REVIEW ARTICLE

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Telomerase: Cancer Detection and Therapy

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Telomerase is a special reverse transcriptase that is expressed in most cancer and immortal cells. It has a unique ability of stabilizing and extending the end portion of the DNA–telomere and thereby maintains the genomic integrity. It provides unlimited division potential to neoplastic cells. Detection of telomerase activity is of diagnostic and prognostic value in many cancers. Since cancer but not normal somatic cells mostly express it, drugs developed against telomerase may prove to be highly selective and free from toxic side effects.

Telomeres are specialized structures at the ends of linear chromosomes of all vertebrate cells. They usually contain a short DNA sequence [$(TTAGGG)_n$ in humans] that is repeated several times along with the associated proteins. At birth, telomeres consist of about 15 to 20 kilo-base pairs of repeated TTAGGG–DNA sequences, which become shorter with each cell division owing to the end replication problem. The mechanism of DNA replication in linear chromosomes is different for the leading and lagging strands. Every time a cell divides it loses 25-200 DNA base pairs off the telomere ends. When this pruning occurs about 100 times, a cell senesces (or ages) and does not continue dividing. Semiconservative DNA replication requires an RNA primer to begin DNA polymerization in the 5′ to 3′ direction. After DNA polymerization, the RNA primers are degraded and replaced by DNA synthesized from an upstream primer. Thus, the 3′ end of lagging strands will lose some nucleotides each time a cell replicates its DNA[1] (Fig. 1). It is well accepted that many cancers are caused by mutations in multiple genes that cumulatively subvert the normal growth controls of a cell. The molecular mechanism of these steps in this process, however, remains unknown. Based on results obtained from studies on normal human fibroblasts and epithelial cells to overcome the mechanisms regulating cellular senescence, at least two separate cellular mechanisms must be overcome or neutralized for immortalization to occur[2]. It was found that the first of the mechanisms, mortality stage 1 or M1 requires functional alterations of the genes encoding two tumor suppressor proteins, p53 and RB [3,4]. The inactivation of the normal function of these genes in many types of cancer is well established. However, this mechanism does not directly result in immortalization of cells. If senescent cells are forced through further rounds of division their telomeres become even shorter, division ceases and programmed cell death occurs. This stage is called as mortality stage M2 or stage of 'crisis'. Majority of cells senesce and die at this stage, however, a few cells avoid crisis by overcoming telomere attrition either by activation of telomerase or recombination mechanisms[6]. Overcoming M2 is almost always concomitant with telomere stabilization (Fig. 2)[7,8].

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Telomere length determination and its significance:

Telomere length has been determined by direct and indirect methods. Presently a direct method, which uses digestion by restriction endonucleases to generate a terminal restriction fragment (TRF) containing the telomeric and subtelomeric sequences, followed by Southern

