

## The role of metabolic reprogramming in -herpesvirus-associated oncogenesis

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**Title:****The role of Metabolic Reprogramming in  $\gamma$ -Herpesvirus-associated Oncogenesis****Angela Kwok-Fung Lo<sup>1\*</sup>, Christopher W. Dawson<sup>2</sup>, Lawrence S. Young<sup>3</sup>, and Kwok-Wai Lo<sup>1</sup>**

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**Abstract**

The  $\gamma$ -herpesviruses, EBV and KSHV, are closely associated with a number of human cancers. While the signal transduction pathways exploited by  $\gamma$ -herpesviruses to promote cell growth, survival and transformation have been reported, recent studies have uncovered the impact of  $\gamma$ -herpesvirus infection on host cell metabolism. Here, we review the mechanisms utilised by  $\gamma$ -herpesviruses to induce metabolic reprogramming in host cells, focusing on their ability to modulate the activity of metabolic regulators and manipulate metabolic pathways. While  $\gamma$ -herpesviruses alter metabolic phenotypes as a means to support viral infection and long-term persistence, this modulation can inadvertently contribute to cancer development. Strategies that target deregulated metabolic phenotypes induced by  $\gamma$ -herpesviruses provide new opportunities for therapeutic intervention.

**Key words:** EBV; KSHV; Metabolism; Transformation

## Introduction

Viruses play a significant role in tumorigenesis, with approximately 12% of the total global cancer incidence attributed to viral infection.<sup>1</sup> Human papillomavirus (HPV), Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV) are the three most significant contributors to virus-associated cancers arising in the population before 50 years of age.<sup>1</sup> EBV and KSHV are the only members of the  $\gamma$ -herpesviridae family associated with human malignancies. They have evolved similar strategies to establish lifelong asymptomatic latent infections with periodic lytic replication in their hosts.<sup>2</sup> As obligate intracellular parasites,  $\gamma$ -herpesviruses depend on host cell metabolism to obtain energy and metabolic precursors for viral replication and propagation. These viruses also rely on host cell proliferation and survival mechanisms for viral amplification and persistence. For these reasons,  $\gamma$ -herpesviruses alter many host cell metabolic pathways to sustain energy production and metabolite synthesis. These metabolic alterations not only support viral persistence but also promote cell growth and survival. Thus, viral infection on a background of underlying genetic abnormalities expedites additional mutations that facilitate cancer development.<sup>3,4</sup> Here, we describe how  $\gamma$ -herpesvirus reprogramming of host cell metabolism contributes to cell survival and proliferation. We also discuss the potential of targeting the metabolic alterations induced by  $\gamma$ -herpesviruses as a viable therapeutic strategy for  $\gamma$ -herpesvirus-associated cancers.

## Epstein-Barr Virus

EBV is a ubiquitous virus, infecting more than 95% of the population worldwide. While infection in most individuals is usually asymptomatic, the virus is linked to the development of a number of human malignancies including Burkitt's lymphoma (BL), Hodgkin's lymphoma (HL), post-transplant lymphoproliferative disease (PTLD), nasopharyngeal carcinoma (NPC) and a subset of gastric carcinoma (GC).<sup>4-7</sup> Approximately, 1.8% of all cancer deaths worldwide are attributable to EBV-associated malignancies.<sup>7,8</sup> Upon infection of host cells, EBV can adopt various forms of latency (Latency I, II and III), which differ in their repertoire of latent genes expressed. *In vitro*, EBV efficiently infects and transforms primary B cells into lymphoblastoid cell lines (LCLs). Both LCLs and PTLN display type III latency, where all latent genes are expressed. This includes six nuclear proteins (EBNA-1, -2, -3a/3b/c, and -5), three membrane proteins (LMP1, LMP2a/b), various non-coding RNAs (EBER1, EBER2 and BART RNAs), as well as the BHRF1 and BART miRNAs. HL and NPC exhibit type II latency, where viral gene expression is limited to EBNA1, LMP1, LMP2a/b, EBER1/2, and the BART RNAs and miRNAs. Endemic BL exhibits type I latency, expressing EBNA1, LMP2a, EBER1/2, and the BART RNAs and miRNAs.<sup>4-7</sup>

## Kaposi's Sarcoma-Associated Herpesvirus

KSHV accounts for approximately 2% of infection-attributable cancers worldwide.<sup>1</sup> KSHV was discovered in Kaposi's sarcoma (KS) but is also linked to primary effusion lymphoma (PEL) and multicentric Cattleman's disease (MCD). KSHV infection is prevalent in sub-Saharan Africa and the

Mediterranean but is rare in most other countries.<sup>1,9</sup> While a small proportion of cells in KS lesions undergo lytic replication, most tumour cells are latently infected, expressing 17 miRNAs and 4 viral proteins: LANA-1, v-cyclin D, v-FLIP, and Kaposin. In PEL lymphoma cells, LANA-2, together with the aforementioned latent genes is also expressed. As in KS, a small proportion of PEL cells undergo spontaneous lytic replication. KSHV can efficiently infect and establish viral latency in B cells, fibroblast, endothelial and epithelial cells *in vitro*; however, KSHV is unable to immortalise these cell types directly.<sup>3,4,9</sup>

### **Metabolic regulators and pathways manipulated by $\gamma$ -herpesviruses**

The activity of metabolic regulators such as HIF-1, Myc and AMPK, in addition to metabolic pathways including aerobic glycolysis, reactive oxygen species (ROS) production, fatty acid synthesis and glutaminolysis, have been shown to be deregulated in  $\gamma$ -herpesvirus-infected cells (Table 1).

