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Telomere Dysfunction and Cancer

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Unlike the circular structure of prokaryotic chromosome, the structure of eukaryotic chromosomes is linear. To prevent the ends of linear chromosomes from being recognized as double-stranded DNA breaks by DNA damage, chromosome end is capped by the special chromatin structure, telomere. Telomere in vertebrate consists of TTAGGG tandem repeats and telosome/shelterin complex including TRF1, TRF2, RAP1, TIN2, TPP1, and POT1. The average length of human telomere is 10～15 kb at birth, and several rounds of cell division lead to telomere shortening in somatic cells due to the absence of telomerase, a reverse transcriptase, adding TTAGGG repeats at chromosome ends. The uncapping telomere by telomere attrition causes genomic instability activating cell cycle checkpoints and inducing cell cycle arrest, senescence, or apoptosis. Highly-proliferative tumor cells that have the impaired checkpoints and short telomeres can escape normal limits on cell proliferation, resulting in massive genome instability and malignancy of tumor cells. In this review, we provide an overview of telomere structure and function, and the relationship between telomere dysfunction and the initiation of human cancer. **(*Cancer Prev Res* 15, 28-33, 2010)**

Key Words: Telomere, Telomerase, Telosome/shelterin, Tumorigenesis

INTRODUCTION

The proliferative capacities of normal human diploid fibroblast are intrinsically limited. After 60～80 population doublings, they stop cell division and start to have senescence phenotypes which are characterized by flat and large cell size, vacuolated morphology, inability of DNA synthesis, and expression of the senescence-associated β-galactosidase marker.[1)] This replicative senescence is due to the 'telomere' attrition. The progressive erosion of telomere by cell division is proposed to represent a 'molecular clock' underlying organismal aging.

The telomerase, a specialized reverse transcriptase, synthesizes and maintains telomere. The expression of telomerase is low or absent in most human somatic tissues, whereas it is high in proliferative progenitor germ and stem cells.[2)] The massive experimental evidences have suggested that telomerase reactivation occurs in at least 85% of human cancers. Because the telomere maintenance by reactivated telomerases also plays an important role in the long-term survival of cancer cells, the therapeutic strategy that is based on targeting telomerase and telomeres might be very attractive to suppress tumorigenesis. In this review, we give an updated summary of telomere biology and describe the current understanding of relationship of telomere dysfunction to genome instability and tumorigenesis.

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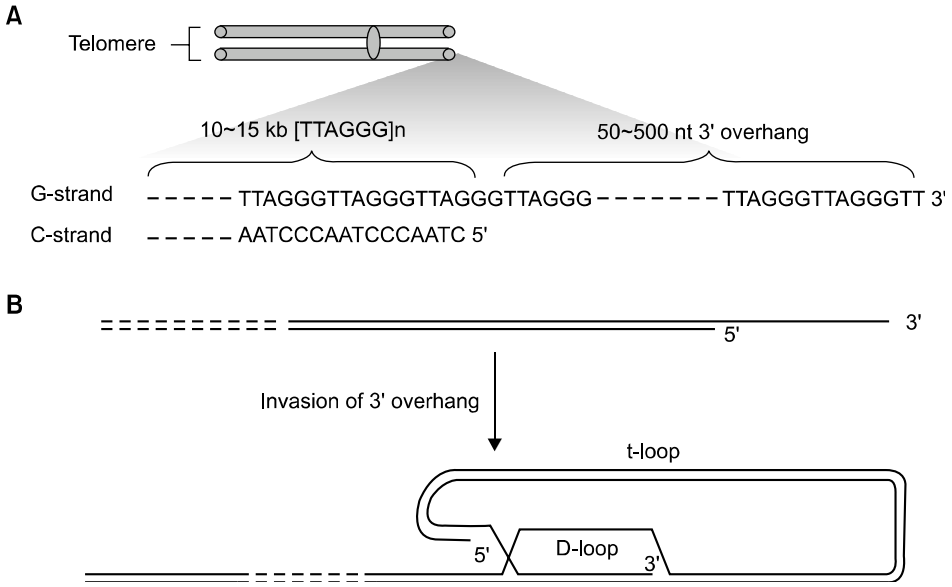