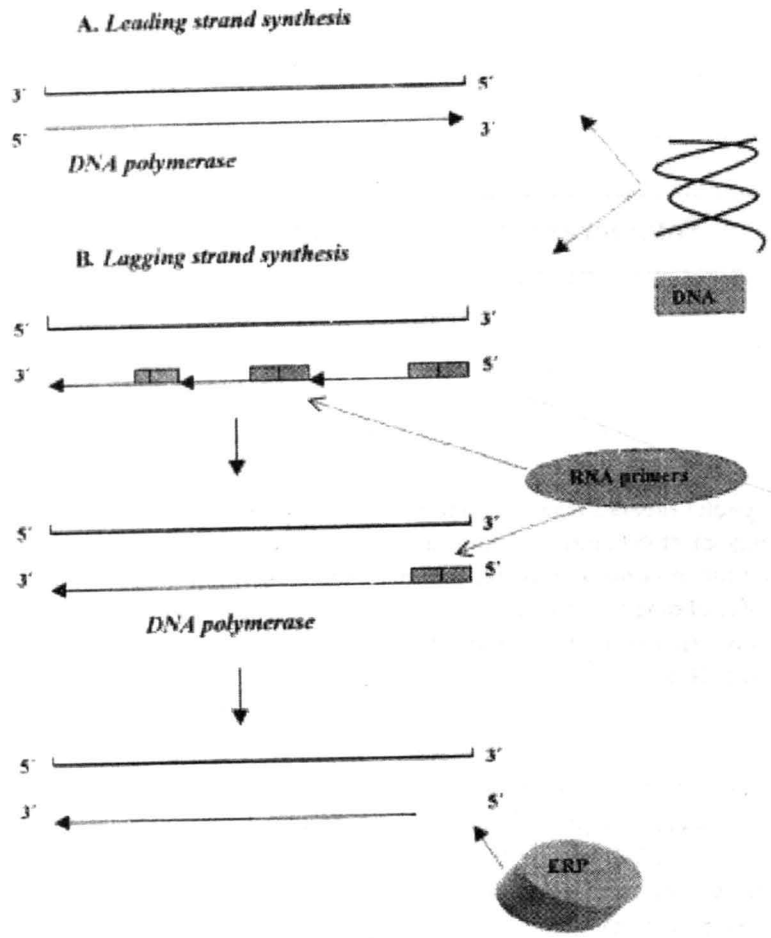

Fig. 1: (A) The leading strand synthesis takes place without any problem by DNA polymerase since it occurs in the 5′-3′ direction. (B) The lagging strand synthesis requires the help of RNA primers to begin DNA polymerization in the 5′-3′ direction. After DNA polymerization, the RNA primers are degraded (the fragments of DNA synthesized are called 'Okazaki fragments') and replaced by DNA synthesized from an upstream primer. Since the fragment at the end cannot be synthesized, replication always results in end (telomere) shortening. This problem is called as end replication problem.

hybridization with a specific telomeric probe[9]. The telomeric length is proportional to the TRF length. More recent methods attempt to measure telomeric length by using fluorescence *in situ* hybridization (FISH) and confocal microscopy[10].

Cancer, Aging and Telomerase:

Cancer is a disease of uncontrolled cellular proliferation (division), and any mechanism that can prevent or stop this process could potentially interrupt its progression. Hence natural induction of senescence or aging mediated by telomere shortening may be an evolutionarily devised way of preventing cancer in species which live for long. Cancers do occur possibly by overcoming senescent controls through lifelong accumulation of mutations of important growth controlling tumor suppressor genes and oncogenes[8].

Of late it is increasingly accepted that there is a strong association between cellular immortalization (cancer) and human telomerase activity both *in vitro* and *in vivo* [11-22].

Telomerase is a ribonucleoprotein enzyme that uses its internal RNA component (complementary to the telomeric single stranded overhang) as a template in order to synthesize telomeric DNA $(TTAGGG)_n$ directly onto the ends of chromosomes in normal germline tissues, in most tumor cells, and in immortal single cell eukaryotes.

Table 1:
TELOMERASE ACTIVITY IN NORMAL AND CANCEROUS TISSUES[11-21]

Tissue type	Telomerase Positivity (%)
Malignant tumors	
Brain	66
Bladder	97
Breast	93-95
Colorectal	93-95
Gastric	85
Gynecologic	88
Head and Neck	87.5
Hepatocellular	80
Leukemias	
Acute myeloid	82
Acute lymphoid	70
Lung	
Small cell	100
Non-small cell	78.4
Neuroblastoma	94
Non-Hodgkin's lymphoma	100
Pancreatic	95
Prostate	84
Oral	
Squamous cell lines	100
Malignant tumor & rinses	100
Ovarian	100
Renal	71
Skin	92
Overall	**>86**
Normal tissue	
Ovary & testis (germ cells)	100
Progenitor stem cells	100
Activated lymphocytes	100
Somatic cells	0
Non malignant (benign and pre-malignant) including benign oral lesions	24(0-75)

