

Chapter 1

Oncogenic Viruses, Cellular Transformation and Human Cancers

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Abstract: It has been a century since the initial discovery of the possible link between viruses and tumors. During the past century, extensive studies have been conducted to understand the relationship between viruses and cancers. The early studies were focused on tumor viruses that do not cause cancers in their natural hosts. These studies provided the basis for the subsequent studies on the six known human oncogenic viruses. These human oncogenic viruses, which include hepatitis B virus, hepatitis C virus, human papillomavirus, Epstein-Barr virus, Kaposi's sarcoma-associated herpesvirus, and human T-cell leukemia virus 1 are associated with nearly 20% of the human cancer cases. In this chapter, we review the historical aspects of oncogenic virus research, human oncogenic viruses, and the molecular mechanisms of cellular transformation by viruses.

1. Introduction and Historical Aspects

Cancer develops from a succession of genetic changes, which cumulatively provide growth advantage for the cancerous cells. Despite the heterogeneity of human cancers in cellular origin, etiology and pathogenesis, they more or less share the same characteristics defined by Hanahan and Weinberg (Hanahan & Weinberg, 2000): self-sufficiency

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in growth stimulation, insensitivity to antigrowth signals, evasion of apoptosis, infinite lifespan, sustained angiogenesis, tissue invasion and metastasis ability, and genetic instability (Hanahan & Weinberg, 2000). These cellular changes may be inherited genetically or acquired as a combinational aftermath of environmental mutagens and infectious agents such as viruses.

The initial observation that viruses may cause cancer was made in 1908 when Vilhelm Ellerman and Oluf Bang found that cell-free filtrates could transfer leukemia from one chicken to another (Ellerman & Bang, 1908). This observation was reinforced in 1911 when Peyton Rous demonstrated that healthy chickens injected with cell-free sarcoma filtrates, in which only viruses could be present, developed sarcoma (Peyton, 1911). It is now clear that the causative infectious agent in chicken leukemia and Rous sarcoma are both retroviruses, with the former named avian leukosis virus and the latter named Rous sarcoma virus. That viruses can induce tumors were reaffirmed in the 1950s and 1960s, when three additional DNA viruses, simian vacuolating virus 40 (SV40), mouse polyomavirus and adenovirus, were found to cause tumors in newborn rodents (Girardi *et al.*, 1962; Stewart *et al.*, 1958; Trentin *et al.*, 1962). These three different viruses, however, did not cause tumors in their natural hosts.

The early research on retroviruses and DNA tumor viruses, which include the aforementioned SV40, mouse polyomavirus and adenovirus, has generated much valuable information for understanding viral tumorigenesis and cancer biology. The discovery of transducing retroviruses, which carry an oncogene (*v-onc*) in their genome, led to the discovery of cellular homologues (*c-onc*) of these viral oncogenes (Stehelin *et al.*, 1976). Retroviruses are now known to induce tumors through three different mechanisms. The first mechanism involves transducing retroviruses. These viruses induce tumors at a high frequency and requires a short latency period. The second mechanism involves *cis*-acting retroviruses. These *cis*-acting retroviruses do not carry a cellular oncogene. However, they can activate cellular oncogenes through the insertion of the provirus into the cellular chromosomes (Hayward *et al.*, 1981). This mechanism induces tumors at an intermediate frequency and requires a longer latency

period. The third mechanism involves *trans*-acting retroviruses. These viruses encode regulatory proteins to affect cell growth and death. These viruses induce tumors at a low frequency and often require a long latency period.

The research on DNA tumor viruses also led to a better understanding of viral oncogenesis and facilitated the later research on human oncogenic viruses. SV40 and mouse polyomavirus, which both belong to the *polyomaviridae* family, encode early gene products that are capable of causing tumors. These gene products were thus termed tumor (T) antigens. The large T antigen of SV40 and mouse polyomavirus is a multifunctional protein that can immortalize primary cells, bind to the tumor suppressor p53 to different degrees and to the retinoblastoma protein RB, and activate gene expressions to promote cell cycle progression (Conzen & Cole, 1995; Imperiale & Major, 2006). The mouse polyomavirus middle T-antigen, which is absent in SV40, possesses additional activities (Ichaso & Dilworth, 2001). This middle T-antigen can bind to and constitutively activate *c-src*, an important tyrosine kinase that activate cellular signaling pathways, and can also serve as a substrate of *c-src* to activate phosphotyrosine-binding proteins such as phosphoinositol-3-kinase and phospholipase C- γ (Dilworth, 2002). SV40 and mouse polyomavirus also produce a small t-antigen that can enhance the transformation activity of the virus (Khalili *et al.*, 2008). Similarly, the research on adenovirus led to the finding that its E1A and E1B proteins have oncogenic potential. These two proteins can bind to RB and p53, respectively. Of particular interest is the highly oncogenic Ad12 adenovirus strain. The E1A protein of this particular adenovirus strain can suppress the expression of the major histocompatibility (MHC) class I antigen and inhibit the cytotoxic T lymphocyte (CTL) response (Schrier *et al.*, 1983; Vasavada *et al.*, 1986). This indicates that the modulation of the immune system is also important for viral oncogenesis.

Despite the research progresses on animal tumor viruses, conclusive evidence on a similar viral etiology in human malignancies was not available until 1964, when Epstein-Barr virus (EBV) was identified in B-cell lines derived from Burkitt's lymphoma samples

(Epstein *et al.*, 1964). The causal relationship between a viral infection and human cancer has always been difficult to determine due to many reasons: the long incubation period between the infection and the onset of tumors; relative rarity of cancer cases among the virus-infected population; requirement of other potent and non-viral related cofactors during oncogenesis; and inconsistent oncogenic potentials among different serological strains of the same virus (Henderson, 1989). To date nearly 20% of human cancer cases worldwide can be attributed to viral infections (Farrell, 2002). Human oncogenic viruses consist of both RNA viruses and DNA viruses and encompass several different taxonomic groups. Human oncogenic RNA viruses that include hepatitis C virus (HCV) and human T-cell leukemia virus 1 (HTLV-1; also known as human T-lymphotropic virus 1), and human oncogenic DNA viruses that include hepatitis B virus (HBV), human papilloma virus (HPV), Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV). The possibility that polyomaviruses may also cause human cancers has also been raised (Khalili *et al.*, 2003), although this topic remains highly controversial (Feng *et al.*, 2008; Lowe *et al.*, 2007; Poulin & DeCaprio, 2006). Recently, Merkel cell carcinoma, a rare skin cancer, was found to contain DNA from a previously unknown polyomavirus (Feng *et al.*, 2008). The DNA of this polyomavirus, which has since been named Merkel cell polyomavirus, is found integrated in the chromosomes of 80% of Merkel cell carcinoma tissues (Feng *et al.*, 2008), indicating that this virus may play a role in Merkel cell carcinogenesis. Further research, however, will be required to confirm this possibility.

