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REVIEW

Telomerase: Biological Function and Potential Role in Cancer Management

Vassilios D CHATZIANTONIOU

Semmelweis University, Budapest, Hungary

Telomeres are the specialized ends of eukaryotic chromosomes, thought to have many functions, most importantly serving as a clock signaling entry into cellular senescence. These structures are maintained by the reverse transcriptase telomerase, a peculiar enzyme in both structure, since it contains its own template RNA and function, since it is inactivated in most normal tissues but activated in the vast majority of malignant tumors. These features have made telomerase a subject of intense investigation, both to understand its cellular role and regulation and to exploit its activation in cancer to develop drugs or diagnostic methods based on telomerase. This work gathers all the information currently available in the biological and clinical fields of telomerase research. (Pathology Oncology Research Vol 7, No 3, 161–170, 2001)

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BIOLOGY OF TELOMERES AND TELOMERASE

Telomeres

The structure of telomeric DNA

Telomeric structure has been conserved in most eukaryotes, and is quite different from the termini of linear viral genomes or non-nuclear plasmids.[1] Telomeric DNA consists of a series of very simple tandem repeats which can be regular, consisting of perfect tandem repeats of a fixed sequence (e.g. TTAGGG in human), or irregular, consisting of length or sequence variations of a basic repeat unit (e.g. TG_{1-3} in the yeast Saccharomyces). A few thousand base pairs of these repeated sequences seem to be sufficient to maintain a stable telomere in vertebrates.

Telomeric DNA has a double-stranded conformation. At the extreme ends of the chromosome, however, the G-rich telomeric DNA strand that runs 5' to 3' towards the terminus protrudes 50-150 nucleotides beyond the complementary C-rich strand.[2] Different cells within a population have different telomere lengths and the length varies even among the chromosomes of a single cell, even though it appears to be regulated within certain boundaries.

Telomeric DNA forms characteristic structures, called G-quadruplexes, as a result of it being rich in G residues (*Figure 1*). These structures are presumably stabilized by non-Watson-Crick G-G base pairing. Newer observations suggest that the telomeric DNA forms a loop (t-loop) with the overhang invading the double-stranded portion and forming a D-loop.[3]

Telomere associated proteins

Telomeres are bound by several proteins, called Telomere Binding Proteins (TBPs). These show sequence specificity and their binding protects telomeric DNA against chemical modification and degradation by nucleases. Such proteins are probably the "cap" which protects telomeric ends ***in vivo.*** These proteins may also participate in the regulation of telomerase ***in vivo.*** TBPs can be divided into those that bind along the length of the double-stranded telomere repeats and those that bind the single stranded repeats at the extreme termini. Many TBPs have been identified to date, including the duplex telomere TTAGGG repeat binding factors TRF1 and TRF2 and the single-strand telomere binding proteins telomerase-associated protein 1 (TEP1), and hnRNP A1.[4]

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Correspondence: Vassilios CHATZIANTONIOU, 2 Alkyonis St. P.Faliro, 17561 Athens, Greece Tel: +30-932-668020, fax: +30-1-3618603; e-mail: vchatzis@hotmail.com

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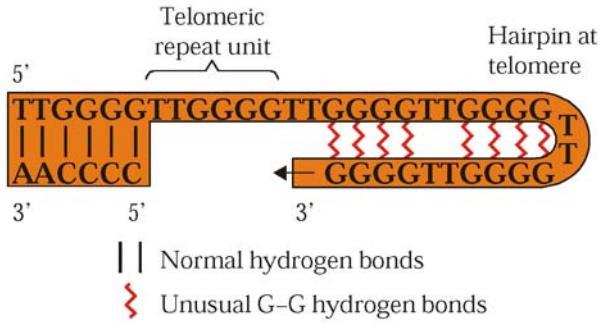

Function of telomeres

Telomeres protect chromosomes against degradation and fusion with other chromosome ends by exonucleases and ligases and from recombination and rearrangement. In addition to maintaining chromosome stability, they also play multiple roles in spatial organization of the cell nucleus and anchor chromosomes with the nuclear machinery to facilitate DNA replication at various stages of the cell cycle by chromosomal recognition and separation during cell division. They can also influence the transcription of genes located near chromosome ends and help in transcriptional repression, heterochromatin formation, and replication timing. Telomeres also appear to play a basic role in the determination of the number of divisions a cell can undergo, cellular senescence and immortalization and malignant transformation of cells.