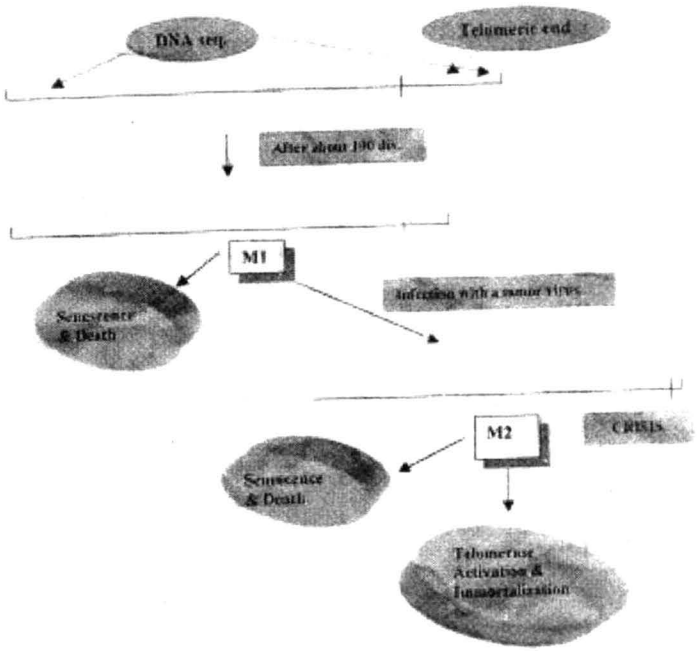

Fig. 2: The telomeric portion of DNA undergoes shortening after a definite number of cell divisions to reach the mortality stage (M1). At this stage cells normally senesce and die. Infection with a tumor virus (say SV40) can force the cell to divide further with shortening of the telomere. The cell is pushed into a second mortality stage (M2). Many cells die at this point of 'crisis' but a few will survive and become 'immortal'.

This extension of the 3′ DNA template end in turn permits additional replication of the 5′ end of the lagging strand, thus compensating for the end replication problem, protecting and stabilizing telomeres (Fig.3). Since DNA chain is synthesized from RNA template with its help telomerase may be considered as a special kind of 'reverse transcriptase enzyme'. It is also called as 'immortalizing enzyme'.

This observation has led to many interesting studies related to various aspects of telomerase production in cancer. Detection of telomerase in tissue material has become a valuable tool for the detection of cancer. One of the most commonly and routinely used methods is a polymerase chain reaction (PCR)-based procedure. This method, which is also known as telomerase repeat amplification protocol (TRAP) has been subjected to different modifications for increased sensitivity and speed[23-24]. A novel quantitative 'stretch PCR assay', shown to be very useful in testing the progression of telomerase activity in leukemias was developed by Tatematsu and

Table 2:
TELOMERE LENGTHS IN NORMAL AND TUMOR TISSUES[9,10,26]

Tissue	Telomere length	
	Normal	Cancerous
Ovarian epithelium	12	4.5
Colorectal	9.3	5
Renal	4.9	6.3
Breast	8.1	9.7
Endometrial	7.2	10.5
Hepatocellular	5.6	12.4
Prostate	4.8-6.0	5.9-7.7

co-workers[25]. Commercial kits are also available for routine detection of telomerase in tissue samples.

Telomerase activity was measured by TRAP method in different normal tissues (somatic and germline), tissues adjacent to tumors, benign and malignant tumors[11,22]. It was observed that in normal somatic tissues telomerase activity was totally absent whereas in germ cells (testis and ovary), all samples expressed the activity. In non-malignant (benign and pre-malignant) and malignant tumors telomerase activity was detectable in 24 (0-75) and 86 (70-100) % of the samples. The malignant tumors included specimens of lung, skin, prostate, oral, gastric, hepatic, breast, colon, uterine, ovary, neuroblastoma, renal, brain and various hematological malignancies. The benign and pre-malignant tumors included intestinal metaplasia, oral lesions, gastric and intestinal adenomas, fibrocystic breast disease and fibroadenomas, benign prostatic hyperplasia and prostatic intraepithelial neoplasia, anaplastic astrocytoma, oligodendroglioma, benign meningioma, ganglioneuroma, and leiomyomas[2,26] (Table 1).