For all the human oncogenic viruses, oncogenic transformation is not the prerequisite for the production of viral progeny. Neither is the productive viral replication required for cellular transformation. In addition, the fact that only a small fraction of the virus-infected population develops malignancy after a long-latency period indicates that viral infection may only contribute to some of the steps of carcinogenesis. In the following sections, we will briefly discuss these human oncogenic viruses. Details of these viruses will be separately discussed in the chapters that follow.

2. Human Oncogenic Viruses

2.1. Hepatitis B Virus (HBV)

In 1965, Baruch S. Blumberg discovered the Australia antigen, later known as the HBV surface antigen (HBsAg), when working with the serum from an Australian aborigine (Blumberg *et al.*, 1965). He soon recognized its relationship (Blumberg *et al.*, 1967) with hepatitis and developed a serological test for it (Melartin & Blumberg, 1966). However, HBV was not identified until 1970 (Dane *et al.*, 1970) and the viral genome was sequenced in 1979 (Galibert *et al.*, 1979).

Classified as a member of the hepadnavirus family, HBV is a DNA virus with a partially double-stranded circular genome about 3.2 Kb in length. Four genes named S, C, P and X genes are encoded by the viral genome. The S gene codes for the surface (envelope) proteins, the C gene codes for the serum e antigen and the core protein that forms the viral core particle, the P gene codes for the viral DNA polymerase, which is also a reverse transcriptase, and the X gene codes for a 16.5 kDa regulatory protein. After infection, the HBV genome is transported to the nucleus of infected cells where it directs the synthesis of viral messenger RNAs. Although HBV DNA is frequently detected in the chromosomes of infected cells, this DNA integration is not an essential step of the viral life cycle.

It is estimated that over 2 billion people worldwide have been exposed to HBV, and approximately 350 million people are chronically infected by this virus (Kane, 1995). HBV infection is primarily through skin puncture and mucous membranes. In West Europe and North America, high-risk sexual activities and intravenous drug abuse are the major transmission pathways for HBV. However, in the developing countries in Asia and Africa where HBV is endemic, vertical transmission from infected mothers to their children is the major cause of HBV infection (Alter, 2003; Milich *et al.*, 1990; Stevens, 1994).

Most of the acutely infected adult HBV patients clear the viral infection, but 5–10% of them fail to clear the virus and become chronically infected (Aldershvile *et al.*, 1980; Fattovich, 2003). This rate of chronic infection becomes higher for younger patient with about 90%

of the babies born to HBV-positive mothers becoming chronic carriers (Stevens *et al.*, 1975).

Chronic HBV infection is associated with multiple liver diseases, including hepatitis, liver cirrhosis and hepatocellular carcinoma (HCC). A causal relationship between HBV infection and HCC was first suggested by several large-scale epidemiology studies. Geographic distributions of the prevalence of HBV infection and incidence of HCC are strikingly similar. HBV carriers are over 200-fold more likely to develop HCC than non-carriers (Beasley, 1988; Beasley *et al.*, 1981; Szmunes, 1978). Since the universal immunization program against HBV was launched, the incidence of pediatric HCC in Taiwan has significantly declined, further indicating a causal role of HBV in the development of HCC (Chang *et al.*, 1997).

2.2. Hepatitis C Virus (HCV)

The major infectious agent for post-transfusion non-A, non-B hepatitis (NANBH) was isolated in 1989 via screening of the cDNA expression library prepared from plasma containing the NANBH agent, the antiserum of a NANBH patient being used (Choo *et al.*, 1989). This infectious agent was named HCV. HCV belongs to the *Hepacivirus* genus in the *Flaviviridae* family. It has a single, positive-stranded RNA genome with a length of approximately 9600 nucleotides. The HCV genome contains a long ORF and is translated by a cap-independent mechanism using a highly conserved internal ribosomal entry site (IRES) located near its 5'-end. The HCV polyprotein synthesized is cleaved by cellular and viral proteases to generate 10 mature viral protein products. The core protein and the E1 and E2 envelope proteins are located at the N-terminus of the polyprotein sequence. These structural proteins are followed by seven non-structural proteins, referred to as p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B. In addition to the polyprotein, an F protein coding sequence overlaps with the core protein coding sequence in the +1 reading frame (Walewski *et al.*, 2001; Xu *et al.*, 2001). This protein is synthesized by translational ribosomal frameshift (Choi *et al.*, 2003; Xu *et al.*, 2001), although other expression mechanisms, including internal initiation of translation have also

been proposed (Baril & Brakier-Gingras, 2005; Fiorucci *et al.*, 2007; Vassilaki & Mavromara, 2003).

HCV is transmitted by the parenteral route. Most patients infected by HCV fail to resolve the infection and become chronic carriers of the virus (Jin, 2007). There are an estimated 170 million HCV carriers in the world. HCV can cause severe liver diseases, including hepatitis, steatosis, and liver cirrhosis and it is the leading cause of liver transplantation in the U.S. The causal relationship between HCV and HCC was first reported in 1990 (Kiyosawa *et al.*, 1990). In addition to causing liver diseases, HCV can also cause several hematopoietic disorders, including mixed cryoglobulinemia and non-Hodgkin's disease.

2.3. Human Papilloma Virus (HPV)

Papilloma virus, as its name indicates, causes papillomas or warts in higher vertebrates. The first papillomavirus was identified in 1932 by Richard Shope who demonstrated the induction of papilloma in cottontail rabbits by a viral agent (Shope, 1932). This viral agent, now named cottontail rabbit papillomavirus, was the first DNA tumor virus identified. To date, more than 130 strains of papilloma viruses have been identified (Damania, 2007). The association of HPV with cervical carcinoma was recognized in the 1980s when HPV-16 and HPV-18 DNA were detected in cervical carcinoma tissues (Boshart *et al.*, 1984; Durst *et al.*, 1983).

Whilst most HPV infections only result in benign lesions and rarely progress into malignancies, HPV DNA is detected in more than 95% of all cervical tumors (Hebner & Laimins, 2006) and HPV infection is widely accepted as the cause of cervical cancer, the second most common cancer among women worldwide (Woodman *et al.*, 2007). Additional human mucosal and epithelial lesions associated with HPV infection range from skin or genital warts, laryngeal papillomatosis, condylomata acuminata, to squamous carcinomas of the skin and head and neck (Sisk & Robertson, 2002). Based on their oncogenic capability, papillomaviruses are divided into high-risk and low-risk groups. The former consists of HPV-16, 18, 31, 33, 35, 39, 45, 51,

52, 56, 58, 59 and 66, whereas the latter group includes HPV-6 and 11 (Bosch *et al.*, 2002).

