BIOLOGY OF NEOPLASIA

Role of Telomeres and Telomerase in the Pathogenesis of Human Cancer

By William C. Hahn

Abstract: Specialized nucleoprotein structures, termed telomeres, cap the ends of human chromosomes. These terminal structures, composed of repetitive arrays of guanine-rich hexameric DNA together with specific telomere-binding proteins, play essential roles in protecting the chromosome from damage and degradation. In addition, several lines of evidence implicate telomere maintenance as an important regulator of cell life span. Activation of telomerase, a dedicated reverse transcriptase that synthesizes telomeric sequences, is strongly associated with cancer, and recent observations confirm that telomeres and telomerase perform important roles in both suppressing and facilitating malignant transformation. These dual functions of telomere biology are evident in the clinical manifestations of the multisystem syndrome, dyskeratosis congenita, forms of which display defects in telomerase function. Recent advances in our understanding of telomere biology indicate that the manipulation of telomeres and telomerase will lead to clinically significant applications in the diagnosis, prevention, and treatment of neoplastic disease.

Cancer encompasses a diverse set of diseases that not only originate from almost every tissue but also display remarkable heterogeneity in presentation and prognosis. Despite this immense range of clinical characteristics, all human tumors share a limited set of behaviors that define the malignant state. Among these hallmarks, unlimited replicative potential and widespread genomic disarray are among the most common characteristics exhibited by human cancer cells. Although several distinct molecular pathways regulate specific aspects of each of these phenotypes, emerging evidence now implicates the maintenance and function of the specialized chromosomal terminal structures, termed telomeres, as essential regulators of both cell life span and chromosomal integrity. This review highlights recent advances in our understanding of mammalian telomere biology as it relates to cancer and discusses current approaches to exploit this knowledge to develop novel antineoplastic therapeutic approaches.

TELOMERE STRUCTURE AND MAINTENANCE

Telomeres are composed of both repeated DNA elements and specific DNA-binding proteins, which together form the ends of eukaryotic chromosomes (Fig 1). Although the sequence of these terminal structures varies among different organisms, all telomeres are composed of large arrays of short guanine-rich sequences, such as those found in mammalian cells, 5'-TTAGGG-3'. The extreme end of the telomere terminates with a single-stranded 3' overhang of variable length that contributes to the secondary structure of the telomere by helping to form a large duplex loop, termed a T-loop, by invading into proximal telomeric sequences.

In addition to these repeated sequences, a number of proteins populate the mammalian telomere and serve to regulate telomere structure. The structurally related telomeric repeat binding factor 1 (TRF1) and TRF2 proteins bind double-stranded telomeric DNA and play important roles in regulating telomere length. By expressing mutant versions of these proteins, van Steensel and de Lange demonstrated that TRF1 is a negative regulator of telomere length, whereas TRF2 plays an essential role in protecting telomeric integrity. Biochemical studies indicate that TRF2 assists in the formation of the T-loop, indicating that TRF2 helps to maintain the secondary structure of the telomere. Recently, a third telomere-binding protein, Pot1, which binds single-stranded telomeric DNA, was identified.

In addition to these telomere-specific DNA-binding proteins, several other proteins aggregate at the telomere through their associations with TRF1 and TRF2. Two proteins bind TRF1, the poly-adenosine diphosphate ribosylase tankyrase and the TRF1-interacting nuclear protein 2, each of these proteins serves to regulate TRF1 function. TRF2 also interacts with several proteins, including the human Rap1 protein and the Mre11 complex, which is implicated in the...
cellular response to agents that damage DNA. In addition to the Mre11 complex, the Ku complex, known to be involved in certain types of DNA double-stranded break repair, localizes to the telomere. 24-26 Although determining the mechanisms by which these proteins coordinate telomere structure and function remains an active area of research, these observations demonstrate that the physiologic maintenance of the telomere requires complex interactions among these proteins, telomeric DNA, and other cellular factors.

