ONCOGENIC HERPESVIRUSES:
MOLECULAR MECHANISMS OF CELLULAR TRANSFORMATION

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ABSTRACT

Tumor-associated viruses, also known as oncogenic viruses, consist of both DNA viruses and RNA viruses and comprise several different taxonomic groups (2). Oncogenic RNA viruses encompass several human viruses, for instance, human T-cell leukemia virus type-I (HTLV-I), and hepatitis C virus (HCV). On the other hand, hepatitis B virus (HBV), human papillomavirus (HPV), Epstein-Barr virus (EBV), and Kaposi’s sarcoma-associated herpesvirus (KSHV) are excellent examples of oncogenic DNA viruses (2,3). These viruses clearly represent an extraordinary burden on global human health. Even though they are not considered as the major causes of cancer, it has been estimated that each year approximately more than 1 million people would develop cancers, caused by these viruses, and majority of them will die (4).

Several mechanisms in regards to the oncogenesis of herpesviruses have been suggested. These mechanisms are perturbation of tumor suppressor proteins activities leading to the evasion of apoptotic pathways, cell immortalization, and induction of genetic and/or chromosomal instability. Nevertheless, most of the mechanisms, apparently, related to the inhibition of tumor suppressor proteins and/or the activation of NF-kB signalling pathways.

Key words: herpesviruses, molecular mechanisms, cellular transformation

INTRODUCTION

Tumor-associated viruses, also known as oncogenic viruses, consist of both DNA viruses and RNA viruses and comprise several different taxonomic groups (2). Oncogenic RNA viruses encompass several human viruses, for instance, human T-cell leukemia virus type-I (HTLV-I), and hepatitis C virus (HCV). On the other hand, hepatitis B virus (HBV), human papillomavirus (HPV), Epstein-Barr virus (EBV), and Kaposi’s sarcoma-associated herpesvirus (KSHV) are excellent examples of oncogenic DNA viruses (2,3). These viruses clearly represent an extraordinary burden on global human health. Even though they are not considered as the major causes of cancer, it has been estimated that each year approximately more than 1 million people would develop cancers, caused by these viruses, and majority of them will die (4).

Among all oncogenic DNA viruses, several viruses – for example, EBV and KSHV – are members of the same family, Herpesviridae (5,6). As one of the largest families in DNA viruses, Herpesviridae is distinguished taxonomically into three subfamilies, α, β, and γ, based on the similarity of their genome sequence and organization, and the biological properties (7). To date, several herpesviruses from different subfamilies have been recognized as a causal agent or, to be likely, a potential causal agent of cancer (7,8,9).

Over the last two decades, oncogenic viruses have been used extensively as a main tool in the revelation of molecular basis of cancer (3). Since viral-mediated cellular transformation has been considered as one of the major causes of cancer, a conceptual outline in viral oncogenesis would improve our understanding in the development of cancer (3,4).

It is the aim of this review to compile and summarise the state-of-art of oncogenic mechanisms of herpesviruses, focusing on four major herpesviruses, EBV, Marek’s disease herpesvirus (MDHV), herpesvirus saimiri (HVS), and KSHV. Other suspected oncogenic herpesviruses, human cytomegalovirus (HCMV) will be addressed to some extent in this review. What is more, understanding the oncogenic mechanisms of these herpesviruses might facilitate the prediction of
Antiviral treatments and improve the application of available vaccines to overcome the incidence of tumor-associated herpesviruses.

**Oncogenic Herpesviruses in Brief**

Herpesviruses are large double-stranded DNA viruses that have been grouped together primarily based on the commonality of biological properties and genome organization (7). A distinctive feature of herpesviruses is a linear double-stranded DNA genome of 125-290 kb contained in an icosahedral nucleocapsid which is surrounded by an envelope containing membrane-associated glycoproteins (10).

Recently, the International Committee on Taxonomy of Viruses (ICTV) has established a new order *Herpesvirales* which comprises three herpesvirus families: *Herpesviridae*, *Alloherpesviridae*, and *Malacocberpesviridae* (11). *Herpesviridae* is a large family of mammal, bird, and reptile viruses and encompasses three major subfamilies, *alphaherpesvirinae* (α-herpesviruses), *betaherpesvirinae* (β-herpesviruses), and *gammaherpesvirinae* (γ-herpesviruses) (11). Several herpesviruses of the *Herpesviridae* family origin have been documented in a wide variety of host including human (11, 12) and have generally been considered as one of the causes of cancer (5, 4).

