Telomeres and Telomerase in Cancer

Keiko Hiyama
Editor

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Preface

Telomerase, an enzyme that elongates telomeres and endows eukaryotic cells with immortality, was first discovered in *Tetrahymena* in 1985 and studied among basic scientists in the 1980s. In the 1990s, it was proven that this enzyme also plays a key role in the development of human cancers and many clinical researchers became involved in this field, and in the twenty-first century, telomeres and telomerase are becoming key factors in "stem cell" research including cancer stem cells, regenerative medicine, and congenital diseases with "stem cell dysfunction." Since telomeres/telomerase biology on ciliates, yeasts, and model mice were studied ahead of humans by basic researchers, existing monographs on telomeres and telomerase have devoted much space to biology in such well-studied species, fundamentally important but somewhat different from humans. They are very informative but sometimes confusing for clinical doctors. Now clinical trials and molecular diagnosis targeting telomeres and telomerase in cancer have been started, and all medical oncologists and medical students are required to have knowledge of telomeres and telomerase biology in humans. So, this book focuses on the telomeres and telomerase in human cancers and may provide a basic understanding of up-to-date topics of these unique and fascinating molecules.

I have been enamored with the scientific mystery of telomeres and telomerase along with my husband Eiso since 1990, and been supported by Dr. Jerry W. Shay and my colleagues and friends, many of them kindly contributed to this book as chapter authors. Our study has been encouraged by the Radiation Effects Research Foundation, Hiroshima University Graduate School of Biomedical Sciences, and Hiroshima University 21st Century COE Program-Radiation Casualty Medical Research Center. I would like to express my sincere gratitude to all contributors in this book, Ms. Rachel R. Warren and Mr. Michael Taylor for editorial support, and Dr. Mieczyslaw A. Piatyszek and all my colleagues, friends, and staff for their valuable suggestions and assistance.

This book is dedicated with gratitude to my family, for their love and encouragement.

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Part I:
Basic Background

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Chapter 1
Telomeres and Telomerase in Humans

Keiko Hiyama, Eiso Hiyama, and Jerry W. Shay

Abstract Telomerase can compensate for telomere shortening and helps prevent cellular senescence in eukaryotic cells. In humans, only specific germline cells and the vast majority of cancer cells have sufficient activity for indefinite proliferation. Lymphocytes and stem/progenitor cells in self-renewal tissues have weak activity for extension of their lifespan, but they still undergo replicative senescence. In contrast, most somatic cells do not have telomerase activity and display a finite replicative lifespan. Heterozygous mutations in either of principal telomerase components, *TERT* or *TERC*, cause telomere dysfunction and unexpectedly early senescence to stem cells of renewal tissues. Thus, restoration of telomere function in regenerative medicine via telomerase expression and inhibition of telomerase as an anticancer strategy is a double-edged sword of telomeres and telomerase in clinical medicine.

Keywords: Telomere, Telomerase, Germline cell, Cancer cell, Stem cell, Cellular immortalization, Telomere dysfunction, End-replication problem, Mortality stage, Hayflick limit, TERT, TERC.

1.1 Introduction

Somatic cells explanted into tissue culture do not divide indefinitely *(1)* because of lack or low levels of telomerase and by progressive telomere shortening each time a cell divides. In contrast, some cells, such as male germline cells, have a greatly extended capacity to divide because of expression of the ribonucleoprotein enzyme telomerase, the sole cellular enzyme that can elongates telomeres (Fig. 1.1). DNA sequences of human daughter cells are not completely identical with those of their parent cell: During DNA synthesis prior to cell division, both ends of each chromosome, "telomeres", are not replicated completely because of the "end-replication problem" *(2, 3)*, oxidative damage, and other poorly defined end processing events.

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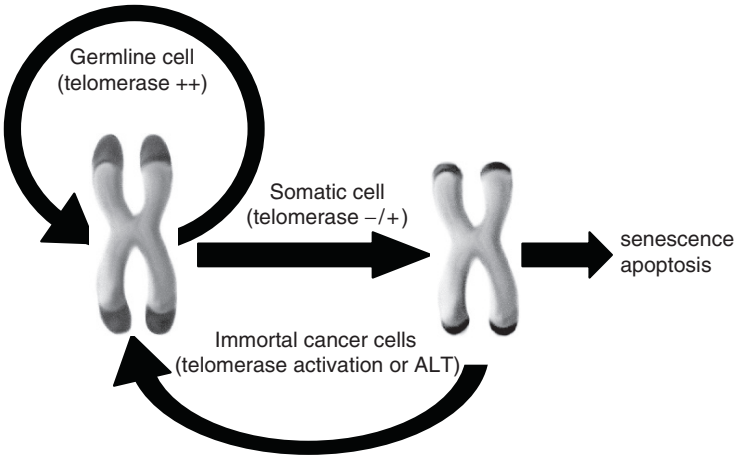

Fig. 1.1 Telomere, telomerase, and cellular lifespan. Telomerase, or much less commonly alternative lengthening telomeres (ALT) mechanism, can compensate telomere shortening and prevent cellular senescence

This progressive telomere shortening is the cellular fate of eukaryotes that have linear chromosomes.