Telomerase

Telomerase is a large ribonucleoprotein complex containing an RNA subunit and several protein components (*Figure 2*). It can be classified as a reverse transcriptase, because its mechanism of action involves the copying of an RNA template into DNA. It is an unusual reverse transcriptase, however, because it contains its own RNA template as an integral part of the enzyme.[5]

The telomerase RNA

Human telomerase RNA (hTR) is 451 nucleotides long, with an 11 nucleotide template sequence (CUAACCCUAAC) coding for the telomeric repeat (TTAGGG)n. The coding gene is localized to the distal part of the long arm of chromosome 3. Specific nucleotides interact with structural components of the DNA primer substrate and protein subunits making the RNA subunit essential in the enzyme active site. When telomerase RNA with an identical template but different non-template base is substituted for the native RNA, a functional but irregular telomerase ensues, stressing the equal importance of non-template domains.[6]

The telomerase protein components

The human telomerase reverse transcriptase, or hTERT, is a 127 kDa polypeptide, encoded by a gene localized in chromosome 5. This protein possesses a unique conserved region called the "T motif" that is amino-terminal to seven conserved reverse transcriptase motifs (RT motifs). *In vitro* reconstitution experiments show that hTERT and hTR constitute the minimum core structure of telomerase.[7]

Several alternative splicing variants of hTERT mRNA have been found[8]. The role of these variants is yet to be defined. It is currently thought they may substitute for full-length hTERT to alter telomerase activity during tissue and organ development of humans.

Telomerase associated protein 1 (TEP1) in human includes an RNA binding domain, four repeats of a 30 amino acid sequence at the NH_2 terminus, a centrally located nucleotide binding motif, and a large number of WD-40 repeats at the carboxyl-terminal region. TEP1 is thought to play a role in coordinating telomerase holoenzyme structure and in the regulation of the enzyme by binding several regulatory factors.

Telomere elongation

Telomere replication occurs late in the cell cycle. Telomeres have been found to elongate by three mechanisms. *De novo* synthesis of telomeres by telomerase is the mechanism used in humans. In *Drosophila,* the transposition of specific retrotransposons to the chromosome end affords for telomere maintenance.[9] Finally in yeast, telomere extension can occur by recombination of telomeric DNA between telomeres of homologous or heterologous chromosomes.[10] Here, only the elongation by telomerase activity will be presented.

The terminal regions of the G-rich strand DNA sequence is synthesized by telomerase. The centromere proximal telomere repeats are copied by the conventional replication apparatus.

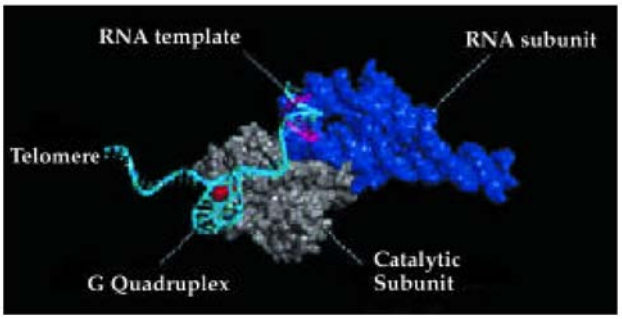

Figure 2. *3D structure of telomerase. The enzyme consists, at least, of an RNA subunit and a protein, catalytic subunit. The RNA subunit contains the template for telomere synthesis. On the telomere, there is a G-quadruplex, as well.*

Telomerase requires a DNA primer, to which telomeric repeats are added in the usual 5' to 3' direction. The G-rich overhang is the most efficient primer since telomerase recognizes G-rich DNA sequences and blunt-ended fully duplex DNAs are not used as primers.

The chemical reaction carried out by telomerase in human, where the primer, as mentioned, is the DNA oligonucleotide TTAGGGTTAGGG, can be depicted as follows:

TTAGGGTTAGGG + 2n dTTP + n dATP + 3n dGTP → TTAGGGTTAGGG(TTAGGG)**n** + 6n pyrophosphate.

The underlined sequence represents the primer supplied to the reaction. No other nucleotides or cofactors are required for reaction and "n" is usually in the hundreds.

Based on the *Tetrahymena* telomerase, the elongation reaction is carried out by the following steps[5] (*Figure 3*):

1. The telomere-complementary sequence in the telomerase RNA base-pairs with the nucleotides of the terminal chromosomal G-rich overhang.
2. The chromosomal end is extended using the RNA as a template.
3. The enzyme proceeds in the 3' direction with the RNA template base-pairing with the extended DNA terminus to begin another polymerization cycle.

After elongation of the G-rich strand, the C-rich strand is believed to be filled in by conventional DNA polymerases, most probably the pol/primase since it can initiate replication *de novo*.

Telomerase regulation

Telomerase seems to be reversibly regulated. Three levels of control are currently believed to be involved in the regulation of the enzyme.

a. Transcriptional regulation

The observation that hTR and hTEP1 are expressed in normal tissues and that expression of hTERT is repressed in most normal somatic tissues after birth and becomes activated in most primary tumors, suggest that hTEP1 and hTR may form an inactive complex requiring the presence of hTERT for the holoenzyme activation.

While the hTERT promoter is inactive in normal tissues, it is activated in immortal cell lines. Several transcription factors bind to the promoter, indicating that expression of hTERT is regulated by different factors in different situations. c-Myc has been shown to activate telomerase.[11] The same holds true for estrogen,[12] whereas methylation of the promoter sequence or deacetylation of associated histones repress the transcription of the gene.