#### **Hypoxia-inducible factor-1 (HIF-1)**

HIF-1 is a heterodimeric transcription factor composed of HIF-1 $\alpha$  and ARNT (also known as HIF-1 $\beta$ ). HIF-1 $\alpha$  mRNA expression is regulated by the NF $\kappa$ B and STAT3 pathways, while HIF-1 $\alpha$  protein synthesis is enhanced by the PI3K/Akt/mTOR and Raf/MEK/MAPK/p70S6K1 pathways.<sup>10,11</sup> Under normoxic conditions, HIF-1 $\alpha$  is hydroxylated by three oxygen-dependent prolyl hydroxylases (PHD1-3), ubiquitinated by the von Hippel-Lindau (VHL) protein, and degraded by the proteasome. HIF-1 transcriptional activity is dependent on the stability of HIF-1 $\alpha$ . In the absence of oxygen, the PHDs cannot hydroxylate HIF-1 $\alpha$ , resulting in the stabilisation of the HIF-1 $\alpha$  protein. HIF-1 regulates the transcription of many genes associated with aerobic glycolysis and fatty acid synthesis in addition to other genes involved in cell growth, survival and angiogenesis (Figure. 1).<sup>10,12</sup> It is evident, therefore, that aberrant HIF-1 activation can impact on cancer initiation and progression.

Both EBV and KSHV have been shown to target and activate HIF-1 $\alpha$ . EBV-infection of B cells is accompanied by an upregulation of HIF-1 $\alpha$  protein expression.<sup>13,14</sup> In epithelial cells, LMP1 stimulates HIF-1 transcriptional activity through the p42/44 MAPK pathway.<sup>13,14</sup> LMP1 also promotes H<sub>2</sub>O<sub>2</sub> production, inducing Siah1, which degrades the PHDs, thereby stabilising HIF-1 $\alpha$  protein.<sup>15</sup> Furthermore, LMP1-mediated p42/44 MAPK and STAT3 signalling increase the stability of HIF-1 $\alpha$  transcripts by downregulating the RNA-destabilizing proteins tristetraprolin (TTP) and pumilio RNA-binding family member 2 (PUM2).<sup>14</sup> Both LMP1 and EBNA-1 upregulate HIF-1 $\alpha$  and its downstream targets, IL-8 and VEGF.<sup>16</sup> EBNA-5 and EBNA-3, which are expressed in LCLs bind PHD1 and PHD2 respectively, blocking the catalytic activity of these enzymes and stabilising HIF-1 $\alpha$ .<sup>17</sup> The expression and transcriptional activity of HIF-1 $\alpha$  and HIF-2 $\alpha$  are elevated in KSHV-infected endothelial cells and KS lesions, implicating a role for HIF in KS pathogenesis.<sup>18</sup> Among the KSHV encoded proteins, vGPCR has been found to increase HIF-1 $\alpha$  transcriptional activity through the p42/44 MAPK and p38 MAPK signalling pathways.<sup>19</sup> vGPCR also increases VEGF secretion which in turn activates multiple

signalling pathways in neighbouring cells, leading to an mTOR-dependent induction of HIF-1 $\alpha$  and HIF-2 $\alpha$ .<sup>20</sup> LANA-1 promotes HIF-1 $\alpha$  protein accumulation. The SOCs motif of LANA-1 targets and degrades the VHL protein, resulting in HIF-1 $\alpha$  protein stabilisation.<sup>21</sup> Furthermore, a specific 43-amino acid domain of LANA-1 interacts with the oxygen-dependent degradation (ODD) domain of HIF-1 $\alpha$ , inhibiting HIF-1 $\alpha$  degradation.<sup>21,22</sup> Similarly, binding of the double  $\alpha$ -helix motifs of LANA-2 to the bHLH domain of HIF-1 $\alpha$  inhibits HIF-1 $\alpha$  degradation.<sup>23</sup> KSHV-encoded miRNAs (miR-K12-1 to 9, and 11) have also been shown to target and downregulate PHD1, leading to an increase in HIF-1 activity.<sup>24,25</sup> Overall, the above findings suggest that multiple gene products of  $\gamma$ -herpesviruses can promote the transcriptional activity of HIF-1 and the induction of HIF-1 $\alpha$  in response to  $\gamma$ -herpesvirus infection can promote cancer development.

