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Advances in Tumor Virology

Tumor Cell Development: A Role for Viruses and Telomerase Activity?

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ABSTRACT: Telomere biology is an important aspect of human cancer, because the telomere dysfunction and telomerase activity are associated with genomic instability and cellular immortalization. In this article, we review the regulation of telomere dynamics and telomerase activity in virus-related cancers. Viruses may exploit these events to favor its replication and, therefore, may differ from non-viral cancers in this regard. Focusing on Epstein–Barr virus (EBV), *human papillomavirus* (HPV), hepatitis B virus (HBV), and hepatitis C virus (HCV), which concentrate the majority of the evidence regarding telomere biology and viral cancers), although other viruses are more briefly mentioned, it is noticeable that regulatory mechanisms of telomere dynamics and telomerase activity are virus specific. Such mechanisms include accelerated telomere shortening because of higher rates of cellular proliferation, telomerase activity regulation, and activation of alternative lengthening of telomeres (ALT). Additionally, there is also some evidence supporting a role of viral non-coding RNAs (ncRNAs) and virus-induced alterations in host ncRNAs in telomerase activity. Clarification of the roles of telomere biology in mediating viral cancers would have implications only for developing telomere-targeting anti-cancer approaches and, possibly, to accelerate advances in other telomere-related diseases, such as non-viral cancers and other aging traits.

KEYWORDS: cancer, virus, telomere length, telomerase, ALT, ncRNAs

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Telomeres and Telomerase in Aging and Cancer: an Overview

Telomeres are 5′-TTAGGG-3′ DNA tandem repeats associated with at least six auxiliary proteins (commonly referred to as the telomere shelterin complex) that compose the capping structure of eukaryotic chromosomes.[1] Such structure has functional implications in several processes, including prevention of recognizing chromosome ends as double-strand DNA breaks and of being fused together.[2] Additionally, telomere integrity has been evidenced to protect against nuclease activity and chemical modifications,[3–5] as well as to be important for chromosomal segregation during mitosis and homolog chromosomes alignment in meiosis.[6–9] A critical aspect of telomere biology is the so-called end replication problem, since the incapacity of the cellular machinery to replicate the very ends of linear chromosomes results in telomere shortening after each cell division.[10] The accumulation of such shortening results in loss of telomeric DNA and, eventually, los of chromosome capping, which triggers p53-mediated senescence and/or apoptosis, thus limiting lifespan at the cellular level.[11]

Telomere shortening is counteracted by telomerase activity. This ribonucleoprotein complex is composed of two main subunits: the telomerase reverse transcriptase (TERT, encoded by *TERT*; GeneEntrez ID: 7015), which promotes telomere elongation by reverse transcription, and the telomerase

RNA component (TERC, encoded by *TERC*; GeneEntrez ID: 7012), which serves as a template for the reaction.[3,12,13] In humans, telomerase activity is absent (or present at undetectable levels) in most cells of the adult body (mainly because of *TERT* down-regulation), although it is present in some cell types, including embryonic stem cells, germ-line stem cells, and adult stem cells. In the last, however telomerase activity levels are insufficient to fully prevent telomere shortening.[14,15] Therefore, even these cells eventually reach a state of critically shortened telomeres, which is one of the best established molecular causes of organism aging as a result of loss of tissue renewal capacity. Furthermore, telomere biology has important implications in cancer. Telomerase activity is the most common mechanism of cellular immortalization in malignancies, being present in approximately 85–90% of human cancers.[16,17] Moreover, there is substantial evidence that failure to sense critically shortened telomeres by the genome surveillance machinery facilitates the maintenance of cells with genomic instability, which favors the accumulation of mutations and is considered a cancer hallmark.[18] The implications of the interplay between telomeres and tumor suppression mechanisms in aging and cancer have been discussed in detail elsewhere.[19]

Telomere Biology in Viral Cancers

Viral influence on telomere biology and the importance of telomere dysfunction in virus-mediated oncogenesis have been extensively described. Tumor viruses reprogram host cells to promote their rapid proliferation, which is often associated with multiple markers and causes of telomere dysfunction, including progressive shortening of the telomeres.[20] Many cancer viruses activate or increase telomerase activity and influence telomere length in a cooperative fashion with other events. These factors are advantageous for the virus because they increase the cellular proliferative capacity of host cells,[21] which favors the proliferation of the virus itself.

