Telomerase flies the coop: the telomerase RNA component as a viral-encoded oncogene

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Telomerase, the enzyme that elongates our telomeres, is crucial for cancer development based on extensive analyses of human cells, human cancers, and mouse models. New data now suggest that a viral telomerase RNA gene encoded by Marek's disease virus (MDV), an oncogenic herpesvirus of chickens, promotes tumor formation. These findings highlight the importance of telomerase in cancer and raise new questions regarding the mechanisms by which the telomerase RNA component supports tumorigenesis.

Marek's disease virus and lymphoma

Many of our first insights into oncogenes and tumor suppressor genes—genes dysregulated in human cancer—came from studies of cancer-causing viruses. The discovery that the potent tumorigenicity of transforming avian retroviruses is caused by capture of cellular oncogenes led to the identification of the protooncogenes c-myc and src, among others. An alternative tumorigenic strategy is used by DNA tumor viruses, such as SV40 virus and human papilloma virus, which encode their own oncoproteins that inactivate key tumor suppressor proteins, including p53 and Rb (1). In humans, herpes family viruses represent an important class of viruses that can induce cancer, principally in patients whose immune systems are suppressed by medications or HIV. Examples include Epstein-Barr virus, which can induce lymphoma, and human herpesvirus (HHV)8, the causative agent of Kaposi's sarcoma. These large DNA viruses can also stably acquire viral versions of host genes and use them to help transform human cells. For example, HHV8 expresses a viral D-type cyclin and a viral version of the growth factor NF-IL6 (2). Given the already impressive progress in understanding the mechanisms of viral transformation, is it likely that there are important clues to understanding cancer development that remain to be discovered through analysis of transforming viruses? The answer is undoubtedly yes, as revealed by recent work on MDV, an oncogenic herpesvirus that infects chickens. Infection with MDV leads to neurologic disease and rapid development of lymphoma (3). The virus was recently found to contain two identical copies of a viral telomerase RNA component (vTR), which were appropriated from the chicken genome, suggesting the tantalizing possibility that vTR acts as an oncogene (4).

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Telomerase, telomere shortening, and cancer

Telomerase comprises two essential subunits: the telomerase reverse transcriptase (TERT), and the telomerase RNA component (TR) (5). TR encodes the template sequence that is reverse transcribed by telomerase onto chromosome ends during telomere elongation. Together, TERT and TR represent the catalytic core of the enzyme required for addition of telomere sequences—TTAGGG nucleotide repeats in mammals and birds. In the absence of sufficient levels of telomerase, telomeres shorten with each cell division because of the inability of DNA polymerase to copy the end of the lagging DNA strand—known as the end replication problem. With continued telomere shortening in human fibroblast cultures, telomeres undergo a conformational change—telomere uncapping—that causes replicative senescence, a form of cell cycle arrest and altered gene expression requiring the p53 and Rb tumor suppressor pathways. Loss of p53 and Rb enables cell proliferation beyond this senescence checkpoint, but continued telomere shortening ultimately results in covalent ligation of telomere ends, as telomeres can no longer suppress recombination. This state—termed telomere-based crisis—is characterized by high rates of programmed cell death and chromosomal instability.

Telomere shortening also occurs in human tissues with advancing age, perhaps caused by restricted expression patterns of telomerase. Telomerase is active in the germline, but is limited in expression in many adult tissues to stem cells and progenitor cells (6). Although telomerase is repressed in most somatic cells, it is reactivated in ~90% of human cancers (7). Much of this regulation appears to center on transcriptional control of the TERT component, which is restricted in its expression. In contrast, TR is expressed more broadly, leading to the generally held belief that TERT is a limiting component for telomerase activity. Telomerase expression is critical for tumor development. For example, expression of TERT is sufficient to immortalize primary human cells (8) and is required for experimental transformation of primary human cells expressing numerous oncogenes (9). In addition, telomerase knockout mice with short telomeres exhibit profound resistance to tumorigenesis (10, 11). Although these and other findings definitively link telomerase to immortal proliferation and cancer, many of the genetic changes associated with classical oncogenes—mutation, amplification, or expression by transforming viruses—have been lacking for telomerase components.

