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Commentary

Telomerase activity, cell proliferation, and cancer

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Telomerase and telomere length have received a lot of recent attention from the biomedical research community. The cancer field started to take an interest in telomerase and telomere length approximately 7 years ago. Early experiments showed that telomeres shorten in primary fibroblasts with increased divisions in culture (1, 2) and that telomerase activity was not detected in these primary cultures. In contrast, after immortalization, telomere lengths are stabilized and telomerase is active (3). This suggested that telomerase might be required for the indefinite growth of immortal cells in culture. Around the same time, other experiments showed that tumors have shorter telomeres than normal tissue (2, 4) and that telomerase is active in many tumors and not in many normal samples (5, 6). Thus, the idea emerged that telomerase might be required for tumor growth *in vivo*. Testing this idea has fed the exponential increase in telomerase papers in the past 5 years.

Telomerase Activity Is Associated with Cell Proliferation in Cultured Cells. Recent evidence suggests that the simple model of telomerase activation in transformed cells and tumors is not strictly accurate. Telomerase activity is present in some normal human tissues, and some immortal cells lack detectable activity (reviewed in ref. 7). In those cells that do express telomerase the activity, it is tightly growth-regulated. One of the first reports that telomerase is correlated to the growth index of cells was from Silvia Bacchetti and coworkers. They showed that extracts from mouse mammary tissue and skin samples that had low levels of telomerase activity had greatly elevated levels when the cells were grown in short term culture (8).

Subsequently, work on the down-regulation of telomerase during differentiation indirectly implicated cell proliferation as important for telomerase activity. Telomerase activity is decreased when a variety of different cultured cell types are induced to differentiate *in vitro* (9–15). Because differentiation led to cell cycle exit in most of these studies, the down-regulation of telomerase activity may be related simply to the proliferation status of the culture: Activity is high in the actively cycling culture and low in the quiescent differentiated cells.

Telomerase Components Are Correlated with Cell Proliferation. The levels of telomerase components in cells are also growth-regulated. The mouse telomerase RNA component is up-regulated in early hyperproliferative stages of tumor progression before telomerase activity is apparent (16, 17). Furthermore, mouse telomerase RNA component levels correlate well with levels of histone H4, a proliferation marker (17). In human tumors, *in situ* hybridization assays show that the levels of the telomerase RNA component are tightly correlated with the proliferation marker MIB-1 in ependymal tumors (18).

Recently, two protein components have been identified that are associated with human telomerase activity, TP-1 and a human homologue of the yeast EST2 protein (hEST2). TP-1 is present in a variety of human tissues (19, 20), and its levels are growth-regulated in lymphocytes. TP1 protein levels increase when T cells are activated and enter the cell cycle (R. Oulton and L. Harrington, personal communication). The hEST2 protein recently was cloned independently by four different groups (21–24). hEST2 expression correlates well with telomerase activity in tumors and established cell lines although it is not yet clear whether hEST2, like the RNA component, is more tightly linked to proliferation than is activity.

Telomerase Activity Is Correlated with Cell Proliferation in Normal Human Tissues Cells and Tumors. Although telomerase was proposed to be expressed in tumor and not normal human tissue, evidence has accumulated that telomerase is expressed in a variety of normal tissues and that it is growth-regulated. The discovery of low levels of telomerase activity in normal human blood samples (25, 26) quickly led to the observation that mitogenic stimulation of lymphocytes causes telomerase up-regulation (27–33). In fact, in normal mature T cells, the activity is specifically up-regulated on entry into S phase (34).

In addition to blood, telomerase has been detected in several other normal human cell types. Typically, it is the mitotically active cells in a given tissue that express telomerase. Skin samples have very low levels of telomerase activity. However, when skin layers were dissected, relatively high levels of telomerase activity were found in the proliferative basal layer whereas the quiescent dermis was telomerase-negative (35). Consistent with this finding, isolated normal epithelial cells and epithelial cell cultures are telomerase-positive (35, 36), as are cultures of normal human endothelial cells (37). In a previous issue of *Proceedings*, Belair *et al.* (38) reported that, although telomerase is not detected in isolated normal human uroepithelial cells, when these cells are grown in culture, telomerase was readily detected. This provides a further example of the regulation of telomerase with cell proliferation in culture. Furthermore, telomerase length did not shorten in these cultures.

Telomerase activity is also growth-regulated in human tissues *in vivo*. Three recent reports indicate that activity is present in endometrial tissue and is correlated tightly with proliferation during the menstrual cycle (39–41). In addition, mitotically active regions of human hair follicles and the proliferative zone of intestinal crypts also have telomerase activity (42, 43).

