Human tumor viruses are responsible for one-fifth of all cancers worldwide. These viruses have evolved multiple strategies to evade immune defenses and to persist in the host by establishing a latent infection. Proliferation is necessary for pretumor cells to accumulate genetic alterations and to acquire a transformed phenotype. However, each cell division is associated with a progressive shortening of the telomeres, which can suppress tumor development by initiating senescence and irreversible cell cycle arrest. Therefore, the ability of virus-infected cells to circumvent the senescence program is essential for the long-term survival and proliferation of infected cells and the likelihood of transformation. We review the multiple strategies used by human DNA and RNA tumor viruses to subvert telomerase functions during cellular transformation and carcinogenesis. Epstein-Barr virus, Kaposi sarcoma–associated herpesvirus, human papillomavirus, hepatitis B virus, hepatitis C virus, and human T-cell leukemia virus-1 each can increase transcription of the telomerase reverse transcriptase. Several viruses appear to mediate cis-activation or enhance epigenetic activation of telomerase transcription. Epstein-Barr virus and human papillomavirus have each developed posttranscriptional mechanisms to regulate the telomerase protein. Finally, some tumor virus proteins can also negatively regulate telomerase transcription or activity. It is likely that, as future studies further expose the strategies used by viruses to deregulate telomerase activity and control of telomere length, novel mechanisms will emerge and underscore the importance of increased telomerase activity in sustaining virus-infected cells and its potential in therapeutic targeting.

Telomeres, which form the ends of eukaryotic chromosomes, are composed of tandem arrays of telomeric repeats (5′-TTAGGG-3′ in humans) and help to preserve genome integrity and to prevent senescence (1,2). Somatic cells have a finite proliferative capacity, due largely to the inability of DNA polymerase to replicate the distal ends of chromosomes, which leads to a progressive shortening of the telomeres after each cell division. Immortal tumor cells overcome this barrier mainly by increasing transcription of telomerase, a cellular reverse transcriptase that can extend telomeric ends (3–5). Increased telomerase activity may contribute indirectly to the transformation process by sustaining the proliferation of pretumor cells indefinitely, thereby increasing the potential risk of cumulative genetic defects. Only 10% of human tumor cells do not express telomerase; these cells use an alternative lengthening of telomeres pathway, referred to as ALT, to maintain telomere length (6). Recent studies show that the ALT pathway involves the DNA repair and homologous recombination machinery (7,8).

Telomerase is a holoenzyme that consists of several subunits, including hTR (human telomerase RNA), TERT (telomerase associated protein 1), hTERT (human telomerase reverse transcriptase), hsp90 (heat shock protein 90), p23, and dyskerin (1). The ability of telomerase to extend telomere length is subject to complicated controls. Not only is the bTERT gene subject to transcriptional, posttranscriptional, and epigenetic control, but access of telomerase to the telomere ends is also regulated by the shelterin complex. Shelterin consists of the double-stranded DNA-binding proteins TRF1 and TRF2 (TTAGGG repeat binding factor-1 and -2), the single-stranded DNA-binding protein POT1 (protection of telomere), and their respective binding partners TIN2 (TRF1-interacting nuclear protein 2) and PTOP (POT1- and TIN2-interacting protein) (9). Shelterin provides telomerase with signals regarding the length of telomeres that allow or prevent telomerase from gaining access to the telomere ends. In addition, the shelterin components can form various subcomplexes that determine the structure of the telomere ends and preserve the integrity of telomeres by preventing DNA damage pathways from becoming activated (10).

Although telomere attrition can lead to chromosomal abnormalities, a direct role for telomeres or telomerase in the transformation process is not likely. Somatic cells expressing telomerase can extend their replicative lifespan but are unable to form tumors in nude mice and do not form colonies in soft agar (11). By contrast, normal human epithelial cells and fibroblasts can be converted to tumorigenic cells with expression of transfected bTERT and Ras oncogenes along with the gene encoding the large T-antigen of simian virus 40 (SV40) (12). Removal of SV40 T-antigen from the transfection mixture for several human cell
lines still led to transformation as long as Rb and p53 were inactivated and Ras, c-Myc, and hTERT were overexpressed (13). Taken together, these findings suggest that overexpression of telomerase by itself is not sufficient to induce a tumorigenic state in normal cells but that it can contribute to tumorogenesis in the presence of additional oncogenes and/or the disruption of tumor suppressors.

There is a strong need for human tumor viruses to regulate telomerase and telomere length. In some cases, enhancement of telomerase activity may be part of a cooperative transformation mechanism. In addition, since tumor viruses regularly reprogram infected cells to rapidly proliferate, if viral regulation of telomerase were not present, telomeres would shorten over the many rounds of cell division, inducing cell senescence and cell cycle arrest. Here, we review the strategies used by human tumor viruses to promote telomerase activity and to maintain telomere length. Many tumor virus proteins act as transcription factors or act by other means to increase telomerase expression. A few viruses have also evolved mechanisms for posttranscriptional regulation of telomerase. Last, some viruses have also evolved an additional mechanism for negative regulation of telomerase, presumably to regulate some aspects of the complex relationships that exist between these viruses and their hosts.