HPV belongs to the *Papillomaviridae* family. It is a small non-enveloped virus about 55 nm in diameter. HPV infection is highly species/tissue specific, infecting only the cutaneous and mucosal epithelia of the anogenital tract or upper respiratory tract (Sisk & Robertson, 2002). Transmission of HPV is believed to be through minor abrasions of the epithelium which expose cells in the basal layer for viral entry. Although heparin sulfate is suggested to be the mediator for the initial viral entry, the identity of the HPV receptor is still unknown (Longworth & Laimins, 2004). Primary HPV infection always begins in the cells of the basal layer of squamous epithelium, where the virus maintains its genome as low-copy episomal DNA. HPV late gene expression and virion production only occur in the nucleus of terminally differentiated keratinocytes. Hence the vegetative HPV DNA production is tightly controlled by keratinocyte differentiation.

The circular double-stranded DNA genome of HPV, about 8 kb in size, is composed of three regions: a non-coding upstream regulatory region (URR) containing transcription promoters and DNA replication elements; an early region encoding six proteins (E1, E2, E4, E5, E6 and E7); and a late region encoding two capsid proteins (L1 and L2). Most HPV genes are transcribed as polycistronic mRNAs from a single DNA strand and the precise identity of the mRNAs for each ORF has not been fully established. HPV uses alternate RNA splicing and alternative RNA polyadenylation to ensure the proper expression of all open reading frames (ORFs) from a compact genome (Zheng & Baker, 2006).

2.4. Epstein-Barr Virus (EBV)

Epstein-Barr virus, named after its discoverers Michael Epstein and Yvonne Barr, was first observed by electron microscopy in B-cell lymphoma cell lines in 1964 (Epstein *et al.*, 1964). Taxonomically, EBV belongs to the *Gammaherpesvirinae* subfamily and the *Herpesviridae* family.

EBV is an enveloped virus with a double-stranded DNA genome about 172 Kb in size. It is also the first large DNA virus whose whole genome has been determined by sequencing (Baer *et al.*, 1984). EBV is orally transmitted and preferentially infects B cells and epithelial cells. This virus affects more than 90% of the population worldwide (Boccardo & Villa, 2007; Kuppers, 2003). Primary EBV infection usually occurs during childhood. The infection remains asymptomatic and the infected individual becomes an EBV carrier for life. In contrast, primary EBV infection during adolescence or young adulthood results in a self-limiting disease, called infectious mononucleosis (IM), in about 30–50% the infected individuals. In some cases, the EBV infection can lead to malignancies including both carcinomas and lymphomas. The former includes gastric carcinoma and nasopharyngeal carcinoma (NPC), and the latter ranges from Burkitt's lymphoma (BL), a subset of Hodgkin's disease (HD), immunoblastic lymphoma to T-cell lymphoma. Most of our current knowledge of EBV comes from studies on lymphoblastoid cell lines (LCLs) derived from *in vitro* infection of primary B-lymphocytes with EBV.

Like other members in the herpesvirus family, EBV may cause either lytic infection or latent infection. Lytic infection can occur in both B cells and epithelial cells within the tongue and oropharynx, whereas latent infection is largely limited to B cells. During the lytic infection, EBV expresses the entire genome, amplifies its DNA, produces high titer of progeny viral particles to initiate a new round of infection, and eventually kills the host cell. During the latent infection, the EBV genome is maintained as an episomal DNA and partially expressed. Since all lytically infected cells die, only latent infection is associated with cellular transformation.

2.5. Kaposi's Sarcoma-associated Herpesvirus (KSHV)

KHSV, also called human herpesvirus 8 (HHV-8), is also a gamma herpes virus. It was first identified in 1994 from Kaposi's

sarcoma samples using representational differential analysis (Chang *et al.*, 1994). The KSHV genome is a double-stranded DNA with a size of about 160 Kb that contains over 80 protein coding sequences. Similar to other herpesviruses, KSHV can cause either lytic infection or latent infection. During latency, the viral genome replicates as a closed circular episome in the nucleus; only a subset of viral genes are expressed and no progeny viral particles are produced. During lytic infection, the viral genome is linearized, nearly the entire genome is expressed, and progeny virions are produced and released upon the death of the host cell. The virus-encoded replication and transcription activator (RTA) protein plays an important role in inducing lytic replication from latency (Sun *et al.*, 1998).

The transmission route of KSHV is largely unknown. Sexual and other parenteral transmission pathways have been suggested. KSHV infection is not as common as the other herpesviral infections. Epidemiological studies have shown great geographic variations of the KSHV prevalence rate. Low level infections (0–20%) occur in North America and northern Europe, intermediate level infections (10–25%) are observed in Mediterranean countries; and high seropositivity rates (>30%) are detected in Africa (Jarrett, 2001). Most KSHV infections are asymptomatic. However, co-infection by human immunodeficiency virus-1 can greatly increase the risk for Kaposi's sarcoma (KS), which is the leading cause of AIDS-associated morbidity and mortality (Ganem, 2006).

KS is a multifocal angioproliferative disorder that often presents as a cutaneous lesion. It is estimated that greater than 95% of Kaposi's sarcoma lesions are positive for KSHV DNA, indicating an association between Kaposi's sarcoma and KSHV infection. Besides Kaposi's sarcoma, primary effusion lymphoma (PEL) and multicentric Castleman's diseases (MCD), which are two types of B-cell lymphomas, are also associated with KSHV infection (Sullivan *et al.*, 2006). KSHV DNA is present in all PEL lesions, which may contain EBV as well. Nearly 100% of AIDS-associated MCD and 50% of non-AIDS-associated MCD are positive for KSHV DNA (Damania, 2007).

2.6. Human T-cell Leukemia Virus-1 (HTLV-1)

HTLV-1 was first discovered in 1980 from patients suffering from a disease at that time thought to be mycosis fungoides, a cutaneous T-cell lymphoma (Poiesz *et al.*, 1980). It is the only human oncogenic retrovirus known at present.

HTLV-1 is a member of the *deltaretrovirus* genus of the *Retroviridae* family and is closely related to HTLV-II, bovine leukemia virus and simian T-cell leukemia virus. The single-stranded RNA genome of HTLV-1 is composed of about 9000 nucleotides and contains several genes, including *env*, *gag*, *pol* and *tax* as well as two long terminal repeats (LTRs). HTLV-1 does not harbor any oncogenes derived from the host cells and thus is not a transducing retrovirus.

HTLV-1 infection is endemic in southern Japan, West Africa, the Caribbean Islands and South America, but rare in North America and Europe. About 10 to 20 million people worldwide have been infected by HTLV-1 (Pagano *et al.*, 2004). HTLV-1 is transmitted either directly from mother to infant, or through sexual contact and other parenteral routes. The ubiquitously expressed glucose transporter 1 protein (GLUT1) and heparin proteoglycans have been suggested to be the viral receptors (Manel *et al.*, 2003; Okuma *et al.*, 2003). Interestingly, this virus cannot be transmitted as free virions and it appears that cell-to-cell contact is required for HTLV-1 transmission.

