Human oncogenic viruses and cancer

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The role of viral infection in cancer was established towards the beginning of 20th century. The study of tumour viruses, their oncogenes and different mechanisms employed by these viruses to subvert the growth-suppressive and pro-apoptotic functions of host tumour suppressor genes has laid the foundation of cancer biology. The human tumour viruses induce malignancies after a prolonged latency and in conjunction with other environmental and host factors. The eight known human tumour viruses contribute to nearly 10–15% of the cancers worldwide. Advancements in research on virus-related cancers offer a plethora of opportunities to fight cancer by preventing viral spread through vaccination and use of antivirals. Besides, recent developments on viral oncogenic mechanisms should allow development of novel and targeted approaches for control and treatment of virus-associated human cancers.

Keywords: Cancer, cell transformation, mitogenic signalling, tumour virus, viral integration.

Introduction

INFECTIOUS agents such as viruses, bacteria and parasites are well-accepted, bona fide etiological factors associated with specific human cancers and account for almost 20% of the global cancer burden[1,2]. It is estimated that up to 15% of all human tumours worldwide are caused by viruses[3]. The infectious nature of viruses distinguishes them from other cancer-causing factors in that viruses establish chronic infections in humans, where cancer development occurs by the accumulation of multiple co-operating events[4]. Such long-term association with hosts provides them ample opportunities to mount mutagenic onslaughts and initiate the cell transformation process ultimately giving rise to malignant disease. Transformed cells often exhibit chromosomal aberrations which may result from integration of viral genome into chromosomes of the host cell. These viruses usually infect host precursor cells in order to exploit their differentiation programme and establish viral replication.

The concept of viruses being cancer-causing agents emerged at the turn of 20th century, with the contemporaneous yet independent discovery of cell-free transmission of human warts, chicken leukaemia and chicken sarcoma by Ciuffo (1907), Ellerman and Bang (1908), and Rous (1911) respectively[3]. However, these observations were met with skepticism as cancer in human was not considered contagious and relegated to the background as scientific curiosities. Interest in virus association in cancer was rekindled in early 1950s following the discovery of a Murine leukaemia virus and a polyomavirus that induced tumours in murines[5]. The first human tumour virus, Epstein–Barr virus (EBV), was reported in 1964 from Burkitts lymphoma cells using electron microscopy, which established the first link between viral infection and cancer[6]. In 1970, the human hepatitis B virus (HBV) was visualized in the human sera positive for Australia antigen (now known as hepatitis B surface antigen or HBsAg)[7]. Since then, six more cancer viruses have been discovered that are considered to be the causal agents for specific cancers in humans. Both DNA and RNA viruses belonging to a broad range of virus families constitute the group of tumour viruses. They encode different types of oncoproteins which may or may not target common regulatory mechanisms in host cells.

Studies on tumour viruses have made enormous impact on our understanding of cancer at the molecular level. It was the analysis of experimental cell transformation by viruses that led to the discovery of oncogenes and tumour suppressor genes[3,8]. Subsequent studies on viral oncogenes led to the finding that these are not unique to viruses and homologous genes are found in all cells known as proto-oncogenes. Normally, the cellular proto-oncogenes are not expressed in a quiescent cell as these are growth and development-related genes. However, these genes may be aberrantly expressed after infection by tumour viruses. Therefore, understanding how viral oncogenes modify the expression of growth-promoting factors has also provided new insights into the basic mechanisms of cancer development. This review limits the discussion to recent developments on various aspects of known human tumour viruses, including their biology, disease pathogenesis and approaches to vaccination and therapy.

Human tumour viruses

Tumour-viruses are known to be associated with discrete human malignancies. They have been broadly classified

into two distinct groups, DNA- and RNA-tumour viruses, on the basis of their genetic make-up. Human DNA tumour viruses include EBV, HBV, Kaposi's sarcoma herpesvirus (KSHV), human papilloma virus (HPV) and Merkel cell polyomavirus (MCV), whereas RNA tumour viruses comprise retroviruses like human T-cell leukaemia virus-1 (HTLV-1) and human immunodeficiency virus-1 (HIV-1), and flavivirus such as hepatitis C virus (HCV). The distinguishing features and biology of the human tumour viruses are summarized in Table 1.

EBV and KSHV

EBV (also known as HHV-4) and KSHV (also known as HHV-8) are both herpesviruses that harbour large linear dsDNA genome. Both these viruses preferentially infect B lymphocytes and epithelial cells. EBV is highly prevalent throughout the world and more than 90% of adults worldwide are infected[2]. The oral route is the primary route of transmission of EBV. However, transmission by transfusion is also documented. The primary infection with EBV is usually asymptomatic, in case it happens during infancy. However, the infected individual is rendered a carrier for lifetime. During adolescence, EBV infection usually results in self-limiting disease called infectious mononucleosis[9]. EBV infection can immortalize primary B cells and establish tumour–viral clonality. Due to its powerful transforming potential, EBV infection in some cases can lead to the development of lymphomas (such as Burkitt lymphoma and non-Hodgkin lymphoma) and carcinomas[2], as listed in Table 1. The virally encoded latent membrane protein 1 (LMP-1) appears to mediate the oncogenic potential of EBV[9]. It mimics the constitutively active form of CD40 receptor, a member of the tumour necrosis factor receptor family and induces several signal transduction pathways resulting in cell proliferation[10,11].

KSHV is highly prevalent in sub-Saharan Africa (>50%), moderately elevated in the Mediterranean region (10–30%) and low in northern Europe, USA and Asia (<10%)[2]. KSHV is primarily transmitted via saliva and infection is usually asymptomatic. However, KSHV and HIV-1 co-infections greatly enhance the risk of Kaposi's sarcoma and other B-cell associated malignancies[12]. The latency-associated nuclear antigen (LANA) seems to play a key role in KSHV associated tumourigenesis[2,12]. Upon expression, it results in cellular proliferation usually via inactivation of tumour suppressors such as p53 and Rb, activation of telomerase reverse transcriptase promoter or accumulation of intracellular notch domain.

HPV

The estimated worldwide HPV prevalence is approximately 10% with the highest in Africa and Latin America (20–30%), and the lowest in southern Europe and South-

east Asia (6–7%)[2]. HPV infections are transmitted mainly through direct skin-to-skin or skin-to-mucosa contact. So far, more than 130 types of HPV have been identified and subsequently classified into low-risk (LR) or high-risk (HR) groups depending on their cervical cancer-causing potential[1,13]. Nearly 70% of the cervical cancers are associated with the HR-HPV types 16 and 18. HPV has also been shown to play a role in the development of other human cancers such as skin cancers in immunosuppressed patients, head and neck tumours and other anogenital cancers[14]. HPVs are small, non-enveloped DNA viruses that cause warts or benign papillomas upon infection in epithelial cells. Cancer development upon persistent infection with HR-HPV subtype is mainly attributable to the expression of two potential oncogenes, E6 and E7, which have been documented to degrade p53 and Rb in a proteasome-dependent manner, thereby promoting genomic instability and cellular transformation[13].

MCV

MCV is rather a recently identified human oncogenic virus and therefore, evidence of the incidence and mortality of Merkel cell carcinoma (MCC) is yet to be established. However, early surveillance, epidemiology and end results (SEER) data collected in USA between 1984 and 1996 showed an annual age-adjusted incidence of 2.3 cases per million among whites and only 0.1 cases per million among blacks[15]. The virus has a circular double-stranded genome similar to other human polyomaviruses. It has been found to be associated with most of the MCCs[16]. The constitutive expression of small and large T antigens upon viral integration results in virus-induced transformation, thus making it an etiological agent of MCC[16].

