Is Telomerase a Universal Cancer Target?

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Perhaps one naive goal of cancer research is the discovery of a cellular component absolutely required for growth of all cancers but not for normal cells. This factor would be a universal target for therapeutic intervention. Could this elusive agent be the enzyme telomerase? Recent reports indicate that telomerase is expressed in more than 85% of all cancers but not in normal tissues (1-3). This editorial examines issues associated with using telomerase and telomeres in cancer therapy. Also in this issue of the Journal, Rhyu (4) more extensively reviews what is known about the roles of telomeres and telomerase in cancer.

The inability of the DNA replication machinery to completely replicate chromosome termini (telomeres) leads to the progressive shortening of chromosomes upon continuous cell division (5). This shortening can ultimately lead to loss of telomeric function and chromosome destabilization. A DNA polymerase called telomerase is required to overcome the end replication problem. Unlike other DNA polymerases, telomerase is a ribonucleoprotein (RNP) composed of an essential RNA and a few proteins (6,7). It synthesizes the G-rich tandem repeats that comprise telomeres [(TTAGGG)$_{150-2000}$ in humans], using a template on the RNA that is complementary to the telomeric repeat.

The interest of cancer researchers was aroused when it was proposed that telomerase is not expressed in human somatic tissues and that telomerase is required for cancer cell growth (8). The telomere hypothesis, as it has come to be called, states that telomerase is only active in the germline cells of adult humans (9-11), where it would be required to ensure the transmission of intact (i.e., full-length) chromosomes to progeny. In contrast, the telomeres of somatic cells would shorten until they reach a length that signals a halt to cell division (senescence). Studies in vitro suggest this barrier, called M1, can be bypassed by transformation with viral oncogenes like SV40 T-antigen (12). However, the telomeres of cells at this point are still functional; but the cells are also telomerase negative, their telomeres continue to shorten, and the cells are not immortal (i.e., capable of indefinite division). Eventually transformed cells enter a second crisis, called M2, where the majority of the cells senesce and later die. In M2, telomere length has decreased to the point where a subset of the chromosomes have nonfunctional telomeres. Only rare cells that have undergone a specific mutational event(s) emerge from M2 and are immortal, and only these cells have stable telomeres and express telomerase (12). Thus, the nonexpression of telomerase in somatic tissues provides a barrier to indefinite cell growth, a supposed hallmark of cancer cells. Inhibition of telomerase in cancer cells might activate the cellular senescence pathway and prevent their propagation.

Numerous studies of telomere length with donor tissues and a variety of primary (mortal) cell lines lend credibility to the model (13-15). Direct measurements of telomerase activity in tumor cells have also supported this theory. A study last year (16) demonstrated telomerase activity in ovarian carcinoma (ascites) cells but not in normal ovarian tissues surrounding the tumors. The telomerase assay used in this study requires enough cells that application of the technique to other cancers is impractical. However, in what represents an important advance, a highly sensitive polymerase chain reaction-based assay for telomerase activity has been developed (1), and many normal human tissues and more than 400 tumor specimens and tumor cell lines have now been tested (1-3). Only testis and ovary express telomerase among somatic tissues, and more than 85% of tumors contain telomerase activity. By using this assay and the newly reported human telomerase RNA gene as a probe, it should be possible to examine other tumors for the presence of telomerase and to test the universality of these results. In the future, only direct measurements of telomerase activity or gene expression should be used, since inferring the presence of telomerase from measurements of telomere length is often misleading, based on the fact that comparisons of telomerase activity and telomere length in cell lines do not indicate that they are associated (1,17). While the evidence strongly supports the telomere hypothesis, only molecular genetic manipulation of the human telomerase RNA gene in cell lines will allow elucidation of the role that telomerase has in the escape from senescence.

Many issues remain to be resolved. What role does cellular immortality have in cancer? Is telomerase activation necessary for cell immortalization? Will prevention or reversal of cancer cell immortality be an effective treatment, and can antitelomerase therapy prevent or reverse cell immortalization? What problems might be associated with this therapy, and what types of cancer would be amenable to this treatment?

There are likely to be several barriers to obtaining cellular immortality, but one barrier is the chromosome end replication problem. The issue of whether cancer cells are immortal is un...
resolved (18), but estimates of the number of cell divisions that occur in primary tumors and their derivative metastases (19) can exceed the in vitro replicative life span of the cells from which the tumor is derived. The exciting finding that the great majority (>85%) of human cancers have activated telomerase (1-3) suggests that cell division beyond what is otherwise limited by their telomeres has occurred. Thus, while proof is lacking that tumor cells are immortal, they are likely to be dependent on telomerase.

While telomerase activation in tumor cells is extremely prevalent, there are rare examples of apparently malignant tumors with no observable telomerase activity (1-3). It is possible that the cells of these tumors are not immortal but are capable of enough cell divisions that they are harmful nonetheless. Alternatively, these tumor cells could be using a different solution to complete telomere replication, which is probably the case in some apparently immortal human cell lines that have no observable telomerase activity (20). There are many solutions to the end replication problem observed in biology, including the retrovirus-based system likely to maintain normal Drosophila telomeres (21) and the recombination-based rescue pathway activated in some Saccharomyces cerevisiae telomere mutants (22).

How effective would a specific telomerase inhibitor be? Because the telomeric repeats provide a buffer of DNA that can be lost from chromosome termini, many cell divisions may be required before telomere function is compromised. Fortunately, the telomere lengths of most cancer cells are quite short (1,13,17), and only experiments that ablate telomerase expression or function will tell if cancer cell telomeres will decrease with enough rapidity to be clinically useful.