HTLV-1 infection is causally associated with adult T-cell leukemia (ATL), a human malignancy with poor prognosis. Integrated proviral DNA are found in the majority of ATL and seropositivity is high among ATL patients. It has been estimated that the cumulative lifetime risk of ATL among HTLV-1 carriers is about 1–4.5% (Levine *et al.*, 1998).

3. Mechanisms of Virus-induced Cellular Transformation

The oncogenic mechanisms used by different viruses differ significantly. We will summarize some of the common mechanisms in this section and the more detailed descriptions of the mechanisms

for each human oncogenic virus can be found in the chapters that follow.

3.1. Perturbation of Signaling Pathways

The behaviors of cells are subjected to regulations by external stimuli. These stimuli are transmitted inside the cell often through specific receptors either on the cell surface or inside the cell. These stimuli are then further passed, integrated, furcated and interpreted by intracellular signaling networks for cell growth, death, differentiation or self-renewal. Human oncogenic viruses have evolved ways to perturb these signal pathways to result in cellular transformation.

3.1.1. *Mimicking the signaling ligands*

IL-6, a pro-proliferative, pro-angiogenic and pro-inflammatory cytokine, plays important roles in many human malignancies, especially those with a B-cell origin, in a paracrine or autocrine manner. KSHV encodes an IL-6 analog (vIL-6). This vIL-6 has about 25% sequence homology with cellular IL-6 (cIL-6) and can functionally substitute for cIL-6 to support the proliferation of IL6-dependent B9 cell proliferation (Moore *et al.*, 1996). Downstream signaling pathways activated by vIL-6 include JAK/STAT and MAP kinase pathways (Nicholas, 2007; Sullivan *et al.*, 2006). In contrast to IL-6, activation by vIL-6 requires only the gp130 cellular receptor and not the gp80 receptor (Chen & Nicholas, 2006). vIL-6 transforms NIH3T3 cells *in vitro*, which form tumor in mice with a high degree of vascularization (Aoki *et al.*, 1999). Increased level of VEGF production stimulated by vIL-6 may be the key player contributing to the angiogenesis and cell proliferation seen in PEL (Aoki *et al.*, 1999; Aoki & Tosato, 1999).

In addition to vIL-6, KSHV also expresses three macrophage inhibitory proteins, vCCL1/2/3 (also called vMIP-1/2/3). These cytokines can bind to the endogenous receptors of MIP, which is postulated to skew host immune response away from Th1 type and thus important for KSHV to evade host immune surveillance

(Nicholas, 2007). vCCLs play active roles during oncogenesis, largely due to their pro-angiogenesis, pro-proliferation and pro-survival activities. For instance, vCCL1 induces the expression of VEGF and promote cell survival in PEL cell lines (Liu *et al.*, 2001).

3.1.2. *Mimicking the cellular signaling receptors*

The EBV LMP1 protein is an integral membrane protein constitutively activating intracellular signaling pathways, mimicking that of activated CD40, a member of the tumor necrosis factor receptor (TNFR) superfamily. Functionally, LMP1 is able to partially replace CD40 *in vivo* to stimulate B cells with activation and differentiation signals (Uchida *et al.*, 1999). LMP1 is universally expressed in several EBV-associated diseases, including Hodgkin's disease, nasopharyngeal carcinoma and immunoblastic lymphoma (Young, 2001). Recombinant EBV deficient in LMP1 is unable to transform B cells (Cahir McFarland *et al.*, 1999). The cytoplasmic region of LMP1 contains two domains referred to as C-terminal activation region 1 and 2 (CTAR1 and CTAR2), which interact with TARFs and TRADDs, respectively, for the activation of NF- κ B signaling pathways in both B-cell and epithelia cells (Eliopoulos *et al.*, 1999a). Other intracellular events activated by LMP1 include the JNK/p38 (Eliopoulos *et al.*, 1999b), MAP kinase and phosphatidylinositol 3 kinase (PI3K) pathways, which are important for regulating cellular apoptosis and transformation. Other pleiotropic effects of LMP1 include upregulation of anti-apoptotic signals, induction of cell adhesion molecules and cytokines, and growth inhibition (Eliopoulos *et al.*, 1996). It has been shown that A20, Bcl-2 (Kenney *et al.*, 1998), ICAM-1, interferon regulatory factor 7 (IRF-7) (Zhang & Pagano, 2000), matrix metalloproteinase-9 (MMP-9) (Takeshita *et al.*, 1999), fibroblast growth factor 2 (FGF2) (Damania, 2007), interleukin 6 and 8 (IL-6 and IL-8) (Eliopoulos *et al.*, 1999b; Eliopoulos *et al.*, 1997), and CD54 are among the downstream targets of LMP1 signaling.

A similar mechanism is used by KSHV, which has a viral G protein-coupled receptor (vGPCR). vGPCR is highly homologous to

CXCR1 and CXCR2, the cellular receptor for IL-8. However, it is constitutively active. Downstream cellular events activated by vGPCR include all three MAP kinase pathways (ERK, JNK and p38MAPK), the PI3K pathway, the NF- κ B pathway as well as the JAK/STAT pathway (Sullivan *et al.*, 2006). vGPCR contributes to the pathogenesis of KS, PEL and MCD through its pro-angiogenesis and cytokine-inducing activities.

3.1.3. *Mimicking the intracellular signaling adaptors*

Several gamma herpesviruses, including KSHV, encode a viral FLICE inhibitory protein (vFLIP). vFLIP is another protein expressed in latently KSHV-infected KS and PEL cells (Sun *et al.*, 2003b). The primary function of vFLIP is to protect cells from both intrinsic and extrinsic apoptotic pathways. As its name suggests, the virus-encoded vFLIPs mimic the function of cellular FLIP (Ganem, 2006). vFLIP interacts with the IKK- γ (NEMO) complex and activates the anti-apoptotic NF- κ B pathway. The NF- κ B activation by vFLIP may also be mapped to even earlier signaling events such as binding to TARF2 and RIP (Ganem, 2006; Nicholas, 2007). This activation of NF- κ B by vFLIP is important for the survival of leukemia cells after serum withdrawal (Sun *et al.*, 2003a) and it is the primary anti-apoptotic signal in latently infected PEL cells (Keller *et al.*, 2000).

3.1.4. *Activation of cell surface receptors*

The HPV E5 protein is a small membrane protein that can induce the dimerization and activation of growth factor receptors, including epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor, and colony-stimulating factor-1 receptor. Over-expression of HPV E5 can result in elevated phosphorylation and decreased endosomal degradation of EGFR (Longworth & Laimins, 2004), possibly through inhibition of the vacuolar proton-ATPase on the endosomal membrane (Straight *et al.*, 1995). The HPV E5 protein moderately transforms rodent fibroblasts *in vitro* (Bedell *et al.*, 1989).