It has, finally, recently been found that chromosome region 3p14.2-p21.3 of chromosome 3 shows hTERT repression activity.[4]

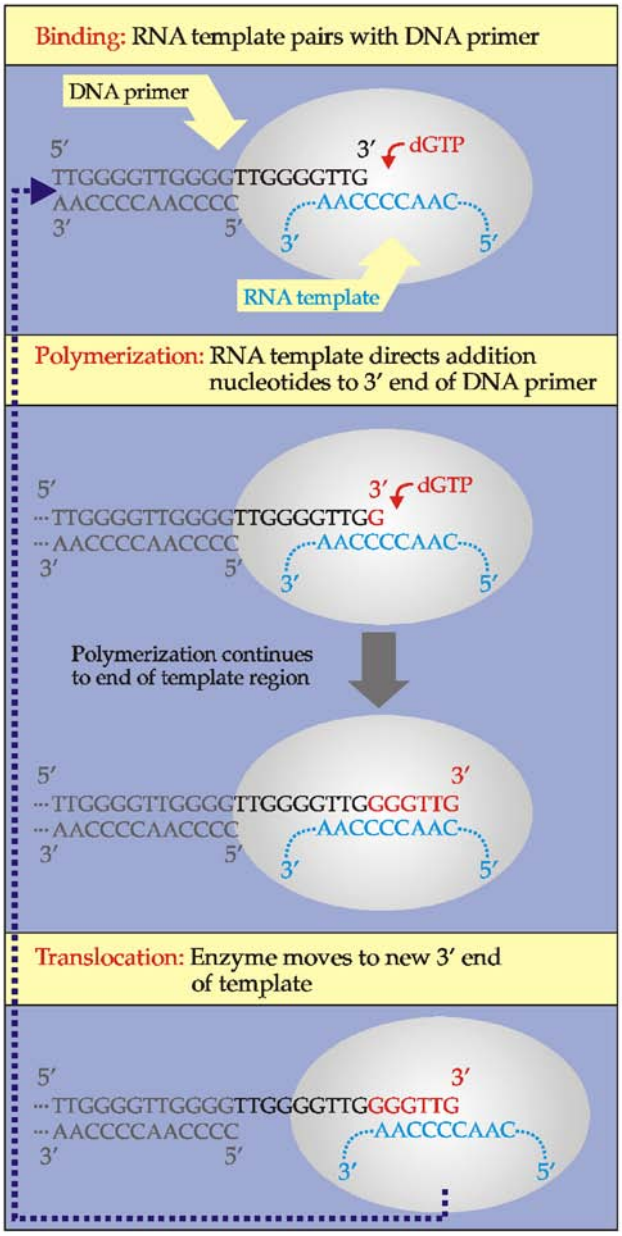

Figure 3. *The telomerase reaction depicted for the Tetrahymena enzyme, where it has been mostly studied. As described in the text, the reaction consists of three steps, which are binding, polymerization and repositioning of the RNA template.*

b. Interaction with other proteins

Post-transcriptional mechanisms are also involved in telomerase regulation. Telomerase holoenzyme optimal conformation and activity probably requires interaction with other proteins. Given that the half-life of the holoenzyme is more than 24h, activity might be regulated by conformational changes by protein interactions. It has been shown that a peptide from the NH_2 region of hTEP1, termed telomerase inhibitory polypeptide 1 or TEIPP1,

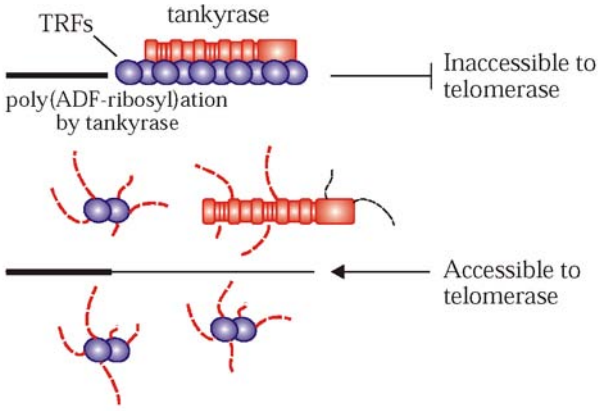

Figure 4. Model of the action of tankyrase. TRFs are bound to telomeres, making them inaccessible to telomerase for elongation. When tankyrase ribosylates them, the TRFs leave the telomere, which is now accessible to telomerase for elongation.

specifically inhibits telomerase activity *in vitro,*[13] further supplying the hypothesis that hTEP1 serves a regulatory role and is probably interacting with regulatory factors and influencing the holoenzyme conformation.