### Myc

Myc is a transcription factor that regulates many cellular processes involved in cell growth and metabolism (Figure. 1). Myc regulates the expression of numerous genes associated with glycolysis, glutaminolysis, and the synthesis of fatty acids and amino acids.<sup>26-28</sup> Given the multiple regulatory functions of Myc, its expression and stability are tightly controlled in normal cells. However, dysregulation of Myc has been found in many types of cancer.<sup>26-28</sup> Both endemic and sporadic BLs, regardless of their EBV association, exhibit high levels of Myc due to the presence of chromosomal translocations that place the Myc gene under the control of either the heavy- or light-chain immunoglobulin loci.<sup>7,26-28</sup> Myc is commonly hijacked by  $\gamma$ -herpesviruses. In EBV-transformed LCLs, EBNA2 binds to two enhancers upstream of the Myc gene to activate Myc transcription.<sup>29,30</sup> Myc expression is driven by the EBV super-enhancers (ESEs). ESEs are clusters of gene-regulatory sites that are bound by all EBNAs, LMP1-activated NF $\kappa$ B subunits and an array of transcription factors critical for LCL growth and survival.<sup>31</sup> ESE enhancer RNAs (eRNAs) activated by EBNA2 is essential for Myc expression and LCL growth.<sup>32</sup> EBNA3c has also been found to enhance Myc protein stability and Myc-dependent transcription by recruiting Myc and its cofactor Skp2 to the promoters of target genes.<sup>33</sup> LMP1 has been shown to induce Myc expression in LCLs.<sup>34,35</sup> The induction of Myc by LMP1 activation of STAT3 in latent EBV-infected or LMP1-transfected nasopharyngeal epithelial cells has also been demonstrated.<sup>36,37</sup> Myc is also upregulated in LMP1 positive NPC lesions.<sup>38</sup> Overall, it is clear that multiple EBV-encoded latent proteins can induce Myc expression; the discovery of Myc ESEs and eRNAs further emphasises the key role of Myc in EBV persistence and oncogenesis. KSHV latent proteins also impact on Myc activity. LANA-1 has been shown to stabilise Myc through a direct interaction<sup>39</sup> and to inhibit Myc protein turnover by suppressing GSK-3 $\beta$ -mediated phosphorylation of Myc at Threonine 58.<sup>40</sup> Myc modulator 1 (MM-1) functions to inactivate Myc. LANA-2 has been found to interact with MM-1, resulting in the activation of Myc transcriptional activity.<sup>41</sup> LANA-2 also recruits Myc and its cofactor, Skp2, to the regulated promoters, enhancing Myc transcriptional activity.<sup>42</sup> A role for Myc in the maintenance of KSHV latency is supported by the demonstration that the inhibition of Myc in PEL cells induces cell cycle

arrest and apoptosis along with the induction of Rta, a viral protein required for initiation of the lytic cycle.<sup>43</sup>

### **AMP-activation protein kinase (AMPK)**

AMPK is an energy sensor that regulates cellular energy homeostasis (Figure. 1). AMPK activates catabolic pathways for ATP generation and blocks ATP-consuming anabolic pathways. AMPK activation inhibits cell growth and proliferation. AMPK also negatively regulates aerobic glycolysis in cancer cells and inhibits tumour growth.<sup>44,45</sup> Loss of AMPK activity has been documented in  $\gamma$ -herpesvirus-associated cancers. In KSHV-infected HUVEC cells, activation of the PI3K/Akt/mTOR pathway is accompanied by inactivation of AMPK. These effects are essential for the survival of KSHV-infected cells following exposure to etoposide, staurosporine or serum deprivation.<sup>46</sup> Furthermore, the KSHV-encoded K1 protein has been found to inactivate AMPK by binding to its  $\gamma$  subunit.<sup>47</sup> A study on HUVEC cells has shown that endogenous AMPK activity restricts KSHV lytic replication and virion production by inhibiting lytic gene expression.<sup>48</sup> Moreover, EBV-encoded LMP1 has been found to inactivate LKB1-AMPK, an effect that contributes to LMP1-mediated cellular proliferation and transformation.<sup>49</sup> Also, a negative correlation has been observed between LMP1 expression and AMPK phosphorylation in NPC lesions.<sup>49</sup> LMP1 also negatively regulates AMPK activity by inhibiting DNA-dependent protein kinase (DNA-PK) phosphorylation and activity, thereby facilitating LMP1-mediated glycolysis and resistance to apoptosis induced by irradiation.<sup>50</sup>

### **Aerobic Glycolysis**

Mammalian cells undergo oxidative phosphorylation (OXPHOS) to produce energy using glucose as fuel. Extracellular glucose is taken up by glucose transporters (GLUTs) and converted to glucose-6-phosphate by hexokinases (HK). Glucose-6-phosphate then undergoes glycolysis to generate pyruvate in the cytoplasm. When oxygen is available, pyruvate translocates to the mitochondria where it enters the Krebs cycle to produce metabolic intermediates and ATP. In the absence of oxygen, pyruvate is converted to lactate by lactate dehydrogenase 1 (LDHA1) in the cytoplasm. However, in rapidly proliferating cells such as cancer cells, pyruvate is primarily converted to lactate despite the presence of oxygen; a phenomenon known as aerobic glycolysis or the Warburg effect (Figure. 1). Aerobic glycolysis is facilitated by several oncoproteins including HIF-1 $\alpha$ , Myc and FGFR1.<sup>12,51,52</sup>

