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Telomerase and cancer

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Telomerase, a eukaryotic ribonucleoprotein (RNP) complex, contains both an essential RNA and a protein reverse transcriptase subunit. By reverse transcription, the telomerase RNP maintains telomere length stability in almost all cancer cells. Over the past few years there has been significant progress in identifying the components of the telomerase holoenzyme complex and the proteins that associate with telomeres, in order to elucidate mechanisms of telomere length regulation. This review covers recent advances in the field including the use of telomerase in cancer diagnostics and an overview of anti-telomerase cancer therapeutic approaches.

INTRODUCTION

A fundamental difference in the behavior of normal versus tumor cells in culture (1–5) is that normal cells divide for a limited number of times (exhibit cellular senescence) whereas tumor cells usually have the ability to proliferate indefinitely (are immortal). There is substantial experimental evidence that cellular aging is dependent on cell division and that the total cellular lifespan is measured by the number of cell generations, not by chronological time (6,7). This means there is an intrinsic molecular counting process occurring during cell growth that culminates in the cessation of cell division. It is now evident that the progressive loss of the telomeric ends of chromosomes is an important timing mechanism in human cellular aging (8–20). Human telomeres contain long stretches of the repetitive sequence TTAGGG (21,22) which are bound by specific proteins. With each cell division, telomeres shorten by ~50–200 bp (23), primarily because the lagging strand of DNA synthesis is unable to replicate the extreme 3′ end of the chromosome (known as the end replication problem) (24,25). When telomeres become sufficiently short, cells enter an irreversible growth arrest called cellular senescence. In most instances cells become senescent before they can accumulate enough mutations to become cancerous, thus the growth arrest induced by short telomeres may be a potent anti-cancer mechanism.

Telomerase, a eukaryotic ribonucleoprotein (RNP) complex (26–33), helps to stabilize telomere length in human stem cells, reproductive cells (34) and cancer cells (35,36) by adding TTAGGG repeats onto the telomeres using its intrinsic RNA as a template for reverse transcription (37). Telomerase activity has been found in almost all human tumors but not in adjacent normal cells (35,36). The most prominent hypothesis is that maintenance of telomere stability is required for the long-term proliferation of tumors (38–42). Thus, escape from cellular senescence and becoming immortal by activating telomerase, or an alternative mechanism to maintain telomeres (43), constitutes an additional step in oncogenesis that most tumors require for their ongoing proliferation. This makes telomerase a target not only for cancer diagnosis but also for the development of novel anti-cancer therapeutic agents.

EVIDENCE THAT TELOMERE SHORTENING LEADS TO REPLICATIVE SENESCENCE

Early in their cultured lifespan, human fibroblasts derived from a young individual have long telomeres and strong signals when examined by *in situ* hybridization (44) using a labeled probe specific for TTAGGG repeats, whereas old passage have considerably shorter telomeres (Fig. 1). In many patients with premature aging syndromes called segmental progerias (e.g. Hutchinson–Gilford syndrome, Werner's syndrome and Trisomy 21) there are tissues that have shorter telomeres compared with age-matched controls, and cells obtained from some of these individuals show a reduced proliferative capacity in culture (45). Most human proliferative tissues and organs, including most somatic cells (even stem cells of renewal tissues), exhibit progressive telomere shortening throughout life. There have been many studies demonstrating correlations between telomere shortening and proliferative failure of human cells (6–17). Evidence that it is causal was demonstrated by introducing the telomerase catalytic protein component [human telomerase reverse transcriptase (hTERT)] into normal human cells (18,19). Normal human cells stably expressing transfected telomerase exhibited telomerase activity, demonstrated telomere maintenance and showed indefinite proliferation, providing direct evidence that telomere shortening controls cellular aging (46–54). The cells with introduced telomerase maintain a normal chromosome complement and continue to grow in a normal manner for hundreds of doublings (46,47). These observations provide direct evidence for the hypothesis that telomere shortening determines the proliferative capacity of human cells.

