Telomerase: anti-cancer target or just a fascinating enzyme?

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Telomerase activity is upregulated in most types of malignant tumor. Highly selective small molecule inhibitors will be needed to understand the biological basis for this observation and to determine if telomerase is a viable target for chemotherapy.

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Chemistry & Biology November 1996, 3:863–867

© Current Biology Ltd ISSN 1074-5521

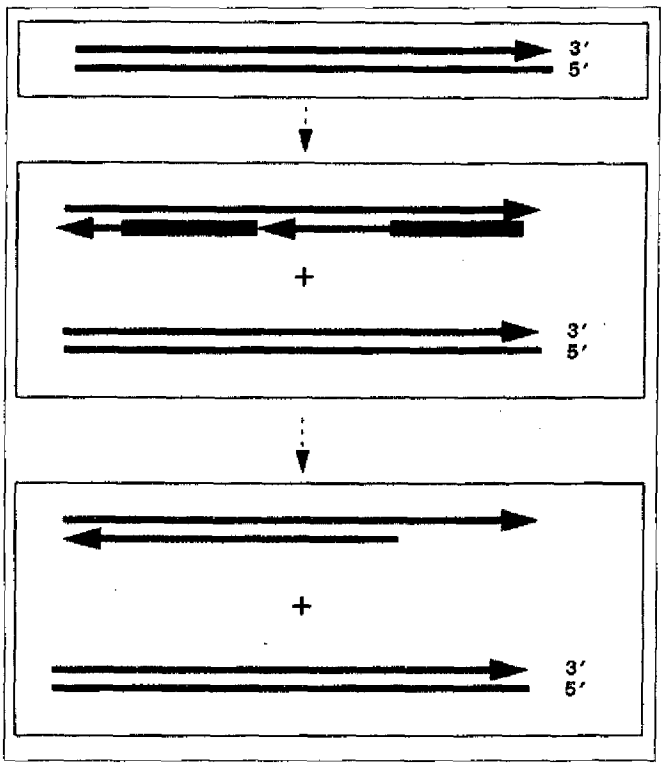
Questions arise whenever an enzyme activity is more prevalent in cancerous cells than normal cells. Why is there a difference? Is the activity necessary for tumor growth? Can it be used as a diagnostic or prognostic marker? Can small molecules selectively inhibit the enzyme in cancerous cells? Recently, a new difference between normal somatic cells and cancerous cells has been noted — the presence or absence of telomerase activity [1–4]. Telomerase is an unusual target for chemotherapy, and deciphering its true potential will challenge chemists and biologists. The link between telomerase and cancer has been reviewed extensively [5–8]. Our goal here is to describe briefly the evidence for and against the telomerase–cancer connection and then to focus on the design of telomerase inhibitors.

Telomerase function and biochemistry

Telomeres are specialized sequences at the ends of chromosomes which are believed to protect adjacent regions of DNA [9]. Human telomeres are composed of the repeated sequence GGGTTA and vary in length from 10–15 kb in germ line cells to 5–12 kb in peripheral blood leukocytes [10,11]. Maintenance of telomere length poses a dilemma for the cellular replication machinery because lagging-strand synthesis cannot fully replicate the telomere end (Fig. 1) [9,12]. Cells must possess mechanisms to solve this 'end-replication problem', to prevent telomeres from becoming progressively shorter during successive cell divisions, an outcome which would eventually reduce cell viability. An answer to this [13] problem was supplied when Blackburn and colleagues discovered telomerase, an enzyme that extends telomeres.

Telomerases from lower eukaryotes have been characterized and consist of two protein components and an RNA component [14–16]. The RNA component has an unusual role: it binds the incoming chromosome end and acts as a template for telomere extension (Fig. 2). These properties make telomerase the only known polymerase that

Figure 1



The end-replication problem. During DNA replication, synthesis of the lagging strand requires an RNA primer (green). The primers that are extended by DNA polymerase are subsequently removed, leaving gaps. DNA polymerase cannot repair these gaps when they occur at the end of the telomere because of the absence of primer. As a result, one daughter strand will lack the DNA previously encoded by the terminal sequence.

carries its own template with it. Protein components bind the RNA and participate in nucleotide polymerization. Two protein components have been identified in *Tetrahymena* [15], neither of which has substantial identity with known polymerases.

The protein components responsible for telomerase activity in mammalian cells have yet to be identified because, unlike *Tetrahymena* and other ciliated protozoans that have thousands of chromosomes and high levels of telomerase activity, each human cell possesses very few molecules of telomerase. What we know about human telomerase is limited to the information from the sequence of its RNA component [17], mechanistic studies [18], and the study of telomerase in lower organisms [16].