Telomerase Growth Regulation and the Telomerase Null Mouse. Studies in mice also suggest that telomerase activity is growth-regulated. Telomerase activity is up-regulated during multi-staged tumorigenesis in mice, although activity was not detected in 100% of the tumors even from genetically identical individuals (8, 16, 17, 44). Unlike with human cells, there was no evidence of telomere shortening that might provide selective pressure for cells that have telomerase activity (17). Furthermore, the ability of cells derived from a telomerase knockout mouse to form tumors suggests that telomerase activation is not an essential event in tumor formation, at least

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in mice (45). The simplest explanation for the high percentage of telomerase-positive tumors in mice is that telomerase activity is high because of the high percentage of cycling cells in some tumors.

Not All Actively Dividing Cells Express Telomerase. If telomerase clearly is growth-regulated in a number of tissues and cell types, does that suggest that telomerase is not activated *de novo* in any cancer? Not necessarily; there is evidence that telomerase must be activated for activity to be seen in some cases. There are numerous reports that some primary cell types, such as fibroblasts (46), mammary epithelium (47), and embryonic kidney cells (48), do not express telomerase activity even when they are cycling actively. When these cells are immortalized in a variety of ways, telomerase often is activated. In some cases, even established cell lines do not express telomerase. Telomere maintenance in these cells is thought to occur through an alternative (perhaps recombination mediated) mechanism (49). In addition, there are a number of benign hyperplastic conditions in which telomerase activity is not detected, for example uterine fibriods (46) and benign prostate hyperplasia (50). In mice, some tissues, such as liver, are telomerase-positive, but in human liver samples, no activity is detected (8, 51, 52).

Further evidence for the activation of telomerase, in at least some tumors, comes from the lack of correlation of enzyme activity levels with proliferation in mouse mammary tumors. Although the mouse telomerase RNA component levels were increased 2-fold and correlated well with proliferation markers, activity levels were increased 10-fold, suggesting a second level of regulation in addition to growth regulation (17). Thus, although telomerase is growth-regulated in tissues and cell types that express it, there may be some cases in which telomerase is truly repressed and activation must occur for telomerase expression.

Because cancer is a collection of different diseases with distinct origins, it may be that telomerase is activated in some cancers and not in others. In fact, even in the telomerase knockout mice, we may find in later generation mice that some types of tumors will be sensitive to the absence of telomerase activity and others will not. Because some kinds of tumors undergo more rounds of cell division than others, caused in part by different rates of apoptosis, we might expect to see an effect first in a tumor type with a high turnover rate. If telomerase is to be a target for anti-cancer therapies, it will be very important to know whether a particular tumor type requires the enzyme. Thus, more sophisticated assays that do more than simply determine the presence or absence of telomerase will be required to determine which—if any—human cancers might require telomerase activity.

Telomerase Activity as a Diagnostic Marker in Cancer. In a variety of cancers, evidence suggests that telomerase activity might be a useful diagnostic marker for disease stage outcome (reviewed in ref. 53). And, there is some indication that activity might be a useful prognostic marker for disease. Studies in colon (54), bladder (55), and thyroid (56) cancer suggest that telomerase may be useful for distinguishing different stages of the disease. In the prostate, where current markers do not distinguish benign hyperplasia from prostate cancer, telomerase apparently does distinguish (50, 57, 58). The ability to detect telomerase from very small samples might increase its utility as a marker. Fine needle aspirates can be used to assay telomerase from very few cells (59–61). In some cases, even a few cells obtained from an oral rinse have been used to detect head and neck squamous cell carcinoma (62), and exfoliated cells from urine or colonic washes have been used to detect bladder and colon cancer (54, 55, 63). In cancers in which few, if any, markers have been identified, such as meningiomas, there is high hope that telomerase activity might provide clinicians with an objective staging of disease progression (64).

Although evidence from several different areas indicates that telomerase activity is often associated with cell proliferation, evidence also suggests that there may be additional regulation in some tissue and cells types. Thus, telomerase might be a better marker than Ki-67, MIB1, or other proliferation markers as a cancer diagnostic. In many instances, telomerase activity may indicate high proliferation rates, and in others it may indicate activation followed by proliferation. Even if telomerase were "just" associated with cell proliferation, if it can be documented that it has practical value in diagnosis in at least some cancers, then the recent boom in clinical telomerase research will have served a very useful purpose.

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