A specialized RNA-dependent, DNA polymerase, called telomerase, maintains telomeric DNA. This ribonucleoprotein enzyme is a reverse transcriptase composed of two essential subunits, human telomerase RNA component (hTERC) and human telomerase catalytic component (hTERT; Fig 2). 28,29 hTERT, the RNA subunit, provides the template for the telomere synthesis reaction and is ubiquitously expressed in mammalian cells. 30 In addition, hTERC is a member of a class of small nuclear RNA molecules termed box H/ACA RNAs. 31 The expression of the catalytic subunit of telomerase, hTERT, is restricted only to cells that exhibit telomerase activity, indicating that hTERT is the rate-limiting component of the telomerase enzyme. 32-34

Recent observations indicate that telomerase exists as a complex tetramer composed of two RNA subunits and two catalytic subunits. 35-37 These subunits act in concert to elongate telomeres by reading from the RNA template sequence carried by the RNA subunit and synthesizing a complementary DNA strand. Mutations in conserved reverse-transcriptase catalytic residues found in telomerase eliminate the enzymatic activity of telomerase. 38-42 Other telomerase-specific motifs are also required for catalytic activity. 43-46 In addition to these core components, several other proteins associate with the telomerase holoenzyme, including TEP1, p23, and Hsp 90 47,48; however, the physiologic function of these other proteins remains undefined because both biochemical and genetic experiments indicate that these other proteins are dispensable for telomerase activity. 41,49

BIOLOGY OF TELOMERES AND TELOMERASE

One of the primary functions of the telomere is to protect linear chromosomes from damage and degradation. During her studies on the effects of radiation on maize chromosomes, McClintock 50 first postulated that telomeres protected chromosome ends from forming fusions. Blackburn and Chiu 51 subsequently identified the molecular structure of the telomere as an array of repeated DNA elements. Later observations in simple eukaryotic organisms, such as Tetrahymena thermophila and yeast, and more recent experiments in mammalian cells confirmed that disruption of telomeric structure directly or through the interruption of the homeostatic mechanisms that maintain telomere length rapidly leads to the accumulation of illegitimate chromosomal associations, confirming McClintock’s postulate 16,52-54. In addition, these studies have demonstrated that the total number of telomeric repeats plays an important role in this protective function.

However, emerging evidence now indicates that the telomeric complex, including both telomere sequences and associated proteins, provides protection to chromosomes in a dynamic fashion rather than serving merely as a passive repository of extra DNA at the chromosome end. 10,55-59 Although some minimum number of telomeric repeats is necessary to protect the chromosome, telomere lengths vary greatly in many different types of human cells. 4,60 and maintenance of telomere length by telomerase can confer end protection even at relatively short telomere lengths. 61 In addition, the observations that the TRF2 protein binds the Mre11 complex and that Ku associates with the telomere raise the possibility that there is an active interplay between the telomere and the cellular response to DNA damage. These types of observations have led some to indicate that telomeres function as a cap that guards the chromosome from recognition as a product of DNA fragmentation. 10,55

In addition to this role of protecting the chromosome, several lines of evidence now implicate that telomere maintenance is an important regulator of human cell life span. 62 It has long been known that normal human cells exhibit a limited proliferative capacity in culture. 63 After extended passage in vitro, normal human cells enter an irreversible growth arrest called replicative senescence (Fig 3). In presenescent human cells, telomerase activity is repressed, 64 and the telomeres in these cells shorten with successive cell divisions. 65,66 In contrast, many cell lines derived from human cancers are capable of unlimited replicative
potential, express telomerase activity, and maintain stable telomere lengths. This correlation between stable telomere lengths and unlimited replicative life span indicated that telomeres might serve as a molecular device that counts cell divisions and limits cell life span.\(^6\) The findings that telomeres were found to be shorter in fibroblasts and leukocytes derived from older individuals, compared with the same types of cells taken from younger patients,\(^6\) and that telomere lengths are dramatically shortened in donor hematopoietic cells after bone marrow transplantation\(^9\) add more evidence that telomere loss occurs with cell proliferation.

To test the hypothesis that telomere shortening regulates cell life span, several groups have introduced the catalytic subunit of telomerase, hTERT, into different types of primary human cells. For some cells, such as fibroblasts and endothelial cells, expression of hTERT in telomerase-null, mortal cells not only halts telomere shortening by conferring telomerase activity but also permits hTERT-expressing cells to bypass replicative senescence and achieve immortalization.\(^7\) Consistent with these observations, inhibition of telomerase in immortal cancer-cell lines by genetic,\(^5\) antisense,\(^6\) or pharmacologic\(^6\) methods results in telomere shortening and eventually halts cell proliferation. These observations provide strong evidence that telomere maintenance plays an important role in governing the life span of human cells.