3.2. Deregulation of the Cell Cycle

With a few exceptions, somatic cells may enter terminally differentiated post-mitotic state by losing their proliferation ability permanently or may be quiescent and proliferate only upon stimulation by mitogenic signals. The cell cycle is divided into four phases named G1, S, G2 and M. Most of the extracellular signals induce cell proliferation by promoting the G1 to S phase transition. Once the cell enters the S phase, the cell cycle will proceed in an autonomous way, independent of extracellular signals. The critical step before the G1/S transition is referred to as the restriction point or R point.

The cell cycle is regulated by multiple protein complexes, each with a catalytic Ser/Thr protein kinase CDK and a regulatory unit of cyclin. Both the kinase activity and the substrate specificity of CDKs are determined by the cyclins associated with them. For example, CDK4 and CDK6 can bind to one of the three G1 cyclins, D1, D2 and D3 to form a functional complex for the progression of the cell cycle during the early-mid G1 phase. The cyclin E/CDK2 complex, in contrast, is critical for the cell to pass the R point. Similarly, cyclin A/CDC2 and cyclin B/CDC2 are important for the S phase and the G2/M phase, respectively. Consistent with the notion that cell cycle is regulated by extrinsic stimuli only during the G1 phase, the G1 cyclins accumulate in response to the extracellular mitogenic signals, leading to the activation of CDK4/6. The kinase activities of CDKs are also subject to negative regulation by inhibitors, which form a ternary complex with CDKs. p16^{Ink4A}, p15^{Ink4B}, p18^{Ink4C}, and p19^{Ink4D} are the inhibitors specific for CDK4 and CDK6, whilst p27^{Kip} and p21^{Cip} are for all CDKs.

The activation of CDK4/6 can promote the cell cycle through the G1 phase just before the R point. For the cell cycle to progress further, it will need to remove the cell cycle blocks imposed by members of the RB protein family, which includes the closely related proteins RB, p107 and p130. RB is a tumor suppressor and is mutated in retinoblastoma, a tumor of the eye. In normal cells, RB is mainly regulated via phosphorylation by CDKs such as cyclinD-CDK4/6 and cyclinE-CDK2. Hypophosphorylated RB binds to E2F transcription factors

(E2F1 to E2F6) and inhibits their transcriptional regulatory activity. However, hyperphosphorylation of RB at the R point dissociates E2F from RB, leading to the functional activation of E2F.

RB is the molecular target of the large T antigen of SV40 and mouse polyomavirus and the E1A protein of adenovirus. For these DNA tumor viruses, their DNA replication requires enzymes and substrates that are produced in the S phase. Therefore, it is critical for these viruses to induce entry of their host cells into the S phase of the cell cycle. Human oncogenic viruses have also developed multiple levels of regulation to promote cell cycle progression and cell proliferation.

3.2.1. *Abrogation of the RB function*

The HPV E7 protein can bind to RB. This protein contains three conserved regions, termed CR1, CR2 and CR3. The LXCXE motif within the CR2 region mediates the interaction with RB and displaces E2F from the Rb/E2F complex to cause deregulation of the cell cycle. Although E7 from both high risk and low risk groups of HPV bind to RB, the affinity of the high-risk HPV E7 for RB can be 10-fold stronger than that of the low-risk HPV E7. Furthermore, the high-risk HPV E7 enhances the degradation of RB by proteasomes via a second low-affinity RB binding motif present in its C-terminus. Studies show that the loss of RB in stratified squamous epithelia in a mouse model is well correlated with the expression of E7 (Balsitis *et al.*, 2003).

3.2.2. *Enhancement of CDK activities*

The cell cycle is negatively regulated by CDK inhibitors. Therefore, several viruses have developed strategies to counteract these inhibitors. HPV E7 is reported to be able to bind to p21^{Cip} and p27^{Kip} (Funk *et al.*, 1997). Importantly, the interaction between E7 and p21 suppresses the p21^{Cip} activity. This interaction with CDK inhibitors may serve as a failsafe mechanism to ensure the complete inactivation of RB to allow entry of the cell into the S phase.

A similar activity is also exhibited by the HTLV-1 *tax* protein, which physically interacts with p16^{Ink4A}/p15^{Ink4B} to suppress their CDK inhibitor activities (Matsuoka & Jeang, 2007). In addition, as a transcription factor, *tax* also suppresses the expression of p18^{Ink4C}, p19^{Ink4D} and p21^{Cip} genes.

3.2.3. Targeting of cyclin

The kinase activities of CDKs drive the progression of the cell cycle. However, the activation of CDKs requires cyclins. Viruses may target cyclins by transcriptional regulation, post-transcriptional modification or by expression of a viral version of cyclin.

Elevated expression of the G1-phase cyclin D2 is seen in HTLV-1 transformed cell. Further studies indicate that the HTLV-1 *tax* protein can transcriptionally activate the promoter of the cyclin D2 (*CCND2*) gene. In contrast, KSHV encodes a viral version of cyclin (*v-cyclin*) during both the latent infection and the lytic replication. The sequence of *v-cyclin* has 58% sequence homology with human cellular cyclin D2, and this protein binds predominantly to CDK6. Similar to its cellular counterpart, *v-cyclin*/CDK6 complex functions at the G1/S transition to promote entry into the S phase. However, this complex has a broader substrate range and is more resistant to the negative regulation by CDK inhibitors including p16^{Ink4a}, p21^{Cip} and p27^{Kip}. The resistance to p27^{Kip} is due to either the phosphorylation-coupled proteasomal degradation or enhanced cytoplasmic localization of p27^{Kip} (Jarviluoma *et al.*, 2004; Sarek *et al.*, 2006). The contribution of *v-cyclin* to cellular transformation seems to be context dependent. The expression of *v-cyclin* in mouse embryonic fibroblasts (MEFs) leads to multinucleation and polyploidy, and triggers apoptosis in the presence of p53. However, in the absence of p53, *v-cyclin* transgenic mice developed lymphomas (Verschuren *et al.*, 2002).

3.3. Escape of Apoptosis

Tissue homeostasis is a balance between cell proliferation and cell attrition. Metazoan organisms actively minimize the number of

malfunctioning cells from their tissues through an important process known as apoptosis. Although uncoordinated growth is a key characteristic of cancer, numerous studies have indicated that proliferation alone is not sufficient to cause cancer. For example, deregulation of the cell cycle may allow cells to proliferate rapidly. However, unless other survival signals exist, these cells, by default, will be eliminated by apoptosis. Therefore, overriding the barrier created by such a failsafe mechanism is required for cellular transformation.

Apoptosis, also called programmed cell death, is executed through a process that involves sensors and effectors. Apoptosis can be provoked by extracellular and intracellular signals. Examples of extracellular death signals include those carried by TNF- α and Fas ligand, which are captured by their corresponding receptors on the cell surface. A wide range of stresses, such as DNA damage and hypoxia, can induce cell death from inside the cells. Regardless of the origin of the death signals, apoptotic pathway converges at the mitochondria, where the Bcl-2 family proteins, either pro-apoptotic or anti-apoptotic, coordinate with one another to decide on the release of cytochrome c from the mitochondria. This release of cytochrome c will activate an array of caspases, including caspases 8 and 9, which elicit the caspase-cleavage cascade to pass death signals to the downstream effector (executioner) caspases, which will target different cellular components such as chromatin, cellular membrane, cytoskeleton, etc. to cause their disintegration.