Normal somatic cells do not express telomerase activity and would therefore eventually grow old and die after a set number of cell divisions. Recently Geron and other scientists at University of Texas Southwestern Medical Center succeeded in developing engineered telomerase-expressing somatic cells that are capable of breaking through the senescence barrier. These cells which would normally senesce at 50-55 divisions, have divided more than 100 times and are "staying youthful". They have been shown to express telomerase and free

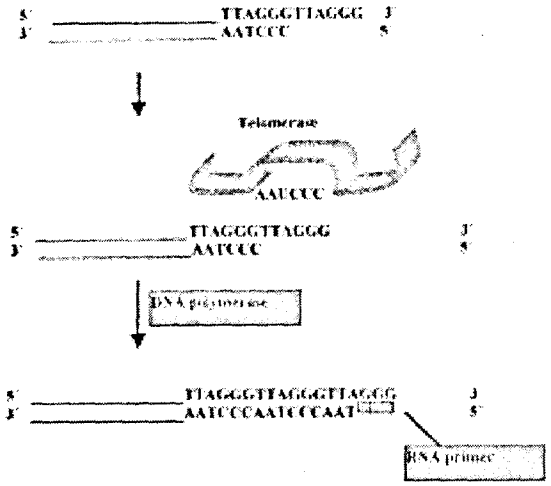

Fig. 3: The extension of 3´ DNA template end by telomerase followed by additional replication of the 5´ end of the lagging strand by DNA polymerase, compensates for the end replication problem

from the signs of aging (accumulation of age pigment lipofucin). These experiments have a number of exciting therapeutic applications such as, rejuvenation of cultured skin cells for treating skin ulceration, retinal pigment epithelial cells for treating macular degeneration and stem cells for bone-marrow transplantation. One would, however, question whether increasing cell life span through engineered expression of telomerase activity could trigger cancer. But it has been suggested that telomerase plays a permissive rather than an initiating role in cancer. Telomerase may be of only minor importance in the initiation of cancer, but its involvement in later stages is more significant[27]. This has paved way to develop telomerase inhibitors as potential anticancer drugs. Such drugs hopefully will stop cancer cell growth without affecting the normal cells since the latter do not contain telomerase.

Telomerase – a potential target for anticancer strategy:

There are quite a few observations, which direct towards the possible role of telomeres/ telomerase as potential targets for anticancer agents. They are.

1. Telomere lengths in most tumors are shorter than in their normal counterparts (Table.2)[9,10,26]. The degree of shortening has been shown to be dependent on the stage of the disease[28]. A direct correlation was also observed between telomere length and progression of myelogenous leukemia (staging and survival chance)[29].

2. Telomerase upregulation that is noticed in most immortalized cells (M2 stage) is needed to preserve the critically shortened telomere lengths[26,30].

3. Telomerase activity increases with a more malignant phenotype. The activity is directly correlated to the stage of tumors[26].

4. Telomerase inhibition can result in further shortening of telomere and induce senescence in immortalized cells. Introduction of chromosome 3 in immortalized renal carcinoma cells restored senescence[31].

Many strategies are being worked out for the development of highly potent and selective inhibitors of human telomerase with minimal toxicity. These include:

A. **Oligonucleotides:** Peptide nucleic acids (PNA) are a class of modified oligonucleotides containing a nonionic backbone in which deoxyribose linkages have been replaced by N- (2-aminoethyl) glycine units. They recognize the RNA component of human telomerase and inhibit activity of the enzyme in pM to nM range. Inhibition depends on targeting exact boundaries of the telomerase template. Phophorothioate (PS) oligomers which are analogous have also been found to inhibit Human telomerase but they are 10-50 fold less efficient than PNA[33]. In a recent experimental study in cell cultures and animals sequence specific telomerase inhibition was demonstrated by 2'-O-methyl – RNA[34].