Cellular glycolysis is a major metabolic pathway hijacked by  $\gamma$ -herpesviruses. EBV-infected LCLs have been shown to produce high levels of lactate. Many HIF-1 $\alpha$  responsive glycolytic genes are also expressed at high levels in LCLs.<sup>17</sup> By following the metabolic changes that accompany the progression of EBV-infected primary B cells to established LCLs, one study has demonstrated high levels of plasma membrane GLUT1 and increased rates of glucose uptake, leading to elevated glycolysis.<sup>53</sup> In BL cells, LMP1 increases glucose uptake through IKK $\beta$ /NF $\kappa$ B/Akt-induced plasma membrane trafficking of GLUT1; interestingly, the NF $\kappa$ B activation appears to have no impact on

GLUT1 and GLUT3 expression.<sup>54</sup> In contrast, an induction of GLUT1 expression in epithelial cells expressing LMP1 has been demonstrated.<sup>55</sup> In nasopharyngeal epithelial cells, we have found that LMP1 promote aerobic glycolysis by promoting GLUT1 translocation to the plasma membrane, increasing the cellular uptake of glucose and glutamine, enhancing LDHA activity and lactate production, but reducing PK activity and the intracellular levels of pyruvate.<sup>56</sup> Furthermore, we have identified a mutual exclusivity relationship between LMP1 expression and genetic inactivation of TRAF3 in NPCs.<sup>57</sup> TRAF3 deficiency has been reported to promote glycolysis.<sup>58</sup> Moreover, we have found that LMP1 upregulates Myc and HIF-1 $\alpha$  expression and induces FGF2/FGFR1 signalling activity.<sup>56</sup> Activation of FGF2/FGFR1 signalling by LMP1 contributes to aerobic glycolysis and cellular transformation. A positive correlation between LMP1 expression and FGFR1 phosphorylation has also been observed in NPC specimens.<sup>56</sup> LMP1-mediated PI3K induction of Myc has been shown to upregulate HK2, while, HK2 silencing in LMP1-expressing cells causes a reduction in glucose consumption and a loss of cell viability. Interestingly, one study has shown that HK2 expression positively correlates with LMP1 expression in NPC tissues and that high levels of HK2 are associated with poor survival rates in NPC patients following radiation therapy.<sup>59</sup> Therefore, it appears that the induction of aerobic glycolysis by EBV-encoded proteins facilitates cancer development.

KSHV also promotes aerobic glycolysis. Infection of endothelial cells with KSHV is associated with increased rates of glucose uptake and lactate production but decreased oxygen consumption. These effects are also accompanied by an upregulation of GLUT3, HK2, PKM2 and the HIF proteins.<sup>18,60</sup> Interestingly, inhibition of aerobic glycolysis by 2DG, an HK2 inhibitor, or oxamate, an LDH inhibitor in KSHV-infected cells leads to higher rates of apoptosis, indicating that aerobic glycolysis is essential for the survival of KSHV-infected cells.<sup>60</sup> Furthermore, inhibition of HIF-1 $\alpha$  translation by digoxin suppresses the expression of HIF-1-regulated metabolic genes and the Warburg effect in infected cells, indicating that aerobic glycolysis induced by KSHV is partially if not exclusively, mediated by HIF-1 activity.<sup>61</sup> Also, aerobic glycolysis in KSHV-infected PEL cells is reportedly associated with the activation of PI3K/Akt/mTOR pathway. Among the KSHV genes, LANA-1 has been demonstrated to facilitate glycolysis through a mechanism involving the degradation of VHL and p53.<sup>62</sup> Also, KSHV miRNA clusters have been reported to target and downregulate PHD1 (an HIF-1 $\alpha$  regulator) and HSPA9 (functions to regulate the transport of mitochondrial proteins), to increase HIF-1 activity and suppress mitochondrial biogenesis and activity. This facilitates the induction of aerobic glycolysis and suppression of OXPHOS in endothelial cells.<sup>24</sup> Interestingly, HSPA9 and the other proteins involved in mitochondrial import machinery have been identified as targets of the EBV BART miRNAs.<sup>63</sup> The possible impact of EBV miRNAs on OXPHOS activity and metabolic transformation is an area worthy of more detailed exploration.

### **Reactive Oxygen Species**

For energy production in the electron transport chain, electrons from NADPH and FADH<sub>2</sub> are oxidised to generate ATP and oxygen molecules are reduced to form water (Figure 1).<sup>12,51,52</sup> However, approximately 1-5% of oxygen molecules are incompletely reduced to produce superoxide radical (O<sub>2</sub><sup>-</sup>) by NADPH oxidases (NOX). O<sub>2</sub><sup>-</sup> can be further converted to H<sub>2</sub>O<sub>2</sub> and water. The O<sub>2</sub><sup>-</sup> and H<sub>2</sub>O<sub>2</sub> are so-called reactive oxygen species (ROS) (Figure. 1). ROS can be detoxified by redox-regulating enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx).<sup>64-66</sup> ROS is a natural product of energy metabolism and is necessary for intracellular signalling, antimicrobial defence and cell survival. However, an imbalance between ROS production and ROS detoxification results in oxidative stress. High levels of ROS cause severe damage to mitochondria and cell structure, leading to apoptosis. In contrast, low levels of ROS can activate growth-promoting signalling pathways including STAT3 and NFκB. ROS also facilitates glycolysis and pentose phosphate pathway flux for rapid cell proliferation. Moreover, ROS causes DNA damage, promoting DNA mutation and genomic instability, a major driving force in tumorigenesis.<sup>64-66</sup>

