 doi:10.20944/preprints202209.0482.v1

Article

The Molecular Interplay between Human Oncoviruses and Telomerase in Cancer Development

Maria Lina Tornesello[1*], Andrea Cerasuolo[1], Noemy Starita[1], Anna Lucia Tornesello[1], Patrizia Bonelli[1], Franca Maria Tuccillo[1], Luigi Buonaguro[2], Maria G Isaguliants[3], Franco Maria Buonaguro[1]

[1] Molecular Biology and Viral Oncology Unit, Istituto Nazionale Tumori IRCCS "Fondazione G. Pascale", Napoli, Italy. m.tornesello@istitutotumori.na.it (M.L.T.), a.cerasuolo@istitutotumori.na.it (A.C☺), n.starita@istitutotumori.na-it (N.S.), a.tornesello@istitutotumori.na.it (A.L.T.), f.buonaguro@istituto-tumori.na.it (F.M.B.)

[2] Cancer Immunoregulation Unit, Istituto Nazionale Tumori IRCCS "Fondazione G. Pascale", Napoli, Italy. l.buonaguro@istitutotumori.na.it (L.B.)

[3] Riga Stradins University, Riga, Latvia. Maria.Issagouliantis@rsu.lv (M.G.I.)

* Correspondence: should be addressed at Molecular Biology and Viral Oncology Unit, Istituto Nazionale Tumori IRCCS "Fondazione G. Pascale", Via Mariano Semmola 80131 Napoli, Italy. Email m.tornesello@istitutotumori.na.it Tel +39.081.17770588

Simple Summary

The expression of the telomerase reverse transcriptase (TERT) gene is commonly repressed in terminally differentiated somatic cells and becomes reactivated in the large majority of tumours. Oncogenic viruses have evolved multiple strategies to subvert the telomerase function in the host cells. Viruses may promote TERT transcription through the binding of their oncoproteins to cis-regulatory elements in the gene promoter or by integrating their genomes nearby TERT locus. Other viruses cause telomerase activation via epigenetic mechanisms that contribute to the maintenance of long telomeres and cellular immortality. This review will outline recent findings on the strategies employed by viruses to deregulate telomerase activity and telomere length and to promote cancer development.

Abstract

Human oncoviruses are able to subvert telomerase function in cancer cells through multiple strategies. The activity of the catalytic subunit of telomerase (TERT) is commonly enhanced in virus-related cancers. Viral oncoproteins, such as high-risk human papillomavirus (HPV) E6, Epstein-Barr virus (EBV) LMP1, Kaposi sarcoma-associated herpesvirus (HHV-8) LANA, hepatitis B virus (HBV) HBVx, hepatitis C virus (HCV) core protein and human T-cell leukemia virus-1 (HTLV-1) tax protein, interact with regulatory elements in the infected cells and contribute to the transcriptional activation of TERT gene. Specifically, viral oncoproteins have been shown to bind TERT promoter, to induce post-transcriptional alterations of TERT mRNA and to cause epigenetic modifications, which have important effects on the regulation of telomeric and extra-telomeric functions of the telomerase. Other viruses, such as herpesviruses, operate by integrating their genomes within the telomeres or by inducing alternative lengthening of telomeres (ALT) in non-ALT cells. In this review, we recapitulate recent findings on virus-telomerase/telomeres interplay and the importance of TERT-related oncogenic pathways activated by cancer causing viruses.

Keywords: Telomerase reverse transcriptase; TERT; TERT promoter; TERTp; human papillomavirus; HPV; Epstein Barr virus (EBV); Kaposi sarcoma-associated herpesvirus; HHV-8; hepatitis B virus; HBV; hepatitis C virus; HCV; human T-cell leukemia virus-1; HTLV-1

1. Introduction

 doi:10.20944/preprints202209.0482.v1

More than 12% of human cancers are caused by seven oncogenic viruses, including high risk human papillomaviruses (HPV), Epstein–Barr virus (EBV), hepatitis B (HBV) and C (HCV) viruses, human T cell lymphotropic virus 1 (HTLV-1), Kaposi's sarcoma herpesvirus (HHV8), and Merkel cell polyomavirus (MCPyV) [1]. Although human oncoviruses belong to diverse virus families and hold specific tissue tropism as well as distinct replication mechanisms, they have similar pathogenic activities [2,3]. All oncoviruses promote cell survival and transformation through several common features that include the ability to establish long-lasting persistent infections, to cause chronic insults and deregulation of metabolic pathways, to trigger the progressive accumulation of genetic damages and immune escape [3].

The telomerase complex, by extending the ends of chromosomes during each cell division, plays a key role in the evasion of cellular senescence and promotion of replicative immortality of cancer cells [4]. The telomerase activity is restored in more than 75% of human tumours via multiple mechanisms including the aberrant expression of transcription factors binding to TERT promoter, TERT gene amplification, post-transcription modifications of TERT mRNA as well as activating mutations and methylation of TERT regulatory regions [5,6].

Tumour viruses have the ability to enhance telomerase activity and telomere length and to contribute to the unlimited proliferation and transformation of chronically infected cells [7]. Of note, several oncoproteins encoded by human tumour viruses, such as HPV E6, EBV LMP1 and HHV-8 LANA, transactivate TERT gene leading to the immortalization and transformation of human epithelial cells [8-10]. The genomes of some viruses, such as HBV, have been frequently found integrated nearby TERT gene locus causing enhanced TERT expression [11]. Furthermore, the insertion of herpesviruses DNA within telomeres of latently infected cells, facilitated by the high homology between virus and human telomere sequences, is crucial for tumor formation and reactivation of latent infections [12,13].

This review summarizes the diverse mechanisms operated by six oncoviruses to enhance telomerase activity in the human cells and the possible oncogenic mechanisms involved in the promotion of cancer.

2. TERT expression and telomerase activities

Telomeric DNA repeats (TTAGGG), located at the ends of chromosomes, are essential to prevent end-to-end chromosomal fusions and to maintain genome integrity during successive cycles of cell division [4,14]. The telomerase complex, which elongates and protect telomeres, is composed of the telomerase reverse-transcriptase holoenzyme (TERT), the RNA template component (TERC) and the dyskerin-NOP10-NHP2-GAR1 tetrameric complex [15,16]. Moreover, the telomere ends are protected from DNA damage response by a shelterin complex formed by six proteins, namely TRF1, TRF2, TIN2, RAP1, TPP1 and POT1 [15]. TERC and other telomerase components are ubiquitously and constitutively expressed in human cells, while TERT expression, which is the rate-limiting catalytic subunit of telomerase, is restricted to embryos and stem cells [17]. TERT gene becomes repressed in the majority of somatic cells leading to progressive shortening of telomerases during successive cell divisions and replicative senescence (Hayflick limit) [18].

The tight regulation of TERT levels is maintained by the complex cooperation of numerous transcription factors acting as repressors or activators and by signalling pathways. However, in cancer cells the stringent control of TERT transcription is disrupted by the aberrant expression of TERT promoter activators, primarily MYC, the first oncoprotein demonstrated to induce the telomerase expression, and by NF-κB signalling that is considered the master regulator of TERT activation in cancer cells [19-21]. Other modalities of telomerase re-activation in tumours include chromosomal rearrangements, TERT gene amplification, and TERT promoter hypermethylation [22,23]. Short telomeres also induce TERT expression by determining the detachment of the 5p sub-telomeric region from the

 doi:10.20944/preprints202209.0482.v1

TERT locus, which is a TERT transcription repression mechanism operated only by long telomeres [24].

More recently, single nucleotide mutations in the proximal promoter of TERT gene, particularly at positions -124A and -146A upstream the ATG start site, have been shown to generate de novo consensus binding motifs (GGAA) for E-Twenty-Six family transcription factors causing the aberrant expression of telomerase [23,25-27]. These mutations are highly frequent in a vast majority of cancers including glioblastoma (80–90%), melanoma (70%), basal cell carcinoma (70%), hepatocellular carcinoma (60%), bladder cancers (60%), penile carcinoma (53%), conjunctival carcinoma (46%) and oral cancer (above 30%), [28-33].

In addition to the telomere-lengthening function, TERT can act as a transcription factor contributing to the regulation of multiple pathways involved in the physiological processes, such as cell renewal and tissue homeostasis, as well as in pathologic conditions, such as tumour formation and resistance to treatments [34]. TERT has shown to bind NF-kBp65 subunit and to regulate NF-kB-dependent genes, including matrix metalloproteinase (MMP) genes as well as to activate Wnt/β-catenin signalling in gastric and prostate cancers [5]. In particular, TERT has been shown to bind SMARCA4 factor, which is a chromatin remodeller, and to induce the expression of Wnt-responsive genes such as MYC and the vascular endothelial growth factor (VEGF) thus promoting cell transformation [35-37]. On the other hand, the Wnt/β-catenin signalling has been reported to regulate the telomerase activity and the acquisition of cancer stem cell-like phenotype in the radioresistant nasopharyngeal carcinoma cell line CNE-2R through a positive feedback loop [38]. Other non-canonical functions of TERT include the ability to induce overexpression of the epidermal growth factor receptor (EGFR) in human mammary epithelial cells and DNA methyltransferases (DNMTs) in human fibroblasts as well as downregulation of pro-apoptotic genes in diverse human cancer derived cell lines [39-42]. Specifically, TERT-induced VEGF expression promotes angiogenesis, while TERT-related DNMTs expression determines the abnormal methylation and silencing of PTEN promoter [42,43]. PTEN is a tumour suppressor that inhibits PI3K/AKT signalling, consequently TERT-induced PTEN silencing causes increased AKT activity and cell survival and proliferation.

In conclusion, the mechanisms underlying telomeric and non-telomeric functions of telomerase in cell transformation processes are regulated by complex mechanisms and have yet to be fully exploited.

2.1. Human Papillomaviruses

The Papillomaviridae family comprises a large group of small, non-enveloped viruses with approximately 8-kilobase double stranded circular DNA genomes that are classified in five genera (α, β, γ, μ, and ν) [44]. The α genus includes 13 HPV genotypes (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 66, and 68), which are the causative agents of near all cases of cervical carcinoma and a significant fraction of cancers arising in the mucosal squamous epithelia of the oropharynx, anus, penis, vagina and vulva [45]. These viruses have been defined as high risk HPVs and classified as carcinogenic to humans (group 1) by the International Agency on Cancer Research (IARC) [46].

The early proteins E6 and E7 encoded by high risk HPVs are the most important factors in virus carcinogenesis for their ability to abrogate the function of tumor suppressors and to interact with multiple cell factors involved in the regulation of cell proliferation and apoptosis [47-50] [51]. Particularly, the HPV E6 forms a complex with E3 Ubiquitin Ligase E6 Associated Protein (E6AP) that targets p53 for degradation, and HPV E7 activates and recruits calpain-1, which cleaves the C-terminal domain (Rb-Lys^{810}) of Rb thus facilitating ubiquitination mediated by cullin E3 ligases [52-55].