TRF1 appears to inhibit telomerase.[14] Tankyrase, a protein capable of interacting with TRF1, has been identified as potentially affecting telomerase (*Figure 4*). Tankyrase catalyzes the ADP-ribosylation of TRF1 at telomeres, leading to the dissociation of both tankyrase and TRF1 from telomeres allowing telomerase to act on telomere extension.[15]

In addition, p53 protein binds to hTEP1 peptide specifically and inhibits telomerase activity *in vitro.* This inhibitory effect is abrogated by TEIPP1. These data imply that telomerase is probably a downstream element of p53, with the tumor suppressor influencing its activity. It has been, consequently, proposed that p53 exerts its tumor suppressing function, at least in part through the inhibition of telomerase, and that down-regulation of p53 or obstruction of its effect on telomerase leaves the enzyme uninhibited and leads to cell immortalization.[16]

c. The role of phosphorylation

Protein phosphatase 2A (PP2A) markedly inhibits telomerase *in vitro*, while protein phosphatase 1 or protein phosphatase 2B, do not exhibit such an effect.[17] Nonspecific alkaline phosphatase shows a similar effect while the PP2A inhibitor okadaic acid prevents telomerase inhibition. It has been demonstrated that both hTERT and hTEP1 are phosphoproteins, dephosphorylated by PP2A, and rephosphorylated by protein kinase C_α (PKC_α).[18] Other isoforms of protein kinase C are also capable to phosphorylate telomerase, even though their role is unclear. Protein kinase B (PKB or Akt) is also found to phosphorylate the hTERT peptide *in vitro* and to stimulate telomerase activity. The phosphorylation is involved in mediating cellular signaling produced by growth factor activation of the PI_3 kinase pathway. These findings propose a model in which phosphorylation activates and dephosphorylation deactivates telomerase.

It has, finally, been reported that telomerase activity is repressed by a high calcium concentration.[19]

Telomere length regulation

Apart from telomerase regulation, other factors interplay to maintain telomere length. These factors include at least the following: telomere shortening (incomplete replication, telomere processing activities, recombination), telomere stabilization (TBPs, telomere chromatin structure), and telomere lengthening (telomerase, C-strand synthesis, recombination). Before proceeding to the interactions between these factors, those acting to shorten telomeres will be presented. The others have already been accounted for in the preceding sections.

a. Factors shortening telomeres

The main factor shortening telomeres is their incomplete replication, referred to as the "end-replication problem" (*Figure 5*). Since DNA polymerases can only polymerize DNA in the 5' to 3' direction and they require the 3' end of a base-paired primer strand on which to add further nucleotides and which is later extruded from the mature DNA strand, the ends of linear chromosomes lose 50–200 nucleotides per replication cycle. When these RNA primers are removed, the double-stranded DNA is left

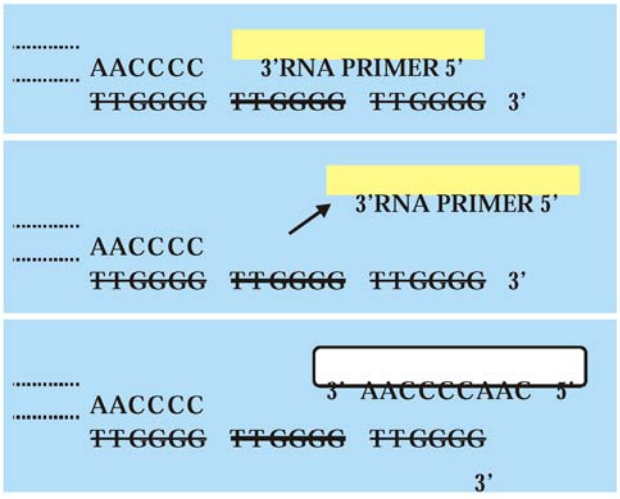

Figure 5. The "end-replication problem". When the RNA primer is removed from the lagging strand, the leading strand is left with a 3' overhang. Telomerase helps to overcome this problem, by elongating the chromosome ends, resulting in the recovery of the terminal parts.

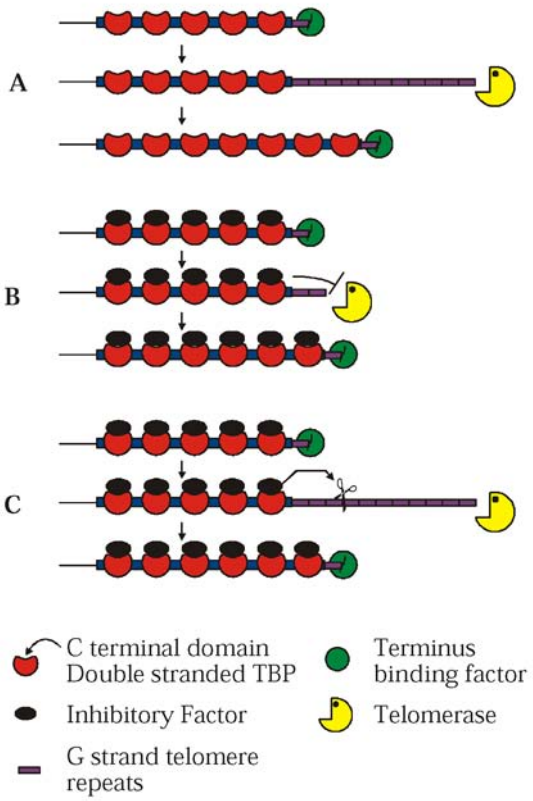

Figure 6. *Models for length regulation. See text. Reproduced from Greider C.W., (Ref. 31)*

with a 3' overhang composed of single-stranded telomeric repeats (G-strand).