Fig. 1. The structure of human telomere. (A) Telomeres are located in the ends of chromosomes. They are composed of double-stranded DNA region of hexameric TTAGGG repeats that varies in length. Telomeres are characterized by a 50～500 nt long single-stranded overhang of the G-strand. Note that the 3'end is not precisely defined whereas 5' end of telomere generally features the sequence ATC-5'. (B) The 3' single-stranded overhang folds back and invades the double-stranded telomeric region, forming the t-loop.[4]

TELOMERE AND TELOMERASE

In vertebrate, the ends of chromosomes are capped by telomeres which are composed of TTAGGG repeats and terminate in 3' single-stranded DNA overhangs (Fig. 1A). The G rich strands that constitute the 3'-end and the other strands are called the G- and C-strands, respectively. Telomeres are typically located adjacent to the gene-rich subtelomeric regions. They span around 20～50 kb in mice and 10～15 kb in human at birth.[3] The 3'-G single-stranded overhang of mammalian telomeres varies between 50～500 nucleotides, which invades into the double-stranded telomere region to form large duplex lariat structure, the telomere-loop (t-loop, Fig. 1B).[4] It is proposed that the t-loop structures provide protective architectures to hide the exposed linear chromosome ends or naturally occurring double-stranded breaks from DNA damage repair machinery.

Because of the incomplete replication of linear chromosomes by conventional DNA polymerases, telomeres are gradually shortened during cell divisions (end-replication problems). DNA polymerases cannot initiate DNA synthesis *de novo*. They add nucleotides at the site of 3'-OH group. As consequence, the 5' to 3' replication of DNA by DNA polymerases requires a short RNA primer for initiation that is subsequently degraded and replaced by DNA. The last RNA primer of the lagging strand on a linear DNA is terminal and it cannot be replaced by DNA after removal. Therefore, every round of DNA synthesis results in loss of terminal sequence and then progressive shortening of the chromosome ends. In mammal, telomeres shorten much faster than predicted by end-replication problem. The rate of telomere shortening in mammal is in a range of 50～200 bp per population doubling.[5] It has been suggested that this higher rate of shortening is resulted from post-replication processing. The most ends of C-strands terminate the sequence ATC-5' (Fig. 1A), and the ends of leading strand have ～two fold shorter 3'-overhang compared to those of lagging strand. This structure is explained by extensive resection of C-strand at chromosome ends by certain nucleases. Therefore, the end-replication problem and 5'-end resection process result in net sequence loss of chromosome ends.[6]

To overcome the problems of telomere shortening, the action of telomerase is essential. Telomerase is ribonucleoprotein complex composed of a catalytic telomerase reverse transcriptase (TERT) and RNA template (TERC).[7] TERT is related to the reverse transcriptases encoded by nonlong terminal repeat retrotransposons and group II introns,[8] which extend the 3'-end of a DNA primer. TERC is composed of 451 nucleotides including an 11-nucleotide-long template in human, and the region, AAUCCCAAUC, serves for both the annealing of the 3'-overhang and the addition of one telomeric repeat per elongation step. The 3' end of TERC contains a Cajal body localization sequence and an H/ACA motif, which is found in small nucleolar RNAs. Other proteins such as dyskerin, NOP10, NHP2, and GAR1 bind to H/ACA motif of TERC, playing a role in stabilization of the complex.[9] The mutations