Telomere length. The association between telomere length regulation and virus-mediated carcinogenesis is well established. Nevertheless, there is no consensus regarding the underlying mechanisms, which have been evidenced to be (at some degree) virus-specific. Indeed, in spite of most viral infections influence tumor development (regarding telomere biology) through telomere shortening, others were evidenced to increase telomere length.[22] For example, Epstein–Barr virus (EBV)-positive Burkitt's lymphoma cell lines were described as having longer telomeres than EBV-negative cell lines, although telomerase activity levels were similar,[23] thus suggesting that EBV infection may have telomerase-independent effects on telomere dynamics. In this regard, there is an induction of telomere abnormalities in cells expressing EBV nuclear antigen 1 (EBNA1), including loss or gain of telomere signals, telomere fusion, and heterogeneous telomere length. These phenomena highlight the importance of this virus in malignant transformation by inducing genomic instability. Additionally, EBNA1 alters the cell phenotype via induction of oxidative stress-dependent telomere uncapping.[24] Importantly, it was shown that EBV-transformed lymphoblastoid cell lines have little telomerase activity and that their telomeres are progressively shortened during their normal life span. However, only cells with stabilized telomeres did not undergo proliferative crisis; these cells also acquired indefinite proliferative potential, thus reinforcing the importance of telomere length maintenance (by telomerase activity up-regulation, for example) for virus-mediated cellular immortalization.[25]

Although many oncogenic viruses can induce or increase telomerase activity, telomere shortening is commonly observed in cells infected by oncogenic viruses during or after malignant transformation. Infected cells that express viral proteins are susceptible to proliferation-dependent telomere attrition, alterations in telomere shelterin complex function, and generation of reactive oxygen species that cause irreparable damage of telomeric DNA.[20] For example, an epidemiological study reported higher odds of HPV16 seropositivity in oropharyngeal squamous cell carcinoma (OPC) patients with shorter telomeres in peripheral blood leukocytes, and short telomeres were associated with *Human papillomavirus* (HPV)-16 (HPV16) seropositivity in OPC.[26] These findings suggest that HPV16 infection and OPC are not caused by short telomeres and that, in isolation, none of these events induce telomere shortening. Indeed, the results are indicative of HPV16-mediated OPC as a cause of telomere shortening, allowing the speculation that the OPC carcinogenic process mediated by HPV16 has a specific regulatory system of telomere dynamics, which is absent in non-OPC HPV16-infected cells and in OPC HPV16-free cells.

In other study with human subjects, shorter telomeres and higher telomerase activity levels were detected in patients with acute or chronic adult T-cell leukemia compared with asymptomatic carriers of human T-lymphotropic virus type 1 and with healthy volunteers. Furthermore, the median survival period of acute adult T-cell leukemia patients was reduced when comparing those with shortened telomeres and high telomerase activity with those with normal telomere length and low telomerase activity.[27] These results are in accordance with the notion that stabilization of short telomeres by telomerase is associated with (most likely as a cause of) cancer initiation and/or progression. There is also some evidence supporting direct binding of viral proteins into proteins of the telomere shelterin complex, thus inducing telomere dysfunction.[20]

Telomerase activity. Table 1 presents a summary of proteins of cancer viruses that have been evidenced to regulate telomerase activity. Such activity is primarily modulated by transcriptional level regulation of *TERT* expression. The *TERT* promoter contains several E-boxes and five GC-rich elements, to which the transcription factors Myc and Sp1, respectively, can bind.[28] In support of this notion, it is well-known that overexpression of Myc can directly induce *TERT* expression.[29,30] It is also well-established that *TERT*

Table 1. Viral proteins and their postulated functions in telomerase activity regulation.