The infection by human oncogenic viruses imposes significant amount of stress to cells. To avoid premature cell death, these viruses use different ways to counteract apoptosis. These countermeasures play important roles in the eventual transformation of the infected cells.

3.3.1. *Inactivation of the “gatekeeper” p53*

p53 was discovered as a tumor suppressor in late 1970s during the studies on cellular transformation mediated by the SV40 T-antigen (Lane & Crawford, 1979; Linzer & Levine, 1979). Mutation of its gene exists in more than 50% of all human cancers (Hanahan & Weinberg, 2000). In human cancers without p53 mutations, the

activity of p53 is frequently lost due to various indirect mechanisms. Up to date, more than 150 p53 target genes have been identified, most of which are implicated in cell-cycle arrest, senescence, DNA repair and apoptosis (Bode & Dong, 2004). Due to its important role in preventing oncogenesis, p53 is referred to as “gatekeeper” or “guardian” for cells.

Most of the p53 functions rely on its transcriptional regulatory activities. Tetrameric p53 binds to p53-specific DNA sequence present in the promoter regions of its target genes and functions either as a transcriptional activator or repressor. The steady state level of p53 within normal unstressed cells is very low due to its short half-life. The degradation of p53 is mediated through its ubiquitination by the ubiquitin E3-ligase MDM2, followed by degradation by the 26S proteasome. Upon sensing stress signals, p53 is stabilized by its dissociation from MDM2 and it activates the expression of proapoptotic genes such as Bax, which in turn triggers the release of cytochrome c and the activation of the caspase cascade. More recently, activated p53 has been shown to be able to translocate to mitochondria, where it directly interacts through its DNA binding domain with anti-apoptotic proteins, such as Bcl-2 and Bcl-XL, to induce apoptosis (Mihara *et al.*, 2003).

HPV E6 is a small protein with approximately 150 amino acids. Two zinc finger domains with four C-X-X-C motifs mediate most of the interactions of E6 with its cellular target proteins. E6 binds to p53 and a cellular ubiquitin E3-ligase, the E6-associating protein (E6-AP), to promote ubiquitination and the proteasomal degradation of p53 (Scheffner *et al.*, 1990). The activities of p53 can also be regulated by acetylation at its C-terminal lysine residues by coactivators such as p300/CBP and PCAF. HPV E6 can also be recruited to the promoter region of the p21^{Cip} gene, where it will interact with both p53 and p300 to inhibit p300-mediated acetylation of p53 (Thomas & Chiang, 2005). This will suppress p53-dependent transcription. Moreover, HPV E6 may further suppress the normal functions of p53 through inhibition of p53 DNA-binding activity or by sequestering p53 in the cytoplasmic compartment (Wise-Draper & Wells, 2008).

3.3.2. Expression of viral version of Bcl-2 (vBcl-2)

The Bcl-2 protein family includes more than 20 closely related proteins. Each of these proteins contains up to four domains termed Bcl-2-homologous regions (BH) arranged in the order BH4-BH3-BH1-BH2. An additional transmembrane domain (TM) at the C-terminus mediates the membrane association. Depending on their structural organization and functions, these proteins can be grouped as pro-apoptotic, anti-apoptotic or BH3-only. The pro-apoptotic members BAX and BAK multimerize at the surface of mitochondria to form a channel to allow the release of cytochrome c and other pro-apoptotic factors. The anti-apoptotic members, such as Bcl-2, are thought to sequester the BH3 only members from interaction with the pro-apoptotic members and thus prevent the activation of these pro-apoptotic proteins. A virus encoded Bcl-2 homolog has been identified in all of the members of the gammaherpesvirus, including the human oncogenic viruses EBV and KSHV (D'Agostino *et al.*, 2005).

BHRF1, the Bcl-2 homolog encoded by EBV, is expressed during the early stage of the lytic replication cycle. It can protect cells from a broad array of extrinsic and intrinsic apoptotic stimuli, such as death factor signaling, DNA damaging factors including drugs and radiation, deprivation of growth signals, or the over-expression of BIK or BOK (Cuconati & White, 2002; D'Agostino *et al.*, 2005). Similarly, KSHV ORF-16 also encodes a viral homolog of Bcl-2. This homolog has about 15–20% sequence homology with its cellular counterpart (D'Agostino *et al.*, 2005).

Yeast two-hybrid screening has identified several cellular proteins that interact with vBcl-2. BHRF1 possibly interacts with the pro-apoptotic Bcl-2 members such as BAK, BAX, BIK, and BOK. KSHV vBcl-2 can bind to Bcl-2 (D'Agostino *et al.*, 2005). However, whether viral Bcl-2 functions through heterodimerization with other Bcl-2 members is still being debated.

Caspase-3 cleavage of anti-apoptotic Bcl-2 family members, such as Bcl-2 and Bcl-X_L, in their loop domain near the N-terminus converts them into potent pro-apoptotic factors upon receiving the right

death signals. In contrast, such a negative regulation mechanism seems to be absent for the viral Bcl-2. They are either resistant to protease cleavage or their C-terminus cleavage products are not pro-apoptotic (Hardwick & Bellows, 2003).

3.4. Immortalization of Cells

Normal mammalian cells have limited propagation potentials, averaging about 60–70 cell divisions (Hanahan & Weinberg, 2000). Once their division numbers reach the “Hayflick limit,” cells will become senescent. Loss of tumor suppressor genes such as p53 or RB may endow cells with additional replicative abilities. However, these cells will eventually enter a “crisis” state, featured by massive apoptotic cell deaths and end-to-end joining of chromosomes. The only exceptions are stem cells, which can self-renew infinitely.

This limited ability of cells to propagate is due to an autonomous cell generation counting device called telomere, which is an array of thousands of copies of a hexanucleotide repeat at the ends of chromosomes. This telomere will be shortened by 50–100 base pairs after each cell division. Telomerase, a ribonucleoprotein composed of an RNA component TERC (telomerase RNA component) and a protein component TERT (telomerase reverse transcriptase), can replenish the telomere repeats at the ends of chromosomes. The expression level of TERT is extremely low in somatic cells and high in stem cells, which explains the different replication potentials between these two types of cells. In contrast to most somatic cells, telomerase reactivation is frequently seen in malignantly transformed cells. Alternatively, telomere may also be maintained by a recombination-based mechanism called ALT (alternative lengthening of telomere). By these mechanisms, cancer cells obtain infinite multiplication potentials similar to that of stem cells.

