Chapter 6
EBV Infection and Glucose Metabolism in Nasopharyngeal Carcinoma

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Abstract To establish persistent infection in cells, viruses evolve strategies to alter host cellular pathways to regulate cell proliferation and energy metabolism which support viral infection. Epstein-Barr virus (EBV) undergoes both lytic and latent infection to achieve persistent and lifelong infection in human. EBV readily infects human B cells, driving their transformation to proliferative lymphoblastoid cell lines (LCL), and eventually establishes lifelong latent infection in memory B cells. In contrary, EBV undergoes lytic replication upon infection into normal epithelial cells which is essential for the replication of EBV genome and production of infectious viral particles for transmission through saliva. EBV shuttles between B cells and epithelial cells to complete its infection cycle. EBV infection is closely associated with nasopharyngeal carcinoma (NPC) and is present in practically 100% of undifferentiated NPC. In contrast to undergo lytic infection of normal pharyngeal epithelium, EBV establishes latent infection in NPC. The switch from lytic infection to latent infection may represent an early and essential step in the development of NPC. Recent studies in both B cells and NPC cells latently infected with EBV reveal alterations in cell metabolism to support persistent and latent EBV infection. Events underlying the switching of lytic to latent EBV infection in NPC cells are largely undefined. Molecular events and alterations of cell metabolism are likely to play crucial roles in switching EBV infection from lytic to latent in NPC cells. Latent EBV infection and expression of viral genes, including LMP1, LMP2, and possibly EBV-encoded micro RNAs, may play essential roles in alterations of cell metabolism to support NPC pathogenesis. Alteration of energy metabolism is an essential hallmark of cancer. The role of altered energy metabolism in host cells in modulating latent and lytic EBV infection in NPC cells is unclear. In this review, we will discuss the impact of genetic alterations in NPC to module cellular metabolism and its influence on latent infection and lytic reactivation of EBV infection in NPC cells. In particular, the role of EBV-encoded genes in driving glucose metabolism and their contribution to NPC pathogenesis will be discussed. This new perspective

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on the interplay between EBV infection and altered host metabolic pathways in NPC pathogenesis may offer novel and effective therapeutic strategies in the treatment of NPC and other EBV-associated malignancies.

Keywords Epstein-Barr virus • Latent infection • Nasopharyngeal carcinoma • Glucose metabolism

6.1 Introduction

The EBV is a type of human γ-herpesvirus infecting over 90% of the populations worldwide. EBV infection is associated with human malignancies of both lymphoid and epithelial origins [1, 2]. Upon infection, EBV effectively transforms and immortalizes B cells into lymphoblastoid cell lines with unlimited proliferative potential, a hallmark property of EBV contributing to B-cell malignancies [3]. Nearly 100% of undifferentiated NPC and a subtype of gastric cancer (about 10%) are latently infected with EBV infection [4]. Compared to B-cell malignancies, the pathogenic roles of EBV in human epithelial cancers are much less understood. In complete contrast to EBV infection of primary B cells, infection of primary epithelial cells by EBV does not induce cell proliferation. In contrast, the EBV-infected primary epithelial cells readily undergo growth arrest. As EBV infection is practically present in all undifferentiated NPC cells, presumably, EBV infection should have selective growth advantages to NPC cells in patients. The nature of growth advantages of EBV infection in NPC remains to be determined but have been postulated to involve immune evasion and survival advantage of infected NPC cells. EBV readily undergoes lytic infection in normal epithelial cells. However, in NPC cells, EBV infection is predominantly latent with expression of restricted number of latent genes including EBER, EBNA1, LMP1, LMP2, and miR-BARTs. An earlier study has shown that LMP2 induces mTOR activation to upregulate c-myc protein translation [5]. Recent studies showed that LMP1 drives mTORC1 signaling and glucose metabolism [6, 7] supporting the important role of EBV infection in energy metabolism in NPC pathogenesis. Recent genomic profiling of NPC also revealed frequent mutations leading to activation of NF-κB [8, 9] and other cell signaling pathways such as ERBB/PI3K signaling [10], which are upstream events involved in mTOR signaling. Activation of mTOR signaling and enhanced glucose metabolism may play a crucial role to support latent infection of EBV and contribute to NPC pathogenesis.