VIRUS	PROTEIN	INFLUENCE ON TELOMERASE ACTIVITY	REF.
HPV	E2	Telomerase activity reduction by *TERT* expression inhibition through interaction with an repressor E2-responsive region of *TERT*	36
HPV	E6	Increased telomerase activity through *TERT* expression induction via interaction with Myc, histone acetylation and dimethylation and Maz binding reduction	37,38
KSHV	LANA	Increased telomerase activity through *TERT* expression induction via Sp1 binding	46
HBV	HBx, HBxΔ127 HBc, preS2	Increased telomerase telomerase activity through *TERT* expression induction	48,49,51
EBV	LMP1	Increased telomerase activity through *TERT* expression induction via Myc	56
EBV	LMP2A	Telomerase activity reduction by silencing the *TERT* promoter	57
HCV	HCV core protein, NS3	Increased telomerase telomerase activity through *TERT* expression induction	60,61
HIV	Vpr	Reduction of telomerase activity via the EDD-DDB1-VPRBP E3 ligase complex	62

expression is increased following Myc binding to E-boxes present in the *TERT* promoter. In fact, in most cells, Myc and Sp1 act cooperatively as the main modulators of *TERT* expression.[28,31] Importantly, many cancer cells express high levels of Myc, which may contribute to the activation or up-regulation of *TERT* expression.[21,32]

Infection by HPV is one of the best established cases of a virus that directly regulates telomerase activity (as well-reviewed elsewhere).[33–35] More specifically, E6 and E7 HPV proteins play important roles in host cell transformation and carcinogenesis.[36] E6 was shown to interact with Myc by facilitating its binding to *TERT* promoter E-boxes (in substitution to the repressive factors USF1 and USF2), thus increasing *TERT* expression.[37] Such interaction between E6 and Myc was also evidenced to modify the chromatin structure of the *TERT* promoter through histone acetylation and dimethylation in an E6AP-dependent fashion, as mentioned elsewhere.[38] More recently, other mechanisms of telomerase activity regulation by E6 were described. For example, an unbiased *in vivo* screen using a LacO–LacI system in human cells identified a novel *TERT* repressor (the Maz protein), whose binding to *TERT* promoter is reduced by E6 expression.[38] There is also evidence suggesting that E6 is more important to telomerase activity up-regulation than E7. Indeed, some studies demonstrated that E6 alone can induce high levels of telomerase expression, while E7 alone cannot.[39] However, there is also evidence supporting that E7-expressing cells can be immortalized by a protein–protein interaction between E7 and TERT, although this immortalization seems to not be independent of telomere lengthening.[40] Importantly, *TERT* promoter activation is a unique feature of oncogenic HPV types according to an investigation of 29 viral types, suggesting that *TERT* plays important roles in HPV-induced carcinogenesis.[41]

An additional feature of HPV-related cervical carcinoma is the loss of expression of E2 viral protein, which is important for extrachromosomal DNA replication and the completion of the virus life cycle.[42] Moreover, E2 inhibits cell growth in HPV-infected cells and triggers apoptosis in HeLa cells.[36] E2 was also evidenced to participate in telomerase activity regulation by repressing the *TERT* promoter by interacting with an E2-responsive region of *TERT* located 185 bp upstream of the translation start site. Of note, HPV18 E2 does not inhibit the transcriptional activity of *TERT* promoter via Myc.[36] The same study showed that, through competition with co-activators, E2 down-regulates the transactivation function of Sp1 in *TERT* promoter activity.[36]

It is also possible that the effects of HPV proteins on *TERT* expression interfere with TERT non-canonical functions, including alteration of apoptotic responses, tumor formation, stem cell migration and renewal, as well as chromatin remodeling.[40] In this regard, the fact that telomere erosion rates in HPV-expressing keratinocytes is similar to the rates observed in normal keratinocytes[40,43] suggests that the effects of *TERT* overexpression in cellular immortalization might involve functions additional to those in telomere maintenance and/or elongation. However, it is important to note that the existence of such non-canonical functions is not universally acknowledged in the literature.[44,45]

While HPV E6 activates *TERT* transcription through a Myc-dependent pathway, latency-associated nuclear antigen (LANA) of Kaposi's sarcoma-associated herpesvirus (KSHV) activates *TERT* promoter through Sp1.[46] LANA serves as an origin binding protein to recruit the cellular replication machinery to the KSHV latency origin of replication. LANA is one of the major latent proteins detected in all forms of Kaposi's sarcoma-associated malignancies, including body-cavity-based lymphomas and multicentric Castleman's disease, an aggressive lymphoproliferative disorder.[46] Importantly, in contrast to up-regulating *TERT* expression, LANA was shown to interact with telomere-related proteins and ablate their binding to telomeric repeats. Moreover, LANA expression resulted in telomere shortening[47] in telomerase-immortalized endothelial cells, but not in U2OS cells, which uses the