Moreover, high risk HPV E6 oncoproteins are able to promote telomerase activity by multiple mechanisms involving the regulation of transcriptional, epigenetic, and post-transcriptional processes [56] [57] [58]. Early studies showed that the expression of HPV16 E6 in early-passage human keratinocytes induced high levels of telomerase and that such

 doi:10.20944/preprints202209.0482.v1

effect was independent from the HPV E6/E6AP degradation of p53 [9,59]. Moreover, TERT levels and telomerase activity were significantly higher in human keratinocytes expressing both HPV E6 and E7 oncogenes compared to cells expressing HPV E6 alone [60]. The minimal E6-responsive region in the TERT promoter was found located within the 258-bp sequence, proximal to the ATG start site [60]. This region, defined as TERT core promoter, contains several E-boxes/X-boxes and GC-boxes, which are cis regulatory sequences preferentially bound by the transcription factors MYC/MAX/MAD1 and SP1, respectively, (Figure 1) [61,62]. Several studies have demonstrated that MYC can activate TERT expression through the formation of MYC/MAX heterodimers, which displace MAD1/MAX repressors on the E-boxes [21,63,64]. The E6/E6AP complex has been shown to cooperate with MYC in the stabilization of MYC/MAX heterodimers, which causes the shift of MAD1/MAX repressor and enhanced TERT transcription and telomerase activity [60,65-68]. The mutation of MYC binding sites did not result in the complete abrogation of TERT expression, which was instead obtained by the disruption of both MYC and SP1 binding sites. These results suggested a complex mechanism of E6-dependent TERT promoter activation involving a cooperative action of transcription factors binding simultaneously to MYC and SP1 cis elements [60,62].

Further studies demonstrated that HPV E6 in concert with E6AP binds NFX1-91, a transcriptional repressor of TERT promoter, causing its ubiquitination and degradation [69-72]. The NFX1-91 interacts with the mSin3A-histone deacetylase complex and its removal causes the overexpression of histone acetyltransferase, the increase of histone protein acetylation and enhancement of TERT transcription [73]. The HPV16 E6 has also been demonstrated to cooperate with a splice variant of NFX1-91, namely NFX1-123, as well as with cellular RNA processing proteins, such as cytoplasmic poly(A) binding proteins (PABPCs), and to cause the increase of telomerase activity through the post-transcriptional stabilization of TERT mRNA in human keratinocytes [74,75]. The study of NFX1-123 transcripts in biological samples showed that it is highly expressed in cervical precancer lesions, invasive carcinoma and derived cell lines [76]. On the other end, transient knockdown of NFX1-123 in HPV16 positive SiHa cells led to pronounced TERT reduction and slowing of cell growth [76].

In addition, HPV16 E6/E6AP complex has been reported to cause the acetylation of histone H3 at TERT promoter causing elevated TERT mRNA levels and telomerase activity particularly in early passages keratinocytes expressing HPV E6 [72]. Long term culture of E6 transduced keratinocytes caused histone acetylation at the TERT promoter and decrease of the coactivator/acetyl transferase p300 which is targeted by E6 [72].

More recently, HPV E6 has been reported to promote TERT expression through the destabilization of the tumour suppressor TIP60, a histone acetyltransferase enzyme that regulates gene transcription [77]. TIP60 is involved in the SP1 acetylation at the residue K639, which inhibits SP1 binding to the TERT promoter and determines repression of TERT gene transcription [77]. TIP60 has also a role in the repression of HPV E6 expression through its binding to the HPV early promoter, the histones acetylation and recruitment of BRD4 factor [78]. However, the E6 proteins encoded by low- and high-risk HPV genotypes interact with E3 ubiquitin ligase EDD1 and cause the polyubiquitination and proteasomal degradation of TIP60, thus determining transcriptional activation of both HPV E6 and TERT gene [79].

Integration of HPV genomes into the human DNA is considered a crucial event in the HPV-related carcinogenesis [80,81]. The process involves linearization of HPV DNA episomes, disruption of E2 genes and concomitant loss of E2 negative regulatory function triggering increased levels of E6 and E7 oncoproteins [82,83]. The HPV integration is frequently associated with chromosomal aberrations, extensive genomic instability and copy number variations [84]. In some cervical cancer biopsies and derived cells lines, the HPV DNA has been shown to integrate near TERT locus with viral enhancers activating TERT promoter and increased telomerase expression [85].

 doi:10.20944/preprints202209.0482.v1

Furthermore, HPV E6 protein has been shown to induce telomerase activation via a post-transcriptional mechanism based on the direct interaction of E6 protein with TERT protein and association with telomerase complexes and telomeric DNA [56].

Of note, TERT expression and telomerase activity in cervical samples of women diagnosed with ASCUS (abnormal squamous cells of undetermined significance), LSIL (low-grade squamous intraepithelial lesion), HSIL (high-grade squamous intraepithelial lesion), and invasive cervical carcinoma have been shown to increase with disease severity [86-88]. Similarly, to cervical neoplasia, head and neck cancers related to HPV infection show higher levels of TERT expression compared to those negative for HPV [89-91]. On the basis of such results telomerase may be considered a useful biomarker for the early detection of cervical lesions as well an ideal target for anti-cancer therapies.

2.2 Epstein-Barr Virus

The EBV or human gammaherpesvirus 4 is a ubiquitous virus of the Herpesviridae family, containing a genome of 184 kb linear double strand DNA, which can infect both epithelial cells and B cells [92]. Following primary infection, EBV performs a short lytic program and then primarily establishes a persistent lifelong infection in almost all human subjects. Few latently infected cells are able to switch from the latent stage into the lytic cycle to produce virus particles [93].

EBV infection can cause Burkitt's lymphoma and nasopharyngeal carcinoma, as well as at lesser extent other human malignancies, such as Hodgkin's disease, gastric cancer, and T-cell lymphoma [94,95]). The EBV-related tumorigenesis is associated with the establishment of latency programs, such as EBV latency I, II or III, and the differential expression of latent viral proteins in virus infected epithelial tumours and lymphoblastoid cell lines [93]. Particularly, the EBV latency I with selective expression of EBV nuclear antigen (EBNA)-1 is frequently identified in Burkitt's lymphoma cells. The EBV latency program II, characterized by the expression of EBNA-1 and latent membrane proteins LMP-1, LMP-2A, LMP-2B, is typical of Hodgkin's lymphoma and nasopharyngeal carcinoma cells. The EBV latency III program, characterized by the expression of all EBNAs and LMPs proteins, is commonly present in tumours developing in immunocompromised patients such as post-transplant lymphoproliferative disorders and AIDS-associated lymphomas [96]. The main activator of EBV lytic program is the viral transcription factor BZLF1 whose expression is under the negative control of the telomerase through the NOTCH2/BATF pathway [97]. On the other end, the inhibition of telomerase expression triggers a complete EBV lytic cycle and death of EBV-infected cells.

The EBV-driven cell transformation strongly depends on the ability of latent viral proteins to activate multiple signalling pathways, including mitogen-activated protein kinase (MAPK), c-Jun N-terminal kinase (JNK), phosphatidylinositol 3-kinase (PI3K)/AKT, and NF-κB, and to promote unlimited cell proliferation [97,98]. TERT expression has a key role in the maintenance of EBV latency by preventing viral lytic cycle in both EBV-immortalized lymphobastoid cell lines and EBV-positive Burkitt lymphoma (BL) cell lines [99]. On the other end, TERT inhibition induces the expression of BZLF1 and activates a complete lytic viral replication [99].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 September 2022 doi:10.20944/preprints202209.0482.v1

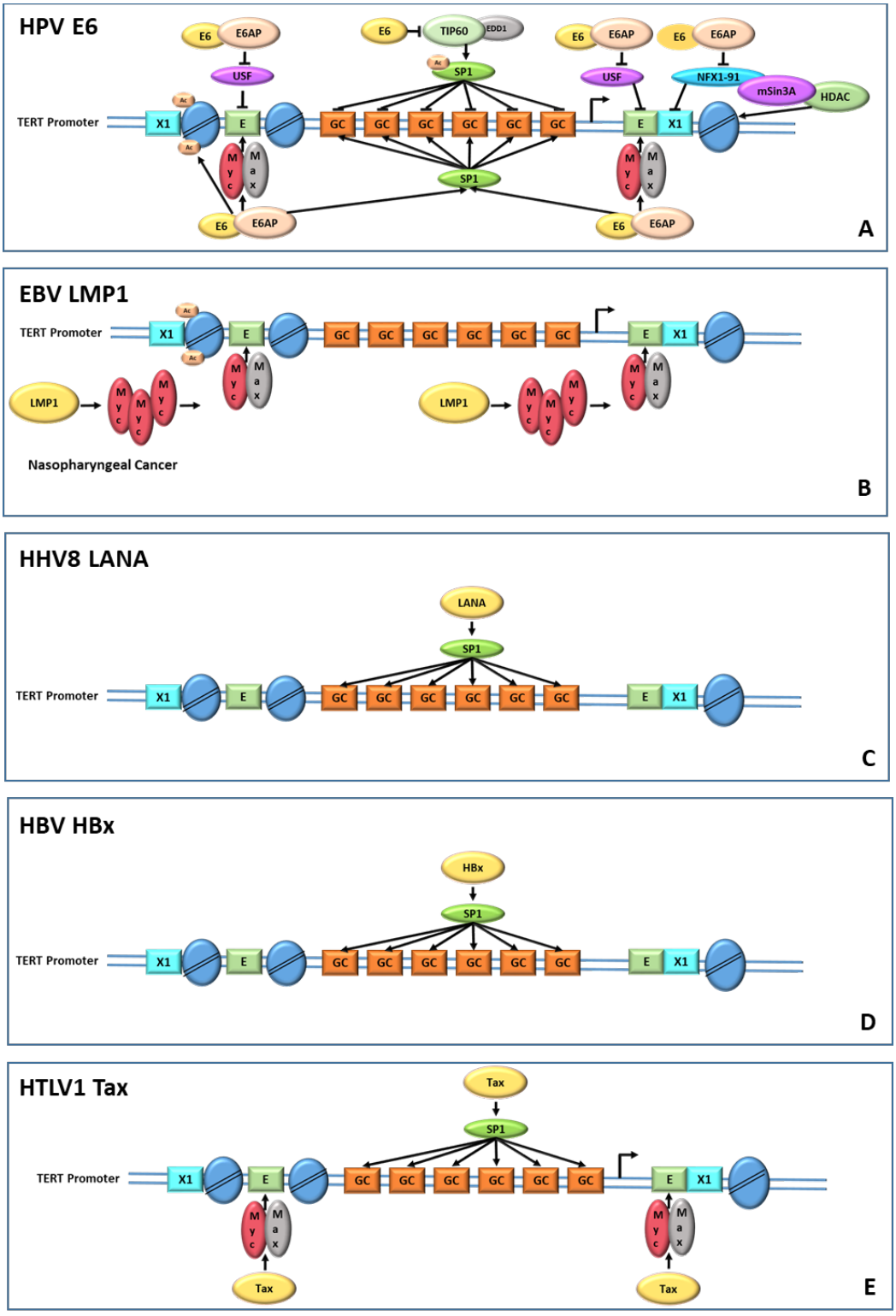

Figure 1. Strategies adopted by oncoviruses to regulate telomerase activity at transcriptional level. A) The HPV E6/E6AP complex stabilizes Myc/Max and Sp1 transcription activators, dislocates USF and NFX1-91 transcription repressors, induces the acetylation of histone H3 and inhibits TIP60-mediated acetylation of SP1. B) The EBV LMP1 recruits MYC on the cognate binding sites on TERT promoter causing telomerase expression in nasopharyngeal carcinoma cells. C) and D) HHV8 LANA sand HBV HBx stabilize the binding of SP1 to GC binding sites.; E) HTLV1 Tax stabilizes Myc/Max and Sp1 transcriptional activators to E-boxes and GC binding sites, respectively.

 doi:10.20944/preprints202209.0482.v1

Importantly, several studies have demonstrated that EBV LMP1 activates TERT at transcriptional level both in epithelial cells and B lymphocytes by distinct mechanisms [10,100]. Indeed, the transfection of LMP1-expressing vectors in primary human nasopharyngeal epithelial cells causes an increase in telomerase activity related to increased MYC expression driven by LMP1. On the other end, LMP-1 expression in B lymphocytes has been demonstrated to activate TERT via the NF-κB and MAPK/ ERK1/2 pathways independently from MYC expression [100,101].