6.2 EBV Establishes Latent Infection in NPC

Both lytic and latent infection of EBV are involved to maintain persistent and life-long infection in human. EBV devises specific strategies to switch its cellular tropism to facilitate the shuttling of virus between epithelial cells and B cells during its infection cycle [11]. EBV establishes latent infection in human memory B cells and is believed to be the reservoir for persistent EBV infection in human. Differentiation of infected B cells to plasma cells will trigger lytic reactivation of EBV resulting in production of infectious viruses to infect epithelial cells. The EBV episomes in infected epithelial cells replicate efficiently and packaged into infectious viruses for transmission. Lytic replication of EBV in infected oropharyngeal epithelial cells has been postulated to be the continuous source of infectious virus shedding into saliva which is the major route of EBV transmission [12]. As mentioned, EBV infection in NPC is predominantly latent [2]. The switching of EBV infection from lytic to latent mode may represent an early and essential step in NPC pathogenesis.

Three types of latent infection program of EBV in human cells have been observed. In NPC, EBV undergoes a specific type of latency infection program (referred as latency type II) where expression of latent EBV genes is limited to EBERs, EBNA1, LMP1, LMP2, and BART transcripts. The BART transcripts are expressed with high abundance in NPC cells, which are further processed to EBV-encoded microRNAs (miR-BARTs). Interestingly, the miR-BARTs are expressed at exceptionally high levels in epithelial cancers, including the NPC and EBV-associated gastric cancer, but at reduced levels in lymphoid malignancies (type I latency) and very low levels in EBV-transformed lymphoblastoid cell lines (type III latency). The high expression of miR-BARTs in NPC as well as EBV-associated gastric cancer suggests the pathogenic roles of miR-BARTs in NPC [13]. Events regulating latent infection of EBV in NPC are largely undefined [11]. Interestingly, overexpression of Cyclin D1 and inactivation of p16, which are common events in NPC, support stable and latent EBV infection in immortalized nasopharyngeal epithelial cells [14]. Additional genetic mutations and alterations of host cell signaling in NPC cells are likely to contribute to the establishment of latent EBV infection.

6.3 Glucose Metabolism and Viral Infection

Alteration in cell signaling pathways to rewire energy metabolism is an essential hallmark of human cancer. In the presence of oxygen, differentiated tissues and normal cells metabolize glucose mainly rely on oxidative phosphorylation to generate energy which is a highly efficient process, resulting in generation of up to 38 ATP molecules per molecule of glucose metabolized. Under low oxygen condition (hypoxia), normal cells switch to anaerobic glycolysis which results in 2 ATP per glucose molecule and generation of lactate. Enhanced glucose consumption, via glycolysis despite the presence of oxygen (referred as aerobic glycolysis), is

common in cancer cells and is referred to as the "Warburg's effect" [15, 16]. Besides ATP, enhanced aerobic glycolysis in cancer cells also generates substantial number of biosynthetic metabolites, which are essential for biomass production associated with cell growth. Enhanced glycolysis also results in high accumulation of lactate which has important influence on the tumor microenvironment. Adaptions of host cell metabolism to viral infection have been observed in cells infected with EBV, KSHV, HPV, and HCV [17–19]. Virus-encoded oncoproteins, such as E6 and E7 of the high-risk HPVs, drive cell proliferation which requires high demand for energy and biosynthetic metabolites to support cell growth [20]. The key pathway involved in balancing cell metabolisms to meet requirement of cell growth is the mTOR signaling pathway, which could sense the levels of energy and nutrients in cells. The viral oncoproteins also regulate metabolic pathways to control the nutrient assimilation required by proliferating cells [21]. In EBV-infected NPC cells, the expression of EBV-encoded LMP1 has been shown to enhance glycolysis by modulating multiple signaling pathways including FGF1 [22], AMPK [23], and mTORC1 [6] to drive up glucose metabolism. The viral oncoproteins may also act as transcription factors to upregulate the enzyme activities directly involved in glucose metabolism [24].

There are much remains to be determined on how EBV infection in NPC alters the glucose metabolism and contributes to the malignant transformation of nasopharyngeal epithelial cells. Recently, the landscape of somatic mutations in NPC has been reported [8–10]. Their involvement in glucose metabolism in NPC and latent EBV infection will be discussed. Altered energy metabolism in NPC may stabilize latent EBV infection. Expression of latent EBV products may further enhance and stabilize energy reprogramming in EBV-infected NPC cells. A close interplay between genetic alterations and EBV infection to enhance energy metabolism may support NPC development.