**Overview of Telomerase Activity and Telomere Length in Human Oncogenic Virus Infections**

**Epstein-Barr Virus**

EBV infection is associated with Burkitt lymphoma, Hodgkin disease, nasopharyngeal carcinoma, and infectious mononucleosis along with approximately 10% of gastric cancers and a subset of T-cell lymphomas (14). EBV mainly infects and immortalizes B lymphocytes, but it can also produce latent infections in T lymphocytes or natural killer cells. Analysis of samples from patients with nasopharyngeal carcinoma revealed that 97.5% were positive for EBV and 94.9% were positive for telomerase activity (15), and analysis of EBV-immortalized B-lymphoblastoid cell lines revealed increased telomerase activity (16). Both findings support an important role for telomerase reactivation in EBV-infected individuals.

The EBV-encoded latent membrane protein-1 (LMP1) displays properties of a constitutively active member of the tumor necrosis factor receptor family. LMP1 is expressed during viral latency and leads to the activation of the NF-κB and Jak/STAT signaling pathways and oncogenic transformation (17). LMP1 is at least partially responsible for the increase in telomerase activity in nasopharyngeal epithelial cells infected with EBV (18). LMP-1 also appears to enhance telomerase activity in B-cell lymphomas, in which expression of small-interfering RNAs (siRNAs) directed against LMP1 substantially reduced hTERT protein levels and telomerase activity (19). Moreover, expression of LMP1 in an EBV-negative nasopharyngeal carcinoma cell line increased hTERT protein expression (19). Although hTERT has not been implicated in regulation of gene expression, a recent study suggests that hTERT expression decreases EBV lytic gene expression and promotes proliferation of latently infected B lymphocytes (20). Other work in B lymphoblasts suggests that immortalization may be associated with the reactivation of telomerase activity, along with aneuploidy and changes in p16, Rb, and p53 status; however, this finding has yet to be confirmed by independent laboratories (21,22).

EBV latent membrane protein 2A (LMP2A) induces constitutive activation of downstream effectors of the B-cell receptor, preventing infected cells from entering the lytic replication cycle (23). LMP2A is also involved in transformation of epithelial cells. Transient transfection assays indicate that LMP2A may act as a negative regulator of the hTERT promoter (24).

Analysis of telomere lengths in EBV-positive Burkitt lymphoma cell lines showed increases in telomere length compared with those in EBV-negative cells (25,26). Although infections by many tumor viruses have been associated with increased telomerase activity, EBV is, to date, the only human oncogenic virus for which infection has been linked to an actual increase in telomere length.

**Human Herpesvirus 8/Kaposi Sarcoma–Associated Herpesvirus**

HHV-8/KSHV is the etiologic agent for Kaposi sarcoma, multifocal Castleman disease, and primary effusion lymphoma (27). KSHV-transformed endothelial cells have elevated telomerase activity compared with uninfected cells (28). The latency-associated nuclear antigen (LANA) protein of HHV-8/KSHV plays an important role in latent episomal persistence of the viral genome in infected cells (29). LANA targets p53 and Rb and plays a role in B-cell lymphoma development (30). LANA has been found to increase expression at the hTERT promoter through modulation of Sp1 in 293 fibroblasts and the B-cell lines BJAB and BCBL1 (31,32).

To date, the influence of HHV-8/KSHV infection on telomere length in HHV-8/KSHV–infected cells has not been reported. However, it has been shown that a gene whose product is associated with angiogenesis and lytic infection, the G protein–coupled receptor oncogene (vGPCR) of KSHV, can immortalize human umbilical vein endothelial cells in which telomere length is maintained by the ALT pathway (33). A direct oncogenic role for vGPCR has not been established as it is an early lytic gene not expressed in HHV-8/KSHV–transformed cells.

**Human Papillomavirus**

Over 100 types of HPV are responsible for a wide array of human diseases, ranging from malignant cervical cancer to benign warts (34). High-risk HPV16 and 18 are the genotypes most commonly associated with cancer (35,36).

Early studies with HPV16 found elevated telomerase activity in precancer human cervical keratinocytes that express the viral protein E6 (37). The HPV E6 protein cooperates with the viral E7 protein to transform infected cells by targeting p53 and the retinoblastoma (pRb), respectively, for proteasomal degradation (38).

Further studies indicated that E6-mediated elevation of telomerase activity requires an additional protein, the E6-associated protein (E6AP) (39). E6AP is a ubiquitin–ligase that is involved in p53 degradation. Because E6AP is also involved in ubiquitination, it has been suggested that E6 targets a cellular inhibitor of telomerase activity for degradation using the ubiquitin machinery that is
also used to degrade p53. A recent study demonstrated that E6 mutants that are defective in E6AP binding retained their ability to stimulate \textit{hTERT} expression, suggesting the existence of both E6AP-dependent and -independent mechanisms for E6-mediated increase in \textit{hTERT} expression (40).

