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CHAPTER

7

Human DNA tumor viruses and oncogenesis

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Summary

Viruses with transforming abilities can change a normal cell into a cancer cell when persist in the infected cells. Tumor viruses are subclassified as either DNA viruses, which include Epstein–Barr virus (EBV), Kaposi's sarcoma–associated herpesvirus (KSHV), human papillomavirus (HPV), hepatitis B virus (HBV), and Merkel cell polyomavirus (MCPyV), or RNA viruses, such as hepatitis C virus (HCV) and human T-cell lymphotropic virus (HTLV-1).

What you can expect to know

Cancer involves the deregulation of multiple cell-signaling pathways that govern fundamental cellular processes such as cell death, proliferation, differentiation, and migration (Abhik Saha et al., 2010). The biological pathways that lead to cancer are more complex and intertwined (Hanahan and Weinberg, 2000). Globally, it is estimated that 15%–20% of all cancers are linked to oncogenic viruses (Parkin, 2006). However, most viral infections do not lead to tumor formation as several other factors influence the progression from viral infection to cancer development. Some of these factors include the host's genetic makeup, mutation occurrence, exposure to cancer-causing agents, and immune impairment. Initially, viruses were believed to be the causative agents of cancers only in animals. It was almost half a century before the first human tumor virus, Epstein–Barr virus (EBV), was identified in 1964 (Moore and Chang, 2010). Subsequently, several human tumor viruses were identified. Tumor viruses are subcategorized as either DNA viruses, which include EBV, Kaposi's sarcoma–associated herpesvirus (KSHV), human papillomavirus (HPV), hepatitis B virus (HBV), and Merkel cell polyomavirus (MCPyV), or RNA viruses, such as hepatitis C virus (HCV) and human T-cell lymphotropic virus (HTLV-1) (Abhik Saha et al., 2010). The normal cell is transformed into a cancer cell on persistent viral infection, either by integrating or retaining its genome as an extrachromosomal entity. The infected cells are regulated by the viral genes, which have the ability to drive the abnormal growth. The virally infected cells are either eliminated via cell-mediated apoptosis or they persist in a state of chronic infection. Importantly, the chronic persistence of infection by tumor viruses can lead to oncogenesis (Damania and Pipas, 2009). This chapter specifically focuses on the major tumor DNA viruses associated with human cancer and their mechanism of oncogenesis.

History and methods

Oncogenesis or tumorigenesis, that is, the development of cancer, begins with the accumulation of disruptions in several normal cellular activities that can eventually transform normal cells into cancer cells. These disruptions upset the normal balance between cell proliferation and death, allowing cells to acquire certain capabilities essential to malignant growth and spread. These general hallmarks of cancer include self-sufficient growth, insensitivity to antigrowth signaling, evasion of apoptosis, limitless replication, tissue invasion/metastasis, and angiogenesis (Hanahan and Weinberg, 2000). The progression stage of oncogenesis occurs when cells acquire a combination of these

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abilities, which allows conversion of a normal cell into a cancer cell (Hahn et al., 1999). As the repertoire of capabilities continues to build, the next stage of oncogenesis is observed when cells acquire the ability to degrade the local basement membrane, allowing them to spread and invade the surrounding tissues. In the case of solid tumors, these now invasive cancer cells can acquire the ability to induce blood vessel growth (i.e., a blood supply) from preexisting vessels *via* angiogenesis (Folkman, 2002). This provides the cancer with nutrients and oxygen needed to further grow and spread. At this point, the cancer cells may concurrently acquire the ability to metastasize to distant sites (such as other organs) as tumor-mediated angiogenesis can provide primary tumor cells with a mode of transport to metastasize. This metastatic process includes the successful intravasation of cancer cells into blood/lymphatic vessels, transit, extravasation out of blood vessels, and finally, establishment of a secondary site of the tumor growth. These cancer cells can then either lie dormant or can aggressively propagate into a secondary tumor. Each of these successive phases of carcinogenesis increases the likelihood of cancer-related morbidity and mortality, the metastasis stage representing the largest contributor. In addition, promoting a permissive microenvironment is crucial to the progression of carcinogenesis, one major example being the modulation of immune responses (Hanahan and Weinberg, 2000). Similar to environmental and host-related oncogenic events, human tumor–associated viruses can lead to malignancies by providing viral mechanisms that promote one or more general hallmarks of cancer (Damania and Pipas, 2009). Furthermore, it is recognized that chronic inflammation and immunosuppression provide a microenvironment more conducive to the progression of oncogenesis (Goedert, 2001). This chapter specifically focuses on human DNA tumor viruses that are known to associate with various cancers and also highlights mechanisms of virus-induced oncogenesis.

Transformation and oncogenesis

Studies on DNA tumor viruses have been instrumental to our understanding of basic cell biology and how the perturbations of cellular pathways contribute to the initiation and maintenance of cancer. DNA tumor virus infection leads to immortalization of the infected cell through deregulation of multiple cellular pathways via expression of many potent oncoproteins (Abhik Saha et al., 2010) as shown in Fig. 7.1. Research on various viral oncoproteins has revealed many of their novel cellular targets that are directly associated with cellular signaling, cell-cycle control, and the host's defense system (Abhik Saha et al., 2010; Stevenson, 2004) (Table 7.1). Tumor viruses reprogram the host quiescent, G0 cell into the S phase of the cell cycle, allowing viral access to the nucleotide pools and cellular machinery that are required for viral replication and transmission. The host cellular innate immune responses respond to viral infection by activating tumor-suppressor proteins, pRB1 and p53, to induce cell death. However, the tumor viruses have evolved the means to inactivate these signaling pathways for their own benefits (Goedert, 2001; Bouvard et al., 2009). Importantly, p53, the "guardian of the genome," and its downstream effectors are inactivated in 50% of human cancers. Herpesvirus family members, EBV- and KSHV-encoded oncoproteins, have been shown to manipulate p53 and pRb functional activity to block apoptosis during tumor progression. The EBV-encoded proteins, EBNA3C and LMP1, modulate p53 function either by repressing its transcriptional activity or by blocking p53-mediated apoptosis (Abhik Saha et al., 2010). Several studies have demonstrated that EBNA3C recruits MDM2 E3-ubiquitin ligase activity for augmenting proteasome-dependent degradation of p53. Recently, EBNA3C has been shown to bind and stabilize Gemin3 expression, which is crucial for inhibiting p53-dependent transcriptional activity and apoptosis. EBNA3C has also been shown to induce pRb degradation, thus leading to an establishment of latent infection (Moore and Chang, 2010). Similarly, KSHV-encoded latency-associated nuclear antigen (LANA) and K8 proteins, block p53-mediated host cell death through their interaction with p53. The KSHV-encoded LANA can also directly interact with pRb and enhance E2F-dependent transactivation activity and contribute to KSHV-induced oncogenesis by targeting the pRb-E2F regulatory pathway (Abhik Saha et al., 2010; Verma et al., 2007). Likewise, other DNA tumor virus-encoded oncoproteins also target tumor-suppressor proteins; HPV-encoded E6 protein has been shown to bind and degrade p53 through the ubiquitin-proteasome pathway (Cai et al., 2010; Enrique et al., 2010). In addition, HPV E7 oncoprotein bypasses cell cycle arrest through binding to the hypophosphorylated form of pRb, thereby inducing the degradation of pRb through a proteasome-mediated pathway (Moore and Chang, 2010). Also, HBV-encoded HBx interacts with p53 to inhibit its functional activity, which leads to the development of human hepatocellular carcinoma (HCC). In addition, the HBV-encoded HBx oncoprotein destabilizes pRb by upregulating the E2F1 promoter activity (Abhik Saha et al., 2010; Damania, 2007). Recent studies show that KSHV-encoded LANA and HBV-encoded HBx can downregulate *von Hippel-Lindau*, a tumor-suppressor gene, along with p53 (Damania, 2007; Colin et al., 2006). The association

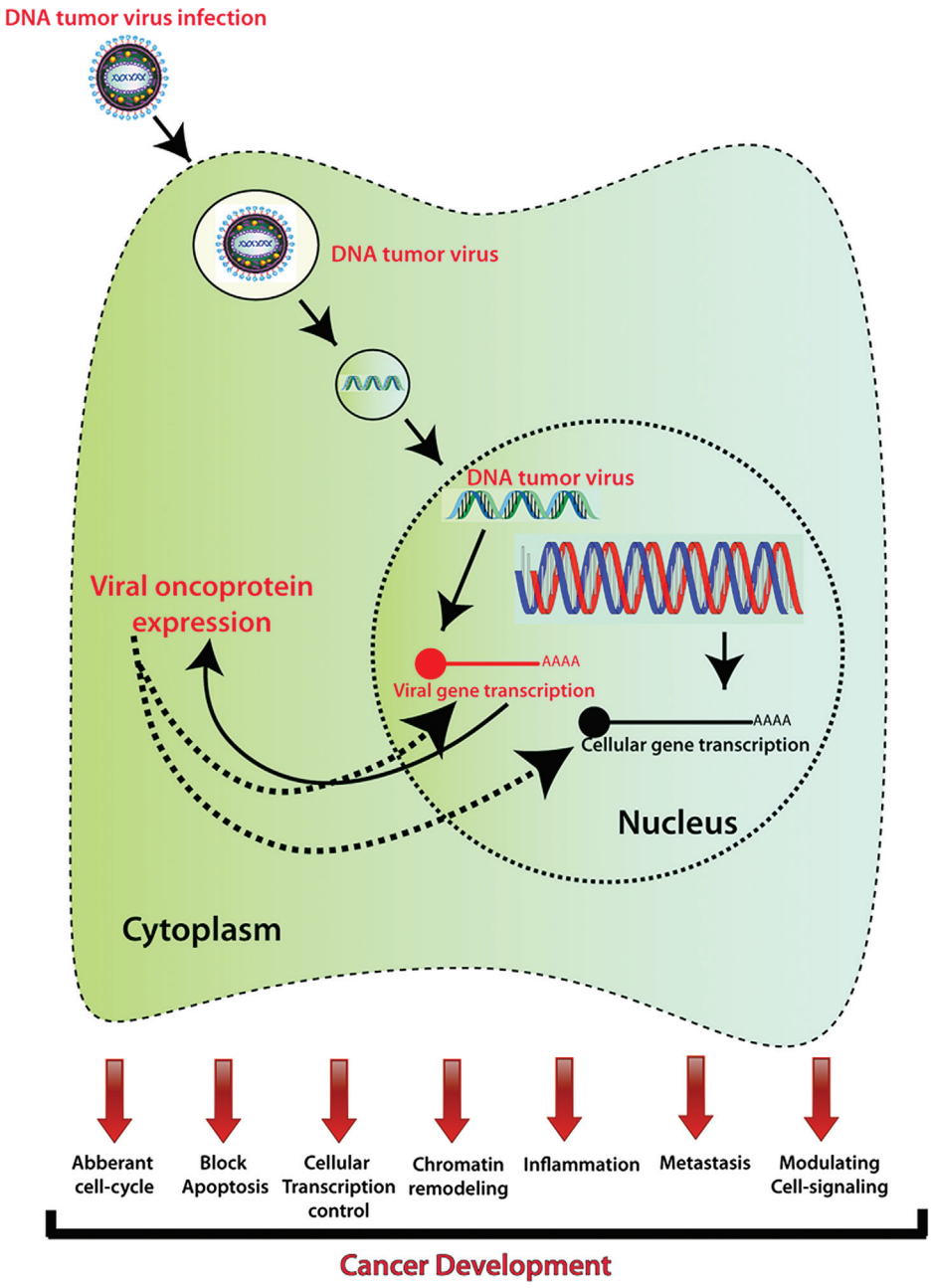

FIGURE 7.1 DNA tumor virus infection leads to immortalization of the infected cell through deregulation of multiple cellular pathways involved in cellular signaling, cell-cycle control, and defense system via expression of many potent oncoproteins.

