VIROLOGICA SINICA 2015, 30 (2): 107-121
DOI 10.1007/s12250-015-3592-5

REVIEW

The role of Epstein-Barr virus infection in the pathogenesis of nasopharyngeal carcinoma

Chi Man Tsang, Sai Wah Tsao✉

Department of Anatomy, Li Ka Shing Faculty of Medicine, University of Hong Kong, Hong Kong SAR, China

Nasopharyngeal carcinoma (NPC) is closely associated with Epstein-Barr virus (EBV) infection. EBV episomes are detected in almost all NPC cells. The role of EBV in NPC pathogenesis has long been postulated but remains enigmatic. In contrast to infection of B lymphocytes, EBV infection does not directly transform nasopharyngeal epithelial cells into proliferative clones with malignant potential. EBV infection of normal pharyngeal epithelial cells is predominantly lytic in nature. Genetic alterations in premalignant nasopharyngeal epithelium, in combination with inflammatory stimulation in the nasopharyngeal mucosa, presumably play essential roles in the establishment of a latent EBV infection in infected nasopharyngeal epithelial cells during the early development of NPC. Establishment of latent EBV infection in premalignant nasopharyngeal epithelial cells and expression of latent viral genes, including the BART transcripts and BART-encoded microRNAs, are crucial features of NPC. Expression of EBV genes may drive further malignant transformation of premalignant nasopharyngeal epithelial cells into cancer cells. The difficulties involved in the establishment of NPC cell lines and the progressive loss of EBV epsiomes in NPC cells propagated in culture strongly implicate the contribution of host stromal components to the growth of NPC cells *in vivo* and maintenance of EBV in infected NPC cells. Defining the growth advantages of EBV-infected NPC cells *in vivo* will lead to a better understanding of the contribution of EBV infection in NPC pathogenesis, and may lead to the identification of novel therapeutic targets for NPC treatment.

KEYWORDS **Epstein-Barr virus (EBV); nasopharyngeal carcinoma; latent infection; pathogenesis; inflammation**

INTRODUCTION

Epstein-Barr virus infection is ubiquitous in humans

Epstein-Barr virus (EBV) belongs to the gamma herpesvirus family that also includes the Kaposi's sarcoma-associated herpes virus (KSHV), which is more commonly associated with immune-deficient diseases. The EBV is a highly successful virus infecting the majority of the human population (< 90%) worldwide. It is also the first human tumor virus identified (Young and Rickinson, 2004). While infection of EBV is ubiquitous, tumorigenesis only occurs in a small fraction of the infected population, suggesting that the tumorigenic transformation of human cells by EBV involves complex virus-host interactions and other additional co-factors. A compromised host immune condition and a chronic inflammatory microenvironment probably play major roles in mediating the pathogenic actions of EBV in human malignancies (Rickinson, 2014).

EBV exhibits dual tropism, infecting both B cells and epithelial cells (Borza and Hutt-Fletcher, 2002). Latently infected memory B cells are believed to be the reservoir

Received: 2 April 2015, Accepted: 16 April 2015
Published online: 21 April 2015
✉ Correspondence:
Phone: +852-2819-9227, Fax: +852-2817-0857,
Email: gswtsao@hku.hk
ORCID: 0000-0001-9082-9717

of EBV, which undergoes lytic reactivation upon stimulation to produce infectious virus. Human B cells, but not epithelial cells, are readily infected by EBV. Infection of B cells is mediated by the complement receptor type 2 (CR2) on the membrane surface. EBV infection in infants and young children is generally asymptomatic; however, in adults, EBV infection induces infectious mononucleosis (glandular fever) involving a proliferation of lymphoid tissue. Symptoms include fever, sore throat, and fatigue. The lymphoproliferation process is self-limiting in healthy individuals with competent immune systems, and will eventually subside. After that, EBV establishes a lifelong infection in the body and generally remains asymptomatic. However, lymphoproliferation will take place if immune function is compromised, as in post-transplantation and HIV (human immunodeficiency virus) patients. Clearly, the body immune system plays a paramount role in checking the proliferation of EBV-infected cells. The cytotoxic T cells (CTL) are constantly checking and regulating the proliferation of EBV-infected B cells in the body.

EBV infection and human cancers

The tumorigenic potential of EBV was first observed in Burkitt's lymphoma, a special type of childhood cancer common among African children. Co-factors are believed to be involved in the pathogenesis of Burkitt's lymphoma (Thorley-Lawson and Allday, 2008). The incidence of Burkitt's lymphoma is closely associated with malaria infection, though the exact contribution of malaria infection to Burkitt's lymphoma remains undefined. The chronic inflammation associated with malaria infection may promote clonal expansion of EBV-infected B cells. Malaria infection may also compromise the host immune system through unknown processes and provide a permissive environment for EBV-infected B cells to evolve into Burkitt's lymphoma cells (Moormann et al., 2011). Translocation of c-myc in infected B cells plays a key role in the etiology of Burkitt's lymphoma.

The EBV was identified directly under electron microscopic observation of cell lines established from Burkitt's lymphoma. The ability of EBV to induce proliferation in infected B cells was later demonstrated by culturing peripheral B cells with filtered supernatant harvested from Burkitt's lymphoma cells, where clusters of proliferative clones of EBV-infected B cells could be readily demonstrated (Henle et al., 1967). The ability of EBV to transform and immortalize human B cells strongly implicates the tumorigenic potential of the virus.

In addition to Burkitt's lymphoma, EBV infection was later observed in other human malignancies, including hematological and lymphatic tumours, such as Hodgkin's disease, T cell lymphoma, and NK cell lymphoma, and epithelial cancers, such as nasopharyngeal and gastric carcinomas (Young and Rickinson, 2004; Tsao et al., 2015). In all cases, the nature of EBV infection in infected cancer cells is predominantly latent.

CLOSE ASSOCIATION OF EBV INFECTION WITH NASOPHARYNGEAL CARCINOMA (NPC)

Nasopharyngeal carcinoma (NPC) is unique in its close association (100%) with EBV infection. It is a rare cancer in Western countries, but a common cancer in the ethnic Chinese population living in the southern provinces of China. NPC is closely associated with Cantonese-speaking populations and is nicknamed "Cantonese cancer". The etiology of NPC is multifactorial, and includes genetic predisposition, EBV infection, and diet (Tsao et al., 2014). The major histological type of NPC in endemic regions is undifferentiated NPC, which is associated with EBV infection. EBV infection could be demonstrated in almost every NPC cell, distinguishing NPC from other squamous carcinomas arising in the head and neck regions, which are all EBV-negative. NPC patients have elevated serological IgA against the EBV lytic protein-viral capsid antigen (VCA) and early antigen (EA). The detection of IgA against EBV VCA and EBV DNA in plasma are important diagnostic tools of NPC, and are used extensively in early screening of NPC in high-risk populations. Clearly, the status of differentiation has a role in persistence of EBV infection in epithelial cancers. Recently, the detection of plasma EBV DNA has been shown to improve the sensitivity and specificity of the diagnosis of NPC (Le et al., 2013). The level of plasma EBV DNA also reflects faithfully the tumor burden in NPC patients, and is a powerful tool for monitoring the disease progression during treatment (To et al., 2003).

Route of EBV entry and infection of B cells and human epithelial cells

As previously mentioned, infection of B cells, but not epithelial cells, by EBV is a highly efficient process. The CR2 receptor, which is present on the surface of B cells and facilitates EBV entry, is generally not expressed in epithelial cells. Infection of oropharyngeal epithelial cells could be achieved via the EBV BMRF2 protein and cellular integrin receptors (Tugizov et al., 2003). Interestingly, EBV adopted an intricate cell entry mechanism by switching its envelope proteins in order to infect B cells and epithelial cells alternatively. The EBV binds to CR2 receptor present on B cell surfaces through the viral envelope protein, gp350. This interaction is augmented by the binding of another viral envelope protein, gp42, to the human leukocyte antigen (HLA) class II protein expressed on the B cell surface, which triggers the fusion of the EBV envelope to the B cell membrane (Chesnokova et al., 2009). This process involves the vi-

ral envelope proteins gB and gHgL. Neither the CR2 and HLA class II are expressed on the surface of epithelial cells. The EBV entry into epithelial cells involves the binding of viral envelope proteins to epithelial surface integrins, αvβ6 and αvβ8, which triggers membrane fusion and viral entry. The presence of gp42 in the viral envelope actually impedes EBV entry into epithelial cells through interaction with the integrin complex. Interestingly, EBV virions emerging from B cells that have been triggered to undergo lytic infection lack gp42, facilitating subsequent EBV binding and entry into epithelial cells. In contrast, virions released from epithelial cells are rich in gp42, which facilitates the infection of B cells. By switching its envelope proteins, EBV is able to shuttle between B cells and epithelial cells, which is crucial for its persistent infection in humans. Recently, the neuropilin1 (NRP1) was identified as an entry receptor for EBV infection of epithelial cells, and found to interact with the EBV envelope protein gB to promote EBV infection of nasopharyngeal epithelial cells (Wang et al., 2015).

It remains unknown whether primary pharyngeal epithelial cells or naïve B cells are the first cellular target of EBV infection. Latently infected memory B cells are believed to be a reservoir of EBV; an estimated 1 in 10^6 circulating blood lymphocytes are latently infected (Babcock et al., 1998). These latently infected B lymphocytes may spontaneously reactivate into lytic cycle. The released virions may then infect a few cells in the oropharyngeal epithelium. EBV infection of primary human epithelial cells is believed to be primarily lytic in nature. A low frequency of lytic EBV infection occurring in the oropharyngeal epithelium may be responsible for the continuous release of infectious viral particles in the saliva for transmission (Hadinoto et al., 2009).

Establishment of latent infection in nasopharyngeal epithelial cells

Similar route of infection of nasopharyngeal epithelial cells may take place *in vivo*. As EBV infection of normal epithelial cells normally results in a lytic infection, establishment of a latent EBV infection in epithelium may be an early step in carcinogenesis. Detection of latent EBV infection in normal epithelium is uncommon in healthy individuals. Nonetheless, a low percentage (0.005 to 0.01%) of EBV-infected cells expressing the EBV latent gene, LMP1, were present in oropharyngeal epithelium explanted to grow in culture (Pegtel et al., 2004). The status of latent infection of normal nasopharyngeal epithelium is unknown. The unique histological properties of the nasopharyngeal epithelium may support latent EBV infection, particularly in dysplastic or precancerous conditions. NPC in endemic regions is predominantly undifferentiated or poorly differentiated in nature. Inactivation of the p16 tumor suppression gene and overexpression of cyclin D1 are common events in NPC, and can be detected in premalignant and dysplastic nasopharyngeal epithelium (Lo et al., 2004b). Inactivation of p16 and activation of the cyclin D1 pathway may confer undifferentiated properties to the nasopharyngeal epithelium to support EBV infection. Using an *in vitro* model of immortalized nasopharyngeal epithelial cells, we showed that EBV infection readily induced growth arrest in nasopharyngeal epithelial cells. However, inactivation of p16 and/or activation of cyclin D1/cdk4/6 could override the growth arrest induced by EBV infection, and supported a stable latent EBV infection in infected nasopharyngeal epithelial cells (Tsang et al., 2012). Overexpression of cyclin D1 in immortalized nasopharyngeal epithelial cells confers resistance to stimulation of differentiation induced by serum and calcium (Tsao et al., unpublished observation). Similarly, other oncogenic alterations, including overexpression of B lymphoma Mo-MLV insertion region 1 homolog (bmi-1), may also support latent EBV infection of nasopharyngeal epithelial cells (Yip et al., 2013). Moreover, EBV-infected immortalized nasopharyngeal epithelial cells remain non-tumorigenic when injected subcutaneously in immunosuppressed mice, indicating that additional events are required for the malignant transformation of EBV-infected immortalized nasopharyngeal epithelial cells.