HPV16 E6–expressing fibroblasts had an increased lifespan and did not enter cellular senescence, unlike control cells that also had a marked decrease in telomere length (41). In these E6–expressing cells, the shortened length of the telomeres was eventually stabilized through an ill-defined mechanism.

A similar pattern—that is, increased telomerase activity following viral infection and initial telomere shortening followed by stabilization—is found in HPV-infected cells, as in nearly all human infections by oncogenic viruses. For example, analysis of patient samples from early stages of cervical intraepithelial neoplasia to later, malignant stages of this disease demonstrated the persistence of shortened telomeres and strong telomerase activity in all stages of disease (42).

HPV early gene E2 is important for viral transcription through E2-responsive elements and replication (43). E2 can also suppress growth of HPV-positive cancer cells through transcriptional repression of E6 and E7 (44). Because E6 is a positive regulator of the \textit{hTERT} promoter, it is expected that E2 would act as a negative regulator in the context of HPV-infected cells by preventing the action of E6.

**Hepatitis B Virus and Hepatitis C Virus**

HBV and HCV infect hepatocytes, leading to chronic liver disease and malignant transformation. Combined, they account for 70% of hepatocellular carcinomas worldwide (45). Tissue biopsies from HBV-positive patients exhibit strong telomerase activity compared with those from normal liver tissue, irrespective of disease stage (46). Short telomeres were present in HBV- and HCV-positive tissues despite elevated telomerase activity (47). Similarly, telomeres have been found to be shorter in hepatocellular carcinoma than in noncancerous liver tissues isolated from the same patient (48,49).

HBV encodes two proteins with transcriptional activator functions, the HBV-X and preS2 activators (large surface proteins and truncated middle surface proteins, respectively) (50). The HBV-X transactivator oncprotein (HBX) stimulates viral gene expression and cellular transformation by altering p53 functions and disrupting multiple cell signaling pathways (51). HBX has been shown to increase telomerase expression and telomerase activity in hepatoma cells (52). Transfection of the \textit{preS2} gene into a hepatocellular carcinoma cell line was also associated with an increase in telomerase expression and activity (53).

**Human T-Cell Leukemia Virus Type 1**

HTLV-1 is the etiologic agent of adult T-cell leukemia/lymphoma, a lymphoproliferative disorder of infected CD4+ T-cells (54). Similar to the pattern seen in HPV, HBV, and HCV infections, telomeres are short in HTLV-1 infected cells despite the presence of strong telomerase activity (55). Telomerase activity was high in both HTLV-1-immortalized and HTLV-1–transformed cell lines as well as lymphocytes from patients with adult T-cell leukemia/lymphoma, including those with acute, smoldering, and chronic disease, compared with peripheral blood mononuclear cells from uninfected individuals and samples from asymptomatic carriers (55–57). Disease progression, from the asymptomatic stages to acute and chronic disease, was associated with increased telomerase activity, such that patients with acute and chronic disease exhibited a higher level of telomerase activity than asymptomatic carriers of the virus (55,57). Multiple other studies have confirmed that HTLV-1–infected cells have elevated telomerase activity (58,59). Additional studies found that increased telomerase activity was not associated with transformation status as measured by IL-2 dependence (55).

A direct role for HTLV-1 in the elevation of \textit{hTERT} expression was demonstrated by in vitro transmission of the virus to human primary T cells, which led to increased \textit{hTERT} promoter expression in infected cells only (60). Similar results were obtained by transduction of lymphocytes with a Tax-expressing vector. Although one study proposed that the HTLV-1–encoded Tax protein had a negative effect on an \textit{hTERT} promoter reporter vector in transient assays (61), current data indicate that Tax is a strong positive regulator of the endogenous \textit{hTERT} promoter (60,62).

The initial report that Tax acts as a repressor of the \textit{hTERT} promoter (61) turned out to be misleading because these experiments were performed in the presence of phytohemagglutinin (PHA), a potent inducer of \textit{hTERT} expression. The results of that article showed that PHA-mediated induction of \textit{hTERT} was indirectly lessened by Tax; however, such effects were not seen when Tax was expressed in the absence of PHA stimulation. Thus, Tax should be considered as a positive regulator of the \textit{hTERT} promoter. It remains to be demonstrated whether, in the context of HTLV-1 infected cells, Tax prevents full induction of \textit{hTERT} expression under physiologic conditions that trigger cell activation (PHA or antigen stimulation).