HBV and HCV

HBV, a DNA tumour virus carrying partially dsDNA genome, is one of the most common infectious viruses with over two billion people infected worldwide. Approximately 360 million of these are chronically infected and nearly one million people die each year from HBV-related chronic liver disease, including liver cirrhosis and hepatocellular carcinoma (HCC)[2]. HBV is highly contagious and is transmitted by percutaneous and permucosal exposure to infected blood and other body fluids (i.e. semen and vaginal fluid). The viral infection is usually asymptomatic or results in acute hepatitis and is normally cleared by adult HBV patients. However, a large fraction of infected neonates or young children who fail to clear the infection become chronic HBV carriers[17]. Chronic HBV infection is usually associated with chronic hepatitis, liver cirrhosis and HCC development. HBV-related HCC is mainly a result of viral genome integration into host chromosome and/or expression of virus-encoded

Table 1. Tumour viruses associated with human malignancies

Tumour virus (family)		Disease	Tropism	Unique biology	Cancer	Oncogene	Genome
DNA tumour viruses							
Epstein-Barr virus (EBV)[2,9] (Herpesviridae)		IM, oral hairy leukoplakia	Oropharyngeal epithelial cells, B cells	Immortalizes B cells	Gastic cancer, nasopharyngeal cancer, Burkitt's lymphoma, Hodgkin's disease	LMP-1	dsDNA ~172 kb (~90 genes)
Hepatitis B virus (HBV)[2,17] (Hepadnaviridae)		Hepatitis, cirrhosis	Hepatocytes, white blood cells	May cause chronic infection and inflammation	Hepatocellular carcinoma (HCC)	HBx	Partially circular dsDNA ~3.2 kb (4 genes)
Human papilloma virus (HPV-16 and 18)[2,13] (Papillomaviridae)		Skin warts, EV, genital warts, LP	Squamous epithelial cells	Highly species and tissue, replication dependent on cell differentiation	Papilloma warts, cervical cancer, penile cancer, anogenital cancer, head and neck cancer	E6 and E7	Circular dsDNA ~8 kb (8–10 genes)
Kaposi's Sarcoma associated herpesvirus (KSHV)[2,12] (Herpesviridae)		Several neoplastic diseases	Vascular endothelial cells, lymphocytes	Contains many cellular genes	Kaposi's sarcoma (KS), primary effusion lymphoma (PEL), multicentric Castleman's disease (MCD)	vGPCR, vIL-6, vFLIP, vBcl-2, vIRF4, LANA, Kaposin B	dsDNA ~160 kb (~90 genes)
Merkel cell polyomavirus (MCV or MCPyV)[16] (Polyomaviridae)		A range of neoplasms	Epidermal cells	Species and tissue specific	Merkel cell carcinoma (MCC)	Large T and small T antigens	Circular dsDNA ~5.4 kb (~180 genes)
RNA tumour viruses							
Hepatitis C virus (HCV)[2,19] (Flaviviridae)		Hepatitis, cirrhosis	Hepatocytes	High rate of chronic infection and inflammation	HCC	HCV core protein, NS5	Single positive RNA ~9.6 kb (9 genes)
Human T-cell leukaemia virus-1 (HTLV-1)[2,20] (Retroviridae)		HAM/TSP	T cells	Immortalizes T cells, encodes trans-acting factors	Adult T-cell leukaemia (ATL)	Tax, HBZ	ssRNA ~9.0 kb (6 genes)
Human immunodeficiency virus-1 (HIV-1)[2,22] (Retroviridae)		AIDS	CD4+ T helper cells, macrophages	Kills T cells and suppresses the host immune system	Immunosuppression-mediated enhanced risk of cancer by other viruses	Tat*	Diploid positive sense RNA ~9.7 kb (9 genes)

AIDS, Acquired immunodeficiency syndrome; EV, Epidermodysplasia verruciformis; HAM, HTLV-I-associated myelopathy; HBZ, HTLV-1 bZIP factor; IM, Infectious mononucleosis; LANA, Latency associated nuclear antigen; LMP-1, Latent membrane protein 1; LP, Laryngeal papillomas; TSP, Tropical spastic paraparesis; vFLIP, Viral FLICE inhibitory protein; vGPCR, viral G protein-coupled receptor. *Putative oncogene.

HBx oncoprotein which mediates cellular proliferation and transformation. HBx interferes with and subverts a number of cellular pathways, including signal transduction via its direct interaction with growth factor receptors, p53, EGR1, Oct1, etc. leading to the progression of HCC[18].

HCV is another major contributor to HCC development. The estimated prevalence of HCV infection worldwide is ~2.2% (ref. 2). HCV can be transmitted by transfusion of blood and blood products, transplantation of solid organs from infected donors and unsafe therapeutic injections. Unlike HBV, HCV is a positive-strand RNA virus which carries an RNA-dependent RNA polymerase activity, but no reverse transcriptase activity[19]. The non-structural proteins (such as NS5) of HCV can disrupt signal transduction pathways leading to uncontrolled cellular proliferation followed by cancerous progression. The core protein of HCV has also been implicated in cell transformation. Global burden of HCC attributable to HBV and HCV is estimated to be more than 80% of all cases[2].

HTLV-1

HTLV-1 was the first human retrovirus which was found to be associated with adult T-cell leukaemia[20]. Even though the geographic distribution of HTLV-1 has been well defined, its global prevalence is less well understood. There are three main modes of transmission in HTLV-1 infection: vertical, sexual and parenteral. The viral-encoded Tax and HBZ proteins appear to play a key role in viral-associated malignancies. Of these two regulatory proteins, Tax serves as a major oncogenic determinant of HTLV-1 owing to its interference with several signalling cascades and cellular DNA repair pathway, thereby augmenting cell survival and transformation[21].

HIV-1

Currently, an estimated 33.3 million people are living with HIV. According to a 2007 estimate, the HIV-1 prevalence ranges from less than 0.5% in most developed countries up to 30% in Central and southern Africa[2]. HIV-1 infection is transmitted through three main routes: sexual intercourse, blood contact and from mother to infant. HIV-1 is a retrovirus and has been recently classified as an oncogenic virus even though it is not a direct cause of cancer. Immunosuppression associated with HIV infection is considered to enhance the susceptibility to carcinogenesis in the presence of other opportunistic infections[2,22].

Viral oncogenes and deregulation of host cell functions

First and foremost, in order to establish itself in the host, it is indispensable for a virus to bring about uncoupling of cellular differentiation and proliferation, thus presenting its own genome an opportunity to replicate in the cycling infected cell. To attain this, oncogenic viruses have evolved a plethora of mechanisms to hijack different cellular processes described below.

Cell signalling

The most fundamental characteristic of a cell is to proliferate in a controlled manner in response to various growth signals and inhibitory stimuli. Tumour viruses through their oncoproteins and other regulatory molecules modulate nearly all major signalling pathways[23], including MAP kinase, JAK-STAT, TGFβ, NF-κB, Notch, TNF, Wnt and Hedgehog. It has been suggested that tumour viruses do so in order to create an ambience conducive for their replication and push host cells to actively divide and proliferate.

The mitogen activated protein kinase (MAPK) pathways are activated in response to growth factors (ERK) or stress signals (JNK and p38), and are found aberrantly activated in cancer. For example, the EBV-encoded LMP1 protein utilizes JNK pathway to auto activate its own promoter and mediate cell transformation and ROS generation – a distinguishing feature of EBV-positive Burkitt's lymphoma via ERK activation[10]. HBx protein of HBV differentially activates the ERK, JNK and p38 MAPK pathways under both transforming and non-transforming conditions. The sustained activation of p38 and JNK MAPK by HBx leads to Fas/FasL and TNFα-mediated apoptosis[24]. Similarly, core protein of HCV activates the ERK, JNK and p38 pathways and NS-3 mediated activation of JNK signalling pathway is crucial for cell growth[25]. Activated JNK and p38 MAPK pathways are crucial for KSHV-mediated primary cell infection as well as KSHV reactivation from latency[26]. HIV1 infection significantly increases the risk of Kaposi sarcoma in patients, where it contributes to KSHV reactivation by activating Ras/c-Raf/AMPK/ERK kinase pathway[27].

JAK-STAT pathway functions to transmit external cytokine signals via cell surface receptor and its constitutive activation correlates with oncogenic transformation. LMP1 and LMP2 of EBV and Tax protein of HTLV reportedly induce STAT transcription factors. There is a positive autoregulatory loop that exists between LMP1 and STAT, whereby STAT activation permits LMP1 expression which in turn induces IL-6-mediated STAT activation[28,29]. Interestingly, KSHV encodes a viral IL-6 (vIL-6) which activates the IL-6 responsive JAK-STAT pathway[30]. HBx constitutively activates JAK/STAT pathway by phosphorylating STAT3/STAT5 and enhancing the kinase activity of JAK1 (ref. 24). HIV-encoded Tat increases the replication of KSHV by inducing IL-4/STAT3 and IL-4/STAT6 signalling[31].