6.4 Genomic Profiling of NPC Reveals Alteration of Multiple Signaling Pathways Involved in Glucose Metabolism

Several comprehensive genomic profiling studies in NPC have revealed unique genetic landscapes relevance to NPC pathogenesis [8–10]. The most common genetic mutations observed in NPC are those involved in NF-κB activation [8, 9]. NF-κB signaling activation could be linked to mTOR activation which is a key driver in glucose metabolism in cancer cells [25]. Other common mutations in NPC alterations that are implicated in glucose metabolism including *PTEN/AKT/PIK3CA* mutations, which activate PI3K/AKT signaling upstream of mTOR activation, were also identified.

6.4.1 Activation of NF-κB Signaling

Two recent genomic studies in NPC employing whole exome sequencing (WES) have identified frequent mutations in multiple upstream negative regulators of NF-κB signaling including *CYLD*, *TRAF3*, *NFKB1A*, *TNFAIP3*, and *NLRC5* [8, 9]. The CYLD cleaves the lysine 63-linked polyubiquitin chains from target proteins, including NEMO (IKKγ) which is involved in phosphorylation and degradation of IκBs, which are inhibitors of NF-κB signaling. The CYLD also deubiquitinates TRAF2 which is an activator of NF-κB signaling. Furthermore, the CYLD inhibits activation of bcl3, which was reported in an early study to be involved in atypical activation of NF-κB in NPC associated with p50 dimmers [26]. The TRAF3 is involved in the suppression of NIK-activating NF-κB signaling. Most mutations of *TRAF3* in NPC are in the domain regions involved in this suppression of NIK activation. The *NFKB1A* encodes IκBα, which belongs to the NF-κB inhibitor family (IκBs). The NLRC5 is a potent inhibitor of NF-κB activation and competes with NEMO for binding to IKKα and IKKβ. Collectively, mutations of all these negative regulators upstream of NF-κB signaling may contribute to the constitutive activation of NF-κB commonly detected in NPC [26, 27]. Interestingly, an exclusive relationship between mutation of these negative regulators of NF-κB and high expression levels of LMP1 in NPC was observed [9]. The potent function of NF-κB activation of the EBV-encoded LMP1 is well documented. Together, LMP1 expression and mutations of upstream negative regulators of NF-κB account for the majority of NPC samples examined. Hence, the genomic landscape of NPC reveals an essential role of NF-κB signaling in NPC pathogenesis either by genomic mutation or expression of EBV-encoded LMP1 [9]. The role of NF-κB signaling contributing to establishment of latent EBV infection in NPC is unclear.

Earlier study has showed that activation of NF-κB signaling by overexpressing p65 in EBV-infected cells inhibits activation of lytic promoter of EBV [28]. In lymphocytes and epithelial NPC latently infected with EBV, treatment with an NF-κB inhibitor (Bay 11–7082) resulted in expression of lytic viral protein [28, 29]. Using a more specific inhibitor of NF-κB, NBD peptide, we also observed lytic reactivation of EBV in infected NPC cells (Tsao SW. unpublished observations). The NBD peptide specifically inhibits NF-κB activation by binding to the NEMO-binding domain of IKK to inactivate the kinase activity of IKK complex upstream of NF-κB signaling [30]. Furthermore, expression of LMP1 or activated CD40 domain, which effectively activates NF-κB signaling in EBV-infected lymphocytes, also suppressed lytic reactivation of EBV [31]. These studies support a role of NF-κB activation in establishment of latent infection of EBV in infected cells. The underlying mechanisms are unclear.

As a key inflammatory modulator, aberrantly activation of NF-κB signaling contributes multiple hallmarks of cancer, including energy metabolism [32–34]. An early study reported that NF-κB activation is directly involved in activation of mTORC1 signaling [25]. The IKK kinase complex is upstream of NF-κB signaling and composed of IKKα, IKKβ, and IKKγ (NEMO). The IKKβ could directly

interact and phosphorylate the TSC1, which is the negative regulator of the mTORC1 complex [25]. Phosphorylation of TSC1 at Ser^{487} and Ser^{522} by IKKβ results in the release of TSC1 suppression on mTORC1 signaling. Furthermore, the IKKα, another key component of the kinase complex in NF-κB activation, could also phosphorylate mTOR at Ser^{1415} to activate mTORC1 signaling [35]. Activation of mTORC1 signaling enhances uptake of glucose through activation of HIF1α to upregulate *Glut-1* transcription which increases the glucose influx to support aerobic glycolysis [21]. We showed that NF-κB activation by LMP1 could directly enhance transcription of *Glut-1*, and another study also showed that LMP1 induced *Glut-1* translocation to plasma membrane, both of which increase the glucose influx into cells to support aerobic glycolysis [6, 7].