TABLE 7.1 KEY VIRAL ONCOPROTEINS AND THEIR CELLULAR TARGETS.

Tumor virus	Viral oncoproteins	Important cellular binding partners	Deregulated signaling pathways
EBV	LMP1	p53, Mdm2, pRb, p300, Chk2, c-Myc, HDAC1, SUMO-1, SUMO-3, Cyclin A, E and D1, TRAFs, TRADD, JAK	Cellular transcription, cell cycle, metastasis, ub-proteasome, apoptosis, inflammation, chromatin remodeling, cellular signaling
KSHV	vGPCR, vIL-6, vBcl2, vCyclin, LANA and vFLIP	p53, pRb, c-Myc, core histones, apoptosis, TRAF2, Transcriptional activators- Sp1, AP-1, and transcriptional inhibitors HP1 and mSin3	Cellular transcription, cell cycle, apoptosis, ub-proteasome, chromatin remodeling, cellular signaling
HPV	E6	p53, p73, c-Myc, pRb, p21CIP1, p27KIP1,	Cell cycle, ub-proteasome
	E7	IRF-1, cyclin A and E	
MCPyV	LT	p53, pRb	Cell cycle
HBV	HBx	NFκB, p53, c-jun, c-fos, PKC, c-myc	Cell cycle, apoptosis, cellular transcription, Cellular signaling, metastasis.

between viral oncoproteins and cell-cycle regulatory factors, that is, cyclin/CDK complexes, also play a crucial role in viral transformation. The EBV-encoded latent antigen, EBV nuclear antigen 2 (EBNA2), transactivates cyclin D2 through the activation of c-Myc in EBV-associated lymphomas. Similarly, KSHV-encoded LANA stabilizes β-catenin, resulting in the increased expression of both β-catenin and cyclin D1 in KSHV tumors (Abhik Saha et al., 2010). Viral oncoproteins also deregulate various cellular signaling pathways that are directly linked to the development of oncogenesis, such as Notch signaling, MAPK, TLR, JAK/STAT, JNK, Wnt, interferon regulatory factors (IRFs), the ubiquitin-proteasome system, tumor necrosis factor (TNF), and nuclear factor (NF)-κB-signaling pathways, to evade host immune responses and facilitate their survival (Abhik Saha et al., 2010).

History of human DNA tumor viruses and cancer

The International Agency for Research on Cancer estimates that one-fifth of cancers worldwide are associated with viral infections (Ignatovich et al., 2002). Viruses have played a central role in the modern cancer research and have been providing profound insights into both infectious and noninfectious cancer cases. This diverse group of viruses revealed unexpected connections among innate immunity, immune sensors, and tumor-suppressor signaling that control both viral infection and cancer (Parkin, 2006). The first human tumor virus, EBV [also known as human herpesvirus-4 (HHV-4)], was identified by Anthony Epstein, Bert Achong, and Yvonne-Barr in 1964 in African pediatric patients with Burkitt's lymphoma. To date, seven viruses—EBV, KSHV [also known as human herpesvirus-8 (HHV-8)], high-risk HPV, HBV, HCV, HTLV-1, and MCPyV—have been classified as type-1 carcinogenic agents, linked to different types of human cancers (Table 7.2). Infectious cancer agents have been divided into two broad categories: direct-acting carcinogens, which are generally found in a monoclonal form within the tumor cells and express either viral or cellular oncogenes that directly contribute to cell transformation into cancer cell and indirect transform carcinogens, which are not conditioned to exist within the cell that forms the tumor. These agents presumably cause cancer by triggering chronic inflammation and oxidative stress or by producing immunosuppression that reduces or eliminates antitumor immune surveillance mechanisms, which in turn eventually leads to carcinogenic mutations in host cells (Moore and Chang, 2010). By definition, a direct viral carcinogen is present in each cancer cell and expresses at least one transcript to maintain the transformed tumor cell phenotype, as occurs with HPV, MCPyV, EBV, and KSHV-related cancers. Evidence supporting this comes from knockdown studies in which the loss of viral proteins results in the loss of host cancer viability. Even in these cases, external factors such as immunity and exposure to other infectious agents directly affect carcinogenesis. Indirect carcinogens could potentially also include "hit-and-run" viruses in which the viral genes are lost as the tumor begins to mature. Several agents such as HBV, HCV, and HTLV-I, which are involved in HCC, partly fit into this category (Moore and Chang, 2010). The oncogenic viruses are generally characterized by prolonged and often lifelong latency and evasion of the host immune surveillance as only a small subset of viral genes are normally expressed that may elicit an immune response

TABLE 7.2 HUMAN ONCOGENIC VIRUSES.

Virus	Genome	Notable cancers	Year identified
Epstein–Barr virus (EBV)	174 kb linear dsDNA	Burkitt's lymphoma, nasopharyngeal Carcinoma, Hodgkin's disease, infectious mononucleosis and X-linked lymphoproliferative disorders	1964
Kaposi's sarcoma herpesvirus (KSHV)	137 kb linear ds DNA	Kaposi's sarcoma, primary effusion lymphoma, and multicentric Castleman's disease	1994
Human papillomaviruses (HPV)	8 kb circular ds DNA	Most cervical cancer, penile cancers, anogenital, head and neck cancers	1983
Merkel cell polyomavirus (MCV)	5 kb circular ds DNA	Merkel cell carcinoma	2008
Hepatitis B virus (HBV)	3–3.3 kb of relaxed circular, partially duplex DNA	Hepatocellular carcinoma	1965

(Moore and Chang, 2010). Among DNA tumor viruses, latency is well studied on herpesvirus family members, EBV and KSHV. During latency, the virus is tumorigenic and persists as multiple copy, extrachromosomal circular episomes in a highly ordered chromatin structure whose propagation is dependent on their ability to hijack the replicative machinery of the host (Moore and Chang, 2010). The most likely explanation for the connection between virus latency and tumorigenesis is that productively replicating viruses initiate cell death, which has long been known to virologists as the cytopathic effect (Cai et al., 2010; Enrique et al., 2010; Ann Arvin et al., 2007). Small DNA tumor viruses, that is, HPV and MCPyV do not encode their own replication proteins, while large DNA tumor viruses, such as EBV, KSHV, and HBV, do encode their own viral DNA polymerase, but still require components of cellular replicative machinery for efficient viral DNA synthesis. A contrasting feature between small and large DNA tumor viruses is that small DNA tumor viruses can integrate into the host chromosomal DNA during or before cancer progression (Moore and Chang, 2010).

Epstein–Barr virus

EBV/HHV-4 is a double-stranded (ds) DNA virus that belongs to the genus *Lymphocryptovirus* of the human γ-herpesvirus family and is found in approximately 95% of the adult population worldwide. EBV is the primary cause of infectious mononucleosis (IM) and is associated with epithelial cell malignancies such as nasopharyngeal carcinoma and gastric carcinoma, as well as lymphoid malignancies including Hodgkin's disease, Burkitt's lymphoma, non-Hodgkin lymphoma, and posttransplant lymphoproliferative disorder (Moore and Chang, 2010; Damania, 2007). Immunocompromised patients, including AIDS or postorgan transplant patients, have a high probability of getting EBV-associated lymphomas. The virus only infects cells expressing the receptor for complement C3d component (CR2 or CD21); these cells include epithelial cells (mainly in the upper digestive tract) and B-lymphocytes. EBV has a powerful transforming potential for B-lymphocytes and establishes a lifelong latent infection in the B-cell of the infected host. The EBV genome is a linear, 175 kb dsDNA genome, which is maintained in the nucleus as an episome via tethering to the host chromosome (Ann Arvin et al., 2007). EBV encodes several viral proteins that have a transforming potential. EBV latency proteins (LMP1, EBNA2, EBNA3A, and EBNA3C) have been shown to express in AIDS-associated lymphoma, posttransplant lymphoma patients and lymphoblastoid cell lines (LCLs) generated from EBV infection of primary B-cells (Ann Arvin et al., 2007). LMP1 and EBNA2 are essential for the ability of EBV to immortalize B-cells because deletion of LMP1 from EBV renders the virus nontransforming (Abhik Saha et al., 2010). EBV-encoded latent protein EBNA-LP functions like a co-stimulator of EBNA2-mediated transactivation of many cellular and viral genes shown to be critical for B-cell immortalization (Damania, 2007). EBV nuclear antigen 1 (EBNA1) is essential for the maintenance and segregation of the EBV genome. EBNA3A and 3C are also critical for B-cell immortalization, while EBNA3B enhances the survival of cells. All three EBNA3 proteins are shown to bind with RBP-Jκ/CBF1 and regulate cellular gene transcription important for transforming B-cells into immortalized LCLs (Abhik Saha et al., 2010).

Kaposi's sarcoma–associated herpesvirus

KSHV/HHV-8 is a member of the γ-herpesvirus family (genus *Rhadinovirus*), which is tightly associated with human cancers, including Kaposi's sarcoma (KS), primary effusion lymphoma (PEL), and multicentric Castleman's disease (MCD) (Abhik Saha et al., 2010). More recently, KSHV-inflammatory cytokine syndrome (KICS) has been identified as a new inflammatory disorder associated with KSHV infection (Ann Arvin et al., 2007). KSHV remains asymptomatic in healthy individuals and establishes a lifelong persistence in B-lymphocytes after primary infection, similar to EBV (Uldrick et al., 2010). KS, originally called "idiopathic multiple pigmented sarcoma of the skin," was first described by Moritz Kaposi, a Hungarian dermatologist (Blake, 2010). KS was initially thought to be an uncommon tumor of Mediterranean populations until it was identified throughout sub-Saharan Africa and later became more widely known as one of the AIDS-defining illnesses in the 1980s (Ann Arvin et al., 2007). The viral cause for this cancer was found by Yuan Chang and Patrick Moore in 1994 by isolation of DNA fragments of a herpesvirus from a KS (Enrique et al., 2010) tumor in an AIDS patient (Hahn et al., 1999). Similar to EBV, KSHV has a linear dsDNA genome of approximately 165–170 kb that encodes for nearly 86 viral open reading frames (ORFs); however, only a small subset of these genes is expressed during latency, which includes LANA, vCyclin, vFLIP/K13, K12/Kaposin, and an micro RNA (miRNA) cluster (Chang et al., 1994). LANA, encoded by ORF73, is a multifunctional nuclear antigen and functional homolog to the EBV EBNA1 protein that plays a central role in deregulating various cellular functions, including maintenance of the viral episome

(Cai et al., 2010; Enrique et al., 2010), degradation of the p53 and pRb tumor suppressors, transactivation of the telomerase reverse transcriptase promoter, promotion of chromosome instability in KSHV-infected B-cells (Verma et al., 2007), and accumulation of the intracellular domain of Notch in KSHV-mediated tumorigenesis (Verma et al., 2007). LANA also plays a crucial role in maintaining latency by regulating the expression of RTA, another critical viral-encoded transcriptional activator required to switch from the latent to the lytic cycle (Abhik Saha et al., 2010; Moore and Chang, 2010). During latency, LANA tethers the viral episomal DNA to the host chromosomes, which helps in the efficient partitioning of the viral DNA in the daughter cells after cell division. Disruptions of LANA expression led to reduction in episomal copies, suggesting the importance of LANA in KSHV-mediated pathogenesis (Verma et al., 2007). Several KSHV genes have oncogenic potential, for instance, modulation of various cellular signaling pathways by K1 and K15, regulation of cell cycle by vCyclin and vIRF3, inhibition of cell death by K1, vFLIP, and vBcl-2, and immune modulation by vIRF3, K3, and K5 (Abhik Saha et al., 2010).