Herpesviruses are highly disseminated among animals (12) and shared a similarity in the establishment of a lifelong period of latency in their host (13). In fact, the establishment of latency has been assumed as one of the critical strategies of the herpesviruses to survive in the nature (14). In regards to the latent tropisms of herpesviruses, the α-subfamily of herpesviruses - for instance, HSV-1 and HSV-2 - are neurotropic while EBV, KSHV, and HVS, as the examples of the γ-herpesviruses, are considered lymphotropic. Meanwhile, the main cellular sites of β-herpesviruses’ latency – for example, HCMV - have not been defined (13).

In general, herpesviruses are widely known as highly successful parasites due to their capability to produce latency following primary infection in their host and their involvement in a range of prominent diseases (10). Within the latency state, their DNA conformation is changed into closed circular form and therefore, retains the DNA replication (10). In addition, since only a small amount of viral genes is expressed in the latent state, the immune evasion could be achieved effectively (15).

Herpesviruses are large DNA viruses that have evolved for more than 400 million years (12). To date, more than 130 herpesviruses have been identified and most animal species can be infected by at least one herpesvirus (7). Some of them, furthermore, have been identified or, at least suspected, could provoke cell transformation that lead to cancer (16, 7). Current evidences indicate that most of tumor-associated herpesviruses, also known as oncogenic herpesviruses, are mainly the members of γ-herpesviruses (13, 7). EBV, KSHV, and HVS for example, are widely known to have the ability to induce cellular transformation in their host (7). In contrast, only MDHV have been identified so far as an oncogenic virus in the α-subfamily. Moreover, three herpesviruses of the β-subfamily – HCMV, HHV-6, and HHV-7 – have been suspected as tumor-associated herpesviruses (18, 19). A summary outline of main characteristics of eminent oncogenic herpesviruses and major lineages within these according to our recent understanding are provided in Table 1.

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**Molecular Oncogenesis of Tumor-Associated Herpesviruses**

A relationship between a number of viral infections and certain types of tumor has been generally established since the initial discovery of EBV within malignant cells of Burkitt lymphoma in 1964 (1, 2). In the last three decades or so, a number of evidences have been reported regarding the role of oncogenic viruses, particularly herpesviruses, in the development of cancer in animals (7, 17) and humans (1, 7).
To date, an extensive amount of research results has been published in regards to explain the mechanisms of viral oncogenesis (6), including herpesviruses-associated malignancies (1). As a result, our understanding in herpesvirus-induced oncogenesis has been considerably increased during the past three decades (1,6).

It is now generally believed that several herpesviruses possess oncogenic properties (7,8). A number of studies in animals and humans revealed the biological pathways applied by EBV, KSHV, and HVS from the γ-subfamily of herpesviruses and MDHV from the α-herpesvirinae to induce cell transformation which lead to cancer (21,22,23,24,25). Some herpesviruses such as HSV-1, HSV-2, HHV-6, HHV-7, and HCMV, have also been suspected as oncogenic herpesviruses due to the fact they are sometimes isolated from tissue biopsies of specific malignancies (8,18).

As mentioned earlier, numerous pathways have been used by oncogenic herpesviruses to control key cellular process and provoke the formation of malignant cells. These strategies, furthermore, will be discussed in detail in the following sections.

**Evasion of Apoptotic Pathways**

The activities of normal cells such as in cell signalling, proliferation, and apoptosis, are tightly regulated by the expression of many proteins which are encoded by tumor suppressor genes (1). The disruption of tumor suppressor proteins by viral oncoproteins might create a critical situation where abnormal cells could initiate the induction of malignancies as well as evade the immune system of the host (1,6). This mechanism has been widely used by several oncogenic herpesviruses for transforming the normal cells into malignant ones (7).

In general, several types of cellular tumor suppressor proteins such as p53, retinoblastoma protein (pRb), von Hippel-lindau (VHL) protein, and promyelocytic leukemia (PML) protein have been identified as a major target of oncogenic herpesviruses (26,27,28). p53, also known as the “guardian of the genome”, is the eminent tumor suppressor protein that responsible to the stability of the genome (29). This protein plays an important role in the regulation of cell cycle, DNA repair, and if necessary, mediates the initiation of programmed cell death (apoptosis) (30,31,32).

Another tumor suppressor protein, pRb, is a protein that controls G1/S cell cycle through its association with the family of E2F transcription factors. In the active form, pRb acts as a transcription repressor by inactivating E2F transcription factors, thereby prevent accidental entry into S phase (33). Furthermore, VHL protein (pVHL), a component of ubiquitin ligase, plays a vital role in cell proliferation and differentiation of human kidney cells whereas PML regulates cell growth in the lympho-hematopoietic compartment (28).