Although these observations support the hypothesis that maintenance of telomere length is the critical parameter that permits cell immortalization, recent evidence indicates that this simple model fails to completely describe the role of telomeres in regulating cell life span. In fact, human cells must bypass two proliferative barriers to achieve immortalization (Fig 3).\(^7\) The first of the barriers is, as described above, replicative senescence, a state of arrested proliferation but continued cell metabolism. Although expression of hTERT leading to activation of telomerase is one method to bypass senescence in some human cell types,\(^7\) simultaneous inactivation of the retinoblastoma (pRB) and p53 tumor-suppressor pathways also allows human cells to avoid replicative senescence.\(^8\) Experimentally, this dual inactivation is usually accomplished by the expression of viral oncogenes, such as SV40 large T antigen or human papillomavirus E6 and E7 oncogenes, which share the ability to bind and inactivate pRB and p53.\(^9\) Such postsenescent cells do not express telomerase and continue to proliferate until they reach a second proliferative barrier termed crisis or M2, which is characterized by extremely short telomeres and widespread apoptosis.\(^10\) At a very low frequency (approximately one in \(10^7\) cells), rare cells survive crisis and are immortal;\(^11\) all of these survivors now exhibit stable telomere lengths with additional passage and most express telomerase.\(^12\) Introduction of hTERT into postsenescent, precrisis cells allows such cells to avoid crisis, confirming that crisis is also dependent on telomere maintenance.\(^12\)

Thus, for some cell types, such as fibroblasts and endothelial cells, expression of telomerase permits direct immortalization. However, in other types of human cells, including many epithelial cells, expression of telomerase is necessary for immortalization, but other factors or conditions are also required.\(^15\) In particular, recent evidence indicates that ablation of both the pRB and p53 tumor-suppressor pathways together with expression of telomerase is required to permit immortalization of many epithelial-cell types under standard culture conditions.\(^15\) Although crisis clearly seems to depend on telomere length, Karlseder et al.\(^15\) recently demonstrated that an alteration in telomere state rather than telomere length is the critical parameter that signals when cells enter replicative senescence. At present, the molecular nature of this telomere state remains undefined; however, these observations have important implications for our understanding of telomere biology and its role in cancer.

Although most immortal cell lines and tumor cells express detectable telomerase activity, a significant minority maintain stable telomere lengths yet do not express hTERT or telomerase.\(^15\) Such cells are believed to use a yet uncharacterized second pathway of telomere maintenance, termed alternative lengthening of telomeres (ALT). In yeast, this telomerase-independent pathway of telomere maintenance is dependent on recombination,\(^16\) and there is some evidence for increased telomeric recombination in some ALT cells.\(^16\) Indeed, telomere maintenance by telomerase can coexist with ALT, indicating that these mechanisms are not mutually exclusive; although some have argued that expression of active telomerase inhibits the ALT phenotype.\(^16\) Because ALT represents an important mechanism to bypass telomerase inhibition, gaining additional understanding of the mechanisms that lead to ALT is important, particularly as development of telomerase inhibitors proceeds.

**TELOMERES, TELOMERASE, AND CANCER**

These observations connecting telomere maintenance to the regulation of replicative life span strongly imply that alterations in telomere biology play an important role during malignant transformation. Supporting this hypothesis, initial surveys of human cell lines and tissue specimens with a sensitive biochemical assay for telomerase activity (telomere repeat amplification protocol [TRAP]) demonstrated readily detectable telomerase activity in the majority of cancer cell lines and tumors.\(^17\) In contrast, most normal human cells lacked telomerase activity.
The only exceptions to this correlation were normal cells whose functions required ongoing proliferation, such as lymphocytes, \(^{98-100}\) basal keratinocytes, \(^{101,102}\) intestinal crypt cells, \(^{103}\) CD34-expressing peripheral-blood stem cells, \(^{104}\) and immortal cells that exhibited the ALT phenotype. \(^{99}\) Subsequent studies have confirmed that telomerase activity and the expression of hTERT correlate strongly with histologic evidence of malignant cells in many different human tissues. \(^{105}\) A model that emerges from these types of observations indicates that nascent cancer cells acquire replicative immortality by acquiring the ability to maintain telomere length, usually through the activation of hTERT and telomerase. Thus, during the early stages of cell transformation, telomere attrition suppresses malignant transformation by limiting cell life span; whereas, telomere maintenance by telomerase or ALT in later stages of cancer development facilitates oncogenesis (Table 1). Recent work using human and rodent models of cancer provides clear experimental support for this paradigm for telomere biology and cancer.