Kaposi's sarcoma

KS is a multifocal vascular tumor of mixed cellular composition that develops from the cells that line lymph or blood vessels and is most often seen as a cutaneous lesion (Hahn et al., 1999). The abnormal cells of KS form purple, red, or brown blotches or tumors on the skin called lesions (Hahn et al., 1999). KSHV is always found in the spindle cells of the lesion, which are thought to be of endothelial origin. In some cases, the disease causes painful swelling, especially in the legs, groin area, or skin around the eyes. KS can cause serious problems or even become life threatening when the lesions are in the lungs, liver, or digestive tract (Ann Arvin et al., 2007). KS in the digestive tract, for example, can cause bleeding, while tumors in the lungs can cause difficulty in breathing. There are four distinct clinical variants of KS, defined on the extent of immunosuppression and the severity of infection:

1. Classic KS is seen in HIV-negative elderly male patients of Mediterranean, Eastern European, and Middle Eastern heritage. Classic KS is more common in men than in women. Patients typically have one or more lesions on the legs, ankles, or the soles of the feet.
2. Endemic KS is a second type of KS, which is seen in Africa and affects HIV-positive and HIV-negative individuals and even children (Damania and Pipas, 2009). Rarely a more aggressive form of endemic KS is seen in children before puberty; it usually affects the lymph nodes and other organs and can lead to death within a year. There could be other factors in Africa (such as environmental cofactor and genetic predispositions in the population) that could contribute to the development of aggressive KS since the disease affects a broader group of people that includes children and women.
3. The third type of KS lesion is an iatrogenic form of KS that develops in posttransplant patients undergoing immunosuppressive therapy to prevent graft rejection after the organ transplantation (Enrique et al., 2010). Greater than 95% of all KS lesions, regardless of type, have been shown to contain KSHV viral DNA, thereby indicating a strong epidemiological link between KSHV infection and KS (Enrique et al., 2010; Damania, 2007).
4. AIDS-associated epidemic KS is a highly aggressive tumor and is primarily detected in HIV-infected individuals whose immune systems are severely damaged. In these individuals, the KS lesion is not restricted to the skin and often disseminates to the liver, spleen, gastrointestinal tract, and lungs. KS is the most frequently detected tumor in AIDS patients.

Primary effusion lymphoma

PEL (also called body cavity-based lymphoma) is a rare, rapidly fatal B-cell lymphoma commonly found in HIV-infected patients and is considered an AIDS-defining illness (Enrique et al., 2010; Damania, 2007). PEL is generally present as a pleural or pericardial effusion without a detectable tumor mass (Enrique et al., 2010; Yi-Bin Chen and Hochberg 2007). PEL cells are morphologically variable, with a null lymphocyte immunophenotype with evidence of HHV-8 infection. In PEL cells, KSHV presents as a single positive, indicating a strong epidemiological link between the presence of KSHV and the induction of PEL; however, 90% of these lymphoma cells often contain EBV as well. PELs are observed in both HIV-positive and HIV-negative individuals, with both types of PELs invariably containing KSHV viral DNA (Yi-Bin Chen and Hochberg, 2007).

Multicentric Castleman's disease

Castleman's disease, also called angiofollicular or giant lymph node hyperplasia, is a clinically heterogeneous entity that can be either localized (unicentric) or multicentric. MCD is an atypical lymphoproliferative disorder of a plasma cell type and is related to immune dysfunction (Damania, 2007; Yi-Bin Chen and Hochberg, 2007). There are two types of MCD, a hyaline vascular form, which presents as a solid mass, and a plasma-cell variant, which is associated with lymphadenopathy. Sometimes a mixture of both hyaline

vascular and plasma cell variants can also be found (Cai et al., 2010; Enrique et al., 2010). KSHV-MCD is characterized by intermittent flares of inflammatory symptoms, including fever, fatigue, cachexia, and edema, together with lymphadenopathy and/or splenomegaly. Nearly 100% of AIDS-associated MCD is positive for KSHV, whereas less than 50% of non−AIDS-associated MCD contains KSHV viral DNA (Damania, 2007). Patients with AIDS-associated MCD often develop malignancies like KS and non-Hodgkin's lymphoma (Damania, 2007).

KSHV inflammatory cytokine syndrome

KICS is a newly described clinical inflammatory condition that is characterized by systemic illness, poor prognosis, high KSHV viral loads, and elevated levels of interleukin 6 (IL-6) and interleukin 10 (IL-10) (Cai et al., 2010; Damania, 2007), comparable to those seen in KSHV-MCD. These features of KICS are comparable to the KSHV-MCD without the characteristic lymphadenopathy (Box 7.1).

Human papillomavirus

The main risk factor for the development of cervical cancer, the second leading cause of cancer death in women worldwide, is the persistent HPV infection (Uldrick et al., 2010). The first century of tumor virology research culminated with the Medicine Nobel prize granted to Harald zur Hausen for demonstrating an association between high-risk HPV infection and the development of cervical cancer (Damania and Pipas, 2009). The HPVs are small, nonenveloped, icosahedral DNA viruses, with a diameter of 52−55 nm. The virus particle contains a single dsDNA genome of approximately 8000 bps. The majority of the known types of HPV are asymptomatic, but some types may cause warts, while others can lead to cancers of the cervix, vulva, vagina, and anus (in women) or cancers of the anus and penis (in men) (Damania, 2007). Nearly, 50%−80% of sexually active population becomes HPV infected at some point in their lives. Most HPV infections in young females are temporary and have little long-term significance. Seventy percent of infections are gone in 1 year and 90% in 2 years. However, when the infection persists, in 5%−10% of infected women, there is high risk of developing precancerous lesions of the cervix, which can progress to invasive cervical cancer (Damania, 2007; Narisawa-Saito and Kiyono, 2007). Cervical screening using a Papanicolaou (Pap) test or liquid-based cytology is used to detect abnormal cells that may develop into cancer (Narisawa-Saito and Kiyono, 2007). Approximately 200 different HPVs have now been characterized and subsequently classified into low- or high-risk groups according to their potential for causing cervical cancer (Damania and Pipas, 2009). More than a dozen HPV types (including types 16, 18, 31, 33, 35, 45, 51, 52, 56, 58, 59, 62, 66, and 68) have been classified as "high-risk" types because they can lead to cervical cancer as well as anal, vulvar, vaginal, and penile cancers (Moore and Chang, 2010; Narisawa-Saito and Kiyono, 2007). HPV subtypes 16 and 18 are the most frequently detected HPV in tumors. Several types of HPV (type 16 in particular) have been found to be associated with HPV-positive oropharyngeal cancer (OSCC), a form of head and neck cancer (Abhik Saha et al., 2010; Moore and Chang, 2010; Damania, 2007; Narisawa-Saito and Kiyono, 2007). HPV-induced cancers often have viral sequences integrated into the cellular DNA (Damania, 2007; Narisawa-Saito and Kiyono, 2007). Low-risk viruses are associated with benign lesions such as condyloma accuminata or with basal cell and squamous-cell carcinomas of the skin (Damania, 2007). Another very rare inherited disease associated with HPV is epidermodysplasia verruciformis (EV), which is caused by an autosomal recessive mutation that leads to abnormal, uncontrolled papilloma virus replication (Damania, 2007). This results in the growth of scaly macules and papules on many parts of the body, but especially on the hands and feet. EV, which is associated with a high risk of skin carcinoma, is typically associated with HPV types 5 and 8

BOX 7.1

1. The Nobel Prize in Medicine (2008) was awarded to Dr. Harald zur Hausen for his discovery of HPV's association with cervical cancer, the second most common cancer among women. His discovery led to the development of two vaccines against cervical cancer. He shared this award with two other French virologists, Françoise Barré-Sinoussi, and Luc A. Montagnier, for discovering HIV.
2. The Nobel Prize in Medicine (2018) was awarded to Dr. James P. Allison and Dr. Tasuku Honjo for their discovery of cancer treatment by inhibition of negative immune recognition.

(other types may also be involved) (Damania, 2007; Narisawa-Saito and Kiyono, 2007).

Primary HPV infection occurs in the basal stem cells of the epithelium. The virus cannot bind to live tissue; instead, it infects epithelial tissues through microabrasions or other epithelial trauma as would occur during sexual intercourse or after minor skin abrasions that exposes segments of the basal membrane (Damania and Pipas, 2009). The virus then traverses upward and replicates in the terminally differentiated keratinocytes and is shed from the stratum corneum (Damania and Pipas, 2009; Narisawa-Saito and Kiyono, 2007). *HPV* genes encode proteins responsible for replication, cellular transformation, control of viral transcription, and those necessary for the generation of viral progeny (Damania, 2007). The main oncogenic proteins, E6 and E7 of high-risk HPV strains, are necessary for the maintenance of the malignant phenotype and have strong transforming abilities. HPV oncoproteins E6 and E7 have been shown to act synergistically to immortalize cells in vitro and induce skin tumors in transgenic animals (Abhik Saha et al., 2010; Damania, 2007). The HPV viral proteins target tumor suppressors, for instance, E6 binds to p53 and E7 binds to the Rb family of proteins, which induces their degradation through ubiquitin-proteasome-mediated degradation and leads to deregulation of the cell cycle and the inhibition of apoptosis (Damania, 2007).

Hepatitis B virus

HBV, a small-enveloped DNA virus, belongs to the hepadnaviruses family, which infects hepatic cells of humans causing inflammatory diseases of the liver (Abhik Saha et al., 2010; Cai et al., 2010; Narisawa-Saito and Kiyono, 2007). It is estimated that about 360 million are infected with the virus, and many of them are chronic carriers (Damania and Pipas, 2009; Martin and Gutkind, 2008) without any identifiable risk factor (Parkin, 2006; Kao, 2011). Most individuals with chronic hepatitis B remain asymptomatic for many years or decades. Patients with HIV infection or postorgan transplant patients taking immunosuppressive drugs are at higher risk of developing chronic infection (Colin et al., 2006). However, patients with chronic hepatitis B are at a risk of developing HCC, the fifth most common cancer and the third leading cause of cancer death worldwide (Damania, 2007; Colin et al., 2006). Infections with HBV and HCV are considered as the major contributors to HCC development, accounting for over 80% of all HCC globally (Damania, 2007; Colin et al., 2006; Kao, 2011).