Potential oncogenic mechanisms of vTR

This discrepancy is what makes the findings of Trapp et al. in this issue (p. 1307) so important to the study of telomerase and cancer (12). The authors set out to address whether the vTR genes in MDV are important in malignant transformation by the virus. The two vTR genes are 88% identical to the chicken TR gene (cTR) (4). The vTR gene, when expressed in mouse cells deficient in mouse TR, reconstituted telomerase activity, indicating that vTR is capable of supporting telomerase enzymatic activity. Trapp et al. deleted both copies of vTR in an oncogenic strain of MDV and found that lymphomagenesis in infected chickens was reduced by 60% in the vTR deletion strains compared with wild-type virus. Furthermore, when animals infected with the vTR deletion strains did develop lymphoma, these cancers were smaller in size and less widely disseminated. Importantly, deletion of vTR did not impair lytic replication of the virus, indicating that vTR serves a role supporting lymphomagenesis, rather than viral replication (12). Thus, the vTR gene exhibits attributes of an oncogene, enhancing the incidence and severity of lymphoma caused by MDV.

These important findings raise a series of new questions centered on the problem of how and why expression of vTR in MDV is oncogenic. The most straightforward interpretation is that vTR enhances telomerase activity leading to stabilization of telomeres. According to this model, a subset of telomeres in lymphoid cells infected with MDV must become critically short. In the absence of vTR, telomere uncapping in these lymphocytes would reduce tumor formation, whereas in wild-type MDV strains that express vTR, lengthening of these short telomeres would allow full malignant potential. Consistent with this idea, vTR was shown to yield increased telomerase activity compared with cTR, when combined in vitro with recombinant chicken TERT protein (13). This model implies that in the target lymphocyte population cTR levels are limiting and TERT protein is in excess, such that telomerase activity rises when vTR is expressed from the virus. Alternatively, another viral gene may lead to stimulation of endogenous TERT levels enabling increased telomerase when coupled with expression of vTR.

Another distinct possibility is that vTR acts through a mechanism that is independent of telomere synthesis. Emerging evidence from several laboratories has indicated that telomerase has additional roles independent of telomere length. Transgenic expression of TERT in mice led to an increased number of carcinogen-induced skin papillomas (14) and to an elevated incidence of spontaneous breast cancers (15). Since mouse telomeres are sufficiently long that telomere uncapping does not occur in mouse tissues, these prooncogenic effects of TERT are not thought to require telomere elongation. In human cells that maintain their telomeres through a telomerase-independent mechanism (known as alternative lengthening of telomeres [ALT]), expression of TERT was necessary for malignant transformation and growth of tumors in immunocompromised mice (16). This effect of TERT in ALT cells appears to extend to TR as well, as overexpression of mouse TR was necessary to enable mouse ALT cells from $TR^{-/-}$ mice to grow efficiently as metastatic nodules in lung (17). These results are reminiscent of the current findings in MDV.

In addition to these cancer-causing activities, telomerase has recently been found to exhibit profound effects on stem cells in mouse skin (18, 19). Conditional expression of TERT in mouse skin led to activation of quiescent stem cells in the hair follicle and a rapid developmental change in the follicle from the resting phase (telogen) to the active phase of the hair follicle cycle (anagen) (18). Induction of anagen by TERT facilitated robust hair growth. These effects of TERT did not require TR and were therefore genetically separable from TERT's well-understood role in elongating telomeres. Could TR also exhibit activities independent of its role in serving as a template for telomere addition that might explain its transforming activity in MDV? Recent loss-of-function data in human cancer cells lines support this idea. Depletion of human TR (hTR) through RNA interference in human cancer cell lines reduced the rate of cell proliferation. This effect of hTR depletion was not caused by telomere uncapping, as there was no evidence of telomere dysfunction, such as an increase in DNA damage foci (20, 21). Instead, cells treated with hTR siRNA showed a marked change in gene expression profiles that may explain the effect of hTR depletion. Thus, the deleterious, telomere length-independent effect of hTR loss on cancer cell proliferation could represent the flip side of the prooncogenic effects of vTR overexpression in MDV.

Future questions

The findings of Trapp et al. suggest several high priority experiments to address the mechanism of vTR's transforming activity (12). It will be critical, for example, to determine if vTR contributes to lymphomagenesis by extending telomeres. One genetic approach to this question would be to determine if chicken TERT is required for vTR's effects. Alternatively, one could dissect the sequence requirements within vTR necessary for complementing vTR-deficient MDV in transformation. Through deletion or mutation approaches, one can test if vTR's templating function is required for lymphomagenesis. An equally interesting question is whether vTR's activities in supporting lymphoma development are shared with its cellular counterpart cTR. If cTR cannot restore the full transforming activity of the vTR-deficient MDV strains, it would argue that the few sequence differences between the two genes are important in transformation. Ultimately, understanding how vTR supports lymphomagenesis in genetic and molecular terms will yield important new insights into telomerase function in cancer.

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