Several human oncogenic viruses target the hTERT to immortalize their infected cells. Since the transcription rate of the hTERT gene is very low in normal somatic cells, most of the reactivation seen in virus infected cells is achieved by transcriptional activation. The hTERT core promoter region contains E and GC boxes, which can

be bound by transcription factors such as myc, Sp1 and USF. Expression of HPV16 E6 in the early passages of human cell cultures leads to an elevated hTERT activity due to the activation of hTERT transcription (Katzenellenbogen *et al.*, 2007; Liu *et al.*, 2005; Veldman *et al.*, 2003). Several distinct mechanisms have been proposed for the E6-mediated hTERT reactivation. Interaction between myc and E6/E6AP will bring the latter to the promoter region and activate the myc-mediated transcription activation of hTERT (Veldman *et al.*, 2003). Alternatively, the E6/E6AP complex may remove the suppressive effect of NTX1, a transcriptional repressor for the hTERT promoter, by ubiquitination and destabilization of NFX1 (Gewin *et al.*, 2004). The functional interaction between E6/E6AP and p300/CBP increases histone acetylation at the hTERT promoter. Such an epigenetic modification can also activate hTERT transcription (James *et al.*, 2006). In a similar way, the LANA oncoprotein encoded by KSHV has also been reported to upregulate the gene transcription of hTERT through its interaction with the ubiquitous transcription factor Sp1 (Verma *et al.*, 2004).

3.5. Induction of Genetic Instability

One of the important hallmarks of cancer cells is their intrinsic genetic instability at either the gene level or the chromosome level. The former results in gene mutations and the latter leads to microsatellite instability (MIN) or chromosomal instability (CIN).

It is well accepted that tumorigenesis is a multi-step event, which requires the accumulation of multiple genetic/epigenetic alterations in the genome throughout the progression of tumors. However, although somatic cells are constantly exposed to mutagens from both outside and inside, they have evolved a wide array of ways to protect the genomic integrity. For instance, high-fidelity DNA polymerases ensure a low error rate during DNA replication. In additions, cells employ multiple mechanisms to monitor DNA damages and to restore the damaged genetic information using a variety of DNA repair systems. DNA alkyltransferase reverses base modifications. Base-excision repair (BER) and nucleotide-excision repair (NER)

take care of DNA lesions originated from endogenous and exogenous sources, respectively. In addition, cells may undergo error-prone DNA repair when massive DNA damages occur. The mitotic check-point during cell cycle further scrutinizes any abnormality of chromosomal segregation during mitosis. The fact that tumors nonetheless occur argues that the genome of cancer cells are highly mutable (Hanahan & Weinberg, 2000; Weiberg, 2007). This elevated mutability provides the tumor-initiating cell a much greater chance to gather enough mutations to reach the stage of malignant transformation.

HCV infection causes a “mutator” phenotype in which higher mutation frequency are observed in multiple cellular genes in HCV-infected B cell lines, peripheral mononuclear cells and HCC tumor tissues (Machida *et al.*, 2004b). Consistent with this phenotype, higher activity of B-cell somatic mutation machinery and error-prone DNA polymerase, as well as increased amount of double strand DNA breaks (DSB) are observed in these samples (Machida *et al.*, 2004b). Further analysis reveals that the expression of HCV core and NS3 proteins leads to elevated level of reactive oxygen species (ROS), which contributes to the formation of DSB (Machida *et al.*, 2006; Machida *et al.*, 2004a). In addition, the binding of HCV E2 envelope protein to the viral receptor CD81 in B-cell lines induces the activation-induced cytidine deaminase (AID) and causes hypermutations in the immunoglobulin gene (Machida *et al.*, 2005). These findings highlight an important role of virus-induced genomic instability in tumorigenesis.

Expression of E6 and E7 protein in HPV infected cells are usually characterized by the loss of p53 and Rb and the deregulated cell cycle control, which in turn leads to the genomic instability of the host cell. High risk HPV E6-expressing cells usually show nuclear abnormality as a result of blockage of cytokinesis due to the loss of p53 (Duensing & Munger, 2003a). Expression of high risk E7 protein can also induce abnormal centrosome synthesis, which subsequently leads to numerical chromosomal instability (Duensing *et al.*, 2001). This activity of E7 is independent of its ability to abrogate the activity of RB (Duensing & Munger, 2003a; Duensing & Munger, 2003b).

Recently, viral replication has also been suggested to be another factor contributing to the genome instability. The replication of HPV DNA from the integrated viral replication origin may be extended to the adjacent cellular DNA. Removal of the replication intermediate by the host DNA repair system may cause recombination and rearrangement of the cellular DNA (Kadaja *et al.*, 2007).

DNA damage and chromosomal abnormality are frequently present in HTLV-1-associated ATL cells. Proper centrosome duplication and segregation are vital to preserve chromosomal integrity. HTLV-1 *tax* affects both by targeting TAX1BP2 and RanBP1 (Matsuoka & Jeang, 2007). HTLV-1 *tax* further engenders the formation of multinucleated cells by down-regulating the mitotic spindle assembly checkpoint (SAC) complex through its interaction with MAD-1 (Jin *et al.*, 1998). It has been shown that *tax* abrogates the DNA damage checkpoint during the G2/M phase. Further studies show that *tax* suppresses DNA damage pathways, including Chk1/Chk2, BER, NER and mismatch repair (Grassmann *et al.*, 2005; Matsuoka & Jeang, 2007).

3.6. Insertional Mutagenesis

The life cycle of retroviruses requires the integration of proviral DNA into the host genome. This integration may occur in the vicinity of or inside important cellular genes, leading to their mutations. The outcome of proviral insertions can be at multiple levels depending on where the integration occurs. For example, transcriptional regulatory elements in the long terminal repeats of the proviral genome may lead to transcriptional activation of cellular genes. Alternatively, the splicing donor sites in the viral genome may generate chimeric or truncated cellular gene transcripts (Uren *et al.*, 2005).

Although all of the human DNA tumor viruses, including HPV, EBV, KSHV and HBV are capable of maintaining their genomes as episomes, the integration of their genomes into the host chromosomes is frequently detected, particularly in tumor samples. The insertional mutagenesis caused by these viral DNA integrations likely do not play major roles in viral oncogenesis, as common integration sites in the host genome have not been identified. It is noteworthy, however, that

the DNA of woodchuck hepatitis B virus (WHV), a virus that is closely related to HBV, is frequently found to integrate next to the cellular *N-myc* oncogene for its activation (Fourel *et al.*, 1990; Hansen *et al.*, 1993). Such an integration pattern is not detected for HBV. HBV DNA integrants are frequently found in the host chromosomes of HCC tissues (Matsubara & Tokino, 1990) and have been detected near cellular *erb-A* and *cyclin A* genes (Dejean *et al.*, 1986; Wang *et al.*, 1990). However, the integration of HBV DNA near genes that may regulate cell proliferation is a rare event. Nevertheless, the possibility that host chromosomal rearrangements and deletions caused by HBV DNA integrations may exert random mutagenic effects and contribute to the process of oncogenesis cannot be ruled out.