Dr. Baruch Blumberg's laboratory discovered the viral cause of HBV in 1965. The genome of HBV is an enveloped virus with approximately 3.3 kb full-length negative-sense strand and a 2.8 kb short-length sense strand (Abhik Saha et al., 2010; Moore and Chang, 2010). One end of the full-length strand is covalently linked to the viral DNA polymerase, and the other short strand has an RNA oligonucleotide at its 5′ end. Thus, neither DNA strand is closed, and the circularity is maintained by cohesive ends (Moore and Chang, 2010; Damania, 2007). Viral DNA enters the nucleus and integrates into the host DNA immediately after infection. HBV replication initiates increased DNA synthesis and interferes with normal cellular detoxification and repair functions, causing chronic liver cell injury that leads to HCC (Damania, 2007). There are three different types of hepatitis B antigens encoded by the HBV genome. These include hepatitis B surface antigens (HBsAg; MHBsAg and LHBsAg), hepatitis B core antigen, and hepatitis B early antigen (HBeAg). HBsAg is most frequently used for diagnosis and is the first detectable viral antigen to appear during infection. There is a strong correlation between HBsAg chronic carriers and the incidence of HCC (Colin et al., 2006; Kao, 2011). It has been shown that HBsAg carriers have a risk of HCC, that is, 217 times than that of a noncarrier, and 51% of the deaths of HBsAg carriers are caused by liver cirrhosis or HCC compared to 2% of the general population (Colin et al., 2006; Kao, 2011). The virus is classified into four major serotypes (adr, adw, ayr, and ayw) based on antigenic epitopes presented on its envelope proteins and into eight genotypes (Kao, 2011) according to overall genomic variation (Vaughn and Elenitoba-Johnson, 2001). The genotypes show a distinct geographical distribution and disease severity and are used in tracing the evolution and transmission of the virus (Kao, 2011). HBV is transmitted by exposure to infectious blood or body fluids through sexual contact, blood transfusions, reuse of contaminated needles and syringes, and vertical transmission from mother to child during childbirth (Kao, 2011). Without intervention, a mother who is positive for HBsAg confers a 20% risk of passing the infection to her offspring at the time of birth (Damania, 2007; Colin et al., 2006; Kao, 2011). This risk is as high as 90% if the mother is also positive for HBeAg (Colin et al., 2006). However, holding hands, sharing utensils or glasses, kissing, hugging, coughing, sneezing, or breastfeeding cannot spread HBVs (Colin et al., 2006).

Approximately 80% of HBV-related HCC shows integrated HBV sequences; however, HBV can also be found integrated in non-HCC tissue (Colin et al., 2006). Integration of HBV leads to severe mutagenic consequences, such as large inverted duplications, deletions, amplifications, and translocations, that result in chromosomal instability (Abhik Saha et al., 2010;

Damania, 2007; Martin and Gutkind, 2008). HBV-encoded oncogene *HBx* is a viral replication protein that participates in transcription and DNA repair, through which it regulates cell cycle, apoptosis, and genomic instability. In addition, HBx transgenic mice develop liver carcinomas. HBx is most commonly found to be integrated in patients with HBV-related cirrhosis and dysplasia (Abhik Saha et al., 2010). HBx has been shown to interact with epidermal growth factor receptor, c-myc, c-jun, c-fos, p53, AP-1, NF-κB, and SP1 in multiple ways to contribute to the molecular pathogenesis of human HCC (Abhik Saha et al., 2010; Moore and Chang, 2010).

Human polyomaviruses

Polyomaviruses are nonenveloped, circular, dsDNA viruses of approximately 5000 bp. Ludwik Gross discovered the first murine polyomavirus in 1953 (Abhik Saha et al., 2010; Moore and Chang, 2010; Martin and Gutkind, 2008). Subsequently, many polyomaviruses have been found to infect birds and mammals. Polyomaviruses were so named because they are potentially oncogenic and cause a wide range of tumors in a number of animal species. Polyomaviruses are icosahedral in shape, with a small circular DNA genome (Damania and Pipas, 2009). For several years after the discovery of leukoencephalopathy [JC virus (JCV)] and nephropathy [BK virus (BKV)] in 1971, it was considered that only these two viruses infected humans, but next-generation sequencing techniques have enabled the identification of at least nine other members in humans, including MCPyV. By using digital transcriptome subtraction and high-throughput genome sequencing techniques, Chang and Moore along with their coworkers identified the presence of MCPyV in Merkel cell carcinoma (MCC) in 2008 (Damania, 2007). Polyomavirus infections via respiratory tract are highly common in childhood and young adult infections. Most of these infections are asymptomatic although the virus persists lifelong in almost all adults (Huichen Feng et al., 2008). Clinical evidence of disease caused by human polyomavirus infection is most common among persons who become immunosuppressed by AIDS, who are of old age or persons taking immunosuppressive drugs after organ transplantation. BKV was identified from the urine of a kidney transplant patient, and JCV was identified from the brain of a Hodgkin's lymphoma patient who developed progressive multifocal leukoencephalopathy (PML). The human JCV and BKV have been linked to several different human cancers. However, whether the role of the virus is causal or incidental has been the subject of much debate (Silva RLd, 2011). JCV DNA has been identified from brain tumors found in patients with or without PML and with glial tumors and pediatric medulloblastomas (Damania, 2007; Silva RLd, 2011). JCV has also been shown to be associated with colon cancer and central nervous system (CNS) lymphoma (Damania, 2007). Similarly, BKV is associated with polyomavirus nephropathy (PVN), a form of acute interstitial nephritis (Damania, 2007), and BKV DNA has also been found in pancreatic islet tumors and brain tumors (Silva RLd, 2011). This indicates that BKV is newly emerged as an opportunistic CNS infectious agent in AIDS and transplant patients, particularly those with a coexistent urologic disease and neurological decline (Damania, 2007).

Merkel cell polyomavirus

In 2008, MCPyV was discovered as the causative agent of MCC, a rare but aggressive skin malignancy, also termed as trabecular carcinoma of the skin (Silva RLd, 2011). Although MCPyV is similar to classical oncogenic polyomaviruses, subtle differences are beginning to emerge. It is found clonally integrated into the majority of MCC tumors. It develops in hair follicles or on (or beneath) the skin (Shailender Bhatia and Nghiem, 2011). MCC occurs most often on the sun-exposed face, head, and neck. MCC can also be found on the trunk and genitals but at a reduced frequency. Epidemiological studies indicate that older, light-skinned people or individuals taking immunosuppressive drugs after organ transplantation are at higher risk to develop MCC (Shailender Bhatia and Nghiem, 2011). MCPyV appears to be widely prevalent among healthy individuals. It has been shown that the prevalence of MCPyV seropositivity was 0% in infants, 43% among children aged 2–5 years, and increased to 80% among adults older than 50 years (Shailender Bhatia and Nghiem, 2011). In addition, MCPyV DNA was detected in cutaneous swabs from clinically healthy individuals with a prevalence of 40%–100%. Although widely prevalent, active MCPyV infection appears to be asymptomatic in healthy individuals, with the exception of MCC. This virus has not been yet convincingly linked with any other human disease (Shailender Bhatia and Nghiem, 2011). The exact mode of transmission remains to be elucidated and could involve cutaneous, fecal-oral, mucosal, or respiratory routes. MCPyV viral DNA has been detected in lower frequencies among respiratory secretions, on oral and anogenital mucosa, and in the digestive tract. Importantly, it appears that the virus is being shed chronically from clinically normal skin in the form of assembled virions (Moore and Chang, 2010; Shailender Bhatia and Nghiem, 2011). The MCPyV large T-antigen transcript has numerous functions in MCPyV infection, including initiation of viral replication and manipulation

of the host cell cycle. It appears to retain the major conserved features of other polyomavirus LT-antigens, such as Rb-binding motif and helicase/ATPase domains (Shailender Bhatia and Nghiem, 2011). MCPyV is the first polyomavirus that has been shown to integrate into human genomic DNA (Moore and Chang, 2010; Shailender Bhatia and Nghiem, 2011). The virus then undergoes at least two mutations (Shailender Bhatia and Nghiem, 2011), the first being a nonhomologous recombination with the host chromosome and then a sequential large T (4)-antigen truncation mutation that eliminates its viral replication functions but spares its Rb-targeting domain. These sequential mutational events result in persistent T-Ag expression, which play a key role in turning asymptomatic viral infection into an aggressive MCC (Moore and Chang, 2010).

Principle

DNA tumor viruses encode proteins with oncogenic potential, which alter the normal growth of cells by deregulating various cellular pathways. Here, we discuss the roles of viral proteins and RNA in inducing tumorigenesis by taking the example of EBV. A diagrammatic representation of the EBV life cycle is depicted in the flowchart **(**Fig. 7.2**)**: (1) The first step in EBV primary infection is the viral entry through the buccal cavity. (2) EBV shows higher affinity to B-cells in primary infection of naïve B-cells that occurs in the oropharyngeal mucosa. (3) Upon entry into the target cells, EBV enters into a short burst of lytic proliferation followed by a well-defined latency program. (4) EBV persists in the B-cells in the peripheral circulation and this latent infection of B-cells leads to various types of lymphomas. (5) Latently infected B-cells mature into plasma cells. (6) Plasma cells undergo lytic reactivation releasing infectious EBV particles. (7) EBV produced thus could reinfect fresh naïve B-cells or epithelial cells to cause nasopharyngeal carcinoma.

Epstein–Barr virus genome structure

A mature EBV viral particle is approximately 120–180 nm in diameter. It is composed of a linear dsDNA genome enclosed by a protein capsid surrounded by a protein tegument, which in turn is surrounded by a lipid envelope. The EBV genome is approximately 184 kb and contains 0.5 kb terminal repeats and internal repeat sequences (Shailender Bhatia and Nghiem, 2011). EBV strain B95-5 (derived from IM) was the first herpesvirus to have its genome completely cloned and sequenced. The virus encodes for approximately 80 proteins; however, not all of them have been fully characterized. The EBV genome was sequenced from a viral DNA BamHI-fragment cloned library; hence, ORFs, genes, and sites for transcription or RNA processing are frequently referenced to specific BamHI fragments, from A to Z, in a descending order of fragment size.

Entry into the cell

B-cells are the main target of EBV infection, although EBV can also infect epithelial cells, mainly in the upper digestive tract, which is thought to occur during viral reactivation process. During primary infection, EBV infects B-lymphocytes through interaction of the glycoprotein gp350/220 with the complement receptor CD21. Following primary infection, EBV persists in the infected host as an episomal DNA and causes lifelong asymptomatic infection (Ann Arvin et al., 2007). To achieve long-term persistence in vivo, EBV colonizes the memory B-cell pool where it establishes latent infection, which is characterized by the expression of a limited subset of virus latent genes (Ann Arvin et al., 2007). These genes affect the normal B-cell growth, leading to the immortalization of the cells. A low level of reactivation during the lytic cycle allows viral shedding into the saliva and transmission of the virus in vivo. In vitro, EBV can transform peripheral human B-lymphocytes into indefinitely proliferating LCLs that allows for genetic manipulation of the virus (Ann Arvin et al., 2007). The mechanisms of EBV entrance into epithelial cells are different from those of B-lymphocytes. To enter epithelial cells, viral protein BMRF-2 interacts with cellular β1 integrins, which in turn triggers fusion of the viral envelope with the epithelial cell membrane, thus allowing EBV to enter the cell. There are several well-described forms of EBV latency, each of which is utilized by the virus at different stages of its life cycle and are also reflected in various EBV-associated malignancies (Blake, 2010).

