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Commentary

Activation of telomerase in a human tumor

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Tumors owe their perverse growth potential to changes in the controls that normally restrain somatic cells. The now-familiar players in this drama include distorted genes for growth factors and their receptors, misdirected signaling molecules, and errant cell-cycle regulators. Through these alterations cells evade many of the growth constraints imposed by their environment. But are such uncontrolled cells licensed for infinite rounds of cell division? Recent advances suggest that telomere loss is yet another hurdle in the progression of human tumors and that this obstacle may be circumvented by the activation of telomerase.

The speculation that telomerase may play a role in human cancer is not new and has been discussed in a variety of contexts (1–4). However, a study by Counter *et al.* (5) in this issue of the *Proceedings* demonstrates that telomerase is activated in ovarian carcinoma. Their data indicate that expression of telomerase and the resulting stabilization of telomeres may be important for the expansion of a human tumor. This commentary reviews the background to these findings and discusses possible contributions of telomere dynamics to the transformation of human cells. More general reviews on telomeres and telomerase can be found in refs. 6–9.

Human Telomeres and Telomerase

Human telomeres look very much like the telomeres of other eukaryotes in that they contain an array of tandem DNA repeats. We share the telomeric sequence $(TTAGGG)_n$ with all other vertebrates as well as with a few distant relatives in other kingdoms (ref. 10; reviewed in refs. 6, 7, and 9). Human chromosomes end in several kilobases of telomeric repeat DNA oriented so that the G-rich strand runs out to the 3′ end of the chromosome. Despite their monotonous sequence the last kilobase pairs of our chromosomes fulfill two important functions (6–9).

First, telomeres hide natural chromosome ends from factors that act on DNA termini. Unlike broken chromosome ends, which either get degraded or fuse to other DNA, telomeres are resistant to exonucleases and ligases. They also escape detection by the DNA damage checkpoints. A case in point is the RAD9 protein in yeast (11). Although this checkpoint responds to a single chromosome break and signals cell-cycle exit, it fails to notice the 32 telomeres in every haploid yeast nucleus (12). The termini of natural chromosome ends are probably concealed by a complex of specialized proteins that bind telomeric DNA. Such telomeric factors have been characterized in yeast and in several ciliates (see ref. 13 for review), and candidate telomere-binding activities have been found in extracts of vertebrate cells (14, 15). How these proteins contribute to the various activities ascribed to telomeres remains to be determined.

Telomeres also solve a problem associated with the replication of chromosomal DNA. The new 5′ end of a replicated linear DNA is predicted to lack the sequence of the RNA primer used to initiate that strand, resulting in a small gap that cannot be filled. Inevitably, this replication strategy leads to a gradual loss of terminal DNA. Although attrition due to primer removal may be modest (≈4 bp per end per division), the end result can be worse, depending on the position of the 5′-most primer. Telomeres can counter this effect by engaging telomerase, a telomere-specific DNA polymerase that adds telomeric repeats to chromosome ends (ref. 16; reviewed in ref. 8).

Telomerases use the 3′ telomeric end as a primer and employ an RNA template for the synthesis of G-rich telomeric repeats (17, 18). In the ciliate telomerases, these template RNAs are 160–200 nt in length and encode 1.5 telomeric repeat copies (8, 17). As RNA-dependent DNA polymerases, the ciliate telomerases may be classified as reverse transcriptases. Once telomerase polypeptides have been cloned, their evolutionary relationship to other, more notorious, reverse transcriptases should be revealed. Mammalian telomerases probably also depend on an RNA component, since they are inactivated by RNase A (19). However, neither the mammalian telomerase RNA with its anticipated $(CCCUAA)_n$ template nor the associated proteins has been isolated so far.

Telomeric Decline in Somatic Cells

Early glimpses of human chromosome ends indicated that something unexpected was happening in somatic cells (20). Somatic (peripheral blood) telomeres appeared significantly shorter than germline (sperm) telomeres from the same individual (20–22). It is now clear that in most (if not all) tissues chromosomes gradually lose their terminal TTAGGG repeats with each division. As a result, skin and blood telomeres diminish by 15–40 bp per year, and the telomeres of *in vitro* cultured fibroblasts, T cells, embryonic kidney cells, mammary epithelium, and cervical cells lose 50–200 bp of TTAGGG repeats per population doubling (1, 23–29). By contrast, sperm telomeres increase in length with donor age, indicating that telomeres are actively maintained and even elongated in the germline (25).