Giunco et al. reported that short-term inhibition of TERT in EBV-infected cells induced a complete viral lytic cycle by activating the expression of lytic viral proteins, including BZLF1, and caused apoptosis via the AKT1/FOXO3/NOXA and ATM/ATR/TP53 pathways [102]. Remarkably, TERT inhibition in EBV-positive Burkitt's lymphomas and lymphoblastoid cell lines enhanced the apoptotic effect induced by antiviral therapy opening opportunities for new therapeutic protocols including TERT inhibitors to treat EBV-related malignancies [102].

Moreover, LMP1 has been shown to modulate telomerase activity at posttranscriptional level by inducing the direct binding of TERT to NF-κB p65 and nuclear translocation of TERT in nasopharyngeal carcinoma cells [103].

The EBV latent membrane protein 2A (LMP2A) has been also implicated in the maintenance of the viral latency, but with a negative regulatory effect on TERT expression [104]. Indeed, epithelial cells expressing LMP2A showed a significant reduction of TERT mRNA levels together with decreased telomerase activity. TERT promoter was inhibited by LMP2A expression both in B cells and epithelial cells, and that the tyrosine-based activation motif ITAM (immunoreceptor tyrosine-based activation motif) in LMPA protein was required for such inhibition. Repression of the TERT gene by LMP2A was suggested to hamper B-cell activation and to promote virus latency [104].

2.3. Kaposi Sarcoma–Associated Herpesvirus/ Human Herpesvirus 8

Kaposi Sarcoma–Associated Herpesvirus (KSHV) or human gamma herpesvirus 8 (HHV8) has a linear double-strand DNA genome of approximately 165 kb containing 100 ORFs. Several viral genes share sequence homology to the closely related EBV as well as to cellular genes, while other ORFs are unique with no similarity to other herpesviruses [105]. The HHV8 is recognized as the causative agent of three different types of malignancies such as Kaposi's sarcoma (KS), multicentric Castelman's disease and a form of AIDS-related primary effusion lymphoma (PEL) [106-108].

Several proteins encoded by conserved or unique genes have been implicated in KS pathogenesis, ncluding K1, K2, vMIPS, K4, K4.1, K5, K9, K12, ORF-6, ORF-71, ORF-72, ORF-73, ORF-74, and K15 [109]. Among these, LANA and v-cyclin, encoded by ORF-72 and ORF-73, respectively, have been shown to play central roles in deregulating various cellular functions. LANA, which is constitutively expressed in HHV8-associated human malignancies, is a functional homolog of EBV EBNA1 protein, acts as an adaptor molecule for an E3 ubiquitin complex via a specific protein motif and causes ubiquitylation and degradation of p53 [110]. LANA inhibits also the Rb tumor suppressor pathway, allowing the infected cells to become resistant to anti-growth signals and cell cycle arrest [111,112].

Early studies reported that the infection of human primary endothelial cells with purified HHV8 particles caused long-term proliferation and cell survival associated with the acquisition of telomerase activity and anchorage-independent growth [113]. LANA has been recognized to act as a transcription factor modulating the regulatory regions of several cellular and viral genes, including TERT promoter [8]. Particularly, LANA has been shown to interact with responsive elements localized in the TERT promoter region containing the five Sp1 transcription factor-binding sites. The interaction of LANA with SP1 binding sites has been determined by electrophoretic mobility shift assays using a probe

 doi:10.20944/preprints202209.0482.v1

containing GC-boxes in the presence of BJAB nuclear extracts. The lack of interaction between LANA and the GC-boxes in the absence of BJAB nuclear extracts suggested that LANA targets the DNA-protein complex bound to GC-boxes to activate TERT promoter [8]. Furthermore, Verma et al. observed that the transactivation of TERT promoter was due to the physical interaction between LANA N-terminal domains and the target DNA binding site [114].

Proteomic screen experiments allowed the discovery of novel interactions between LANA and cell proteins that could be also involved in TERT [115]. Among sixty-one proteins interacting with adenovirus-expressed Flag-LANA three proteins, namely replication protein A1 (RPA1) and RPA2, xeroderma pigmentosum complementation group A (XPA) and telomeric repeat binding factor 1 (TRF1), were confirmed to associate with LANA and to be involved in the regulation of telomerase activity and telomere maintenance [115]. RPA1 and RPA2 bind single stranded DNA by forming a complex with XPA protein and play a critical role in DNA replication, DNA damage response, and telomere length maintenance [116]. In the absence of LANA, the RPA1 and RPA2 proteins localize to telomeric DNA, but are rapidly displaced by the LANA expression suggesting that LANA might inhibit telomere lengthening in cells lacking alternative lengthening of telomeres (ALT). The analysis of telomere length in HHV8-positive primary effusion lymphoma cell lines BCBL1 and in BC3 revealed shorter telomeres compared to telomere length in B cells. Therefore, LANA-dependent telomere shortening may represent an additional mechanism of cell transformation [115].

An additional effect of the HHV8 infection is the induction of ALT-like features, which trigger telomere extension via recombination and break-induced replication, and a concomitant reduction of telomerase levels in non-ALT BJAB, SLK and EA.hy926 cell lines [117]. ALT activity has also been observed in HHV8-associated Kaposi sarcoma. Notably, recombination and break-induced replication mechanisms has been shown to be necessary for survival of HHV8-infected cells, maintenance of viral latency, and for replication of HHV8 [117].

2.4. Hepatitis B Virus

HBV is a small virus belonging to the hepadnavirus family, which contains a 3.2 kb circular double-stranded DNA genome encoding the reverse transcriptase/polymerase (Pol), the capsid protein (core antigen HBcAg), three envelope proteins (L, M, and S), and the transactivating protein x (HBx) [118].

HBV infects more than 2 billion people worldwide, of whom 240 million develop a chronic infection and are at elevated risk of cirrhosis and hepatocellular carcinoma (HCC) [119,120].

Telomerase activity and telomeres length have crucial roles in hepatocarcinogenesis [121]. TERT expression in normal hepatocytes gradually decreases during embryo development and it is fully inhibited in the adult hepatocytes which show non-shortened telomeres and several markers of senescence [121]. During cirrhosis, telomeres become very short as a consequence of liver damage and unceasing cell renewal, thus causing the activation of DNA damage response, cell senescence and activation of telomerase [122].

TERT expression and telomeres elongation is detected in above 90% of HCC and reported to be associated with tumour aggressiveness and worse patient prognosis [123]. Major mechanisms of telomerase activation in HCC involves the occurrence of somatic mutations in TERT core promoter, TERT gene amplification and HBV genome integration nearby TERT loci [30,124-128]. TERT promoter region has been identified as the most frequent site of HBV insertion in HCC (38.5%), but it is rarely observed in non-tumour liver tissues (3%) [129].

Two proteins encoded by HBV, namely HBx and surface (S) gene (preS2), have been shown to increase TERT expression and telomerase activity in hepatoma cells and in HCC

 doi:10.20944/preprints202209.0482.v1

cell lines, respectively[130,131]. Particularly, wild type and truncated forms of HBx proteins have shown to increase telomerase activity and TERT expression in a dose-dependent manner in different HBx-transfected cells. The HBx responsive region has been localized in TERT core promoter (nt -132 to nt +5) and the HBx transactivation mechanism relies on its ability to stabilize SP1 to binding sites, as demonstrated by electrophoretic mobility shift assays [130]. HBx is highly expressed in HBV-related HCC biopsies and it is associated with increased transcription of TERT gene [130].

The HBV preS2 has also been shown to activate TERT expression, telomerase function, and to enhance the malignancy of HepG2 cells in vivo and in vitro [131]. Suppression of preS2 expression caused decrease of TERT mRNA levels and telomerase activity as well as inhibition of cell proliferation and tumorigenicity of HepG2 cells. The preS2 was demonstrated to activate the transcription of TERT gene via a 20 bp long preS2-responsible region (PRR) located between nt -349 and nt -329 bp upstream of TERT transcription start site [131].

Moreover, a component of the telomerase complex, namely NHP2, has been found overexpressed along with TERT enzyme in HBV-related HCC as well as in HBx transduced hepatoma cell lines. The silencing of NHP2 gene caused decreasing of TERT expression, destabilization of telomerase complex and inhibition of proliferation of hepatoma cells overexpressing HBx [132]. The knockdown of NHP2 also suppressed HBx-transduced hepatoma cell growth in xenograft animal model, suggesting that therapeutic approaches targeting NHP2 may provide a novel strategy for treating HBV-related HCC [132].

2.4. Hepatitis C Virus

HCV, classified in the flaviviridae family, has a single-stranded RNA genome encoding a 3000-amino acid polyprotein which is cleaved by viral and cellular proteases into four structural proteins, named capsid protein C, envelope glycoproteins E1 and E2, and protein P7, and six nonstructural proteins, named NS2, NS3, NS4A, NS4B, NS5A, and NS5B [133].

HCV represents a major health problem in the world with over 185 million infected people and about 80% - 85% evolving in chronic infections with the risk to cause cirrhosis, HCC or lymphoproliferative disorders [134]. Similarly to HBV-related HCC, also HCV-related cancers show high TERT expression and telomerase activity which is associated with tumour progression and aggressiveness [135].

The antibody levels against specific peptides of the HCV proteome, including the N-terminal HCV core peptides, have been found significantly high in HCC patients and proposed as useful biomarkers of disease progression among HCV-positive patients [136].

HCV core protein is considered a positive regulator of telomerase function. Indeed, the expression of HCV core protein in human hepatoma cells has been reported to increase TERT gene transcription, telomerase activity and localization of TERT in the nucleus [137]. Although the molecular mechanisms involved in the telomerase regulatory properties of core protein are not known, this pathway may also contribute to the hepatocarcinogenesis mediated by HCV.

An additional effect of HCV core protein on telomerase activation has been shown to work via downregulation of microRNA-138 in HCV-related HCC [138]. TERT mRNA is a direct target of miR-138 in HCC cells, then the suppression of miR-138 by the mature HCV core protein (173 amino acids long) in HCV-related HCC induced an elevated expression of telomerase, inhibition of cell replicative senescence and promotion of hepatocarcinogenesis [138].

An additional mechanism of HCV-related telomerase deregulation is based on the ability of NS3-4A protease-helicase to bind the C-terminal region of TERT. The NS3-4A/TERT complex triggers increased telomerase activity in NS3-4A-transfected cells [139].

 doi:10.20944/preprints202209.0482.v1

Such effect was hindered by NS3-4A helicase and protease inhibitors proving its key role in the regulation of the telomerase and cell transformation.

2.4. Human T-Cell Leukemia Virus Type 1

HTLV1 was the first oncogenic human retrovirus to be discovered. HTLV1 contains a 8.5 kb linear, single-stranded RNA-positive genome, encoding structural proteins and enzymes as well as regulatory and accessory factors [140,141]. HTLV1 causes lifetime infection in about 10 million people worldwide, which following an incubation period lasting 15-30 years may impair the immune system causing lymphoproliferative diseases such as adult T cell leukemia and HTLV1 associated meylopathy/tropical spastic paraparesis [142].

Early studies reported strong telomerase activity, despite the maintenance of short telomeres, in HTLV1-positive adult T-cell leukemia cells compared to asymptomatic carriers or normal donors [143]. Moreover, the telomerase activity was reported to gradually increase with progression from the asymptomatic stages to acute and chronic disease [144,145].

Infection of human primary T cells with HTLV1 has been shown to cause transcriptional activation of TERT gene [146]. Moreover, Tax protein, transiently transduced into primary lymphocytes was able to induce telomerase expression through the nuclear NF-kB signalling pathway. The NFkB-dependent activation of TERT promoter is based on the increased binding of c-Myc and Sp1 to the cognate binding sites in HTLV1 and Tax-expressing cells [146].