The research on telomeres and telomerase was started by a small group of basic researchers in the 1980s, but many clinical researchers and pathologists came in this field after development of the telomerase detection "TRAP" assay in 1994, which enabled scientists to detect telomerase activity in clinical materials. Now we are in the era when the biology of telomeres and telomerase are required knowledge for clinicians, especially for medical oncologists. To encourage newcomers in the field who are not familiar with the milestone discoveries on telomeres and telomerase, we list them in Table 1.1, especially focusing on human research. In addition, we provide a brief overview of some of the key historical findings. We apologize for any major contributions omitted from this table.

1.2 Telomere Structure

Both ends of all chromosomes, "telomeres", end with G-rich repeats in 5′–3′ strand in every eukaryotes *(4, 8, 10)*. Every vertebrate has (TTAGGG)n repeats, while other species have different G-rich sequences, e.g., *Tetrahymena* has (TTGGGG)n and *Schizosaccharomyces pombe* has GGTTAC(A)(C)(G0-6). In humans, (TTAGGG)n repeats are about 15–20 kb in length at birth and about 8∼10 kb in adults, but the length varies among individuals, organs, cells, and even among chromosomes. The extreme end of each telomere is not blunt (Chap. 15): the 3′ single-strand overhang is about 200 nucleotides and loops back with some of the double-stranded telomeric DNA to make a telomere loop called "T loop" *(66)*, so

Table 1.1 Milestones of telomeres and telomerase research focusing on human

1961.12. Hayflick L and Moorhead PS *(1)* proposed a limitation of replicative lifespan of human diploid cells
1971.12. Olovnikov AM *(2)*; 1972.10. Watson JD *(3)* proposed "End-replication problem" hypothesis
1978.3. Blackburn EH and Gall JG *(4)* identified telomeric repeats in Tetrahymena
1981.5. Klobutcher LA et al. *(5)* found 3′ single-stranded overhang of the G-rich strand in ciliates
1984.2. Ide T et al. *(6)* showed lifespan elongation by SV40 mediated transformation in normal human diploid cells
1985.12. Greider C and Blackburn EH *(7)* identified telomerase activity in Tetrahymena
1986. Cooke HJ and Smith BA *(8)* showed longer telomeres in germ cells than in somatic cells
1988.8. Pereira-Smith OM and Smith JR *(9)* proposed 4 genes that regulate cellular immortalization of human cells
1988.9. Moyzis RK et al. *(10)* determined human telomeric repeat sequences as "TTAGGG"
1989.1. Greider C and Blackburn EH *(11)* cloned telomerase RNA component in Tetrahymena
1989.6. Allshire RC et al. *(12)* identified 3 types of repeat at subtelomeres and found TTAGGG repeats longer in sperm than in blood
1989.7. Wright WE et al. *(13)* proposed "two-stage model" for the escape from human cellular senescence
1989.11. Morin GB *(14)* identified telomerase activity in human cells (HeLa)
1990.2. de Lange T et al. *(15)* demonstrated structure of human chromosome ends and telomere shortening in tumors
1990.5. Harley CB et al. *(16)* demonstrated shortening of telomeres during ageing in cultured human fibroblasts
1990.8. Hastie ND et al. *(17)* found shortening of telomeres in colorectal cancers and with aging
1991.4. Zahler AM et al. *(18)* found that telomeric G-quartet structure is a negative regulator of elongation by telomerase in *Oxytricha*
1991.11. Harley CB *(19)* proposed "Telomere hypothesis" as mitotic clock
1992.2. Hiyama E et al. *(20)* proposed clinical association of telomere length in neuroblastoma
1992.5. Counter CM et al. *(21)* demonstrated experimental evidence of "Telomere hypothesis"
1994.7. Shirotani Y et al. *(22)* proposed clinical association of telomere length in lung cancer
1994.12. Kim NW et al. *(23)* developed "TRAP assay" and demonstrated telomerase activity in all cancer cell lines and 90% of cancerous tissues examined as well as the first evidence for the alternative lengthening of telomeres (ALT) pathway
1995.3. Hiyama E et al. *(24)* proposed association of telomerase activity with pathogenesis and prognosis of neuroblastoma
1995.3. Piatyszek MA et al. *(25)* showed telomerase activity in peripheral blood mononuclear cells
1995. 5. Counter CM et al. *(26)* showed upregulation of telomerase activity in leukemia cells
1995.6. Hiyama K et al. *(27)* proposed a clonal selection model of telomerase positive cancer cells in lung cancer development
1995.6. Collins K et al. *(28)* cloned telomerase protein components "p80" and "p95" in Tetrahymena
1995.6. Chadeneau C et al. *(29)* found telomerase activity in colorectal carcinoma but not in adenomatous polyps
1995.7. Tahara H et al. *(30)* showed telomerase activity in hepatitis and cirrhotic tissues in addition to hepatocellular carcinomas
1995.8. Hiyama E et al. *(31)* found associations of telomerase activity with stage, prognosis, telomere length alteration, and aneuploidy in gastric cancer
1995.9. Bryan TM et al. *(32)* identified alternative lengthening of telomeres (ALT) in human cultured immortal cells

