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Activation of cellular metabolism during latent Kaposi's Sarcoma herpesvirus infection

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Herpesviruses can establish latent infections in the host with severely limited viral gene expression. Kaposi's Sarcoma-associated herpesvirus (KSHV) is found predominantly in the latent state in the main KS tumor cell, a cell of endothelial origin. While many viruses alter host cell metabolism during productive infection, latent KSHV infection of endothelial cells activates metabolic pathways that are activated in many cancer cells. Inhibition of these major metabolic pathways leads to apoptotic cell death of the latently infected cells. The study of KSHV activation of metabolism may lead to novel therapeutic options for eliminating latent infection of gamma-herpesviruses and could also lead to a deeper mechanistic understanding of how to target cancer cell metabolism.

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Introduction

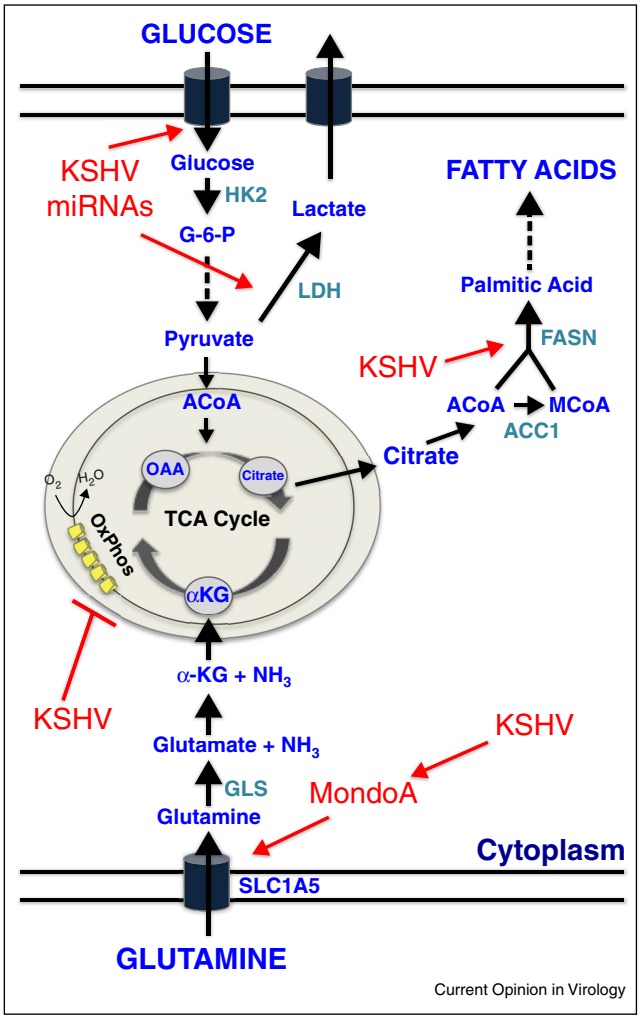
A common feature of all herpesviruses is the ability to establish life long latency in their host. During latent infection, herpesvirus genomes are almost always maintained as episomes where only a very limited number of viral genes are expressed. Latent herpesviruses do not replicate *via* a rolling circle mechanism, as occurs during lytic replication, and new viral particles are not produced. Therefore, it is assumed that latency is a fairly quiescent infection with little damage to the host cell. Herpesviruses have evolved to encode functions to keep the latently infected host cell alive [1,2]. While human alpha-herpesviruses establish latency in non-dividing cells, the beta and gamma herpesviruses establish latency in proliferating cells and therefore, they must encode proteins to maintain the viral genome during cell division [3–5]. Thus the viral genes expressed during latency allow for long-term latency and contribute to the ability of the herpesviruses to maintain infection for the life of the host.

The two human gamma-herpesviruses, EBV and KSHV are both associated with B-cell lymphomas and tumors of other cell types during latent infection. Latent EBV infection is associated with the majority of Burkitt's lymphoma (BL) in Africa and some BL in developed countries. In addition to other B-cell lymphomas, EBV is also associated with nasopharyngeal carcinoma, an epithelial cell cancer common to Southeast Asia. KSHV is associated with primary effusion lymphomas and, as the name suggests, is the etiologic agent of Kaposi's Sarcoma (KS), an endothelial based tumor. KS is the most common tumor of AIDS patients worldwide and is among the most common tumors overall in parts of sub-Saharan Africa [6]. As both latent KSHV and EBV infection can lead to tumor formation they likely encode oncogenic functions during this phase.