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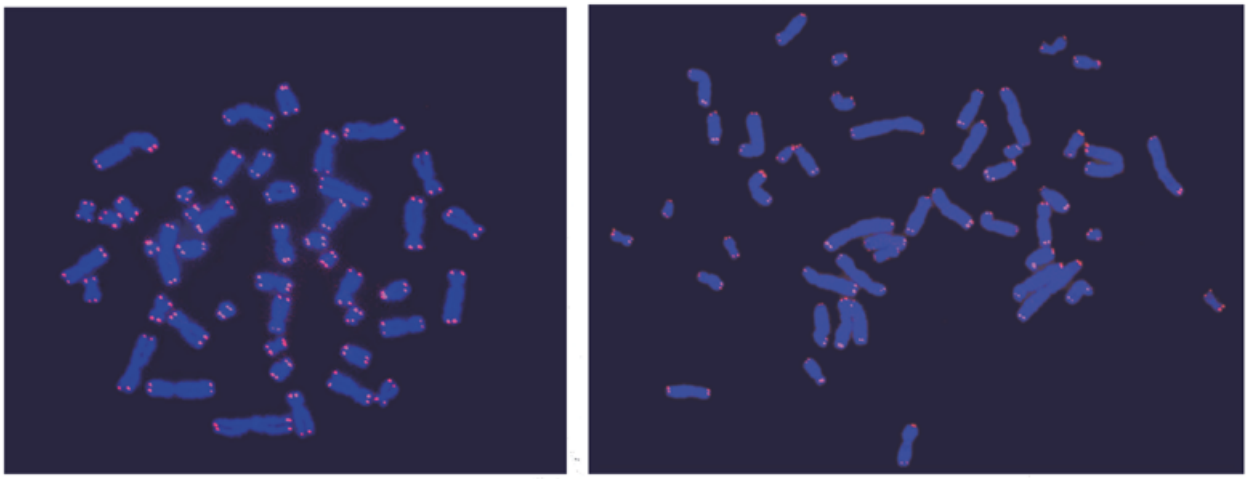

Figure 1. Telomeres are repetitive DNA sequences at the end of linear chromosomes. In most normal cells, progressive telomere shortening is observed each time a cell divides. When telomeres are short, cells stop dividing and undergo a growth arrest (called replicative senescence). Almost all cancer cells are immortal, having overcome cellular senescence by reactivating or upregulating telomerase, a cellular reverse transcriptase that stabilizes telomeres. In this figure, human dermal BJ fibroblasts at low passage, population doubling (PD) 16 and 61, were treated with colcemid to arrest cells in mitosis and chromosome spreads were made. Samples were prepared for quantitative fluorescence *in situ* hybridization (Q-FISH) microscopy using Cy3-labeled peptide nucleic acid probes specific for $(TTAGGG)_n$ telomere sequences (red/pink) and the general DNA dye DAPI (blue/purple). Images of Cy3 and DAPI fluorescence were acquired on a digital image microscopy system to calculate the fluorescence intensity for each telomere. The telomere length is proportional to the number of hybridized probes.

PROTEINS THAT INTERACT WITH TELOMERASE AND TELOMERES

Two central issues are determining how short telomere length signals entry into replicative senescence in normal cells and how telomere length is maintained by the telomerase RNP in tumor cells. To answer these important questions, two overlapping areas are being pursued: (i) identifying and defining the function of the proteins at the telomere and (ii) identifying the components and function of the proteins that associate with the telomerase RNP complex.

