clarified several aspects of the mechanism of action of this novel RNA polymerase and identified the regions from positions 43 to 51 within RNA as having the sequence 5'CAACCCCAA 3'. This sequence was later confirmed by site-specific mutations in this region, which yield telomerase that now synthesized the repeats containing the corresponding change in the cell (20). Greider and Blackburn (46) recognised that the template RNA contains approximately one or one and a half times the complement of the GGGGTT repeat and speculated that the surplus sequence enables telomerase to hybridize to an existing telomeric repeat via several bases and to extend the repeat using the remaining bases as the template. Recent mutational analyses using *in vitro* reconstituted enzyme suggest that the templating RNA bases lie at the 5' end of the 9 nucleotide stretch, whereas the nucleotides at the 3' end of this region serve for alignment, allowing correct positioning of the 3' end of the telomeric DNA at the beginning of each elongation cycle (47). It has been proposed that synthesis of the complementary C-rich sequence strand is carried out by primase-polymerase-mediated discontinuous synthesis, typical of semi-conservative DNA replication mechanisms, using the extended G-rich strand as a template

(48). Isolation of protein components of the telomerase enzyme has proved more elusive. Recently, Collins et al (49) described the purification of *Tetrahymena* telomerase and cloning of the two genes encoding the two protein components of the enzyme.

In normal human tissues, telomerase activity is only observed in germ cells and some activity is also detected in normal bone marrow, peripheral blood leukocytes and hematopoietic progenitor cells (50, 51). All other human tissues appear not to show evidence of active telomerase, even after screening with recently developed, very sensitive PCR-mediated telomeric repeat amplification protocol (TRAP) method (52). Telomerase activity was first demonstrated in ovarian carcinoma (38) and has now been found in approximately 90% of tumors as observed from more than 100 primary biopsies from over a dozen different tumor types, but was absent in 50 normal somatic tissues (52). Recent studies correlate the reactivation of telomerase with progression of various human malignant tumors (53-58).

Hiyama et al (58) reported that neuroblastomas with high telomerase activity had other genetic changes like *N-myc* amplification and an unfavourable prognosis whereas tumors with low telomerase activity were devoid of such genetic changes and were associated with a favourable prognosis. Bednareck et al (59) have observed that the progressive increase in the telomerase activity is associated with the increased level of genomic instability and the phenotypic progression of skin premalignant papillomas into malignant ones.

The expression of telomerase and ensuing stabilization of telomeres appear to be concomittant with the attainment of immortality in human tumor cells (7, 52). Thus telomerase activity appears to be repressed in somatic cells and tissues but is reactivated in immortal cells and human cancers, an indication that in almost all instances tumor growth is maintained by immortal cells. Moreover, it was hypothesized that in normal tissues, telomerase could be physiologically repressed to reduce the chances of cancerous growth (8).

BIOLOGICAL SIGNIFICANCE OF TELOMERES AND TELOMERASE

If telomere loss and telomerase activation are casually involved in cellular immortalization, which in turn contributes to cancer, then telomeres and telomerase present exciting new targets for drug discovery and diagnostics. Inhibition of telomerase could provide a safe and effective therapy for cancer. The strategy taken by Greider and Blackburn (60) for obstructing telomerase RNA activity through an antisense oligonucleotide targeted to the template region, is one approach that could be applied to the human telomerase RNA. Another inhibition strategy with precedent in basic research is generation of mutant telomeric RNA which elongates the wrong sequence of chromosome termini, resulting in telomeres that fail to stabilize chromosomes and that consequently induce senescence in ciliates (20). The protein components of the telomerase would present another viable target for inhibition if the human telomerase protein could be identified.

Studies of subtelomeric DNA may uncover new DNA markers associated with disease-causing genes, for example, the gene responsible for Huntington's disease. It will also help in investigation into the causes of cellular aging. An effective inhibitor of telomerase might induce prompt senescence in rapidly dividing tumor with small telomeres. The drugs aimed at telomerase inhibition could provide a therapy with relatively little side effects as the studies suggest that hematopoietic stem cells may also have low levels of telomerase activity, in addition to the sperm and oocytes. However, some studies propose that stabilization of telomeric length appears to be maintained by some other mechanisms, in addition to telomerase (61, 62). Hence, the elucidation of the dynamics of telomere maintenance and factors which modulate telomere loss and re-

aquisition of telomerase activity will have profound implications towards understanding the fate of a cell. Finding a specific telomerase inhibitor and the cloning of the human telomerase are awaited to gather further insights.

