Available online at www.worldscientificnews.com

World Scientific News

WSN 42 (2016) 87-100 EISSN 2392-2192

Activities of human telomerase in cancer development, detection and therapeutics - A Review

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ABSTRACT

Telomeres are the specialized nucleoprotein structures associated with eukaryotic chromosomal ends, which are essential for maintaining the stability of the linear eukaryotic chromosomes. Progressive telomere shortening is an inevitable occurrence in normal somatic cells due to the end-replication problem leading to limited replication efficiency. The hallmark characteristics of human cancer cells include infinite reproductive potential, uncontrolled proliferation and immortality. These abilities of transformed cancerous cells are mainly due to the maintenance of their telomeres since degradation of chromosomal telomeric ends leads to cellular senescence or death. Thus telomere biology is important in the study of human cancer development. The mechanism by virtue the cancer cells are able to divide indefinitely is by maintaining telomeres. Activity of telomerase, a telomere-elongating ribonucleoprotein reverse transcriptase enzyme, is responsible for inducing the property of immortality to cancer cells. In humans nearly about 80% to 90% cancer cells activate telomerase and elongate their telomeres to overcome the end-replication problem. Telomere shortening suppresses cancer formation in contrast according to certain reports it sometimes promotes genomic instability which leads to enhancement of carcinogenesis and consequently the development of malignancy. Majority of cancer cells activate telomerase, but it remains mysterious as to find the reasons of the fact that certain cancer cells often show shorter telomeres in comparison to the cells in the surrounding normal tissues. This controversial role of telomerase associated with certain transformed cells leading to the cancerous state in relation to its role in normal cells is an interesting field to study which points

out to the fact of development of cancer cells targeting drugs based on telomerase activities as an alternative weapon in combating against this dreaded human disease. The present review focuses on the activity of telomerase in telomere maintenance in the development of cancerous cells in humans, the use of telomerase as an assay technique for cancer detection as well as the anti-cancer therapeutic approach of targeting the telomerase in the current era of treatment of human cancer.

***Keywords*:** Chromosomes; End-replication; Telomere; Telomerase; Cancer; Therapeutic

1. INTRODUCTION

The eukaryotic chromosomal ends are characterised by the presence of specialized structures, the telomeres. The telomeric end of the chromosomes shortens with each round of replication during the synthetic phase of the cell cycle because of the end replication problem which plays critical role in maintaining the cellular integrity and hence the genomic stability. Eventually, critically shortened telomeres in normal somatic cells fail to protect the chromosome ends against the DNA damage response, resulting in cellular senescence or apoptosis. In contrast most cancer cells maintain the telomeres evading senescence or apoptosis by activating telomerase, a telomere-elongating reverse transcriptase enzyme. The telomere position effect influences chromatin status and gene expression which suggests, differences in telomere length may directly affect the behaviour of cancer cells. In normal human cells, telomere shortens with successive cell division (Cooke *et al*., 1986; Harley *et al*., 1990) probably due to loss of terminal sequence during DNA replication (end replication problem). In tumorous growth cells, the telomeric shortening is halted through the activation of telomerase (Counter *et al*., 1992: Counter *et al*., 1994; Kim *et* al., 1994). Thus telomere length is stable in several immortalized cell line (Counter *et al*., 1992), suggesting that a regulatory mechanism exists for limiting telomere elongation by telomerase. This particular behaviour of certain transformed cancer cells is an interesting topic of study. In this report the telomere maintenance in certain cancer cells and the telomerase activities in promoting carcinogenesis are briefly discussed as well as the use of telomerase in cancer detection and the therapeutic approach of targeting the telomerase in the control of cancerous cell growth in human cancers.

2. THE CHROMOSOMAL END-REPLICATION PROBLEM

The $3^{\prime}$ ends of linear chromosome in eukaryotic cells are not replicated by DNA polymerases since it can replicate only in a $5^{\prime}$ to $3^{\prime}$ direction by extending existing polynucleotide chains. The mechanism of DNA replication follows semi-discontinuous mode where the leading and the lagging DNA strands are synthesized simultaneously. The leading strand is replicated continuously. The lagging strand DNA polymerization starts from several RNA primers, which are elongated to create DNA fragments, termed Okazaki fragments and thus this strand of DNA is synthesized discontinuously. These RNA primers are finally degraded and replaced by DNA sequences and both the reactions are catalysed by DNA polymerase having $5^{\prime}$- $3^{\prime}$ exonuclease as well as polymerase activity.

Removal of the terminal RNA primer on the lagging strand by exonuclease activity leaves a gap that ordinarily is filled in by extension of the next Okazaki fragment. The absence of any template strand for the "last" Okazaki fragment beyond the 5´ end of the chromosome the progeny strand for that part cannot be synthesized to its very end. This particular molecular event is the end replication problem that predicts the progressive reduction of chromosomal DNA from the 3´ ends after each round of cell cycle following replication.