Cancer cell metabolism

In the 1920s, Otto Warburg first proposed that metabolic changes play a major role in cancer [7]. He found that cancer cells have increased levels of glucose uptake and glycolysis leading to increased production of lactic acid. Increased glycolysis is commonly accompanied by a decrease in oxygen consumption due to decreased oxidative phosphorylation in the mitochondria. These changes in metabolism, dubbed the Warburg effect, generally occur in most, if not all, cancer cells [8]. Fatty acid synthesis is also commonly increased in cancer cells, where there is a switch to, or increase in, *de novo* fatty acid synthesis generated from citrate [8,9]. In cancer cells, tri-carboxylic acid (TCA) cycle intermediates are depleted by the utilization of glucose for lactic acid production and citrate for fatty acid synthesis. Anapleurosis is a process by which TCA cycle intermediates can be supplemented from other sources, namely glutamine. Glutamine is the second most abundant carbon source in the blood and cancer cells are often glutamine addicted. Many cancer cells increase glutamine uptake and require glutaminolysis, the breakdown of glutamine to provide increased levels of the TCA cycle intermediate alpha-ketoglutarate [8,10]. It has been recently appreciated that changes in cellular metabolism may be a driver in oncogenesis not simply a cellular adaptation [11,12]. While these three pathways, shown in Figure 1, are clearly important for cancer cell growth, the exact requirements for these metabolic switches are not clear. A better understanding of how cells switch to cancer cell metabolism could be helpful in creating novel cancer therapies through the application of metabolic

Figure 1



Depiction of the three major metabolic pathways, glycolysis, glutaminolysis and fatty acid synthesis, that are altered in many cancer cells and by latent KSHV infection. A subset of key metabolites in the three major metabolic pathways and the TCA cycle are shown in blue. During latency, KSHV activates all three major pathways at points indicated by red arrows and inhibits oxidative phosphorylation. Chemical inhibition of the metabolic enzymes shown in light blue has been shown to lead to cell death in latently infected endothelial cells.

inhibitors [13,14]. Studies of how viruses alter cell metabolism may lead to novel findings about cancer cell metabolism. In particular, the study of latent infection, where the cell is kept alive long-term, will be relevant to the study of altered metabolism during oncogenesis.

KSHV induces the Warburg effect during latency

KSHV latent infection of endothelial cells in culture induces the Warburg effect. Latent KSHV infection significantly increases glucose uptake of endothelial cells and induces the production of lactic acid, thereby rapidly acidifying the media of latently infected cells [15••]. There is an increase in the expression of Hexokinase 2, the first rate-limiting step of glycolysis, as well as the expression of glucose transporter 3 [15••]. Oxygen consumption of latently infected endothelial cells decreases compared to mock infected cells, indicating a decrease in oxidative phosphorylation in the mitochondria, as often accompanies the Warburg effect [15••]. Importantly, inhibition of glycolysis with drugs that inhibit steps of glycolysis, including oxamate and 2-deoxyglucose, induce apoptosis in the latently infected cells at much higher rates than in their mock counterparts [15••]. These data indicate that endothelial cells latently infected with KSHV induce and become dependent on the induction of glycolysis. KSHV induction of glycolysis does not occur in Human Foreskin Fibroblast cells, demonstrating there is some cell type specificity to the induction of glycolysis by KSHV [15••]. KSHV induces hypoxia induced factors, HIF-1 and HIF-2 transcriptional activity in endothelial cells [16]. Inhibition of HIF blocked the induction of glycolysis by KSHV indicating that HIFs are responsible for at least some of the KSHV induction of glycolysis [17]. Primary effusion lymphoma cell lines, a KSHV induced B-cell lymphoma, EBV associated Burkitt's lymphoma cell lines and nasopharyngeal carcinoma cells all have activated glycolysis [18,19,20•]. While clouded by the fact that these cells are derived from human cancers, they certainly raise the possibility that KSHV and EBV latency in B-cells and EBV in epithelial cells also activate glycolysis similarly to KSHV latent infection of endothelial cells. There is also evidence that EBV infection of B-cells induces glycolysis in the cells that form the EBV immortalized lymphoblastoid cells [21,22].

Latent genes from both KSHV and EBV are sufficient to induce glycolysis. KSHV expresses numerous microRNAs from 12 loci. Overexpression of a region containing 10 of these miRNA loci was sufficient to induce glycolysis in endothelial cells [23•]. The latent EBV protein, LMP-1, that is expressed in specific types of EBV latency, is also sufficient to induce glycolysis [18,24] though LMP-1 is not expressed in all types of EBV latency. Therefore, both KSHV and EBV have evolved to encode functions that are expressed during latency and are sufficient to activate glycolysis. Further experiments are necessary to demonstrate that these genes are necessary for viral induction of glycolysis during latency in endothelial or B-cells.

KSHV activates fatty acid synthesis during latency

A global screen of metabolite levels revealed that latent KSHV infection induces several additional metabolic changes, including products of fatty acid synthesis [25••]. A mass-spectrometry based metabolomics screen measured the levels of nearly 200 metabolites at 48 and 96 hours post infection when latency is established. At 96 hours post-infection, nearly one-third of all the measured metabolite