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Viral Activation of Cellular Metabolism

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Abstract

To ensure optimal environments for their replication and spread, viruses have evolved to alter many host cell pathways. In the last decade, metabolomic studies have shown that eukaryotic viruses induce large-scale alterations in host cellular metabolism. Most viruses examined to date induce aerobic glycolysis also known as the Warburg effect. Many viruses tested also induce fatty acid synthesis as well as glutaminolysis. These modifications of carbon source utilization by infected cells can increase available energy for virus replication and virion production, provide specific cellular substrates for virus particles and create viral replication niches while increasing infected cell survival. Each virus species also likely requires unique metabolic changes for successful spread and recent research has identified additional virus-specific metabolic changes induced by many virus species. A better understanding of the metabolic alterations required for each virus may lead to novel therapeutic approaches through targeted inhibition of specific cellular metabolic pathways.

Introduction

Viruses are non-living entities and as such do not inherently have their own metabolism. However, within the last decade, it has become clear that viruses dramatically modify cellular metabolism upon entry into a cell. Viruses have likely evolved to induce metabolic pathways for multiple ends. Virus-induced metabolism may provide increased pools of free nucleotides necessary for rapid viral genome replication as well as increased amino acid production for rapid virion assembly. For enveloped viruses, increased lipid material may be needed to provide additional membrane material for envelopment of the viral particles. Alteration of cellular metabolism by viruses could also be necessary to provide specific substrates that are uniquely required at high levels for virion production, for example increased glycoproteins for viral envelopes. In addition to providing direct substrates for virion production, adjustments to metabolic pathways may be required to provide ATP in a rapid fashion for the high energy costs of genome replication and packaging. Altered cellular metabolism may aid in the survival of infected cells during the stress of viral

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infection. Identification of how viruses alter cellular metabolism and where in the virus life cycle these metabolic changes are necessary will provide a deeper understanding of virus replication needs and potentially provide cellular targets for inhibition of viruses.

While there were clear indications from studies in the 1950s and 60s that virus infection require specific metabolic pathways for replication, identification of virus induced metabolic pathways has been facilitated by recent technological advances. Advanced mass spectrometry techniques can concurrently measure the levels of many metabolites and measure carbon flux through the metabolic system. The term metabolomics was coined to describe experiments where large numbers of cellular metabolite levels are measured concurrently. These studies yield a broader comparison of mock- and virus-infected cell metabolite levels and can identify large-scale changes induced by a virus. Additionally, technological advances have led to the ability to measure carbon flux by following isotope labeled carbon that, while difficult to perform, have allowed a better understanding of the fate of extracellular carbon sources.

In 2006, the first eukaryotic virus-infected cell metabolomics study led the resurgence of the virus-induced metabolism field. The study analyzed over 60 metabolites during human Cytomegalovirus infection (HCMV) (Munger et al., 2006). The metabolomics study was followed up with a carbon flux analysis that provided a clearer picture of the flow of carbon from labeled glucose and glutamine following CMV infection, further showing how carbon is utilized in the infected cell (Munger et al., 2008). Since these first studies, a number of viruses have been shown to alter multiple major metabolic pathways and have expanded the number of metabolites measured (Birungi et al., 2010; Delgado et al., 2012; Diamond et al., 2010; Fontaine et al., 2014; Fontaine et al., 2015; Hollenbaugh et al., 2011; Lin et al., 2010; Ritter et al., 2010; Roe et al., 2011; Vastag et al., 2011). A number of core cellular metabolic pathways, including glycolysis, fatty acid synthesis and glutaminolysis, are significantly altered by multiple virus families (Table 1). These core pathways are often similarly activated in many cancer cells. Therefore, it is important that, when possible, metabolomics studies are done in primary or minimally immortalized cells. While information can still be gleaned from studies in transformed cells, many pathways are masked by metabolic switches that are triggered during oncogenesis and in some cases cell lines are immortalized by oncogenic viruses or viral proteins that could contribute to the metabolic profile observed. The importance of glycolysis, fatty acid synthesis and glutaminolysis to viral infections is discussed below.

Glycolysis

Primary mammalian cells under standard growth conditions predominantly utilize glucose for oxidative phosphorylation in the mitochondria. Glucose is metabolized to pyruvate through multiple steps. Pyruvate is then translocated to the mitochondria, where it enters the TCA cycle and ultimately drives the electron transport chain, in a process that requires oxygen. In anaerobic conditions, glucose is primarily utilized for glycolysis where it is metabolized to pyruvate and then is converted to lactate and pumped out of the cell. In most cancer cells glucose is primarily utilized for the production of lactic acid even in the presence of abundant oxygen, a process often referred to as aerobic glycolysis or the

Warburg effect. While oxidative phosphorylation provides significantly more ATP per glucose, glycolysis is a much faster process providing ATP rapidly. However, utilizing glycolysis as the main metabolic pathway for glucose requires increased uptake of extracellular glucose to match the increased metabolic rate. While cancer cells utilize aerobic glycolysis, it is not clear if the switch is needed for faster production of ATP or if the production of lactate and higher levels of glycolytic intermediates are advantageous as a source of biomass.

Early hints that glycolysis was required for viral replication came from viral infection studies in the 1950s and 60s. In 1956, it was shown that the propagation of poliovirus in Hela cells was blocked in minimal media but the addition of glucose recovered a significant portion of the viral titer (Eagle and Habel, 1956). This finding was subsequently confirmed in primary monkey kidney cells where an increase in lactate in the media during the first two hours of infection was also shown (Baron and Levy, 1956; Levy and Baron, 1957). In 1962, it was shown that HSV-1 passage in Hela cells was dependent on the presence of glucose but not glutamine in the media (Lewis and Scott, 1962). In the absence of glucose, HSV-1 penetrated cells equally but did not produce infectious progeny. It was subsequently shown that HSV-1 virion production did not decrease significantly in the presence of 2-DG, a competitive inhibitor of glucose for Hexokinase-2 (Courtney et al., 1973). However, the production of infectious particles was severely impaired in the presence of 2-DG, possibly due to alterations in the viral glycoproteins. Similar studies with pseudorabies, another alphaherpesvirus, found that viral nucleic acid replication did not require glycolysis and viral proteins were produced in the presence of 2-DG but there was a significant decrease in the production of infectious virus (Ludwig and Rott, 1975). These data show that virus production requires glycolysis for a later step in replication, possibly late gene synthesis, virion assembly or egress, and is not due to the simple explanation that cell integrity is compromised by the lack of glucose. Through the 1980s and 1990s studies of glycolysis and metabolism in virus-infected cells were mostly limited to transforming retroviruses where the effect was clouded by the transformation of the cells by the virus. However, there were studies showing that some viruses, including HCMV and HSV, induced glucose uptake (Landini, 1984; Saito and Price, 1984).