From previous reports according to Olovnikov, 1973 (Olovnikov, 1973) and Watson, 1972 (Watson, 1972) who pioneered the implications of this end replication problem, lacking a means to replicate chromosome ends chromosome would shorten with each cell division, ultimately reaching a certain point of chromosomal shortening which leads to cell senescence/death. Earlier Hayflick, 1961 had previously pointed out that most cultured cells could survive only a limited number of cell divisions and suggested that finite cellular lifespans might explain why physiologic function breaks down as an organism ages (Hayflick *et* al., 1961).

3. TELOMERES AND TELOMERASE

The 3´ ends of eukaryotic chromosomes bear special structure the telomeres, which are provide protection from enzymatic end-degradation as well as maintaining chromosomal stability within the nucleus of normal cells. Chromosomes with shortened telomeric tips fuse with other such chromosomal ends or become lost during cell division resulting in the loss of the chromomsome itself. Moreover the telomeres also play major role in nuclear organization of the cellular nucleus by serving as attachment points to the nuclear matrix (de Lange, 1992). The biochemical constituents of telomeres include a DNA component and multiple protein components (Greider, 1996).

The sequence analysis of telomeric DNA showed noncoding tandemly repeated sequences and the exact repeat sequence varies from one species to species. Similar analyses of the chromosomes of humans and other vertebrates hexanucleotide TTAGGG in 5´ to 3´ direction repeat unit is reported. Further genomic studies showed that such repeats are also found in other internal regions of the chromosomes (Katinka and Bourgain, 1992). The humans telomeric ends are 8–14 kilo basepairs (kbp) long, whereas the mean telomeric repeat lengths in some lower forms like ciliates the size is much smaller ranging about 36 bp whereas in mice the range may be as much as 150 kbp (Kalluri, 1996). Thus the telomeric ends vary a lot within different groups of animals.

The eukaryotic telomere which plays a major role in genomic stability is maintained by telomerase which is by nature a reverse transcriptase by virtue of its action of copying the short RNA template sequence within the telomerase RNA into DNA; an enzyme that copies RNA into DNA is by definition a reverse transcriptase. The protein part of human telomerase, telomerase reverse transcriptase (hTERT), is indeed a protein enzyme and its amino acid sequence includes reverse transcriptas.e motifs. However, the RNA is also critically important to the enzyme action, and not only because it provides the template. The template is only a minor part of the entire telomerase RNA molecule. The telomerase RNA is built into the structure of the core ribonucleoprotein complex of telomerase, which contains the protein hTERT and the telomerase RNA component (Feng *et al*., 1995).

4. PROTEINS THAT INTERACTED WITH TELOMERE AND TELOMERASE

4. 1. Telomere associated proteins

Telomere length in human cell lines can be maintained in two ways: either by telomerase-mediated elongation (Morin, 1989) or in a telomerase-independent pathway that may involve recombination (known as alternative lengthening of telomeres or ALT). According to a study it is reported that several immortal cell lines maintain a stable telomeric length and suggested that regulatory mechanism exists for limiting telomere elongation by telomerase (Counter *et al*., 1992).

The identification of two of the major telomeric DNA-binding proteins – telomeric repeat binding factor 1 and 2 (TRF1 & TRF2), the credit goes to the pioneering work by the de Lange laboratory (Chong *et al*., 1995; Broccoli *et al*., 1997; van Steensel *et al*., 1998; van Steensel and de Lange, 1997; Smogorzewska *et al*., 2000; Bianchi *et al*., 1999). TRF 1 and TRF 2 are associated with telomeric repeats throughout the cell cycle and are responsible for the length regulation of human telomere either directly or by their interaction with other regulatory factors. Both of these factors are expressed in all human cell types as reported by various authors.

The factor 1 identified TRF1, which is involved in this regulatory process concerned with telomere length maintenance and it is observed that in telomerase –positive tumor cell line like HT1080 over expression of TRF1 resulted in a gradual and progressive telomere shortening suggesting that TRF has a role in telomere length stability in transformed human cell lines. It is also reported by other group of scientists that TRF1 functions as a suppressor of telomere elongation and also involved in the negative feedback mechanism for stabilizing telomere length. (Shay, 1999; Greider, 1996; Smith *et al*., 1998; Smith and de Lange, 2000; Bilaud *et al*., 1997; Smith and de Lange, 1999; Kim *et al*., 1999; Li *et al*., 2000; Zhu *et al*., 2000; Bianchi and de Lange, 1999; Hsu *et al*., 2000).

The 3´ terminus of the telomeric end of the chromosome has single stranded overhang which varies in length according to the cell types under investigation. Recently electron microscopic analysis of telomere has revealed that the 3´ end forms a higher order structure called the t-loop (Griffith *et al*., 1999).

4. 2. Telomerase associated proteins

The catalytic proteins component of human telomerase RNP consists of a (hTERT, human telomerase reverse transcriptase) and the ribonucleic acid part is a 451 bp integral RNA (hTR, Human Telomerase RNA).