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REFERENCES

1. Hayflick L. The limited *in vitro* lifetime of human diploid cell strains. *Exp Cell Res* 1965; 37 : 614-636.
2. Hayflick L, Moorhead PS. The serial cultivation of human diploid cells. *Exp Cell Res* 1961; 25 : 585-621.
3. Girardi AJ, Jensen FC, Koprowski H. SV40-induced transformation of human diploid cells : crisis and recovery. *J Cell Comp Physio* 1965; 65 : 69-84.
4. Stamps AC, Gusterson BA, O'Hare MJ. Are tumors immortal? *Eur J Cancer* 1992; 29A : 1495-1500.
5. Shay JW, Wright WE, Werbin H. Defining the molecular mechanisms of human cell immortalization. *Biochem Biophys Acta* 1991; 1072 : 1-7.
6. Harley CB, Vaziri H, Counter CM, Allsopp RC. The telomere hypothesis of cellular aging. *Expt Gerontol* 1992; 27 : 375-382.
7. Counter CM, Avilion AA, LeFeuvre CE, Stewart NG, Greider CW, Harley CB, Bacchetti S. Telomere shortening associated with chromosomes instability is arrested in immortal cells which express telomerase activity. *EMBO J* 1992; 11 : 1921-1929.
8. Harley CB, Kim NW, Prowse KR, Weinrich SL, Hirsch KS, West MD, Bacchetti S, Hirte HW, Counter CM, Creider CW, Wright WL, Shay JW. Telomerase, cell immortality, and cancer. *Cold Spring Harb Symp Quant Biol* 1994; 59 : 307-315.
9. Muller HJ. The remaking of chromosomes. The collecting net. *Woods Hole* 1938; 31 : 181-198.
10. McClintock B. The stability of broken ends of chromosomes in *Zea mays*. *Genetics* 1941; 26 : 234-282.
11. Blackburn EH. Structure and function of telomeres. *Nature* 1991; 350 : 569-573.
12. Zakian VA. Structure and function of telomeres. *Annu Rev Gent* 1989; 23 : 579-604.
13. Allshire RC, Dempster M, Hastie ND. Human telomeres contain at least three types of G-rich repeat distributed non-randomly. *Nucleic Acid Res* 1989; 17 : 4611-4627.
14. de Lange T, Shiue L, Myers RM, Cox DR, Naylor SL, Killery AM, Varmus HE. Structure and variability of human chromosome ends. *Mol Cell Biol* 1990; 10 : 518-527.
15. Chan CSM, Tye BK. Organization of DNA sequences and replication origins at yeast telomeres. *Cell* 1983; 33 : 563-573.
16. Corcoran LM, Thompson JK, Walliker D, Kemp DJ. Homologous recombination within subtelomeric repeat sequences generates chromosomes size polymorphisms in *P. falciparum*. *Cell* 1988; 53 : 807-813.
17. Murray AW, Szostak JW. Construction and behaviour of circularly permutated and telocentric chromosomes in *Saccharomyces cerevisiae*. *Mol Cell Biol* 1992; 6 : 3166-3172.
18. Moyzis RK. The human telomere. *Sci Am* 1991; 265 : 34-41.
19. Gilson E, Laroche T, Gasser SM. Telomeres and the functional architecture of the nucleus. *Trends Cell Biol* 1993; 3 : 128-134.
20. Yu CL, Bradley JD, Attardi LD, Blackburn EH. *In vivo* alteration of the telomere sequences and senescence caused by mutated *Tetrahymena* telomerase RNAs. *Nature* 1990; 344 : 126-132.
21. Hanish JP, Yanowitz JL, de Lange T. Stringent sequence requirements for the formation of human telomeres. *Proc Natl Acad Sci USA* 1994; 91 : 8861-8865.
22. Sheng H, Hou Z, Schierer T, Dobbs DL, Henderson E. Identification and characterization of a putative telomere end-binding protein from *Tetrahymena thermophila*. *Mol Cell Biol* 1995; 15 : 1144-1153.
23. de Lange T. Human telomeres are attached to the nuclear matrix. *EMBO J* 1992; 11 : 717-724.
24. Agard DA, Sedat JW. Three dimensional architecture of a polytene nucleus. *Nature* 1983; 302 : 676-681.
25. Olovnikov AM. A theory of marginotomy. *J Theor Biol* 1973; 41 : 181-190.
26. Harley CB. Telomere loss : mitotic clock or genetic time bomb? *Mutat Res* 1991; 256 : 271-282.
27. Shay JW, West MD, Wright WE. Re-expression of senescent markers in deinduced reversibly immortalized cells. *Exp Gerontol* 1992; 27 : 477-492.
28. Allsopp RC, Vaziri H, Patterson C, Goldstein S, Younglai EV, Futcher AB, Greider CW, Harley CB. Telomere length predicts replicative capacity of human fibroblasts. *Proc Natl Acad Sci USA* 1992; 89 : 10114-10118.
29. Harley CB, Futcher AB, Greider CW. Telomere shorten during aging of human fibroblasts. *Nature* 190; 345 : 458-460.
30. Hastie ND, Dempster M, Dunlop MG, Thompson AM, Green TK, Allshire RC. Telomere reduction in human colorectal carcinoma and with aging. *Nature* 1990; 346 : 866-868.
31. Vaziri H, Schachter F, Uchida I, Wei L, Zhu X, Effors R, Cohen D, Harley CB. Loss of telomeric DNA during aging of normal and trisomy 21 human lymphocytes. *Am J Hum Genet* 1993; 52 : 661-667.