**Model for Telomere Maintenance in Virus-Infected Cells**

A list of the tumor virus–encoded regulators of telomerase activity that we have described is presented in Table 1. Current data support a model in which the initial stages of viral infection are associated with progressive telomere shortening despite an increase in \textit{hTERT} expression (Fig. 1). Shortened telomere lengths are eventually stabilized, and maintenance of telomere size becomes dependent on elevated telomerase activity (53). The reasons for the initial telomere shortening and stabilization are unclear. Variations in the

<table>
<thead>
<tr>
<th>Virus</th>
<th>Viral-encoded regulator</th>
<th>Impact on telomerase</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBV</td>
<td>LMP1</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>LMP2A</td>
<td>Negative</td>
</tr>
<tr>
<td>HHV-8</td>
<td>LANA</td>
<td>Positive</td>
</tr>
<tr>
<td>HPV</td>
<td>E6</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>E6AP (through NFX1-123)</td>
<td>Negative (through NFX1-91)</td>
</tr>
<tr>
<td>HCV</td>
<td>E2</td>
<td>Negative</td>
</tr>
<tr>
<td>HTLV-1</td>
<td>HBX</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Core</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Tax</td>
<td>Positive</td>
</tr>
</tbody>
</table>

*EBV = Epstein-Barr virus; HHV-8 = human herpesvirus 8; HPV = human papillomavirus; HBV = hepatitis B virus; HCV = hepatitis C virus; HTLV-1 = human T-cell leukemia virus type 1; LMP1 = latent membrane protein 1; LMP = latent membrane protein 2A; LANA = latency-associated nuclear antigen; E6AP = E6-associated protein; HBX = HBV-X transactivator protein; HCV-C = HCV core protein; NFX1 = nuclear transduction factor, X-box binding 1.*
composition of the shelterin complex or accessibility of telomerase to the telomeres may explain this phenomenon. The presence of shortened telomeres and aneuploidy in cancer cells is intriguing, but, to date, there is no experimental evidence that telomere attrition induces chromosome instability.

In some instances, short telomeres may be markers of poor prognosis in disease progression, as is the case in hepatocellular carcinoma and several leukemias, including chronic myeloid leukemia, chronic lymphocytic leukemia, and HTLV-1–associated adult T-cell leukemia/lymphoma (63–66). However, in other types of cancer, such as HPV-associated cervical cancer, there is no obvious relationship between telomere length and clinical outcome (67). Although telomeres were substantially shortened in all cervical intraepithelial neoplasia samples analyzed in one study (42), no further telomere shortening occurred in the majority of samples from these patients during transition to a malignant phenotype.

Mechanisms of Viral Activation of Telomerase

hTERT expression is regulated primarily at the level of transcription (68). The hTERT promoter contains several E-boxes and five GC-rich elements, which can bind c-Myc and Sp1, respectively (69). It is well established that hTERT gene expression is increased following c-Myc binding to E-box elements present in the hTERT promoter (70,71). In fact, in most cells, c-Myc and Sp1 act cooperatively as the dominant determinants of hTERT expression (69,72). A more complete listing of various factors known to regulate hTERT transcription can be found in Table 2. The most important tumor virus proteins, known to directly regulate hTERT transcription, are shown in boldface type in Table 2, and are discussed below.

trans-Activation of the hTERT Promoter by Viral Oncoproteins

Most studies of viral trans-activation of hTERT gene expression have been done with the papillomavirus system, in which the HPV E6 oncoprotein has been shown to directly trans-activate the hTERT promoter (73). Mutations in either the GC or the E-box elements of the hTERT promoter modestly inhibit telomerase expression, demonstrating that independent binding of c-Myc or Sp1 to the hTERT promoter is not sufficient for E6-induced telomerase activation (73). However, telomerase expression is strongly induced by E6 when c-Myc and Sp1 are cooperatively bound to the hTERT promoter (73). Although E6 had no effect on levels of c-Myc gene expression, immunoprecipitation studies demonstrated that E6 forms a complex with c-Myc on the hTERT promoter (74). The use of E6AP knockout mice and E6AP siRNA has demonstrated that E6AP is important for hTERT promoter activation (75). Both E6 and E6AP are required for binding to the E-box elements and for
the consequent activation of hTERT expression (75). Indeed, a yeast two-hybrid screen identified NFX1 (nuclear transcription factor, X-box binding 1) as a binding partner of the E6/E6AP complex. NFX1 exists in two isoforms, NFX1-123 and NFX1-91. NFX1-123 has been shown to activate the hTERT promoter in cooperation with c-Myc (39).

In the case of papillomavirus infection, one possible model for the mechanism of hTERT trans-activation is that E6 forms a ternary complex with E6AP and c-Myc. Once the complex is bound to E-box elements, E6AP could target the degradation of possible negative regulators of the hTERT promoter through activation of the NF-κB pathway. Chromatin immunoprecipitation assays demonstrated increased binding of c-Myc and Sp1 at the hTERT promoter in response to NF-κB activation in both HTLV-1-infected and Tax-expressing cells (60). In fact, a Tax mutant unable to activate the NF-κB pathway could not stimulate hTERT expression (60).

The LMP1 protein of EBV has been found to induce c-Myc-mediated trans-activation of the hTERT promoter in primary human nasopharyngeal epithelial cells and in a nasopharyngeal carcinoma cell line that stably expresses LMP1 (18). In this case, the C-terminal portion of LMP1, which includes CTAR1 and 2 domains, was found to stimulate hTERT gene expression through NF-κB activation.