Both these two components are essential for normal telomerase activities (Bodnar *et al*., 1998; Weinrich *et al*., 1997). Sequence analysis revealed that the 3´ half of the hTR resembles the box H/ACA family of small nucleolar RNAs (snoRNA) (Mitchell *et al*., 1999; Chen *et al*., 2000). Similarly the 5´ end of the hTR contains the template used for addition of telomeric sequences to the end of the chromosomes (Feng *et al*., 1995; Greider and Blackburn, 1987) as well as a pseudoknot that is likely to be important for telomerase function (Gilley and Blackburn, 1999).

Another study reported that the 5´ end of the hTR also contains a 6bp U-rich tract required for the direct interaction with hnRNPs C1 and C2 (Ford *et al*., 2000). More recently, it was reperted that a La autoantigen which is concerned with the assembly of other RNA

particles (Ford *et al*., 2001; Pannone *et al*., 1998; Kufel *et al*., 2000) and the maturation of the tRNA molecules has been shown directly to interact with the human telomerase RNP; Ford *et* al., concluded that La's expression levels influence telomere length in a telomerase RNA-dependent fashion along with its normal function (Ford *et al*., 2001).

5. TELOMERASE ACTIVITIES IN CANCER DEVELOPMENT

According to the theory of carcinogenesis uncontrolled cell proliferation is the hallmark for development of malignancy, which leads to the immortality of cancerous cells. This particular property of malignant cell lines is linked with the activity of a specialized part of the chromosome the telomere. The controlled cell growth of the normal cells is associated with progressive shortening of the telomeric end of the chromosomes which ultimately results in cell apoptosis. From various studies it is reported that the telomerase activity is associated with maintaining a balance between the telomere shortening after each round of cell cycle and the telomere elongation in malignant cell lines.

This fact can be correlated with the findings such as in one study it was detected that in certain tumor cell type shorter telomeres exist than the cells of normal original tissue (Bacchetti and Counter, 1995). Moreover different studies conducted on telomerase acticvity in different cancer cell types as for example in neuroblastoma, endometrial cancer, breast cancer, leukemias, and lung cancer, the severity of the cancerous state can be correlated with decreasing telomere lengths. (Hiyama *et al*., 1992; Smith and Yeh, 1992; Odagiri *et al*., 1994; Ohyashiki *et al*., 1994; Hiyama *et al*., 1995 (1)) The karyotypic It has already been reported that telomerase reactivation is dependent upon the degree of telomere shortening in normal cells since shortening of telomeres beyond a certain extent leads to karyotypic instability. Although low amounts of telomerase activity are reported in normal human tissues including hematopoietic progenitor cells and activated T- and B- lymphocytes (Hiyama *et al*., 1995 (2)), in germ cells, ovaries, and testes (Wright *et al*., 1996) and in physiologically regenerating epithelial cells (Yasumoto *et al*., 1996).

The activation of telomerase in certain human cancer development can be advocated in the acquisition of malignancy in benign and premalignant tumors, including breast fibrocystic disease and fibroadenomas, benign prostatic hyperplasia, colorectal adenomas, anaplastic astrocytomas, and benign meningiomas and leiomyomas, in general no telomerase activity was detected; however, it was found in malignant tumor stages (Hiyama *et al*., 1996; Sommerfeld *et al*., 1996; Langford *et al.,* 1995; Chadeneau *et al*., 1995).

The activity of telomerase has diagnostic importance in the early detection of progression towards malignancy of certain benign stages of tumors. This approach of telomerase based assay in malignancy detection has been applied in cases of benign prostrate hyperplasia, benign giant tumors of the bone as well as in tissues that may proceed towards malignancy (Sommerfeld *et al*., 1996; Langford *et al.,* 1995; Chadeneau *et al*., 1995; Schwartz *et al*., 1995). This fact has been elaborated in another finding where neuroblastoma cells the telomerase activity increased in the later stages of cancer than the early stages (Hiyama *et al*., 1995 (3)). Another contradictory finding stated that the telomerase activity became weak at a special stage (Stage IV) of neuroblastoma.

The reason stated for this behaviour favourable for patients affected with that type of cancer was based on the assumption that the enzyme activity too weak to sustain in an

immortal cell environment (Hiyama *et al*., 1995 (4)). Moreover evidences from gastric cancer cells it was reported that the telomerase activity has useful diagnostic value since it serve as an indicator in the cancer development. The gastric cancer patients showing detectable telomerase activity in their tumors have a shorter survival rate than those devoid of any telomerase function in their tumors (Hiyama *et al*., 1995 (4)).

6. TELOMERASE ASSAY IN CANCER DETECTION

According to a report the telomerase activity in a normal and malignant cell types can be detected using molecular biological methods including Telomerase Repeat Amplification Protocol (TRAP) assay, which utilizes the Polymerase chain reaction (PCR) technology (Kim *et al*., 1994). TRAP assay detected telomerase activity in almost all tumor samples tested but in contrast to the normal tissues. The germ cells in the ovary and testis showed telomerase activity (Kim *et al*., 1994). In another study conducted using the TRAP assay reported according to the previous study and concluded that around 85% of solid tumor samples have detectable telomerase activity (Shay and Bacchetti, 1997).