32. Kligelhutz AJ, Barber SA, Smith PF, Dyer K, McDougall JK. Restoration of telomeres in human anogenital epithelial cells. *Mol Cell Biol* 1994; 14 : 961-969.
33. Pathak S, Risin S, Brown NW, Berry K. Telomeric associations of chromosomes is an early manifestation of programmed cell death. *Int J Oncol* 1994; 4 : 323-328.
34. Pathak S, Wang Z, Dhaliwal MK, Sachs PC. Telomeric associations: Another characteristic of cancer chromosomes? *Cytogenet Cell Genet* 1988; 47 : 227-229.
35. Adamson DJA, King DJ, Haites NE. Significant telomere shortening in childhood leukemia. *Cancer Genet Cytogenet* 1992; 61 : 204-206.
36. Smith JK, Yeh G. Telomere reduction in endometrial adenocarcinoma. *Am J Obstet Gynecol* 1992; 167 : 1883-1887.
37. Hiyama E, Hiyama K, Yokoyama T, Ichikawa T, Matsuura Y. Length of telomeric repeats in neuroblastomas : correlation with prognosis and other characteristics. *Jpn J Cancer Res* 1992; 83 : 159-164.
38. Counter CM, Hirte HW, Bacchetti S, Harley CB. Telomerase activity in human ovarian carcinoma. *Proc Natl Acad Sci USA* 1994; 91 : 2900-2904.
39. Mehle C, Ljungberg B, Roos G. Telomere shortening in renal cell carcinoma. *Cancer Res* 1994; 54 : 236-241.
40. Shay JW, Wright WE, Brasiskyte D, Van Der Haegan BA. E6 of human papilloma virus type 16 can overcome the M1 stage of immmortalization in human mammary epithelial cells but not in human fibroblasts. *Oncogene* 1993; 8 : 1407-1413.
41. Shay SW, Wright WE, Werbin H. Loss of telomeric DNA during aging may predispose cells to cancer. *Int J Oncol* 1993; 3 : 559-563.
42. Shay JW, Werbin H, Wright WE. Telomere shortening may contribute to aging and cancer : A perspective. *Mol Cell Diff* 1994; 2 : 1-21.
43. Blackburn EH. Telomerases. *Annu Rev Biochem* 1992; 61 : 113-129.
44. Greider CW, Blackburn EH. Identification of a specific telomere terminal transferase activity in *Tetrahymena* extracts. *Cell* 1985; 43 : 405-413.
45. Morin GB. The human telomere terminal transferase enzyme is a ribonucleoprotein that synthesizes TTAGGG repeats. *Cell* 1989; 59 : 521-529.
46. Greider CW, Blackburn EH. A telomeric sequence in the RNA of *Tertrahymena* telomerase required for telomere repeat synthesis. *Nature* 1989; 337 : 331-337.
47. Autexier C, Greider CW. Functional reconstitution of wild-type and mutant *Tetrahymena* telomerase. *Genes Dev* 1994; 8 : 563-575.
48. Shampay J, Szostak JW, Blackburn EH. DNA sequences of telomeres maintained in yeast. *Nature* 1984; 310 : 154-157.