Here, we postulated that metabolic adaption through NF-κB activation to activate mTOR and glucose metabolism may minimize metabolic stress associated with viral infection and play an essential role in establishment of latent EBV infection in NPC cells. In support of this hypothesis, we have also observed enhanced lytic replication in EBV-infected NPC cells upon treatment with rapamycin (a specific inhibitor of mTORC1) (Tsao SW, unpublished observation). Similarly, inhibition of lytic reactivation in BX-1 EBV-infected AGS (gastric cancer cells) by rapamycin has also been reported [36].

6.4.2 Activation of PTEN/PI3K/AKT Signaling Pathway

The PTEN/PI3K/AKT signaling pathway, which is a key signaling pathway upstream of mTOR activation, is frequently activated in cancer cells. Mutation of their negative regulator, *PTEN*, and activation mutations in *PIK3CA* could activate PI3K/AKT signaling [21, 37]. Recent genomic profiling studies of NPC revealed deletion of *PTEN*, amplification, and hot spot mutations of *PIK3CA* [8–10]. The mTORC1, a serine/threonine protein kinase, is the key sensor in cells to regulate cell metabolism by balancing the energy status and controlling the synthesis of essential metabolic resources in cells including proteins, nucleotides, and lipids [38]. The constitutive activation of mTORC1 signaling is commonly observed in human cancers including NPC [39]. Activation of mTORC1 signaling enhances glucose uptake and consumption to ensure sufficient energy and generation of biosynthetic metabolites for growth and proliferation of cancer cells [21]. Interestingly, this adaptive process also confers metastatic potential to cancer cells and their resistance to chemotherapy [40]. PTEN is an inhibitor of AKT, which suppresses PI3K activity, the common cell signaling pathway in mTOR activation. Mutation and deletion of *PTEN* were detected in NPC [9] which may lead to activation of PI3K/AKT and mTOR. Interestingly, loss of function mutation of *PTEN* in mammalian cells may also regulate aerobic glycolysis via a PI3K-independent manner through the E3 ubiquitin ligase activity of APC/C-Cdh1 to enhance aerobic glycolysis [41]. Other mutations detected in NPC including the ERBB2/ERBB3 may also converge to PI3K/MAPK signaling leading to mTOR activation [9, 10].

6.5 Latent EBV Genes Drive Glucose Metabolism in NPC

6.5.1 LMP1

The LMP1 is a well-documented EBV-encoded oncoprotein expressed during latent EBV infection in NPC. LMP1 is a potent activator of multiple signaling pathways. Its role in activation of NF-κB signaling is well documented [42]. The LMP1 has been shown to enhance aerobic glycolysis in NPC cells through alteration of metabolism-associated pathways [6, 7, 22, 23, 43, 44]. A recent study showed that LMP1 induces expression of hexokinase 2 (HK2), a key enzyme to control and enhance the glycolysis process [43]. LMP1 expression is positively correlated with HK2 expression in NPC tissue and poor overall survival of NPC patients following radiation therapy. Enhanced aerobic glycolysis also conferred insensitivity to radiation therapy in LMP1-expressing cells. Suppression of HK2 expression induces apoptosis in NPC cells. The transport of glucose over the plasma membrane by its glucose transporters (Gluts) is the first rate-limiting step of glucose metabolism. We recently reported that LMP1 upregulates *Glut-1* transcription in NPC cells to enhance aerobic glycolysis, a process dependent on activation of mTORC1 and NF-κB signaling [6]. Blocking aerobic glycolysis by specific chemical and genetic inhibitors also suppressed multiple LMP1-mediated malignant phenotypes. An earlier study also showed that LMP1 mediated the relocation of Glut-1 to plasma membrane in EBV-infected B cells involving activation of IKKβ/NF-κB signaling [7]. Localization of the Glut-1 to cell membrane enhances glucose uptake to support cell proliferation and also confers resistance to apoptosis. Hence expression of LMP1 serves as an important driver in EBV-infected cells to accelerate aerobic glycolysis by targeting glycolysis-associated events, particularly activation of Glut-1 and HK2.