(*continued*)

Table 1.1 (continued)

1995. 9. Feng J et al. *(33)* cloned human telomerase RNA component"TERC (hTR)"
1995. 9. Lingner J et al. *(34)* proposed "leading strand problem" instead of "ragging strand problem" as "end-replication problem"
1995.10. Ohmura H et al. *(35)*; 1998.10. Tanaka H, et al. *(36)* proposed existence of telomerase repressor gene in human chromosome 3
1995.10. Hiyama K et al. *(37)* identified activation of telomerase upon proliferation in normal human lymphocytes and hematopoietic progenitor cells
1995.11. Langford LA et al. *(38)* found telomerase activity in a distinct subgroup of brain tumors and reported telomerase correlated with the stage of disease
1995.12. Sharma HW et al. *(39)* showed downregulation of telomerase activity upon differentiation of immortal leukemia cells
1995.12. Chong L et al. *(40)* cloned human telomere binding protein "hTRF1"
1996.1. Hiyama E et al. *(41)* proposed a diagnostic usefulness of detecting telomerase activity in cytologic specimens of breast cancer
1996.1. Holt SE et al. *(42)* found that telomerase is active throughout the cell cycle but repressed in G0
1996.4. Taylor RS et al. *(43)* detected telomerase activity in normal epidermis and inflammatory skin lesions
1996.9. Hiyama E et al. *(44)* detected telomerase activity in normal human intestinal crypts
1996.10. Bodnar AG et al. *(45)* found that increase in telomerase activity during T cell activation is transient and does not prevent telomere shortening in long-term culture
1996.11. Tatematsu K et al. *(46)* developed "Stretch PCR" for quantitative evaluation of telomerase activity
1997.2. Kyo S et al. *(47)* detected telomerase activity in human proliferative-phase endometrium
1997.2. Harrington L et al. *(48)* cloned human telomerase-associated protein "hTEP1 (TP1)"
1997.2. van Steensel B and de Lange T *(49)* identified control of telomere length by TRF1
1997.4. Sun D et al. *(50)* demonstrated inhibition of human telomerase by a synthetic G-quadruplex interactive compound.
1997.6. Ohyashiki K et al. *(51)* developed "*in situ* TRAP assay"
1997.8. Nakamura TM et al. *(52)*; 1997.8. Meyerson M et al. *(53)* cloned human telomerase reverse transcriptase "hTERT (hEST2)"
1997.10. Blasco MA et al. *(54)* developed $mTR^{-/-}$ mice and found viable up to 6th generation
1997.10. Broccoli D et al. *(55)*; 1997.10. Bilaud T et al. *(56)* cloned telomere binding protein "hTRF2"
1997.12. Weinrich SL et al. *(57)* showed reconstitution of *in vitro* telomerase activity only by TERC (hTR) and TERT (hTRT)
1997.11. Bryan TM et al. *(58)* identified alternative lengthening of telomeres (ALT) in human tumors (1771)
1998.1. Bodnar AG et al. *(59)* showed extension of cellular life-span by expression of hTERT in normal human cells
1998.2. van Steensel B et al. *(60)* identified protection of human telomere end-to-end fusion by TRF2
1998.4. Lee HW et al. *(61)* found telomere dysfunction in highly proliferative organs in late-generation $mTR^{-/-}$ mice
1998.9. Ulaner GA et al. *(62)* found alternate splicing of hTERT
1999.1. Cong YS et al. *(63)*; 1991.2. Takakura M et al. *(64)* cloned and characterized hTERT promoter region
1999.1. Morales CP et al. *(65)* showed immortalization without malignant transformation of normal human fibroblasts by expression of hTERT

(*continued*)

Table 1.1 (continued)

1999.5. Griffith JD et al. *(66)* found that mammalian telomeres end in a large duplex loop, "T-loop"

1999.7. Hahn WC et al. *(67)* created human tumor cells with defined genetic elements: TERT, SV40 large-T, and oncogenic H-ras

1999.9. Yeager TR et al. *(68)* found a novel type of PML body in ALT cells.

1999.10. Hahn WC et al. *(69)* showed that dominant negative form of hTERT inhibits telomerase activity and tumorigenicity of immortal cancer cells

1999.12. Kim SH et al. *(70)* identified TIN2 as a new regulator of telomere length.

1999.12. Herbert B et al. *(71)* showed that PNA and 2′-O-MeRNA oligomers reversibly inhibit telomerase activity and induce telomere shortening

1999.12. Mitchell JR et al. *(72)* found dysfunction of telomerase in X-linked dyskeratosis congenita with mutations in dyskerin

2000.1. Thomas M et al. *(73)* demonstrated elongation of bovine adrenocortical cell function with hTERT expression in experimental xenotransplantation