The telomerase–cancer connection

It is generally accepted that multiple genetic alterations must occur for cells to become malignant and that, once

immortal, cancerous cells undergo extensive proliferation during tumor growth and metastasis. As a result, they are more likely to require mechanisms to preserve functional telomeres. Studies showing the presence of telomerase activity in tumor samples but not in adjacent normal tissue support this conclusion, suggesting that cancerous cells must reactivate or upregulate telomerase activity prior to establishment of an aggressive malignancy (Fig. 3) [5–8]. These studies have shown that telomerase activity is an excellent marker for malignancy, providing a valuable tool for tumors where diagnosis by other techniques is difficult. Telomerase activity may also prove to be a useful prognostic indicator for the progression of a given cancer, enabling chemotherapy to be better tailored to the severity of the disease for an individual patient. Beyond use as a marker, telomerase activity may be important, if not required, for sustained tumor growth, leading to the hypothesis that its inhibition may cause tumors to regress.

Whether telomerase is required for cell immortality or tumor growth is in dispute, as some tumors and immortal cell lines do not contain detectable telomerase [19–21]. In addition, yeast appears to be capable of utilizing telomerase-independent pathways to maintain telomere length [22]. Therefore it is likely that telomerase activity will not always be necessary for cell immortality or tumor growth, even though greater than 85 % of all human malignancies studied to date do express the enzyme [8].

Studies of mice which have had their telomerase RNA genes disrupted are ongoing. This much-awaited and currently unpublished work will provide insights into telomere and telomerase biology, but the relevance of these studies to human cancer must be evaluated with caution because murine telomere maintenance may not correlate well with that in humans. *Mus musculus*, the species used, possesses telomeres that are two to three times longer than those found in humans, and it may take several generations for chromosome shortening or phenotypic changes to become apparent. Moreover, normal mouse cells spontaneously immortalize in culture whereas normal human cells do not [23], suggesting that mice and humans have evolved different strategies for telomere regulation. This suggestion is supported by findings that telomerase activity is upregulated in murine tumors despite the presence of extremely long telomeres [24–26].

Telomerase as a target for chemotherapy

A definitive understanding of the role of telomerase in human cancer will require examination of each type of tumor, and the application of selective telomerase inhibitors would be of great value for this purpose. Telomerase is an unusual target for chemotherapy because it is likely that telomerase inhibitors will need to be administered for weeks or months before an effect is observed. Telomerase therapeutics will need to be potent, have mild side effects, and be relatively inexpensive per dose. As telomerase inhibitors will not initially kill cells, they may not be useful as a primary therapy. Their most likely application may be to help keep tumors from recurring after chemotherapy or surgery, especially when used in combination with other drugs. One possible source of side effects is the probable inhibition of telomerase in proliferating male germ cells and proliferative cells of renewal tissues, which also possess this activity. These cell types have, however, longer telomeres than do most tumor cells, and may be more likely to survive treatment with telomerase inhibitors (Fig. 3) [8].

Figure 2

Telomerase binds a telomeric end of a DNA chromosome and aligns it by recognition of the RNA template. The chromosome 3′ end is elongated with six nucleotides complementary to the RNA to create the characteristic telomere repeat. A translocation event repositions the telomere to repeat the polymerization step.