The first eukaryotic virus-infected cell metabolomics study found that glycolytic intermediates were induced following HCMV infection (Munger et al., 2006). This study measured over 60 metabolites following HCMV infection of primary human foreskin fibroblasts (HFF) cells. A number of glycolytic intermediates were significantly increased following infection. Flux analysis demonstrated an increased flux of glucose carbon through glycolysis ultimately leading to an increase in lactate production (Munger et al., 2008). Subsequent studies examined the mechanism of HCMV induction of glucose uptake and glycolysis. Viral protein expression is required for HCMV induced glycolysis, as cyclohexamide blocks induction (McArdle et al., 2011). It appears that early genes are necessary for HCMV induction of glyocolysis as phosphonoacetic acid, an inhibitor of viral replication that prevents the expression of late viral genes, has no effect on HCMV induced glycolysis. This study also analyzed a cellular pathway know to be important for increased glycolysis in cancer cells, the calmodulin-dependent protein kinase family. An inhibitor of CAMKK, but not CaMKII, blocks HCMV induced glycolytic flux (McArdle et al., 2011).

Additionally, the CAMKK inhibitor blocks viral replication and late gene synthesis and the production of viral progeny. Taken together, these data show that HCMV requires glycolysis for replication of the viral genome. Interestingly, the CAMKK inhibitor has little effect on HSV-1, an alphaherpesvirsus, while 2-DG inhibits the two viruses equally (McArdle et al., 2011). Therefore, the mechanism for the virus-induction of glycolysis is virus species specific. HCMV also alters glucose transporter expression on infected HFF cell membranes (Yu et al., 2011b). There are a number of transporters, some of which are ubiquitous and some are differentially expressed between cell types. HCMV downregulates the ubiquitously expressed glucose transporter-1 (glut1) and concurrently increases the expression of glut4, a more efficient glucose transporter that can accelerate glucose uptake (Yu et al., 2011b). Therefore, HCMV induces a shift in glucose transporter expression allowing increased glucose accumulation in infected cells. An inhibitor of glut4 decreased glucose uptake in infected HFF cells, as well as the production of infectious CMV. Though much is known about how HCMV alters glycolysis, the viral genes necessary for viral induction have not been elucidated and there are likely more cellular pathways involved.

Adenovirus, a non-enveloped double stranded DNA virus also induces glycolysis. Adenovirus5 infection of non-tumorigenic breast epithelial cells led to increased glucose consumption and lactic acid production with a concurrent decrease in oxygen consumption (Thai et al., 2014). Expression of the viral gene, E4ORF1, was sufficient to induce glycolysis but did not decrease oxygen consumption, indicating these might be separable functions in Adenovirus infected cells. E4ORF1 induces and co-imunoprecipitates with Myc and its interaction with Myc is required for the induction of glycolysis. An Adenovirus expressing a mutant E4ORF1 that does not induce Myc also does not induce glycolysis supporting the conclusion that activation of Myc is involved in the viral induction of this glycolysis. Adenovirus infection led to increased carbon flux into nucleotides and when radiolabeled glucose was added to infected cell media, adenovirus DNA was labeled (Thai et al., 2014). Carbon flux into nucleotides and Adenovirus DNA replication was blunted when cells were infected with the adenovirus mutant where E4ORF1 could not activate Myc. Therefore, it appears that Adenovirus induces Myc to activate glycolysis and nucleotide synthesis for adenovirus DNA replication. Interestingly, knockdown of Myc with shRNA led to a one-log decrease in virus titer in epithelial cells with low glycolytic rates but not in cells with high glycolytic rates (Thai et al., 2014). Therefore, activation of Myc and glycolysis is required for maximal Adenovirus replication in specific cellular settings where glycolysis is limiting.

Hepatitis C virus (HCV), a positive strand RNA virus, also induces glycolysis. Hepatitis C virus infection of Huh7 cells decreases host cell oxidative phosphorylation and increases dependence on extracellular glucose (Ripoli et al., 2010). HCV infectious virus culture systems are derived from a patient with fulminant hepatitis and these systems require Huh7 cells, a hepatoma cell line derived from a cellular carcinoma, for replication. While not an ideal cell line for metabolism studies as it is derived from a human hepatocellular carcinoma and therefore has many metabolic changes characteristic of most tumor cell lines, the HCV replication systems are severely limited. In addition to an increased requirement for glucose, there is an increase in lactate production in HCV-infected cells (Ramiere et al., 2014). A global proteomic screen showed that there is increased expression of many glycolytic

enzymes in HCV infected Huh7 cells, further indicating that HCV induces glycolysis (Diamond et al., 2010). We are not aware of studies showing that the increased glycolysis is necessary for HCV replication, however, based on the studies described, it is highly likely to be important for some stage of virus production. As described below, fatty acid synthesis is required for many stages of HCV replication and assembly and the induction of glycolysis may be necessary to meet the infected cell needs for increased fatty acid synthesis. While the viral gene or genes necessary for HCV induction have not been determined, the NS5A protein interacts with HK2 to enhance its activity and is sufficient to induce increased glucose uptake and lactic acid production (Ramiere et al., 2014). Future work is required to determine if NS5A is the sole HCV determinant for induction of glycolysis

While a number of early glycolysis studies focused on transforming retroviruses, recent studies of the non-transforming lentivirus, HIV, showed that retroviruses directly alter metabolism as well. Initial proteomic analysis of HIV infected CD4+ T cells indicated positive changes in glycolysis (Chan et al., 2009). However, a metabolomics study of HIV infected CD4+ T-cells and infected macrophages yielded mixed results (Hollenbaugh et al., 2011). Glucose analogs are taken up to higher levels in the HIV-infected CD4+ T-cells while the uptake is depressed in the macrophages. Additionally, metabolites involved in glycolysis were increased by HIV infection of the CD4+ T-cells while they were decreased in infected macrophages (Hollenbaugh et al., 2011). Macrophages generally maintain long-term infection while CD4+ T-cells most often support more acute lytic infection, which may account for the differences. This study shows that the activation of specific metabolic pathways is dependent on the cell type infected and further highlights the importance of the cell type tested for metabolic studies with viruses.