PROFILE OF EBV GENE EXPRESSION DURING LATENT INFECTION

Latent infection with EBV is commonly associated with the development of human cancers. During latent infection, EBV expresses a small number of its genes to evade detection by the host immune system. The latent gene expression in EBV-infected cells is under epigenetic regulation (Tempera and Lieberman, 2014). Several types of latent gene expression profiles have been identified in EBV-infected B cells and human cancer cells (Table 1). Type 0 latency is recognized in memory B cells, where expression of EBV genes is reduced to only EBV-encoded small RNAs (EBERs), with no EBV proteins expressed. The EBV nuclear antigen 1 (EBNA1) is only expressed in memory B cells undergoing division (Hochberg et al., 2004). Type I latency is characteristic of EBV-associated B cell lymphoma, while type II latency is observed in nasopharyngeal and gastric carcinoma. Type III infection represents a full-blown expression of latent EBV genes for growth promotion, and is observed during the *in vitro* transformation of B cells into proliferative lymphoblastoid cell lines (LCL) by EBV. Similarly, type III latency is also observed in lymphoproliferative disorder in immunocompromised patients. The proliferative transformation of B cells during EBV infection is

not observed in EBV infection of epithelial cells. EBNA2 and 3C, which are involved in cell cycle progression in EBV-transformed LCL cells are not expressed in NPC. In contrast, the BamH1 A rightward transcripts (BARTs) and their encoded EBV miRNAs are abundantly expressed in NPC (type II latency) and Burkitt's lymphoma (type I latency). Interestingly, the BamH1 H rightward opening reading frame 1 (BHRF1) encoded miRNAs are abundantly expressed in LCL cells, a type III latency, but not in type I or II latencies. The pathological role of the differential expression of EBV-encoded miRNAs in epithelial malignancies has been proposed (Lo et al., 2012). The expression profiles of EBV genes during different latency program are listed below (Table 1).

Contribution of EBV-encoded genes to epithelial malignancies

A summary of the functions of EBV genes expressed in NPC (type II latency) is reviewed here. Their potential contributions to the pathogenesis of epithelial malignancies are discussed below:

Epstein-Barr nuclear antigen 1 (EBNA1). EBNA1 is required for the persistence of EBV genomes in latently infected cells and is expressed in all EBV-associated cancers, including NPC (Yates et al., 1984; Frappier, 2012). It is involved in the replication of EBV episomes in infected cells, and their segregation into daughter cells during mitosis. The EBNA1 protein binds to the FR element in the *oriP* (origin of replication) of EBV episomes, and tether them to host cell chromosomes to ensure their even segregation during cell division (Lupton and Levine, 1985; Krysan et al., 1989; Lee et al., 1999; Nanbo et al., 2007). Inactivation of EBNA1 function reduces the copy number of EBV episomes in EBV-infected B lymphoma cell lines and inhibits their growth (Kennedy et al., 2003).

EBNA1 affects multiple cellular pathways, including cell proliferation, invasion, survival, and DNA repair. Expression of EBNA1 in EBV-negative gastric carcinoma cell lines (SCM1 and TMC1) enhances their malignant properties when grown as xenografts in nude mice (Cheng et al., 2010). In HONE1 NPC cells, EBNA1 expression also promotes tumorigenicity and metastases in nude mice (Sheu et al., 1996). This is in concordance with the effects of EBNA1 to counteract the suppressive action of Nm23-H1 on cellular proliferation and migration (Murakami et al., 2005; Kaul et al., 2007). Further evidence of EBNA1 to promote metastasis was revealed by profiling the nuclear proteome of NPC cells in response to EBNA1 overexpression (Cao et al., 2012). EBNA1 increases the nuclear levels of the metastasis related proteins, including Nm23-H1, stathmin1 and maspin. Overexpression of EBNA1 has been reported to induce epithelial-mesenchymal transition in NPC cells by inhibiting the expression of miR-200a and miR-200b, hence upregulating their target genes, the zinc finger E-box binding homeobox proteins, ZEB1 and ZEB2 (Wang et al., 2014). Interestingly, EBNA1 also enhances the angiogenic properties of NPC cells by modulating the AP-1 transcriptional pathways to enhance the secretion of VEGF (O'Neil et al., 2008). EBNA1 has been suggested to contribute to epidermal hyperplasia by inhibiting NF-κB signaling through suppressing the phosphorylation of IKK alpha/beta (Valentine et al., 2010).

Another important role of EBNA1 is to promote cell survival with DNA damage. EBNA1-expressing cells have decreased levels of p53 in response to DNA damage, and therefore are more likely to survive with DNA damage. This may be related to the action of EBNA1 to disrupt the promyelocytic leukemia (PML) nuclear bodies (Sivachandran et al., 2008), which are nuclear foci containing many cellular proteins involved in cell survival, p53 activation and DNA repair (Bernardi and Pandolfi, 2007; Salomoni et al., 2008). EBNA1 induces loss of PML nuclear bodies by binding to the CK2 kinase and ubiquitin-specific protease 7 (VSP7). The interaction of EBNA1 with USP7 leads to destabilization of p53. USP7 is known to bind to and stabilize p53 and Mdm2. EBNA1 outcompetes p53 and Mdm2 binding to USP7, leading to their degradation by the ubiquitin/proteasome system (Sivachandran et al., 2012). Upregulation of ROS and NADPH oxidase levels have been identified in EBNA1-expressing NPC cells (Cao et al., 2012), suggesting that EBNA1 promotes oxidative-stress induced DNA damage, but allows the survival of cells with DNA damage by de-

Table 1. Major EBV genes expressed in different types of latent infection.

Latency type	EBV genes expressed	Cell types/associated diseases
Type 0	EBER	Memory B cells
Type I	EBER, EBNA1, BARTs, BART-miRNAs	Burkitt's lymphoma,
Type II	EBER, EBNA1, LMP1, LMP2, BARF1, BARTs, BART-miRNAs	NPC, gastric cancer, lymphoepithelioma, Hodgkin's disease, T/NK lymphoma
Type III	EBER, EBNA1, EBNA2, EBNA3, EBNALP, LMP1, LMP2, BHRF1-miRNAs	Lymphoblastoid cell lines (LCL), lymphoproliferation in immunocompromised patients

stabilizing p53 via disruption of its interaction with USP7.

EBV-encoded small RNA 1/2 (EBER1/2). EBV encodes two small non-polyadenylated RNAs (EBER1 and EBER2), which are 167 and 172 nucleotides long, respectively, and form double-stranded RNA-like structures (Lerner et al., 1981; Takada and Nanbo, 2001). They are the most abundant viral transcripts in EBV-infected NPC and gastric cancer cells, and contribute to oncogenesis by promoting cellular growth and modulating innate immunity (Takada, 2012). A recent study has indicated that EBER may regulate LMP1/LMP2 expression and contribute to the persistence of latent EBV infection in cells (Lee et al., 2015). EBERs can induce insulin-like growth factor 1 (IGF-1) to stimulate autocrine growth of NPC cells (Iwakiri et al., 2005). Induction of IGF-1 is initiated by the activation of retinotic acid inducible gene-1 (RIG-1) and toll-like receptor 3 signaling, leading to the phosphorylation of downstream effector molecules, such as IRF-3, and the release of IGF-1 (Yoneyama et al., 2004; Samanta et al., 2008; Liu and Gu, 2011). EBERs are responsible for the immune system activation by EBV, resulting in the production of antiviral and anti-proliferative cytokines, such as type 1 interferons (IFNs). Interestingly, EBERs can counteract the effects of INFs by inhibiting the major downstream events of IFNs and PKR signaling (Yamamoto et al., 2000; Nanbo et al., 2002; Nanbo et al., 2005). EBERs block the phosphorylation of the cellular substrate of PKR, eIF-2alpha, which signals a translational block of protein synthesis that may protect EBV-infected cells from Fas-mediated apoptosis induced by IFNs (Nanbo et al., 2005).

Latent membrane protein 1 (LMP1). LMP1 is a transmembrane protein displaying numerous oncogenic properties in EBV-infected cells (Dawson et al., 2012). It is one of the earliest proteins identified to transform human B cells and rodent fibroblasts (Wang et al., 1985; Kaye et al., 1993). LMP1 is a potent activator of NF-κB signaling and is believed to play an essential role in promoting NPC development (Tsao et al., 2002; Dawson et al., 2012). However, expression of LMP1 alone could not transform immortalized/premalignant nasopharyngeal epithelial cells *in vitro* (Tsang et al., 2010; Dawson et al., 2012; Tsang et al., 2012). LMP1 acts as a constitutively activated tumor necrosis factor receptor 1, and consists of a cytoplasmic N-terminal domain, six transmembrane spinning regions and a large cytosolic C-terminal domain (Dawson et al., 2012). The transmembrane domain has been recently reported to activate the cdc42, one of the Rho GTPases that signals cytoskeleton rearrangement and invasive properties (Liu et al., 2012). The C-terminal domain contains three activation regions, CTAR1, CTAR2 and CTAR3, which are involved in activation of a panel of signaling pathways, including NF-κB, JNK/p-38, PI3K/AKT, ERK/MAPK and JAK/STAT, to elicit various oncogenic functions (Tsao et al., 2002; Li and Chang, 2003; Zheng et al., 2007; Morris et al., 2009). LMP1 promotes cell survival, proliferation and resistance to apoptosis in NPC cells. It upregulates the growth rate of NPC cells by enhancing the expression of EGFR, a growth-stimulating receptor frequently overexpressed in NPC tissues (Miller et al., 1995; Sheen et al., 1999). It also promotes the expression of anti-apoptotic proteins, such as survivin and Mcl-1, while inactivating pro-apoptotic proteins, such as Bad and Foxo3a (Tsao et al., 2002; Morris et al., 2009; Lo et al., 2010). LMP1-expressing cells exhibit impairment of the G2 checkpoint, which leads to unrepaired chromatid breaks after gamma-ray irradiation, and chromosome instability (Deng et al., 2012). LMP1 also resists the growth suppressive effect of TGF-beta by induction of the inhibitor of differentiation 1 (Id-1) protein (Lo et al., 2010). In addition, LMP1 contributes to chemo-resistance by induction of miR-21 through activation of PI3K/AKT/FOXO3 to resist apoptotic stimuli (Yang et al., 2013). LMP1 also downregulates p16/ p21 and upregulates cyclin D1 to bypass the G1/S cell cycle checkpoint (Yang et al., 2000; Huang and Huang, 2003; Lo et al., 2004a). A recent study reported that LMP1 promotes the binding of both EGFR and STAT3 to the cyclin D1 promoter to drive the expression of cyclin D1 in NPC cells (Xu et al., 2013).