so-called alternative lengthening of telomeres (ALT) mechanism (discussed in the next section). Taken together, these results support the notion that telomerase depends on some proteins of the telomere shelterin complex to promote telomere lengthening, which is not the case for the telomerase-independent mechanism. According to this speculative view, it is possible that *TERT* up-regulation is advantageous to the virus in a telomere-independent manner or, alternatively, such up-regulation has minor implications in the aforementioned context. In this regard, a critical aspect related to KSHV-mediated carcinogenesis is the mechanism of telomere stabilization (which is required to evade cell crisis-related death, as previously mentioned) since, although there is evidence suggesting that KSHV may favor the emergence of the ALT mechanism,[21] there is no evidence that this is the universal telomere maintenance mechanism in Kaposi's sarcoma.

Another example of telomerase activity regulation by viral proteins is observed in hepatitis B virus (HBV), and such regulation was evidenced to have important implications for hepatocellular carcinoma (HCC). Upon HBV infection, HBV X protein (HBx) increases telomerase activity by up-regulating TERT expression, favoring malignant transformation and prolongation of the life span of infected hepatocytes.[48] Of note, a natural HBx mutant termed HBxΔ127 (with a COOH-terminal portion truncation of 27 amino acids) was shown to play an important role in hepatocarcinogenesis.[49] Compared with wild type HBx, HBxΔ127 was shown to remarkably increase the proliferation and migration of hepatoma cells through up-regulating *TERT* in addition to other targets, including NF-κB, survivin, proliferating cell nuclear antigen, 5-lipoxygenases, fatty acid synthase, osteopontin and Myc.[50] Other two HBV proteins, HBV core protein (HBc) and preS2 protein, are also up-regulators of *TERT* expression, which correlates with increased hepatocyte proliferation.[51] Furthermore, it is postulated that chronic inflammation induced by the hepatitis virus is an important risk factor for the accumulation of genetic alterations and promotion of carcinogenesis.[52,53] This is because of telomere shortening in hepatocytes during the progression of chronic inflammation in the liver,[54,55] but this relationship between telomere length and hepatocarcinogenesis is yet to be completely elucidated.

A third case of *TERT* regulation by proteins of oncogenic viruses occurs in EBV-related nasopharyngeal carcinoma. More specifically, latent membrane protein 1 (LMP1) down-regulation reduces *TERT* expression and influences TERT phosphorylation mediated by Akt phosphorylation, thus leading to telomerase activity inhibition, which was also evidenced to enhance radiosensitivity of these cancer cells.[56] Another EBV protein that modulates telomerase activity is the latent membrane protein 2A (LMP2A), which reduces *TERT* expression by silencing its promoter in tumor cell lines.[57] In spite of the aforementioned studies, the roles of TERT in EBV-induced immortalization remain unclear, especially given that it was observed that newly established EBV-transformed lymphoblastoid cell lines often exhibit very low telomerase activity without apparent telomere shortening.[58] This suggests that an alternative mechanism of telomere maintenance may occur in such cells (as discussed below).

Additional examples of viruses that have been evidenced to affect telomerase activity are hepatitis C virus (HCV) and human immunodeficiency virus (HIV). HCV infection status has been associated with high-grade bladder tumors and invasive squamous cell carcinomas, as well as with high levels of *TERT* expression and telomerase activity.[59] Regarding HCV proteins specifically, exogenous HCV core protein expression was shown to increase *TERT* promoter activity and mRNA levels, resulting in increased telomerase activity and longer telomeres in Huh7 cells when compared to cells transfected with the vector alone.[60] Moreover, exogenous NS3 protein expression in NIH37T3 cells was shown to increase telomerase activity, which was higher under the expression of NS3 with a C-terminal deletion when compared to an N-terminal deletion.[61] Regarding HIV, a viral protein named Vpr was evidenced to inhibit telomerase activity by reducing TERT levels through the EDD–DDB1–VPRBP E3 ligase complex.[62]

Alternative lengthening of telomeres. There are two known mechanisms of telomere-maintenance in human cancers: telomerase activation and a telomerase-independent mechanism termed ALT, which occurs in telomere-negative cells. ALT occurs mainly through homologous recombination of telomeric sequences and is more prevalent in tumors arising from non-epithelial tissues than in those of epithelial origin.[63–65] Although there is relatively few studies assessing the frequency and importance of ALT for viral cancers, it may be important for telomere length stabilization in some cases, as discussed in the previous section.