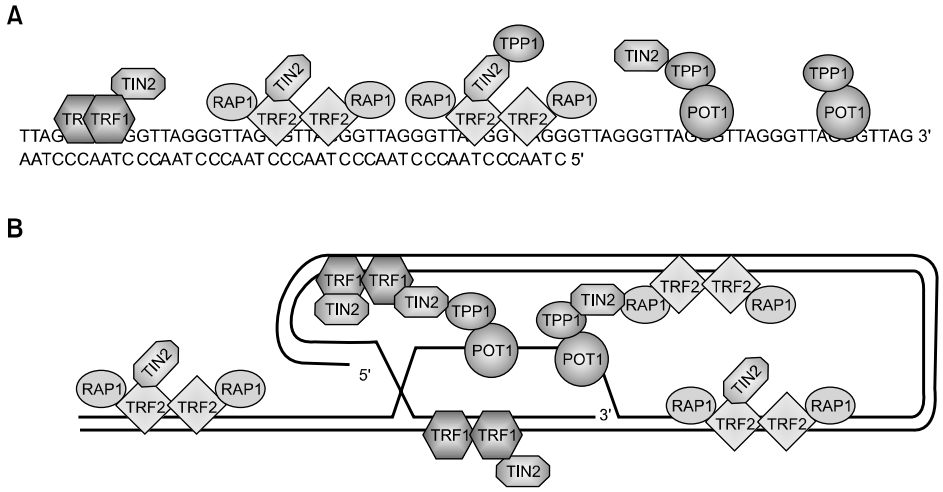

Fig. 2. Telomere-binding protein complex, telosome/shelterin. (A) A schematic of telosome/shelterin. TRF1 and TRF2 can form homodimer and bind to double stranded region of telomere. POT1 is a single-strand binding telomeric protein and recruits TPP1 by protein-protein interaction. RAP1 is constitutive binding partner of TRF2. (B) TIN2 occupies a central position in telosome/shelterin and is able to bind to TRF1, TRF2, and TPP1, then providing a bridge between double-stranded and single-stranded telomeric components.[4]

in dyskerin or NOP10 and deletion of the H/ACA motif in TERC result in reduced or diminished telomerase activity, which are associated with several human premature ageing syndromes. One of these syndromes is dyskeratosis congenita (DC). DC patients have mutations in either the *Terc* gene or *dyskerin* gene, showing premature aging phenotypes such as short stature, defects of the skin and haematopoietic system, bone marrow failure, and premature death.[10~12] Both mutations result in decreased telomerase activity, shorter telomeres compared with healthy individuals, and increased chromosomal instability with age. DC patients also show an elevated incidence of spontaneous cancer.

THE TELOMERE-BINDING PROTEIN COMPLEX, TELOSOME/SHELTERIN

Telomeres associate with the six telomere-specific protein complex, telosome/shelterin that is essential for protection of these regions from DNA damage repair machinery and telomere length maintenance (Fig. 2).[4,13] The components of telosome/shelterin localize specifically to telomeres. They include the double-stranded DNA binding proteins, telomeric repeat binding factor-1 and 2 (TRF1 and TRF2),[14,15] the single-stranded telomeric DNA binding protein, protection of telomeres 1 (POT1),[16] and three associated proteins, TRF1-interacting nuclear protein 2 (TIN2),[17] RAP1 (human ortholog of the yeast repressor/activator protein 1),[18] and TPP1 (previously TINT1/PTOP/ PIP1)[19,20] (Fig. 2). TIN2, RAP1, and TPP1 do not have DNA binding activity, but they form telomere protein complexes by interacting with other telomere proteins. TIN2 interacts with TRF1, TRF2, and TPP1. RAP1 associates with TRF2. TPP1 is recruited into telomere by binding to TIN2 or POT1.