Another known function of LMP1 is to enhance the invasive and metastatic potential of NPC cells. NPC is a highly metastatic cancer (Tao and Chan, 2007). LMP1 induces epithelial-mesenchyme-transition (EMT) by downregulating E-cadherin, and upregulating Twist, Snail and Slugs (Fahraeus et al., 1992; Horikawa et al., 2007; Horikawa et al., 2011; Dawson et al., 2012). LMP1 can transcriptionally induce TNF-alpha-induced protein 2 (TNFAIP2), which correlates with metastasis and poor survival in NPC patients (Chen et al., 2014). Interestingly, LMP1 increases the levels of HIF-1alpha in exosomes, which are then delivered to surrounding tumor cells for EMT induction and pro-metastatic effects (Aga et al., 2014). LMP1 can also induce the secretion of matrix metalloproteinases (MMPs) and suppress the expression of tissue inhibitor of metalloproteinases (TIMPs) to facilitate the degradation of extracellular matrix for cellular invasion or metastasis development in NPC (Horikawa et al., 2000; Yoshizaki, 2002; Lee et al., 2007; Chang et al., 2008). C-Met, an important invasive promoting protein, could be upregulated by LMP1 and has a positive association with cervical lymph node metastasis developed from primary NPC (Horikawa et al., 2001). LMP1 also regulates the expression of microRNAs, such as miRNA 203 and miRNA 10b, to promote tumor incidence and metastasis, respectively (Li et al.,

2010; Yu et al., 2012). Furthermore, LMP1 induces cancer stem/progenitor cell-like properties in NPC cells, and thereby upregulates their *in vitro* self-renewal and *in vivo* tumor initiation ability (Kondo et al., 2011). LMP1 also enhances the expression of cancer stem cell-like markers, such as CD44, by activating the Hedgehog pathway and an autocrine activation of the SHH ligand (Port et al., 2013). The CD44 high cells are more radioresistant than the CD44 low cells, which may be due to the suppressed DNA damage response and p53-induced apoptosis (Yang et al., 2014). Recent publications suggest that LMP1 also modulates the cellular metabolism to promote proliferation and transformation of NPC cells (Lo et al., 2013; Xiao et al., 2014). Upregulation of hexokinase 2 and inhibition of LKB-AMPK in LMP1-expressing cells are shown to be responsible for reprogramming of glycolysis and energy metabolism, which contribute to radioresistance. Angiogeneis is another important biological process regulated by LMP1. A higher density of microvessels can be observed in NPC tumors with high expression of LMP1 (Tsuji et al., 2008). This could be attributed to the reduced degradation of hypoxia inducible factor alpha (HIF-1alpha) and induced expression of VEGF by LMP1.

Latent membrane protein 2 (LMP2). The LMP2 proteins, LMP2A and LMP2B, are transcribed from two distinct mRNAs encoding 54-kDa and 40-kDa proteins, respectively. LMP2A/B is an integral membrane protein with 12 transmembrane spanning regions. Their mRNAs share the same exons (2 to 9) (Sample et al., 1989; Pang et al., 2009; Dawson et al., 2012). While the exon 1 of LMP2B (exon 1B) is non-coding, the exon 1 of LMP2A (exon 1A) encodes an additional cytosolic N-terminus, which mediates multiple signaling processes (Sample et al., 1989; Pang et al., 2009; Dawson et al., 2012). The N-terminal domain contains multiple signaling domains, including an immunoreceptor tyrosine-based activation motif (ITAM) recognized by the Lyn/Syk kinases to transduce BCR signaling, and a PY motif that interacts with the NEDD4 family of ubiquitin ligases (Ikeda et al., 2000; Portis et al., 2002; Ikeda et al., 2003). Other signaling pathways downstream of these domains include PI3k/akt, RhoA, and MAPK/ERK (Heussinger et al., 2004; Pang et al., 2009; Dawson et al., 2012).

Genetic studies have revealed that LMP2A and LMP2B are not required for EBV-dependent transformation of B cells; however, LMP2A is required for the successful outgrowth of EBV-infected epithelial cells *in vitro* (Speck et al., 1999). LMP2 also induces anchorage-independent growth in soft agar and inhibits differentiation through activation of PI3 kinase and the Akt kinase (Scholle et al., 2000; Fukuda and Longnecker, 2007). In epithelial cells, LMP2 can promote β-catenin signaling through the activation of Akt and phosphorylation of GSK3 (Morrison and Raab-Traub, 2005). Activation of β-catenin is common in the development of carcinoma through genetic mutations, suggesting that activation of this pathway may mediate the effects of EBV on epithelial cell growth. LMP2A was also shown to inhibit cellular differentiation and promote cell survival through the PI3K/Akt-mediated stabilization of ΔNp63 (Fotheringham et al., 2010). Other roles of LMP2A-activated PI3K/akt signaling include the counteraction of the growth inhibitory and pro-apoptotic effects of TGF-beta1 during epithelial carcinogenesis (Fukuda and Longnecker, 2004), and the proliferation and protein synthesis in cells via the activation of mTOR pathway (Moody et al., 2005). LMP2A and LMP2B have also been shown to limit the anti-viral response against EBV-infected cells by modulating IFN signaling (Shah et al., 2009). Response to IFN was downregulated in LMP2A or LMP2B-expressing epithelial cells due to an increased turnover of IFN receptors (Shah et al., 2009). Lastly, similar to LMP1, LMP2A can promote the invasive/migratory properties of epithelial cells, which may relate to the metastatic phenotype of NPC (Allen et al., 2005; Pegtel et al., 2005; Lu et al., 2006; Kong et al., 2010). Studies have suggested that LMP2A and LMP2B modulate the interaction and focal adhesion formation with the extracellular matrix (Allen et al., 2005; Pegtel et al., 2005). Cells overexpressing LMP2A/B have an increased rate of attachment, spreading and migratory movement on the extracellular matrix (Allen et al., 2005). This possibly involves the regulation of integrin-mediated processes. A recent study has shown that the ITAM signaling domain of LMP2A can activate the Syk tyrosine kinase and Akt to stabilize alphaV-integrin and FAK activation (Fotheringham et al., 2012). Another study showed that LMP2A expression is positively associated with integrin alpha6 in NPC biopsies (Pegtel et al., 2005). Antibodies blocking the integrins abrogate LMP2A-induced invasion (Pegtel et al., 2005). These reports suggest an interaction of LMP2A with integrins to govern the migratory, invasiveness and metastasis of the epithelial cancers. LMP2A was reported to be localized at the tumor invasive front (Kong et al., 2010). It can also potentiate cancer stem cell-like properties through activation of the Hedgehog signaling pathway (Port et al., 2013). Exogenous expression of LMP2A induces EMT, stimulates the expression of stem cell markers, and enhances the acquisition of side populations in the NPC cells (Kong et al., 2010).

BamH1-A fragment rightward reading frame 1 (BARF1). The BARF1 is considered a lytic EBV protein and is expressed early during lytic infection. However, a high level of BARF1 expression could be detected at high levels in NPC (Decaussin et al., 2000). BARF1 is encoded in the BamH1-A fragment of EBV and is a homolog of

the human colony stimulating factor 1 (CSF-1) receptor (Strockbine et al., 1998). The signaling axis of CSF-1 and the CSF-1 receptor is known to be involved in promoting tumorigenicity in various types of epithelial cancer, including gastric and breast cancer (Sapi et al., 1995; Lin et al., 2001). It was also shown to have oncogenic activity, as evidenced by its malignant transforming property in rodent fibroblasts and inhibitory effects on apoptosis by activating bcl-2 (Wei and Ooka, 1989; Sheng et al., 2003). Expression of BARF1, in addition to the H-Ras and SV40 T antigens, can transform non-malignant human nasopharyngeal epithelial NP69 cells (Jiang et al., 2009). BARF1 is expressed as a latent protein in NPC and EBV-associated gastric cancer (Decaussin et al., 2000; Seto et al., 2005; Takada, 2012). It can be detected by RT-PCR and immunohistochemical assays in clinical biopsies of NPC and by a BARF1-specific nucleic acid sequence-based amplification assay in gastric tumors (Decaussin et al., 2000; zur Hausen et al., 2000). BARF1 is expressed in EBV-associated epithelial malignancies, but not in lymphoid malignancies (Takada, 2012). Detection of BARF1 mRNA in nasopharyngeal brushings has been suggested to be a promising noninvasive method for NPC diagnosis (Stevens et al., 2006).

The role of BARF1 in NPC development has been investigated by infecting CNE2 NPC cells with a recombinant EBV with BARF1 constitutively expressed under the SV40 promoter (Seto et al., 2008). Compared to control NPC cells infected with wild-type EBV, NPC cells infected with EBV constitutively expressing BARF1 have higher growth rates and are more resistant to apoptosis in serum-deprived conditions. BARF1 was detected in the culture medium, which promoted growth of the cancer cells. NPC cells infected with recombinant EBV constitutively expressing BARF1 have greater rates of tumorigenicity in the nude mice model (Seto et al., 2008). In EBV-associated gastric cancer, BARF1 enhanced the expression of cyclin D1 *in vitro* (Wiech et al., 2008). Analysis of a tissue array consisting of 170 gastric tumors and 11 EBV-associated gastric tumors revealed a significant overexpression of cyclin D1 in EBV-associated tumors but not in EBV-negative tumors (Wiech et al., 2008). A recent study showed that overexpression of BARF1 in gastric cancer cells promotes cellular proliferation, likely through the upregulation of expression of NF-κB, RelA, cyclin D1, and reduced expression of cell cycle inhibitor, p21 (Chang et al., 2013).

Bam HI A rightward transcripts (BARTs). The BARTs are multi-spliced RNAs transcribed rightwards from the BamH1 A region of EBV (Hitt et al., 1989; Smith et al., 2000; Zhang et al., 2001). The exceptional abundance of BART expression in NPC and EBV-associated gastric cancer strongly implicates an important role in these cancers (Smith et al., 2000; Al-Mozaini et al., 2009). BARTs comprise more than 96% of all EBV reads in a recent RNA-sequencing analysis of EBV-associated gastric carcinoma (Strong et al., 2013). Several ORFs in the spliced cDNA transcripts, including RPMS1, A73, BARF0, CST, vIL, and BLLF1, have been postulated to have functional roles (Kienzle et al., 1999; Al-Mozaini et al., 2009). In particular, recombinant RPMS1 and A73 expressed in *E. coli* were found to modulate the Notch and RACK1 signaling pathways, respectively (Smith et al., 2000; Al-Mozaini et al., 2009). RPMS1 can act as effective antagonist of Notch-IC transcription activation, and therefore may suppress the differentiation of epithelial cells (Smith et al., 2000). A73 binds with RACK1 and possibly regulates calcium release from intracellular stores by enhancing the affinity of IP3 receptor binding for IP3 (Smith et al., 2000; Al-Mozaini et al., 2009). These reports suggest that BARTs may encode for proteins having biochemical functions related to oncogenesis. However, evidence for the endogenous expression of potential BART-proteins, such as RPMS1, A73 and BARFO, in EBV-infected cells is lacking (Kienzle et al., 1999; Al-Mozaini et al., 2009). Moreover, the BARTs are expressed extensively in the nucleus, but not in the cytoplasm, suggesting they are not transcribed as mRNAs (Al-Mozaini et al., 2009; Jang et al., 2011). Nevertheless, the possibility remains that these BART-proteins may be expressed under certain conditions to augment the development of cancer (Al-Mozaini et al., 2009). Another possibility is that these BARTs may act as long non-coding RNAs (lncRNA), which are involved in repressive complexes to regulate cellular gene expression (Strong et al., 2013). Interestingly, the expression patterns or levels of BARTs vary in different infection states, such as lytic and latent infections (Yamamoto and Iwatsuki, 2012). Notably, the expression of BARTs is under the regulation of c-myc and C/EBP (Chen et al., 2005) and possibly NF-κB (HL Chen, personal communication). This highlights the potential importance of local inflammation and the role of inflammatory cytokines in affecting the expression of BARTs. All this warrants the future investigation of potential functional roles of BARTs in contributing to human malignancies, particularly in NPC.