Oncogenic virus also targets TGF-β pathway regulated by TGF-β cytokine via cell-surface receptors and intracellular SMADs. Its target genes are involved in cell growth, cell differentiation, apoptosis and cellular homeostasis. Interestingly, the normal tumour suppressive pSMAD3C signalling function shifts to oncogenic and fibrogenic pSMAD3L/pSMAD2L/C signalling in both HBV and HCV infections[32]. Alternatively, the core, NS3 and NS5A proteins of HCV disrupt the SMAD3/SMAD4 complex formation and prevent its binding to DNA[33]. The LMP-1 protein of EBV, and E6 and E7 oncoproteins of HPV impair TGF-β signalling by different mechanisms such as down regulation of TGF-β receptor, interference with nuclear translocation or DNA binding of SMADs[34,35]. The HTLV-1 Tax protein too provides resistance to TGF-β-mediated inhibition by disrupting the interaction of SMADs with co-activator CREB-binding protein/p300, preventing SMAD3/SMAD4 complex or promoting c-Jun/SMAD3 complex formation[36]. KSHV LANA protein epigenetically silences TGF-β receptor II promoter through methylation and deacetylation[12]. Besides, the KSHV-encoded micro RNA miR-K-12-11 down-regulates the TGF-β pathway by targeting SMAD5, while miR-K10 confers resistance against TGF-β-induced apoptosis[37].

NF-κB signalling plays a dominant role in the evasion of apoptosis and thus provides a critical link between inflammation and cancer. The NF-κB pathway is stimulated by a variety of signals and modifies the expression of many host proteins. Most tumour viruses tinker with NF-κB signalling to have a profound effect on host physiology. Regulation of NF-κB by HBx is well documented and includes activation of protein kinase B and Raf-1, and inhibition of IκB-α. While E6 protein of HPV stimulates the expression of NF-κB inducible genes and pathway proteins such as p50, NIK and TRAF, E7 protein associates with IκB kinase complex and impairs its phosphorylation[38]. EBV-encoded LMP1 and KSHV-encoded vFLIP activate NF-κB pathway to maintain latency, which is central to tumour formation and maintenance of the transformed phenotype[39]. Besides KSHV encodes a microRNA, called miR-K1, that upregulates NF-κB by directly targeting the IκBα transcript[39]. While HTLV-1 Tax activates both canonical and non-canonical NF-κB pathways, HBZ inhibits the activity of NF-κB subunit c-Rel/p65 and promotes T-cell transformation by acting at different stages in oncogenesis[40]. HCV core and NS5A employ two distinct mechanisms to modulate NF-κB signalling, including binding to TNFR1 to prevent both TNF-α and FasL-induced apoptosis. Besides, these induce endoplasmic reticulum stress-related phosphorylation of IκB-α leading to activation of NF-κB pathway[19,41].

Numerous viral oncoproteins target the Notch signalling pathway emphasizing its significance in regulating normal cell growth and differentiation. LMP2A of EBV alters B cell identity and autoregulates its own expression in Hodgkin's lymphoma by constitutively activating Notch1 pathway. EBNA2 acts as a biological equivalent of activated Notch receptor RBP-Jκ, the master regulator of Notch signalling pathway. In contrast, E6/E7 oncoproteins of HPV are known to down-regulate Notch 1 expression[23]. KSHV replication and transcription activator (RTA), a major lytic cycle transactivator, contributes to the development of latency by inducing LANA expression during early stages of infection by targeting RBP-Jκ[42]. HBx protein of HBV activates the Notch signalling pathway by upregulating the expression of ligands Jagged-1, Notch-1 and Hes-1 and blocking the Notch pathway partially reverses the effects of HBx in cell growth and prolonged S phase of cell cycle[43].

Tumour viruses have evolved strategies to manipulate TNF signalling, which has a profound effect on cell proliferation, differentiation and apoptosis. The TNF receptor associated factor (TRAF) acts as a key effector of TNF signalling. Interestingly, LMP-1 of EBV mimics CD40 (a TNF receptor) and activates downstream NF-κB and c-Jun kinases[9,11]. The KSHV oncoprotein vFLIP activates NF-κB pathway for cell survival via TRAF2 and TRAF3, which is crucial for KSHV-associated lymphomagenesis[12]. HPV-mediated TNF resistance is a key event in the multi-step process leading to cervical cancer[13,14]. Activation of the TNFα system also has a pivotal role in the inflammatory process linked to chronic liver diseases associated with HCV and HBV infections[18,24].

Aberrant upregulation of Wnt pathway is a prevalent theme in cancer biology. Wnt signalling, with β-catenin as its central modulator, controls embryonic development and tissue homeostasis in adult organisms. LMP1 stabilizes β-catenin through transcriptional repression of E3 ubiquitin ligase Siah1 contributing towards elevated levels of β-catenin in nasopharyngeal carcinomas[9,44]. Also, KSHV-encoded LANA activates the β-catenin pathway by increasing the nuclear accumulation of GSK-3β (ref. 44). The HBx protein of HBV, and core and NS5A proteins of HCV also activate Wnt pathway by suppressing GSK-3β activity via different mechanisms in order to promote hepatocarcinogenesis[19,45]. HPV E6/E7 proteins prevent SIAH1-mediated degradation of β-catenin to promote Wnt signalling[46].

Hedgehog (Hh) signalling is another key pathway reportedly activated in cancers of brain, skin and liver. HBx increases the stability and nuclear translocation of Gli1 – a key transcription factor of Hh signalling pathway. Blockade of Hh signalling impairs HBx ability to promote cell migration, anchorage-independent growth and tumour development[47]. Likewise, in chronic HCV infections, Hh ligands are upregulated and increased Hh signalling is associated with cirrhosis and HCC[48]. Hh-activating mutations are selected in cells immortalized by HPV. Inhibition of Hh pathway in cervical cancer cells renders them susceptible to apoptosis[49].

Regulation of transcription[50–52]

Viral oncoproteins usually reprogramme the host cells by hijacking and repurposing host regulatory components of transcription network. Since a major requirement for induction of cell cycling is to overcome the Rb-mediated repression of cell cycle-regulated genes, most tumour viruses deploy a vast repertoire of viral strategies to modulate Rb function. These may include its hyper-phosphorylation and thus inactivation, degradation and decrease in half-life of Rb, eventually leading to activation of E2F transcriptional activity. Virus-induced unscheduled inactivation of Rb triggers strong engagement of p53-mediated cell cycle arrest and cell death. Hence viruses have evolved elaborate mechanisms to circumvent p53-driven anti-proliferative response. Different strategies used by tumour viruses to overcome p53 activities include E6-induced degradation of p53, ablation of transactivation function by HBx, prevention of its phosphorylation-dependent activation by Tax and inhibition of transcriptional co-activators by KSHV oncoproteins. Oncogenic viruses display exquisite predilection for NF-κB-dependent transcription to upregulate the expression of its target genes. c-Myc and AP1 are other key transcription factors targeted by viral oncoproteins.