TRF1 and TRF2 are constitutively present at telomeres and share a common domain structure composed of the TRF homology (TRFH) domain and a C-terminal Myb DNA-binding domain. In contrast to the N-terminus of TRF2 that contains Gly/Arg-rich basic domain, TRF1 has acidic amino acid at its N-terminus. Both TRF1 and TRF2 bind to double-stranded region of telomeres as homodimer or oligomers through homotypic interactions in the TRFH domain.[14,15] TRFH domain of TRF1 and TRF2 also functions as molecular hub through the recognition of F/YxLxP motif on target proteins such as Apollo, MCPH1, PNUTS, and TIN2.[21,22]

TRF1 and TRF2 are extremely abundant, estimated to cover each telomere with thousands of dimmers. Despite of variation in the length of individual telomeres, the average telomere length is kept within a narrow species-specific range, indicating an equilibrium between telomere erosion and elongation. One of the explanations is protein-counting model for telomerase inhibition. For example, TRF1 and TRF2 have inhibitory effect on telomere elongation. Longer telomeres by carrying a larger number of TRF1 and TRF2 binding sites allow increased association of telomerase repressors, and as a consequence, telomerase is largely inhibited at these ends. PINX1, an inhibitor of telomerase[23] and tankyrase, a protein with poly (ADP-ribose) polymerase activity[24] are known to interact with TRF1 and inhibit telomerase activity.

TRF2 is also emphasized owing to its essential roles of the end protection. Deletion of TRF2 from mouse cells or its inhibition with a dominant negative mutant in human cells enhances a robust DNA damage response at telomere[25,26] and forms telomere dysfunction-induced foci (TIF) showing the presence of 53BP1, MDC1, and γ-H2AX at telomere. This DNA damage response is mediated by the ATM kinase pathway. It is possible that TRF2 interacts with negative regulators of DNA damage response and blocks the downstream step of DNA damage sensing pathway even after the natural double stranded DNA breaks of chromosome ends are sensed. In addition to TRF2, all the components of telosome/shelterin act as negative regulators of DNA damage response.

POT1 has two OB folds in its N-terminus that are oligonucleotide- or oligosaccharide-binding domains and are used for recognition of single-stranded telomeric region, 3'-overhang. When POT1 levels are diminished by siRNA knockdowns in cells or *Pot1*-knockout in mice, TIF is induced by activation of ATR pathway,[26] the clear preference for ATC-5' in C-strand terminal nucleotides is lost,[27] and telomere length maintenance are broken. POT1 is also connected with TIN2 through TPP1. POT1 mutants that lack the TPP1 interaction domain are largely excluded from nucleus and the knockdown of TPP1 diminishes the association with POT1 to telomere as well as the amount of nuclear POT1.[28] TIN2 occupies a central position in telosome/shelterin through the multi-interactions with TRF1, TRF2, and TPP1. Therefore, TIN2 provides a bridge between the components that bind to double-stranded and single-stranded telomeric regions (Fig. 2).

Through the association with non-telosome/shelterin proteins, telosome/shelterin contributes to regulation of the telomere length and resection of telomere ends during cell cycle, and protect the telomere from the DNA damage repair systems.[4,13] For example, TRF1 negatively regulates telomere length via its direct interaction with PINX1. TRF1 and TRF2 also recruit a variety of DNA transactions factors such as nucleases ERCC1/XPF[29] and Apollo;[30] DNA damage signaling ATM[4,31] and MRN complex;[32] helicases BLM and WRN.[33]

TELOMERE DYSFUNCTION, GENOME INSTABILITY AND TUMORIGENESIS

The progenitor and germ cells have high telomerase activity to maintain telomere length over the age. However, most somatic cells do not express sufficient telomerase and therefore undergo progressive telomeres shortening with age (Fig. 3).[34] This telomere attrition may lead to significantly short and uncapped telomeres, which finally induce genome instability, apoptosis, and senescence. The minimal number of telomere repeats is required for prevention of telomere fusions which is observed in *Trf2*-deficient cells.[35,36] In yeast telomerase-null model, telomeres become dysfunctional and the mutation rates are increased as a result of an induced frequency of terminal deletions,[37] suggesting the relationship between telomere dysfunction and genetic instability. In late generation of *Terc*-knockout mice, short telomeres cause instability of chromosomes by end-to-end fusions and up-regulate the expression of p53, a tumor-suppressor. However, age-related human diseases such as cancer and atherosclerosis are not observed in the telomerase-deficient mice, indicating that development of these diseases requires further gene alteration. Thus, various tumors develop in *Terc*-null mice with *p53*-deficiency.[38] The progressive telomere shortening in early-phase tumor, which possesses low activity of telomerase, finally triggers genomic instability and DNA damage response, impairs cell division, and increases apoptosis within the tumor (Fig. 3). With the additional acquirement of mutation (for examples, p53 deficiency), short or uncapped telomeres can give high chromo-