Another shortening mechanism, telomere processing, has been observed in ciliates; the conservation of telomere structure and function, however, suggests that processing activities are possibly present in other eukaryotes as well. The best example of telomere processing is found during macronuclear development in ***Euplotes.***[31] In this process, the initially oversized telomeres produced after ciliate conjugation are trimmed to their final length and structure, probably by a telomere-specific endo- or exonuclease activity.

b. Models for length regulation

As the exact functions and interactions of these factors are still hypothetical, telomere length regulation is incompletely understood and only models of such interactions are yet available. Greider[31] proposed 3 models of increasing complexity, based on previously described models (*Figure 6*).

In the simplest model, TBPs are assumed to be the limiting factor in telomere elongation. Telomerase is not regulated, but only the portions of telomeres bound by TBPs are stabilized and protected from degradation. TBP availability, thus, is the main factor influencing telomere length. Another model suggests that TBPs pose negative feedback on telomerase, limiting the number of added repeats. A third model, finally, suggests that telomere processing rather than telomerase regulation plays the main role in length regulation. The two latter theories are more consistent with present data, even though other explanations can also evolve.

Role of telomeres in normal cells, aging and tumorigenesis

Several factors are implicated in aging, but the process seems to be mainly genetically determined. Telomeres seem to play a central role in this process, at least in the cellular level. As mentioned, telomeres shorten with each cell division and it has been observed that *in vivo* telomeres are shorter in certain human tissues in older than younger people.

a. Telomerase activity in normal cells

Most human tissues do not possess telomerase activity. High levels of activity, however, have been found in the cells of the reproductive system and lower levels in normal human white blood cells, stem cells and some noncancerous liver diseases, such as hepatitis and liver cirrhosis, suggesting that telomerase may be reactivated during liver regeneration or other regenerative processes. Telomerase activity has also been observed in various human fetal tissues such as muscle, lung, skin, and adrenal gland, implying that telomerase might be active during development and then turned off in mature tissues.

b. Telomeres and cellular senescence

Normal cells in culture have a finite doubling capacity, termed the Hayflick limit, and the number of divisions is correlated with telomere length. When telomeres become critically short they signal a halt to cell division. It has been suggested that the telomeres on chromosome 17p are critical for this function.[20] At this time, termed mortality stage 1 (M1) tumor suppressor proteins such as p53 and retinoblastoma gene product (pRb) are probably activated. Cell cycle inhibitor p21, a gene known to be induced by p53, is found to be elevated in senescent cells, further supporting this view. Cells with critically short telomeres become senescent, although they remain viable at an arrested G0- or G1-like state.

In some cells, the inactivation of p53 and pRb by viral oncoproteins or by mutations allows them to bypass the Ml stage and continue to divide, until their telomeric length reaches a critical level known as mortality stage 2 (M2). Most cells that enter M2, or crisis, undergo apopto-

sis. Those that survive possess a salvage mechanism for the loss of telomeric length and are capable of indefinite proliferation becoming immortal.

c. Telomeres and organismal senescence

Evidence from mice indicates that telomere length does not correlate with organismal life span, since mice possess telomeres about 5-10 times longer than humans, even though their life span is much shorter than in humans. Despite this, it seems likely that telomeres might influence indirectly aging, as short telomeres have been observed in Hutchinson-Gilford progeria and Down syndrome, diseases characterized by premature aging.[32,21] It can be proposed therefore, that other aging-related diseases such as atherosclerosis and Alzheimer's disease may result from early cellular senescence in the relevant tissues.

d. Telomerase activation in cancer

Telomerase activity is detected in about 85% of malignant tumors. Gradients of increasing telomerase activity between early (benign) and late stage (malignant) tumors have been observed as well. Telomerase activation appears to be a crucial event in tumorigenesis, rather than an coincidental finding. It has been reported by Hahn et al[22] that ectopic expression of three genes, namely large-T, Ras and hTERT is enough to change normal into cancerous cells and that regardless of the cellular pathways activated by the expression of these genes, the expression of hTERT was necessary to achieve tumorigenesis. This result, which marks for the first time the *in vitro* conversion of normal to cancerous cells is in agreement with the current view that telomere stabilization provide cells with indefinite doubling capacity and if further oncogenic stimuli are presented to the cell, malignant transformation can occur.

Some tumors, however, do not show telomerase activity, complicating things further. It might be that some other mechanism ("the alternative mechanism") is salvaging cells from senescence, or that telomerase is activated only at a critical time in tumor progression or even a false-negative result. It has been observed that telomeres from tumor tissues are generally shorter than those from normal adjacent tissues, probably because malignant cells have undergone more doublings than their normal counterparts, further implying that telomerase might be activated only at a critical point, as telomeres get shorter.