Many other viruses have been shown to induce glycolysis or there are strong indications that glycolysis is induced by infection. An early study in 1961 showed that Influenza A infection of chick embryo cell monolayers increased glucose uptake, glycolysis and lactic acid production by 3 hours post infection (Klemperer, 1961). A recent targeted metabolomics study showed that two strains of InfluenzaA H1N1 induced glucose uptake, glycolytic enzymes and lactic acid production between 8 and 12 hours post infection (Ritter et al., 2010). As replication had occurred, they concluded that the increase in glycolysis coincided with apoptosis of the cells and may be related. However, it is not clear if Influenza A virus induced apoptosis leads to increased glycolysis or if the induction of glycolysis delays the course of apoptosis. Other viruses appear to induce glycolysis without the induction of apoptosis. Dengue virus, a flavivirus, distantly related to HCV, also induces and requires glycolysis for efficient replication (Fontaine et al., 2015). Dengue virus induces glycolysis at relatively early times post infection but continues to replicate and produce virus at 48 hours post infection indicating that apoptosis does not appear to occur early. A virus of invertebrates has also been shown to induce glycolysis. Metabolism of shrimp hemocytes was examined following infection with White spot syndrome virus, a natural pathogen of shrimp. Studies showed that glucose uptake and lactic acid production were increased during the replication phase of infection in shrimp (Chen et al., 2011). These changes occurred prior to virion production and cell death. A subsequent metabolomics/proteomics study further showed the induction of aerobic glycolysis at 12 hours post infection of shrimp (Su

et al., 2014). It is likely that as more viruses are examined, many will induce and require glycolysis for replication and production of infectious virus progeny.

While most viruses tested have a requirement for the induction of glycolysis for viral replication, there are exceptions. Our metabolomics study of HFF cells infected with Vaccinia virus, a model member of the Poxviridae, indicated that viral infection did not identify any induction of glycolytic intermediates (Fontaine et al., 2014). Interestingly, there was an increase in intracellular sorbitol and fructose. When glucose is in excess of the cellular needs, it can be metabolized to fructose through sorbitol. The metabolomic data led to the interesting hypothesis that glucose is not utilized by Vaccinia virus-infected cells. In accordance with this hypothesis, when glucose was removed from the media, Vaccinia virus replicated and produced virions at near wild type levels. While many viruses induce glycolysis, induction and utilization of glycolysis for viral replication is clearly not universal.

As described above, early studies showed that HSV-1 requires glucose in the media (Lewis and Scott, 1962) and that the hexokinase2 inhibitor, 2-DG, inhibits the production of infectious virus (Courtney et al., 1973). However, a recent metabolomics study examined the global metabolic changes induced by two strains of HSV-1 in multiple cell types and found a very different metabolic pattern from that of HCMV infected cells (Vastag et al., 2011). While glycolysis is induced by HCMV in multiple cell types, HSV-1 does not induce glucose uptake or lactic acid production and the alteration of glycolytic metabolites has a very different pattern as compared to HCMV. During infection with HSV-1, glucose-carbon is not utilized for the TCA cycle to the same extent as HCMV, but is shunted toward nucleotide synthesis. This study concluded that HSV-1 does not induce aerobic glycolysis (Vastag et al., 2011). HCMV has a relatively long replication cycle with maximal virion production at 96 hours post infection while HSV-1 begins to produce virus within 6 hours and has maximal virion production by 24 hours post infection. Thus, two herpesviruses with different replication kinetics have very different metabolic needs and therefore have divergent effects on glycolysis. In contrast to the metabolomic findings, a subsequent study of HSV-1 infection of a different strain of HSV-1 in Vero green monkey kidney cells found that HSV-1 infection induces glucose uptake and lactic acid production dependent on phosphofructokinase (Abrantes et al., 2012). Additional studies found that HSV-1 is inhibited by the glycolytic inhibitor 2-DG (Courtney et al., 1973; McArdle et al., 2011). Taken together, these studies show that HSV-1 appears to require glycolysis for replication but it may not induce this pathway similarly to HCMV. The requirement for the glycolytic pathway during HSV-1 infection may lie in the induction of nucleotide synthesis by HSV-1 that is increased by HSV-1 infection (Vastag et al., 2011).

Unexpectedly, latent viral infections can also induce glycolysis. During latent herpesvirus infection there is little or no production of virions and limited viral gene expression and therefore less obvious need for rapid metabolic changes. Kaposi's Sarcoma-associated herpesvirus (KSHV) is the etiologic agent of Kaposi's Sarcoma, an endothelial based tumor. Upon infection of endothelial cells, KSHV establishes a predominantly latent infection. A metabolomics study of endothelial cells latently infected with KSHV found that glycolytic metabolites are induced during latency (Delgado et al., 2012). KSHV induces the uptake of

glucose at least in part through induction of glucose transporter3 (Delgado et al., 2010). Hexokinase 2, the first rate-limiting step of glycolysis is upregulated during latent infection by KSHV and there are also increases in the production of lactic acid leading to increased acidification of the media following infection. Oxygen utilization is decreased in latently infected endothelial cells indicating that oxidative phosphorylation is diminished. The induction of glycolysis is essential for the survival of latently infected cells as inhibition of this pathway led to cell death of latently infected cells due to apoptosis (Delgado et al., 2010). Therefore, the switch to glycolysis induced by viruses is not solely required for nucleic acid replication or virion assembly and egress but may play a role in cell survival as well. KSHV is also the etiologic agent of primary effusion lymphomas (PEL), a pleural cavity lymphoma. The level of glycolysis is very high in PEL cells as compared to primary B-cells (Bhatt et al., 2012). PEL cells are isolated from a human tumor so it is formally difficult to separate out if glycolysis is altered due to viral infection or to tumor formation in general. However, the PEL cell data it is consistent with viral induction of glycolysis.