Different strategies have been successfully implemented by oncogenic herpesviruses in the inhibition of these tumor suppressor proteins (26,34,35). According to Friberg et al. (34), KSHV, also known as human herpesvirus-8 (HHV-8), prevents cell death by inhibit p53 protein through the latency-associated nuclear antigen (LANA). In addition, evidence suggests that LANA also inactivate pRb and thus, increase the transcription of E2F promoter in SAOS-2 cells which would lead to the disruption of G1/S phase in cell cycle (35).

LANA is primarily expressed during the latency state in Kaposi’s sarcoma (KS), Multicentric Castleman’s disease (MCD), and primary effusion lymphomas (PEL) (36). To date, LANA has been distinguished into two main forms : LANA1 and LANA2 (37). LANA1, a highly immunogenic protein encoded by open reading frame (ORF) 73 of the KSHV genome, has been suggested could inhibit two tumor suppressor proteins, p53 and VHL, via its E3 ubiquitin ligase activity (27,34). This process, furthermore, would prevent apoptosis and induce the increase of factor-1 α levels which leads to cell growth impairment (27). Another KSHV latent protein, LANA 2 also inhibits p53-mediated apoptosis (37) and disrupts PML oncogenic domains (POD), a combination of multiple tumor suppressor proteins such as Sp100, Sp140, ISG20, CBP, pRb, Daxx, BLM, and p53, which are involved in gene transcription, genetic stability, cell cycle control, and apoptosis (28).

Another γ-herpesvirus that has been identified could provoke cancer by blocking p53-mediated apoptosis pathway is EBV (38). EBV, also called human herpesvirus-4, was the first virus in the Herpesviridae family recognized to be associated with human malignancies (39). Since then, a massive number of researches has been conducted to explain the mechanisms of how EBV contribute to cancer (40). In 1991 for example, Henderson et al. proposed a mechanism by which EBV oncoprotein, Latent Membrane Protein 1 (LMP-1), contributes to the inhibition of p53-mediated apoptosis in B-lymphocytes. They suggested that the stimulation of bcl-2, one of the anti-apoptotic genes, by LMP1 could protect B-lymphocytes from apoptosis (41). This mechanism is probably achieved through the up-regulation of bcl-2 expression (42). It is remarkable, however, this mechanism alone might be inadequate to protect B-cells from p53-mediated apoptosis since Fries et al. (1996) showed that the levels of two bcl-2 family members, bcl-xL (anti-apoptotic) and bax (pro-apoptotic), were not affected by LMP1. Noteworthy, they found that the induction of A20, a tumor necrosis factor (TNF) inducible gene product, would protect epithelial cells from p53-mediated apoptosis. This mechanism could be achieved through CD40 cross-linking (43) and/or the activation of the NF-kB transcription factor (44,45).

EBV, named after its discoverers Michael Epstein and Yvonne Barr, also encodes several nuclear proteins that could interfere with tumor
suppressor proteins' function, designated as EBV nuclear antigen 1-6 (EBNA 1-6) (46). Within these EBV specific proteins, EBNA-2 is able to trans-activate p53 through the induction of NF-κB transcription factor (47) whilst EBNA-5, alternatively called EBNA-LP, has been known for its capability to bind to pRb and p53 (46). In theory, the activation of NF-κB pathways stimulates interleukin (IL)-6 production leading to the activation of the signal transducers and activators of transcription (STAT) (48) and promotes cell growth and survival (49). A recent study showed that EBNA-5 also binds to a nucleolar protein, P14ARV, which is widely known as an upstream regulator of the p53 pathway resulting in the evasion of apoptosis and prolonged survival rate of P14ARF-expressing cells (50).

To date, several oncoproteins that are responsible to the T-cell transformation have also been detected in HVS (51). These proteins are called saimiri transforming proteins (STPs) and classified into three subgroups: A, B, and C, based on the transformation potential and on the sequence variability (52,53). However, it has been generally accepted that only HVSs of subgroup C are able to induce oncogenesis in human and rabbit T-cells (53,54). According to Jung et al. (55), STP-C shows higher transformation potential in fibroblasts compare to STP-A. Interestingly, STP-B does not confirm any oncogenic properties (56).

In regards to the oncogenic properties of STP-C in fibroblasts, Jung and Desrosiers (1995) have suggested that STP-C might stimulate the activation of Ras and the serine-protein kinase Erk and thus, initiates DNA transcription and translation. However, it is arguable since they do not provide direct evidence concerning this mechanism. Furthermore, recent findings showed that the potential oncogenic mechanism of HVS in the T-cell transformation is depend on the ability of STP-C's in the activation of NF-κB signalling pathways leading to the evasion of apoptosis (7,51).