POTENTIAL ROLE OF TELOMERASE IN CANCER MANAGEMENT

Since telomerase is active in the vast majority of malignant tumors, but only in a small percentage of normal tissues, hopes are raised that it can be exploited as both a marker of tumor detection and prognostication and as a target for drug chemotherapy. Several ways of telomerase detection have been developed and these will be mentioned first.

Measurement of telomerase activity and telomeric length

a. Detection of telomerase activity

The introduction of the very sensitive TRAP (Telomeric Repeat Amplification Protocol) assay in 1994 by Kim et al revolutionized telomerase detection as it provided both great sensitivity and fast results and replaced previous primer extension assays which were problematic in result interpretation and needed many times more cells to detect activity. The TRAP assay is a one-tube, two step procedure, able to detect telomerase activity from a single cell. In the first step, a telomere-specific oligonucleotide primer is provided as a substrate to the lysates of the studied cells. If telomerase is present the oligonucleotide is elongated and in the second step the product is amplified by the Polymerase Chain Reaction (PCR) technique.[23] Afterwards, gel electrophoresis is utilized to visualize the products, the whole procedure requiring about 5-7 h to obtain a result.

The main problem of the TRAP assay is its poor quantitation. Other artifacts, such as inhibitors of the Taq polymerase in the sample or sensitivity to heat are other shortcomings, making the method somehow difficult to use and standardize. Several variations of the original assay, however, have already been developed to improve some of its aspects. One such method uses an internal standard oligonucleotide both to provide some quantitative results by comparing the intensity of the telomerase ladder signals with that of the internal standard and to rule out false-negatives as a control.

hTR provides another possibility by measuring the enzymatic expression in clinical samples. Alternatively, a radioactive in situ hybridization assay for expression of hTR has been proposed, where hTR can be directly visualized and localized in the cell (*Figure 7a,b*).

b. Assessment of telomeric length

This is achieved by the use of restriction endonucleases followed by Southern blot with a telomere-specific probe. Since telomeric repeats do not contain cleavage sites for restriction enzymes, these are removed together with a portion of subtelomeric DNA to form terminal restriction fragments (TRF), which greatly vary in length among different chromosomes in a cell. The average TRF length, however, provides a fair assessment of telomeric length. In situ hybridization is used in this case as well to assess length, by evaluating the fluorescence intensity by using either flow cytometry or image analysis methods[24] (*Figure 7c,d*).

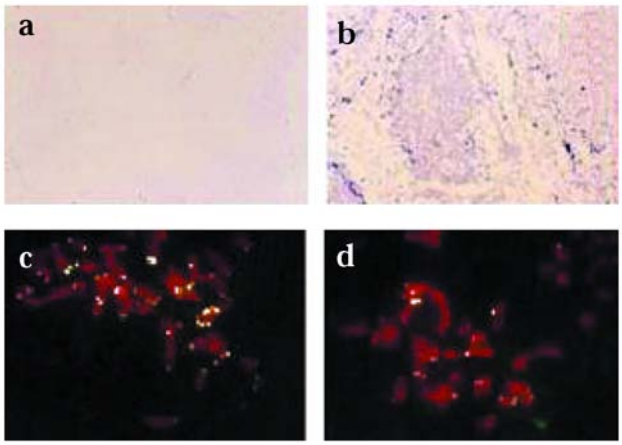

Figure 7. In situ hybridization to detect telomerase. In contrast to normal breast tissue (a), cells from breast cancer (b) react to a probe for the RNA component of human telomerase, staining telomerase positive cells purple. Telomeres from young skin cells (c), after doubling 24 times, are stained bright yellow, while the rest of the chromosome is red. Chromosomes from senescent cells (d), after doubling 82 times, show a marked loss of terminal sequences, such that numerous chromosomes have no detectable telomeric DNA.

Potential role of telomerase in the diagnosis of cancer

Telomerase is currently regarded as an auxiliary tool to histopathology, whenever cytologists fail or are unable to make a final decision. Telomerase especially helps in certain tumor types and diagnostic techniques, such as fine needle aspirates (FNA) and PAP (cervical) smears, where indeterminate cases are common. According to a study, telomerase based diagnostic methods appear to have a specificity of 91% and sensitivity of 85%[25]. These early estimations show that telomerase has a promising potential as a useful diagnostic marker for cancer. These methods, apart from their highly successful results seem to be advantageous for other reasons as well. Since enzyme activity can be detected in a small proportion of benign and premalignant lesions,[25] it might be that activity in these samples is derived from histologically benign cells that have undergone malignant transformation setting the possibility that cancer can be diagnosed at an earlier stage. Telomerase detection may, also, be a more sensitive method for detecting infiltrating cancer cells in tumor margins. Telomerase-based methods can, finally, be applied in easily obtained body fluids and washes, making sample collection easier than the currently used biopsies. This, however, might pose the difficulty of incomplete sampling or sampling error. Another possible problem is the presence of infiltrating lymphocytes and in the case of genital tumors, ovarian or testicular cells, giving false positive results.