Increased ROS production and oxidative stress have been documented in a number of γ-herpesvirus-associated cancers. EBV-infected BL cells have been shown to exhibit high levels of ROS compared to EBV-negative BL cells, and that directly activates NFκB pathway.<sup>67</sup> Likewise, the induction of STAT3 and NFκB, two signalling pathways associated with oxidative stress has been found in EBV-infected nasopharyngeal epithelial cells.<sup>37</sup> EBV infection of B lymphocyte, epithelial and lymphoblastoid cell lines leads to increased levels of oxidative stress, and an accompanying reduction of CAT and SOD expression.<sup>68</sup> Interestingly, sera from NPC patients has been shown to contain decreased SOD activity.<sup>69</sup> Mass spectrometry analysis has identified the induction of two antioxidant enzymes, SOD1 and peroxiredoxin 1 (Prx1) in EBNA1-expressing NPC cells in which substantially increased expression of NOX1 and NOX2, and large induction of ROS levels were detected.<sup>70</sup> These findings suggest that the overall levels of ROS are determined by the differential expression of enzymes responsible for ROS production and detoxification, respectively. In lymphoid cell lines, EBNA1 has been shown to increase ROS production via transcriptional activation of NOX2, causing oxidative DNA damage, genomic instability and telomere dysfunction.<sup>71,72</sup> In addition to EBNA1, EBNA2 has been shown to increase ROS levels in BL cells.<sup>67</sup> In epithelial cells, LMP1 induces p22phox NOX subunit expression and NOX activity, resulting in ROS accumulation.<sup>67,73</sup> Interestingly, a correlation between p22phox and LMP1 expression in NPCs has been demonstrated.<sup>73</sup> Overall, these findings suggest that induction of ROS by EBV plays a role in maintaining viral latency and inadvertently facilitates malignant transformation. In the case of KSHV, the initiation of lytic cycle increases viral vGPCR expression and ROS production in virus-infected cells.<sup>74</sup> Overexpression of vGPCR in uninfected KS cells also causes ROS induction through an enhancement of NOX activity.<sup>74</sup> Interestingly, treatment of KSHV-infected KS cells with the antioxidant, N-acetyl cysteine (NAC) inhibits proliferation, angiogenesis, tumour formation and viral gene expression.<sup>74</sup> Therefore, it

appears that KS oncogenesis is associated with ROS-mediated cell proliferation and angiogenesis induced during the KSHV lytic cycle.

### **Fatty acid synthesis**

For fatty acid synthesis (FAS), Krebs-cycle-derived citrate is firstly converted to acetyl-coenzyme A (acetyl-CoA), which is then converted to malonyl-CoA by acetyl-CoA carboxylase (ACC). Malonyl-CoA is then repeatedly condensed with acetyl-CoA by fatty acid synthase (FASN) to generate fatty acids (palmitate) (Figure. 1). Normal cells obtain fatty acids from the diet, while cancer cells prefer to engage FAS to produce palmitate.<sup>75,76</sup> Activation of FAS is also common in  $\gamma$ -herpesvirus-infected cells. Overexpression of FASN has been reported in oral hairy leukoplakia (OHL) biopsies, with FASN expression induced through BRLF1-mediated p38MAPK signalling.<sup>77</sup> OHL is caused by lytic EBV infection, initiated by the immediate-early (IE) lytic proteins, BZLF1 and BRLF1. However, inhibition of FAS by FASN inhibitors prevents the expression of IE lytic and early lytic gene expression in EBV-infected B cells in response to lytic reactivation.<sup>77</sup> Moreover, the EBERs, which are expressed in NPC cells have been shown to upregulate FASN and promote cell proliferation. Interestingly, these effects can be inhibited by quercetin, an FASN inhibitor.<sup>78</sup> Overall, these findings suggest that FAS is necessary for the initiation of EBV lytic cycle and the growth of latently infected cells. In KSHV infected endothelial cells, increased levels of FAS-associated metabolites have been reported in addition to large numbers of lipid droplets.<sup>79</sup> Interestingly, the FASN inhibitor, C75 or the ACC inhibitor, TOFA have been shown to induce apoptosis in KSHV-infected cells, while, the addition of palmitate protects KSHV-infected cells from apoptosis induced by TOFA treatment.<sup>79</sup> Similarly, latent KSHV infection in PEL cells induces FAS and increases cell sensitivity to C75 treatment compared to the uninfected primary B cells.<sup>80</sup> Collectively, these findings indicate that FAS induced by KSHV is essential for the survival of latently infected cells.