2000.10. Hooijberg E et al. *(74)* established immortal CD8 + T cell clones by hTERT expression

2000.12. Dunham MA et al. *(75)* demonstrated that ALT occurs by means of homologous recombination and copy switching

2001.2. Shin-ya K et al. *(76)*; 2002. 3. Kim MY et al. *(77)* demonstrated the effects of telomestatin as a telomerase inhibitor

2001.6. Baur JA et al. *(78)* demonstrated telomere position effect in human cells

2001.7. Hemann MT et al. *(79)* found that telomere dysfunction is recognized at the onset of meiosis and triggers germ cell apoptosis in mice

2001.9. Vulliamy T et al. *(80)* found mutations in hTR in autosomal dominant DKC

2002.6. Vulliamy T et al. *(81)* found mutations in hTR in aplastic anemia

2002.7. Yatabe N et al. *(82)* demonstrated the effects of 2–5A antisense therapy directed against hTR in cervical cancer cells

2002.7. Seimiya H et al. *(83)* demonstrated the effects of telomerase inhibitors MST-312, -295, and -1991 in human cancer cells

2002.10. Stewart SA et al. *(84)* demonstrated that telomerase contributes to tumorigenesis by a telomere length-independent mechanism

2002.11. Seger YR et al. *(85)* transformed a normal human cell by adenovirus E1A, Ha-RasV12, and MDM2 expression without telomerase activation

2003.3. Hakin-Smith V et al. *(86)* found that ALT phenotype is a good prognosis indicator in glioblastoma multiform

2003.4. Zhang A et al. *(87)* found deletion of hTERT and haploinsufficiency of telomere maintenance in Cri du chat syndrome

2003.4. Stewart SA et al. *(88)* proposed that erosion of single-strand telomeric overhang, rather than overall telomere length, serves to trigger replicative senescence

2003.4. Ulaner GA et al. *(89)* found that telomerase activation and ALT are comparably poor prognosis indicators in osteosarcoma

2003.5. Colqin LM et al. *(90)* proposed that human POT1 protein can act as a telomerase-dependent positive regulator of telomere length

2003.6. Loayza D and de Lange T *(91)* proposed that POT1 interacts with TRF1 complex and transmits information of telomere length to the telomere terminus

2003.6. Lin SY et al. *(92)* proposed 3 tumor suppressor pathways involved in hTERT repression: Mad1/c-Myc, SIP1, and Menin

2003.7. Masutomi K et al. *(93)* proposed that hTERT is expressed even in normal human somatic cells maintaining telomere structure such as 3′ single-stranded overhang

2003.7. Asai A et al. *(94)* developed a telomerase template antagonist GRN163 and demonstrated its anticancer effects *in vitro* and in xenograft model

(*continued*)

Table 1.1 (continued)

2003.8. Tauchi T et al. *(95)* demonstrated effects of G-quadruplex-interactive telomerase inhibitor telomestatin (SOT-095) in leukemia cells

2004.2. der-Sarkissian H et al. *(96)* proposed that the chromosomes with shortest telomeres are the first to become unstable in telomerase-negative-transformed cells

2004.5. Preto A et al. *(97)* demonstrated that telomere erosion triggers growth arrest in thyroid cancer cells with wild p53 while it cause crisis by abrogation of p53

2005.1. Seimiya H et al. *(98)* demonstrated that tankyrase 1 inhibition enhances telomere shortening by telomerase inhibitor

2005.4. Fasching CL et al. *(99)*; Marciniak RA et al. *(100)*; 2005.11. Cerone MA, et al. *(101)* proposed that ALT-associated promyelocytic leukemia bodies (APBs) are not always essential for ALT-mediated telomere maintenance

2005.5. Yamaguchi H et al. *(102)* found mutations in TERT in aplastic anemia

2005.6. Sun B et al. *(103)* demonstrated a minimal set of genetic alterations required for fibroblast transformation

2005.7. Nakamura M et al. *(104)* demonstrated that hTERT KO by siRNAs sensitizes cervical cancer cells to ionizing radiation and chemotherapy

2005.8. Herbert BS et al. *(105)* demonstrated a superiority of lipid modification of GRN163 (GRN163L) in telomerase inhibition

2005.8. Zaug AJ et al. *(106)* found that human POT1 disrupts telomeric G-quadruplexes allowing telomerase extension

2005.8. Flores I et al. *(107)* found that mobilization of stem cells out of their niche was inhibited by telomere shortening and promoted by Tert overexpression in mice

2005.9. de Lange T *(108)* proposed a concept of "Shelterin" as telomere binding proteins consisting of TRF1, TRF2, POT1, TIN2, TPP1, and RAP1

2005.11. Djojosubroto MW et al. *(109)* demonstrated *in vitro* and *in vivo* effects of hTR antagonist GRN163 and GRN163L on hepatoma cells

2005.11. Armaninos M et al. *(110)*; Goldman F et al. *(111)* demonstrated TERC haploinsufficiency on the inheritance of telomere length in autosomal dominant dyskeratosis congenital

2005.11. Verdun RE et al. *(112)* found that telomeres of telomerase-negative cells recruit Mre11, phosphorylated NBS1, and ATM in every G2 phase of the cell cycle