In a different experimental study certain modification of TRAP assay has been utilized including, TRAP-ELISA assay and using another technique non-radioisotopic silver staining telomeric repeat amplification protocol (Wei *et al.*, 1997; Wei *et al*., 1998) telomerase activity was reported in a large number of cells and also in liver carcinomas (Mu and Wei, 2002).

According to Langford *et al*., (Langford *et al.*, 1995) the telomerase activity in certain cell lines are variable as observed in glioblastoma and retinoblastoma where 50% activity was noticed in comparison to 90% in usual cancerous cases. In meningiomas and astrocytomas telomerase activity was not even detectable (Langford *et al.*, 1995).

Research on telomerase based diagnosis of stomach cancer it was reported that the telomerase activity in neoplastic cells is dependent upon the human telomerase reverse transcriptase mRNA (hTERT). The upregulation of hTERT mRNA is responsible for telomerase activity in human tumor cell lines which acts as a genomic marker in the detection of cancerous state. Moreover it was also reported that independent of telomerase activity the expressions of human telomerase RNA (hTR) and telomerase-associated proteins were noticed in telomerase-negative cells and tissues. Scientists used modern technique of enzyme assay such as immunohistochemical method in the detection of hTERT in gastric carcinomas. The result of their experiments detected telomerase activity in neoplastic gastric carcinomas whereas in non-neoplastic cell lines no telomerase activity was detectable (Wataru *et al*., 1998).

The study based on immunohistochemical assay for the detection of hTERT in cancer cell lines will serve as a novel approach for cancer diagnosis (Wataru *et al*., 1998). A modified application based on immunohistchemical was conducted by another group of scientists using anti-hTERT antibody-reactive protein (Poreboma *et al*., 2000).

The potentiality of telomerase assays in cancer diagnosis remains an area is a novel approach and still further investigations are of utmost need in the present era. The assay based on the detection of hTERT or other telomerase-associated will complement the approach for the determination of telomerase activity in neoplastic cell lines which may help in the quick and early detection of malignancy, the hallmark of oncogenesis.

7. TARGETING TELOMERASE AS ANTI-CANCER THERAPEUTIC APPROACH

In contrast to telomerase activities for telomere maintenance in immortal cell lines there are alternative pathways in isolated cancer cell lines where the telomere length is maintained based on recombination events. This view was advocated by Bryan *et al*., (Byran *et al*., 1995). Recent research on anti-telomerase drugs revealed the fact that inhibition of telomerase might lead to the erosion of the telomeres in cancerous cell lines potentiating the belief that drugs targeting telomerase has the potentiality to reduce cancer cell growth (Shay and Keith, 2008; Harley, 2008).

Telomeres consist of tandem arrays of a short DNA sequence, TTAGGG in vertebrates and telomerase repeatedly adds the sequence repeats to chromosome ends as a solution for the end replication problem of eukaryotic cells (Lodish *et al*., 2013). It was reported that the G-rich sequence is capable for the synthesis of G –quadruplex which has to be unfolded before telomerase can function (Parkinson *et al*., 2002). These findings led to the concept that molecules promoting the formation of quadruplex may function as telomerase inhibitors in cancer cells leading to erosion of the telomeric ends. Previously many quadruplex-stabilizing effects have been identified with antiproliferative properties (Sun *et al*., 1997; Riou *et al*., 2002). One of the drawbacks of targeting the G –quadruplex resides in the fact that those quadruplex interferes with other G-rich sequences in the genome leading to unexpected effects (Siddiqui –Jain *et al*., 2002).

For the identification of genetic alternations related with tumors can be exploited for new therapeutic approaches and thus modern research relies on RNAi technologies to identify genes that when inactivated induces death of tumor cells but not normal cells (Lodish *et al*., 2013). Antisense oligonucleotides are sequenced and cloned to identify the RNA of human telomerase (Feng *et al*., 1995). Demidov *et al*., (Demidov *et al*., 1994) identified an interesting type of antisense agent, peptide nucleic acid (PNA) which is a DNA mimic having the entire deoxyribose phosphodiester backbone replaced by N-(2- aminoethyl) glycine units. The agent PNA is highly resistant to degradation by protease and nuclease and capable of forming double helical complexes with single-stranded nucleic acids either DNA or RNA. Another group of scientists working in this field studied the effect of 19-mer antisense oligonucleotide linked to 2,5-oligoadenylate against telomerase isolated from human malignant glioma cell lines which resulted in the activation of endoribonuclease RNAase L. The authenticity of the conjugate was verified by researchers when *in vitro* tumor cell growth was reduced which was further correlated when *in vivo* analysis using nude mouse xenograft gave similar result as well as *in vitro* prostate cancer cell lines (Kondo *et al*., 1998).

In another experimental study using the ribozyme technology which is a modification and extension of the antisense technology telomerase activity was inhibited in the endometrial cancer cell lines AN3CA (Yokoyan *et al*., 1998). They designed a hammer-head ribozyme that can bind to its target RNA sequence and induce a specific chemical cleavage at the 44-46 GUC residue of the enzyme telomerase. This is a novel technology and in near future it will be a handy technology for cancer detection based on telomerase.