Potential role of telomerase in the treatment of cancer

New drugs targeting telomerase are being developed and evaluated for their ability to inhibit telomere elongation by tumor cells and minimize systemic side effects. Appropriate tumor targets are also sought, with cells shortening their telomeres before tumor burden kills the patient. It is also probable that blocking telomerase activity might induce regulatory mechanisms, such as apoptosis, or block other vital processes that could have a more immediate therapeutic effect. It is currently believed that following telomerase inhibition apoptosis, differentiation or senescence should be expected[26]. It is further assumed that tumors whose cells have short telomeres are likely to undergo apoptosis, while tumors with longer telomeres probably will follow a senescence pathway. As previously noted, cancer cells, on average, possess shorter telomeres than normal cells. Thus, if telomerase is blocked in the rapidly dividing tumor cells, cancer cells should approach critically short telomere lengths faster than normal cells. This factor suggests that potential side-effects will be minimized.

a. Possible targets and mechanisms of action

Several targets have been proposed in order to inhibit telomerase. Prevention of hTERT and hTR transcription and RNA processing; post-transcriptional targeting of the RNAs; blockade of the translation and post-translational modification of hTERT; prevention of telomerase holoenzyme assembly; prevention of its translocation into the nucleus; inhibition of its active site and interference with its interaction with the telomeres are possible targets.[26] There might be other points to intervene as well, based upon how telomerase is normally regulated in the cell. A vaccine has also been proposed, based on the observation that the generation of endogenously processed telomerase peptides bound to Class I MHC molecules could target cytotoxic lymphocytes to tumors of different origins. The study on this application also found a minimal occurrence of side-effects.[27]

b. Agents developed so far

Depending on the site of action telomerase inhibitors can be divided into two categories, namely the telomerase-interacting compounds and the telomere-interacting compounds. A short description of each available drug class will follow.

Telomerase-interacting compounds

I. ANTISENSE OLIGONUCLEOTIDES: These are modified deoxyribose oligonucleotides, which mimic telomeric sequences and are directed against the template region of hTR. The most studied agent in this category is phospho-

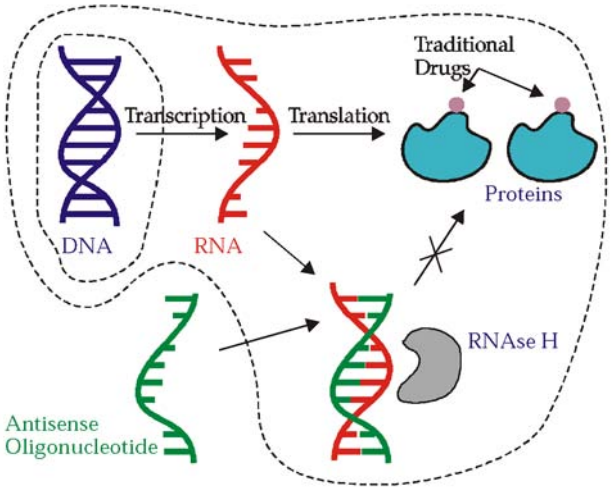

Figure 8. Action of antisense oligonucleotides. The oligonucleotide binds to the newly transcribed RNA and mediates cleavage by the enzyme RNAse H, thereby preventing translation and production of proteins, unlike traditional drugs that act at a later step, binding to and inhibiting the produced proteins.

rothioate. When bound to RNA, they mediate cleavage of the RNA:oligonucleotide hybrid by the cellular endonuclease RNase H and disrupt translation *(Figure 8)*. In order to increase the oligonucleotide interaction with other nucleic acids, intercalators (e.g. acridine) have been tested, attached to the nucleotide. It was found that the addition of the acridine moiety to the nucleotides enhanced the effectiveness of the system by an order of magnitude when compared to the antisense oligonucleotide alone.[24]

II. PEPTIDE NUCLEIC ACIDS (PNAs): PNAs are modified oligonucleotides that contain a non-ionic backbone. The uncharged nature of the bonds between the nucleotides of PNAs, increases their affinity and rate of hybridization with targeted nucleic acids and can withstand degradation by proteases and nucleases more efficiently. PNAs exert their antisense effects by blocking RNA processing, cytoplasmic transport, or translation. PNAs are directed against the RNA component of telomerase (hTR) and thereby specifically inhibit its activity.

In a study comparing the extent of telomerase inhibition achieved by PNAs and phosphorothioate oligonucleotides in melanoma cell extracts, PNAs inhibited the enzyme activity more efficiently and at lower concentrations.[21]

III. 2'-O-METHYL-RNA MOLECULES: These molecules are emerging as powerful inhibitors of telomerase. Their chemical structure is similar to that of DNA, which is hoped to give them an advantage in substrate recognition by the telomerase complex. These agents seem to be even more effective than PNAs with identical sequences.[26] The availability of protocols for delivery of negatively charged oligonucleotides into cells makes 2'-O-methyl-RNAs promising candidates for initial ***in vivo*** anti-telomerase studies.