Regulation of replication and DNA damage

Replication of tumour viruses is intrinsically linked to their ability to drive cell proliferation. Most of these viruses infect quiescent cells driving their re-entry into cell cycle to promote an environment conducive for viral genome replication. Such aberrant induction of cell proliferation results in replicative stress and elicits a DNA damage response (DDR). While the effect of viral onslaught on host DDR response is well documented, much work needs to be done to understand the mechanistic link between virus-induced tumourigenesis and host DNA replication machinery. Nevertheless, replication factors like PCNA, Cdt1, CDC6 and geminin have been found to be dysregulated by viral oncoproteins like E7 and HBx, which have been correlated with induction of re-replication[50,53–55]. Few reports also suggest inappropriate activation of origins of replication by viral oncoproteins like Tax and E7. The DNA damage response, despite being growth-suppressive, is beneficial for the virus since activation of host DDR exerts S-phase arrest, thus creating an S-phase like cellular milieu of replication factors, allowing viruses to replicate. Hence oncogenic viruses have developed mechanisms to directly activate specific components of DDR, while stringently inhibiting downstream triggering of cell death. ATM arm of DDR pathway is frequently activated following HBV, KSHV, MCV and EBV infection[56–58]. Additionally, DDR pathway can also be activated indirectly through induction of mitotic defects by HTLV-1, KSHV and HPV oncoproteins[56,59,60], and elevation of reactive-oxygen species by oncoproteins like Tax and EBNA1[57]. Interestingly, despite activating DDR components, viral oncoproteins ensure mitigation of growth suppressive and cell death-inducing effects of DDR pathways. p53, a DDR downstream target, is a common target of viral oncoproteins such as E6, LANA, HBx, EBNA3C and Tax, which inactivate it using a multitude of mechanisms ensuring prevention of cell death induction. Activities of DNA damage sensing and signal-relaying kinases such as Chk1, Chk2 and DNA-PK upstream kinases are found to be attenuated by oncoproteins like Tax, EBNA3C, etc.[56,58,60]. The final consequence of perturbation of various DDR pathways is accumulation of aneuploid cells that promote tumourigenesis by amplification of oncogenes or loss of tumour suppressor genes. Table 2 summarizes the effects of viral oncoproteins on host DDR pathways and replication machinery components.

Epigenetic reprogramming of tumour virus-infected cell

Orderly progression of DNA transcription, replication, recombination and repair requires spatial and temporal changes in the structure of the chromatin, which in turn governs the availability of gene regulatory elements, controlling their tissue-specific expression. It is increasingly becoming clear that viral oncoproteins promote widespread remodelling of chromatin organization, contributing to both up- and down-regulation of a large number of genes. Table 3 outlines information available on epigenetic mechanisms used by human oncogenic viruses in tumourigenesis. Tumour virus infections are associated with inactivation of tumour suppressor genes by DNA hypermethylation of their CpG island-rich promoters[50]. Consistent with this concept, most viral oncoproteins exhibit interaction, enzyme activity stimulation and/or transcriptional upregulation of DNMTs, e.g. DNMT1, DNMT3A and DNMT3B[61,62]. Additionally, alteration of histone modifying machinery by viral oncoproteins has been widely reported, with p300/CBP and HDACs like HDAC1 and 2, being frequent targets[23,50–52,61,62]. It is noteworthy that p300/CBP activity is sabotaged by almost all tumour viruses using multiple mechanisms, exemplifying for functional convergence of oncoproteins of distinct viral origins. Other histone modifiers like histone methyltransferases for instance KDM6A, KDM6B and EZH2, are targeted by tumour viruses, thus altering levels of specific histone methylation marks[62]. Furthermore, several lines of evidence have elucidated SWI/SNF chromatin remodelling complex as an important target of a number of viral oncoproteins[23,61]. In conclusion, virus-encoded oncoproteins hijack host epigenetic machinery to promote viral replication and expression of viral genes, in the process altering epigenetic signature of the host cell and triggering oncogenesis.

Table 2. Perturbation of host DNA replication and DNA damage response pathway by tumour viruses – the effects of crosstalk between oncoproteins of viral origin and host players of replication and DDR pathways are reviewed

Viral oncoprotein	Cellular replication/DNA damage and repair (DDR) related factor/activity affected	Reference
Tax	• Transcriptional upregulation of *PCNA*.	50, 51,
	• Inappropriate activation of origins and increase in number of supplementary origins of replication.	56, 59,
	• Antagonizes p53, downstream target of DDR.	
	• Inhibition of Chk1 and Chk2 checkpoint kinases signalling and upstream DNA-damage sensing DNA-PK.	
	• Sequesters DDR components MDC1 and BRCA1 at artificial tax-induced foci of pseudo-DNA damage.	
	• Attenuates ATM-downstream signalling leading to faster release of G1/S checkpoint in response to ionizing radiation.	
	• Abolishes mitotic checkpoints causing aneuploidy.	
E7	• Induces DNA synthesis and *PCNA* and *Pol α* upregulation in suprabasal cells.	53, 56,
	• Overexpression of Cdt1 and induction of re-replication.	60, 128
	• Interaction with p21 blocks inhibition of *PCNA*-dependent DNA replication.	
E6/E7	• Activated DDR characterized by ATM, Chk1, Chk2 and H2AX phosphorylation.	
	• Interaction of E7 with Ser1981-phosphorylated ATM.	
	• Upregulation of PLK4 resulting into centriole multiplication by E7.	
E6	• Degradation of p53, a downstream target of DDR.	
HBx	• Upregulation of replication factor CDC6 transcriptionally and posttranscriptionally.	54–57
	• Induction of DNA re-replication and polyploidy.	
	• Increase in Cdt1–Geminin ratio.	
	• Activation of ATR arm of DDR, induction of S-phase arrest.	
	• Sequesters p53 in cytoplasm and inhibits its DNA-binding activity, suppressing apoptosis.	
EBNA proteins	• Cellular hyper-proliferation and activation of ATM and downstream DDR checkpoints by EBNA2.	50, 56,
	• EBNA3C interacts and interferes with the DDR activity of p53.	58
	• Direct interaction with and attenuation of Chk2 activity and DDR signalling by EBNA3C.	
	• EBNA3 proteins inhibit canonical G2/M checkpoint through p27 suppression.	
	• EBNA3C decreases levels of spindle assembly checkpoint protein BubR1.	
v-cyclin D	• Activates ATM, centrosomal amplification and intra-S-phase growth arrest.	56
LANA	• Direct association with and modulation of p53 activity.	56
LMP1	• Transcriptional down-regulation of ATM.	56

Translational machinery

Cellular protein translational machinery that strongly correlates with cellular metabolic activities is often hijacked by viruses for their own protein synthesis and perpetuation. Different steps of translational machinery are reported to be targets of viral interference. Viruses such as MCV, EBV and HPV can affect the initiation phase of translation. For example, the small T antigen of MCV targets rapamycin (mTOR) signalling pathway to maintain the hyperphosphorylation status of eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1). This process results in dysregulation of cap-dependent translation[63]. Some tumour viruses encode proteins that inhibit the translation inhibitory kinase PKR signalling and promote autophagy such as EBV BILF1, KSHV viral interferon regulatory factors 2 and 3 (vIRF2/3) and HCV non-structural protein 5A[64–66]. PKR phosphorylates the α-subunit of the eukaryotic translation initiation factor 2 (eIF2), leading to inhibiting of translation and stimulation of autophagy[67]. The mRNA cap-binding factor eIF4E which is essential for recruitment of mRNA to the ribosome is another major target for translation regulation[68]. The initiation of Cap-dependent translation by 4E-BP1 in association with eIF4E is a phosphorylation-dependent process. The E7 oncoprotein of HPV targets 4E-BP1 and maintains this in a constantly active form to facilitate its own translation[69].

Notably, some tumour viruses can alter the ribosomal biogenesis regulation, the main core of protein synthesis. For instance, NS protein 5A (NS5A) of HCV is able to transduce signals into the nucleoplasm via UBF hyperphosphorylation leading to rRNA transcription activation, which links to cell growth in cancer[70]. Furthermore, polyomaviruses enhance phosphorylation of some ribosomal proteins like RPS6 in transformed cells[71]. Some viruses like KSHV target nucleophosmin – a nucleolar phosphoprotein involved in ribosomes[72]. These reports point towards a plethora of mechanisms employed by tumour viruses to hijack and overtake the host translational machinery for their own survival and spread.

Nucleolar functions and ribosome biogenesis

Nucleolus is regarded as the primary site for rRNA synthesis, which plays a major role in ribosome biogenesis. The nucleolar integrity is dependent upon RNA polymerase I activity and the presence of key nucleolar antigens like NPM, fibrillarin and nucleolin. Of late, several new functions have been assigned to the nucleolus, including activities such as cell-cycle regulation, gene silencing, senescence, innate immune response and stress sensing[73].