Lastly, the LANA protein of KSHV has been shown to trans-activate the hTERT promoter in various cell lines (31). LANA activates hTERT gene expression through interactions with Sp1 (32).

Much less is known about the mechanisms used by other viral oncoproteins to trans-activate hTERT gene expression. The Tax protein of HTLV-1 is able to stimulate the hTERT promoter through activation of the NF-κB pathway. Chromatin immunoprecipitation assays demonstrated increased binding of c-Myc and Sp1 at the hTERT promoter in response to NF-κB activation in both HTLV-1-infected and Tax-expressing cells (60). In fact, a Tax mutant unable to activate the NF-κB pathway could not stimulate hTERT expression (60).

Table 2. Tumor virus interactions with transcriptional activators and repressors of the hTERT promoter*

<table>
<thead>
<tr>
<th>Regulator</th>
<th>Impact on hTERT</th>
<th>Mode of action</th>
<th>EBV</th>
<th>HHV-8</th>
<th>HPV</th>
<th>HBV</th>
<th>HCV</th>
<th>HTLV-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bmi-1</td>
<td>Positive</td>
<td>—</td>
<td>(+) LMP1</td>
<td>—</td>
<td>(+) E6</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>c-Fos/c-Jun</td>
<td>Negative</td>
<td>Direct</td>
<td>(+) LMP1</td>
<td>(+) LANA</td>
<td>(+) E6</td>
<td>(+) X</td>
<td>(+) NS5B</td>
<td>(+) Tax</td>
</tr>
<tr>
<td>c-Fos/JunD</td>
<td>Negative</td>
<td>Direct</td>
<td>(+) vFLIP</td>
<td>(+) LANA</td>
<td>(+) E6</td>
<td>(+) X</td>
<td>(+) NS5A</td>
<td>(+) Tax</td>
</tr>
<tr>
<td>c-Myc</td>
<td>Positive</td>
<td>Direct</td>
<td>(+) LMP1</td>
<td>(+) LANA</td>
<td>(+) E6/E6AP</td>
<td>(+) X</td>
<td>(+) Core</td>
<td>(+) Tax</td>
</tr>
<tr>
<td>E2F-1</td>
<td>Negative</td>
<td>Sp1</td>
<td>(+) BZLF1</td>
<td>(+) LANA</td>
<td>(+) E7</td>
<td>(+) X</td>
<td>(+) Core</td>
<td>(+) Tax</td>
</tr>
<tr>
<td>IRF-1</td>
<td>Negative</td>
<td>—</td>
<td>—</td>
<td>(−) vFLIP</td>
<td>(−) E7</td>
<td>—</td>
<td>(−)</td>
<td>—</td>
</tr>
<tr>
<td>p16INK4A</td>
<td>Negative</td>
<td>—</td>
<td>(−) LMP1</td>
<td>(−) (+)</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>(−) Tax</td>
</tr>
<tr>
<td>p72BMI-1</td>
<td>Negative</td>
<td>Direct</td>
<td>—</td>
<td>(−) vIRF</td>
<td>(−) E7</td>
<td>—</td>
<td>(−)</td>
<td>(−) NS5A</td>
</tr>
<tr>
<td>p53</td>
<td>Negative</td>
<td>p21 and E2F</td>
<td>(−) LANA</td>
<td>(−) E6</td>
<td>(−) X</td>
<td>(−) NS5</td>
<td>(−) NS5A</td>
<td>(−) NS5A</td>
</tr>
<tr>
<td>p73</td>
<td>Negative</td>
<td>c-Myc</td>
<td>(−)</td>
<td>—</td>
<td>(−) E6</td>
<td>(−) p73α/Core</td>
<td>(−) Tax</td>
<td></td>
</tr>
<tr>
<td>Smad3</td>
<td>Negative</td>
<td>c-Myc</td>
<td>(−) LMP1</td>
<td>(−) vIRF</td>
<td>(−) E7</td>
<td>(−)</td>
<td>(−) Core</td>
<td>(−)</td>
</tr>
<tr>
<td>Sp1</td>
<td>Positive</td>
<td>Direct</td>
<td>(+) EBNA2</td>
<td>(+) LANA</td>
<td>(+) E6</td>
<td>(+) X</td>
<td>(+) Core</td>
<td>(+) Tax</td>
</tr>
<tr>
<td>Survivin</td>
<td>Positive</td>
<td>c-Myc/Sp1</td>
<td>(+) LMP1</td>
<td>(+) (+)</td>
<td>(+) E6</td>
<td>(+) X</td>
<td>(+) NS5A</td>
<td>(+) Tax</td>
</tr>
<tr>
<td>USF-1/2</td>
<td>Negative</td>
<td>—</td>
<td>—</td>
<td>(−)</td>
<td>(−) E6</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>WT1</td>
<td>Negative</td>
<td>Direct</td>
<td>(+)/</td>
<td>−(−)</td>
<td></td>
<td>(−) X</td>
<td>(−) NS5</td>
<td>(−)</td>
</tr>
</tbody>
</table>