Epstein–Barr virus lytic replication

It has been shown that in newly infected cells before the establishment of latency, EBV undergoes a short burst of lytic DNA replication. EBV expresses a small set of viral genes that were previously classified as immediate-early or early genes of the lytic cycle. During this prelatent stage of infection, the immediate expression of these genes activates resting B-cells and protects them from immediate activation of apoptosis (Damania, 2007). The cascade of events in the lytic phase of the EBV life cycle is divided into three phases

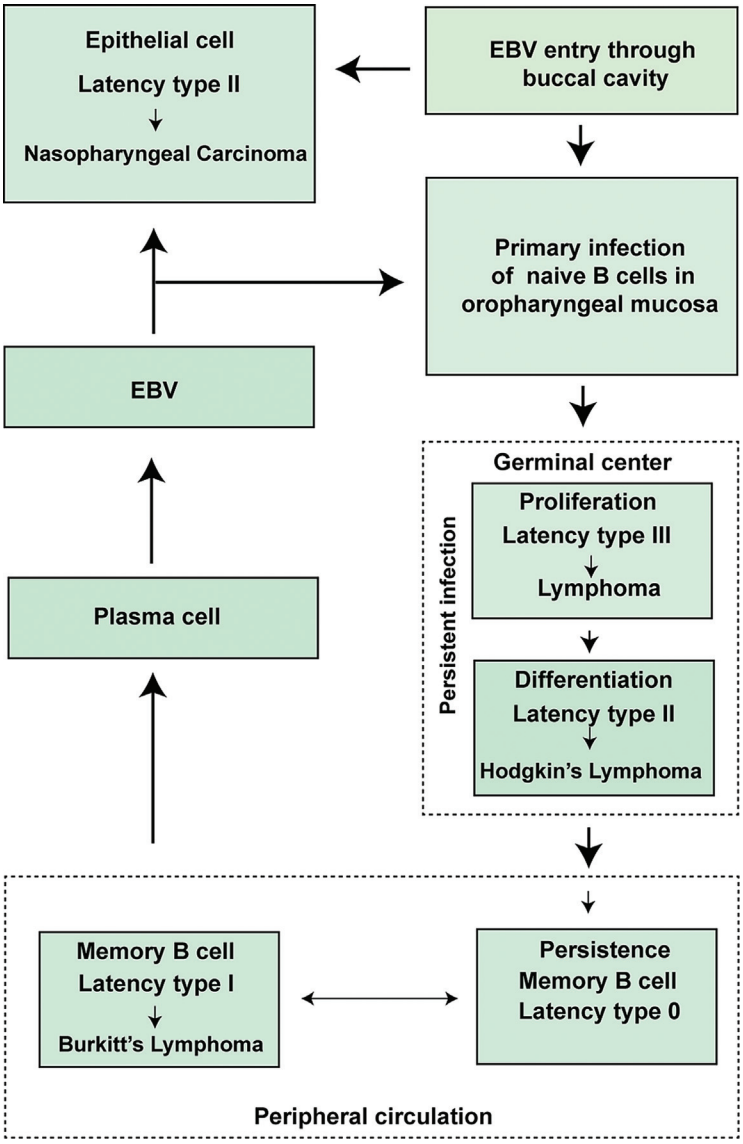

FIGURE 7.2 Flowchart of various stages of EBV life cycle.

of regulated gene expression: immediate-early, early, and late. The immediate-early gene products are transactivator proteins that trigger the expression of the early genes, the products of which include enzymes that are required for viral DNA replication (Blake, 2010). In turn, amplification of EBV DNA defines the boundary between early and late gene expression. During the late phase, viral structural proteins are expressed and assembled into virus particles into which the DNA is packaged before release of infectious virions. The principal switch from latency to productive infection involves activation of the immediate-early genes *BZLF1 (Zta)* and *BRLF1 (Rta)*. These two proteins can be expressed from a major 2.9 kb and a minor 3.8 kb bicistronic R-Z RNA transcribed from the R-promoter (Rp) (Blake, 2010). BZLF1, a viral transactivator protein, is involved in triggering the expression of the lytic genes and downregulation of latent genes, culminating in cell death and the release of infectious virions. These proteins upregulate the expression of other immediate early genes as well as their own expression. Both Zp and Rp are activated by ZEBRA, whereas Rta can upregulate Zp and autoactivate its own synthesis. However, synergistic effects of ZEBRA and Rta induce the maximum activation of the upstream Rp promoter. This synergism suggests that low levels of the two proteins are sufficient to trigger the lytic cascade. These immediate-early gene expressions in turn upregulate the expression of early genes such as viral DNA polymerase (BALF5) and thymidine kinase. The major proteins of the lytic phase are the EBV DNA polymerase, BALF5, and the late lytic cascade major capsid protein, BcLF1 (Ann Arvin et al., 2007). The lytic DNA replication of virion DNA start

from the lytic origin of replication (ori-lyt), which is distinct from the plasmid DNA replication origin (ori-P) that is used to maintain the episomal virus during latency. EBV ori-lyt lies within the BamHI H region of EBV DNA and contains two essential cis-acting regions, the BHLF1 promoter, and a 0.5 kb distant region required for replication (Ann Arvin et al., 2007). The ZEBRA response elements (ZREs) present within the BHLF1 promoter are essential for ori-lyt-directed replication. The six-core replication protein together with ZEBRA is absolutely required for the amplification and replication of the viral genome from ori-lyt. ZEBRA is a member of the basic zipper family of transcription factors and binds as a homodimer to ZREs within early gene promoters (Ann Arvin et al., 2007). Moreover, ZEBRA is an essential component of lytic DNA replication, and its association with the EBV helicase targets ZEBRA to the viral DNA replication compartments within the nucleus. Generally, herpesviruses replicate their DNA in G1-phase of the host cell cycle, which has been suggested to be advantageous to the virus due to the lack of competition with cellular DNA replication. As the key regulatory element, ZEBRA plays a critical role in the EBV lytic cycle by interacting with C/EBPα, which leads to an accumulation of p21CIP-1 and G1 cell-cycle arrest. Further studies suggest that apart from inhibiting cellular DNA synthesis, EBV induces an S-phase-like cellular environment during lytic replication. Latent EBV in B-cells spontaneously reactivates to switch to lytic replication, but the precise trigger for the induction is unknown. Many changes in physiological conditions or other nonrelated infection has been attributed to the triggering of spontaneous reactivation (Ann Arvin et al., 2007). In vitro, latent EBV in B-cells can be reactivated by stimulating the B-cell receptor (BCR) or by treating the cells with sodium butyrate or phorbol esters. EBV lytic replication does not inevitably lead to the lysis of the host cell because the virions are produced by budding from the infected cell.

Epstein–Barr virus latency

Latently infected B-cells maintain EBV genomes tethered to the host chromosome as 174-kb circular plasmids referred to as episomes and express only a limited number of viral latent gene products (such as EBNA1, EBNA2, EBNA3A, EBNA3B, EBNA3C, and EBNA-LP) and three latency-associated membrane proteins (LMP1, LMP2A, and LMP2B) (Ann Arvin et al., 2007). Transcripts referred to as BARTs (BamHI A rightward transcripts) from the BamHI A region (Bam A) of the EBV genome, as well as small nonpolyadenylated RNAs, EBV-encoded RNAs (EBERs) 1 and 2, are abundantly expressed during latency (Blake, 2010). Four patterns of EBV latency programs are recognized at present. In type I latency, represented mainly in Burkitt lymphoma cells, viral gene expression is restricted to the two EBER genes, the BART transcripts, and EBNA1. In latency II, additional expression of three latent-membrane proteins (LMP-1, LMP-2A, and LMP-2B) is observed and is most frequently seen in Hodgkin's lymphoma. Latency III is seen in lymphoproliferative diseases (LPDs) developed in immunocompromised individuals and EBV-transformed LCLs. In this group, all six EBNAs, all three LMPs, and the two EBERs are expressed. Type IV latency is less strictly defined and pertains to IM patients and to posttransplant LPD. Some individuals also present putative latency program (latency 0), which shows no detectable level of latent gene. The principal mediators of EBV-induced growth and cellular transformation of B-lymphocytes in vitro include EBNA2, EBNA3A, 3 C, and LMP1 proteins (Blake, 2010). The *EBNA* genes are important for the transformation of primary B-lymphocytes, leading to transactivation and regulation of other cellular and viral genes. These proteins are involved in augmentation of the expression of genes coding for CD21, CD23, LMP1, and LMP2 proteins in B-lymphocytes. Growing evidence shows that EBV primarily persists in B-lymphocytes. Studies of EBV strains in donor–recipient pairs before and after bone marrow transplantation have shown that the recipient's strain disappears from the oropharynx and is replaced by the donor's strain, thus indicating that the bone marrow B-cells harbor EBV. Furthermore, patients with X-linked agammaglobulinemia, who are deficient in mature B-cells, are found to be free of EBV infection, suggesting that they are not able to maintain a persistent infection. Although much of the evidence described earlier implicates B-cells as the site of persistence, a role for infection of squamous epithelial cells is also suggested by the detection of EBV in oral hairy leukoplakia (Ann Arvin et al., 2007; Blake, 2010).

Epstein–Barr virus latent genes

An understanding of EBV latent gene function is significant both to the factors contributing to the establishment of persistent infection and to the role of the EBV oncogenesis. Recent research on EBV unraveled the essential roles of EBNA2 and LMP1 in an in vitro transformation of B-cells and highlighted roles for EBNA-LP, EBNA3A, EBNA3C, and LMP2A in this process. These studies confirm that EBV-induced B cell transformation requires the cooperative effect of several latent genes. A brief description of EBV latent gene function involved

in virus persistence and cellular transformation is as follows (Ann Arvin et al., 2007).

Epstein–Barr virus nuclear antigen 1

EBV-encoded multifunctional latent protein EBNA1 is a DNA-binding protein that plays an important role in the continued proliferation or survival of EBV-positive tumor cells. EBNA1 is expressed in all forms of latency and has been shown to be important for efficient EBV-mediated immortalization of B-cells in vitro. Several studies showed that EBNA1 is required for the replication and maintenance of the episomal EBV genome. EBNA1 binds to ori-P, the episomal origin of viral replication. EBNA1 is a transcriptional transactivator and upregulates Cp and the LMP1 promoter. The Gly–Gly–Ala repeat domain of EBNA1 is a cis-acting inhibitor of major histocompatibility complex class I-restricted presentation and regulates antigen processing via the ubiquitin-proteosome pathway. Targeting expression of EBNA1 to B-cells in transgenic mice results in B-cell lymphomas, suggesting that EBNA1 might also have a direct role in oncogenesis. EBNA1 acts as a transcriptional activator of several viral and cellular genes (Ann Arvin et al., 2007). In vivo, EBNA1 has been shown to lower p53 levels and apoptosis in response to DNA damage in U2OS cells.