49. Coolins K, Kobayashi R, Greider CW. Purification of *Tetrahymena* telomerase and cloning of genes encoding the two protein components of the enzyme. *Cell* 1995; 81 : 677-686.
50. Broccoli D, Young JW, de Lange T. Telomerase activity in normal and malignant hematopoietic cells. *Proc Natl Acad Sci USA* 1995; 92 : 9082-9086.
51. Hiyama K, Hirai Y, Kyoizumi S, Akiyama M, Hiyama E, Piatyszek MA, Shay JW, Ishioka S, Yamakido M. Activation of telomerase in human lymphocytes and hematopoietic progenitor cells. *J Immunol* 1995; 155 : 3711-3715.
52. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PLC, Coviello GM, Wright WE, Weinrich SL, Shay JW. Specific association of human telomerase activity with immortal cells and cancer. *Science* 1994; 266 : 2011-2015.
53. Tahara T, Nakanishi T, Kitamoto M, Nakashio R, Shay JW, Tahara E, Kajiyama G, Ide T. Telomerase activity in human liver tissues: Comparison between chronic liver disease and hepatocellular carcinomas. *Cancer Res* 1995; 55 : 2734-2736.
54. Hiyama K, Hiyama E, Ishioka S, Tamakido M, Inai K, Gazdar AF, Piatyszek MA, Shay JW. Telomerase activity in small-cell and non-small-cell lung cancers. *J Natl Cancer Inst* 1995; 87 : 895-902.
55. Chadeneau C, Hay K, Hirte HW, Gallinger S, Bacchetti S. Telomerase activity associated with acquisition of malignancy in human colorectal cancer. *Cancer Res* 1995; 55 : 2533-2536.
56. Sommerfeld HJ, Meeker AK, Piatyszek MA, Bova GS, Shay JW, Coffey DS. Telomerase activity : A prevalent marker of malignant human prostate tissue. *Cancer Res* 1996; 56 : 218-222.
57. Hiyama E, Gollahon L, Kataoka T, Kuroi K, Yokoyama T, Gazdor AF, Hiyama K, Piatyszek MA, Shay JW. Telomerase activity in human breast tumors. *J Natl Cancer Inst* 1996; 88 : 116-122.
58. Hiyama E, Hiyama K, Yokoyama T, Matsuura Y, Piatyszek MA, Shay JW. Correlating telomerase activity levels with human neuroblastoma outcomes. *Nature Med* 1995; 1 : 249-255.
59. Bednarek A, Budunova I, Slaga TJ, Aldaz CM. Increased telomerase activity in mouse skin premalignant progression. *Cancer Res* 1995; 55 : 4566-4569.
60. Greider CW, Blackburn EH. The telomere terminal transferase of *Tetrahymena* is a ribonucleoprotein enzyme with two kinds of primer specificity. *Cell* 1987; 51 : 887-898.
61. Bryan TM, Englezou A, Gupta J, Bacchetti S, Reddel RR. Telomere elongation in immortal human cells without detectable telomerase activity. *EMBO J* 1995; 14 : 4240-4248.
62. Strahl C, Blackburn EH. Effects of reverse transcriptase inhibitors on telomere length and telomerase activity in two immortalized human cell lines. *Mol Cell Biol* 1996; 16 : 53-65.