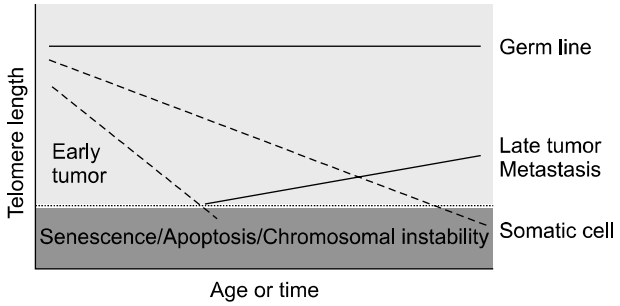

Fig. 3. Telomerase, telomere length and cancer. In contrast to germline cells, the most somatic cells show progressive telomere shortening over time due to low or absent telomerase activity. This progressive telomere loss eventually leads to critically short telomeres, which induces a DNA damage response that results in senescence and apoptosis. The cancer cells have shorter telomeres than normal cells owing to a longer proliferative history. In the absence of the appropriate checkpoints, short telomeres can contribute to the high genomic instability. Telomerase reactivation is occurred in more than 85% of all types of human tumor. Solid line, high telomerase activity; Dot line, low or absent telomerase activity.[3]

somal instability that is fundamental event in tumor. Further selection for tumor cells that have reactivated telomerase confers their indefinite growth and malignancy.

Reactivation of telomerase is frequently occurred in most tumors (85～90%), providing rational reasons for the development of therapeutic strategies based on telomere. The key advantage of targeting telomerase in comparison with most other cancer drugs is its relative university and specificity for cancer cells. Direct telomerase inhibition and active telomerase immunotherapy are under clinical trials.[39] The modified oligonucleotides, GRN163L, is telomere substrate antagonist that can binds to the complementary template sequence in TERC. GRN163L is taken into clinical trials after numerous preclinical studies including mouse xenograft models representing a range of human tumor types such as lung, breast, prostate, brain, and bladder cancers. However, this therapy might not work in ALT tumors that do not express telomerase and use recombination pathway to maintain the telomere length.

CONCLUSION

Telomeres play a crucial role for maintaining genomic integrity by distinguishing natural and damaged chromosome ends and providing a molecular mechanism for complete end replication. Telomere dysfunction by telomere shortening is a biological determinant in the pathobiology of human disease. It is considered that the cancer at the time of disease presentation has short telomeres, which in turn can actively contribute to loss of cell viability due to DNA damage response. In fact, both telomere dysfunction and DNA damage response are activated at the earliest stages in various human cancers, implicating that an intact DNA damage repair response triggered by telomere dysfunction in premalignant lesions can engage cellular senescence and apoptosis to suppress further progression of tumor. However, genome instability is increased in tumor cells that stochastically inactivate components of the DNA damage response pathway leading to tumor progression. Further selection of tumor cells that have reactivated telomerase would then promise indefinite growth by rescuing short telomere. Telomere shortening, therefore, can act good or bad in dependence of the developmental stages of tumor. Senescence or apoptosis by telomere shortening in the initiation step of tumorigenesis which has normal guardians to check genomic instability will suppress tumor progression. In contrast, genomic instability by telomere erosion can be used as a mechanism by which the premalignant cells acquire the high threshold of changes required for malignancy. Although anti-telomerase therapy is promising to cure human cancers, we have further consideration in several limitations for using this kind of drugs. For example, an intact p53 pathway is required to effectively inhibit cancer cell growth. The advanced progression in our understanding of the telomere biology might be essential for the rational development of new therapeutic strategies for cancer that are based on telomeres.