Telomere associated proteins

Human telomeres are hidden from the cellular machinery that would normally treat the end of a linear DNA molecule as a broken strand needing repair. Pioneering work by the de Lange laboratory (55–60) has identified two of the major telomeric DNA binding proteins, telomeric repeat binding factor (TRF)1 and TRF2. Both TRF1 and TRF2 are expressed in all human cell types, are associated with telomeric repeats throughout the cell cycle and influence the length regulation of human telomeres either directly or by their interactions with other factors (61–72). TRF1 interacts with tankyrase (63–65) and TRF1 interacting protein 2 (TIN2) (66) (Table 1), and TRF2 interacts with hRap1 (67) and the Mre11/Rad50/Nbs1 DNA repair complex (68). Other factors involved in the detection and repair of DNA damage, such as Ku70/80 heterodimer, also interact with TRF2 and bind to telomeric DNA ends (69,70). In addition, in certain situations, heterogeneous nuclear RNPs (hnRNPs) (71–74), ATM kinase (75–77) and poly(ADP-ribose) polymerase (PARP) (78) may influence telomere length homeostasis. The very terminus of the telomere has a 3′ single-stranded overhang (which varies in length depending on the cell type). Electron microscopic analysis of telomeres has revealed that the end forms a higher order structure called the t-loop (79). It is thought, but not proven, that the several kilobase-long t-loop is generated by strand invasion of the single-stranded overhang into the duplex part of the telomere repeat, forming a displacement or d-loop (79). Collectively these components and structures are likely to be involved in the protection and the maintenance of the ends.

Telomerase associated proteins

The human telomerase RNP consists of both a catalytic protein component (hTERT) and a 451 bp integral RNA [human telomerase RNA (hTR)] that are essential for telomerase activity (18,33). The 3′ half of the hTR resembles the box H/ACA family of small nucleolar RNAs (snoRNAs) (80,81), and although the box H/ACA motif is not required for *in vitro* assembly of telomerase, it is essential for proper 3′-end processing, stability and nucleolar targeting *in vivo* (82). The 5′ end of hTR contains the template used for the addition of telomeric sequences to the ends of the chromosomes (37,83), as well as a pseudoknot that is likely to be important for telomerase function (81,84). The 5′ end of hTR also contains a 6 bp U-rich tract required for a direct interaction with hnRNPs C1 and C2 (85). Although several regions of hTR interact with the catalytic protein component of telomerase (86–88), it is unclear whether these interactions are mediated by auxiliary proteins, direct contacts or both.

Table 1. Major human telomere proteins and telomerase components

Human telomere proteins[a]	
TRF1 (telomeric repeat binding factor 1: binds duplex TTAGGG)	Tankyrase (TRF1 interacting protein: poly (ADP-ribose) polymerase)
	TIN2 (TRF1 interacting protein 2)
TRF2 (telomeric repeat binding factor 2: binds duplex TTAGGG and end protection)	hRap1 (TRF2 interacting protein: end protection)
	Mre11/Rad50/Nbs1 (DNA repair; modulate t-loop formation
Human telomerase components	
hTERT (human telomerase reverse transcriptase)	p23/hsp90 (chaperone)
hTR (human telomerase RNA: intrinsic template RNA)	SnoRNA binding proteins (dyskerin, hGar1)
	TEP1 (vault protein)
	hnRNPs C1/2 (heterogeneous nuclear ribonucleoproteins)
	La (autoantigen, RNA processing)
	L22, hStau (ribosomal protein, double-stranded RNA binding protein)

[a]Other proteins with putative function in telomere biology are Ku, ATM, hnRNPA1, PARP, BLM, WRN, Rad51 and RPA.

Many auxiliary proteins have been identified that associate with the human telomerase RNP (89–98). The vault protein TEP1 was the first to be described (93,94). The snoRNA binding proteins dyskerin and hGAR1 bind the snoRNA motif at the 3′ end of hTR (80,95). The chaperone proteins p23/hsp90 are involved in the assembly of telomerase activity (96). Members of the hnRNP family of RNA binding proteins interact with telomeric DNA as well as telomerase (85,97,98). More recently, the La autoantigen, which is important for the assembly of other RNA particles (99–101) and the maturation of tRNAs (102), has been shown to interact directly with the human telomerase RNP; La's expression levels also influence telomere length in a telomerase RNP-dependent fashion (99).