Regarding oncogenic viruses, the strongest evidence supporting the occurrence of ALT was obtained from studying EBV-infected cells. It was reported that within the first month of culture EBV-infected cells of rapid proliferation exhibited multiple signs of telomere dysfunction and ALT activation, including the accumulation of extra-chromosomal telomeres and ALT-associated promyelocytic leukemia nuclear bodies containing telomeric DNA (APBs), increased telomere length, telomere length heterogeneity, and telomeric-sister chromatid exchange (T-SCE).[58] Another example of ALT activation possibly mediated by a virus was described in simian virus 40-immortalized cell lines, although telomerase activity was also reported in some cell lines.[66]

Non-coding RNAs in Telomerase Regulation by Oncogenic Viruses

Although some recent evidence was reviewed in the above section, the importance of viral proteins in telomere biology modulation has been appreciated for a relatively long time and is well-reviewed in the literature.[20,21] Besides the well-evidenced roles of viral proteins in telomerase activity regulation (as well as in the acquisition of other tumorigenic

properties), viral non-coding RNAs (ncRNAs) are arising as key players in infection, host-immune system evasion, and carcinogenesis.[67] More specifically, a type of ncRNAs named microRNAs (miRNAs) has been extensively investigated in recent years.

miRNAs. miRNAs are small non-coding single-stranded RNAs of 19–24 nucleotides in length which regulate the expression of many target genes at the post-transcriptional and/or translational levels.[68] Viral miRNAs can be grouped into two main categories: host analogs or virus-specific, which regulate host or viral genes, respectively.[69] Importantly, despite different types of ncRNAs have been investigated,[70–72] the major focus is on viral miRNAs and virus-induced alterations in host miRNAs. These events have been shown to be involved in very sophisticated miRNA-related mechanisms of gene expression regulation.[73] In Table 2, the main miRNAs implicated in telomerase regulation by cancer viruses are summarized.

Viral miRNAs. Among DNA viruses, which express the majority of currently known virus-encoded miRNAs, 95% of viral miRNAs identified to date are of *herpesvirus* origin.[74] Indeed, the first report of virus-encoded miRNAs dates back to 2004 and describes the cloning of viral miRNAs from EBV-infected cells.[75] Since that, viral miRNAs were evidenced to have roles in a variety of pro-tumorigenic process,[67,76,77] although there are relatively few published investigations directly linking viral miRNAs and telomerase regulation. Nevertheless, the existence of such mechanism is plausible based on the evidence available.

EBV is commonly detected in both high-grade nasopharyx dysplastic lesions and invasive nasopharyngeal carcinoma, suggesting that EBV infection favors malignant transformation of nasopharyngeal epithelial cells and/or facilitates clonal expansion of malignant cells.[78] Regarding miRNAs, EBV may contribute to cancer development possibly by two viral genomic regions: *BART* (Bam HI-A region rightward transcript) and *BHFR1* (Bam HI fragment H rightward open reading frame 1).[67] There are 21 miRNA precursors (i.e., miR-BART1 to miR-BART22) that can originate 40 mature miRNAs in the intronic regions of the abundantly expressed *BART* transcript. Additionally, three other precursors (miR-BHRF1-1, miR-BHRF1-2, and miR-BHRF1-3) that can originate four mature miRNAs are located within *BHRF1* mRNA.[76] An example of the importance of miR-BARTs in the acquisition of malignant properties is the fact that they constitute up to 23% of the total of miRNAs expressed in nasopharyngeal carcinoma,[76] but are not detectable in non-cancer EBV-infected cells. Moreover, these miRNAs are up-regulated in Hodgkin's and Burkitt's lymphoma, as well as in other EBV-associated cancers.[77]

Evaluation of miR-BARTs expression profile in nasopharyngeal carcinoma allowed the identification of miR-BART-22, which targets the EBV protein LMP2A.[79] This protein is not only essential for viral latency, but is also a potent immunogenic antigen that is recognized by cytotoxic T-cells. Therefore, down-regulation of *LMP2A* expression by miR-BART22 may favor evasion of EBV-infected cells from host-immune surveillance.[79] Moreover, LMP2A transcriptionally down-regulates *TERT* expression in human tumor cells,[57] suggesting that miR-BART22 may be implicated in telomerase activity regulation by targeting LMP2A. Given that telomerase up-regulation and host-immune evasion are two cancer hallmarks,[18] miR-BART22 is likely to be a mediator of EBV-induced carcinogenesis.