The simplest explanation for these dynamics is that human telomerase is active in germline cells but somehow switched-off in the soma (Fig. 1). In agreement with this model, telomerase activity is not detected in extracts of embryonic kidney cells, peripheral blood leukocytes, and normal ovary epithelium (2, 5, 29). However, future molecular probes for telomerase will be required to establish this expression pattern more rigorously.

Does the decline of telomeric DNA limit the lifespan of human somatic cells? At first glance human telomeres seem long enough (6–10 kb at birth) to endure a lifetime of replicative neglect. However, it is not known how many kilobase pairs of TTAGGG repeats are actually required for full telomere function, the exact amount of TTAGGG repeats at young telomeres is hard to establish, and the rate of telomere loss may vary in different cell types. The strongest indication that telomeric decline could play a role in cellular aging comes from the analysis of primary human fibroblasts grown in culture (1, 25). These cells lose telomeric DNA at a rate of ≈50 bp per doubling and eventually stop dividing at a senescence stage called M1. Remarkably, there is a good correlation between the number of divisions the fibroblasts execute before they senesce and their initial telomere length (25). Based on this and other correlative data, it has been

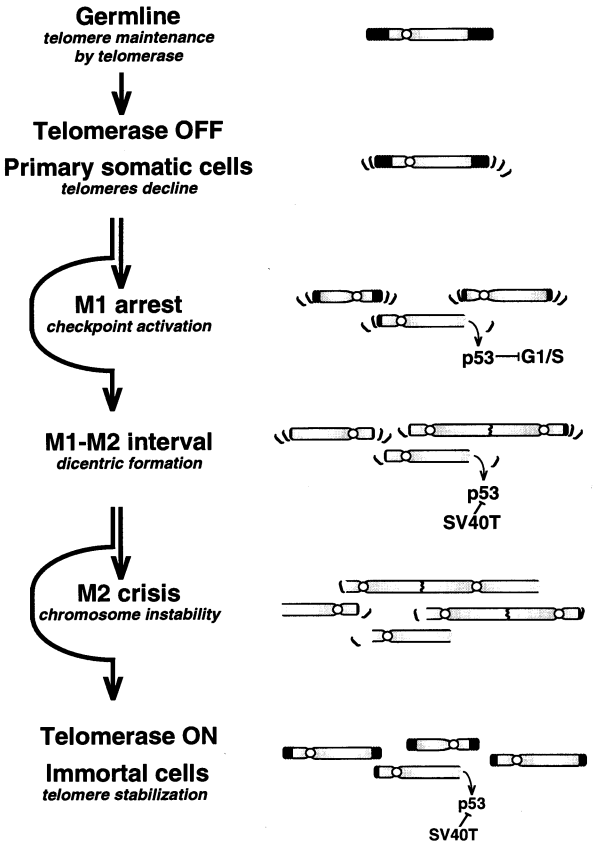

FIG. 1. Speculative model for the role of telomere loss and telomerase expression in senescence and immortalization of human cells. SV40T, simian virus 40-encoded large tumor antigen.

proposed that telomeric decay represents a molecular clock that counts cell divisions and limits the replicative potential of primary cells (1, 3).