Recently, it was shown that the KSHV encoded microRNAs are sufficient to induce aerobic glycolysis (Yogev et al., 2014). KSHV encodes over 17 or more distinct microRNA species from 12 loci. The microRNAs are encoded in the major latent locus and are expressed during latent infection. Ten of the 12 KSHV miRNA loci are intergenic. When these 10 intergenic viral microRNAs were overexpressed in endothelial cells there was an increase in lactic acid production and a decrease in oxygen utilization. The microRNA cluster also induces hypoxia induced factor 1 and upregulates the expression of glucose transporter 1. These effects appear to be due to the microRNA repression of two metabolic regulatory genes, EGLN2 and HSPA9 (Yogev et al., 2014). It remains to be seen if the KSHV miRNAs are necessary for the induction of glycolysis in the context of KSHV infection or if other latently expressed genes are also able to induce glycolysis. Regardless, these results show that KSHV has evolved functions to induce glycolysis and the induction of glycolysis is not a simple cellular response to infection.

Epstein-Barr virus is the causative agent of infectious mononucleosis and also causes a number of malignancies including Burkitt’s lymphoma and nasopharyngeal carcinoma (NPC). EBV infected NPC cell lines have high levels of glycolysis, an effect recapitulated by the expression of a known EBV oncogene expressed during many forms of latency, latent membrane protein 1 (LMP-1) (Xiao et al., 2014). LMP-1 induces the expression of HK2, leading to the induction of glycolysis. Knockdown of HK2 in the LMP expressing NPC cells leads to an increase in cell death, indicating that the induction of glycolysis is necessary for survival of the cells. The importance of glycolysis during latent infection is highlighted by the fact that both KSHV and EBV induce glycolysis during latent infection. In both cases latently expressed genes are sufficient to induce the effect indicating that latent viral infection directly induces glycolysis. Both KSHV and EBV are oncogenic viruses in specific environments and both viruses induce metabolic pathways induced in most cancer cells while keeping the infected cell alive, leading to speculation as to the role of virus induced metabolism in viral oncogenesis.

While many viruses induce and require glycolysis, its exact role in viral replication and during latent infections is not entirely clear. If glucose uptake and utilization is increased,

ATP can be produced more rapidly through aerobic glycolysis. Therefore, viruses may have evolved to induce glycolysis for a rapid source of ATP for replication. In the cancer field, it has been postulated that ATP is not generally limiting and that aerobic glycolysis increases biomass for a growing cell (DeBerardinis et al., 2008). Viruses may require this biomass for replication or for the maintenance of latently infected cells. Increased glucose uptake may also be required to feed other metabolic pathways during viral infection. Importantly, fatty acid synthesis is required for the replication of many viruses and increased glucose may feed this pathway in many virus-infected cells. The need for glycolysis for different viruses ranges widely as exemplified by the comparison of HSV-1 and CMV in the same cell type (Vastag et al., 2011). Determining which aspects of glycolysis are necessary for each virus studied will be important for understanding the different requirements for the induction of glycolysis by distinct viruses.

Fatty Acid Synthesis

Fatty acid synthesis supports the creation of lipid material in the cell and is important for increased membrane production as well as other cellular needs. The core of fatty acid synthesis is the production of palmitate from acetyl-CoA and malonyl-CoA in a reaction that requires NADPH and is catalyzed by fatty acid synthase (FASN). In mammalian cells, the carbon substrates for fatty acid synthesis are generally derived from citrate, an intermediate of the TCA cycle. Once synthesized, palmitate can be further metabolized into a number of long chain fatty acids that can then be used in lipid production for membrane biosynthesis and lipid droplet formation. Lipid droplets are storage organelles for lipids, triacylglycerides and sterol esters and are also beneficial as energy storage for cells. Lipid droplet formation can be indicative of increased fatty acid synthesis and prepares the cell for rapid membrane generation and maintains an energy cache. Fatty acids can also be broken down by beta-oxidation to produce energy. Importantly, many viruses induce and require fatty acid synthesis at some stage of their lifecycle.

Carbon flux studies of HCMV infected cells indicated that during lytic replication HCMV induces the flux of glucose-derived carbon into fatty acid synthesis in primary HFF cells (Munger et al., 2008). There is a marked increase in flux of carbon into citrate and into malonyl-CoA flux and therefore glucose is feeding fatty acid synthesis as well as the TCA cycle in HCMV infected cells as described above. Radiolabeled glucose carbon is also found in lipid material at higher levels in HCMV infected cells as compared to mock directly demonstrating that HCMV induces fatty acid synthesis (Munger et al., 2008). Fatty acid synthesis is required for HCMV production inasmuch as inhibitors of enzymes critical for fatty acid synthesis, both acetyl-coA carboxylase (ACC) and FASN, lead to a significant decrease in the release of infectious HCMV particles (Munger et al., 2008). FASN and ACC inhibitors did not have any significant effect on early or late viral gene expression and therefore fatty acid synthesis must be necessary for a post gene expression step (Munger et al., 2008). Therefore, the inhibition of HCMV by fatty acid synthesis inhibitors was not simply damaging the cell but was required for the production of infectious virus.

The mechanism of HCMV induced fatty acid synthesis is not entirely clear. However, infection of fibroblast cells induces enzymes involved in fatty acid synthesis, in particular

ACC1 (Spencer et al., 2011). Early viral gene expression is required for the induction of ACC1. Activation of the sterol regulatory element binding protein 1 (SREBP-1) via protein cleavage is necessary for the induction of ACC and other FAS enzymes by HCMV (Yu et al., 2012). Inhibition of SREBP-1 inhibits HCMV induced fatty acid synthesis and the production of infectious virions but does not inhibit production of viral early or late proteins. This confirms that the block in virion production is not due to a lack of viral gene expression. It was subsequently shown that SREBP-1 is activated by PKR-like endoplasmic reticulum kinase (PERK) (Yu et al., 2013). PERK is activated by cellular stress raising the possibility that cellular stress due to infection is part of the induction of fatty acid synthesis in HCMV infected cells and that the virus has evolved to utilize this response for production of infectious virus. The induction of glycolysis by HCMV induces relocalization of carbohydrate-response binding element protein (ChREBP) (Yu et al., 2014). In turn, ChREBP activates fatty acid synthesis. Therefore, HCMV induction of glycolysis may also play a role in HCMV induction of fatty acid synthesis. Glut4 and ChREBP can also be controlled by viperin, an interferon inducible protein and restriction factor of HIV. Viperin relocalization to the mitochondria is required for HCMV induced fatty acid synthesis and knockdown of viperin leads to an increase in non-enveloped virus particles within HCMV infected cells (Seo and Cresswell, 2013). Therefore, HCMV-induced fatty acid synthesis may be required for virion envelopment within the cell. Alternatively, it has also been postulated that HCMV induction of fatty acid synthesis may be necessary for the massive increase in the size of the cell nucleus in HCMV infected cells (Yu et al., 2011a). Future work will tease out these possibilities.