DETECTION OF TELOMERASE IN CANCER DIAGNOSTICS

Most human cancers have short telomeres and express high levels of telomerase, whereas in most normal somatic tissues telomerase is absent (35,36). Telomerase has been examined in hundreds of studies as a potentially sensitive biomarker for screening, early cancer detection, prognosis or in monitoring as an indication of residual disease (103–133). The detection of telomerase activity has been evaluated using commercially available research assays (106–108) on fresh or fresh frozen tumor biopsies, fluids and secretions. With few exceptions, these have shown that reactivation or upregulation of telomerase activity and its template RNA (hTR) and catalytic protein component (hTERT) are associated with all cancer types investigated.

The catalytic protein of the telomerase RNP, hTERT, is believed to be a critical if not rate limiting step in the production of telomerase activity (32). We have examined hTERT protein distribution by immunohistochemistry not only in cultured cells (Fig. 2) but also in tissue sections (Fig. 3). Cancer cells (HeLa, HT1080) and normal fibroblasts expressing an introduced hTERT cDNA express high levels of telomerase protein (Fig. 2), but this protein is not detected in normal cells (Fig. 2). Cells with telomerase activity have positive nuclear signals whereas cells without telomerase activity do not (132). In most normal epithelial tissues, hTERT expression is limited to stem cells and their immediate descendants. The immunolocalization of hTERT in specimens of adult cancers reveals that the level of telomerase activity mainly depends on the number of tumor cells in a specimen (132). In cancers with high telomerase activity, hTERT is detected in almost all cells, whereas cancers with low telomerase activity have fewer hTERT positive cells. The signal intensity per nucleus of hTERT positive cells does not differ substantially between tumors with various levels of telomerase activity, suggesting that relative telomerase activity of tissue specimens from cancer patients may be a surrogate indicator of overall tumor burden.

ANTI-TELOMERASE CANCER THERAPY

The telomerase RNP and telomere complex present multiple potential targets for the design of new anticancer strategies (134–169). Telomerase may be a challenging target since its inhibition should exhibit a lag phase: the lack of telomerase should not affect cell growth rates until progressive telomere shortening with each cell division eventually causes cells to die or undergo growth arrest. Although it has been correctly suggested that this approach would not be sufficient by itself in patients with a large tumor burden (138–141), it may be a unique approach to patients with minimal residual disease. Importantly, normal somatic cells that lack telomerase expression should be largely unaffected by anti-telomerase therapy. Although telomerase inhibitors should possess great specificity, it is hoped they will also display low toxicity and few side effects. The most likely use of telomerase inhibitors would be as an adjuvant treatment in combination with surgery, radiation treatment and typical chemotherapy, when tumor burden is minimal. It is also possible that telomerase inhibitors could be