Additional signaling pathways or upstream modulators have been identified in driving glucose metabolism in cancer cells. The LMP1 is also involved in modulating these pathways. The LMP1 was reported to induce FGF expression and secretion to promote FGFR1 signaling which drive aerobic glycolysis [22]. Blockade of FGFR signaling by small molecules suppressed the LMP1-induced transformed phenotypes. HoxC8 is a negative regulator of aerobic glycolysis commonly downregulated in NPC. LMP1 could suppress the expression of HoxC8 via stalling the activity of RNA polymerase II (RNA Pol II) [44]. The AMPK-mTOR axis activity is well known for its involvement in energy metabolism. LMP1 was reported to inhibit AMPK activity and signaling in immortalized nasopharyngeal epithelial cells [45]. Inhibition of AMPK suppressed LMP1-induced proliferation and transformation of immortalized nasopharyngeal epithelial cells. The inhibition of AMPK signaling also accelerated glucose uptake and lactate production and conferred resistance of NPC cells to apoptosis induced by irradiation [46]. In B cells, infection with EBV induced cell proliferation which demands increased supply of energy and metabolites. These observations are concordant with the close association of proliferation of EBV-infected B cells with AMPK inhibition and mTOR activity [47]. A

role of latent EBV infection and expression of the EBV-encoded LMP1 in driving glucose metabolism to meet the increased demand of energy and metabolites for cell proliferation is emerging.

6.5.2 LMP2

LMP2A is another latent EBV-encoded protein expressed in NPC that could activate mTORC1 signaling. Expression of LMP2A in HONE1 cells leads to mTOR activation as revealed by the phosphorylation of 4E–BP1 which is the downstream effector of mTOR [5]. Activation of PI3K/AKT signaling pathway is the upstream in mediating mTOR activation and may provide growth advantage to LMP2A-expressing cells. Activation of mTOR by LMP2A may also contribute to the enhanced glucose metabolism in latent EBV-infected NPC cells.

6.5.3 miR-BARTs

EBV-encoded miRNAs have been identified to be highly expressed in EBV-associated cancers and contribute to viral latency, cell survival, as well as immune escape. High expression of miR-BARTs is found in epithelial malignant cells with latency type II EBV infection, including NPC and gastric carcinoma, but low in EBV-infected B cells which strongly indicates a specific role of miR-BARTs in the pathogenesis of EBV-associated epithelial malignancies [13].

The role of NF-κB in driving latent EBV infection and expression of miR-BARTs has been reported [28, 48]. Expression of miR-BARTs is known to be deregulated in EBV-associated tumors and associated with NPC pathogenesis [49, 50]. Interestingly, constitutively activation of NF-κB upregulates LMP1 and miR-BART expression in EBV-infected NPC cells [48]. Multiple miR-BARTs can downregulate LMP1 expression by targeting its 3′-UTR and thereby form a negative feedback loop to modulate the level of LMP1 in NPC. The miR-BARTs have also been demonstrated to suppress EBV lytic replication in both B cell and epithelial cell lines induced by TPA. miR-BART6 was reported to target DICER, and miR-BART20-5p has the ability to stabilize EBV latency by directly targeting BZLF1 and BRLF1; both of them can govern the EBV entry into the lytic replication phase [51, 52]. These studies are in agreement with the view that miR-BARTs can support latency infection.

At present, the evidence for EBV microRNA in glucose metabolism is limited. A recent report showed that the miR-BART-1 may be involved in enhancing aerobic glycolysis through upregulation of a panel of genes involved in cell metabolism [53]. Based on RNA deep sequencing analysis, overexpression of miR-BART-1 leads to the upregulation of *G6PD*, *PHGDH*, *PAST1*, *IDH2*, *and PISD* and downregulation of *UGT8*, *LDHB*, *SGPL1*, *and DHRS3* [53]. It remains to be determined

whether the effects of miR-BART in glucose metabolism are direct or indirect through modulation of metabolism-related pathways.