EBV-encoded microRNAs (miRNAs). EBV encodes at least 44 miRNAs transcribed from the BHRF1 and BART regions (Klinke et al., 2014). miRNAs can regulate the expression of various proteins by blocking the translation of mRNAs or degrading mRNAs (Bartel, 2009). The expression pattern of miRNAs depends on the latency type and cellular context of the EBV-infected cells. BHRF1 miRNAs are only expressed in EBV-infected B cells exhibiting latency type III infection, and are shown to mediate B cell transformation by protecting

the cells from apoptosis (Amoroso et al., 2011; Vereide et al., 2014). BART miRNAs are expressed in all EBV-infected cells, but the levels are 8 to 13 fold higher in epithelial cells than in B cells (Qiu et al., 2011). In NPC and gastric cancer samples, most of the BART miRNAs are expressed. Although they are processed from the same BART transcript, they are expressed at various levels due to different biogenesis and cellular processing (Zhu et al., 2009; Chen et al., 2010; Lung et al., 2013).

The functions of BART miRNAs have been evaluated in various high-throughput studies. Recent reviews have summarized the results from different research groups in searching for high-confidence targets of EBV miRNAs (Marquitz and Raab-Traub, 2012; Cullen, 2013; Klinke et al., 2014). A few key targets of the BART miRNAs have been identified and validated for their functions. For example, miR-BART3-5p targets the tumor-suppressor gene, DICE1 (Lei et al., 2013). This is in agreement with the finding that DICE1 is usually inversely correlated with the expression of miR-BART3-5p in NPC. The miR-BART2-5p targets the stress-induced immune ligand MICB to facilitate the escape from recognition by natural killer cells (Nachmani et al., 2009). In addition, BART miRNAs can target pro-apoptotic genes and thus promote host cell survival (Choy et al., 2008; Marquitz et al., 2011). Expression of PUMA-beta is regulated by miR-BART5-5p (Choy et al., 2008), and expression of Bim is regulated by miR-BART1, 3, 9, 11 and 12 (Marquitz et al., 2011). A recent study also showed that miR-BART9 promotes the invasiveness and metastatic ability of NPC cells *in vivo* through specific targeting of E-cadherin, a membrane protein crucial for mesenchymal-like phenotype (Hsu et al., 2014).

EBV genes can also be targets of EBV miRNAs (Lo et al., 2007; Barth et al., 2008; Lung et al., 2009). The viral DNA polymerase BALF5 is targeted by miR-BART2-5p (Barth et al., 2008), the EBV latent membrane protein 1 (LMP1) by miR-BART17-5p, -1-5p or -16 (Lo et al., 2007), and LMP2A by miR-BART22 (Lung et al., 2009). This implicates a role for BART miRNAs in the modulation of EBV gene expression to optimize the functions of various EBV proteins in infected cells.

GENETIC ALTERATIONS IN EBV-ASSOCIATED EPITHELIAL MALIGNANCIES

EBV infection in NPC was shown to be a clonal event and occurs during the early stages of NPC (Pathmanathan et al., 1995). Later studies have demonstrated that genetic alterations in the premalignant nasopharyngeal epithelium may precede EBV infection (Lo et al., 2004b; Tsang et al., 2012). Genetic alterations in the premalignant nasopharyngeal epithelium may confer susceptibility to latent EBV infection, which otherwise would support lytic EBV infection.

By comparing changes in EBV-infected and uninfected cancers, evidence for the pathogenic mechanism of EBV may be revealed. In NPC, this is not possible as most cases of undifferentiated NPC are associated with EBV infection. However, gastric cancer provides a unique opportunity to examine the genomic differences that may be related to EBV infection. Recent genomic profiling in EBV-associated gastric cancer reveals a distinct signature of genome wide hypermethylation compared to non-EBV gastric cancer (TCGA, 2014). Hypermethylation is commonly used in the inactivation of tumor suppressor genes. It remains to be determined if the increase of methylation in EBV-associated gastric cancer is a direct result of EBV gene expression or an adaptive response of host cells to EBV infection.

A recent genomic profile of NPC reveals multiple pathways present in NPC, and reveals a similar signature of genomic hypermethylation (Lin et al., 2014). Similar to EBV-associated gastric cancer, fewer genetic alterations were identified in NPC compared to other epithelial cancers. Presumably, EBV infection may play an important role in altering cellular pathways to promote survival of infected NPC cells, which facilitates the selection and expansion of tumorigenic clones *in vivo*. These reports support a causal role of EBV infection in the development of NPC.

EBV strains and NPC

It is postulated that specific EBV strain may be involved in the development of NPC. Multiple strains of EBV can be isolated from the blood and saliva of healthy individuals. Interestingly, only one strain of EBV was detected in a large cohort of NPC samples (JX Bei, Sun Yat Sen Cancer Center, personal communication). The presence of a single strain of EBV in NPC is not surprising, given the clonal origin of EBV infection at the early stages of NPC development. The NPC-associated EBV strains cluster into a distinct family that could be separated from EBV strains isolated from patients with infectious mononucleosis. In the endemic area of NPC in southern China, a specific EBV strain has been proposed to be associated with NPC. Recently, an NPC-derived EBV strain, M81, was isolated with distinct properties in host tropism and other biological properties (Tsai et al., 2013). The M81 EBV strain has a reverse tropism compared to common EBV strains, exhibiting a reduced ability to infect B cells but an increased propensity to infect epithelial cells. M81 spontaneously enters lytic replication upon infection of B cells. It remains to be determined if a specific NPC EBV strain with distinct biological properties may be involved in the pathogenesis of NPC. With further research on the genomic and biological properties of EBV isolated from NPC, the role of EBV in the devel-

opment of NPC may be better understood.

Contribution of lytic EBV infection in human malignancies

The master switch of EBV from latent to lytic infection is triggered by the expression of BZLF1, which turns on a cascade of events that target EBV gene transcription to initiate EBV replication, packaging and release of infectious virus (Kenney and Mertz, 2014). The BRLF1 protein is also involved in the switching of EBV infection from latent to lytic mode, and may play a more important role in the lytic EBV infection of epithelial cells (Reusch et al., 2015). The physiological signals triggering lytic infection are not clearly defined, but may involve signals of differentiation and cellular stress. Lytic EBV infection was observed in non-keratinized squamous epithelial cells on the lateral side of the tongue epithelium of immunocompromised patients (Greenspan et al., 1985). Lytic replication of EBV could be demonstrated in the upper layers of the stratified squamous epithelium undergoing terminal differentiation, but not at the basal or immediate suprabasal layers, where undifferentiated epithelial cells are located. In a 3-dimensional reconstructed epithelium model of telomerase-immortalized oral keratinocytes cultured at the air-liquid phase, expression of lytic EBV genes was observed in the upper layers of differentiated epithelium but not in the undifferentiated basal layers (Kenney and Mertz, 2014). While latent EBV infection is characteristic of human malignancies, lytic EBV infection may also be involved. Interestingly, EBV defective in the lytic EBV gene, BZLF1, was found to have lower tumorigenic ability to transform B cells into lymphoma in the humanized mice model (Hong et al., 2005). A low level of expression of lytic EBV genes is often observed in NPC with predominantly latent infections (Feng et al., 2000). The significance of this low level of lytic gene expression is unclear. The late lytic genes involved in packaging of EBV for infection are not expressed, suggesting that the lytic infection is largely abortive in nature. The role of abortive lytic EBV infection in human malignancies is unclear. The BZLF1 and other lytic genes, including BGLF5, have been shown to induce DNA instability and may be involved at the initiation stage of carcinogenesis of nasopharyngeal epithelium (Sato et al., 2009; Wu et al., 2010). Promotion of premalignant nasopharyngeal epithelial cells harboring genetic mutations may be dependent on the inflammatory environment commonly present at the nasopharyngeal mucosa. It remains to be determined if these lytic genes invoke a local immune and inflammatory response to promote tumor progression in NPC (Rickinson, 2014).

Contribution of host stromal factors to the persistence of EBV infection in NPC

Despite the close association of EBV infection observed in NPC, NPC cell lines established *in vitro* readily lose their EBV episomes upon prolonged propagation. This would suggest that EBV infection *per se* has no advantage in cell proliferation. Careful monitoring of replication of EBV episomes in infected cells suggests 16% of EBV episome are not replicated during each cell cycle (Nanbo et al., 2007). Hence, EBV episomes would be lost in cell culture if EBV-infected cells were not actively selected either by drugs or cell sorting. In contrast to EBV infection of human B cells, which efficiently promotes cell growth and transformation, EBV infection of primary nasopharyngeal epithelial cells induces growth arrest, probably due to the cellular stress associated with viral infection (Tsang et al., 2012). No immediate growth advantage was observed in immortalized nasopharyngeal epithelial cells after infection with EBV. The universal presence of EBV in NPC and the ability of NPC xenografts to retain their EBV episomes after repeated passage (> 25 years) in athymic nude mice suggest an advantage for EBV-infected epithelial cells grown *in vivo*. A recent report shows that expression of EBV-encoded miRNA was upregulated in EBV-infected NPC and gastric cancer cells grown *in vivo*. Furthermore, expression of BARTs enhances the growth and tumorigenicity of these cells, supporting a role for BART expression and BART-miRNA in the growth of EBV-infected epithelial cells *in vivo* (Qiu et al., 2015). Similarly, EBV infection and expression of EBV genes may induce inflammatory responses, which may enhance angiogenesis and growth of cancer cells *in vivo*. These cytokines may also attract migration of inflammatory cells, including activated macrophages and T regulatory cells, to support the growth of EBV-infected cells. Furthermore, we have also observed that stromal fibroblasts isolated from NPC synthesize and secrete IL-6 to stimulate EBV-infected NPC cells (Tsang et al., 2013; Zhang et al., 2013). EBV-associated gastric cancer is also associated with inflammatory components. A distinguishing feature of EBV-associated epithelial malignancies is the undifferentiated property of infected epithelial cells associated with the heavy infiltration of lymphoid elements; hence, the term lymphoepithelioma-like carcinoma (LELC) has been used to distinguish this group of cancer, which could also be observed in lung cancer, tonsillar and cholangiocarcinoma (Tsao et al., 2015). The stromal factors that support growth of NPC cells *in vivo* are not present in *in vitro* conditions. The postulation is that growth of NPC cells is highly dependent on the presence of inflammatory stromal elements in the nasopharyngeal mucosa, which is in a state of chronic inflammation. In concordance with this hypothesis, EBV-positive NPC cell lines are known to be difficult to establish in culture, likely due to the loss of

growth promoting cytokines released from stromal cells present in NPC *in vivo*. The delineation and characterization of the growth requirement for these stromal elements in NPC cell lines would contribute to our understanding of the development of NPC and other EBV-infected malignancies.