The shelterin complex is known to regulate telomeres length by controlling the access of telomerase to the ends of chromosomes [147]. The TRF1, TRF2, and TIN2 factors of the shelterin complex are overexpressed in HTLV1- infected adult T-cell leukemia cells and likely are responsible for the maintenance of short telomeres and apoptotic inhibition [143,148].

An alternative mechanism of regulation of TERT promoter activity is based on the balance between the inhibitory function of Tax and the activating effect of the HBZ proteins (HTLV1 basic leucine zipper) [149]. In this model, the expression of Tax early after infection is supposed to inhibit TERT expression favouring telomeres attrition, genomic instability and neoplastic progression, while a later stage Tax may be repressed and the HBZ expression reactivates telomerase function allowing progression toward fully cell transformation. Furthermore, HBZ has been shown to cooperate with JunD to enhance TERT transcription in adult T-cell leukemia cells [150]. The tumour suppressor menin is able to interact with JunD and to negatively regulate telomerase expression. However, in HBZ-expressing cells the JunD/menin complex binds to HBZ and activate the histone acetyltransferase p300 leading to a reduced JunD/menin suppressor activity and increased TERT transcription [150]. Furthermore, the HBZ has been found to trigger proteasome-mediated degradation of T-cell acute lymphoblastic leukaemia 1 (TAL1) factor, which is a major TERT gene repressor in T lymphoblasts, thus inducing TERT expression and telomerase activity at later stages of infection [149].

3. Conclusions

The human oncoviruses share similar molecular mechanisms to promote cell transformation [151]. The activation of telomerase represents a common strategy operated by almost all oncoviruses to evade replicative senescence and to increase proliferative capacity of infected cells. In addition, telomerase has a crucial role in the regulation of the replicative program of some viruses and in the maintenance of cell immortality. Indeed, TERT

 doi:10.20944/preprints202209.0482.v1

expression is a main regulator of EBV latency and cell transformation, whereas TERT inhibition induces the lytic cycle of EBV and cell death. The regulation of telomerase activity by virus-encoded factors is complex and occurs at multiple levels, such as transcription, alternative splicing, post-transcription modifications and subcellular localization. Numerous studies have shown that TERT, beyond its telomere-lengthening function, concurs to cancer development via multiple activities including also the regulation of T cell function particularly in virus infected cells [152,153].

Virus related tumours develop in a small percentage of infected subjects which fail to produce effective immune response against infected cells. Telomerase expression has been recently implicated in the promotion of the immune suppressive tumour microenvironment. Indeed, the gene expression profiling of more than 9,000 tumours, including virus related cancers, showed that TERT mRNA levels were associated with immune suppressive signatures via TERT-dependent activation of endogenous retroviruses, which by forming double-stranded RNAs induce the RIG-1/MDA5-MAVS signalling pathway, interferon signalling, chemokines expression and recruitment of suppressive T cells in the tumour [154]. Therefore, it is possible to hypothesize that telomerase may potentiate the oncogenic effect of oncoviruses by a positive feedback loop in which viral factors induce TERT activation and TERT in turn causes the expression of endogenous retrovirus mRNAs that inhibit the immune surveillance on cancer-infected cells.

The Merkel cell polyomavirus is the only oncovirus which has not been implicated in TERT reactivation. However, the early proteins encoded by a recently discovered polyomavirus, namely Lyon IARC polyomavirus (LIPyV), have been demonstrated to activate TERT gene expression, via recruitment of the Sp1 transcription factor to the TERT promoter [155]. Therefore, TERT activation may be considered a common path in virus-related tumorigenesis.

We and others have shown that TERT promoter mutations, causing hyperactivation of telomerase, are highly frequent in HPV-related cancers and HCV-related hepatocellular carcinoma [28,31,32,125,156]. Particularly, all TERT promoter mutations are G>A transitions, which generate novel E-twenty-six binding sites, affect chromatin looping, interfere with the interaction of telomeres with the TERT loci as well as destabilize the DNA secondary structures and G-quadruplexes formed within the TERT promoter [156]. It is not yet known whether these mutations are also capable of potentiating the transactivation of the TERT promoter by oncoviral proteins. Of note, a recent study showed comparable levels of HPV16 E6 and a significant increase in TERT mRNA in cervical squamous cell carcinoma specimens mutated in the TERT promoter versus those not mutated, suggesting a synergistic effect of mutations and viral oncoproteins in telomerase expression [32].

Further investigation on the telomerase and oncoviruses interplay is conceivable to yield new advances and new therapeutic strategies to treat virus-related human cancers.

Author Contributions: M.L.T. conceived the study and wrote the manuscript; A.C., N.S., A.L.T, P.B., F.M.T., L.B., M.G.I, F.M.B. analyzed the collected material and wrote the manuscript.

Funding: This work was supported by the Italian Ministry of Health Ricerca Corrente 2022 Grant L1/10.

Data Availability Statement: The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

 doi:10.20944/preprints202209.0482.v1

1. de Martel, C.; Georges, D.; Bray, F.; Ferlay, J.; Clifford, G.M. Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis. *Lancet Glob Health* **2020**, *8*, e180-e190, doi:10.1016/s2214-109x(19)30488-7.
2. Tornesello, M.L.; Annunziata, C.; Tornesello, A.L.; Buonaguro, L.; Buonaguro, F.M. Human Oncoviruses and p53 Tumor Suppressor Pathway Deregulation at the Origin of Human Cancers. *Cancers (Basel)* **2018**, *10*, doi:10.3390/cancers10070213.
3. Mesri, E.A.; Feitelson, M.A.; Munger, K. Human viral oncogenesis: a cancer hallmarks analysis. *Cell Host Microbe* **2014**, *15*, 266-282, doi:10.1016/j.chom.2014.02.011.
4. Jafri, M.A.; Ansari, S.A.; Alqahtani, M.H.; Shay, J.W. Roles of telomeres and telomerase in cancer, and advances in telomerase-targeted therapies. *Genome Med* **2016**, *8*, 69, doi:10.1186/s13073-016-0324-x.
5. Yuan, X.; Larsson, C.; Xu, D. Mechanisms underlying the activation of TERT transcription and telomerase activity in human cancer: old actors and new players. *Oncogene* **2019**, *38*, 6172-6183, doi:10.1038/s41388-019-0872-9.
6. Zaug, A.J.; Crary, S.M.; Jesse Fioravanti, M.; Campbell, K.; Cech, T.R. Many disease-associated variants of hTERT retain high telomerase enzymatic activity. *Nucleic Acids Res* **2013**, *41*, 8969-8978, doi:10.1093/nar/gkt653.
7. Bellon, M.; Nicot, C. Regulation of telomerase and telomeres: human tumor viruses take control. *J Natl Cancer Inst* **2008**, *100*, 98-108, doi:10.1093/jnci/djm269.
8. Knight, J.S.; Cotter, M.A., 2nd; Robertson, E.S. The latency-associated nuclear antigen of Kaposi's sarcoma-associated herpesvirus transactivates the telomerase reverse transcriptase promoter. *J Biol Chem* **2001**, *276*, 22971-22978, doi:10.1074/jbc.M101890200.
9. Klingelhutz, A.J.; Foster, S.A.; McDougall, J.K. Telomerase activation by the E6 gene product of human papillomavirus type 16. *Nature* **1996**, *380*, 79-82, doi:10.1038/380079a0.
10. Yang, J.; Deng, X.; Deng, L.; Gu, H.; Fan, W.; Cao, Y. Telomerase activation by Epstein-Barr virus latent membrane protein 1 is associated with c-Myc expression in human nasopharyngeal epithelial cells. *J Exp Clin Cancer Res* **2004**, *23*, 495-506.
11. Sze, K.M.; Ho, D.W.; Chiu, Y.T.; Tsui, Y.M.; Chan, L.K.; Lee, J.M.; Chok, K.S.; Chan, A.C.; Tang, C.N.; Tang, V.W.; et al. Hepatitis B Virus-Telomerase Reverse Transcriptase Promoter Integration Harnesses Host ELF4, Resulting in Telomerase Reverse Transcriptase Gene Transcription in Hepatocellular Carcinoma. *Hepatology* **2021**, *73*, 23-40, doi:10.1002/hep.31231.
12. Osterrieder, N.; Wallaschek, N.; Kaufer, B.B. Herpesvirus Genome Integration into Telomeric Repeats of Host Cell Chromosomes. *Annu Rev Virol* **2014**, *1*, 215-235, doi:10.1146/annurev-virology-031413-085422.
13. Wallaschek, N.; Sanyal, A.; Pirzer, F.; Gravel, A.; Mori, Y.; Flamand, L.; Kaufer, B.B. The Telomeric Repeats of Human Herpesvirus 6A (HHV-6A) Are Required for Efficient Virus Integration. *PLoS Pathog* **2016**, *12*, e1005666, doi:10.1371/journal.ppat.1005666.
14. Olovnikov, A.M. [Principle of marginotomy in template synthesis of polynucleotides]. *Dokl Akad Nauk SSSR* **1971**, *201*, 1496-1499.
15. Smith, E.M.; Pendlebury, D.F.; Nandakumar, J. Structural biology of telomeres and telomerase. *Cell Mol Life Sci* **2020**, *77*, 61-79, doi:10.1007/s00018-019-03369-x.
16. Zhao, Y.; Sfeir, A.J.; Zou, Y.; Buseman, C.M.; Chow, T.T.; Shay, J.W.; Wright, W.E. Telomere extension occurs at most chromosome ends and is uncoupled from fill-in in human cancer cells. *Cell* **2009**, *138*, 463-475, doi:10.1016/j.cell.2009.05.026.
17. Cristofari, G.; Lingner, J. Telomere length homeostasis requires that telomerase levels are limiting. *Embo j* **2006**, *25*, 565-574, doi:10.1038/sj.emboj.7600952.
18. Shay, J.W.; Wright, W.E. Hayflick, his limit, and cellular ageing. *Nat Rev Mol Cell Biol* **2000**, *1*, 72-76, doi:10.1038/35036093.
19. Dratwa, M.; Wysoczańska, B.; Łacina, P.; Kubik, T.; Bogunia-Kubik, K. TERT-Regulation and Roles in Cancer Formation. *Front Immunol* **2020**, *11*, 589929, doi:10.3389/fimmu.2020.589929.