Table 3. Epigenetic re-programming caused by tumour viruses – the main features of epigenetic alterations brought about by viral oncoproteins and the host components of epigenetic machinery targeted by them are summed up

Chromatin modification/chromatin modifier affected	Viral onco-protein	Nature of association/ interaction	Effect of association	Reference
Hyper-methylation of cellular promoters	HBx EBNA3A, EBNA3C	Indirect	• Promoter hyper-methylation of *p16*. • Promoter hyper-methylation of pro-apoptotic gene *Bim*.	23, 50
p300/CBP	Tax LANA1, LANA2, vCyclin, k-bZIP, RTA E7		• Sequestration of p300/CBP, transcriptional co-activators of p53. • Transcriptional regulation of p300/CBP. • p300-mediated acetylation of Tax at K346, boosting NF-$\kappa\beta$-dependent transcription. • Inhibition of p300/CBP activity to bring about abrogation of p53-induced apoptosis. • Interaction of RTA and LANA with CBP.	50–52, 61
	EBNA3C	Direct	• Down regulation of transcriptional co-activation function of p300/CBP.	
	EBNA2 E6 HBx	Direct Direct Direct	• Interaction with and modulation of HAT activity of transcription co-activator complex p300/prothymosin α. • Binds and inhibits HAT activity of p300. • Inhibits p300-mediated HAT activity on p53 and core histones. • Interacts with p300.	
DNMT	E7 LMP-1 LANA HBx		• Binds to and stimulates DNMT-1 activity. • DNMT-1-dependent suppression of *E-cadherin*. • Upregulation of DNMTs via JNK/AP1-signalling pathway, causing hypermethylation of cellular promoters. • Activates *DNMT3a*. • Activates DNMT1, regulates expression of *DNMT3a* and *DNMT3b*.	61, 62
Polycomb represser complex	E7 E6, E7 HBx EBNA3A, EBNA3C		• Decreases complex-formation between E2F6 and PcG, relieving repressive effect on transcription of E2F1-target genes. • Interaction with PRC components, e.g. BMI1, PCGF2, CBX4, RING1, MGA1, L3MBTL2 • Stimulates EZH2 H3K27 methyltransferase • Transcriptional activation of *EZH2* through E2F-dependent pathway. • Down-regulation of SUZ12, essential for H3K27me3 mark. • Cooperativity with co-repressor CtBP for epigenetic silencing of $p16^{INK4A}$ promoter.	61, 62
KDM6A/B	E7		• Transcriptional upregulation of *KDM6A/B*, projected in dysregulated *HOX* gene expression.	62
H3K27 chromatin modification	E7		• Dramatic decrease in H3K27me3 mark leading to up-regulation of $p16^{INK4A}$.	62
SWI/SNF	Tax KSHV K8 E7		• Binds BRG1 and induction of SWI/SNF complex PBAF component *Baf170* expression • Functions as transcriptional activator via interaction with hSNF5. • Inhibits BRG-1-mediated transcriptional repression of *c-fos* promoter. • Interacts with BRG-1, abolishing cell cycle control.	23, 61
HDAC	Tax EBNA3C E7 HBx		• Mis-recruitment of HDAC leading to inhibition of p53 transactivation function. • Binding to transcriptional co-repressor complexes, including HDAC1 and HDAC2. • Association of several HDACs with KSHV *RTA* promoter during latency for efficient repression. • Binds to pRb and HDAC, promotes expression of pro-proliferative genes. • Interaction with HDAC1, increasing levels of *E2F2* transcription in differentiating cells.	23, 61, 62

Further, nucleolus now is considered as an important target for cancer therapy[74]. Recent reports suggest that trafficking of viral proteins to nucleolus and disruption of the nucleolar functions. Viral proteins change nucleolar dynamics in two ways: first, these get localized into nucleolus and regulate nucleolar export of viral mRNA required for efficient replication and infection. Secondly, they affect redistribution of nucleolar proteomics. For example, the HIV regulatory proteins – Rev and Tat, EBV protein EBNA-5, HCV core and NS5B protein and HTLV fall under the category of notable viral proteins that get localized to the nucleolus[75]. However, reportedly each serves a different function inside the nucleolus. For example, Tat protein of HIV-1 transactivates proviral DNA transcription, whereas Rev promotes nuclear export of viral RNAs and hence is critical for infection. Nucleolin exhibits abnormal intranuclear distribution in the HPV-infected cells, where it binds specifically to enhancer regions of E6 and E7 oncoprotein and also facilitates the binding of other transcription factors and controls cell proliferation by regulating the expression of E6 and E7. Likewise, the interaction of nucleolin with NS5B protein of HCV causes its redistribution in cytoplasm. Further, nucleolin may have a role in the IRES-mediated HCV translation also[75].

The nucleolar modification could also have a major impact on Pol I-mediated transcription. For example, KSHV infection induces the entry of angiogenin into the nucleolus. Angiogenin binds to rDNA promoter and increases the rate of transcription, which may have an anti-apoptotic role[75]. The HCV core protein is also known to activate Pol I transcription via increased recruitment of basal machinery and hyper phosphorylation of UBF[75]. It also facilitates the translocation of protein kinase-R (PKR) into nucleoli affecting the interferon response. A recent report on HBV oncoprotein – HBx conferring resistance against nucleolar stress suggests that HBx interferes with key cellular check points which are upregulated in stress conditions[76].

Ubiquitin proteasomal system

Ubiquitin proteasomal system (UPS) regulates the intracellular stability and activity of proteins in cells by post-translationally attaching ubiquitin moieties to proteins. While ubiquitination of protein is mediated by E3 ubiquitin ligases, deubiquitination is catalysed by a set of proteases called deubiquitinases (DUBs)[77]. Viruses often modulate or adopt the activity of E3 ubiquitin ligases or deubiquitinases to meet their requirements[78]. Therefore, UPS is now considered as an important target for therapeutic intervention for various diseases. The viral oncoproteins like EBNA3C of EBV and HBx of HBV are reported to stabilize transcription factors like c-Myc, pituitary tumour transforming gene 1 and nuclear receptor co-activator AIB to promote oncogenesis. Tumour suppressors and associated factors are also regulated in the same manner to facilitate cancer. For example, the p53–Mdm2 axis is an important target of many viral oncoproteins such as BZLF1 and EBNA3C of EBV, E6 of HPV, and LANA and vIRF4 of KSHV[78]. Similarly, pRb is regulated by NS5B of HCV and E7 of HPV, and CDK inhibitor p27[Kip1] is regulated by EBNA3C. Viral interference of these important cell-cycle regulators contributes towards deregulation of cell cycle. E6 oncoprotein of HPV targets cellular DUB cyclindromatosis (CYLD) and degrades it to activate NFκB pathway[78].

Most interestingly, the BPLF1 of EBV acts as a viral DUB which prevents DNA repair and inactivates viral ribonucleotide reductase 1. EBV also encodes two more DUB-like molecules, BSLF1 and BXLF1, but their functions are yet unknown. On the contrary, the E6 and E7 oncoproteins of HPV are stabilized by the activity of two cellular DUBs, USP15 and USP11 (ref. 79). The oncogenic activity of HTLV-1 Tax is maintained by inactivating cellular DUBs or by reducing the expression of cellular DUBs – CYLD and USP20 respectively. Tax also translocates cellular DUB STAMBPL1 from nucleus to cytoplasm in order to activate NFκB pathway and promote cell survival[79].

Exosome pathway

Discovery of exosomes, tiny vesicles secreted out of most cells, containing bioactive information, has attracted attention of not just cell biologist but also virologist, as this secretory pathway seems quite susceptible to viral manipulation[80]. Key evidences on viral hijacking of this pathway and its repercussions on overcoming host human immune response, latent survival and access to non-target cells have come from in-depth studies on HIV1 and EBV.

The first report on immune suppression by a DNA tumour virus was based on the major oncoprotein LMP-1 of EBV. The LMP-1 expressing cell lines were found to induce T cell anergy resulting in suppression of tumour-infiltrating lymphocytes. Later the secretion of viral oncoprotein was shown to be mediated by exosomes[81]. Further, exosomes secreted by EBV-infected cells also carry viral miRNAs called binder of Arl two (BART) miRNAs, whose functional transfer to dendritic cells down-regulates CXCL11/ITAC, an immune-regulatory gene involved in immune suppression in EBV-associated lymphomas[82].