Telomere Loss and Cellular Senescence

If telomere loss is involved in the M1 senescence of primary human fibroblasts, what is the mechanism of this arrest? Three hints point to a possible answer. First, one of the factors required for the M1 arrest is p53, a cell-cycle checkpoint that can be induced by DNA damage, including DNA breaks (ref. 30; reviewed in ref. 4). Second, the arrest is reversible (31–33). By and large, M1 cells are not dying; they are arrested in the G_1 phase of the cell cycle. A third hint comes from the structure of M1 telomeres. Although telomeric decay is evident, the M1 arrest occurs at an early point when the telomeres are not yet critically shortened. For instance, the telomeres of embryonic kidney cells at M1 are at least 3 kb longer than the telomeres of 293, an immortal human cell line derived from the same tissue (2). Based on this data, it seems unlikely that the senescent cells are experiencing massive loss of telomere function. However, since human telomeres are extremely heterogeneous in length, each M1 cell could harbor one or more telomeric tracts that deviate from the average and fall short of telomere function. Although infrequent, such uncapped chromosome ends probably resemble a DNA break and may activate the p53-dependent cell-cycle arrest pathway. This view of the role of telomere loss in M1 arrest (Fig. 1) combines aspects of models discussed previously (2–4); alternative models invoke changes in expression of subtelomeric senescence genes due to modulation of telomeric silencing (4, 34).

M1 arrest can be bypassed by a variety of viral agents. For instance, the expression of simian virus 40-encoded large tumor antigen will allow fibroblasts and embryonic kidney cells to progress beyond M1; human papillomavirus and adenovirus have much the same effect in other cell types (2, 31–33). Such transformed cells continue to divide for as many as 50 divisions before they face a second crisis, called M2, which is characterized by a balance of cell divisions and cell death. M2 is not circumvented by viral transformation and the transformed cells are not immortal. Progression beyond the M2 crisis occurs at low frequency, apparently through the mutational alterations of unidentified cellular gene(s) that help to avoid this crisis.

Cells that have bypassed the M1 arrest continue to lose their telomeric DNA, resulting in telomeres that appear severely worn down by the time cells reach M2 (2) (Fig. 1). Since the cis-acting requirements for the various functions of human telomeres are not known, it is difficult to gauge at what point the continued decline might disable telomere function. An average telomeric tract size shorter than that found in M2 cells [≈1.5 kb or less (2)] may not be compatible with telomere function; telomeres with less than 1.5 kb of TTAGGG repeats have never been seen in dividing human cells. In addition, due to the size heterogeneity of human telomeres, many chromosome ends in M2 cells will carry considerably less than 1.5 kb of TTAGGG repeats or lack telomeric DNA altogether. According to this reasoning, it is not unlikely that M2 cells suffer from a widespread loss of functional telomeres and a concomitant drop in chromosome stability. Degradation of uncapped chromosome ends may eventually become a serious threat to essential genes. Perhaps more importantly, the unprotected chromosome ends may form dicentric chromosomes through end-to-end fusions, a chromosomal aberration that is typical of cells with extremely short telomeres (2, 29, 35, 36). With abundant dicentric chromosomes, cells may experience an intolerable rate of chromosome loss through nondisjunction or have mechanical problems in anaphase. In agreement with these predictions, the frequency of dicentric chromosomes appears to rise sharply *en route* to M2 (2, 29). Thus, both M1 and M2 can be explained on the basis of known effects of telomere loss. However, our understanding of human telomeres is still rudimentary, and the causal role of telomeric decline in either M1 or M2 will need to be tested directly, preferably by altering the length of telomeres in human cells.

Telomerase in Human Tumors

On occasion, virally transformed cultures yield immortal cells that have bypassed the M2 crisis. Interestingly, unlike senescent cells, the immortal cell lines express telomerase and their telomeres have stopped shortening (2, 28). Counter *et al.* (2) have proposed that telomerase activation is an obligatory step in the immortalization of human cells. Since telomerase is also active in human tumor cell lines, the possibility arises that telomerase might contribute to tumor formation as well.

But do the cells in a primary human tumor actually need functional telomeres? Since many human tumors are thought to lack the ability to detect DNA damage, tumors may pass beyond M1 into the M1–M2 interval. In this growth

phase, the instability of telomere-deficient chromosomes might contribute to tumor progression by facilitating hemizygosing, translocations, amplification, and other rearrangements. In agreement, the telomeres of most human cancers are drastically shortened compared with their precursors in normal neighboring tissue (refs. 22, 23, and 36; reviewed in ref. 9). Furthermore, human tumors often show dicentric chromosomes formed by end-to-end associations (refs. 35–37; see refs. cited in ref. 35). Clearly, these primary human tumors continue to expand while losing telomeric DNA and possibly telomere function. However, eventually the viability of any human cell, transformed or not, should be jeopardized by unchecked telomere loss. At the time of diagnosis, most primary human tumors have probably undergone 30–40 cell divisions, sufficient to grind down an average-size human somatic telomere. The deleterious consequences of this attrition could select out those cells that have activated telomerase.