B. **Antisense RNA:** Apoptosis (programmed cell death) in human malignant glioma cells was demonstrated through expression of high levels of interleukin 1ß-converting enzyme by transfecting the antisense vector against telomerase RNA[35]. In another interesting experiment, Fujimoto and Takahashi exposed three human leukemic cell lines HL60, U937 and K562 to 15-mer antisense c-myc antisense oligonucleotides[36]. All treated cells showed profound decrease in telomerase activity. Kanazawa and coworkers prepared a hammerhead ribozyme (teloRZ) directed against RNA component of human telomerase, which produced specific cleavage in synthesized portion of telomerase RNA. Further it also inhibited telomerase when added to cell extracts from HepG2 (human hepatocarcinoma cell lines)[37]. Similarly Wan and co-workers synthesized 2'-O-methyl modified hammerhead ribozymes that showed potent inhibition of telomerase[38].

C. **G-Quadruplex interacting agents:** Salazar and coworkers demonstrated that the synthesis of telomeric DNA by telomerase is facilitated through formation of stable hairpins or G-quadruplexes that are stabilized by K^+ ions[39]. Compounds like 7-deaza d GTP are incorporated into the telomeric DNA by telomerase and they inhibit translocation since they lack the nitrogen at position 7, which is essential for the formation of G-quartets[40]. Sun and coworkers reported a series of 2,6-diamidoanthraquinones that are considered to mimic K^+ and interact with the G-quadruplex structure by "threading" intercalation[41]. These further stabilize the quadruplex structure and consequently inhibit telomerase activity[42]. Perry and co-workers synthesized three distinct series of regioisomeric difunctionalized amidanthracene-9, 10-diones that showed excellent telomerase inhibitory activity[43].

D. **Reverse transcriptase inhibitors:** Telomerase is a special kind of reverse transcriptase enzyme. Many workers demonstrated that reverse transcriptase inhibitors like azidothymidine (AZT) and dideoxyguanosine inhibited telomerase and induced senescence[44-51]. Preferential localization of AZT to the telomeric regions of CHO cell chromosomes has been demonstrated by immunofluorescence using anti-AZT antibodies[46]. Chen and coworkers observed that continuous growth of the human tumor cells in 800uM AZT resulted in the progressive shortening of telomeres at an approximate rate of 100 bases per generation[44]. Similarly a nucleotide analog ddTTP (2′, 3′-deoxythymidine triphosphate) showed potent inhibitory effect on human processive telomerase[47]. Pai and coworkers isolated and partially purified telomerase from blast cells of an AML (acute myleoblastic leukemia) patient. Three charecteristic forms (two processive and one less processive) of the enzyme were separated. They observed that ddGTP exhibited most potent telomerase inhibitory effect amongst the different dideoxynucleosides tested[49].

E. **Cytotoxic drugs:** Burger and co-workers noticed that cisplatin inhibited telomerase through G-P-G adduct formation in DNA/RNA regions. However, the effect produced by cisplatin in human testicular cancer cells was noticed only when intact cells were incubated in a drug free medium for at least 20 h

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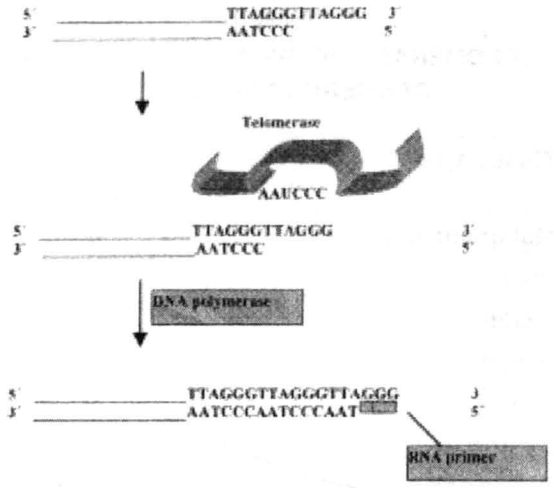

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