Retroviruses such as HIV1 exhibit striking similarities in their particle biogenesis and secretion outside the cell. Discovery of HIV1 virion in cells of central nervous system supports the existence of a non-canonical mode of viral transmission independent of viral specific receptors and co-receptors. HIV1 is reported to enter mature dendritc cells (mDC) via exosomes as these cells exhibit greater ability to capture incoming virions and maintain

these in infectious form[83]. The gag and Nef proteins of HIV1 have been well documented to be secreted into exosomes. Nef protein in particular utilizes exosome-mediated secretion to suppress the host immune response against the virus by depleting CD4$^+$ T cells – a hallmark of AIDS[84]. Interestingly, exosomes-mediated transfer of the two most important chemokine co-receptors, CCR5 and CXCR4, are crucial for HIV1 infection to non-target cells[85,86]. More recently, the assembly and secretion of virions of HCV is shown to be dependent on the Hrs-dependent exosome pathway[87].

Viral oncogenes and cell transformation

Virus-mediated carcinogenesis is a multi-step process that involves a series of diverse complementary events in order to transform a normal cell into a cancerous cell. This involves initiation, promotion and progression events. Tumour viruses change cells by integrating their genetic material into the genomic DNA of host cell. The presence of at least part of the viral genome within the cell is essential for cell transformation. These genes normally interfere with mitogenic signalling and cell-cycle processes in infected cells causing some characteristic phenotypic changes, including anchorage-independent growth, loss of contact inhibition and immortalization. The transformed cells also exhibit increased cell division, which may favour viral propagation. The insertion mechanism can differ depending on whether the viral genome is DNA or RNA. In DNA viruses, the genetic material can be directly inserted into the host genome. RNA viruses must first reverse transcribe RNA to DNA, before its insertion into the host genome.

Inactivation of tumour suppressors

Tumour suppressor proteins that protect the cells from malignant transformation either by inducing apoptosis or cell-cycle arrest can also be targeted by viral oncoproteins. Viral oncoproteins interfere with the function of tumour suppressors by deregulating cell growth leading to continuous cell proliferation[3,23]. The first tumour suppressor identified was p53, which plays a major role to eliminate or inhibit abnormal cell proliferation, thereby preventing neoplastic development. In more than 50% of cancer cases, mutations in p53 gene have been identified[8,88]. The HPV-encoded E6 oncoprotein binds to ubiquitin ligase E6-AP and induces the degradation of p53, thus inhibiting the p53-dependent function. In addition, HBV-encoded HBx oncoprotein contributes in HBV-mediated HCC by inactivating p53 by forming a complex with it in the cytoplasm and preventing its entry to the nucleus and may also block p53-mediated apoptosis. The HCV-encoded NS5A is known to interfere with the DNA-binding activity of p53 and abrogate the p53-mediated transactivation function. ORF K8 protein encoded by KSHV also blocks p53-mediated cell death by transcriptional repression and by interaction with p53. Interestingly, the transcriptional activator Tax of HTLV1 can control p53 activity either by inducing p53–p65/RelA interaction, which leads to the transcriptional repression of p53 through CBP sequestration or by phosphorylating p53 at certain serine residues, which requires the hyper-activation of NFκB.

Retinoblastoma (pRB) is another negative regulator of cell-cycle progression by blocking the cells from entering into the S phase from G1. The hypophosphorylated form of pRb forms a complex with E2F transcription factor resulting in transcriptional repression[8,88]. On the other hand, the E7 oncoprotein of HPV disrupts the binding of transcription factor E2F to pRb and induces their proteasomal degradation[23]. This allows cells to progress into S phase because free E2F transcription factors promote the cell-cycle progression by inducing the expression of genes required for DNA synthesis. Different from others, the KSHV-encoded LANA takes control over the cells by recruiting EC5S ubiquitin ligase to degrade p53 and von-Hippel-Lindau (VHL), and blocking apoptosis[89]. The EBV-encoded proteins, EBNA3C and LMP1, like other viral oncoproteins, have been shown to interfere with p53 and pRb functions. EBNA3C, like LANA, recruits an E3 ubiquitin ligase SCF^{Skp2} to facilitate the degradation of pRb and modulates the p53 function by augmenting Mdm-2-mediated p53 ubiquitination and degradation[90,91]. On the other hand, HBx of HBV binds to and inhibits Skp2 resulting in stabilization of key cell-cycle regulator c-Myc[92].

Deregulation of cell-cycle

Cell-cycle progression is a complex network of regulatory signals which ensures accurate duplication of DNA and proper chromosome segregation. Cyclins, cyclin-dependent kinases (CDKs) and CDK inhibitors (CKIs) are the major regulators of cell-cycle progression. Tumour viruses have evolved numerous strategies to overcome this regulated progression and ensure continuous proliferation of the infected cells. The HPV-encoded E6 oncoprotein has been shown to decrease p21^{Waf1} expression in both p53-dependent and independent manner. Inhibition in p53 activity, which is a transactivator for p21 promoter, leads to decrease in p21^{Waf1} expression. E6 binds to p150 (sal2) and prevents its binding to cis element on p21 promoter without inhibiting p53 activity[93]. On the other hand, the E7 oncoprotein of HPV, blocks the interaction of p21^{Waf1} with the cyclin/CDK complex as well as alters its sub-cellular localization to bypass the p21-mediated arrest in cell cycle[94]. The HTLV-1-encoded Tax protein increases the levels of negative cell-cycle regulator p27^{Kip1} by increasing the activity of anaphase

promoting complex which further degrades and inactivates Skp2, the E3 ligase that targets p27[Kip1] (ref. 95). EBV protein kinase encoded by the viral BGLF4 gene is a Ser/Thr protein kinase which phosphorylates p27[Kip1] and induces its degradation[96]. HCV core protein is known to stabilize the p27[Kip1] in order to enforce cell-cycle arrest[97]. Interestingly, the HBx oncoprotein of HBV induces proteasomal degradation of p27[Kip1] by increasing the CDK2 activity[98]. c-Myc oncoprotein considerd as a master regulator of cell proliferation and cell-cycle division targeted by some viruses like EBV-encoded latent antigen, EBNA2, directly activates c-Myc, which upregulates the expression of its target genes, D-type cyclin and cyclin E, and down-regulates p21[Waf1] and p27[Kip1] (ref. 99). HBx indirectly upregulates the c-Myc protein level by interfering with its degradation mediated by SCF[Skp2] (ref. 92). Tax stabilizes p21 through the inhibition of CDK2 (ref. 100). Another report also suggested that Tax increases the transcription activation and mRNA stabilization of p21[Waf1] without affecting its turnover rate, suggesting a post-translational regulation[95].

Tax also blocks the interaction of p16INK4A with CDK4 or CDK6 and suppresses the expression of p15INK4B, p18INK4C and p19INK4D[23]. EBNA3C can also bypass p16INK4A-mediated suppression of CDKs by regulating the pRb and targeting the G1/S checkpoint[101]. EBNA3C attenuates the p53-mediated apoptosis either by interacting with inhibitors of growth family protein ING4 and ING5 (ref. 91) or by interacting and stabilizing Gemin3 (ref. 102).

EBNA3C can form a complex with cyclin A/CDK2 and stimulate its kinase activity. EBNA3C facilitates the G1/S transition by enhancing cyclin D1 activity via inhibiting its polyubiquitination by blocking GSK3β activity[103]. Tax either directly transactivates the cyclin D2 gene[104], or by the cooperation of phosphorylated CREB activates cyclin D1 transcription in order to bypass the G1/S checkpoint[105]. KSHV encodes v-cyclin which shows homology to cyclin D1. Like its cellular counterparts, v-cyclin can bind and activate CDK6, phosphorylate Rb and facilitate G1-S transition[106]. This association also induces phosphorylation of components of transcription as well as DNA replication[106].