Vonderheide *et al*., 1999, suggested that partial peptides derived from the telomerase reverse transcriptase hTERT were capable to elicit a cytotoxic T-lymphocyte response which could lyse hTERT positive tumor cell lines (Vonderheide *et al*., 1999).

Researches based on the transfection experiments using the tumor suppressor genes *p53* and *RB* and utilizing recombinant adenovirus as vectors showed recognisable cancerous cell

growth arrests as well as apoptosis (Kanaya *et al*., 200). They proposed the possibility of affecting the promoter of hTERT by the incorporation of the *p53* at the binding site for the transcription factor SP 1 (Kanaya *et al*., 2000). Moreover the limitations of the telomerase targeting in cancerous cell telomere shortening leading to cell death depends on the fact that the process requires quite a number of generations and it is reported that it often exceeds the number of generations required for the cell to reach malignancy and lethality. This drawback can be counteracted by the use of telomerase as a tumor antigen (Kanaya *et al*., 2000).

Although the telomerase based cancer therapeutic approaches are being extensively used in the recent research activities the previous investigations had pointed out the limitations of this technique. Bryan *et al*., in 1995 pointed out that approximately 10% – 15% of certain cancerous tumor cells are independent of telomerase activation for maintaining their telomere length (Byran *et al*., 1995). Thus for these tumors, the inhibitor of the telomerase activity may be unuseful. Moreover it was also reported that the inhibitors worked best in cultured tumor cells when telomeres were shortest, but little is known about the length of telomeres in primary human tumors (Bryan *et al*., 1995). Another interesting fact about telomerase inhibitors was pointed out by Morin in 1995 that an effective inhibitor of telomerase might impair the function of normal cells, which have low levels of telomerase activity (Morin, 1995). However further researches in this field is of utmost need in considering the therapeutic potential of telomerase inhibitors which can serve as a useful tool for cancer therapy.

8. DISCUSSION

Recent work suggests that telomerase play a key role that go beyond telomere maintenances (Cong and Shay, 2008; Maida *et al*., 2009). Similarly the discovery of noncoding transcription at telomeres (Luke and Lingner, 2009; Schoeftner and Blasco, 2009) suggests that they may be even more complex than had been originally imagined. Telomerase activities are highly diversified and are very much necessary for genomic stability of normal cells. The activies of telomerase in cancerous cells are varied and in some cancer types the immortatlity of cells are due to continued expression of telomerase in contrast to normal cells. In certain cancer cell types the shortened telomeric ends are maintained and the mechanism by which the telomerase action is controlled is still to find out in detail molecular analysis.

9. CONCLUSION

The positive aspect of telomerase based therapeutic approach in cancer treatment over the conventional chemotherapy relies on the consideration that it is much less toxic to the normal cells of the patients and consequently undesirable side effects are less pronounced. The human haematopoietic stem cells, germ cells and activated T –and B -lymphocytes are telomerase positive and incorporation of telomerase targeting inhibitors block telomere synthesis in patients undergoing the therapy. Another problem is in the variability of telomere lengths among tumors. Telomerase inhibition seems to be useful only in malignant cells with short telomeres; tumor cells with long telomeres would require a prolonged treatment, with possible toxic side-effects. Moreover alternative pathways besides telomerase may exist in

some cancer cell lines for regulating/maitaining telomere length. Further research in this field is of urgent need in the present decade to get an insight into the cellular basis of telomerase action in human cancer development and to develop novel molecular methods in early detection of cancer cells using telomerase based assay along with the use of anti-telomerase treatment procedures as an alternative method to fight against this life threatening disease of humans.

References

[1] Bacchetti S, Counter C (1995). Telomeres and telomerase in human cancer [Review], *Int J Oncol*, 7: 423-432.

[2] Bianchi A, de Lange T (1999). Ku binds telomeric DNA in vitro, *J Biol Chem*, 274: 21223-21227.

[3] Bianchi A, Stansel RM, Fairall L, Griffith JD, Rhodes D, de Lange T (1999). TRF1 binds a bipartite telomeric site with extreme spatial flexibility, *EMBO J*, 18: 5735-5744.

[4] Bilaud T, Brun C, Ancelin K, Koering CE, Laroche T, Gilson E (1997). Telomeric localization of TRF2, a novel human telobox protein. *Nature Genet*, 17: 236-239.

[5] Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, Harley CB, Shay JW, Lichsteiner S, Wright WE (1998). Extension of life-span by introduction of telomerase into normal human cells, *Science*, 279: 349-352.

[6] Broccoli D, Smogorzewska A, Chong L, de Lange T (1997). Human telomeres contain two distinct Myb-related proteins, TRF1 and TRF2, *Nature Genet*, 17: 231-235.

[7] Byran TM, Englezou A, Gupta J, Bachetti S, Reddel RR (1995). Telomere elongation in immortal human cell without detectable telomerase activity. *EMBO J*, 14: 4240-4248.