Another EBV-encoded miRNA that might be involved in telomerase activity regulation in nasal NK T-cell lymphomas is miR-BART9. This viral miRNA was shown to indirectly up-regulate LMP1 protein likely by targeting a

Table 2. Virus-regulated miRNAs and their postulated functions in telomerase activity regulation.

VIRUS	miRNA	TARGET	POSTULATED INFLUENCE ON TELOMERASE ACTIVITY	REF.
EBV	miR-BART22*	LMP2A	Abrogates LMP2A-mediated telomerase inhibition	57,79
EBV	miR-BART9*	unknown	Indirectly up-regulates LMP1, a *TERT* up-regulator	80,81
EBV	miR-BHRF1-1*	p53	Facilitates telomerase activation	83–85
EBV, HCV, HPV	miR-193b, miR-106b, miR-24 miR-223, miR-21, miR-17	ESF1	Down-regulate ESF1, a *TERT* promoter repressor	93–102
HBV	miR-148a	HPIP	Down-regulation of miR-148a by HBx protein may Increase mTOR-mediated TERT activation	103,104
HBV	let-7a	STAT3, MYC	Let-7a down-regulation by HBx protein promotes STAT3 and MYC-mediated *TERT* up-regulation	105,108
HBV	miR-125a	p53	HBx-induced miR-125a up-regulation facilitates Telomerase activation through p53 repression	85,105 109
HBV	miR-29a	PTEN	Reduces PTEN-mediated TERT repression	110,113
HCV	miR-21	PTEN, SPRY2	Abrogates *PTEN* expression induction by SPRY2s and PTEN-mediated TERT repression	115–118

Notes: *Viral (EBV) miRNAs. The remaining miRNAs shown in the table are human.

LMP1 repressor.[80] miR-BART9 might be involved in *LMP1* mRNA stability, therefore increasing its half-life. Indeed, when miR-BART9 is inhibited, *LMP1* mRNA is down-regulated and is evidenced to become more susceptible to degradation.[80] Regarding telomerase activity, LMP1 up-regulates *TERT* through NF-kB- and ERK-dependent pathways in B lymphocytes,[81] suggesting that miR-BART9 may increase telomerase activity in a LMP1-dependent manner. Interestingly, individual expression of either LMP1 or TERT has limited ability to extend the life span of primary cultures of nasopharyngeal epithelial cells, while concomitant expression results in greater life span extension of these cells.[82]

An additional example of an EBV miRNA that may increase telomerase activity is miR-BHRF1-1, which is encoded from *BHRF1* mRNA and directly targets p53.[83] By mechanisms such as degradation of phosphorylated p53 by the BZLF1–ECSE3 ligase complex, the EBV functionally inhibits p53, thus facilitating virus infection and proliferation.[84] Furthermore, cell lines lacking functional p53 have accelerated telomerase activation followed by immortalization, although further events are necessary to activate *TERT* expression.[85] This observation indicates that p53 can suppress telomerase activity, proposing an inhibitory role of p53 in *TERT* transcriptional expression and/or direct association with other proteins in the telomerase complex.[86] The aforementioned evidence supports the notion that, by targeting p53 through miR-BHRF1-1 and reinforced by others mechanisms, EBV may maintain p53 down-regulated, therefore favoring telomerase activation.