Epstein–Barr virus nuclear antigen 2

EBNA2 is the earliest latent cycle protein of EBV and is essential for primary B-cell growth transformation, immortalization, proliferation, and survival. The role of EBNA2 in growth transformation was first revealed with studies in EBV-infected Burkitt's lymphoma cell line, P3HR-1, a nontransforming mutant form of EBV. EBNA2 is a transcriptional activator of both cellular and viral genes and upregulates the expression of various B-cell antigens, including CD21 and CD23, as well as LMP1 and LMP2. EBNA2 also transactivates the Cp promoter and thereby induces the switch from Wp to Cp detected early in B-cell infection. EBNA2 also transactivates *c-myc* oncogene, which is essential for EBV-induced B-cell proliferation and transformation (Ann Arvin et al., 2007).

Epstein–Barr virus nuclear antigen 3 family

Genetic studies have revealed that both EBNA3A and EBNA3C are essential latent antigens for efficient B-cell transformation in vitro, whereas EBNA3B is completely dispensable. Importantly, EBNA3B-mutated B-cell lymphomas were frequently found and evident that EBNA3B inactivation drives lymphomagenesis and immune evasion. Both EBNA3A and EBNA3C interact with a variety of cellular proteins that may mediate transcriptional activation, repression, or affect cell proliferation. Several evidences strongly suggest that EBNA3A and EBNA3C together inhibit the initiation of Bim transcripts. EBNA3C has been shown to induce the upregulation of both cellular (CD21) and viral (LMP1) gene expression. EBNA2 and the EBNA3 proteins work together to precisely control RBP-Jκ activity, thereby regulating the expression of cellular and viral promoters containing RBP-Jκ cognate sequence. EBNA3C has been shown to interact with human histone deacetylase 1, which contributes to the transcriptional repression of Cp promoter. In addition, EBNA3C has been shown to make stable complexes with several transcriptional cofactors, including mSin3A, prothymosin-expressing, and NCoR in EBNA3C-expressing B-cells. EBNA3C has been shown to repress the Cp promoter and interacts with the retinoblastoma protein, pRb, to promote cell transformation (Ann Arvin et al., 2007). EBNA3C can promote metastasis in EBV-positive tumors by modulating Nm23-H1 activities.

EBNA-LP

EBNA-LP, a critical regulator of EBV-induced B-cell immortalization, is encoded as a variable size protein depending on the number of BamHI W repeats contained in a particular EBV isolate. The role of EBNA-LP in an in vitro B-cell transformation is not clear, but EBNA-LP is required for an efficient outgrowth of LCLs. EBNA-LP binds with pRb in LCLs and with both pRb and p53 in in vitro assays, but its expression appears to have no effect on the regulation of the pRb and p53 pathways (Ann Arvin et al., 2007). EBNA-LP has also been observed to interact with several cellular proteins, including tumor suppressors (pRb, p53, p14ARF, and Fte1/S3a), heat shock proteins (hsp70 and hsp72/hsc73), cell-cycle regulatory molecules (DNA-PKcs and HA95), and anti-apoptotic (HAX-1) protein.

LMP1

LMP1, a latent-membrane protein, is the major transforming protein of EBV behaving as a classical oncogene in rodent fibroblast transformation and being essential for EBV-induced B-cell transformation in vitro. LMP1 has pleiotropic effects when expressed in cells resulting in induction of cell surface adhesion molecules and activation antigens, upregulation of antiapoptotic proteins (Bcl-2 and A20), and stimulation of cytokine production (IL-6 and interleukin 8). Studies have shown that the immortalization effect of LMP-1 on B-lymphomas is mediated by the possible cooperation between Bcl-2 and MCL-1. Recent studies have demonstrated that LMP1 can contribute to neoplastic transformation and tumor progression by modulation of the TNF receptor pathway through its

association with the CTAR1 and CTAR2 domains in a ligand-independent manner (Ann Arvin et al., 2007).

LMP2

The gene encoding LMP2 yields two distinct proteins, LMP2A and LMP2B. Neither LMP2A nor LMP2B is essential for B-cell transformation. The LMP2A amino-terminal domain contains eight tyrosine residues, two of which (Y74 and Y85) form an immunoreceptor tyrosine-based activation motif (ITAM) (Ann Arvin et al., 2007) (Matsuo and Itami, 2002). ITAM phosphorylation in the BCR plays a central role in mediating lymphocyte proliferation and differentiation by the recruitment and activation of the src family of protein tyrosine kinases. Expression of LMP2A in the B-cells of transgenic mice abrogates normal B-cell development, thus allowing immunoglobulin-negative cells to colonize peripherally to lymphoid organs. This indicates that LMP2A can drive the proliferation and survival of B-cells in the absence of signaling through the BCR. Together this suggests that LMP2 can modify the normal program of B-cell development to favor the maintenance of EBV latency and to prevent activation of the EBV lytic cycle (Matsuo and Itami, 2002). Other studies have demonstrated that LMP2A can bypass the entire p53 pathway in lymphomagenesis involving c-Myc.

EBERs

EBERs are EBV-encoded two small nonpolyadenylated (noncoding) RNAs, referred to as EBERs 1 and 2, and are most abundant viral transcripts expressed in all forms of latency. However, the EBERs are not essential for EBV-induced transformation of primary B-lymphocytes. The EBERs assemble into stable ribonucleoprotein particles and bind the interferon (IFN)-inducible, dsRNA-activated protein kinase, PKR. EBER-mediated inhibition of PKR function is important for viral persistence, perhaps by protecting cells from IFN-induced apoptosis (Ann Arvin et al., 2007). In addition, EBER expression can confer an apoptotic-resistant phenotype in immortalized nasopharyngeal epithelial cells.

BARTs

EBV is the first human virus in which the expression of miRNAs, such as MIR-BARTs, was identified. MIR-BARTs are derived from the BamHI A region (Bam A) of the EBV genome. BARTs were first identified in nasopharyngeal cancer (NPC) tissue and subsequently in other EBV-associated malignancies such as Burkett's Lymphoma and nasal T-cell lymphoma. Studies have demonstrated that MIR-BART5 promotes cell survival by targeting PUMA expression, leading to latent infection in NPC and germinal center B-cells. MIR-BARTs may play an important role in epithelial cells carcinogenesis as they are abundantly expressed in latently infected epithelial cells compared to B-cells. BARTs encode a number of potential ORFs, including BARF0, RK-BARF0, A73, and RPMS1; however, protein products of these ORFs have not been identified (Ann Arvin et al., 2007).

Micro RNAs

miRs are small, noncoding RNA molecules of only 21–24 nucleotides in length and have been shown to play a role in the posttranscriptional downregulation of target mRNAs. The EBV miRs are arranged in two clusters within the viral genome, that is, the BHRF1 cluster and the BART cluster, which comprises the remaining 20 miRs located in the introns of the BART transcripts. These two clusters of EBV-encoded miRs are differentially expressed in cells exhibiting different forms of EBV latency. The BART cluster is predominantly expressed during latency I or latency II, whereas the BHRF1 miRs are associated with latency III. The expression levels of several miRs from both clusters are enhanced following induction of lytic infection. However, the precise function of these miRs remains unclear (Ann Arvin et al., 2007).

Clinical correlation

Clinical symptoms and molecular diagnostic approaches differ according to the immune status of the patients. In healthy individuals, primary infection of EBV is most often asymptomatic. However, in immunocompromised individuals, EBV is associated with disorders with high rates of morbidity and mortality. The spectrum ranges from benign B-cell hyperplasia resembling IM to more classic malignant lymphomas. Molecular diagnostics is increasingly important for diagnosis and monitoring of patients affected by EBV-related diseases. As virus-specific treatments continue to be investigated, it becomes important to recognize these EBV-associated malignancies so that proper clinical management decisions can be made. New molecular tests [quantitative real-time polymerase chain reaction or EBER-RNA in situ hybridization] combined with traditional serological (heterophile antibody testing) or histochemical assays [immunofluorescence assay (IFA), in situ hybridization, or Southern blot] are helpful for diagnosis and monitoring of EBV-related diseases depending on the clinical setting and the types of samples available for testing. In situ hybridization for EBER-RNA on biopsy samples, and more recently EBV viral load testing of blood samples, provides an accurate measure of the clinical status in patients. So far, the methods of first choice in routine EBV diagnostics are

the IFA, still the gold-standard method, and different enzyme immunoassay (EIA) techniques, including solid-phase ELISAs and western blot analysis. While IFA or EIA is often used for screening, western blot analysis is mainly performed for confirmation. Today, a number of manufacturers provide commercially available EBV-specific IFAs and EIAs. Recently, in situ hybridization of EBER-RNA has become standard for EBV diagnosis in tumor cells, while quantitative polymerase chain reaction (qPCR) procedures are used for EBV typing. Investigations are underway to better define the utility of these assays across the full spectrum of EBV-associated diseases. In addition, gene expression profiling and array technology will likely improve our ability to subclassify these diseases and predict responses to therapy. Some of the most common EBV-associated malignancies are listed as follows.

Burkitt's lymphoma

The association between EBV and Burkitt's lymphoma (BL) has long been established (Ann Arvin et al., 2007). BL is an aggressive B-cell malignancy, classified in three forms, referred to as endemic-associated BL, sporadic-associated BL, and HIV-associated BL, on the basis of geographical distribution and EBV-association. BL is a tumor of the jaw and face found in children of equatorial Africa, which has rare occurrence elsewhere. The exact cause of this tumor is unclear, but there is probably a genetic factor as well as a malarial cofactor. Only 5% of BLs in the United States is associated with EBV, whereas in endemic areas such as eastern Brazil or Africa, nearly 90% of pediatric BLs harbor EBV. The BL cells show evidence of EBV DNA and tumor antigens, and the patients carry a much higher level of anti-EBV antibodies than other members of the population, although the exact role of EBV in BL pathologies remains to be elucidated. Tumor cells are monoclonal and show a characteristic translocation between chromosomes 8 and 14 that places the c-myc oncogene under the control of the immunoglobulin heavy or light chain promoters, resulting in the upregulation of c-myc oncogene in these cells (Bellan et al., 2003). BL is also associated with HIV infection or occurs in postorgan transplant patients undergoing immunosuppressive therapies to prevent graft rejection. BL may be one of the diseases associated with the initial manifestation of AIDS (Damania, 2007).

Nasopharyngeal cancer

NPC is a rare type of head and neck cancer of epithelial cells of the upper respiratory tract. The majority of nasopharyngeal carcinoma cases from individuals in Southern China, Southeast Asia, Mediterranean, Africa, and the United States are associated with EBV infection. The occurrence of NPC in the rest of the world is low, indicating that there may be a genetic predisposition for the development of EBV cancers in these populations, or there may be an involvement of environmental cofactors, such as dietary components (salted fish), creating low-grade, pre-invasive lesions that become susceptible to EBV infection (Damania, 2007; Bellan et al., 2003). As for the association of EBV with NPC, the contribution of the virus is less clear and appears to be a consequence of the aberrant establishment of virus latency in epithelial cells that have undergone premalignant genetic changes. The presence of monoclonal EBV episomes in NPC indicates that viral infection precedes the clonal expansion of malignant cells.