A screen utilizing siRNAs to knock down specific cellular enzymes involved in metabolism, identified a number of fatty acid synthesis enzymes that were important for the production of infectious HCMV (Koyuncu et al., 2013). Knockdown of a number of enzymes involved in the production of very long chain fatty acids (VLCFA) led to significant inhibition of HCMV. HCMV infection leads to increased expression of many enzymes involved in VLCFA synthesis, including acyl-CoA synthetases and the elongases, enzymes that elongate long chain fatty acids. Labeled very long chain fatty acids were found at high levels in the virion envelope (Koyuncu et al., 2013). Drug inhibition of the enzymes involved in VLCFA production and induced by HCMV did not inhibit early or late HCMV gene expression but led to a 15-fold decrease in virion production. However, the VLCFA synthesis inhibitors led to an approximately 350-fold increase in the particle to pfu ratio, demonstrating that the VLCFAs in the virion envelope are required for the virion to become infectious (Koyuncu et al., 2013). It is unknown if the VLCFAs are necessary for binding to subsequent host cells or for virion envelope integrity. It also remains to be determined if fatty acid synthesis is solely required for the creation of VLCFA or if it is necessary for additional facets of HCMV virion production.

Other herpesviruses also appear to require fatty acid synthesis during lytic replication. A FASN inhibitor, cerulinin, blocks the production of Varicella Zoster virus without affecting protein synthesis (Namazue et al., 1989). In VZV-infected cells fatty acid synthesis may be necessary for the increased glycoprotein expression as labeled palmitate added to VZV infected cells could be found in virion glycoproteins (Namazue et al., 1989). EBV also induces FASN in human epithelial tongue cells from a patient with oral hairy leukoplakia

(Li et al., 2004). An EBV immediate early protein, BRLF1 was shown to induce FASN (Li et al., 2004). The reactivation of EBV lytic replication is blocked by FASN inhibitors, apparently in a BRLF dependent fashion at early times after the induction. However, not all herpesviruses induce overt fatty acid synthesis. The metabolomics study of HSV-1 found that infection does not induce metabolites involved in fatty acid synthesis and the production of infectious HSV-1 was unaffected by FASN inhibitors (Vastag et al., 2011). Therefore, while common, induction of fatty acid synthesis is not universal among viruses with differences even within the same family.

RNA viruses that replicate in the cytoplasm alter lipids in the cytoplasm to create a beneficial environment for replication (Chukkapalli et al., 2012; Heaton and Randall, 2011). Virtually all aspects of the HCV lifecycle, from entry to egress and even extracellular virion particles are lipid associated (Schaefer and Chung, 2013). HCV requires low-density lipoprotein receptor as a co-factor for entry. HCV replication occurs on lipid raft-like domains, often referred to as membranous webs, and HCV assembly appears to occur on lipid droplets. To create a replication compartment and increase assembly sites, HCV induces and requires fatty acid synthesis and lipid droplets. The role of fatty acid synthesis in these steps of the HCV lifecycle has been reviewed extensively elsewhere and will not be described at length here (Herker and Ott, 2011; Schaefer and Chung, 2013; Syed et al., 2010; Targett-Adams et al., 2010). HCV was originally shown to induce enzymes involved in fatty acid synthesis *in vivo* in chimpanzees (Su et al., 2002). Subsequent studies showed that fatty acid synthesis is necessary for HCV replication (Kapadia and Chisari, 2005). HCV induces activation of the SREBPs and also induces FASN to increase fatty acid synthesis (Waris et al., 2007; Yang et al., 2008). Lipidomic and metabolomics studies of HCV-infected Huh7 cells show that HCV induces specific lipid moieties including several phosphatidylcholine and phosphatidyethanolamine species as well as cholesterol and sphingolipids (Diamond et al., 2010; Roe et al., 2011). Further studies will likely determine the need for these specific lipids in HCV replication and assembly. HCV assembly is associated with lipid droplets (Herker and Ott, 2011; McLauchlan, 2009). The HCV core protein co-localizes with lipid droplets in cell culture and in chimpanzee liver bioposies (Barba et al., 1997). When the HCV core protein was expressed in some strains of transgenic mice it led to the induction of lipid droplets and ultimately caused steatosis, also known as fatty liver disease (Moriya et al., 1997). Importantly, steatosis occurs in more than half of HCV patients. Therefore, in addition to being required for replication and assembly, the induction of fatty acid synthesis by HCV appears to directly lead to a major disease induced by the virus.

Dengue virus also rearranges specific membrane structures for replication and requires fatty acid synthesis for replication. A directed siRNA screen showed that FASN and ACC1 were required for efficient Dengue virus replication (Heaton et al., 2010). Inhibition of fatty acid synthesis by drugs that inhibit FASN also leads to significant decreases in Dengue virus replication. Dengue virus does not appear to increase the expression level of FASN but rather causes a relocalization of FASN to the novel membrane structures induced by the virus (Heaton et al., 2010). The Dengue virus nonstructural protein, NS3 drives relocalization of FASN (Heaton et al., 2010) and this relocalization appears to involve

Rab18 binding to NS3 (Tang et al., 2014). Dengue virus infection increases overall fatty acid synthesis in host cells as determined by increased uptake of radiolabelled acetate with the highest amount of label found in the subcellular fractions that contained Dengue virus RNA (Heaton et al., 2010). The increase in fatty acid synthesis may lead to the increased lipid droplet formation found in Dengue virus infected baby hamster kidney cells (Samsa et al., 2009). Inhibitors of FASN also decrease the recovery of infectious Dengue virus in mosquito cells without apparent effects on cell viability (Perera et al., 2012; Samsa et al., 2009). Therefore, in both human cells and in its arthropod host, Dengue virus requires fatty acid synthesis for production of infectious virus. Lipidomics of Dengue virus infected mosquito cells showed that, similarly to HCV, sphingolipids and a number of phospholipids were upregulated by Dengue virus infection (Perera et al., 2012). Palmitic acid, among other primary fatty acids, was also elevated by Dengue virus infection. An inhibitor of FASN prevented the increase of these fatty acids in Dengue virus infected cells, suggesting that the induction of fatty acids is most likely due to a requirement for biosynthesis, not breakdown. However, there is evidence that Dengue virus induced autophagy leads to a break down of fatty acids by beta-oxidation to increase available ATP (Heaton and Randall, 2010). Future studies will be necessary to determine under which conditions Dengue virus leads to induction and accumulation of fatty acids and when it leads to breakdown for energy.