20. Li, Y.; Zhou, Q.L.; Sun, W.; Chandrasekharan, P.; Cheng, H.S.; Ying, Z.; Lakshmanan, M.; Raju, A.; Tenen, D.G.; Cheng, S.Y.; et al. Non-canonical NF-κB signalling and ETS1/2 cooperatively drive C250T mutant TERT promoter activation. *Nat Cell Biol* **2015**, *17*, 1327-1338, doi:10.1038/ncb3240.
21. Wang, J.; Xie, L.Y.; Allan, S.; Beach, D.; Hannon, G.J. Myc activates telomerase. *Genes Dev* **1998**, *12*, 1769-1774, doi:10.1101/gad.12.12.1769.
22. Lee, D.D.; Leão, R.; Komosa, M.; Gallo, M.; Zhang, C.H.; Lipman, T.; Remke, M.; Heidari, A.; Nunes, N.M.; Apolónio, J.D.; et al. DNA hypermethylation within TERT promoter upregulates TERT expression in cancer. *J Clin Invest* **2019**, *129*, 1801, doi:10.1172/jci128527.
23. Barthel, F.P.; Wei, W.; Tang, M.; Martinez-Ledesma, E.; Hu, X.; Amin, S.B.; Akdemir, K.C.; Seth, S.; Song, X.; Wang, Q.; et al. Systematic analysis of telomere length and somatic alterations in 31 cancer types. *Nat Genet* **2017**, *49*, 349-357, doi:10.1038/ng.3781.
24. Kim, W.; Ludlow, A.T.; Min, J.; Robin, J.D.; Stadler, G.; Mender, I.; Lai, T.P.; Zhang, N.; Wright, W.E.; Shay, J.W. Regulation of the Human Telomerase Gene TERT by Telomere Position Effect-Over Long Distances (TPE-OLD): Implications for Aging and Cancer. *PLoS Biol* **2016**, *14*, e2000016, doi:10.1371/journal.pbio.2000016.
25. Bell, R.J.; Rube, H.T.; Kreig, A.; Mancini, A.; Fouse, S.D.; Nagarajan, R.P.; Choi, S.; Hong, C.; He, D.; Pekmezci, M.; et al. Cancer. The transcription factor GABP selectively binds and activates the mutant TERT promoter in cancer. *Science* **2015**, *348*, 1036-1039, doi:10.1126/science.aab0015.
26. Vinagre, J.; Almeida, A.; Pópulo, H.; Batista, R.; Lyra, J.; Pinto, V.; Coelho, R.; Celestino, R.; Prazeres, H.; Lima, L.; et al. Frequency of TERT promoter mutations in human cancers. *Nat Commun* **2013**, *4*, 2185, doi:10.1038/ncomms3185.
27. Stern, J.L.; Theodorescu, D.; Vogelstein, B.; Papadopoulos, N.; Cech, T.R. Mutation of the TERT promoter, switch to active chromatin, and monoallelic TERT expression in multiple cancers. *Genes Dev* **2015**, *29*, 2219-2224, doi:10.1101/gad.269498.115.
28. Starita, N.; Pezzuto, F.; Sarno, S.; Losito, N.S.; Perdonà, S.; Buonaguro, L.; Buonaguro, F.M.; Tornesello, M.L. Mutations in the telomerase reverse transcriptase promoter and PIK3CA gene are common events in penile squamous cell carcinoma of Italian and Ugandan patients. *Int J Cancer* **2022**, *150*, 1879-1888, doi:10.1002/ijc.33990.
29. Huang, D.S.; Wang, Z.; He, X.J.; Diplas, B.H.; Yang, R.; Killela, P.J.; Meng, Q.; Ye, Z.Y.; Wang, W.; Jiang, X.T.; et al. Recurrent TERT promoter mutations identified in a large-scale study of multiple tumour types are associated with increased TERT expression and telomerase activation. *Eur J Cancer* **2015**, *51*, 969-976, doi:10.1016/j.ejca.2015.03.010.
30. Pezzuto, F.; Buonaguro, L.; Buonaguro, F.M.; Tornesello, M.L. Frequency and geographic distribution of TERT promoter mutations in primary hepatocellular carcinoma. *Infect Agent Cancer* **2017**, *12*, 27, doi:10.1186/s13027-017-0138-5.
31. Starita, N.; Buonaguro, L.; Buonaguro, F.M.; Tornesello, M.L. Telomerase promoter mutations in human immunodeficiency virus-related conjunctiva neoplasia. *J Transl Med* **2018**, *16*, 77, doi:10.1186/s12967-018-1456-0.
32. Annunziata, C.; Pezzuto, F.; Greggi, S.; Ionna, F.; Losito, S.; Botti, G.; Buonaguro, L.; Buonaguro, F.M.; Tornesello, M.L. Distinct profiles of TERT promoter mutations and telomerase expression in head and neck cancer and cervical carcinoma. *Int J Cancer* **2018**, *143*, 1153-1161, doi:10.1002/ijc.31412.
33. Giunco, S.; Boscolo-Rizzo, P.; Rampazzo, E.; Tirelli, G.; Alessandrini, L.; Di Carlo, R.; Rossi, M.; Nicolai, P.; Menegaldo, A.; Carraro, V.; et al. TERT Promoter Mutations and rs2853669 Polymorphism: Useful Markers for Clinical Outcome Stratification of Patients With Oral Cavity Squamous Cell Carcinoma. *Front Oncol* **2021**, *11*, 782658, doi:10.3389/fonc.2021.782658.
34. Romaniuk, A.; Paszel-Jaworska, A.; Totoń, E.; Lisiak, N.; Hołysz, H.; Królak, A.; Grodecka-Gazdecka, S.; Rubiś, B. The non-canonical functions of telomerase: to turn off or not to turn off. *Mol Biol Rep* **2019**, *46*, 1401-1411, doi:10.1007/s11033-018-4496-x.

35. Zhang, X.; Gaspard, J.P.; Chung, D.C. Regulation of vascular endothelial growth factor by the Wnt and K-ras pathways in colonic neoplasia. *Cancer Res* **2001**, *61*, 6050-6054.
36. He, T.C.; Sparks, A.B.; Rago, C.; Hermeking, H.; Zawel, L.; da Costa, L.T.; Morin, P.J.; Vogelstein, B.; Kinzler, K.W. Identification of c-MYC as a target of the APC pathway. *Science* **1998**, *281*, 1509-1512, doi:10.1126/science.281.5382.1509.
37. Park, J.I.; Venteicher, A.S.; Hong, J.Y.; Choi, J.; Jun, S.; Shkreli, M.; Chang, W.; Meng, Z.; Cheung, P.; Ji, H.; et al. Telomerase modulates Wnt signalling by association with target gene chromatin. *Nature* **2009**, *460*, 66-72, doi:10.1038/nature08137.
38. Chen, K.; Chen, L.; Li, L.; Qu, S.; Yu, B.; Sun, Y.; Wan, F.; Chen, X.; Liang, R.; Zhu, X. A positive feedback loop between Wnt/β-catenin signaling and hTERT regulates the cancer stem cell-like traits in radioresistant nasopharyngeal carcinoma cells. *J Cell Biochem* **2020**, *121*, 4612-4622, doi:10.1002/jcb.29681.
39. Smith, L.L.; Coller, H.A.; Roberts, J.M. Telomerase modulates expression of growth-controlling genes and enhances cell proliferation. *Nat Cell Biol* **2003**, *5*, 474-479, doi:10.1038/ncb985.
40. Young, J.I.; Sedivy, J.M.; Smith, J.R. Telomerase expression in normal human fibroblasts stabilizes DNA 5-methylcytosine transferase I. *J Biol Chem* **2003**, *278*, 19904-19908, doi:10.1074/jbc.M301685200.
41. Ségal-Bendirdjian, E.; Geli, V. Non-canonical Roles of Telomerase: Unraveling the Imbroglio. *Front Cell Dev Biol* **2019**, *7*, 332, doi:10.3389/fcell.2019.00332.
42. Yu, J.; Yuan, X.; Sjöholm, L.; Liu, T.; Kong, F.; Ekström, T.J.; Björkholm, M.; Xu, D. Telomerase reverse transcriptase regulates DNMT3B expression/aberrant DNA methylation phenotype and AKT activation in hepatocellular carcinoma. *Cancer Lett* **2018**, *434*, 33-41, doi:10.1016/j.canlet.2018.07.013.
43. Liu, N.; Ding, D.; Hao, W.; Yang, F.; Wu, X.; Wang, M.; Xu, X.; Ju, Z.; Liu, J.P.; Song, Z.; et al. hTERT promotes tumor angiogenesis by activating VEGF via interactions with the Sp1 transcription factor. *Nucleic Acids Res* **2016**, *44*, 8693-8703, doi:10.1093/nar/gkw549.
44. de Villiers, E.M. Cross-roads in the classification of papillomaviruses. *Virology* **2013**, *445*, 2-10, doi:10.1016/j.virol.2013.04.023.
45. de Martel, C.; Plummer, M.; Vignat, J.; Franceschi, S. Worldwide burden of cancer attributable to HPV by site, country and HPV type. *Int J Cancer* **2017**, *141*, 664-670, doi:10.1002/ijc.30716.
46. Bouvard, V.; Baan, R.; Straif, K.; Grosse, Y.; Secretan, B.; El Ghissassi, F.; Benbrahim-Tallaa, L.; Guha, N.; Freeman, C.; Galichet, L.; et al. A review of human carcinogens--Part B: biological agents. *Lancet Oncol* **2009**, *10*, 321-322, doi:10.1016/s1470-2045(09)70096-8.
47. Phelps, W.C.; Yee, C.L.; Münger, K.; Howley, P.M. The human papillomavirus type 16 E7 gene encodes transactivation and transformation functions similar to those of adenovirus E1A. *Cell* **1988**, *53*, 539-547, doi:10.1016/0092-8674(88)90570-3.
48. Zanier, K.; Charbonnier, S.; Sidi, A.O.; McEwen, A.G.; Ferrario, M.G.; Poussin-Courmontagne, P.; Cura, V.; Brimer, N.; Babah, K.O.; Ansari, T.; et al. Structural basis for hijacking of cellular LxxLL motifs by papillomavirus E6 oncoproteins. *Science* **2013**, *339*, 694-698, doi:10.1126/science.1229934.
49. Saidu, N.E.B.; Filić, V.; Thomas, M.; Sarabia-Vega, V.; Đukić, A.; Miljković, F.; Banks, L.; Tomaić, V. PDZ Domain-Containing Protein NHERF-2 Is a Novel Target of Human Papillomavirus 16 (HPV-16) and HPV-18. *J Virol* **2019**, *94*, doi:10.1128/jvi.00663-19.
50. Hoppe-Seyler, K.; Bossler, F.; Braun, J.A.; Herrmann, A.L.; Hoppe-Seyler, F. The HPV E6/E7 Oncogenes: Key Factors for Viral Carcinogenesis and Therapeutic Targets. *Trends Microbiol* **2018**, *26*, 158-168, doi:10.1016/j.tim.2017.07.007.
51. Estêvão, D.; Costa, N.R.; Gil da Costa, R.M.; Medeiros, R. Hallmarks of HPV carcinogenesis: The role of E6, E7 and E5 oncoproteins in cellular malignancy. *Biochim Biophys Acta Gene Regul Mech* **2019**, *1862*, 153-162, doi:10.1016/j.bbagrm.2019.01.001.