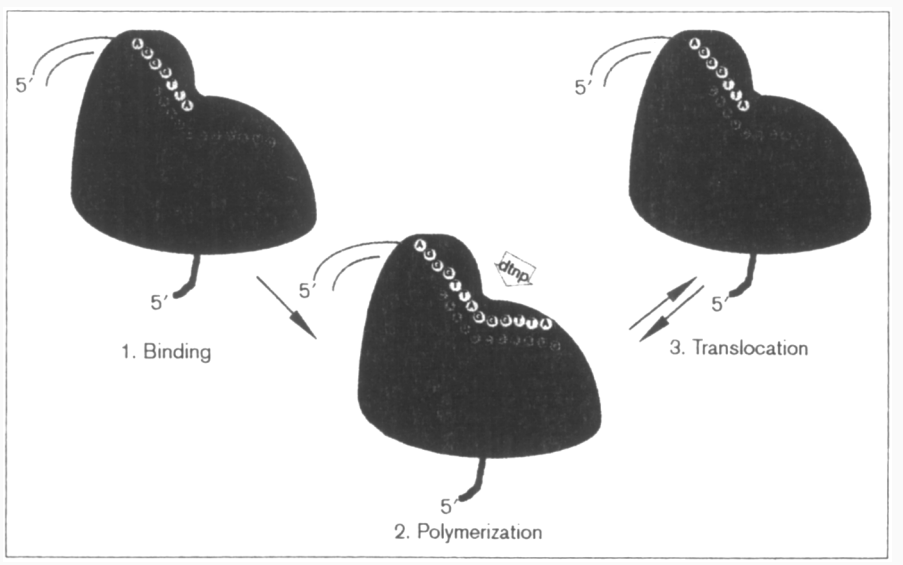
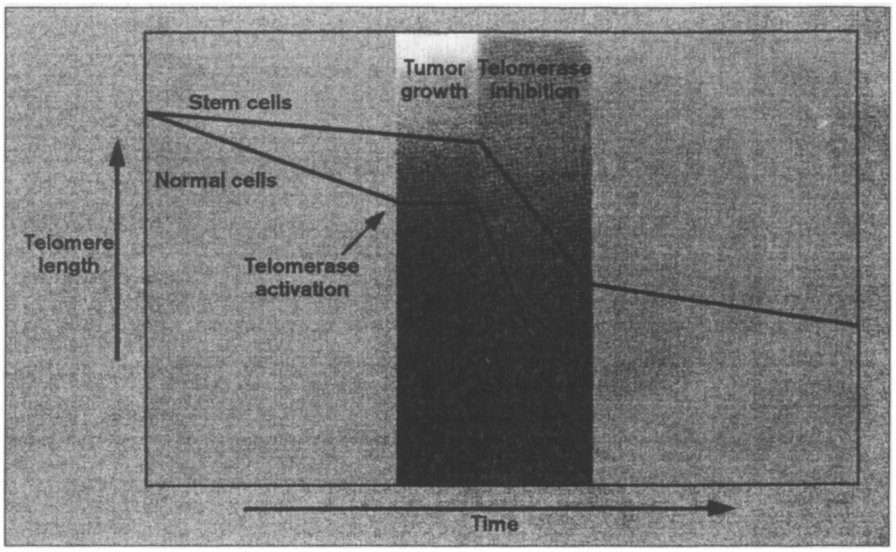


Figure 3

Potential effect of telomerase inhibition [8]. Stem and germ cells typically have longer telomeres than cancer cells, so if telomerase is inhibited, shortening of telomeres may first lead to growth arrest in tumor cells.



Strategies for the rational design of telomerase inhibitors

The rational design of human telomerase inhibitors has been complicated by the lack of pure enzyme, the failure to identify the amino-acid sequence of the protein components and the very limited sequence identity between the protein components of telomerase from lower organisms and known polymerases. However, the RNA sequence provides a characterized target, and studies of the enzyme have provided valuable mechanistic information. Inhibition of telomerase activity can be assayed either by monitoring the elongation of an oligonucleotide primer directly or by a PCR-based assay. The PCR-based assay is 1000-fold more sensitive than the direct assay [2], but care must be taken that added inhibitors do not block any step in the amplification process. Potential molecular targets for telomerase inhibitors include the following (Fig. 4).

The RNA template

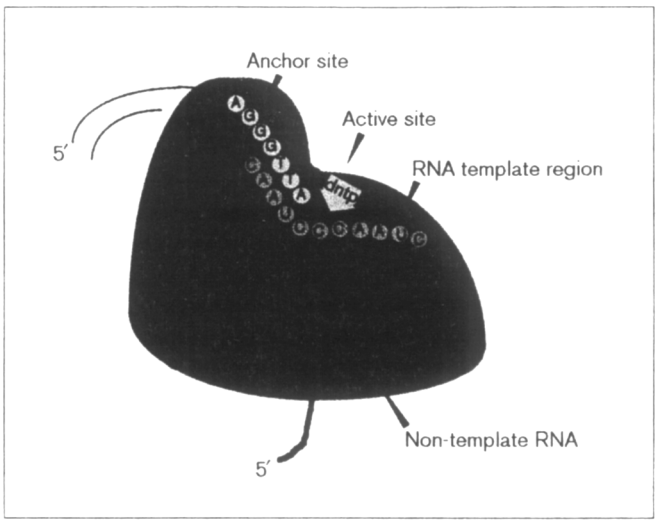
This is an attractive target for inhibitors because the sequence of the human telomerase RNA is known [17]. The dependence on an RNA template suggests that complementary oligonucleotides should be inhibitors, and DNA oligonucleotides have been shown to inhibit ciliate [14] and mammalian [17,27] telomerases. Inhibition of other cellular processes by antisense oligonucleotides directed against mRNA have met with limited success. However, unlike mRNA targets, the telomerase RNA is intrinsically accessible because of its need to bind the exposed telomere end, making it an ideal target for recognition by oligonucleotides.