[8] Chadeneau C, Hay K, Hirte H, Gallinger S, Bacchetti S (1995). Telomerase activity associated with acquisition of malignancy in human colorectal cancer. *Cancer Res*, 55: 2533-2536.

[9] Chen JL, Blasco MA, Greider CW (2000). Secondary structure of vertebrate telomerase RNA. *Cell*, 100: 503-514.

[10] Chong L, van Steensel B, Broccoli D, Erdjument – Bromage H, Hanish J, Tempst P, de Lange T (1995). A human telomeric protein. *Science*, 270: 1663-1667.

[11] Cooke HJ, Smith BA (1986). Variability at the telomeres of the human X/Y pseudoautosomal region. *Cold Spring Harbor Symp Quant Biol*, L1: 213-219.

[12] Cong Y, Shay JW (2008). Actions of human telomerase beyond telomeres. *Cell Re*., 18: 725-732.

[13] Counter CM, A vilion AA, LeFeuvre CE, *et al*., (1992). Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. *EMBO J*, 11: 1921-1929.

[14] Counter CM, Hirte HW, Bacchetti S, Harley C. (1994). Telomerase in human ovarian carcinoma. *Proc Natl Acad Sci USA*, 91: 2900-2904.

[15] de Lange T (1992). Human telomeres are attached to the nuclear matrix. *EMBO J*, 11: 717–24.

[16] Demidov VV, Potaman VN, Frank-Kamenetskii MD, Egholm M, *et al*., (1994). Stability of peptide nucleic acids in human serum and cellular extracts. *Biochem Pharmaco*, 48: 1310-1313.

[17] Feng J, Funk WD, Wang SS, Weinrich SL, Avilion AA, Chiu CP, Adams RR, Chang E, *et al*., (1995). The RNA component of human telomerase. *Science*, 269: 1236-1241.

[18] Ford LP, Myoung J, Wright WE, Shay JW (2000). Heterogeneous nuclear ribonucleoproteins C1 and C2 associate with human telomerase. *Mol Cell Biol*, 20: 9084-9091.

[19] Ford LP, Shay JW, Wright WE (2001). The La antigen associates with human telomerase ribonucleoprotein and influences telomere length *in vivo*. RNA. In press.

[20] Gilley D, Blackburn EH (1999). The telomerase RNA pseudoknot is critical for the stability for the stable assembly of a catalytically active ribonucleoprotein. *Proc Natl Acad Sci USA*, 96: 6621-6625.

[21] Greider CW, Blackburn EH (1987). The telomere terminal transferase of *Tetrahymena* is a ribonucleoprotein enzyme with two kinds of primer specificity. *Cell*, 51: 87-898.

[22] Greider CW (1996). Telomere length regulation. *Annu Rev Biochem*, 65: 337-365.

[23] Griffith JD, Comeau L, Rosenfield S, Stansel RM, Bianchi A, Moss H, de Lange T (1999). Mammalian telomeres end in a large duplex loop. *Cell*, 97: 503-514.

[24] Harley CB (2008). Telomerase and cancer therapeutics. *Nat Rev Cancer*, 8: 167-179.

[25] Harley CB, Futcher AB, Greider CW (1990). Telomeres shorten during aging of human fibroblasts. *Nature*, 345: 458-460.

[26] Hayflick L, Moorhead LS (1961). The serial culture of diploid and transformed human fibroblasts. *Exp Cell Res*, 25: 585-621.

[27] Hiyama E, Hiyama K, Yokoyama T, Ichikawa T, Matsuura Y (1992). Length of telomeric repeats in neuroblastoma; correlation with prognosis and other biological characteristics. *Jpn J Cancer Res*, 83: 159-164.

[28] Hiyama K, Hiyama E, Ishioka S, Yamakido M, Inai K, Gazdar A, *et al*., (1995). Telomerase activity in small-cell and non-small-cell lung cancers. *J Natl Cancer Inst*, 87: 895-902.

[29] Hiyama K, Hirai Y, Kyoizumi S, Akiyama M, Hiyama E, Piatyszek M, *et al*., (1995). Activation of telomerase in human lymphocytes and hematopoietic progenitor cells. *J Immunol*, 155: 3711-3715.

[30] Hiyama E, Hiyama K, Yokoyama T, Matsuura Y, Piatyszek M, Shay J (1995). Correlating telomerase activity levels with human neuroblastoma outcomes. *Nature Med*, 1: 249-255.

[31] Hiyama E, Yokoyama T, Tatsumoto N, Hiyama K, Imamura Y, Murakami Y, *et al*., (1995). Telomerase activity in gastric cancer. *Cancer Res*, 55: 3258-3262.

[32] Hsu HL, Gilley D, Galande SA, Hande MP, Allen B, Kim SH, Li GC, Campisi J, Kohwi-Shigematsu T, Chen DJ (2000). Ku acts in a unique way at the mammalian telomere to prevent end joining. *Genes Dev*, 14: 2807-2812.

[33] Hiyama E, Gollahon L, Kataoka T, Kuroi K, Yokoyama T, Gazdar AF, *et al*., (1996). Telomerase activity in human breast tumors. *J Natl Cancer Inst*, 17: 116-122.