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REFERENCES

1) Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, Medrano EE, Linskens M, Rubelj I, Pereira-Smith O, Peacocke M, Campisi J. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proc Natl Acad Sci U S A* 92, 9363-9367, 1995.
2) Wright WE, Piatyszek MA, Rainey WE, Byrd W, Shay JW. Telomerase activity in human germline and embryonic tissues and cells. *Dev Genet* 18, 173-179, 1996.
3) Blasco MA. Telomeres and human disease: ageing, cancer and beyond. *Nat Rev Genet* 6, 611-622, 2005.
4) Palm W, de Lange T. How shelterin protects mammalian telomeres. *Annu Rev Genet* 42, 301-334, 2008.
5) Huffman KE, Levene SD, Tesmer VM, Shay JW, Wright WE. Telomere shortening is proportional to the size of the G-rich telomeric 3'-overhang. *J Biol Chem* 275, 19719-19722, 2000.
6) Shore D, Bianchi A. Telomere length regulation: coupling DNA end processing to feedback regulation of telomerase. *EMBO J* 28, 2309-2322, 2009.
7) Greider CW, Blackburn EH. The telomere terminal transferase of Tetrahymena is a ribonucleoprotein enzyme with two kinds of primer specificity. *Cell* 51, 887-898, 1987.
8) Nakamura TM, Cech TR. Reversing time: origin of telomerase. *Cell* 92, 587-590, 1998.
9) Calado RT, Young NS. Telomere maintenance and human bone marrow failure. *Blood* 111, 4446-4455, 2008.
10) Mitchell JR, Wood E, Collins K. A telomerase component is defective in the human disease dyskeratosis congenita. *Nature*