### **Glutaminolysis**

Aerobic glycolysis diverts citrate away from the mitochondrial Krebs cycle. However, citrate is necessary for FAS. To promote aerobic glycolysis and FAS in rapidly proliferating cells, glutamine is usually replenished into the TCA cycle. Glutamine is transported into cells through glutamine transporter SLC1A5 and then converted to  $\alpha$ -ketoglutarate, providing TCA intermediates for the production of biosynthetic and bioenergetics precursors<sup>81,82</sup> (Figure. 1). Many cancers cells are addicted to using glutamine for OXPHOS, FAS and protein synthesis. The induction of glutaminolysis by  $\gamma$ -herpesviruses has been documented. We have found that LMP1 increases glutamine uptake and elevates levels of intracellular glutamate in nasopharyngeal epithelial cells.<sup>56</sup> Similarly, latent infection of endothelial cells with KSHV has been shown to enhance glutamine uptake, elevate the intracellular levels of glutamine, and increase SLC1A5 expression.<sup>83</sup> The dependency on glutamine for cell growth has been proven, as KSHV-infected cells undergo apoptosis in response to glutamine deprivation, treatment with an inhibitor of SLC1A5 or siRNA targeting of SLC1A5. These effects can be rescued

by supplementing cells with TCA intermediates.<sup>83</sup> KSHV has been shown to increase the expression of MondoA, a member of Myc superfamily, to promote cell survival. siRNA knocking down of MondoA results in the death of KSHV-infected cells, an effect that can be rescued by the addition of TCA intermediates.<sup>83</sup> These findings suggest that glutaminolysis induced by MondoA for the replenishment of TCA intermediates is essential for the survival of KSHV-infected cells.

### **Therapeutic implications**

As outlined above, metabolic reprogramming facilitates both  $\gamma$ -herpesvirus infection and virus-associated tumour development. As such, treatments that target metabolic alterations may provide a novel therapeutic strategy for  $\gamma$ -herpesvirus-associated cancers (Figure 2). The profound effects of Myc on metabolic reprogramming makes this regulator an attractive therapeutic target. It has been reported that KSHV-infected PEL cells undergo G<sub>0</sub>/G<sub>1</sub> cell cycle arrest, apoptosis and senescence after treatment with (+)-JQ1 and I-BET151, inhibitors of the BET proteins which function to prevent interaction of the BET proteins with Myc. These inhibitors suppress Myc expression, resulting in a genome-wide perturbation of Myc-dependent gene expression.<sup>84</sup> In a xenograft model of PEL, (+)-JQ1 has also been shown to inhibit tumour growth and improve survival.<sup>84</sup> The application of BET inhibitors for the treatment of  $\gamma$ -herpesvirus-associated cancers is clearly worthy of further exploration. HIF-1 $\alpha$  is critical for cellular transformation. Many  $\gamma$ -herpesvirus-encoded proteins have been shown to upregulate HIF-1 $\alpha$ .<sup>13-24,61</sup> HIF-1 $\alpha$  is, therefore, an attractive therapeutic target for the  $\gamma$ -herpesvirus-associated cancers. Topotecan and bortezomib, two drugs that inhibit HIF-1 $\alpha$  translation and transactivation respectively, have been FDA-approved for the treatment of lymphoid and solid cancers.<sup>85,86,87,88</sup> Ganetespib, a drug that inhibits HIF-1 $\alpha$  stability is being evaluated in a Phase III trial for solid cancers.<sup>87,88</sup> These promising findings encourage further investigation into the therapeutic potential of HIF-1 inhibitors in  $\gamma$ -herpesvirus-associated cancers. AMPK is frequently inactive in oncovirus-infected malignancies. The AMPK activator, AICAR, has been shown to inhibit NPC cell growth and potentiates the cytotoxic effects of chemotherapeutic drugs.<sup>49</sup> An additional AMPK activator, metformin, a well-known oral anti-diabetic drug, has been found to induce G<sub>1</sub> cell-cycle arrest and inhibit the proliferation of NPC cells.<sup>89</sup> The therapeutic value of metformin in solid tumours has been evaluated in a number of clinical trial and is worthy of further examination in the context of  $\gamma$ -herpesvirus-associated cancers.<sup>90,91</sup> The glycolytic HK2 enzyme has been proposed as a potential target for cancer therapy. 2-deoxy-D-glucose (2DG) which inhibits HK2 activity, induces apoptosis in KSHV-infected endothelial cells.<sup>60</sup> Similar growth inhibitory effects of 2DG have also been observed in PEL cells.<sup>80</sup> Furthermore, 2DG stimulates ER stress and inhibits viral replication and lytic reactivation in KSHV-infected endothelial cells.<sup>92</sup> These findings suggest that inhibition of HK2 by 2DG not only induces cell apoptosis but also inhibits viral replication. However, owing to its significant systemic toxicity in clinical studies, 2DG has been discontinued for clinical use. As an alternative, lonidamine, the most advanced HK inhibitor has been tested in phase II clinical trials for

the treatment of solid tumours.<sup>93</sup> The possible application of lonidamine in the therapy of  $\gamma$ -herpesvirus-associated cancers is also worthy of examination.