2005.12. Horikawa I et al. *(113)* found that a GC-box within the hTERT promoter is responsible for the human-specific *TERT* repression

2006.1. Anderson CJ et al. *(114)* found that hypoxia induces the transcriptional activity of both hTR and hTERT gene promoters and increase of active hTERT splice variant

2006.2. Compton SA et al. *(115)* found NOS-dependent telomere shortening and apoptosis of prostate cancer cells by inhibition of Hsp90

2006.2. Chai W et al. *(116)* demonstrated different overhang sizes at leading versus lagging strands of human telomeres

2006.3. Tahara H et al. *(117)* found that telomestatin induces loss of 3′ telomeric overhang through TRF2 protein dissociation from telomeres in cancer cells

2006.3. Gellert GC et al. *(118)*; 2006.5. Hochreiter AE et al. *(119)* demonstrated *in vitro* and *in vivo* effects of hTR antagonist GRN163L on breast cancer cells

2006.5. Trapp S et al. *(120)* demonstrated tumor-promoting effects of vTR in a chicken natural virus-host infection model

2006.5. Ambrus A et al. *(121)* proposed "mixed parallel/antiparallel G-strands" as intact telomeric G-quadruplex structure

2007.2. Xin H et al. *(122)*; 2007.2. Wang F et al. *(123)* proposed POT1-TPP1 complex as a processivity factor for telomerase

2007.3. Cohen SB et al. *(124)* showed that human telomerase exists as a complex of two molecules each of hTERT, hTR, and dyskerin

(*continued*)

Table 1.1 (continued)

2007.3. Armanios MY et al. *(125)*; 2007.5. Tsakiri KD et al *(126)* identified mutations in TERT/TERC and short telomeres as etiology of familial and/or adult-onset pulmonary fibrosis
2007.7. Jiang WO et al. *(127)* identified 8 candidate ALT genes as PML, TRF1, TRF2, TIN2, RAP1, MRE11, RAD50, and NBS1
2007.10. Xu L and Blackburn EH *(128)* proposed "T-stumps" in immortal cancer cells as the minimal telomeric unit that can be protected by telomere binding proteins
2007.11. Azzalin CM et al. *(129)*; 2008. 2. Schoeftner S et al. *(130)* identified active transcription of human telomeres into "telomeric repeat-containing RNA (TERRA or TelRNAs)" that regulate telomerase activity
2007.11. Takahashi K et al. *(131)* found that human iPS cells derived from fibroblasts activated intrinsic telomerase
2008.3. Venteicher AS et al. *(132)* found that additional enzymes (ATPases pontin and reptin) are required for telomerase assembly
2008.3. Stadtfeld M et al. *(133)* demonstrated that activation of endogenous telomerase is one of late events during fibroblast reprogramming to iPS cells in mouse

that the chromosome end is distinguished from bona fide dsDNA breaks and protected from exposure to DNA repair system (Chap. 4).

1.3 Why do Telomeres Gradually Shortened?

Until the early 1960s, cultured normal human cells were believed to be able to replicate indefinitely as long as good culture conditions were maintained. In 1961, Hayflick reversed this concept and demonstrated convincingly that normal human cells have a limit in the number of possible cell divisions: lung fibroblast 55 times, heart 26, kidney 40, and skin 43 *(1)*. So, this phenomenon of replicative senescence in normal cells is often called the "Hayflick limit". In 1971 and 1972, Olovnikov and Watson reported the mechanism of this limit in Russian and English, respectively, as an "end-replication problem". When they originally proposed this problem, "lagging (discontinuous) strand" was considered to be responsible for telomere shortening, because DNA polymerase replicates only in the 5′–3′ direction, and requires an RNA primer in starting DNA replication and a complementary strand for replication. Then, after removal of the RNA primer, the 5′ end of the lagging strand locates inside of the extreme 3′ end of the complement strand, i.e., "lagging strand problem" *(134)*. However, considering the structure of 3′ telomere overhang at the end of telomeres, the "end-replication problem" mechanism was then proposed as a "leading strand problem", i.e., inability of leading strand DNA synthesis to produce the 3′ overhang (Fig. 1.2), and then "lagging strand problem" may occur in the next round of replication *(34)*. The "end-replication problem" and resulting limit of cellular lifespan exists in all eukaryotes that have linear chromosomes but not in prokaryotes that have circular chromosomes without chromosomal "ends".