CONCLUSION

The role of EBV in the pathogenesis of NPC is still unknown. There are selective advantages of EBV infection in NPC *in vivo*, which may be facilitated by inflammatory elements in the mucosa. The understanding of these selective advantages will contribute to our understanding of the pathogenic role of EBV infection in human malignancies. A close interaction between EBV infection, host genetic alterations, defective immune recognition and stromal inflammation are believed to be intricately involved in the pathogenesis of NPC. Defining the contribution of these parameters in the growth of

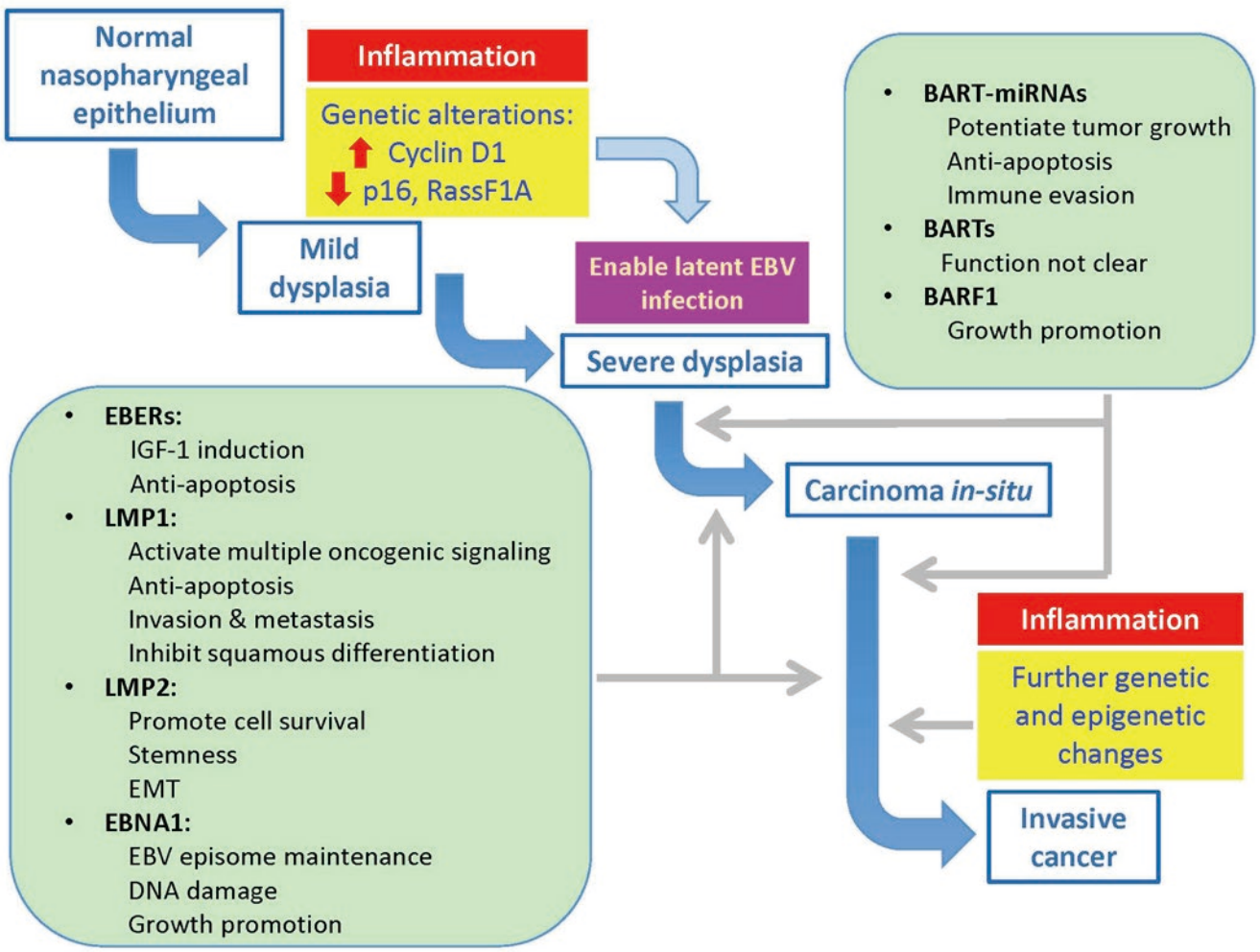

Figure 1. The role of EBV infection in the pathogenesis of NPC. The normal nasopharyngeal epithelium is refractory to EBV infection. Similar to EBV infection of oropharyngeal epithelial cells, EBV infection in the normal nasopharyngeal epithelium is presumably lytic in nature. EBV infection induces growth arrest in normal nasopharyngeal epithelial cells. Genetic alterations in the premalignant nasopharyngeal epithelium, such as cyclin D1 overexpression and p16 inactivation, override the growth arrest induced by EBV infection and support latent infection. EBV infection and expression of EBV-encoded latent genes, including BART-miRNAs, support the growth and progression of premalignant nasopharyngeal epithelial cells. Further genetic and epigenetic changes may drive the clonal expansion of EBV-infected premalignant cells and their transformation to cancer cells. Stromal inflammation is postulated to play a crucial role in modulating EBV gene expression, supporting latent EBV infection and malignant transformation of premalignant nasopharyngeal epithelial cells to cancer cells.

NPC *in vivo* will provide novel therapeutic targets in the prevention and treatment of NPC. A summary of key events is included in Figure 1 to illustrate the postulated roles of EBV in NPC pathogenesis.

ACKNOWLEDGMENTS

The authors acknowledge the generous funding sources for the above study: the Health and Medical Research Fund (Grant No HMRF: 12110942 and 13120872) to CMT; and GRF grants from the Hong Kong Research Grant Council (17120814, 779713, 779312, 780911, 779810), AoE NPC (Grant No. AoE/M-06/08) and the Theme-Based Research Scheme (Grant No. T12-401/13-R) to SWT.

COMPLIANCE WITH ETHICS GUIDELINES

The authors declare that they have no conflict of interest. This article does not contain any studies with human or animal subjects performed by any of the authors.

REFERENCES

Aga M, Bentz GL, Raffa S, Torrisi MR, Kondo S, Wakisaka N, Yoshizaki T, Pagano JS, Shackelford J. 2014. Exosomal HIF1alpha supports invasive potential of nasopharyngeal carcinoma-associated LMP1-positive exosomes. Oncogene, 33: 4613–4622.

Al-Mozaini M, Bodelon G, Karstegl CE, Jin B, Al-Ahdal M, Farrell PJ. 2009. Epstein-Barr virus BART gene expression. J Gen Virol, 90: 307–316.

Allen MD, Young LS, Dawson CW. 2005. The Epstein-Barr virus-encoded LMP2A and LMP2B proteins promote epithelial cell spreading and motility. J Virol, 79: 1789–1802.

Amoroso R, Fitzsimmons L, Thomas WA, Kelly GL, Rowe M, Bell AI. 2011. Quantitative studies of Epstein-Barr virus-encoded microRNAs provide novel insights into their regulation. J Virol, 85: 996–1010.

Babcock GJ, Decker LL, Volk M, Thorley-Lawson DA. 1998. EBV persistence in memory B cells *in vivo*. Immunity, 9: 395–404.

Bartel DP. 2009. MicroRNAs: target recognition and regulatory functions. Cell, 136: 215–233.

Barth S, Pfuhl T, Mamiani A, Ehses C, Roemer K, Kremmer E, Jaker C, Hock J, Meister G, Grasser FA. 2008. Epstein-Barr virus-encoded microRNA miR-BART2 down-regulates the viral DNA polymerase BALF5. Nucleic Acids Res, 36: 666–675.

Bernardi R, Pandolfi PP. 2007. Structure, dynamics and functions of promyelocytic leukaemia nuclear bodies. Nat Rev Mol Cell Biol, 8: 1006–1016.

Borza CM, Hutt-Fletcher LM. 2002. Alternate replication in B cells and epithelial cells switches tropism of Epstein-Barr virus. Nat Med, 8: 594–599.

Cancer Genome Atlas Research N. 2014. Comprehensive molecular characterization of gastric adenocarcinoma. Nature, 513: 202–209.

Cao JY, Mansouri S, Frappier L. 2012. Changes in the naso-pharyngeal carcinoma nuclear proteome induced by the EBNA1 protein of Epstein-Barr virus reveal potential roles for EBNA1 in metastasis and oxidative stress responses. J Virol, 86: 382–394.

Chang MS, Kim DH, Roh JK, Middeldorp JM, Kim YS, Kim S, Han S, Kim CW, Lee BL, Kim WH, Woo JH. 2013. Epstein-Barr virus-encoded BARF1 promotes proliferation of gastric carcinoma cells through regulation of NF-kappaB. J Virol, 87: 10515–10523.

Chang SH, Chang HC, Hung WC. 2008. Transcriptional repression of tissue inhibitor of metalloproteinase-3 by Epstein-Barr virus latent membrane protein 1 enhances invasiveness of nasopharyngeal carcinoma cells. Oral Oncol, 44: 891–897.

Chen CC, Liu HP, Chao M, Liang Y, Tsang NM, Huang HY, Wu CC, Chang YS. 2014. NF-kappaB-mediated transcriptional up-regulation of TNFAIP2 by the Epstein-Barr virus oncoprotein, LMP1, promotes cell motility in nasopharyngeal carcinoma. Oncogene, 33: 3648–3659.

Chen H, Huang J, Wu FY, Liao G, Hutt-Fletcher L, Hayward SD. 2005. Regulation of expression of the Epstein-Barr virus BamHI-A rightward transcripts. J Virol, 79: 1724–1733.

Chen SJ, Chen GH, Chen YH, Liu CY, Chang KP, Chang YS, Chen HC. 2010. Characterization of Epstein-Barr virus miRNAome in nasopharyngeal carcinoma by deep sequencing. PLoS One, 5.

Cheng TC, Hsieh SS, Hsu WL, Chen YF, Ho HH, Sheu LF. 2010. Expression of Epstein-Barr nuclear antigen 1 in gastric carcinoma cells is associated with enhanced tumorigenicity and reduced cisplatin sensitivity. Int J Oncol, 36: 151–160.

Chesnokova LS, Nishimura SL, Hutt-Fletcher LM. 2009. Fusion of epithelial cells by Epstein-Barr virus proteins is triggered by binding of viral glycoproteins gHgL to integrins alphavbeta6 or alphavbeta8. Proc Natl Acad Sci U S A, 106: 20464–20469.

Choy EY, Siu KL, Kok KH, Lung RW, Tsang CM, To KF, Kwong DL, Tsao SW, Jin DY. 2008. An Epstein-Barr virus-encoded microRNA targets PUMA to promote host cell survival. J Exp Med, 205: 2551–2560.

Cullen BR. 2013. MicroRNAs as mediators of viral evasion of the immune system. Nat Immunol, 14: 205–210.

Dawson CW, Port RJ, Young LS. 2012. The role of the EBV-encoded latent membrane proteins LMP1 and LMP2 in the pathogenesis of nasopharyngeal carcinoma (NPC). Semin Cancer Biol, 22: 144–153.

Decaussin G, Sbih-Lammali F, de Turenne-Tessier M, Bouguermouh A, Ooka T. 2000. Expression of BARF1 gene encoded by Epstein-Barr virus in nasopharyngeal carcinoma biopsies. Cancer Res, 60: 5584–5588.

Deng W, Pang PS, Tsang CM, Hau PM, Yip YL, Cheung AL, Tsao SW. 2012. Epstein-Barr virus-encoded latent membrane protein 1 impairs G2 checkpoint in human nasopharyngeal epithelial cells through defective Chk1 activation. PLoS One, 7: e39095.

Fahraeus R, Chen W, Trivedi P, Klein G, Obrink B. 1992. Decreased expression of E-cadherin and increased invasive capacity in EBV-LMP-transfected human epithelial and murine adenocarcinoma cells. Int J Cancer, 52: 834–838.

Feng P, Ren EC, Liu D, Chan SH, Hu H. 2000. Expression of Epstein-Barr virus lytic gene BRLF1 in nasopharyngeal carcinoma: potential use in diagnosis. J Gen Virol, 81: 2417–2423.

Fotheringham JA, Coalson NE, Raab-Traub N. 2012. Epstein-Barr virus latent membrane protein-2A induces ITAM/Syk- and Akt-dependent epithelial migration through alphav-integrin membrane translocation. J Virol, 86: 10308–10320.

Fotheringham JA, Mazzucca S, Raab-Traub N. 2010. Epstein-Barr virus latent membrane protein-2A-induced DeltaNp63alpha expression is associated with impaired epithelial-cell differentiation. Oncogene, 29: 4287–4296.

Frappier L. 2012. Role of EBNA1 in NPC tumourigenesis. Semin Cancer Biol, 22: 154–161.

Fukuda M, Longnecker R. 2004. Latent membrane protein 2A inhibits transforming growth factor-beta 1-induced apoptosis through the phosphatidylinositol 3-kinase/Akt pathway. J Virol, 78: 1697–1705.