In addition, Rhadinoviruses such as KSHV and HVS encode a viral oncoprotein referred to as FLICE (Fas-associated death domain-like interleukin 1 beta-converting enzyme) inhibitory protein (vFLIP) (57,58). This protein inhibits the activation of caspase-3, -8, and -9 and therefore, protects cells from Fas/APO1-mediated apoptotic pathway (59). In addition, KSHV vFLIP also induce the activation of NF-κB pathway following association with TRAF2 and RIP (60,61).

**Immortalization of Cells**

One of specific properties that clearly distinguished normal cells from cancerous cells is the existence of limited propagation potential. Once normal cells reach a certain number of doublings, referred to as Hayflick limit, cells become replicative-senescenct (62). This phenomenon is, apparently, regulated by a large ribonucleoprotein complex that responsible for the preservation of telomeres, called telomerase. Telomeres, on the other hand, are the term used to describe specific structures at the ends of eukaryotic chromosomes (63). It provides protection to chromosome ends from recombination, fusion and degradation, and controlling cell proliferation (64). Therefore, failed in maintaining both the telomeres and the telomerases could lead to the initiation of cancer (9).

Shay and Wright (31) suggested that telomerase activation is important in cellular immortalization and malignancies. Telomerase, an RNA-dependant DNA polymerase composed of an RNA subunit TERC (telomerase RNA component) and a protein component TERT (telomerase reverse transcriptase), have been studied extensively regarding its function in cancer cells survival (9,63). KSHV LANA, for instance, has been recognized to upregulate the gene transcription of human TERT (hTERT) via SP1 pathway (65). In similar to the effect of KSHV LANA on hTERT, Liu et al. (63) found that EBV LMP1 also transactivate hTERT gene and promote the entry of hTERT in nucleus through the NF-κB signalling pathway which would lead to the immortalization of cells. Noteworthy, a recent study has suggested that HCMV could modulate oncogenesis through the activation of telomerase (9).

The upregulation of telomerase activity is also detected in MDHV and basically associated with the increase of viral telomerase RNA subunit (vTR) gene expression in infected chickens (24). This finding suggests that MDHV could trigger T-cell immortalization in the host. Yet, the molecular mechanism of interaction between the hTERT and NF-κB and the consequence of this interaction in the maintenance of telomerises still unclear (63).

**Induction of Genetic Instability**

One of the important characteristics of cancer cells is their genetic instability at either the gene level or the chromosome level. The former leads to gene mutations and the latter results in chromosomal instability (CIN). Chromosomal abnormalities, also known as aneuploidy, are distinguished consequences following telomere dysfunction, and apparently, some malignant cells might experience a variable episode of telomere shortening prior to the immortalization of cells (63). According to Pan et al. (2009), the disruption of mitotic checkpoint by EBV EBNA2 could promote chromosomal imbalances. In this study, they found that EBNA2-infected cells disrupts cellular cycle by performing a “hit-and-run” mechanism and leaves the mitotic cycle in several different ways without triggering the release of cytokines. Furthermore, in Burkitt's lymphoma (BL) patients, chromosomal instability caused by EBV has been associated with the role of c-myc (Kamranvar et al. 2007). However, this is not quite accurate since the defects were between 3 and 10 times more frequent in EBV+ cells. In addition, since EBV LMP1 protein has been recognized as the inhibitor of p53-mediated DNA repair (1), it is
CONCLUDING REMARKS AND FUTURE PERSPECTIVES

The relationship between oncogenic herpesviruses; EBV, KSHV, HVS, and MDHV and their associated cancer were established mostly in the last quarter of the 20th century. It is likely that the current list of oncogenic herpesviruses will be further expanded in the near future.

In conclusion, several mechanisms in regards to the oncogenesis of herpesviruses have been suggested. These mechanisms are perturbation of tumor suppressor proteins activities leading to the evasion of apoptotic pathways, cell immortalization, and induction of genetic and/or chromosomal instability. Nevertheless, most of the mechanisms, apparently, related to the inhibition of tumor suppressor proteins and/or the activation of NF-κB signalling pathways.

The revelation of mechanisms by which EBV, KSHV, HVS, MDHV, and other potential oncogenic herpesvirus, HCMV, contribute to the development of cancer has significantly increased our understanding in the cancer biology field. Further research on oncogenic herpesviruses and their interaction with their host cells will lead to the discovery of novel therapeutic treatments for viral-associated cancer.

REFERENCES