Although it has long been possible to transform murine cells with specific pairs of oncogenes, \(^{106,107}\) introduction of these same oncogenes into human cells failed to yield transformed cells but, instead, led to cells that either entered senescence or crisis. \(^{108}\) These findings led some to postulate that the limited life span of human cells was a mechanism to suppress tumor formation. \(^{109,110}\) Recent experiments have demonstrated that coexpression of telomerase with pairs of transforming oncogenes permits transformation of a wide variety of primary human cells. \(^{111-116}\) These observations confirm that activation of telomerase and subsequent telomere maintenance play important roles in human cell transformation.

Although significant differences exist in the biology of human and murine telomeres, \(^{117}\) similar observations have also been reported in murine experimental systems. Unlike human cells, the expression of telomerase is poorly repressed in murine cells, and telomerase is expressed in most tissues. \(^{118,119}\) In addition, murine telomeres are maintained at much longer lengths than are found in human cells. \(^{120}\) These differences in telomere biology explain, in part, the discrepancy in cell transformation noted above. Despite these differences, several laboratories have created genetically altered mice that lack the gene for the telomerase RNA subunit, mTERC. Because the telomeres in such animals begin at very long telomere lengths, interbreeding for six generations was necessary to create mice harboring shortened telomeres. \(^{53}\) In such sixth-generation mice, defects in highly proliferative organs such as the liver, skin, and hematopoietic system appear, indicating that telomere attrition does limit the proliferation of cells in vivo. \(^{121}\) Furthermore, when such mice are interbred with tumor-prone mice carrying a deletion of the CDKN2A locus, the mice lacking both telomerase and CDKN2A demonstrated a diminished rate of tumor formation. \(^{122}\) Similar observations were also found when such telomerase-deficient mice were treated with carcinogens known to induce a predictable progression of skin cancers. \(^{123}\) These observations in both human and rodent systems indicate that telomere attrition limits the proliferation of cells and serves as a mechanism to suppress tumor formation.

In addition to this role in permitting cell immortalization, telomeres play a second critical role in promoting malignant transformation. As described previously, telomere attrition in postsenescent human cells eventually initiates crisis, which is accompanied by chromosomal fusions, providing evidence of increased genomic instability. \(^{67}\) Indeed, a rare consequence of these changes in genomic structure is the activation of telomerase or ALT, which facilitates immortalization. However, this increased genomic instability caused by telomere shortening and loss of the protective function of telomeres may also drive malignant transformation under certain conditions. In mice lacking telomerase and heterozygous for p53, a situation that mimics postsenescent human cells, Artandi et al. \(^{124}\) noted an increased incidence of cancers, particularly epithelial malignancies. The karyotypes observed in tumors derived from these mice exhibited a high rate of nonreciprocal translocations common to human epithelial cancers but rare in murine tumors. Similar observations have also been made in mice deficient for both telomerase and adenomatous polyposis coli. \(^{125}\) These observations indicate that loss of chromosomal protection by telomere attrition may drive the formation of epithelial cancers.

Thus, these observations confirm that telomere maintenance plays a complex role in human cancer development. Telomere loss limits cell proliferation and serves as a mechanism for tumor suppression. However, sufficient loss of telomere length eventually leads to genomic disarray that drives tumor formation both through the activation of telomerase and through the generation of other mutations necessary for tumor progression. These opposing roles of telomeres and telomerase operate to both suppress and facilitate cancer formation (Table 1).