### **Concluding Remarks**

The human  $\gamma$ -herpesviruses reprogram host cell metabolism to support viral persistence and the proliferation of virus-infected cells. These actions of the virus on a background of pre-existing genetic mutations may facilitate malignant transformation. Future studies will investigate how metabolic dysregulation in response to viral infection contributes to tumour progression, and how these metabolic changes influence viral gene expression. Such investigations may aid the development of novel therapeutic strategies to target and destroy virus-infected cells prior to the onset of cancer progression. Currently, a number of metabolic inhibitors are being evaluated in clinical trials, some of which have been approved by the FDA for cancer treatment. There are high hopes that combinations of conventional chemotherapeutic agents and selected metabolic inhibitors will become highly effective and less toxic anti-cancer drugs for the treatment of  $\gamma$ -herpesvirus-associated malignancies.

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### **Conflict of Interest**

There are no competing financial interests in relation to this work.

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## Legends:

### Figure 1.

The depiction of the major metabolic pathways: aerobic glycolysis, Krebs cycle, oxidative phosphorylation, ROS production, fatty acid synthesis, glutaminolysis, and amino acid synthesis as well as primary metabolic regulators: HIF-1 $\alpha$ , Myc and AMPK that are altered by EBV and KSHV. Key metabolites are shown in black, while key metabolic enzymes are labelled in black with pink boxes. Metabolic pathways are labelled in black with blue boxes. Metabolic regulators are shown in black with green boxes. GLUT: glucose transporter; HK: Hexokinase; PKF: phosphofructokinase; PK: pyruvate kinase; LDHA1: lactate dehydrogenase 1; MCT: monocarboxylate transporter; PDH: pyruvate dehydrogenase; PDHK: pyruvate dehydrogenase kinase; SLC1A5: glutamine transporter;

GLS: glutaminase; ACLY: ATP-citrate lysase; ACC: acetyl-CoA carboxylase; FASN: fatty acid synthase.

**Figure 2.**

A summary of studies describing the use of small molecules to target Myc, HIF-1 $\alpha$ , AMPK and HK2 in cancer treatment.

**Table 1:**

Alterations of metabolic pathways and regulators by  $\gamma$ -herpesviruses.

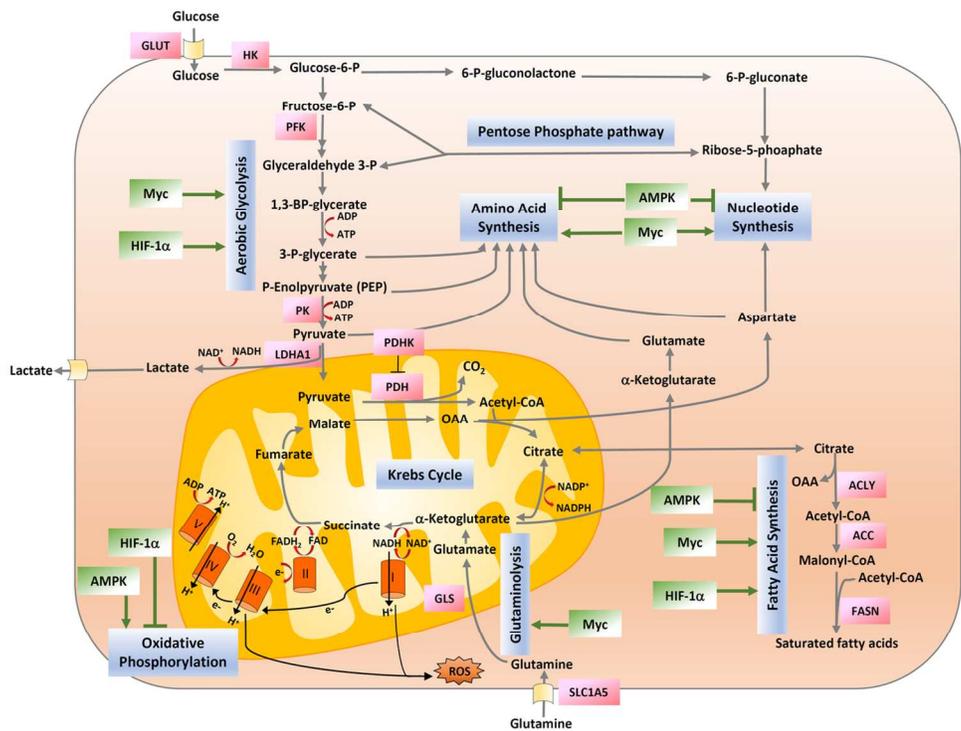


Figure 1

127x94mm (300 x 300 DPI)

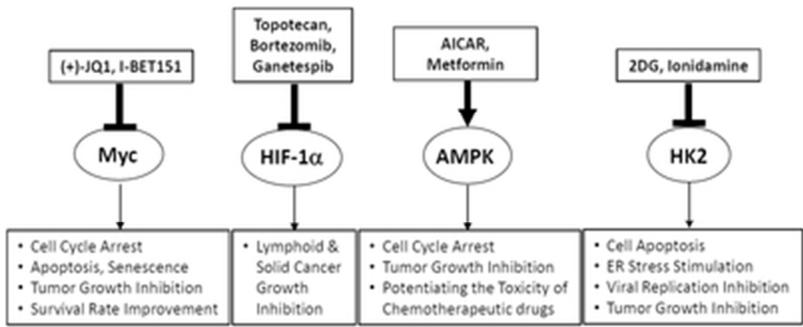


Figure 2

34x13mm (300 x 300 DPI)