Oncogenic cooperation

Tumour viruses harbour one or more oncogenes in their genome that participate in viral carcinogenesis. The viral oncogenes usually act by stimulating other cellular proto-oncogenes. Thus, cooperative and sequential activation of functionally different oncogenes is a major way of achieving step-wise carcinogenesis[107]. For example, KSHV encodes a number of viral oncogenes such as LANA, K1, vFLIP, vIRF-1, vGPCR, vIL-6 and Kaposin B, which cooperate with each other to promote cell

proliferation; but cooperation of LANA with H-Ras and vIRF-1 with Myc proto-oncogene in infected cells plays an important role in the progression of KSHV-associated malignancies[108,109]. Likewise, the sequential activation of Tax and HBZ proteins helps in establishment of leukaemia upon HTLV-1 infection. Tax is required initially to induce cell proliferation and neoplastic transformation. Subsequently, HBZ suppresses its expression in order to evade immune surveillance thereby providing the second oncogenic signal required for viral persistence and maintenance of leukaemic state[20]. Similarly, LMP-1 and EBNA proteins of EBV cooperate to promote the efficient proliferation of lymphocytes upon viral infection. EBNA-2-mediated LMP-1 expression results in the constitutive activation of c-Myc, which in turn activates a pro-apoptotic *Bim* gene. However, the activity of *Bim* is blocked by two other EBNA proteins (EBNA-3A and 3C) without affecting c-Myc stimulation, thereby contributing significantly in EBV-associated lymphogenesis[110]. A direct interaction between E6 protein of HPV and c-Myc is required for transcriptional activation of hTERT promoter, which results in the immortalization and increased proliferation of infected cells[111]. Similarly, HBx oncoprotein of HBV is known to transcriptionally upregulate cellular oncogenes such as c-Myc and hTERT[112,113]. Further, HBx and core protein of HCV are known to cooperate with c-Myc and Ras oncogenes leading to increased cell transformation and HCC[114,115].

Chromosomal integration and destabilization

To evade host immune responses, some tumour viruses integrate into the host chromosome and enter latency. Integration of viral DNA in the infected host genome serves as a key stage in the progression of neoplasias to invasive carcinomas and tumourigenesis[116]. The malignant progression results from deregulated expression of viral oncogenes and the host chromosomal instability upon viral genome integration. Classic examples of viral integration-induced cellular transformation come from in-depth studies on HBV, HPV, MCV and EBV. HPV and HBV normally integrate in the regions of DNA known as chromosome fragile sites that are distributed throughout the genome[117,118]. HPV integration results in constitutive expression of *E6* and *E7* oncogenes as well as disruption of several tumour suppressor genes such as *APM-1*[117]. Also, it has been observed that it integrates mostly within or near *MYC* and *hTERT* gene loci resulting in their increased expression followed by chromosomal breaks and centrosome aberrations causing aneuploidy in cells[119,120]. Like HPV, HBV integration is also random nature and may occur near/within *ANGPT1*. *MLL4*, *hTERT*, *PDGFRβ* and *MAPK* genes[118,121]. In some cases, insertion of *HBx* gene promoter within cellular genes results in virus-driven transcriptional upregulation as well as
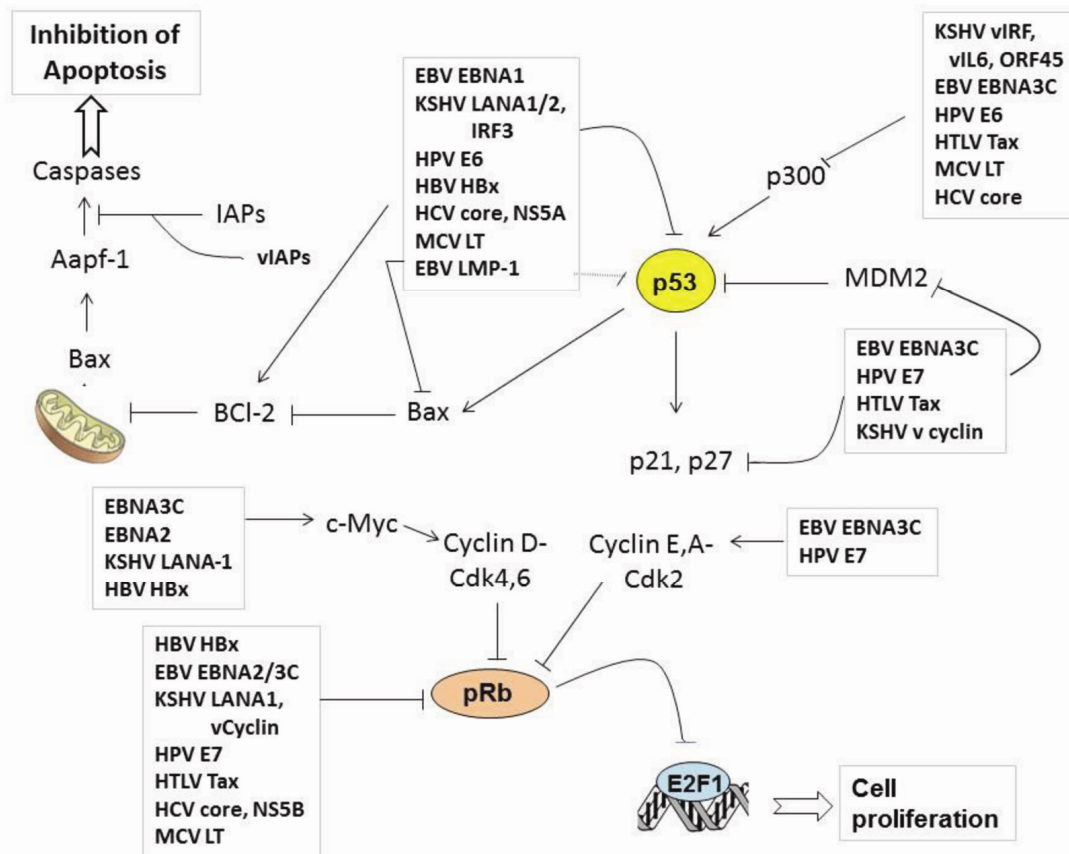
Figure 1. Cellular targets of viral oncoproteins. Cell-cycle regulators such as CDK, CDKI, p53, RB1 are the main targets.

production of hybrid proteins, such as retinoic acid receptor β (RARβ) and cyclin A2 which favour hepatocellular growth[118].

Genomes of large DNA viruses such as EBV, KSHV and MCV are also found integrated into the host chromosomes. Lately however, EBV and MCV DNAs have been found integrated in the genome of infected individuals[122,123]. EBV usually integrates close to the repeat regions of DNA that are devoid of functional genes. However, the integration sites have been shown to overlap with cellular genes such as *BACH2* (tumour suppressor), *MACF1* (cell motility factor), *REL* and *BCL-11A* (proto-oncogenes in myeloid and B-cells)[122]. EBV infection usually results in chromosomal translocation of *c-MYC* oncogene, placing it under the control of immunoglobulin gene promoter. This results in the deregulated expression of c-Myc in these cells, which is the major cause of EBV-associated Burkitt's lymphomas[124]. Integration of MCV in the host genome (~80% of MCC cases) results in deletion of C-terminal fragment of large T antigen, which usually decreases its proliferative potential[123]. The chromosomal abnormalities associated with MCV integration remain to be explored.

Tumour retroviruses such as HTLV-1 and HIV represent an outlier class vis-à-vis integration as their insertion in the host genome is a normal part of the viral life cycle. HTLV-1 integration sites are usually randomly dispersed in the non-transcribing regions of host genome, whereas HIV integrates within genes or active transcription units[125]. Interestingly, no correlation has been observed till date between their integration sites and oncogenic potentiation.

Despite the variation in insertion sites among different DNA tumour viruses, the integration event adversely affects human genome via deletions, duplications, chromosomal translocations, viral promoter-driven transcriptional enhancement, insertional mutagenesis and induction of genomic instability. Taken together, viral integration into the host genome significantly widens the oncogenic opportunities in infected individuals.