Inhibition of telomerase activity by DNA oligonucleotides requires relatively high concentrations of oligomer and relatively long sequences [14,17,27]. Shorter (11–13 nucleotides) peptide nucleic acids (PNAs) bind to complementary sequences with much higher melting temperatures than analogous DNA oligomers because of their lack of charge, and PNAs inhibit telomerase activity *in vitro* when present in concentrations as low as 1 nM [28]. Methods for the delivery of PNAs across cell membranes have not been demonstrated, but if this can be done, PNAs or similar oligomers should be excellent tools for studying the cellular role of telomerase and may be useful lead compounds for therapy.

The primer anchor site

Like other polymerases, telomerase activity depends on interactions between one or more of its protein components and DNA [16]. These interactions stabilize the complex prior to initiation of polymerization, and help translocate the newly extended telomere so that another round of synthesis can begin. Evidence for the strength of these non-Watson–Crick DNA–telomerase interactions is provided by the finding that telomerase uses an artificial primer with an apparent K_m of 10 nM [28], even though the primer has only a four-base complementarity with the RNA template. Although guidelines for design of specific inhibitors at the anchor site are more obscure than those for inhibition at the RNA template, the importance of the site suggests that the design of selective inhibitors should be possible. Indeed, phosphorothiorate oligonucleotides with no complementarity to the RNA template effectively inhibit telomerase activity in the nanomolar range [28], presumably by exploiting electrostatic interactions normally used to stabilize protein binding to the phosphodiester backbone of DNA primers. Small, structured polyanionic compounds may be similarly effective inhibitors, although their intracellular delivery may present problems.

Figure 4



Potential targets for telomerase inhibition. Targets include the RNA template, which binds the telomere by Watson–Crick base pairing, the anchor site, which binds the telomere by non-sequence-specific recognition, the protein active site responsible for nucleotide polymerization, and non-template RNA.

The polymerase active site

Like DNA polymerase or reverse transcriptase, telomerase uses DNA nucleotides to extend a primer. Therefore, in spite of the lack of substantial homology between telomerase and other proteins, nucleotide-based inhibitors will block its action as they do with other polymerases. Moreover, the dissimilarity between telomerase and other polymerases may be advantageous, since structural differences may allow the design of inhibitors that are selective for telomerase. Blackburn and Strahl [29] have already shown that nucleotide analogs inhibit telomerase activity *in vitro*, although evidence for *in vivo* effects is unclear and may even suggest the presence of a mechanism for telomerase-independent telomere maintenance in mammalian cell lines [30]. Screening for more potent and more specific inhibitors is necessary to define the potential for active-site directed molecules.

The RNA–protein interface

As a ribonucleoprotein, telomerase requires proper assembly of its essential RNA and protein components, and disruption of this assembly would inhibit activity. This approach has already been attempted, and cells expressing an antisense transcript complementary to the telomerase RNA in HeLa cells underwent cell crisis [17]. Similarly, small molecules that obstruct proper assembly, perhaps analogs of regions of the RNA, might also block this association and prevent the synthesis of active telomerase. Alternatively, molecules that bind the RNA component may distort the template and prevent its proper functioning. The secondary structure of *Tetrahymena* telomerase RNA has been predicted [31], and this prediction suggests the identity of a domain that may be involved in protein binding [32]. Although such predictions await structural confirmation, they afford a useful starting point for inhibitor design.

Outlook for telomerase inhibition

The uniqueness of telomerase as an enzyme and as a target for chemotherapy make it impossible to predict whether its inhibition will lead to improved outcomes for cancer patients. What is certain is that the discovery of inhibitors is critical for understanding the basic cell biology and biochemistry of telomerase. This knowledge will facilitate the synthesis of optimized inhibitors combining high potency and selectivity with good pharmacokinetic properties. Only with these molecules in hand will it be possible to test definitively the link between telomerase and cancer. Finally, it should be noted that telomerase may be a target for parasitic and fungal diseases as well, leading to even wider application of selective telomerase inhibitors.

Acknowledgements

The authors thank members of the Corey laboratory and Dr Jerry Shay for their thoughtful comments on this manuscript. Support for this work was provided by the Robert A Welch Foundation (I-1244) and by a CaP Cure research award. DRC is an Assistant Investigator with the Howard Hughes Medical Institute.

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