It has been known for some time that Poliovirus, a non-enveloped virus, increases the production of phosphatidylcholine (Mosser et al., 1972) and it was subsequently shown that Poliovirus requires fatty acid synthesis for replication (Guinea and Carrasco, 1990). Recently, it was shown that Poliovirus induces not only fatty acid synthesis but also cellular uptake of extracellular long chain fatty acids (Nchoutmboube et al., 2013). The rapid uptake of extracellular fatty acids does not lead to lipid droplets as expected, but instead these fatty acids are incorporated into phosphatidylcholine. Knockdown of a single Acyl-CoA synthetase, Acsl3, a protein involved in fatty acid uptake, led to inhibition of poliovirus replication (Nchoutmboube et al., 2013). A single poliovirus protein, 2A, was necessary but not sufficient for induced fatty acid uptake. Other picornaviruses also induce uptake of extracellular fatty acids as well, thus, it may be fruitful to determine the need for uptake of extracellular fatty acids for other virus families.

Poxviruses are DNA viruses that replicate in the cytoplasm, and therefore, like RNA viruses might require alteration of cellular membranes. The production of Vaccinia virus is inhibited by a FASN inhibitor (Punjabi and Traktman, 2005). It was subsequently shown that inhibition of FASN could be overcome by the addition of palmitate, a metabolite downstream of FASN in the fatty acid synthesis pathway (Greseth and Traktman, 2014). However, inhibition of phospholipid synthesis did not inhibit virus production. Vaccinia virus appears to require fatty acid synthesis for beta-oxidation of palmitate to increase energy in the cell by feeding the TCA cycle (Greseth and Traktman, 2014). As described above, Vaccinia virus does not induce or require glycolysis and therefore may need alternative pathways to generate metabolic intermediates in the TCA cycle (Fontaine et al., 2014). Further studies are necessary to understand why Vaccinia virus requires fatty acid synthesis for energy via subsequent beta-oxidation rather than inducing glycolysis as many other viruses do.

An RNA virus that replicates in the nucleus also requires fatty acid synthesis. Influenza is in one of the few RNA virus families, Orthomyxoviridae, that replicates in the cytoplasm. A metabolomics study showed increases in fatty acid synthesis metabolites and cholesterol metabolism following influenza A infection of two different transformed cell lines (Lin et al., 2010). It was also shown that influenza A virus production is decreased by inhibitors of fatty acid synthesis (Munger et al., 2008). Therefore, regardless of the replication compartment in the cell, many viruses require fatty acid synthesis for replication or the production of infectious virus particles.

Latent herpesvirus infection can also induce fatty acid synthesis. As described above, infection of cultured endothelial cells with KSHV leads to a predominantly latent infection. In our metabolomics study of latently infected endothelial cells, most of the long chain fatty acids detected are upregulated by infection at 48 hours post infection and this increase is maintained at 96 hours post infection (Delgado et al., 2012). There is also a significant increase in the staining of lipid droplets in the latently infected cells. Inhibition of FASN or ACC leads to death by apoptosis for the majority of the latently infected cells (Delgado et al., 2012). Therefore, during latent infection, where there is limited viral replication, fatty acid synthesis is required for cell survival. Fatty acid synthesis is also induced in the primary effusion lymphoma cell lines and this was specific to PELs versus other non-hodgekin lymphomas indicating this effect may be virus related as well (Bhatt et al., 2012). Lipid droplets are also induced in Burkitt's lymphomas that contain latent EBV suggesting that latent EBV infection may also induce fatty acid synthesis. Therefore, latent infection by herpesviruses appears to induce and, at least in the case of KSHV, require fatty acid synthesis for infected cell survival. Upon latent infection of endothelial cells, KSHV does not induce rapid cell growth; therefore, fatty acid synthesis is not likely to be necessary for rapid membrane production. It is possible that induced fatty acid synthesis is necessary for energetic needs during latency. Further studies are needed to determine the role of induced fatty acid synthesis for cell survival during latency.

Fatty acid synthesis is induced and required for replication and virus production of most but not all viruses examined. For all RNA viruses where metabolism has been studied, fatty acid synthesis is induced and required for replication. Most RNA viruses replicate in the cytoplasm and require remodeling of membrane compartments for replication. Induction of fatty acid synthesis is likely to aid in membrane remodeling to create specific niches for viral RNA replication. Vaccinia virus, a DNA virus that replicates in the cytoplasm, also requires induction of FAS, but this induction may be required for energy through beta-oxidation (Greseth and Traktman, 2014). With the exception of HSV-1, both RNA and DNA viruses that replicate in the nucleus also require fatty acid synthesis. However, it is less clear what the exact need for newly synthesized lipids is required for during virus replication. Both HCMV and VZV appear to utilize induced fatty acid synthesis to create species present in their virion envelope or in the virion glycoproteins, indicating that induction of fatty acid synthesis can provide extra virion components for rapid replication of viruses (Koyuncu et al., 2013; Namazue et al., 1989). Many normal cells can survive for some time when fatty acid synthesis is blocked. Therefore, the inhibition of fatty acid synthesis could provide novel therapeutic targets for blocking viral infection.