52. Zimmermann, H.; Degenkolbe, R.; Bernard, H.U.; O'Connor, M.J. The human papillomavirus type 16 E6 oncoprotein can down-regulate p53 activity by targeting the transcriptional coactivator CBP/p300. *J Virol* **1999**, *73*, 6209-6219, doi:10.1128/jvi.73.8.6209-6219.1999.
53. Huh, K.; Zhou, X.; Hayakawa, H.; Cho, J.Y.; Libermann, T.A.; Jin, J.; Harper, J.W.; Munger, K. Human papillomavirus type 16 E7 oncoprotein associates with the cullin 2 ubiquitin ligase complex, which contributes to degradation of the retinoblastoma tumor suppressor. *J Virol* **2007**, *81*, 9737-9747, doi:10.1128/jvi.00881-07.
54. White, E.A.; Sowa, M.E.; Tan, M.J.; Jeudy, S.; Hayes, S.D.; Santha, S.; Münger, K.; Harper, J.W.; Howley, P.M. Systematic identification of interactions between host cell proteins and E7 oncoproteins from diverse human papillomaviruses. *Proc Natl Acad Sci U S A* **2012**, *109*, E260-267, doi:10.1073/pnas.1116776109.
55. Tomita, T.; Huibregtse, J.M.; Matouschek, A. A masked initiation region in retinoblastoma protein regulates its proteasomal degradation. *Nat Commun* **2020**, *11*, 2019, doi:10.1038/s41467-020-16003-3.
56. Liu, X.; Dakic, A.; Zhang, Y.; Dai, Y.; Chen, R.; Schlegel, R. HPV E6 protein interacts physically and functionally with the cellular telomerase complex. *Proc Natl Acad Sci U S A* **2009**, *106*, 18780-18785, doi:10.1073/pnas.0906357106.
57. Pańczyszyn, A.; Boniewska-Bernacka, E.; Głąb, G. Telomeres and Telomerase During Human Papillomavirus-Induced Carcinogenesis. *Mol Diagn Ther* **2018**, *22*, 421-430, doi:10.1007/s40291-018-0336-x.
58. Katzenellenbogen, R.A. Activation of telomerase by HPVs. *Virus Res* **2017**, *231*, 50-55, doi:10.1016/j.virusres.2016.11.003.
59. Kiyono, T.; Foster, S.A.; Koop, J.I.; McDougall, J.K.; Galloway, D.A.; Klingelhutz, A.J. Both Rb/p16INK4a inactivation and telomerase activity are required to immortalize human epithelial cells. *Nature* **1998**, *396*, 84-88, doi:10.1038/23962.
60. Oh, S.T.; Kyo, S.; Laimins, L.A. Telomerase activation by human papillomavirus type 16 E6 protein: induction of human telomerase reverse transcriptase expression through Myc and GC-rich Sp1 binding sites. *J Virol* **2001**, *75*, 5559-5566, doi:10.1128/jvi.75.12.5559-5566.2001.
61. Takakura, M.; Kyo, S.; Kanaya, T.; Hirano, H.; Takeda, J.; Yutsudo, M.; Inoue, M. Cloning of human telomerase catalytic subunit (hTERT) gene promoter and identification of proximal core promoter sequences essential for transcriptional activation in immortalized and cancer cells. *Cancer Res* **1999**, *59*, 551-557.
62. Kyo, S.; Takakura, M.; Taira, T.; Kanaya, T.; Itoh, H.; Yutsudo, M.; Ariga, H.; Inoue, M. Sp1 cooperates with c-Myc to activate transcription of the human telomerase reverse transcriptase gene (hTERT). *Nucleic Acids Res* **2000**, *28*, 669-677, doi:10.1093/nar/28.3.669.
63. Gabay, M.; Li, Y.; Felsher, D.W. MYC activation is a hallmark of cancer initiation and maintenance. *Cold Spring Harb Perspect Med* **2014**, *4*, doi:10.1101/cshperspect.a014241.
64. Casillas, M.A.; Brotherton, S.L.; Andrews, L.G.; Ruppert, J.M.; Tollefsbol, T.O. Induction of endogenous telomerase (hTERT) by c-Myc in WI-38 fibroblasts transformed with specific genetic elements. *Gene* **2003**, *316*, 57-65, doi:10.1016/s0378-1119(03)00739-x.
65. Veldman, T.; Horikawa, I.; Barrett, J.C.; Schlegel, R. Transcriptional activation of the telomerase hTERT gene by human papillomavirus type 16 E6 oncoprotein. *J Virol* **2001**, *75*, 4467-4472, doi:10.1128/jvi.75.9.4467-4472.2001.
66. McMurray, H.R.; McCance, D.J. Human papillomavirus type 16 E6 activates TERT gene transcription through induction of c-Myc and release of USF-mediated repression. *J Virol* **2003**, *77*, 9852-9861, doi:10.1128/jvi.77.18.9852-9861.2003.
67. Veldman, T.; Liu, X.; Yuan, H.; Schlegel, R. Human papillomavirus E6 and Myc proteins associate in vivo and bind to and cooperatively activate the telomerase reverse transcriptase promoter. *Proc Natl Acad Sci U S A* **2003**, *100*, 8211-8216, doi:10.1073/pnas.1435900100.
68. Khattar, E.; Tergaonkar, V. Transcriptional Regulation of Telomerase Reverse Transcriptase (TERT) by MYC. *Front Cell Dev Biol* **2017**, *5*, 1, doi:10.3389/fcell.2017.00001.

69. Gewin, L.; Myers, H.; Kiyono, T.; Galloway, D.A. Identification of a novel telomerase repressor that interacts with the human papillomavirus type-16 E6/E6-AP complex. *Genes Dev* **2004**, *18*, 2269-2282, doi:10.1101/gad.1214704.
70. Liu, X.; Yuan, H.; Fu, B.; Disbrow, G.L.; Apolinario, T.; Tomaic, V.; Kelley, M.L.; Baker, C.C.; Huibregtse, J.; Schlegel, R. The E6AP ubiquitin ligase is required for transactivation of the hTERT promoter by the human papillomavirus E6 oncoprotein. *J Biol Chem* **2005**, *280*, 10807-10816, doi:10.1074/jbc.M410343200.
71. Kelley, M.L.; Keiger, K.E.; Lee, C.J.; Huibregtse, J.M. The global transcriptional effects of the human papillomavirus E6 protein in cervical carcinoma cell lines are mediated by the E6AP ubiquitin ligase. *J Virol* **2005**, *79*, 3737-3747, doi:10.1128/jvi.79.6.3737-3747.2005.
72. James, M.A.; Lee, J.H.; Klingelhutz, A.J. HPV16-E6 associated hTERT promoter acetylation is E6AP dependent, increased in later passage cells and enhanced by loss of p300. *Int J Cancer* **2006**, *119*, 1878-1885, doi:10.1002/ijc.22064.
73. Xu, M.; Luo, W.; Elzi, D.J.; Grandori, C.; Galloway, D.A. NFX1 interacts with mSin3A/histone deacetylase to repress hTERT transcription in keratinocytes. *Mol Cell Biol* **2008**, *28*, 4819-4828, doi:10.1128/mcb.01969-07.
74. Katzenellenbogen, R.A.; Vliet-Gregg, P.; Xu, M.; Galloway, D.A. Cytoplasmic poly(A) binding proteins regulate telomerase activity and cell growth in human papillomavirus type 16 E6-expressing keratinocytes. *J Virol* **2010**, *84*, 12934-12944, doi:10.1128/jvi.01377-10.
75. Billingsley, C.L.; Chintala, S.; Katzenellenbogen, R.A. Post-Transcriptional Gene Regulation by HPV 16E6 and Its Host Protein Partners. *Viruses* **2022**, *14*, doi:10.3390/v14071483.
76. Vliet-Gregg, P.A.; Robinson, K.L.; Levan, J.; Matsumoto, L.R.; Katzenellenbogen, R.A. NFX1-123 is highly expressed in cervical cancer and increases growth and telomerase activity in HPV 16E6 expressing cells. *Cancer Lett* **2019**, *449*, 106-113, doi:10.1016/j.canlet.2019.02.024.
77. Rajagopalan, D.; Pandey, A.K.; Xiuzhen, M.C.; Lee, K.K.; Hora, S.; Zhang, Y.; Chua, B.H.; Kwok, H.S.; Bhatia, S.S.; Deng, L.W.; et al. TIP60 represses telomerase expression by inhibiting Sp1 binding to the TERT promoter. *PLoS Pathog* **2017**, *13*, e1006681, doi:10.1371/journal.ppat.1006681.
78. Jha, S.; Vande Pol, S.; Banerjee, N.S.; Dutta, A.B.; Chow, L.T.; Dutta, A. Destabilization of TIP60 by human papillomavirus E6 results in attenuation of TIP60-dependent transcriptional regulation and apoptotic pathway. *Mol Cell* **2010**, *38*, 700-711, doi:10.1016/j.molcel.2010.05.020.
79. Subbaiah, V.K.; Zhang, Y.; Rajagopalan, D.; Abdullah, L.N.; Yeo-Teh, N.S.; Tomaić, V.; Banks, L.; Myers, M.P.; Chow, E.K.; Jha, S. E3 ligase EDD1/UBR5 is utilized by the HPV E6 oncogene to destabilize tumor suppressor TIP60. *Oncogene* **2016**, *35*, 2062-2074, doi:10.1038/onc.2015.268.
80. Dürst, M.; Croce, C.M.; Gissmann, L.; Schwarz, E.; Huebner, K. Papillomavirus sequences integrate near cellular oncogenes in some cervical carcinomas. *Proc Natl Acad Sci U S A* **1987**, *84*, 1070-1074, doi:10.1073/pnas.84.4.1070.
81. Tornesello, M.L.; Buonaguro, L.; Giorgi-Rossi, P.; Buonaguro, F.M. Viral and cellular biomarkers in the diagnosis of cervical intraepithelial neoplasia and cancer. *Biomed Res Int* **2013**, *2013*, 519619, doi:10.1155/2013/519619.
82. Choo, K.B.; Pan, C.C.; Han, S.H. Integration of human papillomavirus type 16 into cellular DNA of cervical carcinoma: preferential deletion of the E2 gene and invariable retention of the long control region and the E6/E7 open reading frames. *Virology* **1987**, *161*, 259-261, doi:10.1016/0042-6822(87)90195-4.
83. Ren, S.; Gaykalova, D.A.; Guo, T.; Favorov, A.V.; Fertig, E.J.; Tamayo, P.; Callejas-Valera, J.L.; Allevato, M.; Gilardi, M.; Santos, J.; et al. HPV E2, E4, E5 drive alternative carcinogenic pathways in HPV positive cancers. *Oncogene* **2020**, *39*, 6327-6339, doi:10.1038/s41388-020-01431-8.
84. Integrated genomic and molecular characterization of cervical cancer. *Nature* **2017**, *543*, 378-384, doi:10.1038/nature21386.