Fukuda M, Longnecker R. 2007. Epstein-Barr virus latent membrane protein 2A mediates transformation through constitutive activation of the Ras/PI3-K/Akt Pathway. J Virol, 81: 9299–9306.

Greenspan JS, Greenspan D, Lennette ET, Abrams DI, Conant MA, Petersen V, Freese UK. 1985. Replication of Epstein-Barr virus within the epithelial cells of oral "hairy" leukoplakia, an AIDS-associated lesion. N Engl J Med, 313: 1564–1571.

Hadinoto V, Shapiro M, Sun CC, Thorley-Lawson DA. 2009. The dynamics of EBV shedding implicate a central role for epithelial cells in amplifying viral output. PLoS Pathog, 5: e1000496.

Henle W, Diehl V, Kohn G, Zur Hausen H, Henle G. 1967. Herpes-type virus and chromosome marker in normal leukocytes after growth with irradiated Burkitt cells. Science, 157: 1064–1065.

Heussinger N, Buttner M, Ott G, Brachtel E, Pilch BZ, Kremmer E, Niedobitek G. 2004. Expression of the Epstein-Barr virus (EBV)-encoded latent membrane protein 2A (LMP2A) in EBV-associated nasopharyngeal carcinoma. J Pathol, 203: 696–699.

Hitt MM, Allday MJ, Hara T, Karran L, Jones MD, Busson P, Tursz T, Ernberg I, Griffin BE. 1989. EBV gene expression in an NPC-related tumour. EMBO J, 8: 2639–2651.

Hochberg D, Middeldorp JM, Catalina M, Sullivan JL, Luzuriaga K, Thorley-Lawson DA. 2004. Demonstration of the Burkitt's lymphoma Epstein-Barr virus phenotype in dividing latently infected memory cells *in vivo*. Proc Natl Acad Sci U S A, 101: 239–244.

Hong GK, Gulley ML, Feng WH, Delecluse HJ, Holley-Guthrie E, Kenney SC. 2005. Epstein-Barr virus lytic infection contributes to lymphoproliferative disease in a SCID mouse model. J Virol, 79: 13993–14003.

Horikawa T, Sheen TS, Takeshita H, Sato H, Furukawa M, Yoshizaki T. 2001. Induction of c-Met proto-oncogene by Epstein-Barr virus latent membrane protein-1 and the correlation with cervical lymph node metastasis of nasopharyngeal carcinoma. Am J Pathol, 159: 27–33.

Horikawa T, Yang J, Kondo S, Yoshizaki T, Joab I, Furukawa M, Pagano JS. 2007. Twist and epithelial-mesenchymal transition are induced by the EBV oncoprotein latent membrane protein 1 and are associated with metastatic nasopharyngeal carcinoma. Cancer Res, 67: 1970–1978.

Horikawa T, Yoshizaki T, Kondo S, Furukawa M, Kaizaki Y, Pagano JS. 2011. Epstein-Barr Virus latent membrane protein 1 induces Snail and epithelial-mesenchymal transition in metastatic nasopharyngeal carcinoma. Br J Cancer, 104: 1160–1167.

Horikawa T, Yoshizaki T, Sheen TS, Lee SY, Furukawa M. 2000. Association of latent membrane protein 1 and matrix metalloproteinase 9 with metastasis in nasopharyngeal carcinoma. Cancer, 89: 715–723.

Hsu CY, Yi YH, Chang KP, Chang YS, Chen SJ, Chen HC. 2014. The Epstein-Barr virus-encoded microRNA MiR-BART9 promotes tumor metastasis by targeting E-cadherin in nasopharyngeal carcinoma. PLoS Pathog, 10: e1003974.

Huang H, Huang PC. 2003. Effects of two LMP1 variants on resistance of CNE1 cell strain to TGF-beta1. Ai Zheng, 22: 1254–1259. (In Chinese)

Ikeda A, Caldwell RG, Longnecker R, Ikeda M. 2003. Itchy, a Nedd4 ubiquitin ligase, downregulates latent membrane protein 2A activity in B-cell signaling. J Virol, 77: 5529–5534.

Ikeda M, Ikeda A, Longan LC, Longnecker R. 2000. The Epstein-Barr virus latent membrane protein 2A PY motif recruits WW domain-containing ubiquitin-protein ligases. Virology, 268: 178–191.

Iwakiri D, Sheen TS, Chen JY, Huang DP, Takada K. 2005. Epstein-Barr virus-encoded small RNA induces insulin-like growth factor 1 and supports growth of nasopharyngeal carcinoma-derived cell lines. Oncogene, 24: 1767–1773.

Jang BG, Jung EJ, Kim WH. 2011. Expression of BamHI-A Rightward Transcripts in Epstein-Barr Virus-Associated Gastric Cancers. Cancer Res Treat, 43: 250–254.

Jiang R, Cabras G, Sheng W, Zeng Y, Ooka T. 2009. Synergism of BARF1 with Ras induces malignant transformation in primary primate epithelial cells and human nasopharyngeal epithelial cells. Neoplasia, 11: 964–973.

Kaul R, Murakami M, Choudhuri T, Robertson ES. 2007. Epstein-Barr virus latent nuclear antigens can induce metastasis in a nude mouse model. J Virol, 81: 10352–10361.

Kaye KM, Izumi KM, Kieff E. 1993. Epstein-Barr virus latent membrane protein 1 is essential for B-lymphocyte growth transformation. Proc Natl Acad Sci U S A, 90: 9150–9154.

Kennedy G, Komano J, Sugden B. 2003. Epstein-Barr virus provides a survival factor to Burkitt's lymphomas. Proc Natl Acad Sci U S A, 100: 14269–14274.

Kenney SC, Mertz JE. 2014. Regulation of the latent-lytic switch in Epstein-Barr virus. Semin Cancer Biol, 26: 60–68.

Kienzle N, Buck M, Greco S, Krauer K, Sculley TB. 1999. Epstein-Barr virus-encoded RK-BARF0 protein expression. J Virol, 73: 8902–8906.

Klinke O, Feederle R, Delecluse HJ. 2014. Genetics of Epstein-Barr virus microRNAs. Semin Cancer Biol, 26:52-9.

Kondo S, Wakisaka N, Muramatsu M, Zen Y, Endo K, Murono S, Sugimoto H, Yamaoka S, Pagano JS, Yoshizaki T. 2011. Epstein-Barr virus latent membrane protein 1 induces cancer stem/progenitor-like cells in nasopharyngeal epithelial cell lines. J Virol, 85: 11255–11264.

Kong QL, Hu LJ, Cao JY, Huang YJ, Xu LH, Liang Y, Xiong D, Guan S, Guo BH, Mai HQ, Chen QY, Zhang X, Li MZ, Shao JY, Qian CN, Xia YF, Song LB, Zeng YX, Zeng MS. 2010. Epstein-Barr virus-encoded LMP2A induces an epithelial-mesenchymal transition and increases the number of side population stem-like cancer cells in nasopharyngeal carcinoma. PLoS Pathog, 6: e1000940.

Krysan PJ, Haase SB, Calos MP. 1989. Isolation of human sequences that replicate autonomously in human cells. Mol Cell Biol, 9: 1026–1033.

Le QT, Zhang Q, Cao H, Cheng AJ, Pinsky BA, Hong RL, Chang JT, Wang CW, Tsao KC, Lo YD, Lee N, Ang KK, Chan AT, Chan KC. 2013. An international collaboration to harmonize the quantitative plasma Epstein-Barr virus DNA assay for future biomarker-guided trials in nasopharyngeal carcinoma. Clin Cancer Res, 19: 2208–2215.

Lee DC, Chua DT, Wei WI, Sham JS, Lau AS. 2007. Induction of matrix metalloproteinases by Epstein-Barr virus latent membrane protein 1 isolated from nasopharyngeal carcinoma. Biomed Pharmacother, 61: 520–526.

Lee MA, Diamond ME, Yates JL. 1999. Genetic evidence that EBNA-1 is needed for efficient, stable latent infection by Epstein-Barr virus. J Virol, 73: 2974–2982.

Lee N, Moss WN, Yario TA, Steitz JA. 2015. EBV noncoding RNA binds nascent RNA to drive host PAX5 to viral DNA. Cell, 160: 607–618.

Lei T, Yuen KS, Xu R, Tsao SW, Chen H, Li M, Kok KH, Jin DY. 2013. Targeting of DICE1 tumor suppressor by Epstein-Barr virus-encoded miR-BART3* microRNA in nasopharyngeal carcinoma. Int J Cancer, 133: 79–87.

Lerner MR, Andrews NC, Miller G, Steitz JA. 1981. Two small RNAs encoded by Epstein-Barr virus and complexed with protein are precipitated by antibodies from patients with systemic lupus erythematosus. Proc Natl Acad Sci U S A, 78: 805–809.

Li G, Wu Z, Peng Y, Liu X, Lu J, Wang L, Pan Q, He ML, Li XP. 2010. MicroRNA-10b induced by Epstein-Barr virus-encoded latent membrane protein-1 promotes the metastasis of human nasopharyngeal carcinoma cells. Cancer Lett, 299: 29–36.

Li HP, Chang YS. 2003. Epstein-Barr virus latent membrane protein 1: structure and functions. J Biomed Sci, 10: 490–504.

Lin DC, Meng X, Hazawa M, Nagata Y, Varela AM, Xu L, Sato Y, Liu LZ, Ding LW, Sharma A, Goh BC, Lee SC, Petersson BF, Yu FG, Macary P, Oo MZ, Ha CS, Yang H, Ogawa S, Loh KS, Koeffler HP. 2014. The genomic landscape of nasopharyngeal carcinoma. Nat Genet, 46: 866–871.

Lin EY, Nguyen AV, Russell RG, Pollard JW. 2001. Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. J Exp Med, 193: 727–740.

Liu F, Gu J. 2011. Retinoic acid inducible gene-I, more than a virus sensor. Protein Cell, 2: 351–357.

Liu HP, Chen CC, Wu CC, Huang YC, Liu SC, Liang Y, Chang KP, Chang YS. 2012. Epstein-Barr virus-encoded LMP1 interacts with FGD4 to activate Cdc42 and thereby promote migration of nasopharyngeal carcinoma cells. PLoS Pathog, 8: e1002690.

Lo AK, Dawson CW, Jin DY, Lo KW. 2012. The pathological roles of BART miRNAs in nasopharyngeal carcinoma. J Pathol, 227: 392–403.

Lo AK, Dawson CW, Lo KW, Yu Y, Young LS. 2010. Upregulation of Id1 by Epstein-Barr virus-encoded LMP1 confers resistance to TGFbeta-mediated growth inhibition. Mol Cancer, 9: 155.

Lo AK, Huang DP, Lo KW, Chui YL, Li HM, Pang JC, Tsao SW. 2004a. Phenotypic alterations induced by the Hong Kong-prevalent Epstein-Barr virus-encoded LMP1 variant (2117-LMP1) in nasopharyngeal epithelial cells. Int J Cancer, 109: 919–925.

Lo AK, Lo KW, Ko CW, Young LS, Dawson CW. 2013. Inhibition of the LKB1-AMPK pathway by the Epstein-Barr virus-encoded LMP1 promotes proliferation and transformation of human nasopharyngeal epithelial cells. J Pathol, 230: 336–346.

Lo AK, To KF, Lo KW, Lung RW, Hui JW, Liao G, Hayward SD. 2007. Modulation of LMP1 protein expression by EBV-encoded microRNAs. Proc Natl Acad Sci U S A, 104: 16164–16169.

Lo KW, To KF, Huang DP. 2004b. Focus on nasopharyngeal carcinoma. Cancer Cell, 5: 423–428.