Although these observations identify two important mechanisms by which telomere maintenance contributes to transformation, several important questions about the role of telomerase in the pathogenesis of human cancer require further elucidation. Initial surveys of telomerase activity using the telomere repeat amplification protocol assay indicated that telomerase activity strongly correlated with malignant disease. \(^{64,97}\) In addition, a number of laboratories have reported that increased telomerase activity correlates with increased malignant potential and stage. \(^{105,126-131}\) and that genomic instability associated with loss of telomere sequences correlates with a late stage in the development of colonic adenomas. \(^{125}\) These findings indicate that telomere attrition drives genomic instability and the acquisition of hTERT expression and telomerase activity in the later stages of cancer development.

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<th>Table 1. Dual Roles for Telomeres and Telomerase in Cancer Development</th>
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**NOTE.** Depending on telomere length, telomeres and telomerase serve to suppress or promote malignant transformation.
However, several other studies, particularly those using in situ hybridization for hTERT, indicate that hTERT expression and telomerase activity are readily detected in a number of precancerous lesions, benign tumors, and other disease states. These observations indicate that the activation of telomerase may occur early in tumor development, perhaps even before sufficient telomere attrition has occurred. Consistent with these observations, several laboratories have recently demonstrated that overexpression of telomerase induces tumor formation in both animal and human models, indicating that the acquisition of telomerase activity may serve yet another role in malignant transformation.

Although it is clear that telomere maintenance and telomerase play critical roles in the pathogenesis of human cancer, much additional work is necessary to understand the temporal sequence of telomerase activation, telomere shortening, and malignant transformation.

**TELOMERASE DEFICIENCY AND HUMAN DISEASE**

The observations summarized in the previous section indicate that telomeres play important roles in mammalian cell physiology. In particular, the effects of telomere attrition in the absence of functional telomerase observed in mice deficient for the telomerase RNA subunit indicate that disruption of telomerase function in vivo leads to adverse effects on the cell physiology of highly proliferative tissues. As described previously, the telomerase RNA subunit is a small nucleolar box H/ACA RNA. These RNA molecules share a common posttranscriptional processing pathway, which is perturbed in dyskeratosis congenita, a rare disease characterized by abnormal skin and hair growth, aplastic anemia, and susceptibility to cancer. Two types of inheritance for dyskeratosis congenita have been described, an X-linked, autosomal recessive form and an autosomal dominant form. Recent work indicates that the manifestations of both forms of dyskeratosis congenita result from defective telomerase metabolism.

In the X-linked form of the disease, mutations occur in the protein dyskerin, a nucleolar uridine synthase that associates with the box H/ACA class of small nucleolar RNAs including hTERC. Cells derived from patients afflicted with this X-linked form of the disease show abnormal expression of dyskerin. This altered expression of dyskerin leads to defects in the processing of H/ACA RNAs, including the telomerase RNA subunit, leading to subsequent loss of normal telomere homeostasis. In the autosomal dominant form of dyskeratosis congenita, Vulliamy et al recently described three kindreds that harbor mutations that delete the 3' end of hTERC. These observations implicate abnormalities in the expression of hTERC as an important factor in the pathophysiology of dyskeratosis congenita. Importantly, the clinical features of this syndrome, particularly premature organ failure and cancer predisposition, highlight the important role of normal telomere homeostasis in human physiology.

**DIAGNOSTIC AND THERAPEUTIC CONSIDERATIONS**

The emerging understanding of the role of telomeres and telomerase in the pathophysiology of human cancer indicates that strategies directed against telomeres and telomerase hold much promise for both diagnostic and therapeutic uses. In particular, the restricted expression of telomerase and hTERT make this molecule an especially attractive target for intervention. However, exploiting telomere biology for these uses will require integrating the evolving understanding of the multiple roles of telomeres and telomerase in the physiology of normal and malignant human cells.