85. Ferber, M.J.; Montoya, D.P.; Yu, C.; Aderca, I.; McGee, A.; Thorland, E.C.; Nagorney, D.M.; Gostout, B.S.; Burgart, L.J.; Boix, L.; et al. Integrations of the hepatitis B virus (HBV) and human papillomavirus (HPV) into the human telomerase reverse transcriptase (hTERT) gene in liver and cervical cancers. *Oncogene* **2003**, *22*, 3813-3820, doi:10.1038/sj.onc.1206528.
86. Reddy, V.G.; Khanna, N.; Jain, S.K.; Das, B.C.; Singh, N. Telomerase-A molecular marker for cervical cancer screening. *Int J Gynecol Cancer* **2001**, *11*, 100-106, doi:10.1046/j.1525-1438.2001.011001100.x.
87. Barbosa, L.C.; da Silva, I.D.; Corrêa, J.C.; Ribalta, J.C. Survivin and telomerase expression in the uterine cervix of women with human papillomavirus-induced lesions. *Int J Gynecol Cancer* **2011**, *21*, 15-21, doi:10.1097/IGC.0b013e318203d42b.
88. Branca, M.; Giorgi, C.; Ciotti, M.; Santini, D.; Di Bonito, L.; Costa, S.; Benedetto, A.; Bonifacio, D.; Di Bonito, P.; Paba, P.; et al. Upregulation of telomerase (hTERT) is related to the grade of cervical intraepithelial neoplasia, but is not an independent predictor of high-risk human papillomavirus, virus persistence, or disease outcome in cervical cancer. *Diagn Cytopathol* **2006**, *34*, 739-748, doi:10.1002/dc.20554.
89. Boscolo-Rizzo, P.; Rampazzo, E.; Polesel, J.; Giunco, S.; Menegaldo, A.; Mantovani, M.; Stellin, M.; Bandolin, L.; Spinato, G.; Del Mistro, A.; et al. Predictive and prognostic significance of telomerase levels/telomere length in tissues and peripheral blood in head and neck squamous cell carcinoma. *Sci Rep* **2019**, *9*, 17572, doi:10.1038/s41598-019-54028-x.
90. Boscolo-Rizzo, P.; Da Mosto, M.C.; Rampazzo, E.; Giunco, S.; Del Mistro, A.; Menegaldo, A.; Baboci, L.; Mantovani, M.; Tirelli, G.; De Rossi, A. Telomeres and telomerase in head and neck squamous cell carcinoma: from pathogenesis to clinical implications. *Cancer Metastasis Rev* **2016**, *35*, 457-474, doi:10.1007/s10555-016-9633-1.
91. Boscolo-Rizzo, P.; Rampazzo, E.; Perissinotto, E.; Piano, M.A.; Giunco, S.; Baboci, L.; Spinato, G.; Spinato, R.; Tirelli, G.; Da Mosto, M.C.; et al. Telomere shortening in mucosa surrounding the tumor: biosensor of field cancerization and prognostic marker of mucosal failure in head and neck squamous cell carcinoma. *Oral Oncol* **2015**, *51*, 500-507, doi:10.1016/j.oraloncology.2015.02.100.
92. Young, L.S.; Yap, L.F.; Murray, P.G. Epstein-Barr virus: more than 50 years old and still providing surprises. *Nat Rev Cancer* **2016**, *16*, 789-802, doi:10.1038/nrc.2016.92.
93. Murata, T.; Tsurumi, T. Switching of EBV cycles between latent and lytic states. *Rev Med Virol* **2014**, *24*, 142-153, doi:10.1002/rmv.1780.
94. Young, L.S.; Murray, P.G. Epstein-Barr virus and oncogenesis: from latent genes to tumours. *Oncogene* **2003**, *22*, 5108-5121, doi:10.1038/sj.onc.1206556.
95. Chang, M.S.; Kim, W.H. Epstein-Barr virus in human malignancy: a special reference to Epstein-Barr virus associated gastric carcinoma. *Cancer Res Treat* **2005**, *37*, 257-267, doi:10.4143/crt.2005.37.5.257.
96. Dolcetti, R.; Dal Col, J.; Martorelli, D.; Carbone, A.; Klein, E. Interplay among viral antigens, cellular pathways and tumor microenvironment in the pathogenesis of EBV-driven lymphomas. *Semin Cancer Biol* **2013**, *23*, 441-456, doi:10.1016/j.semcancer.2013.07.005.
97. Giunco, S.; Petrara, M.R.; Zangrossi, M.; Celeghin, A.; De Rossi, A. Extra-telomeric functions of telomerase in the pathogenesis of Epstein-Barr virus-driven B-cell malignancies and potential therapeutic implications. *Infect Agent Cancer* **2018**, *13*, 14, doi:10.1186/s13027-018-0186-5.
98. Young, L.S.; Rickinson, A.B. Epstein-Barr virus: 40 years on. *Nat Rev Cancer* **2004**, *4*, 757-768, doi:10.1038/nrc1452.
99. Giunco, S.; Celeghin, A.; Gianesin, K.; Dolcetti, R.; Indraccolo, S.; De Rossi, A. Cross talk between EBV and telomerase: the role of TERT and NOTCH2 in the switch of latent/lytic cycle of the virus. *Cell Death Dis* **2015**, *6*, e1774, doi:10.1038/cddis.2015.145.
100. Terrin, L.; Dal Col, J.; Rampazzo, E.; Zancai, P.; Pedrotti, M.; Ammirabile, G.; Bergamin, S.; Rizzo, S.; Dolcetti, R.; De Rossi, A. Latent membrane protein 1 of Epstein-Barr virus activates the hTERT promoter and enhances telomerase activity in B lymphocytes. *J Virol* **2008**, *82*, 10175-10187, doi:10.1128/jvi.00321-08.

101. Mei, Y.P.; Zhu, X.F.; Zhou, J.M.; Huang, H.; Deng, R.; Zeng, Y.X. siRNA targeting LMP1-induced apoptosis in EBV-positive lymphoma cells is associated with inhibition of telomerase activity and expression. *Cancer Lett* **2006**, *232*, 189-198, doi:10.1016/j.canlet.2005.02.010.
102. Giunco, S.; Dolcetti, R.; Keppel, S.; Celeghin, A.; Indraccolo, S.; Dal Col, J.; Mastorci, K.; De Rossi, A. hTERT inhibition triggers Epstein-Barr virus lytic cycle and apoptosis in immortalized and transformed B cells: a basis for new therapies. *Clin Cancer Res* **2013**, *19*, 2036-2047, doi:10.1158/1078-0432.Ccr-12-2537.
103. Ding, L.; Li, L.L.; Yang, J.; Tao, Y.G.; Ye, M.; Shi, Y.; Tang, M.; Yi, W.; Li, X.L.; Gong, J.P.; et al. Epstein-Barr virus encoded latent membrane protein 1 modulates nuclear translocation of telomerase reverse transcriptase protein by activating nuclear factor-kappaB p65 in human nasopharyngeal carcinoma cells. *Int J Biochem Cell Biol* **2005**, *37*, 1881-1889, doi:10.1016/j.biocel.2005.04.012.
104. Chen, F.; Liu, C.; Lindvall, C.; Xu, D.; Ernberg, I. Epstein-Barr virus latent membrane 2A (LMP2A) down-regulates telomerase reverse transcriptase (hTERT) in epithelial cell lines. *Int J Cancer* **2005**, *113*, 284-289, doi:10.1002/ijc.20594.
105. Edelman, D.C. Human herpesvirus 8--a novel human pathogen. *Virol J* **2005**, *2*, 78, doi:10.1186/1743-422x-2-78.
106. Chang, Y.; Cesarman, E.; Pessin, M.S.; Lee, F.; Culpepper, J.; Knowles, D.M.; Moore, P.S. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science* **1994**, *266*, 1865-1869, doi:10.1126/science.7997879.
107. Cesarman, E.; Chang, Y.; Moore, P.S.; Said, J.W.; Knowles, D.M. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas. *N Engl J Med* **1995**, *332*, 1186-1191, doi:10.1056/nejm199505043321802.
108. Buonaguro, F.M.; Tornesello, M.L.; Beth-Giraldo, E.; Hatzakis, A.; Mueller, N.; Downing, R.; Biryamwaho, B.; Sempala, S.D.; Giraldo, G. Herpesvirus-like DNA sequences detected in endemic, classic, iatrogenic and epidemic Kaposi's sarcoma (KS) biopsies. *Int J Cancer* **1996**, *65*, 25-28, doi:10.1002/(sici)1097-0215(19960103)65:1<25::Aid-ijc5>3.0.Co;2-3.
109. Neipel, F.; Fleckenstein, B. The role of HHV-8 in Kaposi's sarcoma. *Semin Cancer Biol* **1999**, *9*, 151-164, doi:10.1006/scbi.1999.0129.
110. Suzuki, T.; Isobe, T.; Kitagawa, M.; Ueda, K. Kaposi's sarcoma-associated herpesvirus-encoded LANA positively affects on ubiquitylation of p53. *Biochem Biophys Res Commun* **2010**, *403*, 194-197, doi:10.1016/j.bbrc.2010.11.004.
111. Radkov, S.A.; Kellam, P.; Boshoff, C. The latent nuclear antigen of Kaposi sarcoma-associated herpesvirus targets the retinoblastoma-E2F pathway and with the oncogene Hras transforms primary rat cells. *Nat Med* **2000**, *6*, 1121-1127, doi:10.1038/80459.
112. Si, H.; Robertson, E.S. Kaposi's sarcoma-associated herpesvirus-encoded latency-associated nuclear antigen induces chromosomal instability through inhibition of p53 function. *J Virol* **2006**, *80*, 697-709, doi:10.1128/jvi.80.2.697-709.2006.
113. Flore, O.; Rafii, S.; Ely, S.; O'Leary, J.J.; Hyjek, E.M.; Cesarman, E. Transformation of primary human endothelial cells by Kaposi's sarcoma-associated herpesvirus. *Nature* **1998**, *394*, 588-592, doi:10.1038/29093.
114. Verma, S.C.; Borah, S.; Robertson, E.S. Latency-associated nuclear antigen of Kaposi's sarcoma-associated herpesvirus up-regulates transcription of human telomerase reverse transcriptase promoter through interaction with transcription factor Sp1. *J Virol* **2004**, *78*, 10348-10359, doi:10.1128/jvi.78.19.10348-10359.2004.
115. Shamay, M.; Liu, J.; Li, R.; Liao, G.; Shen, L.; Greenway, M.; Hu, S.; Zhu, J.; Xie, Z.; Ambinder, R.F.; et al. A protein array screen for Kaposi's sarcoma-associated herpesvirus LANA interactors links LANA to TIP60, PP2A activity, and telomere shortening. *J Virol* **2012**, *86*, 5179-5191, doi:10.1128/jvi.00169-12.
116. Kobayashi, Y.; Sato, K.; Kibe, T.; Seimiya, H.; Nakamura, A.; Yukawa, M.; Tsuchiya, E.; Ueno, M. Expression of mutant RPA in human cancer cells causes telomere shortening. *Biosci Biotechnol Biochem* **2010**, *74*, 382-385, doi:10.1271/bbb.90496.

117. Lippert, T.P.; Marzec, P.; Idilli, A.I.; Sarek, G.; Vancevska, A.; Bower, M.; Farrell, P.J.; Ojala, P.M.; Feldhahn, N.; Boulton, S.J. Oncogenic herpesvirus KSHV triggers hallmarks of alternative lengthening of telomeres. *Nat Commun* **2021**, *12*, 512, doi:10.1038/s41467-020-20819-4.

118. McClain, S.L.; Clippinger, A.J.; Lizzano, R.; Bouchard, M.J. Hepatitis B virus replication is associated with an HBx-dependent mitochondrion-regulated increase in cytosolic calcium levels. *J Virol* **2007**, *81*, 12061-12065, doi:10.1128/jvi.00740-07.

119. Venook, A.P.; Papandreou, C.; Furuse, J.; de Guevara, L.L. The incidence and epidemiology of hepatocellular carcinoma: a global and regional perspective. *Oncologist* **2010**, *15 Suppl 4*, 5-13, doi:10.1634/theoncologist.2010-S4-05.

120. Nicolini, L.A.; Orsi, A.; Tatarelli, P.; Viscoli, C.; Icardi, G.; Sticchi, L. A Global View to HBV Chronic Infection: Evolving Strategies for Diagnosis, Treatment and Prevention in Immunocompetent Individuals. *Int J Environ Res Public Health* **2019**, *16*, doi:10.3390/ijerph16183307.

121. Nault, J.C.; Ningarhari, M.; Rebouissou, S.; Zucman-Rossi, J. The role of telomeres and telomerase in cirrhosis and liver cancer. *Nat Rev Gastroenterol Hepatol* **2019**, *16*, 544-558, doi:10.1038/s41575-019-0165-3.

122. In der Stroth, L.; Tharehalli, U.; Günes, C.; Lechel, A. Telomeres and Telomerase in the Development of Liver Cancer. *Cancers (Basel)* **2020**, *12*, doi:10.3390/cancers12082048.

123. Jang, J.W.; Kim, J.S.; Kim, H.S.; Tak, K.Y.; Lee, S.K.; Nam, H.C.; Sung, P.S.; Kim, C.M.; Park, J.Y.; Bae, S.H.; et al. Significance of TERT Genetic Alterations and Telomere Length in Hepatocellular Carcinoma. *Cancers (Basel)* **2021**, *13*, doi:10.3390/cancers13092160.

124. Nault, J.C.; Mallet, M.; Pilati, C.; Calderaro, J.; Bioulac-Sage, P.; Laurent, C.; Laurent, A.; Cherqui, D.; Balabaud, C.; Zucman-Rossi, J. High frequency of telomerase reverse-transcriptase promoter somatic mutations in hepatocellular carcinoma and preneoplastic lesions. *Nat Commun* **2013**, *4*, 2218, doi:10.1038/ncomms3218.