Lu J, Lin WH, Chen SY, Longnecker R, Tsai SC, Chen CL, Tsai CH. 2006. Syk tyrosine kinase mediates Epstein-Barr virus latent membrane protein 2A-induced cell migration in epithelial cells. J Biol Chem, 281: 8806–8814.

Lung RW, Tong JH, Sung YM, Leung PS, Ng DC, Chau SL, Chan AW, Ng EK, Lo KW, To KF. 2009. Modulation of LMP2A expression by a newly identified Epstein-Barr virus-encoded microRNA miR-BART22. Neoplasia, 11: 1174–1184.

Lung RW, Tong JH, To KF. 2013. Emerging roles of small Epstein-Barr virus derived non-coding RNAs in epithelial malignancy. Int J Mol Sci, 14: 17378–17409.

Lupton S, Levine AJ. 1985. Mapping genetic elements of Epstein-Barr virus that facilitate extrachromosomal persistence of Epstein-Barr virus-derived plasmids in human cells. Mol Cell Biol, 5: 2533–2542.

Marquitz AR, Mathur A, Nam CS, Raab-Traub N. 2011. The Epstein-Barr Virus BART microRNAs target the pro-apoptotic protein Bim. Virology, 412: 392–400.

Marquitz AR, Raab-Traub N. 2012. The role of miRNAs and EBV BARTs in NPC. Semin Cancer Biol, 22: 166–172.

Miller WE, Earp HS, Raab-Traub N. 1995. The Epstein-Barr virus latent membrane protein 1 induces expression of the epidermal growth factor receptor. J Virol, 69: 4390–4398.

Moody CA, Scott RS, Amirghahari N, Nathan CO, Young LS, Dawson CW, Sixbey JW. 2005. Modulation of the cell growth regulator mTOR by Epstein-Barr virus-encoded LMP2A. J Virol, 79: 5499–5506.

Moormann AM, Snider CJ, Chelimo K. 2011. The company malaria keeps: how co-infection with Epstein-Barr virus leads to endemic Burkitt lymphoma. Curr Opin Infect Dis, 24: 435–441.

Morris MA, Dawson CW, Young LS. 2009. Role of the Epstein-Barr virus-encoded latent membrane protein-1, LMP1, in the pathogenesis of nasopharyngeal carcinoma. Future Oncol, 5: 811–825.

Morrison JA, Raab-Traub N. 2005. Roles of the ITAM and PY motifs of Epstein-Barr virus latent membrane protein 2A in the inhibition of epithelial cell differentiation and activation of {beta}-catenin signaling. J Virol, 79: 2375–2382.

Murakami M, Lan K, Subramanian C, Robertson ES. 2005. Epstein-Barr virus nuclear antigen 1 interacts with Nm23-H1 in lymphoblastoid cell lines and inhibits its ability to suppress cell migration. J Virol, 79: 1559–1568.

Nachmani D, Stern-Ginossar N, Sarid R, Mandelboim O. 2009. Diverse herpesvirus microRNAs target the stress-induced immune ligand MICB to escape recognition by natural killer cells. Cell Host Microbe, 5: 376–385.

Nanbo A, Inoue K, Adachi-Takasawa K, Takada K. 2002. Epstein-Barr virus RNA confers resistance to interferon-alpha-induced apoptosis in Burkitt's lymphoma. EMBO J, 21: 954–965.

Nanbo A, Sugden A, Sugden B. 2007. The coupling of synthesis and partitioning of EBV's plasmid replicon is revealed in live cells. EMBO J, 26: 4252–4262.

Nanbo A, Yoshiyama H, Takada K. 2005. Epstein-Barr virus-encoded poly(A)- RNA confers resistance to apoptosis mediated through Fas by blocking the PKR pathway in human epithelial intestine 407 cells. J Virol, 79: 12280–12285.

O'Neil JD, Owen TJ, Wood VH, Date KL, Valentine R, Chukwuma MB, Arrand JR, Dawson CW, Young LS. 2008. Epstein-Barr virus-encoded EBNA1 modulates the AP-1 transcription factor pathway in nasopharyngeal carcinoma cells and enhances angiogenesis *in vitro*. J Gen Virol, 89: 2833–2842.

Pang MF, Lin KW, Peh SC. 2009. The signaling pathways of Epstein-Barr virus-encoded latent membrane protein 2A (LMP2A) in latency and cancer. Cell Mol Biol Lett, 14: 222–247.

Pathmanathan R, Prasad U, Sadler R, Flynn K, Raab-Traub N. 1995. Clonal proliferations of cells infected with Epstein-Barr virus in preinvasive lesions related to nasopharyngeal carcinoma. N Engl J Med, 333: 693–698.

Pegtel DM, Middeldorp J, Thorley-Lawson DA. 2004. Epstein-Barr virus infection in *ex vivo* tonsil epithelial cell cultures of asymptomatic carriers. J Virol, 78: 12613–12624.

Pegtel DM, Subramanian A, Sheen TS, Tsai CH, Golub TR, Thorley-Lawson DA. 2005. Epstein-Barr-virus-encoded LMP2A induces primary epithelial cell migration and invasion: possible role in nasopharyngeal carcinoma metastasis. J Virol, 79: 15430–15442.

Port RJ, Pinheiro-Maia S, Hu C, Arrand JR, Wei W, Young LS, Dawson CW. 2013. Epstein-Barr virus induction of the Hedgehog signalling pathway imposes a stem cell phenotype on human epithelial cells. J Pathol, 231: 367–377.

Portis T, Cooper L, Dennis P, Longnecker R. 2002. The LMP2A signalosome--a therapeutic target for Epstein-Barr virus latency and associated disease. Front Biosci, 7: d414–426.

Qiu J, Cosmopoulos K, Pegtel M, Hopmans E, Murray P, Middeldorp J, Shapiro M, Thorley-Lawson DA. 2011. A novel persistence associated EBV miRNA expression profile is disrupted

in neoplasia. PLoS Pathog, 7: e1002193.
Qiu J, Smith P, Leahy L, Thorley-Lawson DA. 2015. The Epstein-Barr virus encoded BART miRNAs potentiate tumor growth *in vivo*. PLoS Pathog, 11: e1004561.
Reusch JA, Nawandar DM, Wright KL, Kenney SC, Mertz JE. 2015. Cellular differentiation regulator BLIMP1 induces Epstein-Barr virus lytic reactivation in epithelial and B cells by activating transcription from both the R and Z promoters. J Virol, 89: 1731–1743.
Rickinson AB. 2014. Co-infections, inflammation and oncogenesis: future directions for EBV research. Semin Cancer Biol, 26: 99–115.
Salomoni P, Ferguson BJ, Wyllie AH, Rich T. 2008. New insights into the role of PML in tumour suppression. Cell Res, 18: 622–640.
Samanta M, Iwakiri D, Takada K. 2008. Epstein-Barr virus-encoded small RNA induces IL-10 through RIG-I-mediated IRF-3 signaling. Oncogene, 27: 4150–4160.
Sample J, Liebowitz D, Kieff E. 1989. Two related Epstein-Barr virus membrane proteins are encoded by separate genes. J Virol, 63: 933–937.
Sapi E, Flick MB, Gilmore-Hebert M, Rodov S, Kacinski BM. 1995. Transcriptional regulation of the c-fms (CSF-1R) proto-oncogene in human breast carcinoma cells by glucocorticoids. Oncogene, 10: 529–542.
Sato Y, Shirata N, Kudoh A, Iwahori S, Nakayama S, Murata T, Isomura H, Nishiyama Y, Tsurumi T. 2009. Expression of Epstein-Barr virus BZLF1 immediate-early protein induces p53 degradation independent of MDM2, leading to repression of p53-mediated transcription. Virology, 388: 204–211.
Scholle F, Bendt KM, Raab-Traub N. 2000. Epstein-Barr virus LMP2A transforms epithelial cells, inhibits cell differentiation, and activates Akt. J Virol, 74: 10681–10689.
Seto E, Ooka T, Middeldorp J, Takada K. 2008. Reconstitution of nasopharyngeal carcinoma-type EBV infection induces tumorigenicity. Cancer Res, 68: 1030–1036.
Seto E, Yang L, Middeldorp J, Sheen TS, Chen JY, Fukayama M, Eizuru Y, Ooka T, Takada K. 2005. Epstein-Barr virus (EBV)-encoded BARF1 gene is expressed in nasopharyngeal carcinoma and EBV-associated gastric carcinoma tissues in the absence of lytic gene expression. J Med Virol, 76: 82–88.
Shah KM, Stewart SE, Wei W, Woodman CB, O'Neil JD, Dawson CW, Young LS. 2009. The EBV-encoded latent membrane proteins, LMP2A and LMP2B, limit the actions of interferon by targeting interferon receptors for degradation. Oncogene, 28: 3903–3914.
Sheen TS, Huang YT, Chang YL, Ko JY, Wu CS, Yu YC, Tsai CH, Hsu MM. 1999. Epstein-Barr virus-encoded latent membrane protein 1 co-expresses with epidermal growth factor receptor in nasopharyngeal carcinoma. Jpn J Cancer Res, 90: 1285–1292.
Sheng W, Decaussin G, Ligout A, Takada K, Ooka T. 2003. Malignant transformation of Epstein-Barr virus-negative Akata cells by introduction of the BARF1 gene carried by Epstein-Barr virus. J Virol, 77: 3859–3865.
Sheu LF, Chen A, Meng CL, Ho KC, Lee WH, Leu FJ, Chao CF. 1996. Enhanced malignant progression of nasopharyngeal carcinoma cells mediated by the expression of Epstein-Barr nuclear antigen 1 *in vivo*. J Pathol, 180: 243–248.
Sivachandran N, Dawson CW, Young LS, Liu FF, Middeldorp J, Frappier L. 2012. Contributions of the Epstein-Barr virus EBNA1 protein to gastric carcinoma. J Virol, 86: 60–68.
Sivachandran N, Sarkari F, Frappier L. 2008. Epstein-Barr nuclear antigen 1 contributes to nasopharyngeal carcinoma through disruption of PML nuclear bodies. PLoS Pathog, 4: e1000170.
Smith PR, de Jesus O, Turner D, Hollyoake M, Karstegl CE, Griffin BE, Karran L, Wang Y, Hayward SD, Farrell PJ. 2000. Structure and coding content of CST (BART) family RNAs of Epstein-Barr virus. J Virol, 74: 3082–3092.
Speck P, Kline KA, Cheresh P, Longnecker R. 1999. Epstein-Barr virus lacking latent membrane protein 2 immortalizes B cells with efficiency indistinguishable from that of wild-type virus. J Gen Virol, 80: 2193–2203.
Stevens SJ, Verkuijlen SA, Hariwiyanto B, Harijadi, Paramita DK, Fachiroh J, Adham M, Tan IB, Haryana SM, Middeldorp JM. 2006. Noninvasive diagnosis of nasopharyngeal carcinoma: nasopharyngeal brushings reveal high Epstein-Barr virus DNA load and carcinoma-specific viral BARF1 mRNA. Int J Cancer, 119: 608–614.
Strockbine LD, Cohen JI, Farrah T, Lyman SD, Wagener F, DuBose RF, Armitage RJ, Spriggs MK. 1998. The Epstein-Barr virus BARF1 gene encodes a novel, soluble colony-stimulating factor-1 receptor. J Virol, 72: 4015–4021.
Strong MJ, Xu G, Coco J, Baribault C, Vinay DS, Lacey MR, Strong AL, Lehman TA, Seddon MB, Lin Z, Concha M, Baddoo M, Ferris M, Swan KF, Sullivan DE, Burow ME, Taylor CM, Flemington EK. 2013. Differences in gastric carcinoma microenvironment stratify according to EBV infection intensity: implications for possible immune adjuvant therapy. PLoS Pathog, 9: e1003341.
Takada K. 2012. Role of EBER and BARF1 in nasopharyngeal carcinoma (NPC) tumorigenesis. Semin Cancer Biol, 22: 162–165.
Takada K, Nanbo A. 2001. The role of EBERs in oncogenesis. Semin Cancer Biol, 11: 461–467.
Tao Q, Chan AT. 2007. Nasopharyngeal carcinoma: molecular pathogenesis and therapeutic developments. Expert Rev Mol Med, 9: 1–24.
Tempera I, Lieberman PM. 2014. Epigenetic regulation of EBV persistence and oncogenesis. Semin Cancer Biol, 26: 22–29.
The Cancer Genome Atlas Research Network (TCGA). 2014. Comprehensive molecular characterization of gastric adenocarcinoma. Nature, 513: 202–209.
Thorley-Lawson DA, Allday MJ. 2008. The curious case of the tumour virus: 50 years of Burkitt's lymphoma. Nat Rev Microbiol, 6: 913–924.
To EW, Chan KC, Leung SF, Chan LY, To KF, Chan AT, Johnson PJ, Lo YM. 2003. Rapid clearance of plasma Epstein-Barr virus DNA after surgical treatment of nasopharyngeal carcinoma. Clin Cancer Res, 9: 3254–3259.
Tsai MH, Raykova A, Klinke O, Bernhardt K, Gartner K, Leung CS, Geletneky K, Sertel S, Munz C, Feederle R, Delecluse HJ. 2013. Spontaneous lytic replication and epitheliotropism define an Epstein-Barr virus strain found in carcinomas. Cell Rep, 5: 458–470.
Tsang CM, Cheung YC, Lui VW, Yip YL, Zhang G, Lin VW, Cheung KC, Feng Y, Tsao SW. 2013. Berberine suppresses tumorigenicity and growth of nasopharyngeal carcinoma cells by inhibiting STAT3 activation induced by tumor associated fibroblasts. BMC Cancer, 13: 619.
Tsang CM, Yip YL, Lo KW, Deng W, To KF, Hau PM, Lau VM, Takada K, Lui VW, Lung ML, Chen H, Zeng M, Middeldorp JM, Cheung AL, Tsao SW. 2012. Cyclin D1 overexpression supports stable EBV infection in nasopharyngeal epithelial cells. Proc Natl Acad Sci U S A, 109: E3473–3482.
Tsang CM, Zhang G, Seto E, Takada K, Deng W, Yip YL, Man C, Hau PM, Chen H, Cao Y, Lo KW, Middeldorp JM, Cheung AL, Tsao SW. 2010. Epstein-Barr virus infection in immortalized nasopharyngeal epithelial cells: regulation of infection and phenotypic characterization. Int J Cancer, 127: 1570–1583.
Tsao SW, Tsang CM, To KF, Lo KW. 2015. The role of Ep-