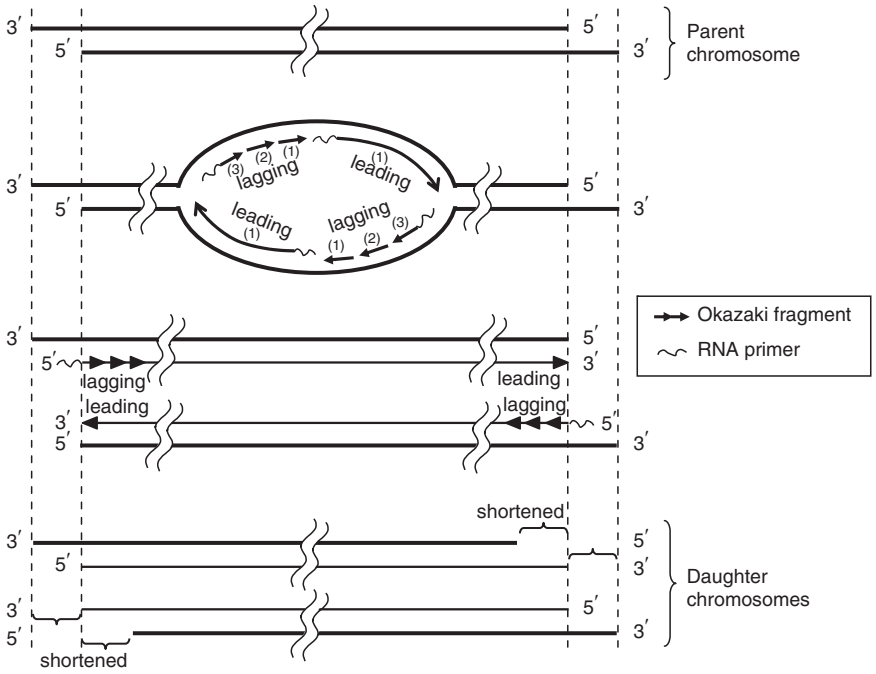

Fig. 1.2 Renewed "end-replication problem." Incomplete replication of leading strand due to formation of 3′ overhang, as well as that of lagging strand due to RNA primer as a prerequisite for DNA synthesis, is responsible for telomere shortening

1.4 Functions of Telomeres and Telomerase

Telomeres consist of noncoding TTAGGG repeats (but the reader should be aware that it was recently reported that telomeric repeats are transcribed and this telomeric RNA may regulate telomerase activity *(129)*). Telomeres protect chromosome ends from DNA degradation, DNA repair mechanisms, and fusion. Uncapped telomeres activate the DNA damage response and cause end-to-end fusions resulting in cellular senescence, apoptosis, and further chromosomal instability (Table 1.2). Since genes near telomeres may be reversibly silenced in a telomere length-dependent manner by telomere position effect (TPE) *(78)*, telomere shortening may result in restoration of expression of such silenced genes. Moreover, telomeres appear to play an important role in "bouquet" formation at the beginning of meiosis, and telomere dysfunction results in germ cell death *(79)*.

The well known function of telomerase is elongation of telomeres, so that cells can increase their replicative capacity, sometimes indefinitely (Table 1.3). However, it may be that even in some normal fibroblasts, telomerase is expressed at low levels. However, this amount of telomerase cannot maintain telomere length but during each S phase may play a role in maintaining chromosomal structure *(93)*.

Table 1.2 Function of telomeres and dysfunction due to telomere shortening

Function	Consequence of telomere shortening/dysfunction
Prevention of erosion of genes in subtelomeres	Cellular senescence, cell death, and/or carcinogenesis
Telomere position effects (TPE)	Reactivation of the silenced genes near telomeres
Protection of chromosomal end-to-end fusion	Chromosome fusion, anaphase bridge
T-loop formation and chromosome stability	Disruption of T-loop inducing p53 mediated cellular senescence and apoptosis, chromosome fusion
"Bouquet" formation at the beginning of meiosis	Impaired meiosis and germ cell apoptosis

Table 1.3 Function of telomerase and consequence of its activation

Function	Consequence examples of telomerase activation
Elongation of telomeres	Elongation of cellular lifespan or immortalization
Maintenance of chromosomal structure	Telomerase is transiently expressed in each S phase in normal cells
Addition of malignant potential	Tumor formation with nontumorigenic ALT cells
Promotion of stem cell proliferation	e.g., increased hair growth
DNA repair?	Required to form DNA damage foci following irradiation
Self-renewal capacity?	Required to reprogram fibroblasts to iPS cells

Telomerase may also have roles in stem cell proliferation *(107)*, and reprogramming of iPS cells *(131, 133)*. The mechanisms of these functions of telomerase may or may not be related to maintenance of telomere length.

1.5 Two Mortality Stage Mechanisms and Telomere Hypothesis

Two mortality stage mechanisms, "M1" and "M2", must be overcome for normal cells to escape from cellular senescence and become immortal *(13, 19)*.

Normal cells stop dividing at the "Hayflick limit," i.e., mortality stage 1 (M1), where p16/pRb and TP53 recognize perhaps a single uncapped telomere as broken or damage DNA. To bypass this potent tumor suppressor mechanism, cells can divide beyond M1 and continue replication by inactivating these tumor suppressor genes (termed extended lifespan). However, the cells again stop dividing at the mortality stage 2 (M2), also called "crisis". At this stage, many telomeres are critically shortened, end-end fusions occur, and cells stop dividing. The escape from M2 in human cells is extremely rare and almost universally involves the upregulation or reactivation of telomerase as a telomere-maintenance mechanism *(19)*. Much less commonly other telomere maintenance mechanisms such as alternative lengthening telomeres (ALT, See Chap. 5) *(58)* are engaged. The cells that have activated telomerase can overcome the M2, and become immortal (Fig. 1.3).