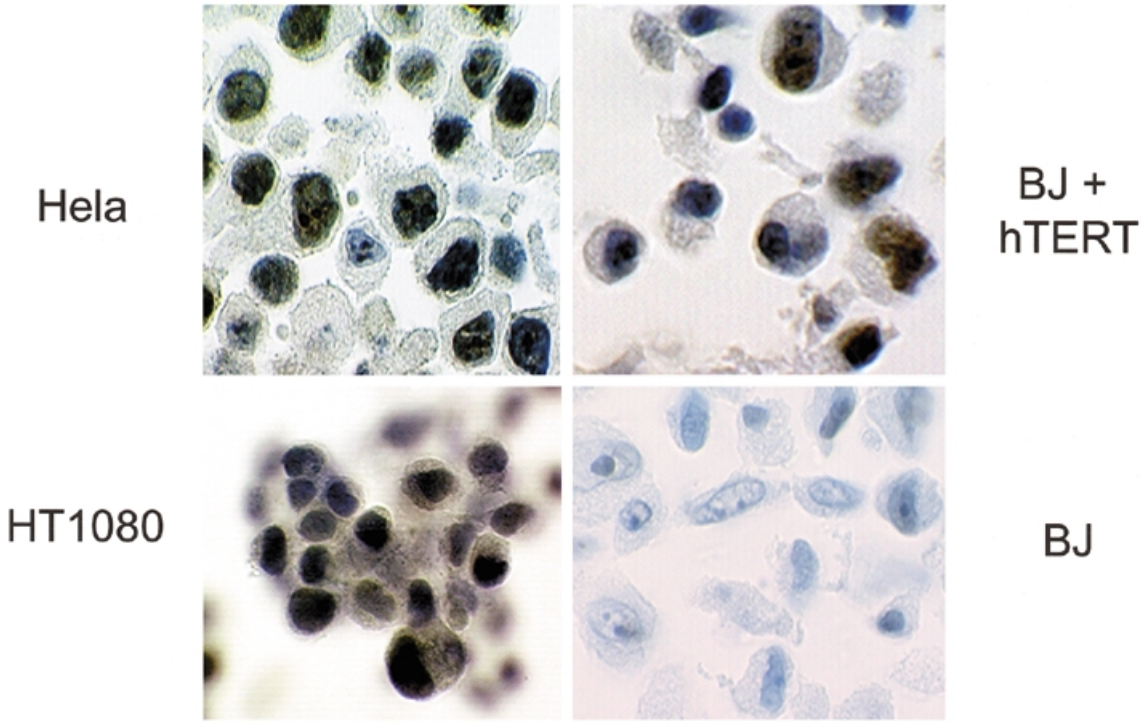

Figure 2. The catalytic reverse transcriptase protein component of telomerase, hTERT, is required for the production of telomerase activity. These images represent immunohistochemical localization of hTERT protein in cells. Cancer cells such as HeLa and HT1080 and normal fibroblasts expressing an introduced hTERT cDNA express high levels of telomerase protein but this protein is not detected in normal cells (BJ). Cells with telomerase activity have positive nuclear signals whereas cells without telomerase activity do not.

used following standard therapies in which there is no clinical evidence of residual disease in order to treat possible micrometastases, and thus prevent cancer relapse. These situations will require prolonged treatment, so it will be important that the drugs have a low toxicity profile and are easily administered.

The primary unwanted effect of telomerase inhibition therapy may be on telomerase-positive reproductive cells and other proliferative cells of renewal tissues (38–42). Cells from such tissues generally have much longer telomeres than most tumor cell populations. Furthermore, stem cells of renewal tissues should be much less affected than dividing tumor cells; they proliferate only occasionally, and telomere shortening should not occur in the absence of cell division. Because the most primitive stem cell populations only rarely divide, their telomeres should shorten at a much slower rate than telomerase-inhibited, proliferating cancer cells. After the cancer cells have shortened their telomeres and died, anti-telomerase therapy could be discontinued and telomerase activity in reproductive and stem cells would be restored. Thus, anti-telomerase therapy is likely to eliminate the proliferative potential of cancer cells before the telomere lengths in normal reproductive and stem cells shorten sufficiently to disrupt their function.

Another avenue is to kill telomerase-expressing cells (146–148,156–160). Immunotherapy directed against telomerase positive cells is currently under investigation (146–148). This approach has the advantage of abolishing the lag phase that is required with the classic mode of telomerase inhibition. However, this treatment might also prove to be toxic to normal stem cells expressing telomerase.

It is still too early to know with certainty whether telomerase inhibitors will become a treatment option against cancer. There is concern about the emergence of alternative mechanisms of telomere maintenance and whether there will be side effects on normal hematopoietic and germline cells. These and other questions will only be answered when anti-telomerase drugs are moved into animal and human clinical trials.