Hodgkin's lymphoma

Hodgkin's lymphoma, formerly known as Hodgkin's disease, is a B-cell LPD of the lymphatic system in which 1% of the tumor population is composed of Hodgkin/Reed-Sternberg (HRS) cells, which are derived from germinal center B-cells. The HRS cells are multinucleated giant cells that have distinct nucleoli (Damania and Pipas, 2009; Damania, 2007). The first sign of Hodgkin's lymphoma is often a swollen lymph node, which appears without a known cause. The disease can spread to the nearby lymph nodes and later may spread to the spleen, liver, bone marrow, and other organs. There are three types of Hodgkin's lymphoma: lymphocyte-depleted, nodular sclerosis, and mixed cellularity. Each of these differs in their association with EBV infection; 20% of nodular sclerosis Hodgkin's lymphomas are linked to EBV, whereas 100% and 70% of lymphocyte-depleted Hodgkin's lymphomas and mixed-cellularity Hodgkin's lymphomas, respectively, are associated with EBV infection (Damania, 2007; Ann Arvin et al., 2007). EBV is reported to play a direct or indirect role in the pathogenesis of Hodgkin's lymphoma, either by activating several pathogenic mechanisms or by regulating the process of immune recognition that supports the malignancy and reactivation of the virus. Immunosuppressed individuals, either due to HIV infection or immunosuppressive drugs in solid organ transplant patients, are at higher risk than the general population (Damania, 2007).

Infectious mononucleosis

IM (also called "the kissing disease") occurs through the exchange of saliva containing EBV from

infected individuals. Most people are exposed to the virus during their childhood, which is asymptomatic. However, the infected person sheds the virus from time to time throughout life. Infection acquired in adolescents and young adults leads to the development of IM after 1–2 months of infection. The disease is characterized by malaise, lymphadenopathy, fever, and enlarged spleen and liver (Damania, 2007). The severity of disease often depends on age, with younger patients resolving the disease more quickly. Although IM is usually benign, there can be complications. These include neurological disorders such as meningitis, encephalitis, myelitis, and Guillain-Barrè syndrome (Damania and Pipas, 2009; Ann Arvin et al., 2007). Secondary infections, autoimmune hemolytic anemia, thrombocytopenia, agranulocytosis, and aplastic anemia may also occur. In IM, infected B-cells are transformed, which proliferates and activates the suppressor CD8 T-cells. These T-cells differ from normal T-cells in appearance and are known as Downey cells (Ann Arvin et al., 2007). This T-cell response results in enlarged lymph nodes as well as an enlarged liver and spleen. The activation of T-cells limits the proliferation of B-cells, and the disease resolves. Uncontrolled viral replication can lead to a severe syndrome with B-cell lymphoproliferation, leukopenia, and lymphoma. In patients with T-cell deficiency, X-linked lymphoproliferative (XLP) disorder may occur. Patients with HIV infection or postorgan transplant patients who are under immunosuppressive therapies are at high risk to develop lymphoproliferative disorder (Damania and Pipas, 2009).

X-linked lymphoproliferative disease

XLP syndrome is a rare immunodeficiency disease that is characterized by a susceptibility for fatal or near-fatal EBV–induced IM in childhood and a markedly increased risk for lymphoma or other LPDs (Damania and Pipas, 2009). There is a mutation on the X-chromosome that has been found to be associated with a T- and natural killer (NK)-cell lymphoproliferative disorder. The mutation is denoted as Xq25 on the long arm of the X chromosome. This mutation creates a deletion in the *SH2D1A* gene that codes for signaling lymphocytic activation molecule–associated protein (SAP). The SAP protein is important in the signaling events that activate T- and NK-cells and result in the modulation of IFN-γ. Persons with XLP disorder have an impaired immune response to EBV infection, which often leads to death from bone marrow failure, irreversible hepatitis, and malignant lymphoma (Damania and Pipas, 2009).

Research methods and protocols

EBV infection during childhood is usually asymptomatic; however, it establishes a lifelong latent infection (Damania and Pipas, 2009). EBV transforms peripheral human B-lymphocytes into indefinitely proliferating LCLs that allow for genetic manipulation of the virus. LCLs are generated by EBV transformation of the B-lymphocytes within the peripheral blood lymphocyte (PBL) population as shown in the flow chart **(**Fig. 7.3**)**: (1) Latently, EBV-infected cells are grown in culture. (2) These cells are treated with 1 mM sodium butyrate and 20 ng/mL of 12-*O*-tetradecanoylphorbol 13-acetate to induce reactivation. (3) Culture supernatant is collected and filtered through a 0.4-μM filter to harvest the secreted virus followed by ultracentrifugation to concentrate the virus. (4) Purified EBV is then used for infecting human peripheral blood mononuclear cells (PBMCs). (5) Transformed B-cells presenting EBV antigens are then selected. (6) The transformed B-cells are further treated with interleukin 2 for clonal expansion. (7) The selected EBV-LCLs are analyzed for specificity and cryopreserved for further research. These LCLs, derived from B-lymphocytes, are extensively used for in vitro research on gene expression studies and surrogate models to study genotype–phenotype relationships in humans.

Conventionally, the bacterial artificial chromosome (BAC) system is efficiently used to generate herpesvirus mutants by homologous recombination. In the BAC system, the entire EBV genome is cloned as a plasmid and propagated in *Escherichia coli*, and any mutation can be rapidly and precisely introduced into viral genes. The EBV genome was first cloned as a BAC from EBV strain B95-5 derived from IM (Enrique et al., 2010). The establishment of this system enabled studies on epithelial cell background as well as on virus infection on other B-lymphocytes like Ramos and Raji (Ann Arvin et al., 2007). These virus-producing cell lines provided the required tools to study the latent and lytic virus replication, virus production, and regulatory role of viral and cellular proteins in establishing virus latency, transformation, and tumorigenesis (Bellan et al., 2003). In vitro molecular biology methods that reveal gene expression levels in LCLs are fairly comparable with the naturally occurring gene expression in primary B-cells.

Turning point: modeling Epstein–Barr virus infection and pathogenesis

Mouse models are considered primary in vivo tools and play a central role in biomedical researches, including infectious diseases, to identify molecular

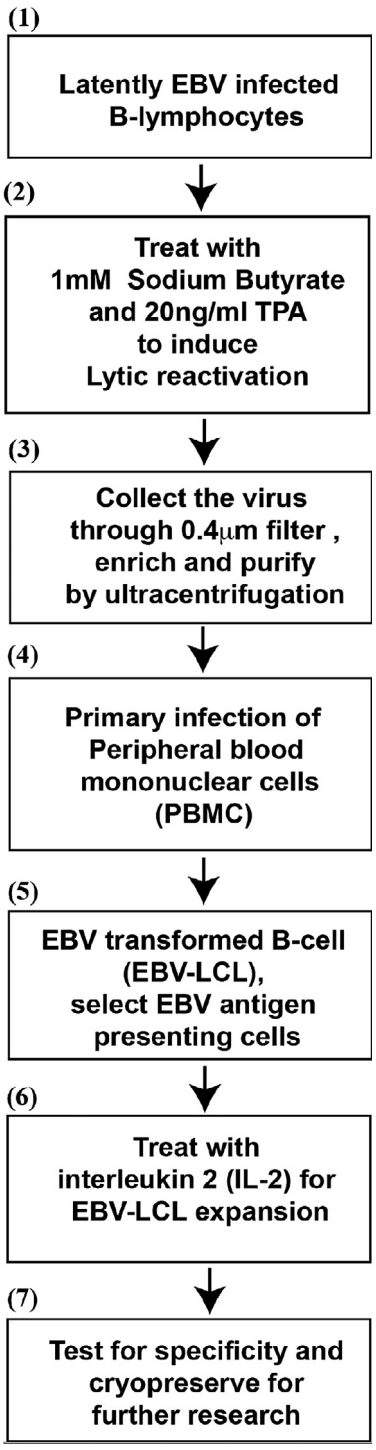

FIGURE 7.3 Flowchart of transformation by EBV.

targets and pathways implicated in tumor progression. In addition, mouse models help validate the efficacy and safety of antiviral therapies before they are tested in clinical trials.

A breakthrough in the generation of a highly immunodeficient mouse model of EBV infection was brought by the development of the *scid*-hu PBL mouse in 1988, based on the C.B-17 *scid* mouse. C.B-17 *scid* mice lack both B and T cells because of a mutation in the gene coding for a subunit of DNA-dependent protein kinase and do not have ability to reject human tissues and cells. Scid-hu PBL mice were prepared by intraperitoneal injection of PBMCs derived from healthy EBV carriers into C.B-17 *scid* mice, which resulted in the development of EBV-positive B-cell LPD. Analyses of the histology, marker expression, and EBV gene expression revealed LPD to be similar to the representative type of EBV-associated LPD in immunocompromised hosts. Scid-hu PBL mice were also used for modeling of HIV-1 and HTLV-1 infections (Enrique et al., 2010).

Since the *scid*-hu PBL mice lacked human immune responses to EBV, the new generation humanized mice were prepared based on novel immunodeficient mouse strains. Transplantation of human hematopoietic stem cells to highly immunodeficient mouse strains such as NOD/Shi-*scid* $Il2rg^{null}$ (Fujiwara et al., 2013) (Bouvard et al., 2009), BALB/c $Rag2^{-/-}Il2rg^{-/-}$, and NOD/LtSz-*scid* $Il2rg^{-/-}$ (NSG) resulted in the reconstitution of functional human immune system components, such as B cells, T cells, NK cells, dendritic cells, and macrophages. Thereafter, these new-generation humanized mice have been extensively used for studying the development and function of human immune system components in vivo and for modeling infections with EBV (Herrman et al., 2015), HIV-1, HTLV-1, dengue virus, HSV-2, and KSHV. In addition, immunodeficient mice carrying functional human hepatocytes were developed for modeling infections with HBV and HCV.

Current research perspectives

Understanding the different mechanisms by which DNA tumor viruses alter signal transduction pathways in malignant cells is important for detecting novel targets for cancer therapies. In addition to viral factors, host factors, genetic makeup, and geographical and environmental factors have also been found to interfere with the normal activities of signaling pathways leading to virus-induced cancers (Abhik Saha et al., 2010). Conventionally, for patients with AIDS-KS, highly active antiretroviral therapy is effectively used to reduce HIV infection together with up to a 90% reduction in KS occurrence (Damania, 2007). Furthermore, antiviral treatment, including acyclovir, ganciclovir, cidofovir, and foscarnet, has been shown to be moderately effective against EBV- and KSHV-associated infections in the lytic phase (Cai et al., 2010; Enrique et al., 2010). Although our knowledge on viral oncoproteins and their associated cellular factors that