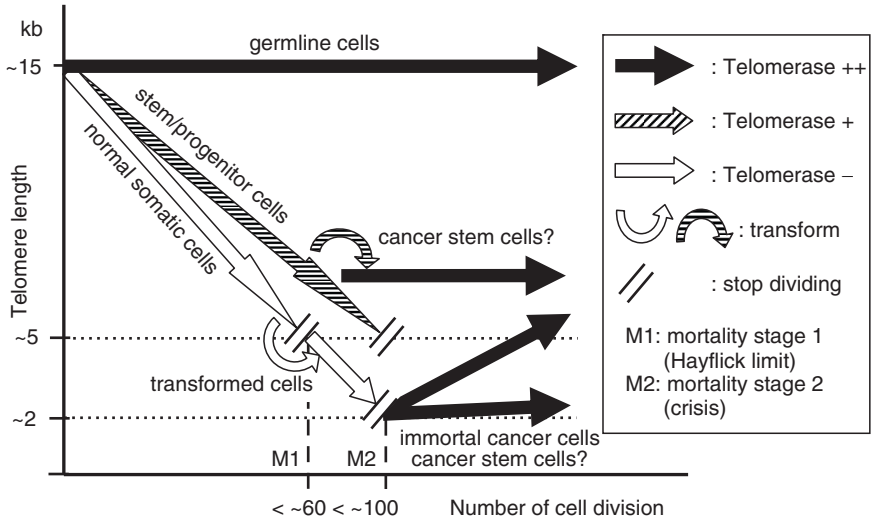

Fig. 1.3 Telomere hypothesis and two independent mortality mechanisms controlling cellular senescence and immortalization. Normal stem cells have elongated lifespan but are not immortal. Cancer stem cells may arise from cells that have bypassed M2 as well as from normal stem cells. The origin of cancer stem cells is still an hypothesis and it is possible that they may have the same, shorter, or longer telomeres compared to the bulk population of tumor cells

Thus both M1 and M2 may be thought of as initial anti-cancer protection mechanisms and only when both have been bypassed are cells immortal and then can progress to advanced malignancies. Thus some preneoplastic lesions may be arrested at M1, some early stage clinical cancers may have overcome M1, but not M2 (transformed but mortal cancer cells), while advanced cancers probably have overcome M2 (transformed and immortal cancer cells). All cancer cell lines and "cancer stem cells" have likely overcome both M1 and M2. Meanwhile, normal lymphocytes and stem/progenitor cells in self-renewal tissues have highly regulated telomerase activity, but gradually senesce and thus while telomerase may partially extend their lifespan, the cells are mortal, since they have not overcome M1 nor M2 (Fig. 1.3, Table 1.2) *(37, 43, 44, 47)*.

1.6 Telomerase is a Conserved Reverse-Transcriptase

Human telomerase is a ribonucleoprotein enzyme composed of catalytic component TERT, telomerase reverse-transcriptase *(52, 53)*, and RNA template TERC (or hTR), telomerase RNA component (or human telomerase RNA) *(33)*. Telomerase can elongate the G-rich 3′ telomere overhang using the TERC as template. Since telomeric DNA is synthesized according to the complementary RNA sequence, telomerase is a reverse-transcriptase. The catalytic component gene *TERT* is evolutionally conserved *(52)*, and has been alternatively called as hTERT (meaning

"human TERT"), *hTRT (52)*, *hEST2 (53)*, *hTCS1 (135)*, or *TP2 (136)* by researchers who independently cloned this gene in 1997. *In vivo*, Cohen SB et al. purified the catalytically active human telomerase complex (650–670 kD) and proposed that telomerase may exist as a dimer consisting of two molecules each of hTERT (127 kD), hTR (153 kD), and dyskerin (57 kD) *(124)*. The telomerase core enzyme may complex with additional telomerase binding proteins (Chap. 2), and its function is regulated transcriptionally and posttranscriptionally (Chap. 3).

1.7 Telomerase Activity and Cellular Immortalization

Expression of TERT is a prerequisite but not always sufficient for cellular immortalization in most human cells (especially when the cell culture conditions are not optimized). Among the core components of telomerase, TERC is constitutively expressed in most cells regardless of their telomerase activity level, and TERT expression levels determine the telomerase activity qualitatively and quantitatively. Ectopic expression of TERT induces telomerase activity in telomerase-negative somatic cells *in vitro (57)*, but cellular immortalization is still a relatively rare event with many clones expressing telomerase potentially remaining mortal with or without elongation of lifespan *(59, 65)*. Thus, activation of telomerase is a prerequisite, except for rare ALT cells, but not necessarily a sufficient condition for cellular immortalization.