6.6 The Impact of Glucose Metabolism to EBV Infection in NPC

As aforementioned, EBV infection readily immortalizes and transforms primary B cells both in vitro and in vivo but not in primary epithelial cells. Primary epithelial cells have finite life span in culture and readily undergo senescence upon passages. EBV infection may induce cellular stress and proliferation arrest in primary epithelial cells. EBV could infect and undergo lytic replication in oral keratinocyte grown as three-dimensional organotypic culture [54]. However, neither latent infection of EBV nor proliferation of EBV-infected epithelial cells was observed in EBV-infected stratified keratinocytes in the organotypic culture. This supports the postulation that EBV undergoes lytic infection in normal epithelium to amplify the EBV genomes and generate infectious viral particles for transmission.

Establishment of latent EBV infection requires modification of host cell signaling. Latent EBV infection could be established in telomerase-immortalized nasopharyngeal epithelial cells [14, 55]. Immortalization is a prerequisite property of cancer cells and is regarded as an early event in human carcinogenesis [15]. Metabolic stress is a major barrier for cell immortalization. The high demand for energy and biosynthetic metabolites to sustain continuous proliferation requires metabolic adaptation in both immortalized and cancer cells. Our recent study showed that mTORC1, as well as NF-κB signaling, is commonly activated during the immortalization of nasopharyngeal epithelial cells mediated by telomerase [56]. Another barrier to immortalization is cellular senescence induced by reactive oxygen species (ROS), which are generated during cell proliferation. Primary cells propagated for extended period of culture will accumulate ROS to induce cellular senescence and apoptosis [57]. Enhanced glycolysis could protect cells from apoptosis due to ROS induced oxidative stress and facilitate immortalization [58].

Metabolic stress has been characterized as a major barrier for immortalization and latency establishment of B cells mediated by EBV infection [47]. Only a small population of EBV-infected B cells could be immortalized by EBV. Analysis of these EBV-immortalized B cells revealed activation of aerobic glycolysis with high glucose metabolism which is associated with suppression of AMPK and activation of mTOR signaling. Accordingly, activation of AMPK and a decrease of mTOR activity were detected in the growth-arrested B cells that further failed to be immortalized upon EBV infection [47]. Furthermore, inhibition of mTORC1 in the EBV-infected epithelial cells with specific inhibitor, rapamycin, effectively elevated the lytic EBV replication in a dose- and time-dependent manner as evidenced by the increase of *Zta* and *Rta* transcripts and their translated proteins in rapamycin-treated cells [36]. As aforementioned, blockade of mTORC1 by rapamycin induces lytic reactivation in NPC and gastric cancer cells. Blockage of mTORC1 activation by

rapamycin may induce a starvation status by slowing down the glucose uptake, which may be a physiological signaling for EBV to switch into lytic replication. Enhanced glucose metabolism may represent essential cellular properties to support latent EBV infection which warrants further investigations.

6.7 Enhanced Glucose Metabolism Alters the Tumor Microenvironment

The tumor microenvironment is known to play an important function for modulation of tumor growth, progression and metastasis to distant sites, and development of acquired treatment resistance and accounts for poor patient prognosis [59]. The tumor microenvironment contains multiple elements, including immune cells, stromal fibroblasts, and tumor-associated endothelial cells, all of which are known to be involved in modulating malignant behaviors of cancer cells. A typical feature of tumor microenvironment is high acidity, which plays key roles for tumor progression. As a result of enhanced aerobic glycolysis, accumulation of lactate is a major contributor for the high acidity of tumor microenvironment. Lactate has been shown to promote angiogenesis, cell migration, metastasis, and growth sufficiency in cancer cells [15, 60–62]. High concentrations of lactate are associated with development of distant cancer metastasis [62–65]. Expression of the EBV-encoded LMP1 in NPC has been postulated to enhance malignant properties of NPC by inducing angiogenesis [66–68], cell motility, and immune escape [69–71]. Some of these malignant properties of NPC cells may be accounted for by the enhanced aerobic glycolysis and accumulation of elevated level of lactate in the tumor microenvironment. Interestingly, treatment of LMP1-expressing nasopharyngeal epithelial cells with aerobic glycolysis inhibitors, STF-31 and 2-DG, suppressed the LMP1-induced cell migration and invasion supporting a role of EBV gene-driven aerobic glycolysis in NPC metastasis (Tsao's unpublished data).

Lactate may also contribute to immune escape of tumor cells through suppressing monocyte migration and release of cytokines [72, 73], inhibiting activation of T cells and natural killer cells [74–76]. NPC is characterized by substantial infiltration of immune cells in the tumor microenvironment including dendritic cells, monocytes, T cells, and B cells. The contribution of aerobic glycolysis driven by EBV-infected NPC cells to modulate host immune responses remains to be determined.