Glutaminolysis

While glutamine is a non-essential amino acid, extracellular glutamine is often imported for multiple cellular metabolic pathways. Glutamine can be utilized for glutathione production, ammonia production, and purine synthesis through nitrogen donation among other uses. Importantly, glutamine can be utilized in glutaminolysis. In glutaminolysis, glutamine is converted to glutamate and then to alpha-ketoglutarate. Alpha-ketoglutarate can enter the mitochondria where it can be utilized as an intermediate of the TCA cycle. Cancer cells often become glutamine addicted. In many cancer cells, glucose carbon is shunted away from the TCA cycle both into lactic acid production as well as fatty acid synthesis. Glutamine is then required as an anaplerotic substrate to replenish the TCA cycle. A number of viruses have also been shown to require glutamine for replication.

In the 1950s, it was shown that glutamine, as well as glucose, was necessary for maximal poliovirus replication in Hela cells (Darnell and Eagle, 1958; Eagle and Habel, 1956). Supplementing glutamine to the media actually restored more virus production than adding back glucose. Glutamic acid produced the same effect, the first hint that glutaminolysis might be necessary for viral replication (Darnell and Eagle, 1958). In 2008, it was first shown that HCMV infection led to increased uptake of glutamine (Munger et al., 2008). Interestingly, in the absence of glucose in the media, HCMV-infected HFF cells live longer than uninfected cells (Chambers et al., 2010). This finding led to the hypothesis that in HCMV-infected cells, glutamine could supplant glucose as a primary energy source. As predicted, HCMV induces the consumption of glutamine and the production of ammonia, and the specific activity of multiple enzymes involved in glutaminolysis is increased (Chambers et al., 2010). In the absence of glutamine in the media, HCMV infected cells have decreased levels of ATP and there is a significant loss of virus production (Chambers et al., 2010). Both ATP levels and virus production can be rescued by TCA cycle intermediates, further demonstrating that glutamine is needed as an anaplerotic substrate for the TCA cycle (Chambers et al., 2010). As described above, HCMV induces glucose uptake and glycolysis but the glucose carbon is used for fatty acid synthesis and nucleotide synthesis and, therefore, glutamine is necessary to provide metabolites for the TCA cycle, similarly to many cancer cells.

Vaccinia virus is one of the few viruses that does not induce or require glycolysis for replication in cultured cells. A metabolomics study of primary HFF cells infected with Vaccinia showed that there is no increase in glycolytic metabolites but there is an increase in intracellular glutamine and glutamate (Fontaine et al., 2014). Removal of glutamine, but not glucose, from the media led to a significant drop in virus production. In the absence of glutamine, late genes were expressed at low levels though the maturation of processed late genes occurred. Electron microscopy studies showed that in the absence of glutamine, immature and mature virus particles are produced but at drastically reduced levels, with only small virus factories in the cytoplasm (Fontaine et al., 2014). Virus factory levels and the production of infectious virus can be restored by supplementation with alpha-ketoglutarate as well as other TCA cycle intermediates. Therefore, as with HCMV, glutamine is required as an anaplerotic substrate for the TCA cycle. The glutamine dependence of Vaccinia virus was subsequently confirmed in transformed African green monkey BSC40 cells (Greseth

and Traktman, 2014). Vaccinia virus increases the metabolites in glutaminolysis at very early time points in human fibrosarcoma cell line, though less so in Hela cells, highlighting the cell specificity of some virus -induced metabolic changes (Mazzon et al., 2014). In both cell lines, a virus deleted for the C16 protein, a protein that stabilizes HIF-1, has lower levels of glutamine metabolites compared to wild type infection indicating that the induction of glutaminolysis may, at least in part, be due to this viral protein.

Why Vaccinia virus has evolved to rely solely on glutamine uptake, while both poliovirus and HCMV require induction of both glycolysis and glutaminolysis is unknown. Perhaps that answer lies in the differential utilization of fatty acid synthesis, Vaccinia relies on beta-oxidation for energy while HCMV relies on longer chain fatty acids (Greseth and Traktman, 2014; Koyuncu et al., 2013). Further work is required to fully understand how glutamine is utilized in the Vaccinia virus infected cell. In contrast, Dengue virus relies on glycolysis for replication with only a small drop in titer in the absence of glutamine (Fontaine et al., 2015). Therefore, viruses have evolved to utilize different carbon sources and future studies of carbon utilization for each virus species is warranted.

Viruses appear to induce glutaminolysis when glucose carbon is shunted away from the TCA cycle. It remains to be seen if the requirement for the TCA cycle lies solely in energy production or if TCA cycle intermediates are needed for virus replication. During latent KSHV infection of endothelial cells, carbon from glucose is utilized for aerobic glycolysis and the production of lactate while fatty acid synthesis is also induced (Delgado et al., 2010; Delgado et al., 2012). Our previously described metabolomics study showed that the latently infected cells have higher levels of glutamine as compared to their mock counterparts (Delgado et al., 2012). In preliminary studies we found that endothelial cells latently infected with KSHV induce glutamine uptake and that glutaminolysis is required for the survival of latently infected cells (Sanchez and Lagunoff, submitted). These data support the hypothesis that glutaminolysis is required for cellular energy during infection.

Looking Forward

In the last decade, great strides have been made in the understanding of the metabolic pathways that are altered by viruses for genome replication, virion production and for enhanced survival of infected cells. While there are common metabolic changes induced by most viruses studied, there are also unique metabolic perturbations for each virus species necessitating the study of each virus species individually. There is also likely to be additional metabolic pathways that are commonly affected by many viruses. For example, a number of the large-scale metabolomics studies have identified increases in amino acid metabolism and nucleotide synthesis. The alteration of metabolites in the pentose phosphate pathway (PPP) has been noted in a number of metabolomic studies (Delgado et al., 2012; Hollenbaugh et al., 2011; Vastag et al., 2011). The PPP metabolizes glucose to ribulose-5-phosphate, an important substrate for nucleotide synthesis (fig. 1). Given the need for rapid viral genome replication, the PPP may provide a source of free nucleotide pools as was shown for Adenovirus infection (Thai et al., 2014). Additionally, the PPP generates net NADPH. NADPH is an essential substrate for fatty acid synthesis. Therefore, virus induction of one metabolic pathway may require induction of other pathways to provide all

the necessary substrates. As many metabolic pathways are inter-related, it is not surprising that the activation of one pathway requires the activation of other pathways. Therefore, future virus-induced metabolism studies will likely identify novel inter-related metabolic pathways that will advance our understanding of cellular metabolism in general.