**Diagnostics**

A large body of literature now exists describing the use of telomerase assays and the in situ expression of hTERT as diagnostic tools. One potential use for such assays is to improve the sensitivity and specificity of detecting malignant cells in cytology specimens. Particularly in urine, pleural, and peritoneal effusions, and bronchial alveolar lavage, some studies have shown promise in improving the sensitivity of cytologic examination (reviewed in Hiyama and Hiyama). However, because some normal, differentiated cell types express telomerase, the specificity of these types of tests must be evaluated closely. Furthermore, in lymphocytes, the regulation of hTERT and, therefore, telomerase may also involve posttranscriptional modifications. Recent observations demonstrating the existence of catalytically inactive versions of hTERT derived from alternative splicing only adds to this complexity. These issues raise significant concerns for the simple interpretation of telomerase activity assays and hTERT expression for diagnostic purposes.

In addition, the questions of how tight repression of hTERT is accomplished and when during cancer initiation and progression do hTERT and telomerase become activated remain unanswered. Indeed, as discussed previously, whether hTERT is expressed primarily in specimens derived from advanced cancers or whether this activation occurs in premalignant lesions is not yet clear. Although recent methods to detect telomere length in tissue sections will aid in determining how to best use telomerase expression as a diagnostic tool, additional work is required to validate hTERT as a diagnostic marker. Studies that simultaneously compare histologic evaluation, telomerase activity, hTERT protein expression, and telomere length are required to elucidate the relationships among these parameters and to define what role telomeres and telomerase play at each stage of cancer development.

**Therapeutics**

Despite these concerns for the use of telomerase expression as a diagnostic tool, the observations that implicate telomerase as essential for the unlimited proliferative capacity of human cancer cells indicate that strategies that inactivate telomerase will prove to have clinical utility. As described previously, inhibition of telomerase by genetic and antisense methods in human cancer cells provided an important confirmation that targeting telomer-
ase in human cancers may be an effective antineoplastic strategy. The prospects for developing small molecular inhibitors of the telomerase reverse transcriptase are promising because inhibitors already exist for viral reverse transcriptases.

In addition to specific inhibitors that target the active site of telomerase, several other groups have reported that other approaches to inhibit telomerase may also have clinical utility. A novel small molecule that inhibits telomerase by preventing telomerase processivity has been shown to inhibit telomerase activity in cancer-cell lines with a soy inhibitory concentration of 100 to 500 nmol/L, indicating that blocking other functions of the telomerase enzyme may prove effective. Another approach to inhibit telomerase is to block the access of telomerase to the telomere. In vitro, telomeric G-rich DNA forms compact structures most easily explained by the formation of guanosine (G) quartets. Experimentally, telomeric DNA folded in this configuration is a poor substrate for elongation by telomerase despite acting as a substrate for telomerase binding. Treatment of cells with specific oligonucleotides that form such G quartets inhibits the access of telomerase to the telomere, resulting in telomerase inhibition in enzymatic assays and telomeric shortening in treated cells. Additional work is necessary to determine whether this approach will have practical utility and to ascertain whether these types of oligonucleotides have effects on telomeres in normal cells. Recently, two groups demonstrated that expression of altered hTERC mutants into human cancer cells resulted in immediate telomere degradation and apoptotic cell death, indicating that specific alteration of telomere structure may also be a strategy to target cancer cells. However, because the expression of such hTERC mutants in cells expressing telomerase would be expected to have dramatic consequences, proper targeting of this type of agent will be required to avoid undesirable side effects.

Because telomerase and, in particular, hTERT seem to be expressed primarily in malignant tissues, several groups have developed strategies to exploit this restricted expression pattern. Although our understanding of the mechanisms that control hTERT expression is still rudimentary, several laboratories have begun to test whether the hTERT promoter can be exploited to deliver neoplastic cell-specific gene expression. Using fragments of the hTERT promoter linked to bax, the herpes simplex virus thymidine kinase gene, and the bacterial diphtheria toxin A chain, these groups have shown that expression of these constructs is limited to telomerase-expressing cells and, in some cases, leads to regression of tumors in vitro and in animal models. Importantly, introduction of identical constructs into cells lacking detectable telomerase activity did not result in cell death. Taken together, these observations indicate that this strategy may indeed confer cancer-cell–specific gene expression. Although additional work will be needed with vectors suitable for gene therapy trials, these observations provide evidence that this approach may achieve therapeutic efficacy with the correct combination of expression and delivery.