are involved in carcinogenesis has greatly advanced, we are still lacking specific drugs that inhibit these viral proteins (Cai et al., 2010; Enrique et al., 2010). Today, most antitumor therapies against virus-induced cancers target cellular proteins that have a role in these processes rather than the viral proteins. Several companies have efforts underway to develop small molecule drugs that target host proteins involved in cell-cycle regulation, inflammatory response, proteasome, and signal transduction pathways (Damania, 2007). Small molecule drugs such as nutlin-3a that target the p53-Mdm2 interaction and bortezomib (Velcade), a proteasome inhibitor, can be used against EBV as well as KSHV-mediated cancer cell lines. Bortezomib has been shown to inhibit proliferation and to induce apoptosis in KSHV-infected PEL cells (Abhik Saha et al., 2010). In addition, PI3K inhibitor (LY294002), mTOR inhibitor (rapamycin), and interleukin-12 that negatively regulate the viral G-protein-coupled receptor pathway have shown promising results against KS (Abhik Saha et al., 2010). Other strategies like inhibition of matrix metalloproteinases and inhibitors for vascular endothelial growth factor are also found to be effective against KS and HBV-associated HCC (Abhik Saha et al., 2010). Interestingly, natural phenolic compounds like resveratrol (found in plants such as grapes) have been shown to be potent antiviral compounds against numerous viruses, including EBV, HSV, HIV, and influenza (Abhik Saha et al., 2010). The use of molecular drugs against cellular factors very often creates many undesirable off-target effects. Thus, developing vaccines or therapeutic drugs that specifically target DNA tumor viral proteins will be essential for effective protection against the virus with reduced drug cytotoxicity (Abhik Saha et al., 2010). Currently, promising vaccines are available only against HPV and HBV (Damania, 2007), and there are no vaccines available against EBV, KSHV, and human polyomaviruses (Lowy and Schiller, 2006; Chang, 2009). Significant efforts have been made to develop vaccines against EBV infection, and the current EBV vaccine development is focused on the most abundant envelope protein, EBV gp350, which is the major target of neutralizing antibodies in human sera (Moore and Chang, 2010; Damania, 2007). In a phase 2 trial, a gp350-based vaccine significantly reduced the incidence of IM but failed to decrease the overall infection rate. Other therapeutic vaccines have also targeted the LMP2 and EBNA-1 viral proteins. Efforts to develop KSHV vaccine are limited, but mouse infection with murine gammaherpesvirus-68 has been exploited as an experimental model for an effective vaccination strategy against the long-term viral latency. A vaccine to increase the immune control of KSHV lytic replication and to decrease the KSHV viral load in people already infected may reduce the risk of KSHV infection. For a therapeutic vaccine, incorporation of epitopes derived from KSHV latent proteins, LANA and Kaposin, will likely increase its efficacy (Herrman et al., 2015).

Ethical issues

EBV infection and tumorigenesis are a complex processes and heavily affected by genetic constitution, socioeconomic background, geographical locations, and subpopulation levels (Damania and Pipas, 2009). Generally, the prognosis and selection of the most appropriate treatment are assessed using both patient-related and standard tumor-related characteristics. The high mortality rate among patients with EBV-associated tumors is partly due to delays in diagnosis that result from the complexity of its initial clinical presentation. EBV-specific qPCR is mostly employed to detect EBV from infected PBLs or biopsy samples (Robertson, 2005). A better understanding of viral gene expression in the context of EBV infections from diverse populations will prove useful in diagnosis and treatment of EBV-associated malignancies. Clinical research on patient samples or animal models involves an array of ethical issues and should be in accordance with World Health Organization criteria. According to this, all clinical research involving patient samples should be approved and continuously monitored by a university or institutional ethical committee. In addition, EBV has strict host specificity, that is, it infects only humans; therefore, it is not practical to study the dynamics of protein expression during EBV infection and pathogenesis. However, an alternative method has been developed to study in vivo infection of EBV by generating a murine model known as "humanized mice." These mice have the potential to maintain human hematopoiesis, including human CD4 + leukocytes that can thereby support persistent EBV infection in vivo. Similar to clinical research, studies involving animal models also require the approval from the institutional ethical committee. Above all, participating patients or their guardians should provide informed consent according to the institutional guidelines. Institutional ethical committees annually review the protocols and progress made in clinical research and have the authority to disapprove of a study if it is deviating from the original guidelines (Robertson, 2005).

Translational significance

The effective early detection of tumor as well as efficient treatment methods could greatly reduce the incidence and mortality of EBV-associated cancers. Currently, there is no effective vaccine available to

prevent EBV infection. Newer formulations of phase 2 trial EBV gp350 that include additional glycoproteins, viral latent, and lytic proteins might improve the efficacy of the currently known EBVgp350 vaccine, making it a promising vaccine candidate against EBV-induced malignancies. Thus, a better understanding of mechanisms of oncogenesis by EBV is important for identifying novel therapeutic targets for the success of EBV-mediated cancer treatment and increase of patient's quality of life.

World Wide Web resources

http://www.virology.net/

All the virology on the worldwide web seeks to be the best single site for virology information on the Internet. It has a collection of all the virology-related web sites that might be of interest to our fellow virologists and others interested in learning more about viruses.

http://www.microbe.tv/twiv/

TWiV (This Week in Virology): This site is an independent podcast network for people who are interested in latest discoveries in the field of science, viruses, and microbes.

https://www.nature.com/subjects/virology

It is the world's foremost international weekly scientific journal that publishes peer-reviewed state-of-art research in all fields including virology.

http://www.ncbi.nlm.nih.gov/books

NCBI Bookshelf provides free access to books and documents in life sciences and healthcare. A vital node in the data-rich resource network at NCBI Bookshelf enables users to easily browse, retrieve, and read content, and spurs discovery of related information.

http://www.ncbi.nlm.nih.gov/pubmed/

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Glossary

Cytopathic effect Refers to degenerative changes in cells, especially in tissue culture.
Deregulation The act of freeing from regulation.
Hit-and-run viruses Viruses that can initiate cancers or play a role in their development, but then can disappear from host.
Immunesurveillance A process in which immune system continually recognizes and removes malignant cells.
Intravasation The invasion of cancer cells through the basal membrane into a blood or lymphatic vessel.
Knockdown Gene knockdown refers to techniques by which the expression of one or more of an organism's genes is reduced.
Oncogene A gene that in certain circumstances transforms a cell into a tumor cell.
Onco proteins A gene product that causes the transformation of normal cells into cancerous tumor cells.
Secondary tumor Metastasis is one of the hallmarks of malignancy. This new tumor is known as a metastatic or secondary tumor.
Oncogenesis The process of tumor formation/induction.

Abbreviations

AIDS	Acquired immunodeficiency syndrome
BAC	Bacterial artificial chromosome
BCR	B-cell receptor
BL	Burkitt's lLymphoma
CNS	Central nervous system
EBER	EBV-encoded small RNA
EBNA	Epstein–Barr nuclear antigen
EBV	Epstein–Barr virus
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
EV	Epidermodysplasia verruciformis
HAX-1	HCLS1-associated protein X-1
HBV	Hepatitis B virus
HBeAg	Hepatitis B early antigen
HBsAg	Hepatitis B surface antigen
HCC	Human hepatocellular carcinoma
HCV	Hepatitis C virus
HHV-4	Human herpesvirus-4
HHV-8	Human herpesvirus-8
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
HPyV	Human polyomavirus
HRS	Hodgkin/Reed-Sternberg
HSC	Hematopoietic cells
HSV-2	Herpes simplex virus-2
HTLV-1	Human T-cell lymphotrophic virus
IFA	Immunofluorescence assay
IL-6	Interleukin 6
IL-10	Interleukin 10
IM	Infectious mononucleosis
IRF	Interferon regulatory factors
ITAM	Immunoreceptor tyrosine-based activation motif
JAK/STAT	Janus kinase-signal transducer and activator of transcription
JNK	c-Jun *N*-terminal kinase
KICS	KSHV-inflammatory cytokine syndrome
KS	Kaposi's sarcoma
KSHV	Kaposi's sarcoma–associated herpesvirus
LANA	Latency-associated nuclear antigen
LCL	Lymphoblastoid cell lines
LMP1	Latent membrane protein1
LPD	Lymphoproliferative disease
MAPK	Mitogen-activated protein kinases
MCC	Merkel cell carcinoma
MCD	Multicentric Castleman's disease
MCPyV	Merkel cell polyomavirus
MDM2	Mouse double minute 2 homolog
miRs	microRNAs
MTCT	Mother-to-child transmission
NPC	Nasopharyngeal cancer
ORFs	Open reading frames
PBMCs	Perinuclear blood mononuclear cells
PEL	Primary effusion lymphoma
PKR	Protein kinase RNA dependent
PML	Progressive multifocal leukoencephalopathy
PVN	Polyomavirus nephropathy
qPCR	Quantitative polymerase chain reaction
SAP	Signaling lymphocytic activation molecule–associated protein
TLR	Toll-like receptors
TNF	Tumor necrosis factor
vFLIP	Viral Fas-associated death domain-like interleukin-1β-converting enzyme-inhibitory protein
vIRF3	Viral interferon regulatory factor-3
XLP	X-linked lymphoproliferative
ZREs	ZEBRA response elements

Long answer questions

1. What is virus transformation? How do DNA tumor viruses transform cells?

2. How small DNA tumor viruses differ from large DNA tumor viruses?
3. Which are the different DNA tumor viruses infecting humans?
4. What are viral oncoproteins? How do they contribute to tumorigenesis?
5. A person with AIDS is diagnosed with acute B-cell lymphoma, what could be the causative agent and why?

Short answer questions

1. Which of the DNA tumor viruses can cause B-cell lymphoma in humans?
2. Which tumor virus has been associated with cervical carcinomas?
3. What is the necessary prerequisite for defining an infection as latent?
4. Which DNA tumor virus has been associated with hepatocellular carcinomas?
5. Why excessive exposure to sun can cause Merkel cell carcinoma?

Answers to short answer questions

1. DNA tumor virus belongs to the family gammaherpesvirus, Epstein–Barr virus (EBV) and Kaposi's sarcoma–associated herpesvirus (KSHV) primarily causes B-cell lymphoma in humans.
2. Infection by human papilloma virus is the most common sexually transmitted disease and can lead to the development of cervical cancer.
3. During latency, the virus remains dormant and persists in a highly ordered chromatin state as episome, whose propagation is dependent on their ability to hijack the replicative machinery of their host. During latency, virus encodes only a few latency-associated proteins that are known to be tumorigenic. Among DNA tumor viruses, latency is well studied on herpesvirus family members, EBV and KSHV.
4. HBV, a member of the Hepadnavirus family, causes an infectious inflammatory disease of the liver. Patients with chronic hepatitis B are at high risk of developing hepatocellular carcinoma.
5. Merkel cell carcinoma (MCC) occurs most often on the sun-exposed face, head, and neck. MCPyV is the first polyomavirus, which has been shown to integrate into human genomic DNA. Virus then undergoes two sequential mutational events resulting in persistent T-Ag expression, which plays a key role in turning asymptomatic viral infection into an aggressive Merkel cell carcinoma. Excessive exposure to sun can induce these mutational events leading to MCC.

Yes/no type questions

1. Do virus-induced tumors retain part of the viral genome?
2. Is HCV a RNA tumor virus?
3. Is coronavirus associated with cancer?
4. Can latent infections persist in an individual without causing any symptoms?
5. Is integration of viral genes required for cell transformation in Burkitt's lymphoma?
6. Does HPV belong to the gammaherpesvirus subfamily?
7. The Nobel Prize in Physiology or Medicine was granted to Harald zur Hausen for his discovery of HBV causing cervical cancer?
8. Is KSHV associated with nasopharyngeal carcinoma?
9. Are the tumor viruses primarily subclassified according to the type of life cycle in the infected host cell?
10. Are the viruses extremely specific to the host and type of cells they infect?

Answers to yes/no type questions

1. Yes
2. Yes
3. No—They are believed to cause common colds in human adults.
4. Yes
5. No
6. No—Only EBV and KSHV does.
7. No—He was awarded the Nobel Prize for discovering the association of HPV with cervical cancer.
8. No—EBV is known to cause nasopharyngeal carcinoma.
9. No—They are classified on the basis of their nucleic acid.
10. Yes.