Another critical issue concerns the potential side effects of antitelomerase therapy. These side effects arise not from the possibility that inhibitors could affect other normal cell proteins but from the possibility that critical cells in the human body might express telomerase. Theoretically, telomerase needs to be expressed in cells that routinely divide. Telomerase is expressed in testis, but sperm telomeres are much longer than those in cancer cells and would be expected to survive a treatment regimen sufficient to kill cancer cells. Regardless, any side effects in testis could be minimized because patients with cancer are usually past reproductive age and might be willing to forgo reproduction or could store sperm for later use. However, stem cells, such as those of the immune system and the hematopoietic system, might also express telomerase; weak telomerase activity has been detected in candidate hematopoietic stem cell populations with the new, more sensitive telomerase assay, suggesting that a subset of the cells express telomerase (23). As in the case of germ cells, stem cell telomeres are likely to be long and the consequences of telomerase inhibition in stem cells would depend on the length of their telomeres and their rate of cell division relative to cancer cells. Providing that cancer cells lose telomere function before stem cells, the side effects may be minimal.

Another consideration is the fate of sperm cells or any stem cells expressing telomerase that survive antitelomerase therapy. Those cells will have shortened telomeres and face at least two possible outcomes. First, their telomere lengths may not recover. The consequences of inheritance of chromosomes with shortened telomeres is unknown, and stem cells with shortened telomeres may not be capable of enough cell divisions during expansion to produce the desired cell populations. Thus, long-term antitelomerase therapy might be ill-advised. Second, their telomeres could recover their former lengths and regain normal function. However, removing telomerase inhibition may not be sufficient for restoration of telomere length because telomerase does not regulate telomere length by itself, as illustrated by a comparison of the telomeres and telomerases of different organisms. Although the telomeres of Tetrahymena are relatively short (<600 base pairs), its telomerase can synthesize thousands of base pairs in vitro (6); however, in mouse cells, where telomeric DNA can be greater than 100 kilobases (24), telomerase adds only a few repeats in vitro (25). Telomere function is mediated by proteins that specifically bind telomeric DNA, and a system must exist for detecting telomere length, regulating telomerase activity, and protecting telomeres. The biology of yeast telomeres suggests the existence of this maintenance system (26,27). The entire system must be active for telomeres to be stable. Thus, telomerase activation in tumor cells cannot solely involve the activation of a single telomerase gene, since even telomerase is a multisubunit enzyme derived from several genes; the activation of a multiple-component telomere maintenance system is probably required. This realization provides additional targets for intervention. Because the phenotypic lag of telomerase inhibition may be problematic, targeting the system that senses telomere length or that regulates telomerase activity may lead to a much more rapid loss of telomere function. Also, nonontelomerase targets in the telomere maintenance system might affect the telomere function of those few cancer cells that use a nonontelomerase solution to the end replication problem.

Telomerase fulfills many of the criteria for an ideal cancer target: the almost universal activation in tumors and a nearly ideal developmental and tissue-expression pattern. What remains is to show that tumors require telomerase for growth and that loss of telomere function will be clinically useful. While these tasks and finding a specific telomerase inhibitor remain daunting, the present optimism appears justified.

References

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The classical paradigm for epidemiology is to relate reported exposures in groups of individuals to disease occurrence. Exposures are often measured crudely by surrogates such as occupation, and disease is dichotomized by the occurrence of a clinically observable late manifestation, such as invasive cancer. Genetic epidemiology acknowledges that disease occurrence is not completely determined by external factors, but that individual differences in susceptibility also exist. Classically, these are manifested through familial clustering of disease.

In recent decades, epidemiology has made great strides forward by obtaining better estimates of exposure from biochemical or serological measurements. These measurements have also had a major impact on genetic epidemiology, notably via studies of the human leukocyte antigen–disease association and differences in drug metabolism. Cancer is a disease of genetic dysregulation, however, and it is appropriate, and likely to be useful, to try to measure effective exposures and host factors at the DNA level.

This naturally leads to consideration of the following progression: exposure, DNA adducts, persistent mutations, neoplastic transformation, preinvasive lesions, and invasive cancer. Ultimately, the goal of molecular epidemiology is to pinpoint the agents and mechanisms responsible for the transition through each of these stages and the host factors that block progression when functioning correctly. Identifying these agents and mechanisms could provide a more accurate measure of exposure at the DNA level while also providing markers for preinvasive lesions likely to become cancer in subsequent years or predicting the aggressiveness of very early lesions. Such an understanding would provide a more rational basis for chemoprevention and screening. Notable successes to date include the following: an understanding of the genetic changes associated with the adenoma–carcinoma sequence in bowel cancer (1); establishment that certain types of human papillomavirus are the key causative agents in cervical cancer (2); demonstration that the 4-aminobiphenyl adducts produced by dark tobacco, but in much lower quantities by blond tobacco, appear to be a key factor in tobacco-induced urinary bladder cancer (3); and polymorphisms in the NAT2 gene leading to an increased susceptibility to urinary bladder cancer in individuals who are slow acetylators (4).

In this issue of the Journal, Kato et al. (5) examine the host factors that influence the first transition from external exposure to the formation of specific DNA adducts. Their study is based on autopsy material from 90 donors who did not have lung cancer. They assessed exposure to cigarette smoking by measuring serum cotinine, which was dichotomized as positive/negative, and by measuring two specific DNA adducts in lung biopsies — 7-methyl-deoxyguanosine adducts, which are a metabolism product of N-nitrosamines, and a specific polycyclic aromatic hydrocarbon–DNA adduct (PAH–dGMP). They also assessed host metabolic factors by polymorphisms in three P450 alleles (CYP1A1, CYP2D6, and CYP2E1) and glutathione S-transferase M1 (GSTM1). A notable achievement reported in their study is the development of assays for 7-methyl-deoxyguanosine adducts and PAH–dGMP that appear to be sensitive