According to these considerations, selection for cells with active telomerase might be expected at a late stage in the progression of human tumors. This prediction is not easily tested, because telomerase cannot yet be identified on protein or RNA blots and the activity assay is fairly demanding of the quality of the extract. Despite these problems, Counter *et al.* (5) were able to detect telomerase activity in extracts from metastasized ovarian carcinoma cells. All seven ascitic fluids that they tested for the synthesis of labeled $(TTAGGG)_n$ strings *in vitro* contained telomerase activity. By contrast, normal control cells, including healthy ovarian epithelium, did not yield detectable telomerase activity. Although the sensitivity of the assay was limited, the specific telomerase activity in the ovarian carcinoma samples appeared to be at least 10 times that in normal cells. As expected for cells with telomerase, the length of ovarian carcinoma telomeres, although very short, did not decline over many months of growth *in vivo* (in consecutive ascites samples) and *in vitro* (in cultured cells). These data show that telomerase is consistently activated in this late-stage tumor type.

Perspectives

Several issues are of immediate concern. We need to know which tumors have active telomerase, at what point the activity appears, and whether continued telomerase expression is required for tumor expansion. These questions are particularly relevant to the prospect of developing telomerase inhibitors as chemotherapeutic agents in cancer management. If normal telomerase activity is indeed limited to the germ line, such treatment strategies may have the advantage of targeting a tumor-specific enzyme. A disadvantage of telomerase inhibitors may be their predicted phenotypic lag, especially in tumors with long telomeres.

More basic questions beg to be answered as well. The idea that telomerase and telomere dynamics play a role in human cancer is entirely based on correlation. A rigorous assessment of the merits of these models will require molecular genetic approaches, most importantly the manipulation of telomerase activity in primary and immortal cells. The cloning of human telomerase components should go a long way toward these goals. Telomerase genes are also required to address the many questions about telomerase mechanism, expression, and regulation that have piled up over recent years.

Another puzzle that deserves a closer look is the structure and dynamics of mouse telomeres. Some species of wild mice have telomeres that are similar in length to human telomeres (38). However, the telomeres of other mice, notably all the *Mus musculus* research strains, are ≈10 times longer and do not show a concomitant increase in the rate of telomeric decline (38, 39). Should this observation put an end to speculations about the role of telomere dynamics in cellular senescence and immortalization? Various *ad hoc* explanations have been offered. For instance, these mice could contain a few telomeres that are much shorter, or their telomeres could be interrupted by nontelomeric DNA near the tips. However, in the absence of factual information on the structure of mouse telomeres, it remains possible that telomere loss as a means to suppress tumor formation is not conserved in all mammals. In this regard, it is provocative that spontaneous immortalization of mouse cells occurs at least 10^6 times more frequently than in human cells, indicating that not all barriers to immortalization are conserved between mouse and man.

Since telomeres are highly conserved, it is of interest to consider lessons from organisms more distantly related as well. Yeast is particularly suitable in this context. In an ingenious molecular amputation, Sandell and Zakian (12) removed a single telomere from a yeast chromosome. Similar to what may be happening in M1, these cells arrested in a RAD9-dependent fashion. A second experiment seems to mimic the proposed contribution of telomere loss to M2. Cells of the *est1* mutant strain lose telomeric DNA (est stands for ever-shorter telomeres), display chromosome instability, and eventually perish (40). Similarly, a lethal cell division crisis occurs when *Tetrahymena* cells add the wrong sequence to their telomere termini (18). Interestingly, the senescent *est* cultures yield occasional survivors whose chromosome ends seem to be repaired by the addition of subtelomeric repetitive DNA (41). This and other examples (42) should be a warning that unconventional solutions to the telomere problem may arise in experimental settings, in evolution, and perhaps also in the clinic.

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