**Table 1:** Alterations of metabolic pathways and regulators by  $\gamma$ -herpesviruses.

Metabolic Targets	Virus	Viral products	Type of Induction	Mechanism(s) involved	Ref.
<b>HIF-1<math>\alpha</math></b>	EBV	LMP1	Protein expression, stabilization & activity	Activation of the p42/44 MAPK pathway; Induction of H <sub>2</sub> O <sub>2</sub> production & induction of Siah1 to degrade PHD1 & 2, preventing HIF-1 $\alpha$ hydroxylation.	13-15
	EBV	LMP1	mRNA transcription & transcript stabilization	Reduction of TTP & PUM2 through p42/44 MAPK & STAT3 pathways.	14
	EBV	EBNA1	mRNA & protein expression	Not known.	16
	EBV	EBNA3 & 5	Protein stabilization	Inactivation of the HIF-1 regulators, PHD1 and PHD2.	17
	KSHV	vGPCR	Transcriptional activity, protein expression	Activation of the MEK/AMPK & p38 MAPK signaling pathways. Increased VEGF secretion, causing HIF-1 $\alpha$ & HIF-2 $\alpha$ induction in neighboring cells.	19-20
	KSHV	LANA1	Protein stabilization & transcriptional activity	Targeting & degrading the VHL protein. Binding to the ODD domain of HIF-1 $\alpha$ to enhance its activity; binding to the bHLH domain of HIF-1 $\alpha$ to inhibit its degradation.	21-23
<b>Myc</b>	EBV	EBNA2	mRNA transcription	Binding to Myc enhancers & increased expression of Myc ESE enhancer RNAs.	29,30,32
	EBV	EBNA3c	Protein stabilization & transcriptional activity	Interaction with Myc, promoting the binding of Myc and its cofactor Skp2 to target gene promoters.	33
	EBV	LMP1	mRNA transcription	Activation of the STAT3 pathway.	34-37
	KSHV	LANA1	Protein stability	Suppression of G3K-3 $\beta$ -mediated Myc phosphorylation at Threonine 58.	39,40
	KSHV	LANA2	Transcriptional activity	Inhibition of MM-1 binding to Myc. Recruitment of Myc & Skp2 to target gene promoters.	41,42
<b>AMPK</b>	KSHV	Latency	AMPK inactivation	Activation of the PI3K/Akt/mTOR pathway.	46
	KSHV	K1	AMPK inactivation	Binding to the $\gamma$ subunit of AMPK.	47
	EBV	LMP1	AMPK inactivation	Inhibition of LKB1 through the MEK/MAPK pathway; inhibition of DNA-PK phosphorylation & activity.	48;49
<b>Aerobic Glycolysis</b>	EBV	LMP1	The Warburg Effect	Increased GLUT1 trafficking through activation of the IKK $\beta$ /NF $\kappa$ B/Akt pathway; increased uptake of glucose & glutamine; enhanced LDHA activity & lactate production; reduced PK activity & intracellular pyruvate; induction of HIF-1 $\alpha$ , Myc & FGFR1 signaling activity. Upregulation of HK2 through PI3K induction of Myc.	53-56,59
	EBV	Latency	The Warburg Effect	Increased lactate levels; increased expression of HIF-1 $\alpha$ responsive glycolytic genes.	17
	KSHV	Latency	The Warburg Effect	Increased glucose uptake & lactate production; reduced oxygen consumption; increased expression of GLUT3, HK2, HIF-1 & HIF-2; activation of the PI3K/Akt pathway.	18,60-62
	KSHV	miRNA	The Warburg Effect	Targeting EGLN2/PHD1 and HSPA9 to increase HIF-1 activity. Increased glucose uptake & lactate production; reduced mitochondria copy number & oxygen consumption; stabilization of HIF-1 $\alpha$ .	24
	KSHV	LANA1	The Warburg Effect	Degradation of VHL & p53	63
<b>ROS</b>	EBV	EBNA1	Induction of ROS levels	Increased expression of NOX1 & NOX2; transcriptional activation of the catalytic subunit of NOX2.	71-73
	EBV	EBNA2	Induction of ROS levels	Increased expression of LMP1.	68
	EBV	LMP1	Induction of ROS levels	Induction of the NOX subunit p22phox; increased NOX activity and ROS accumulation.	68,74
	KSHV	vGPCR	Induction of ROS levels	Enhanced NOX activity.	75
<b>FAS</b>	EBV	BRLF1	Induction FASN	Activation of the p38MAPK pathway.	78
	EBV	EBERs	Induction FASN	Not known.	79
	KSHV	Latency	Induction of FASN	A number of fatty acid species and lipid droplets were detected. Mechanism not known.	62,80
<b>Glutaminolysis</b>	KSHV	Latency	Induction of MonoA	Enhanced glutamine uptake; increased intracellular levels glutamine; expression of the glutamine transporter, SLC1A5.	83