Viral modulation of host miRNAs. Compared with virus-encoded miRNAs, the roles of host miRNAs in virus infection and virus-associated carcinogenesis are better characterized. An example is miR-122, whose expression was identified as a required mechanism for HCV replication.[87] miR-122 is liver-specific and is the most expressed miRNA in this organ. It was shown that miR-122 protects HCV RNA from cytoplasmic sensors of viral RNA by binding to the 5′ terminus of the HCV RNA with 3′ overhanging nucleotides. Moreover, this binding mechanism to the 5′ terminus of HCV RNA was suggested to promote protection against the action of 5′ exonucleases.[88] Owing to the essential role of the miRNA for HCV replication, miR-122 inhibitors are being tested to treat HCV infection. An example of such inhibitors is named miravirsen, which was shown in a phase 2 randomized trial to reduce HCV RNA levels that persisted beyond the end of active therapy in a dose-response manner.[89]

Host miRNA deregulation mediated by oncogenic viruses may also facilitate the immortalization process through telomerase activation. This is the case of ESF1-targeting miRNAs. Under normal conditions, E2F1 is necessary to maintain proper cell proliferation, but its overexpression can induce apoptosis in normal cells.[90] Transcription factors of the E2F family are usually responsible for activating genes involved in cell-cycle progression, but their roles extend to down-regulation of *TERT* expression.[91] An example is ESF1, which was shown to represses *TERT* expression by directly binding to two non-canonical E2F-binding sites in the proximal portion of *TERT* promoter.[92] Several ESF1-targeting miRNAs were validated in different contexts and many of them are deregulated in viral cancers. For example, miR-193b, miR-106b, miR-24, miR-223, miR-21, and miR-17 are known *ESF1* mRNA repressors[93–98] and are overexpressed in some viral cancers: miR-24 and miR-17 in EBV-associated post-transplant smooth muscle tumors (EPTSMT);[99] miR-21 in HCV-associated HCC[100] and EPTSMT;[99] miR-223 in HPV-associated cervical neoplasia and cancer (HPVCNC)[101] and in EPTSMT;[99] miR-193b in HCC[102] and EPTSMT;[99] and miR-106b in EPTSMT,[99] HCC[102] and HPVCNC.[101] Given the *TERT* expression repression effects of ESF1, overexpression of these miRNAs may favor tumorigenesis due (at least partially) to telomerase up-regulation.

In HBV-related cancers, HBx is an important modulator of host miRNAs. This protein has been associated with the development and progression of HCC by inhibiting p53-mediated induction of miR-148a expression. Such down-regulation of miR-148a increases HPIP protein levels, which activates the mTOR pathway, thus sustaining tumor progression and favoring the occurrence of metastasis.[103] Regarding telomerase regulation, mTOR and TERT cooperate to form a protein complex that is necessary for cancer cell survival and/or proliferation. The formation of this complex is initiated by mTOR-mediated TERT up-regulation, which favors cellular immortality.[104] Additional implications of HBx in host miRNA modulation include down-regulation of let-7a, which targets STAT3 protein, an important member of the JAK/STAT pathway that is involved in cellular proliferation[105] and increases *TERT* expression.[106] Importantly, let-7a was also reported to target Myc,[107] a well-know *TERT* transcriptional activator.[108] Together, this evidence suggests that HBx-mediated down-regulation of let-7a increases telomerase activity and, therefore, may favor cellular immortalization in HBV-infected cells. HBx also up-regulates miR125a, which may contribute to tumor progression and telomerase homeostasis by targeting p53,[109] as this event may facilitate telomerase activation in a cancer context, as already mentioned.[85]

miR-29a also plays important roles in HBV-related HCC development. This miRNA was observed to be overexpressed in HBx-transfected HCC cells and HepG2 cells that constitutively replicate HBV, as well as in p21-HBx transgenic mice, and such up-regulation favored migration of HepG2 cells. In the same study, miR-29a was shown to target *PTEN* mRNA, thus reducing PTEN levels and favoring the activation AKT and MMP2 proteins,[110] which are involved in cell proliferation[111] and degradation of extracellular matrix,[112] respectively. Regarding telomerase modulation, there is evidence supporting that PTEN inhibits telomerase activity by reducing *TERT* mRNA levels,[113] while AKT up-regulates telomerase

by phosphorylation of the TERT subunit.[114] Therefore, miRNA-29 may favor telomerase activity by direct targeting of *PTEN* mRNA and indirect up-regulation of AKT, which may contribute to the important roles of this miRNA in HCC development. PTEN is also implicated in HCV-associated HCC, where miR-21 overexpression was observed.[115] Importantly, this miRNA was evidenced to directly target *PTEN* mRNA,[116] therefore favoring telomerase activity directly and indirectly,[117] as already mentioned. Furthermore, miR-21 overexpression reduces the function of *SPRY2* mRNA (another validated target of this miRNA), which was shown to up-regulate PTEN.[118]