SUMMARY AND FUTURE CHALLENGES

Telomere biology is important in human cancer. Cancer cells need a mechanism to maintain telomeres if they are going to divide indefinitely, and telomerase solves this problem. Although we are beginning to identify an increasing number of telomere- and telomerase-associated proteins, the key is to understand how the telomerase holoenzyme and telomere complex interact to maintain telomere length. The challenge is to learn how to intervene in these processes and exploit our increasing knowledge of telomere biology for the diagnosis and treatment of malignancies.

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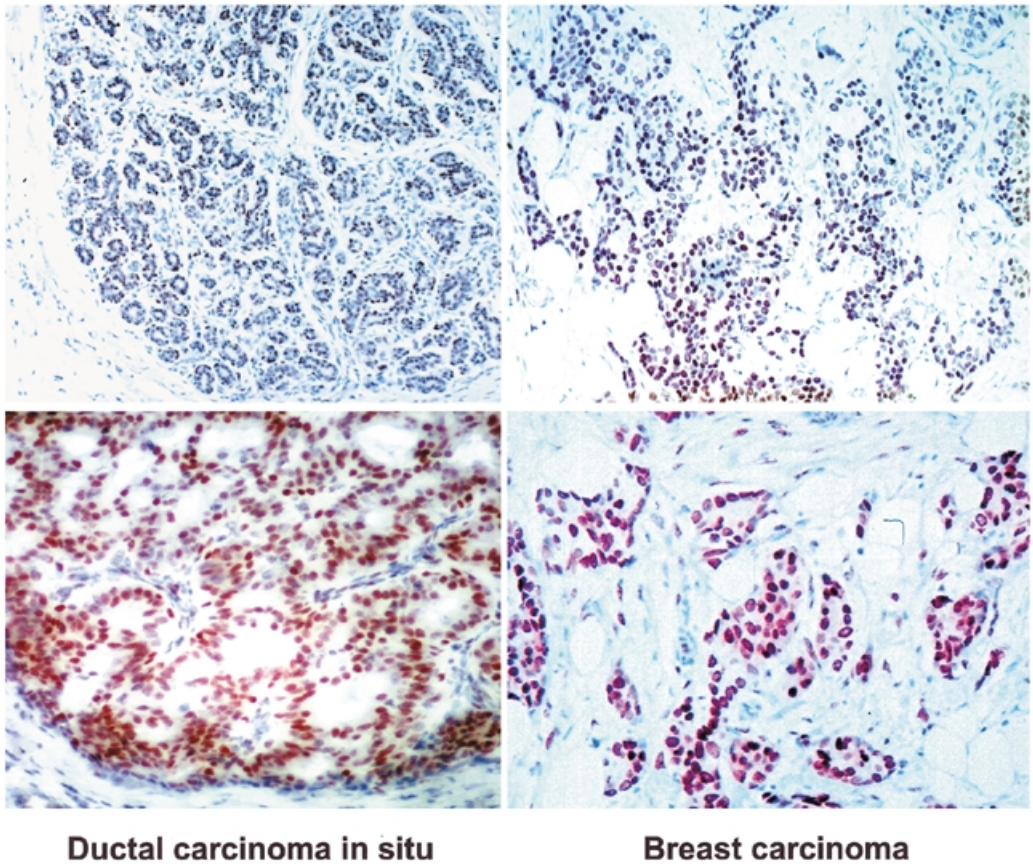

Figure 3. Immunohistochemical localization of hTERT protein in archival paraffin embedded breast tissue. The immunolocalization of hTERT was in the tumor cells of ductal cell carcinoma *in situ* (DCIS) and of invasive breast carcinoma but not in the stromal elements. Telomerase activity was detected in both of these tissues (data not shown) but there was considerably more activity in the advanced cancer compared to the DCIS. Thus the level of telomerase activity in tissue specimens may depend on the number of tumor cells in a specimen.

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