Cell transformation and migration

Cell transformation is a multistep process of oncogenesis that results from certain alterations in the cell cycle leading to immortalization and unperturbed cell growth. Oncogenic viruses have evolved various mechanisms to bring about malignant transformation. Viral transformation may be associated with integration of viral genome into host and/or continuous expression of some of the

Table 4. Mechanism of transformation by DNA viruses

Virus	Transformed cells		Immortalizing proteins and perturbed pathways	Reference
EBV	B cells	*EBNA1*	Destabilizes p53, disruption of PML antibodies, modulation of signalling pathways, induction of oxidative stress.	134, 135
		EBNA2	Potent transcriptional activator, interacts with basal transcription factors.	
		EBNA3A–C		
		LMP1	Mimics B cell activation receptor CD40.	
		LMP2A	Maintenance of EBV latency, deactivation of BCR signalling.	
HPV	Cervical epithelial cells	*E7*	Deregulates cell cycle by inhibiting RB and Cdk inhibitors, stimulating cyclins and cdk2.	128
			Induces DNA damage and activation of ATM–ATR pathways, interact with HDACs.	
		E6	Degrades p53 and activation of HATs, activates hTERT expression, interact with TNF-a receptor inhibiting apoptotic signalling.	
		E5	Contributes to the action of E6 and E7, activates EGFR signalling and MAPK pathway.	
KSHV	Primary endothelial cells	*LANA*	Destabilizes p53, inactivates RB, increases hTERT expression and cooperate with H-Ras.	12
		vFLIP	Inhibits interaction between FADD and caspase 8, activates NF-κB survival signalling.	
		Kaposin	Stabilize cytokine expression.	
		K1	Contains ITAM motif, activation of NF-κB and NF-AT signalling.	
HBV	Primary hepatocytes	*HBx*	Activation of basal transcription factors and signalling pathways like PI3K, TGF-B, JAK STAT. Inactivation of tumour suppressors.	136
MCV	Merkel cell in hair mucosa	*sTAg*	Binds to PP2A, preserves 4E-BP1 phosphorylation and hence active cap-dependent translation.	63, 137
		LTAg	Disrupts RB-E2F balance and destabilizes p53.	

Table 5. Various transformation mechanisms employed by RNA viruses

Virus	Mechanism of action	Example	Reference
Transducing retrovirus	Acquisition of cellular proto-oncogene. Acquired genes are components of signalling networks, receptors, transcription factors. Single hit oncogenesis	Rous sarcoma virus carries homolog of cellular proto-oncogene *v-src* which encodes a tyrosine kinase	138, 139
Cis-acting non-transducing retrovirus	Do not carry oncogenes. Insertional activation of proto-oncogene. Rare and slow-growing	ALV is integrated upstream of c-myc. Thus activation of c-myc a major proto-oncogene is under the control of viral promoter	140
Trans-acting non-transducing long latency retrovirus	No *cis* activation of proto-oncogenes. Involves *trans*-activation of cellular proteins via viral protein. Long latency very rare	Tax protein of HTLV-1 is a potent transactivator and activates NF-κB and AKT pathways.	141
		Core protein of HCV regulates cell cycle by binding to p53 and pRB.	142

viral proteins which alters host cell genetics and cell physiology.

DNA tumour viruses usually fiddle with two main cellular processes – signal transduction and cell cycle. Continuous activation of signal transduction cascades as well as disruption of cell-cycle regulation by these viruses result in uncontrolled cell proliferation. As elaborated in previous sections, viral proteins can alter cellular signalling pathways by mimicking the action of their cellular homologues such as receptors or adapter proteins, resulting in perpetual activation of these pathways. For example, the EBV immortalizing protein LMP-1 is an integral membrane protein acting as a functional homologue of the TNF family. It is known to activate several signalling pathways, NF-κB, Ap1 and JAK-STAT[126]. It also upregulates the transcription of cyclin D1 via NF-κB signalling, resulting in G1 to S transition[127]. Most of the viral oncoproteins tinker with cell-cycle regulation by disrupting two major tumour suppressors – RB and p53 (Figure 1). HPV oncoproteins – E6 and E7 – emerge as classic examples for the same. E7 primarily binds to pRb1 protein disrupting the pRb–E2F complex formation, resulting in activation of E2F responsive genes such as cyclin and cdks promoting unchecked G1 to S phase transition. On the other hand, HPV E6 triggers the proteasome-dependent degradation of p53, evading apoptosis. E6 also activates telomerase expression[128]. Oncoproteins from other DNA tumour viruses and their molecular mechanisms

are given in Table 4. Unlike DNA tumour viruses, RNA viruses mainly target protooncogenes rather than disrupting tumour suppressor functions of cell. Further, these viruses also employ different mechanisms to transform the host cell depending upon the virus type, as summarized in Table 5.

After the host cells are transformed, they acquire the ability to invade the tissue and then metastasize to distant locations. Some viral proteins have the ability to induce the secretion of enzymes that favour cell migration and decrease cell-to-cell adhesion and allow cancer progression. Besides, some viral proteins inhibit the metastatic suppressor proteins and/or alter the level of cell adhesion molecules like integrins and matrix metalloproteinases[23]. A highly studied metastatic suppressor Nm23-H1 is known to interact with EBV oncoproteins EBNA-1 and EBNA3C and E7 protein of HPV, resulting in the abrogation of their activities[129]. LMP-1 of EBV also leads to increase in expression of MMP-9, a type-IV collagenase[9]. In HBV-induced HCC, HBx plays an important role in promoting cell detachment and migration. For example, HBx can down-regulate E-cadherin expression and at the same time induce the expression of MMP-1, MMP-9 and integrins to promote cell migration[24]. Some viruses like KSHV can alter VEGF signalling by allowing its sustained release and promoting EC invasion[130].

Diagnostics and therapeutics

Most diagnostic tests for oncoviral infections available today are based on the detection of viral antigens or their antibodies. However, these tests do not always constitute the golden standard for diagnosis and therefore, necessitate the use of other confirmatory tests. Based on the outcome of these tests, the grade and complexity of the viral diseases are determined and the course of customized therapy is planned.

Some of the currently used methods for detecting viral infections are outlined below[2]:

(a) Serological test – WBC and platelet counts in case of EBV infection.
(b) ELISA for the detection of viral antigens and host antibodies against virus – EBV, KSHV, HBV, HCV, HSV and HTLV-1.
(c) Nucleic acid detection tests and *in situ* hybridization assay – RT-PCR for KSHV, HCV, HPV, HSV, HTLV-1 and MCV.
(d) Immuno-histochemistry – KSHV and MCV.

Therapies for oncoviral infections and virus-associated cancers[131]:

(a) Antivirals – Ganciclovir – for KSHV; Entecavir, Telbivudine and Tenofovir for HBV.

(b) Nucleoside/nucleotide inhibitor – HBV and HCV.
(c) Vaccines – EngerixB and ShantaVacB for HBV[132]; Gardasil and Cervarix for HPV[133].
(d) Chemotherapy – for KSHV and MCV.
(e) Drugs – Salicylic acid, imiquimod, podofilox and trichloroacetic acid for HPV warts.
(f) Steroids – EBV.
(g) Surgical and radiation therapy – KSHV, HBV, HPV and MCV.
(h) Protease inhibitors – Boceprevir and Telaprevir for HCV.
(i) Other therapies – Highly active antiretroviral therapy for AIDS and KSHV infections; transplantation of allogenic hematopoietic stem cells and CHOP chemotherapy for HTLV-1 (CHOP – cyclophosphamide, hydroxydaunomycin (Doxorubicin), oncovin (Vincristine) and prednisone).

Concluding remarks

Viral infections now tail behind smoking as the second highest preventable cause of cancer. Oncogenic viruses were identified and studied in the latter half of the last century. The revelation that cancer can be caused by infectious etiological agents such as viruses opened new avenues for cancer prevention, detection and treatment. Extensive studies have revealed numerous strategies adopted by viruses for hijacking host cellular pathways, crucial for the onset and progression of the oncogenic programme. Further insight into the mechanism of oncoviral subversion of molecular and cellular machineries of the host may help in designing effective targeted therapies for cancers.

Concerted efforts are essential for the discovery of previously unknown viruses directly associated with certain cancers. Also of importance is to pursue the already known viruses such as HIV-1 (which promotes KSHV-mediated oncogenesis) for their involvement in tumourigenesis, which might have otherwise gone unnoticed. It is crucial to design preventive strategies against these viruses to help reduce the cancer burden. The success stories of HBV and HPV vaccines in the market must motivate researchers to come up with similar life-saving vaccines for the remaining oncoviruses as well. Further, studies targeted at interference with the viral–host interface should lead us to futuristic first line of therapeutics.

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