IV. ANTIBIOTICS: Recent reports showed that some antibiotics might also possess an inhibitory effect against hTR.[24] Aminoglycosides, such as neomycin, and quinolone antibiotics at high concentrations, produced growth inhibition and decreased telomerase activity in human transitional carcinoma cell lines.

V. PROTEINS: Several well characterized proteins, such as HIV gp120, TGF-β1 and pRb, have been shown to inhibit telomerase.[26] Prostaglandin A1 has been reported to inhibit telomerase, as well. Proteins are difficult to deliver into cells and may have potential pleiotropic effects, making these factors unlikely to find near-term clinical application in telomerase inhibition. Nonetheless such observations might appear useful for the better understanding of telomerase regulation and even play a role in the development of modified agents in the future.

VI. GENE MODULATORS: Mutant telomerase RNA introduced into cells with telomerase activity may compete with endogenous wild-type RNA for assembly into complexes with telomerase proteins. These manipulated telomerases might then attach incorrect nucleotide sequences to chromosomal ends, resulting in unstable and incompetent telomeres.

Based on the same principle but using a different approach, a study used retroviral vectors to introduce a gene encoding for an inactive mutant of hTERT (termed DN-hTERT, for Dominant Negative-hTERT, since its expression dominated over the normal enzyme) in tumor cells.[28] This technique resulted in disruption of existing telomerase activity in many types of immortal and malignant cells ***in vitro***, results that were also confirmed ***in vivo***.

It was also shown that telomere shortening induced by DN-hTERT led to apoptosis in a p53-independent manner, suggesting that antineoplastic therapies targeting hTERT will still be effective even in cells that lack functional p53.

Telomere-targeting agents

I. NUCLEOSIDE AND NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS: These agents interfere with the polymerase active site of telomerase and are well known for their effectiveness against HIV. Studies evaluated AZT and ddG for their effects in long-term cultures of two immortalized human B- and T-cell lines. Both agents inhibited telomerase in both cell lines, but no senescent phenotype was detected even with long-term cultures, suggesting that counteractive regulation may be present to prevent further losses of telomeric DNA. Furthermore, other known reverse transcriptase inhibitors such as

dideoxyinosine, dideoxyadenosine or phosphonoformic acid (foscarnet) failed to produce either telomere shortening or reduced cell growth or viability.[24]

II. G-QUADRUPLEX INTERACTIVE AGENTS: G-quadruplexes need to be dissociated in order for telomerase to act on the telomere. This has been exploited to develop molecules that stabilize the G-quadruplex structure, effectively preventing the enzyme from using it as a substrate. Other agents, such as cationic porphyrins and other compounds, bind to the G-quadruplex and prevent proper substrate recognition by telomerase. An alternative approach used 7-deazanucleotides, which are thought to disrupt G-quadruplex formation.

c. Possible obstacles of anti-telomerase treatment

The main anticipated problem of telomerase inhibitors is their late effect, since inhibition of tumor cell proliferation will require continued cell division until their telomeres reach a critically short length. Thus, such therapies should be coupled with other therapeutic modalities, particularly those that result in the debulking of the tumor mass. The fact, however, that the effects of telomerase inhibition are still incompletely understood leaves open the possibility of more direct effects following the activation of some as yet unknown pathways.

Side effects are expected from the tissues that normally express telomerase. Lymphopenia and immunosuppression are possible, as is infertility, since lymphocytes and germ cells possess enzyme activity. Furthermore, unpredictable toxicities might occur, taking into account that telomerase biology is not yet fully understood.

Currently developed agents suffer serious drawbacks concerning their usefulness *in vivo* and prevent their introduction in clinical trials. Most have been found to be susceptible to degradation in the circulation, they have large molecular weights and are acid labile.[24] Another problem is their poor membrane permeability, especially of the PNAs. Strategies to facilitate cellular uptake of PNAs, such as conjugation to carrier molecules or incorporation into liposomes, are under investigation. Prolonged exposure has, also, been proposed as a solution to improve the resulting telomerase inhibition.[29]

It is, finally, possible, that telomerase inhibition might not have the expected results. Since telomerase activity is observed in about 85% of human cancers suggests either that telomerase need only be activated at a critical time in tumor development, or that some tumors may maintain telomere length through an undefined alternative mechanism. It is also possible that this mechanism might come into play as a backup in case telomerase is blocked or otherwise fails to provide the required telomere maintenance and provide drug-resistance to tumor cells.

Potential role of telomerase in the prognosis of cancer and premalignant conditions

The analysis of telomerase might provide insights into three general stages of cancer progression. First, it seems that assessment of telomerase activity may be useful for early detection of cancer and the prediction of which lesions are destined to become malignant through evaluation of premalignant lesions and benign tumors with malignant potential. The second application is based on the observation that in certain tumors telomerase activity levels correlated with disease outcome. Measuring the level of telomerase activity could, therefore, provide useful prognostic information. Results on this field are conflicting, however. Finally, telomerase could be used in the same way as conventional tumor markers are used today for monitoring patients for residual or minimal disease after treatment. The evaluation of radiotherapy effectiveness by utilizing telomerase detection has been proposed as well.[30]

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