* A selection of known regulators of hTERT promoter activity. (+) = positive interaction; (−) = negative interaction. The identity of the viral interacting protein is given when known. Those viral proteins that have an established role in direct hTERT regulation are discussed in the text and shown in bold. Bmi-1 = polycomb-group gene; c-Myc = myelomonocytic leukemia gene; E2F-1 = E2F transcription factor-1; IRF-1 = interferon-regulatory factor-1; Sp1 = specificity protein-1; WT1 = Wilms’ tumor protein-1; USF-1/2 = upstream stimulatory factor-1/2; BZLF1 = bZIP transactivator of EBV [initiates the switch between latent and productive infection in B cells (124)]; vFLIP = viral FLICE inhibitory protein [activates NF-κB and protects from apoptosis (125)]; vIRF = viral homolog of the interferon regulatory factors [represses interferon antiviral response (126)]; NS4B = nonstructural protein of HCV [part of the replication complex of HCV (127)]; NS5A = nonstructural protein of HCV [aids in viral invasion of the immune system (128)]; NS3 = nonstructural protein of HCV [signals the immune response (128)]. Dashes reflect a lack of available information, not negative data. See Table 1 notes for definitions of other abbreviations.
could occur by AP1-mediated repression or by JNK-mediated activation. In HTLV-1 and EBV-infected cells in which both AP1 and JNK pathways are activated, JNK activation appears to have a dominant effect (83–86).

Epigenetic Control of hTERT in Virally Infected Cells
Activation of bTERT transcription may involve histone acetylation, and, conversely, repression may involve histone deacetylation. Trichostatin A, which inhibits the family of mammalian histone deacetylases, activates bTERT gene expression in multiple cell types (87). However, trichostatin A may also activate additional genes that directly or indirectly derepress the bTERT gene, including c-Myc itself (88). HPV E6 and E6AP expression promote acetylation of histone H3, providing epigenic control of the bTERT gene (89). Acetylation of histone H3 at the bTERT promoter was increased in late passage E6- and E7-immortalized keratinocytes, whereas p300 expression was decreased (89). E6 has been shown to target the coactivator/acyetyl transferase p300, and cells that expressed E6 and p300 antisense RNA showed increased acetylation of histone H3 and activation of the bTERT gene (89). Therefore, p300 may act as a repressor of telomerase activation in the context of E6 expression. Other examples of this regulatory mechanism include adenovirus E1A binding to p300/CBP and the subsequent recruitment of its histone acetyltransferase activity to the bTERT promoter (90).

Further control of bTERT gene expression involves histone H3 phosphorylation mediated by the mitogen-activated protein kinase (MAPK) cascade. In normal human T lymphocytes and fibroblasts, growth or stress stimuli that are known to drive H3 phosphorylation through MAPK signaling induce bTERT gene expression, whereas inhibition of MAPK-triggered H3 phosphorylation substantially abrogates bTERT induction (91). MAPK-mediated control of H3 phosphorylation may have important consequences for bTERT gene expression during viral infection because c-Jun kinase (JNK) and extracellular signal-regulated kinases 1 and 2 (ERK1/2), members of the MAPK family, are constitutively activated in HTLV-1-infected, EBV-infected, and KSHV-infected cells (84,92,93).

Finally, the bTERT promoter was more frequently methylated in cervical cancer specimens than in normal cervical tissue isolated from different patients (94); however, there was no correlation between methylation status and bTERT gene expression. A similar phenomenon is seen in bTERT-expressing cells, in which a demethylated bTERT promoter does not necessarily correlate with an increase in bTERT gene expression and both methylation-dependent and -independent mechanisms are in place to modulate bTERT gene expression (95–97).

cis-Activation of the hTERT Promoter Through Viral Integration
In addition to encoding telomerase regulators that act in trans on the bTERT promoter, the HPV and HBV genomes have been found in sporadic cases to integrate in proximity to the bTERT gene (98–100). In a subset of cervical and hepatocellular carcinoma tumors, the integration resulted in the placement of viral enhancers near the bTERT promoter without perturbing the bTERT coding region. Telomerase expression increased in all cases, although it was not clear whether the increase was due to viral integration or to the action of viral oncoproteins in transformed cells. Analysis of the hepatocellular carcinoma cell line huH-4 also showed that HBV integration acted as an enhancer for cis-activation of the bTERT gene (101). Many different patterns of karyotypic abnormality have been reported in cervical carcinomas, but the most recurrent structural chromosomal aberration in cervical cancer is a gain of chromosome 3q copy number (102). A recent study demonstrated that the gene encoding the RNA component of telomerase (hTR) at 3q26 was progressively amplified with the development of HPV-associated cervical intraepithelial neoplasias to advanced invasive carcinomas (103). This gain of the bTR gene was predominantly associated with integration of oncogenic HPV.

Therefore, it appears that expression of the genes for hTERT and/or hTR may be specifically activated by virus integration in a population of infected cells. In concert with other viral proteins, enhanced telomerase expression and the resultant increase in telomerase activity could provide a selective advantage by promoting transformation.