6.8 Conclusions

The role of EBV in NPC pathogenesis has been enigmatic. The underlying mechanism supporting latent EBV infection and growth advantage in NPC are not well defined. Genomic profiling revealed that mutations involved in activation of NF-κB and PTEN/PI3K/AKT may drive mTOR signaling to support latent infection of

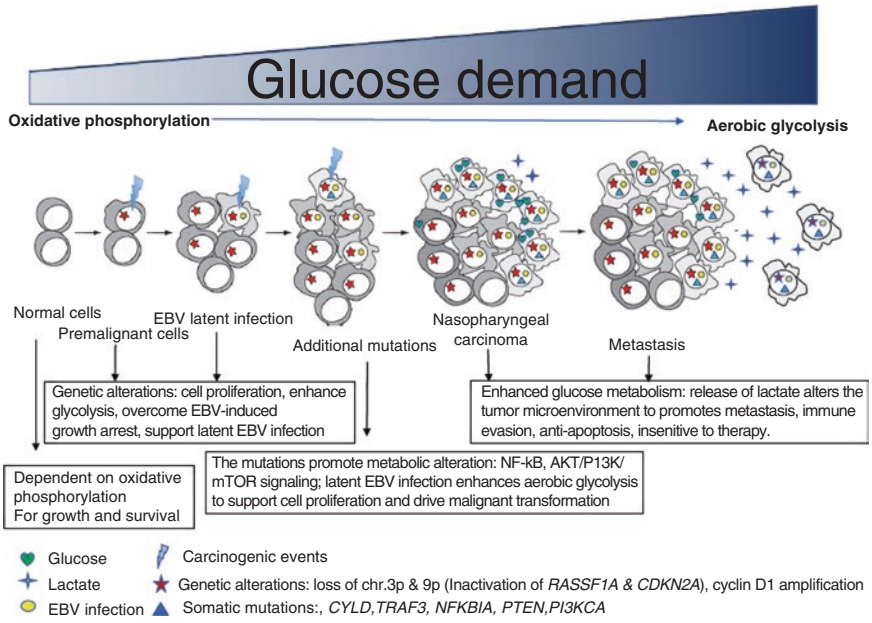

Fig. 6.1 Schematic diagram showing how EBV infection and glucose metabolism may contribute to pathogenesis and progression of nasopharyngeal carcinoma (NPC). The glucose demand increases during malignant transformation of nasopharyngeal epithelial cells which serves as a selective force for latent EBV infection and NPC development. EBV initially establishes latent infection in premalignant nasopharyngeal epithelial cells harboring genetic alterations, e.g., cyclin D1 amplification and p16 deletion. Expression of latent EBV genes, e.g., LMP1 and LMP2, drives glycose metabolism and supports clonal expansion of EBV-infected premalignant nasopharyngeal epithelial cells harboring additional mutations. Genetic alterations involved in activation of NF-κB signaling have selective advantage growth in EBV-infected nasopharyngeal epithelial cells for its ability to activate mTOR and glucose metabolism which support latent EBV infection. Positive feedback of latent EBV genes further enhances glucose metabolism to support malignant transformation of premalignant nasopharyngeal epithelial cells. Enhanced glucose metabolism and accumulation of metabolites of aerobic glycolysis, e.g., lactate, further modulate the tumor microenvironment to promote NPC progression including immune evasion, angiogenesis, anti-apoptosis, resistance to treatment, invasion, and metastasis

EBV in NPC cells. Latent infection of NPC cells and expression of latent EBV genes further drive glucose metabolism and modify the tumor microenvironment to enhance malignant properties of NPC including immune evasion and antiapoptosis. Enhanced glucose metabolism is a common hallmark of human cancer. In NPC, enhanced glucose metabolism has been demonstrated by the EBV-encoded LMP1. A schematic diagram illustrating how latent EBV infection and enhance glucose metabolism may drive the development of NPC and its progression is shown in Fig. 6.1. An interactive interplay between glucose metabolism and EBV gene-driven glucose metabolism may modulate malignant properties of NPC cells including angiogenesis, invasion, metastasis, and resistance to therapy. Understanding the key

events involved in altered glucose metabolism and the role of latent EBV infection in NPC pathogenesis may reveal novel therapeutic targets to suppress NPC metastasis and potentially reverse resistance to treatment therapy in NPC patients.

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