[34] Kalluri R (1996). Telomere (telomerase) hypothesis of aging and immortalization. *Indian J Biochem Biophys*, 33: 88-92.

[35] Kanaya T, Kyo S, Hamada K, *et al*., (2000). Adenoviral expression of p53 represses telomerase activity througy down- regulation of human telomerase reverse transcriptase transcription. *Clin Cancer Res*, 6: 1239-1247.

[36] Katinka M, Bourgain F (1992). Interstitial telomeres are hotspots for illegitimate recombination with DNA molecules injected into the macronucleus of *Paramoecium primaurelia*. *EMBO J*, 11: 725-732.

[37] Kim SH, Kaminker P, Campisi J (1999). TIN2, a new regulator of telomere length in human cells. *Nature Genet*, 23: 405-412.

[38] Kim NW, Piatyszek MA, Prowse KR, *et al*., (1994). Specific association of human telomerase activity with immortal cells and cancer. *Science*, 266: 2011-2015.

[39] Kondo S, Kondo S, Tanaka Y, *et al*., (1998). Targeted therapy of human malignant glioma in a mouse model by 2, 5-A antisense directed against telomerase RNA. *Oncogene*, 16: 3323-3330.

[40] Kufel J, Allmang C, Chanfreau G, Petfalski E, Lafontaine DL, Tollervey D (2000). Precursors to the U3 small nucleolar RNA lack small nucleolar RNP proteins but are stabilized by La binding. *Mol Cell Biol*, 20: 5415-5424.

[41] Langford L, Piatyszek M, Xu R, Schold S, Shay J (1995). Telomerase activity in human brain tumours. *Lancet*, 346: 1267-1268.

[42] Li BB, Oestreich S, de Lange T (2000). Identification of human Rap1: Implications for telomere evolution. *Cell*, 101: 471-483.

[43] Lodish H, Kaiser CA, Bretscher A, Amon A, Berk A, Krieger M, Ploegh H, Scott MP (2013). Molecular cell biology. W. H. Freeman and Company New York, Macmillan. 7th Edition.

[44] Luke B, Lingner J (2009). TERRA: telomeric repeat containing RNA. *EMBO J*, 28: 2503-2520.

[45] Maida Y, Yasukawa M, Furuuchi M, Lassmann T, Possemato R, Okamoto N, Kasim V, Hayashizaki Y, Hahn WC, Mashutomi K (2009). An RNA-dependent RNA polymerase formed by TERT and the RMRP RNA. *Nature*, 461: 230-235.

[46] Mitchell JR, Cheng J, Collins K (1999). A box H/ACA small nucleolar RNA-like domain at the human telomerase RNA 3′ end. *Mol Cell Biol*, 19: 567-576.

[47] Morin GB (1989). The human telomere terminal transferase enzyme is a ribonucleoprotein that synthesizes TTAGGG repeats. *Cell*, 59: 521-529.

[48] Morin GB (1995). Is telomerase a universal cancer target? *J Natl Cancer Inst*, 859-860.

[49] Mu J, Wei LX (2002). Telomere and telomerase in oncology. *Cell Res*, 12: 1-7.

[50] Odagiri E, Kanda N, Jibiki K, Demura R, Aikawa E, Demura H (1994). Reduction of telomeric length and c- erbB-2 gene amplification in human breast cancer, fibroadenoma and gynecomastia. *Cancer*, 73: 2978-2984.

[51] Ohyashiki J, Ohyashiki K, Fujimura T, Kawakubo K, Shimamoto T, Iwabuchi A, Toyama K (1994). Telomere shortening associated with disease evolution patterns in myelodysplastic syndromes. *Cancer Res*, 54: 3557-3560.

[52] Olovnikov AM (1973). A theory of marginotomy : the incomplete copying of template margin in enzymatic synthesis of polynucleotides and biological significance of the phenomenon. *J Theor Biol*, 41: 181-190.

[53] Pannone BK, Xue D, Wolin SL (1998). A role for the yeast La protein in U6 snRNP assembly: evidence that the La protein is molecular chaperone for RNA polymerase III transcripts. *EMBO J*, 17: 7442-7453.

[54] Parkinson GN, Lee MPH, Neidle S (2002). Crystal structure of parallel quadruplexes from human telomeric DNA. *Nature*, 417: 876-880.

[55] Poreboma C, Scheel C, Hero B, *et al*., (2000). Telomerase activity and telomerase subunit gene expression patterns in neuroblastoma:a molecular and immunohistochemical study establishing prognostic tools for fresh-frozen and paraffin-embedded tissues. *J of Clin Oncol*, 18(13): 2582-2592.

[56] Riou JF, Guitat L, Mailliet P, Laoui A, Renou E, Petitegenet O, Megnin-Chanet F, Helene C, Mergny JL (2002). Cell senescence and telomere shortening induced by a new series of specific G-quadruplex DNA ligands. *Proc Natl Acad Sci USA*, 99: 2672-2677.