stein-Barr virus in epithelial malignancies. J Pathol, 235: 323–333.
Tsao SW, Tramoutanis G, Dawson CW, Lo AK, Huang DP. 2002. The significance of LMP1 expression in nasopharyngeal carcinoma. Semin Cancer Biol, 12: 473–487.
Tsao SW, Yip YL, Tsang CM, Pang PS, Lau VM, Zhang G, Lo KW. 2014. Etiological factors of nasopharyngeal carcinoma. Oral Oncol, 50: 330–338.
Tsuji A, Wakisaka N, Kondo S, Murono S, Furukawa M, Yoshizaki T. 2008. Induction of receptor for advanced glycation end products by EBV latent membrane protein 1 and its correlation with angiogenesis and cervical lymph node metastasis in nasopharyngeal carcinoma. Clin Cancer Res, 14: 5368–5375.
Tugizov SM, Berline JW, Palefsky JM. 2003. Epstein-Barr virus infection of polarized tongue and nasopharyngeal epithelial cells. Nat Med, 9: 307–314.
Valentine R, Dawson CW, Hu CF, Shah KM, Owen TJ, Date KL, Maia SP, Shao J, Arrand JR, Young LS, O'Neil JD. 2010. Epstein-Barr virus-encoded EBNA1 inhibits the canonical NF-kappa B pathway in carcinoma cells by inhibiting IKK phosphorylation. Molecular Cancer, 9.
Vereide DT, Seto E, Chiu YF, Hayes M, Tagawa T, Grundhoff A, Hammerschmidt W, Sugden B. 2014. Epstein-Barr virus maintains lymphomas via its miRNAs. Oncogene, 33: 1258–1264.
Wang D, Liebowitz D, Kieff E. 1985. An EBV membrane protein expressed in immortalized lymphocytes transforms established rodent cells. Cell, 43: 831–840.
Wang HB, Zhang H, Zhang JP, Li Y, Zhao B, Feng GK, Du Y, Xiong D, Zhong Q, Liu WL, Du H, Li MZ, Huang WL, Tsao SW, Hutt-Fletcher L, Zeng YX, Kieff E, Zeng MS. 2015. Neuropilin 1 is an entry factor that promotes EBV infection of nasopharyngeal epithelial cells. Nat Commun, 6: 6240.
Wang L, Tian WD, Xu X, Nie B, Lu J, Liu X, Zhang B, Dong Q, Sunwoo JB, Li G, Li XP. 2014. Epstein-Barr virus nuclear antigen 1 (EBNA1) protein induction of epithelial-mesenchymal transition in nasopharyngeal carcinoma cells. Cancer, 120: 363–372.
Wei MX, Ooka T. 1989. A transforming function of the BARF1 gene encoded by Epstein-Barr virus. EMBO J, 8: 2897–2903.
Wiech T, Nikolopoulos E, Lassman S, Heidt T, Schopflin A, Sarbia M, Werner M, Shimizu Y, Sakka E, Ooka T, zur Hausen A. 2008. Cyclin D1 expression is induced by viral BARF1 and is overexpressed in EBV-associated gastric cancer. Virchows Arch, 452: 621–627.
Wu CC, Liu MT, Chang YT, Fang CY, Chou SP, Liao HW, Kuo KL, Hsu SL, Chen YR, Wang PW, Chen YL, Chuang HY, Lee CH, Chen M, Wayne Chang WS, Chen JY. 2010. Epstein-Barr virus DNase (BGLF5) induces genomic instability in human epithelial cells. Nucleic Acids Res, 38: 1932–1949.
Xiao L, Hu ZY, Dong X, Tan Z, Li W, Tang M, Chen L, Yang L, Tao Y, Jiang Y, Li J, Yi B, Li B, Fan S, You S, Deng X, Hu F, Feng L, Bode AM, Dong Z, Sun LQ, Cao Y. 2014. Targeting Epstein-Barr virus oncoprotein LMP1-mediated glycolysis sensitizes nasopharyngeal carcinoma to radiation therapy. Oncogene, 33: 4568–4578.
Xu Y, Shi Y, Yuan Q, Liu X, Yan B, Chen L, Tao Y, Cao Y. 2013. Epstein-Barr Virus encoded LMP1 regulates cyclin D1 promoter activity by nuclear EGFR and STAT3 in CNE1 cells. J Exp Clin Cancer Res, 32: 90.
Yamamoto N, Takizawa T, Iwanaga Y, Shimizu N, Yamamoto N. 2000. Malignant transformation of B lymphoma cell line BJAB by Epstein-Barr virus-encoded small RNAs. FEBS Lett, 484: 153–158.
Yamamoto T, Iwatsuki K. 2012. Diversity of Epstein-Barr virus BamHI-A rightward transcripts and their expression patterns in lytic and latent infections. J Med Microbiol, 61: 1445–1453.
Yang CF, Peng LX, Huang TJ, Yang GD, Chu QQ, Liang YY, Cao X, Xie P, Zheng LS, Huang HB, Cai MD, Huang JL, Liu RY, Zhu ZY, Qian CN, Huang BJ. 2014. Cancer stem-like cell characteristics induced by EB virus-encoded LMP1 contribute to radioresistance in nasopharyngeal carcinoma by suppressing the p53-mediated apoptosis pathway. Cancer Lett, 344: 260–271.
Yang GD, Huang TJ, Peng LX, Yang CF, Liu RY, Huang HB, Chu QQ, Yang HJ, Huang JL, Zhu ZY, Qian CN, Huang BJ. 2013. Epstein-Barr Virus_Encoded LMP1 upregulates microRNA-21 to promote the resistance of nasopharyngeal carcinoma cells to cisplatin-induced Apoptosis by suppressing PDCD4 and Fas-L. PLoS One, 8: e78355.
Yang X, Sham JS, Ng MH, Tsao SW, Zhang D, Lowe SW, Cao L. 2000. LMP1 of Epstein-Barr virus induces proliferation of primary mouse embryonic fibroblasts and cooperatively transforms the cells with a p16-insensitive CDK4 oncogene. J Virol, 74: 883–891.
Yates J, Warren N, Reisman D, Sugden B. 1984. A cis-acting element from the Epstein-Barr viral genome that permits stable replication of recombinant plasmids in latently infected cells. Proc Natl Acad Sci U S A, 81: 3806–3810.
Yip YL, Pang PS, Deng W, Tsang CM, Zeng M, Hau PM, Man C, Jin Y, Yuen AP, Tsao SW. 2013. Efficient immortalization of primary nasopharyngeal epithelial cells for EBV infection study. PLoS One, 8: e78395.
Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M, Taira K, Akira S, Fujita T. 2004. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. Nat Immunol, 5: 730–737.
Yoshizaki T. 2002. Promotion of metastasis in nasopharyngeal carcinoma by Epstein-Barr virus latent membrane protein-1. Histol Histopathol, 17: 845–850.
Young LS, Rickinson AB. 2004. Epstein-Barr virus: 40 years on. Nat Rev Cancer, 4: 757–768.
Yu H, Lu J, Zuo L, Yan Q, Yu Z, Li X, Huang J, Zhao L, Tang H, Luo Z, Liao Q, Zeng Z, Zhang J, Li G. 2012. Epstein-Barr virus downregulates microRNA 203 through the oncoprotein latent membrane protein 1: a contribution to increased tumor incidence in epithelial cells. J Virol, 86: 3088–3099.
Zhang G, Tsang CM, Deng W, Yip YL, Lui VW, Wong SC, Cheung AL, Hau PM, Zeng M, Lung ML, Chen H, Lo KW, Takada K, Tsao SW. 2013. Enhanced IL-6/IL-6R signaling promotes growth and malignant properties in EBV-infected premalignant and cancerous nasopharyngeal epithelial cells. PLoS One, 8: e62284.
Zhang J, Chen H, Weinmaster G, Hayward SD. 2001. Epstein-Barr virus BamHi-a rightward transcript-encoded RPMS protein interacts with the CBF1-associated corepressor CIR to negatively regulate the activity of EBNA2 and NotchIC. J Virol, 75: 2946–2956.
Zheng H, Li LL, Hu DS, Deng XY, Cao Y. 2007. Role of Epstein-Barr virus encoded latent membrane protein 1 in the carcinogenesis of nasopharyngeal carcinoma. Cell Mol Immunol, 4: 185–196.
Zhu JY, Pfuhl T, Motsch N, Barth S, Nicholls J, Grasser F, Meister G. 2009. Identification of novel Epstein-Barr virus microRNA genes from nasopharyngeal carcinomas. J Virol, 83: 3333–3341.
zur Hausen A, Brink AA, Craanen ME, Middeldorp JM, Meijer CJ, van den Brule AJ. 2000. Unique transcription pattern of Epstein-Barr virus (EBV) in EBV-carrying gastric adenocarcinomas: expression of the transforming BARF1 gene. Cancer Res, 60: 2745–2748.