Posttranscriptional Regulation of Telomerase Activity
Telomerase activity can also be regulated at the posttranscriptional level. For example, protein kinase C (PKC)-mediated phosphorylation of telomerase results in the interaction of telomerase and hsp90, a protein that is necessary to maintain the integrity of the telomerase holoenzyme (104). Inhibitors of PKC substantially reduced telomerase activity present in human nasopharyngeal and head and neck cancer cells (104). Moreover, PKC has been shown to modulate telomerase activity in human cervical cancer cells (105). Thus, EBV and HPV may activate PKC to increase telomerase enzymatic activity.

Some viruses have evolved mechanisms to exploit the posttranscriptional regulation of telomerase. These mechanisms include blocking access of the enzyme to its substrate by regulating the phosphorylation and nuclear translocation of the hTERT catalytic subunit and promoting increased translation of hTERT by regulating the poly-A-binding proteins that stabilize hTERT mRNA transcripts.

In uninfected cells, AKT has been shown to elevate telomerase activity by phosphorylating the hTERT protein, leading to its accumulation in the nucleus (106). Similarly, the RelA/p65 subunit of NF-κB has been shown to bind directly to hTERT and to facilitate its translocation to the nucleus (107). On the other hand, protein phosphatase 2A (PP2A) has been shown to dephosphorylate and inactivate telomerase (108). The LMP1 protein of EBV has evolved to exploit this pathway. In nasopharyngeal carcinoma cells, LMP1 can increase telomerase activity posttranscriptionally, by promoting NF-κB RelA/p65–mediated binding to and nuclear localization of hTERT (109). To date, EBV is the only oncogenic virus that has been shown to regulate hTERT nuclear translocation.

In HPV E6–expressing cells, the E6AP-binding protein NFX1-123—in addition to its role as a transcriptional activator of bTERT—can also act as a posttranscriptional regulator of hTERT (110). NFX1-123 has been shown to interact with cytoplasmic poly-A-binding proteins affecting RNA stability in the nucleus and cytoplasm, and thereby elevating telomerase activity.
Table 3. Tumor virus regulation of telomerase activity and telomere length*

<table>
<thead>
<tr>
<th>Virus</th>
<th>Telomerase activity</th>
<th>Telomere length</th>
<th>Epigenetic control</th>
<th>cis-Activation of the hTERT promoter</th>
<th>trans-Activation of the hTERT promoter</th>
<th>Posttranscriptional modification of hTERT</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBV</td>
<td>Elevated</td>
<td>Long</td>
<td>–</td>
<td>–</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>HHV-8</td>
<td>Elevated</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Yes</td>
<td>–</td>
</tr>
<tr>
<td>HPV</td>
<td>Elevated</td>
<td>Short</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>HBV</td>
<td>Elevated</td>
<td>Short</td>
<td>–</td>
<td>Yes</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HCV</td>
<td>Elevated</td>
<td>Short</td>
<td>–</td>
<td>–</td>
<td>Yes</td>
<td>–</td>
</tr>
<tr>
<td>HTLV-1</td>
<td>Elevated</td>
<td>Short</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

* EBV = Epstein-Barr virus; HHV-8 = human herpesvirus 8; HPV = human papillomavirus; HBV = hepatitis B virus; HCV = hepatitis C virus; HTLV-1 = human T-cell leukemia virus type 1. Dashes reflect a lack of available information.

Protection of Short Telomeres in Virus-Induced Tumor Cells

As discussed above, in most tumor cells telomere lengths are shortened despite strong telomerase activity. This situation may seem paradoxical; however, several positive and negative regulators of telomere length can bind and form the shelterin complex, which can protect or sustain telomere length (10). Telomerase extension is often negated because shelterin blocks access of the telomerase reverse transcriptase to the now “closed” ends of telomeric DNA (111).

Three components of the shelterin complex—TRF1, TRF2, and TIN2—are overexpressed in tumor cells of HTLV-1–infected adult T-cell leukemia/lymphoma patients (55). Overexpression of either TRF1 or TRF2 results in rapid telomere shortening (112). In addition, TRF1, TRF2, and TIN2 can inhibit apoptosis and cell-cycle arrest due to DNA damage (113,114). It is possible that the telomeres in adult T-cell leukemia/lymphoma cells reach a critically short length but that the cells are prevented from initiating apoptosis and senescence due to the overexpression of key members of the shelterin complex. Samples from multiple stages of human hepatocarcinogenesis and from patients with non-Hodgkin B-cell lymphoma also showed differential expression of shelterin components, but whether overexpression of shelterin was specific to viral infection was not elucidated (115).

A cellular protein, PINX1, has been shown to negatively regulate telomerase activity by binding to and inhibiting hTERT directly (116). However, samples from HTLV-1– and HBV-infected patients showed no substantial differences in PINX1 mRNA levels compared with those in uninfected control subjects (55,117).