1.8 Telomerase is Activated in >80% of Human Malignancies

Every type of human malignancy examined to the present time has evidence of telomerase activation with the average being detected in >80% of overall cancer tissues *(137)* (Chap. 8). In general, the incidence and level of telomerase activation are higher in advanced stages than early stages, in metastatic lesions than primary lesions, in poor prognosis cases than good prognosis cases, and in malignant lesions than in precancerous lesions, indicating that continuous progression of cancers may ultimately depend on telomerase in all human malignancies. Thus, telomerase components and associated proteins are becoming not only a diagnostic marker of cancer but also the molecular targets of anticancer strategies and some of them are under clinical trials (*see* Part II).

1.9 Telomerase Activity in Normal Somatic Cells and Stem Cells

In most human normal somatic cells, telomerase activity is undetectable. However, lymphocytes and most, but not all, stem/progenitor cells in self-renewal tissues can express telomerase upon mitogenic stimulation *(37)*. These cells have elongated lifespan so that humans can retain immune reactivity to each antigen and maintain

Table 1.4 Telomere length, telomerase expression, and cellular lifespan in human cells

Telomere length (cellular lifespan)	Telomerase expression (detectable level by usual analysis)[a]	
	+	–
Stable (immortal)	Germline cells, immortal cancer cells	Part of *in vitro* immortalized cells[b], a few cancers (e.g. part of sarcoma)[a]
Slowly shortened (mortal with elongated lifespan)	Lymphocytes (activated), renewing stem/progenitor cells (e.g. hematopoietic, intestinal, epidermal, hair follicle), endometrial cells (proliferative)	Mesenchymal stem cells
Shortened (mortal)	Stem cells in progeria, stem cells with dysfunction (e.g. a part of aplastic anemia, dyskeratosis congenital, IPF[c])	Most somatic cells, part of cancer cells

[a]At very low levels, telomerase is expressed during each S phase even in normal somatic cells *(93)*
[b]ALT (alternative lengthening of telomeres) cells
[c]IPF idiopathic pulmonary fibrosis

the function of important organs, such as the bone marrow, intestine, skin, etc. However, when these cells stop dividing and/or are differentiated, telomerase activity disappears. Thus, normal somatic cells never become immortal *in vivo*, even those with regulated telomerase activation (Table 1.4) unless there is loss of tumor suppressor genes or activation of oncogenes. In contrast, human mesenchymal stem cells (hMSCs) may have very low or no detectable telomerase activity *(138–140)*, whereas they can maintain longer telomeres than those in usual somatic cells. hMSCs lack characteristics of ALT cells such as PML bodies and may have unique telomere maintaining mechanism *(141)*.

In the mouse, telomerase activity is detectable in normal somatic cells in addition to stem cells, and telomere length is around fivefold longer than human (~50 kb vs. ~10 kb). Since their lifespan is much shorter than human, telomeres in murine cells are not shortened to reach the Hayflick limit within a lifetime even without telomerase, and $mTR^{-/-}$ (telomerase RNA knockout) mice can survive until the sixth generation *(54)*. At this late generation, telomere dysfunction is manifested as stem cell dysfunction and infertility in $mTR^{-/-}$ mice (Chap. 6).

1.10 Telomere-Binding Proteins

In addition to the core telomere-binding proteins "Shelterin" (TRF1, TRF2, POT1, TIN2, TPP1, and RAP1), DNA repair proteins are also involved in telomere maintenance: Ku complex, MRN complex (MRE11, RAD50, NBS1), XPF/ERCC1, ATM, BLM/WRN, RAD51D, and RAD54 *(108)*. Mutations or absence of these genes cause short telomeres, end-to-end chromosomal fusions, premature aging phenotypes, and/or cancer predisposition (Chap. 2).

1.11 Telomere Dysfunction and Human Diseases

Mutations in telomerase component genes, *TERT* and *TERC*, cause stem cell dysfunction, resulting in dyskeratosis congenita *(80)*, aplastic anemia *(81)*, and idiopathic pulmonary fibrosis *(125, 126)*. This suggests that individuals with heterozygous mutations in *TERT* or *TERC* have reduced telomerase activity in their stem cells and suffer accelerated telomere shortening possibly due to haploinsufficiency, except for mutations in the template domain of *TERC*, which can show dominant negative effects *(142)*. Stem cell dysfunction due to accelerated telomere shortening is caused also by mutations in telomerase binding proteins, such as dyskerin *(72)*. This suggests that telomerase is not in excess (e.g., individuals need long telomeres for a full lifespan). Importantly these telomere-associated genetic diseases suggest that inhibition of only 50% of telomerase in human cancer may be sufficient to drive cancer cells with short telomeres into apoptotic cell death leading to durable cancer responses prior to affecting normal stem cells.

1.12 Concluding Remarks

Telomeres and telomerase dysfunction causes unexpected early senescence to stem cells in renewal tissues or graft tissues, while maintenance of telomeres via activation of telomerase is the critical offender for the indefinite proliferation of immortal cancer cells. Restoration of telomere function in regenerative medicine and inhibition of telomerase (e.g., induction of telomere dysfunction) as an anticancer strategy, respectively, is the double-edged sword in clinical medicine.

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