An alternative approach to telomerase inhibition is to use telomerase as a target for immunotherapy. Because telomerase is expressed in the majority of human tumors and seems to be essential for tumor-cell proliferation, telomerase is an ideal antigen for cellular immunotherapy. Although telomerase is an intracellular molecule, the protein component of telomerase can be processed by the intracellular proteosomes and presented in the context of major histocompatibility molecules as antigens recognized by cytotoxic T lymphocytes. Several groups have shown that cytotoxic lymphocytes that are able to lyse human cancer-cell lines and primary tumor samples can be isolated from normal donors and from cancer patients that recognize hTERT-derived peptides. In a murine immunotherapy model, mice producing such hTERT-restricted cytotoxic lymphocytes were able to reject the growth of a subcutaneous tumor without evidence of autoimmunity. At present, several clinical trials involving telomerase vaccination are now open that will test the feasibility, toxicity, and, eventually, efficacy of antitelomerase immunotherapy.

INTEGRATION AND PRACTICAL CONSIDERATIONS

Although several specific antitelomere- or antitelomerase-based approaches hold promise as targets for antineoplastic therapy, one must consider several aspects of telomere biology that make such approaches different than conventional cytotoxic drug therapy. Some of these aspects of telomere biology will require unique solutions to find methods to test antitelomerase biology–based therapies in a manner that will permit the identification of promising approaches while anticipating potential mechanisms of resistance.

In particular, telomere lengths vary widely among different cancer cells, and the mechanisms that control the length of telomeres in cancer cells are not yet understood. In most cells, complete telomerase inhibition does not have an immediate effect on cell proliferation but, instead, allows telomere shortening to occur with additional cell division. Thus, even if a telomerase inhibitor were capable of complete enzymatic inhibition of telomerase, treatment of a tumor-harboring long telomere would permit clinically significant cancer progression. This aspect of telomere biology presents a unique challenge to the design of clinical trials to test telomerase inhibitors. Because measuring telomere length in human tumor samples remains problematic, telomere length is an impractical inclusion criterion for clinical trials. These issues make the integration of surrogate biologic end points (such as inhibition of enzyme activity in the tumor) into early clinical trials necessary, but the inclusion of such end points requires that patients submit to rebiopsy of their tumors to assess efficacy.

Another important consideration is selection of non–telomerase-based mechanisms of telomere maintenance after prolonged treatment with telomerase inhibitors. The emergence of cells that use ALT to maintain telomere length represents a major predicted mechanism of resistance. Although ALT cells have not been observed after treatment of human cancer-cell lines with genetic or antisense methods, exposure of cancer cells in patients to effective inhibitors may provide a more permissive environment for the selection of ALT. This possibility remains an important potential mechanism of therapy resistance, particularly if treatment with telomerase inhibitors will be required chronically to control cancer.
If such obstacles can be surmounted and promising telomerase inhibitors identified, such agents will likely prove to have the greatest utility in the adjuvant setting or in combination with other conventional therapies. In such scenarios, the majority of the tumor burden will have been eliminated by other approaches, making the continued proliferation of the remaining cancer cells while telomere shortening occurs less likely to affect clinical outcome. In addition, recent work from several groups indicates that tumor cells in which telomerase has been inhibited are more sensitive to modalities that induce DNA damage, including many traditional cytotoxic therapies and radiotherapy. Thus, combining strategies that target telomeres or telomerase with traditional cytotoxic therapies or with other molecularly targeted therapies may lead to synergistic therapeutic interactions.

Finally, because some normal cells, including those with stem cell–like properties, retain the ability to activate telomerase physiologically, there may be significant side effects of long-term treatment with an effective telomerase inhibitor or immunotherapy. Indeed, the effects of long-term telomerase inhibition might mimic the clinical features of dyskeratosis congenita. Fortunately, no impairment of stem-cell function has been observed in murine models of immunotherapy; however, because mouse and human telomere biology differ in significant ways, careful monitoring during clinical trials will be required to determine whether targeting telomerase affects normal tissues.

Despite these considerations, the promise of antitelomerase- and antitelomere-based cancer therapeutics merits additional development and testing of potential drugs and strategies. Increasing our understanding of telomere biology will not only identify targets for drug development but will also aid the efficient design of clinical trials to identify effective antitelomere- and antitelomerase-based therapies.

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