[57] Schoeftner S, Blasco MA (2009). A higher order of telomere regulation : telomeric heterochromatin and telomeric RNAs . *EMBO J*, 28: 2323-2336.

[58] Schwartz H, Juliao S, Sciadini M, Miller L, Butler M (1995). Telomerase activity and oncogenesis in giant cell tumors of bone. *Cancer*, 75: 1094-1099.

[59] Shay JW (1999). At end of the millennium a view of the end. *Nature Genet*, 23: 382-383.

[60] Shay JW, Bacchetti S. A survey of telomerase activity in human cancer. *Eur J Cancer* 1997; 33: 787-91.

[61] Shay JW, Keith WM (2008). Targeting telomerase for cancer therapeutics. *Br J Cancer*, 98: 677-683.

[62] Siddiqui–Jain A, Grand CL, Bearss DJ, Hurley LH (2002). Direct evidence for G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription. *Proc Natl Acad Sci USA*, 99: 11593-11598.

[63] Smith S, de Lange T (2000). Tankyrase promotes telomere elongation in human cells. *Curr Biol*, 10, 1299-1302.

[64] Smith S, de Lange T (1999) Cell cycle dependent localization of the telomeric PARP, tankyrase to nuclear complexes and centrosomes. *J. Cell Sci.* 112: 3649-3656.

[65] Smith S, Giriat I, Schmitt A, de Lange T (1998). Tankyrase, a poly(ADP-ribose) polymerase at human telomeres. *Science*, 282: 1484-1487.

[66] Smith J, Yeh G (1992). Telomere reduction in endometrial adenocarcinoma. *Am J Obstet Gynecol*, 167: 1883-1887.

[67] Sommerfeld HJ, Meeker A, Piatyszek M, Bova S, Shay J, Coffey D (1996). Telomerase activity. A prevalent marker of malignant human prostate tissue. *Cancer Res*, 56: 218-222.

[68] Smogorzewska A, van Steensel B, Bianchi A, Oelmann S, Schaefer MR, Schnapp G, de Lange T (2000). Control of human telomere length by TRF1 and TRF2. *Mol Cell Biol*, 20: 1659-1668.

[69] Sun D, Thompson B, Cathers BE, Salazar M, Kerwin SM, Trent JO, Jenkins TC, Neidle S, Hurley LH (1997). Inhibition of human telomerase by a G-quadruplex-interactive compound. *J Med Chem*, 40: 2113-2116.

[70] van Steensel B, Smogorzewska A, de Lange T (1998). TRF2 protects human telomeres from end-to-end fusion. *Cell*, 92: 402-413.

[71] van Steensel B, de Lange T (1997). Control of telomere length by the human telomeric protein TRF1. *Nature*, 385: 740-743.

[72] Vonderheide RH, Hahn WC, Schultze JL, *et al*., (1999). The telomerase catalytic subunit is a widely expressed tumor-associated antigen recognized by cytotoxic T lymphocytes. *Immunity*, 10: 673-679.

[73] Wataru Y, Hidetoshi T, Eiji T, *et al*., (1998). Expression of telomerase catalytic compontent, telomerase reverse transcriptase, in human gastric carcinomas. *Jpn J Cancer Res*, 89: 1099-1103.

[74] Watson JD (1972). Origin of concatemeric T7 DNA. *Nat New Biol*, 239: 197-201.

[75] Wei Lixin, Wu Mengchao, Guo Yajun, *et al*., Detection of human telomerase activity by telomerase TRAP-ELISA assay. *Chinese Journal of Cancer Research* 1997; 9(4): 277-80.

[76] Wei Lixin, Guo Yajun, Wu Mengchao, *et al*., Detection of telomerase activity in human cells with telomeric repeat amplification protocol by silver staining. *Med Cell PLA* 1998; 13(2): 140-142.

[77] Weinrich SL, Pruzan R, Ma L, Ouellette M, Tesmer VM, Holt SE, Bodnar AG, Lichsteiner S, Kim NW, Trager JB, *et al*., (1997). Reconstitution of human telomerase with the catalytic protein subunit hTRT. *Nature Genet*, 17: 498-502.

[78] Wright W, Piatyszek M, Rainey W, Byrd W, Shay J, (1996). Telomerase activity in human germline and embryonic tissues and cells. *Dev Genet*, 18: 173-179.

[79] Yasumoto S, Kunimura C, Kikuchi K, Tahara H, Ohji H, Yamamoto H, *et al*., (1996). Telomerase activity in normal human epithelial cells. *Oncogene*, 13: 433-439.

[80] Yokoyama Y, Takahashi Y, Shinohara A, *et al.*, (1998). Attenuation of telomerase activity by a hammerhead ribozyme targeting the template region of telomerase RNA in endometrial carcinoma cells. *Cancer Res*, 58: 5406-5410.

[81] Zhu XD, Kuster B, Mann M, Petrini JHJ, de Lange T (2000). Cell-cycle-regulated association of RAD50/MRE11/NBS1 with TRF2 human telomere. *Nature Genet*, 25: 347-352.

(Received 06 February 2016; accepted 19 February 2016)