125. Pezzuto, F.; Izzo, F.; De Luca, P.; Biffali, E.; Buonaguro, L.; Tatangelo, F.; Buonaguro, F.M.; Tornesello, M.L. Clinical Significance of Telomerase Reverse-Transcriptase Promoter Mutations in Hepatocellular Carcinoma. *Cancers (Basel)* **2021**, *13*, doi:10.3390/cancers13153771.

126. Totoki, Y.; Tatsuno, K.; Covington, K.R.; Ueda, H.; Creighton, C.J.; Kato, M.; Tsuji, S.; Donehower, L.A.; Slagle, B.L.; Nakamura, H.; et al. Trans-ancestry mutational landscape of hepatocellular carcinoma genomes. *Nat Genet* **2014**, *46*, 1267-1273, doi:10.1038/ng.3126.

127. Sung, W.K.; Zheng, H.; Li, S.; Chen, R.; Liu, X.; Li, Y.; Lee, N.P.; Lee, W.H.; Ariyaratne, P.N.; Tennakoon, C.; et al. Genome-wide survey of recurrent HBV integration in hepatocellular carcinoma. *Nat Genet* **2012**, *44*, 765-769, doi:10.1038/ng.2295.

128. Paterlini-Bréchot, P.; Saigo, K.; Murakami, Y.; Chami, M.; Gozuacik, D.; Mugnier, C.; Lagorce, D.; Bréchot, C. Hepatitis B virus-related insertional mutagenesis occurs frequently in human liver cancers and recurrently targets human telomerase gene. *Oncogene* **2003**, *22*, 3911-3916, doi:10.1038/sj.onc.1206492.

129. Jang, J.W.; Kim, H.S.; Kim, J.S.; Lee, S.K.; Han, J.W.; Sung, P.S.; Bae, S.H.; Choi, J.Y.; Yoon, S.K.; Han, D.J.; et al. Distinct Patterns of HBV Integration and TERT Alterations between in Tumor and Non-Tumor Tissue in Patients with Hepatocellular Carcinoma. *Int J Mol Sci* **2021**, *22*, doi:10.3390/ijms22137056.

130. Liu, H.; Shi, W.; Luan, F.; Xu, S.; Yang, F.; Sun, W.; Liu, J.; Ma, C. Hepatitis B virus X protein upregulates transcriptional activation of human telomerase reverse transcriptase. *Virus Genes* **2010**, *40*, 174-182, doi:10.1007/s11262-009-0441-3.

131. Luan, F.; Liu, H.; Gao, L.; Liu, J.; Sun, Z.; Ju, Y.; Hou, N.; Guo, C.; Liang, X.; Zhang, L.; et al. Hepatitis B virus protein preS2 potentially promotes HCC development via its transcriptional activation of hTERT. *Gut* **2009**, *58*, 1528-1537, doi:10.1136/gut.2008.174029.

132. Tang, S.; Wu, W.; Wan, H.; Wu, X.; Chen, H. Knockdown of NHP2 inhibits hepatitis B virus X protein-induced hepatocarcinogenesis via repressing TERT expression and disrupting the stability of telomerase complex. *Aging (Albany NY)* **2020**, *12*, 19365-19374, doi:10.18632/aging.103810.

133. Tabata, K.; Neufeldt, C.J.; Bartenschlager, R. Hepatitis C Virus Replication. *Cold Spring Harb Perspect Med* **2020**, *10*, doi:10.1101/cshperspect.a037093.

134. Gower, E.; Estes, C.; Blach, S.; Razavi-Shearer, K.; Razavi, H. Global epidemiology and genotype distribution of the hepatitis C virus infection. *J Hepatol* **2014**, *61*, S45-57, doi:10.1016/j.jhep.2014.07.027.

135. Ningarhari, M.; Caruso, S.; Hirsch, T.Z.; Bayard, Q.; Franconi, A.; Védie, A.L.; Noblet, B.; Blanc, J.F.; Amaddeo, G.; Ganne, N.; et al. Telomere length is key to hepatocellular carcinoma diversity and telomerase addiction is an actionable therapeutic target. *J Hepatol* **2021**, *74*, 1155-1166, doi:10.1016/j.jhep.2020.11.052.

136. Tornesello, A.L.; Reimer, U.; Holenya, P.; Knaute, T.; Pezzuto, F.; Izzo, F.; Buonaguro, L.; Megna, A.S.; Buonaguro, F.M.; Tornesello, M.L. Profiling the HCV Immune Response in Patients with Chronic Liver Diseases and Hepatocellular Carcinoma by Peptide Microarray Analysis. *Curr Med Chem* **2022**, *29*, 2736-2747, doi:10.2174/0929867328666211104093718.

137. Zhu, Z.; Wilson, A.T.; Gopalakrishna, K.; Brown, K.E.; Luxon, B.A.; Schmidt, W.N. Hepatitis C virus core protein enhances Telomerase activity in Huh7 cells. *J Med Virol* **2010**, *82*, 239-248, doi:10.1002/jmv.21644.

138. Shiu, T.Y.; Shih, Y.L.; Feng, A.C.; Lin, H.H.; Huang, S.M.; Huang, T.Y.; Hsieh, C.B.; Chang, W.K.; Hsieh, T.Y. HCV core inhibits hepatocellular carcinoma cell replicative senescence through downregulating microRNA-138 expression. *J Mol Med (Berl)* **2017**, *95*, 629-639, doi:10.1007/s00109-017-1518-4.

139. Zhu, Z.; Tran, H.; Mathahs, M.M.; Moninger, T.O.; Schmidt, W.N. HCV Induces Telomerase Reverse Transcriptase, Increases Its Catalytic Activity, and Promotes Caspase Degradation in Infected Human Hepatocytes. *PLoS One* **2017**, *12*, e0166853, doi:10.1371/journal.pone.0166853.

140. Verdonck, K.; González, E.; Van Dooren, S.; Vandamme, A.M.; Vanham, G.; Gotuzzo, E. Human T-lymphotropic virus 1: recent knowledge about an ancient infection. *Lancet Infect Dis* **2007**, *7*, 266-281, doi:10.1016/s1473-3099(07)70081-6.

141. Coffin, J.M. The discovery of HTLV-1, the first pathogenic human retrovirus. *Proc Natl Acad Sci U S A* **2015**, *112*, 15525-15529, doi:10.1073/pnas.1521629112.

142. Ahmadi Ghezeldasht, S.; Shirdel, A.; Assarehzadegan, M.A.; Hassannia, T.; Rahimi, H.; Miri, R.; Rezaee, S.A. Human T Lymphotropic Virus Type I (HTLV-I) Oncogenesis: Molecular Aspects of Virus and Host Interactions in Pathogenesis of Adult T cell Leukemia/Lymphoma (ATL). *Iran J Basic Med Sci* **2013**, *16*, 179-195.

143. Bellon, M.; Datta, A.; Brown, M.; Pouliquen, J.F.; Couppie, P.; Kazanji, M.; Nicot, C. Increased expression of telomere length regulating factors TRF1, TRF2 and TIN2 in patients with adult T-cell leukemia. *Int J Cancer* **2006**, *119*, 2090-2097, doi:10.1002/ijc.22026.

144. Kubuki, Y.; Suzuki, M.; Sasaki, H.; Toyama, T.; Yamashita, K.; Maeda, K.; Ido, A.; Matsuoka, H.; Okayama, A.; Nakanishi, T.; et al. Telomerase activity and telomere length as prognostic factors of adult T-cell leukemia. *Leuk Lymphoma* **2005**, *46*, 393-399, doi:10.1080/10428190400018349.

145. Uchida, N.; Otsuka, T.; Arima, F.; Shigematsu, H.; Fukuyama, T.; Maeda, M.; Sugio, Y.; Itoh, Y.; Niho, Y. Correlation of telomerase activity with development and progression of adult T-cell leukemia. *Leuk Res* **1999**, *23*, 311-316, doi:10.1016/s0145-2126(98)00170-2.

146. Sinha-Datta, U.; Horikawa, I.; Michishita, E.; Datta, A.; Sigler-Nicot, J.C.; Brown, M.; Kazanji, M.; Barrett, J.C.; Nicot, C. Transcriptional activation of hTERT through the NF-kappaB pathway in HTLV-I-transformed cells. *Blood* **2004**, *104*, 2523-2531, doi:10.1182/blood-2003-12-4251.

147. Evans, S.K.; Lundblad, V. Positive and negative regulation of telomerase access to the telomere. *J Cell Sci* **2000**, *113 Pt 19*, 3357-3364, doi:10.1242/jcs.113.19.3357.

148. Smogorzewska, A.; van Steensel, B.; Bianchi, A.; Oelmann, S.; Schaefer, M.R.; Schnapp, G.; de Lange, T. Control of human telomere length by TRF1 and TRF2. *Mol Cell Biol* **2000**, *20*, 1659-1668, doi:10.1128/MCB.20.5.1659-1668.2000.

149. Terme, J.M.; Mocquet, V.; Kuhlmann, A.S.; Zane, L.; Mortreux, F.; Wattel, E.; Duc Dodon, M.; Jalinot, P. Inhibition of the hTERT promoter by the proto-oncogenic protein TAL1. *Leukemia* **2009**, *23*, 2081-2089, doi:10.1038/leu.2009.131.

150. Borowiak, M.; Kuhlmann, A.S.; Girard, S.; Gazzolo, L.; Mesnard, J.M.; Jalinot, P.; Dodon, M.D. HTLV-1 bZIP factor impedes the menin tumor suppressor and upregulates JunD-mediated transcription of the hTERT gene. *Carcinogenesis* **2013**, *34*, 2664-2672, doi:10.1093/carcin/bgt221.

151. Krump, N.A.; You, J. Molecular mechanisms of viral oncogenesis in humans. *Nat Rev Microbiol* **2018**, *16*, 684-698, doi:10.1038/s41579-018-0064-6.

152. van Baarle, D.; Nanlohy, N.M.; Otto, S.; Plunkett, F.J.; Fletcher, J.M.; Akbar, A.N. Progressive telomere shortening of Epstein-Barr virus-specific memory T cells during HIV infection: contributor to exhaustion? *J Infect Dis* **2008**, *198*, 1353-1357, doi:10.1086/592170.

153. van de Berg, P.J.; Griffiths, S.J.; Yong, S.L.; Macaulay, R.; Bemelman, F.J.; Jackson, S.; Henson, S.M.; ten Berge, I.J.; Akbar, A.N.; van Lier, R.A. Cytomegalovirus infection reduces telomere length of the circulating T cell pool. *J Immunol* **2010**, *184*, 3417-3423, doi:10.4049/jimmunol.0903442.

154. Mao, J.; Zhang, Q.; Wang, Y.; Zhuang, Y.; Xu, L.; Ma, X.; Guan, D.; Zhou, J.; Liu, J.; Wu, X.; et al. TERT activates endogenous retroviruses to promote an immunosuppressive tumour microenvironment. *EMBO Rep* **2022**, *23*, e52984, doi:10.15252/embr.202152984.

155. Venuti, A.; Romero-Medina, M.C.; Melita, G.; Ceraolo, M.G.; Brancaccio, R.N.; Sirand, C.; Taverniti, V.; Steenbergen, R.; Gheit, T.; Tommasino, M. Lyon IARC Polyomavirus Displays Transforming Activities in Primary Human Cells. *J Virol* **2022**, *96*, e0206121, doi:10.1128/jvi.02061-21.

156. Sharma, S.; Chowdhury, S. Emerging mechanisms of telomerase reactivation in cancer. *Trends Cancer* **2022**, *8*, 632-641, doi:10.1016/j.trecan.2022.03.005.