Other types of ncRNAs. Another group of ncRNAs that may play important roles in virus-induced carcinogenesis is termed small nuclear RNAs (snRNAs). This type of ncRNA has been mainly studied in *Herpesvirus saimiri* (HVS), a gammaherpesvirus that causes aggressive T-cell leukemias and lymphomas in some non-human New World primates.[119] This virus encodes seven Sm-class snRNAs named HSURs, being the most abundantly expressed gene products in HVS-transformed T-cells.[120] HSURs are implicated in RNA processing: they are transcribed by RNA polymerase II, acquire a trimethylguanosine cap, and assemble with Sm core proteins. Of note, HSURs show no extensive sequence similarity to any cellular snRNA and are the only Sm-class snRNAs known to be encoded by a virus.[121]

HSURs were demonstrated to function as miRNA "sponges" by capturing multiple complementary miRNAs. There is evidence of interactions of HSURs1 and HSURs2 with host miR-142-3p and miR-16 in HVS-transformed T-cells.[121] Down-regulating the activity of miR-142-3p and miR-16 might be advantageous for the virus, particularly in the case of miR-16, which is reported to target cell cycle and apoptosis regulators such as Bcl-2 and Cyclins D1 and E1.[121–123] Also, miR-16 was shown to target WT1 mRNA,[124] which is known to negatively regulate *TERT* expression in renal cell carcinoma[125] and has been identified as a cell type-specific *TERT* transcriptional repressor, acting through the WT1-binding site in *TERT* promoter.[126] Therefore, miR-16 capture by HSURs may interfere with telomerase activity, although such role is yet to be demonstrated. Importantly, this function of acting as miRNAs "sponges" can be extended to others types of ncRNAs, such as small nuclear ribonucleoproteins (snRNPs)[127] and long non-coding RNAs (lncRNAs).[128] These ncRNAs may be important for the elucidation on how viruses regulate host miRNA expression to induce tumorigenisis and to regulate *TERT* expression and telomerase activity. Nevertheless, such function of viral lncRNA in virus-induced tumors has not been well evidenced to date, which represents an important research field to be explored.

Final Remarks

Cancer-related viruses have evolved numerous strategies to constrain tumor-suppressor pathways, evade immune system surveillance, and promote cellular transformation. Among them, *TERT* transcription and/or telomerase activity up-regulation may act as mechanisms that facilitate to bypass replicative senescence and to increase proliferative capacity. These events may, in turn, increase the risk of accumulating genetic alterations and acquiring other pro-tumorigenic properties. Accelerated cell proliferation is often accompanied by the induction of telomere shortening and this leads, in turn, to DNA damage and genomic instability if not properly sensed by the genomic surveillance machinery. Tumor viruses might affect the telomeres by promoting both telomere dysfunction and/or activating telomerase-dependent or independent (i.e., ALT) pathways for telomere lengthening.

Viral regulation of telomerase activity is likely to be complex, and there are indications that at specific stages of viral infection some viral proteins can also negatively regulate *TERT* transcription or telomerase activity, which is an important consideration regarding the adequacy of telomerase-based therapies for viral cancers. Furthermore, there is emerging evidence supporting the notion that ncRNAs participate in the acquisition of pro-tumorigenic features, including *TERT* expression and/or telomerase activity regulation. Indeed, the detailed roles of telomerase in mediating the tumorigenic effects of viral ncRNAs are yet to be elucidated, which may contribute to better understand the complexity of telomerase regulation and function. Such elucidation is critical to develop new therapeutic approaches to treat virus-related cancers and may also contribute to biomedical advances in other telomerase-related phenotypes, including age-related impairments and other types of cancer.

Author Contributions

FPH conceived the manuscript, made critical revisions and approved the final version. FPH, LG, MSW, and ES wrote the first draft of the manuscript, contributed to the writing of the manuscript, and jointly developed the structure and arguments for the paper. All authors reviewed and approved the final manuscript.

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