Viral DNA integration itself may help to preserve or enhance the expression of viral oncoproteins. The integration of the HBV DNA has been found to produce a truncated middle surface antigen protein (MHBst), which perturbs cellular signaling pathways and has been shown to have oncogenic potential (Meyer *et al.*, 1992). The integration of the HPV genome commonly results in the disruption of the E2 region and abolishes the expression of E2, which negatively regulates the transcription of E6 and E7 oncoproteins (Boccardo & Villa, 2007; Shirasawa *et al.*, 1987; Woodman *et al.*, 2007). It has also been reported that E6 and E7 transcripts derived from the DNA integrants have longer half-lives than those from the episome (Jeon & Lambert, 1995). Consequently, increased expression of E6 and E7 oncoproteins may provide cells with growth advantages (Jeon *et al.*, 1995).

3.7. Induction of Chronic Inflammation

A link between inflammation and tumorigenesis was proposed about 150 years ago, when Rudolf Virchow noted the development of cancers at the sites of chronic inflammation (Moss & Blaser, 2005). Indeed, inflammation, which precedes wound healing, may also lead to cancer. During inflammation, inflammatory cells (both leukocytes and mast cells) are recruited by cytokines as well as chemokines to the site of infection or irritation. A respiratory burst then follows, in which leukocytes release free radicals derived from the oxygen uptake.

The inflammation during wound-healing is “self-limiting,” i.e. cell proliferation and inflammation stop after the tissue regenerates. For cancer development, however, the inflammatory response sustains and provides the initiated tumor cells with an environment rich in growth/survival factors, activated stroma and DNA-damaging agents, which together promote cell proliferation as well as neoplastic transformation (Coussens & Werb, 2002).

Many human cancers, for instance, smoking-associated lung cancers and inflammatory bowel disease-associated colon cancers, are usually preceded by chronic inflammation. Furthermore, epidemiology studies showed that long-term usage of non-steroid anti-inflammatory drugs, such as aspirin, protects people from colon cancer and pancreatic cancer (Baron & Sandler, 2000; Coussens & Werb, 2002). Chronic inflammation in response to viral infection can also lead to the development of cancer. HCC caused by HBV and HCV is one such example.

Chronic liver inflammation caused by HBV and HCV infections frequently precedes the development of HCC in patients. The most direct evidence to support the role of chronic inflammation in the development of HBV-associated HCC comes from the studies on transgenic mice that expressed a non-cytopathic amount of HBV envelope protein known as the surface antigen (HBsAg). Due to the congenital expression of HBsAg from the transgene, there was immunotolerance to this viral protein in the mice. Immune response against HBsAg was reintroduced into thymectomized and lethally irradiated transgenic mice by adoptive transfer of bone marrow and spleen cells from syngeneic nontransgenic mice that had been previously immunized with a recombinant vaccinia virus that expressed HBsAg. This reconstitution of the immune system in transgenic mice induced chronic liver inflammation and liver injury and eventually the development of HCC in these mice (Nakamoto *et al.*, 1998).

Leukocyte (especially macrophage) infiltration is frequently observed in neoplastic tissues. These tumor-associated leukocytes are important for the removal of neoplastic cells. However, these immune cells also secrete many pro-angiogenic factors, cytokines and extracellular proteases, which promote angiogenesis, cell proliferation and metastasis (Coussens & Werb, 2002).

Free radicals are other key carcinogenic factors produced during inflammation. These free radicals can be generated from either reactive oxygen intermediate or reactive nitrogen intermediate during inflammation (Federico *et al.*, 2007). Reactive free radicals, such as hydroxyl radicals (OH^{\bullet}), peroxynitrite (ONOO^{-}) and nitric oxide (NO^{\bullet}), can damage DNA directly to cause single- or double-stranded DNA breaks, nucleotide modifications, DNA adducts and DNA-protein crosslinks (Hussain *et al.*, 2003). Normal cells respond to these damages by arresting the cell cycle for DNA repair to complete or by eliminating themselves through apoptosis. However, if mutations occur in crucial cancer-causing genes during the repairing process, neoplastic transformation of the cell may follow.

Studies have also pointed to an association between p53 mutation and elevated expression of iNOS, the major inducible enzyme for the production of NO^{\bullet} in ulcerative colitis, hemochromatosis, as well as cancers in stomach, brain and breast. Moreover, NO^{\bullet} and its derivatives may promote angiogenesis through its regulation of VEGF expression in the absence of wild-type p53. Therefore, free radicals may function as both tumor initiator and promoter during carcinogenesis (Hussain *et al.*, 2003).

Over-expression of HBV large envelope protein in transgenic mice led to necroinflammatory diseases that progressed to HCC (Chisari *et al.*, 1987). Further analysis indicated that sustained hepatocyte proliferation was accompanied by elevated production of ROS as well as oxidative damage long before the development of HCC (Hagen *et al.*, 1994). These studies lend further support to a causal role of ROS in the development of HCC.

Free radicals and oxidative stress are clearly induced by HCV infection, which is evidenced by decreased levels of hepatic and plas-matic glutathione, an important reductant, and increased levels of 4'-hydroxynonenal and 8-hydroxyguanosine, markers for oxidative DNA damage (Choi & Ou, 2006). Interestingly, free radicals are not only generated during HCV infection as a consequence of chronic inflammation, but can also be generated by HCV core protein and NS5A, which can directly affect mitochondrial electron transport, or by a secondary effect of endoplasmic reticulum (ER) stress induced by

HCV. It is not clear why HCV elicits hepatic oxidative stress as ROS suppresses HCV RNA replication *in vitro* (Choi *et al.*, 2004). Nonetheless, ROS and oxidative stress are critical factors involved in HCV pathogenesis.

4. Concluding Remarks

The relationship between human oncogenic viruses and their associated cancers were established mostly in the last quarter of the 20th century. It is likely that the current list of human oncogenic viruses will be further expanded in the near future. The recent discovery that a polyomavirus may be the causative factor of human Merkel cell carcinoma is a good example (Feng *et al.*, 2008).

Research on oncogenic viruses has significantly advanced the cancer biology research field. Discoveries of Rous sarcoma virus, reverse transcriptase and cellular homologues of viral oncogenes are examples of milestones of cancer research. Further research on human oncogenic viruses and their interaction with their host cells will likely lead to novel therapeutic treatments for virus-associated cancers. The presence of viral proteins in some of the virus-associated cancers may be the Achilles' heel of these cancers, for which the treatments can be developed (Farrell, 2002).

Viral infection is now considered as the second major preventable cancer risk factor after tobacco use. The universal immunization program against HBV in Taiwan and Gambia has successfully lowered the incidence of HCC in the vaccinated population. The first HPV vaccine approved in 2006 are expected to reduce deaths from cervical cancer by 75%, a number that should be a great inspiration for similar research on other human oncogenic viruses as well.

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