The exact stage in the virus life cycle that each metabolic pathway is required for is a fertile ground for future studies. The metabolites or metabolic pathways required for specific virion components have been identified for a couple of viruses thus far (Koyuncu et al., 2013; Namazue et al., 1989). Recently, HCMV was shown to induce pyrimidine biosynthesis to increase UDP-sugars that are utilized for increased synthesis of viral glycoproteins (DeVito et al., 2014). Herpesviruses encode for multiple glycoproteins present in the virus envelope and these glycoproteins are used for binding to host cells. Inhibition of the pyrimidine biosynthesis led to a decrease in the glycosylation of a viral envelope protein, gB (DeVito et al., 2014). The inhibitor of pyrimidine biosynthesis also led to an increase in the particle to pfu ratio as would be expected if there are decreased levels of viral glycoproteins on the virion envelope. These examples show that viral induction of certain metabolic pathways may benefit the virus by producing increased levels of specific virion components. Many of the metabolic pathways induced by viruses, however, are likely to be for efficient energy production and substrates less directly involved in the formation of infectious virions. Delineating the need for induction of each metabolic pathway for each virus species will increase our understanding of why viruses have evolved to target distinct metabolic pathways.

There are surprisingly few studies describing the mechanisms of virus induced cellular metabolism. The cellular signaling pathways necessary for viral induction of metabolic pathways have been identified in a few cases. However, a deeper understanding of these signaling pathways is critical for the goal of therapeutically targeting viral infections through inhibition the specific pathologic metabolic pathways. There are only a couple examples where the viral gene products necessary for changes in each specific metabolic pathway have been identified and these studies have generally followed the cancer cell metabolism field. Future studies to identify the viral genes driving metabolic changes will advance the mechanistic studies beyond current levels.

Many similarities between metabolic pathways induced by viral infection and the pathways induced in cancer have been noted. The viral metabolism field has drawn heavily on what is known in cancer cell metabolism to identify cellular mechanisms of metabolic induction. Further identification of virus-activated host signaling pathways that control cellular metabolism will not only lead to a deeper understanding of the importance of viral induced metabolism but will also facilitate discoveries in cancer cell metabolic control. Therefore a deeper understanding of virus induced metabolic pathways will have the potential to identify novel antivirals as well as new ways to target cancer metabolism.

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Virus infection dramatically alters host cell metabolism

Many viruses induce glycolysis, fatty acid synthesis and/or glutaminolysis

Viruses require induction of specific host metabolic pathways for replication and spread

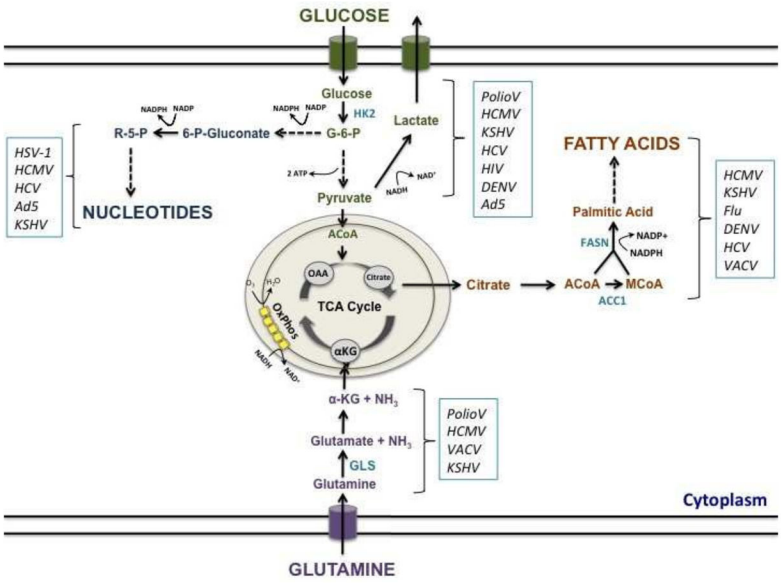

Figure 1. Virus infection alters host cell metabolism

Major metabolic pathways altered by virus infection are highlighted, glycolysis (Green), Fatty Acid Synthesis, FAS (Orange), Glutaminolysis (Purple), Pentose Phosphate Pathway, PPP (Dark Blue). Glucose enters the cell and is metabolized to glucose-6-phosphate (G-6-P) which can be shunted to the Pentose Phosphate Pathway (PPP) to support nucleotide synthesis, or to pyruvate. Pyruvate can be converted to lactate via glycolysis, which is secreted from the cell, or Acetyl-CoA (ACoA), which enters the TCA cycle. Citrate can be shunted out of the mitochondria to enter FAS. Glutamine enters the cell and is deaminated twice to form aKG and ammonia (NH_3). αKG can then enter the TCA cycle. Major enzymes of carbon metabolism are in turquoise and discussed in main text.

Table 1

Virus	Essential Carbon Source/important metabolic pathway	Major publications
Poliovirus	Glucose Glutamine Fatty Acid Synthesis	Eagle, H. and Habel, K., J. (1956) Darnell, JE. And Eagle, H. (1958) Nchoutmboube, JA. (2013)
HCMV	Fatty Acid Synthesis Glucose and Glycolysis Glutamine and Glutaminolysis	Munger, J., et al. (2006) Munger, J., et al. (2008) Chambers, J., et al. (2009) Vastag, L., et al. (2011)
DENV	Fatty Acid synthesis Glycolysis	Heaton, NS., et al. (2010) Perera et. al. (2012) Fontaine, KA., et al. (2014)
HCV	Fatty Acid Synthesis Glycolysis	Kapadia and Chisari (2005) Ripoli et. al. (2010) Diamond, DL., et al. (2010) Ramière, C., et al. (2014)
VACV	Glutaminolysis Fatty Acid Synthesis	Fontaine, KA., et al (2014) Greseth, M. and Traktman, P. (2014)
HSV-1	Glucose and Glycolysis?	Lewis, VJ. and Scott, LV. (1962) Courtney, RJ. et al. (1973) McArdle, J., et al.(2011) Vastag, L., et. al. (2011)
Ad5	Glycolysis	Thai, M., et. al. (2014)
KSHV (Latency)	Glycolysis Fatty Acid Synthesis Glutamine and Glutaminolysis	Delgado, T., et al. (2010) Bhatt, AP., et al. S(2012) Delgado, T. et al. (2012) Yogev, O., et al. (2014)