402, 551-555, 1999.
11) Vulliamy T, Marrone A, Goldman F, Dearlove A, Bessler M, Mason PJ, Dokal I. The RNA component of telomerase is mutated in autosomal dominant dyskeratosis congenita. *Nature* 413, 432-435, 2001.
12) Vulliamy T, Marrone A, Szydlo R, Walne A, Mason PJ, Dokal I. Disease anticipation is associated with progressive telomere shortening in families with dyskeratosis congenita due to mutations in TERC. *Nat Genet* 36, 447-449, 2004.
13) Xin H, Liu D, Songyang Z. The telosome/shelterin complex and its functions. *Genome Biol* 9, 232, 2008.
14) Bianchi A, Smith S, Chong L, Elias P, de Lange T. TRF1 is a dimer and bends telomeric DNA. *EMBO J* 16, 1785-1794, 1997.
15) Broccoli D, Smogorzewska A, Chong L, de Lange T. Human telomeres contain two distinct Myb-related proteins, TRF1 and TRF2. *Nat Genet* 17, 231-235, 1997.
16) Baumann P, Cech TR. Pot1, the putative telomere end-binding protein in fission yeast and humans. *Science* 292, 1171-1175, 2001.
17) Kim SH, Kaminker P, Campisi J. TIN2, a new regulator of telomere length in human cells. *Nat Genet* 23, 405-412, 1999.
18) Li B, Oestreich S, de Lange T. Identification of human Rap1: implications for telomere evolution. *Cell* 101, 471-483, 2000.
19) Liu D, Safari A, O'Connor MS, Chan DW, Laegeler A, Qin J, Songyang Z. PTOP interacts with POT1 and regulates its localization to telomeres. *Nat Cell Biol* 6, 673-680, 2004.
20) Ye JZ, Hockemeyer D, Krutchinsky AN, Loayza D, Hooper SM, Chait BT, de Lange T. POT1-interacting protein PIP1: a telomere length regulator that recruits POT1 to the TIN2/TRF1 complex. *Genes Dev* 18, 1649-1654, 2004.
21) Kim H, Lee OH, Xin H, Chen LY, Qin J, Chae HK, Lin SY, Safari A, Liu D, Songyang Z. TRF2 functions as a protein hub and regulates telomere maintenance by recognizing specific peptide motifs. *Nat Struct Mol Biol* 16, 372-379, 2009.
22) Chen Y, Yang Y, van Overbeek M, Donigian JR, Baciu P, de Lange T, Lei M. A shared docking motif in TRF1 and TRF2 used for differential recruitment of telomeric proteins. *Science* 319, 1092-1096, 2008.
23) Zhou XZ, Lu KP. The Pin2/TRF1-interacting protein PinX1 is a potent telomerase inhibitor. *Cell* 107, 347-359, 2001.
24) Smith S, de Lange T. Tankyrase promotes telomere elongation in human cells. *Curr Biol* 10, 1299-1302, 2000.
25) Karlseder J, Broccoli D, Dai Y, Hardy S, de Lange T. p53- and ATM-dependent apoptosis induced by telomeres lacking TRF2. *Science* 283, 1321-1325, 1999.
26) Denchi EL, de Lange T. Protection of telomeres through independent control of ATM and ATR by TRF2 and POT1. *Nature* 448, 1068-1071, 2007.
27) Hockemeyer D, Sfeir AJ, Shay JW, Wright WE, de Lange T. POT1 protects telomeres from a transient DNA damage response and determines how human chromosomes end. *EMBO J* 24, 2667-2678, 2005.
28) Chen LY, Liu D, Songyang Z. Telomere maintenance through spatial control of telomeric proteins. *Mol Cell Biol* 27, 5898-5909, 2007.
29) Zhu XD, Niedernhofer L, Kuster B, Mann M, Hoeijmakers JH, de Lange T. ERCC1/XPF removes the 3' overhang from uncapped telomeres and represses formation of telomeric DNA-containing double minute chromosomes. *Mol Cell* 12, 1489-1498, 2003.
30) Lenain C, Bauwens S, Amiard S, Brunori M, Giraud-Panis MJ, Gilson E. The Apollo 5' exonuclease functions together with TRF2 to protect telomeres from DNA repair. *Curr Biol* 16, 1303-1310, 2006.
31) Celli GB, de Lange T. DNA processing is not required for ATM-mediated telomere damage response after TRF2 deletion. *Nat Cell Biol* 7, 712-718, 2005.
32) Zhu XD, Kuster B, Mann M, Petrini JH, de Lange T. Cell-cycle-regulated association of RAD50/MRE11/NBS1 with TRF2 and human telomeres. *Nat Genet* 25, 347-352, 2000.
33) Opresko PL, von Kobbe C, Laine JP, Harrigan J, Hickson ID, Bohr VA. Telomere-binding protein TRF2 binds to and stimulates the Werner and Bloom syndrome helicases. *J Biol Chem* 277, 41110-41119, 2002.
34) Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. *Nature* 345, 458-460, 1990.
35) Farr C, Fantes J, Goodfellow P, Cooke H. Functional reintroduction of human telomeres into mammalian cells. *Proc Natl Acad Sci U S A* 88, 7006-7010, 1991.
36) Hanish JP, Yanowitz JL, de Lange T. Stringent sequence requirements for the formation of human telomeres. *Proc Natl Acad Sci U S A* 91, 8861-8865, 1994.
37) Hackett JA, Feldser DM, Greider CW. Telomere dysfunction increases mutation rate and genomic instability. *Cell* 106, 275-286, 2001.
38) Artandi SE, Chang S, Lee SL, Alson S, Gottlieb GJ, Chin L, DePinho RA. Telomere dysfunction promotes non-reciprocal translocations and epithelial cancers in mice. *Nature* 406, 641-645, 2000.
39) Harley CB. Telomerase and cancer therapeutics. *Nat Rev Cancer* 8, 167-179, 2008.
40) Calado RT, Young NS. Telomere diseases. *N Engl J Med* 361, 2353-2365, 2009.