Mechanisms of Viral Negative Regulation of Telomerase

Telomerase activity is inhibited by cellular differentiation and by reduction of bTERT transcription, and high levels of hTERT are a characteristic of immortalized cells (3). Several tumor suppressor pathways, such as Mad-1/c-Myc or TGF-β, repress bTERT in somatic cells, thereby preventing a critical component of tumorigenesis from becoming activated (118).

Thus, it may be surprising that several oncogenic viruses encode negative regulators of telomerase activity. In fact, in addition to the positive regulators, there are two known viral-encoded negative regulators of bTERT gene expression. The EBV-encoded transmembrane protein, LMP2A, like LMP1, is expressed in the latent stages of disease and during cellular transformation. Surprisingly, LMP2A has been shown to reduce telomerase expression and activity in epithelial cells by repressing bTERT promoter activity through its tyrosine-based activation motif, ITAM (24). Repression of the bTERT gene was not associated with changes in cell-cycle progression. It has been suggested that LMP2A reduces bTERT gene expression to prevent B-cell activation and promote the viral latent state (24).

Reduced activity of E2, an HPV regulatory protein that represses E6/E7 expression, is obligatory for HPV-mediated carcinogenesis (119,120). In fact, expression of E2 induces senescence via pRb- and p21-associated pathways and E2 represses bTERT gene expression through Sp1 (121,122). E2 may disrupt the interaction of positive regulators, such as histone-deacetylases, at the bTERT promoter, thereby leading to a decrease in telomerase activity. The ability of E2 to repress E6/E7 and bTERT gene expression may have an important role in the induction of senescence.

NFX1 was identified as a novel telomerase target and as a possible candidate for E6/E6AP-mediated telomerase regulation (39). The NFX1-91 isoform represses promoter activity in HPV16 E6–or c-Myc–expressing keratinocytes by binding to an X-box motif adjacent to the E-box (123). NFX1-91 is also ubiquitinated by the E6/E6AP complex. Why NFX1-123, and not NFX1-91, stimulates telomerase expression is still unknown. Possible explanations include the rapid turnover of NFX1-91, the fact that NFX1-91 lacks a C-terminal domain similar to that of NFX1-123, or the fact that NFX1-91 is expressed predominantly in the nucleus whereas NFX1-123 is mostly cytoplasmic (110). All of these factors may affect the binding of NFX1-91 to stimulatory elements at the bTERT promoter. Whether NFX1-91 is necessary for the overall regulation of telomerase expression during HPV infection and whether additional proteins that are required for telomerase trans-activation are ubiquitinated by E6AP still remains to be demonstrated.

Thus, although it may seem counterintuitive, both EBV and HPV have been shown to encode such negative regulators of telomerase expression. In addition, the Tax protein of HTLV-1 is a potent positive regulator of bTERT expression yet was able to limit bTERT gene activation following antigen stimulation. Thus, a balance between transcriptional activation and repression of telomerase may be important in viruses that require a latent infection. Elevation of telomerase expression ensures that the infected cells can proliferate indefinitely in the absence of senescence and...
apoptosis, whereas repression of telomerase may play an important role in preventing an immune response against activated infected cells or create a state of transient genetic instability. It would be important to know whether these viral inhibitors are expressed constitutively during latency or whether they repress telomerase gene expression during a particular stage of disease progression.

Conclusions

The human tumor viruses have evolved numerous strategies to constrain tumor suppressor pathways and to promote cellular transformation. Among these, elevation of telomerase transcription and/or activity can be used as mechanisms to bypass replicative senescence and to increase proliferative capacity, and these mechanisms, in turn, increase the cumulative risk of genetic alterations. A summary of the data concerning the relationships of six human tumor viruses to telomere length and telomerase activity is shown in Table 3. Malignant cells infected by all six human tumor viruses studied exhibited elevated telomerase activity, yet in all but EBV-infected cells, telomere length was found to be shorter than that in uninfected control cells. In the case of EBV, HHV-8/KSHV, HPV, and HTLV-1, elevated telomerase activity can be clearly ascribed, at least in part, to direct trans-activation of the hTERT promoter by viral proteins. As discussed above, the LMP1 protein of EBV, the LANA protein of HHV-8/KSHV, the E6 protein of HPV, the X transactivator of HBV, and the Tax transactivator of HTLV-1 each can increase transcription of the telomerase reverse transcriptase. Whereas HPV and HBV may mediate cis-activation of telomerase transcription, HPV appears to enhance its epigenetic activation. EBV and HPV may regulate telomerase posttranscriptionally by activation of its phosphorylation through PKC, promotion of its nuclear translocation through NF-κB, or stabilization of hTERT mRNA. However, viral regulation of telomerase activity is likely to be complex, and there are indications that at some stages of viral infection, some tumor virus proteins can also negatively regulate telomerase transcription or activity.

The diversity of strategies used by tumor viruses underscores the complexity of hTERT promoter and telomerase regulation. Further investigation in this area is likely to yield many new advances and to shed light on potential new therapeutic approaches for the treatment of human cancers.

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