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Oncogenic Viruses and Tumor Glucose Metabolism: Like Kids in a Candy Store

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Abstract

Oncogenic viruses represent a significant public health burden in light of the multitude of malignancies resulting from chronic or spontaneous viral infection and transformation. Though many of the molecular signaling pathways underlying virus-mediated cellular transformation are known, the impact of these viruses on metabolic signaling and phenotype within proliferating tumor cells is less well understood. Whether the interaction of oncogenic viruses with metabolic signaling pathways involves enhanced glucose uptake and glycolysis, both hallmark features of transformed cells, or dysregulation of molecular pathways regulating oxidative stress, viruses are adept at facilitating tumor expansion. Through their effects on cell proliferation pathways, such as the PI3K and MAPK pathways, the cell cycle regulatory proteins, p53 and ATM, and the cell stress response proteins, HIF-1 α and AMPK, viruses exert control over critical metabolic signaling cascades. Additionally, oncogenic viruses modulate the tumor metabolomic profile through direct and indirect interaction with glucose transporters, such as GLUT1, and specific glycolytic enzymes, including pyruvate kinase, glucose 6-phosphate dehydrogenase, and hexokinase. Through these pathways, oncogenic viruses alter the phenotypic characteristics of transformed cells and their methods of energy utilization, and it may be possible to develop novel anti-glycolytic therapies to target these dysregulated pathways in virus-derived malignancies.

Virus involvement in cellular transformation

The course of cellular transformation begins with dysregulation of cell signaling processes induced by genetic, environmental, and/or infectious processes. These events can be set in motion by oncogenes encoded by viruses that are known to induce malignant transformation. In a broad sense, virus-encoded genes disrupt normal cellular function by interfering with processes that regulate the cell cycle, growth signaling, oxidative stress development, and biosynthetic processes. On a molecular level, these events involve the interaction of oncogenes with nearly every aspect of cell signaling pathways. For example, disruption of the cell cycle checkpoint regulator, p53, by viral proteins, such as JC virus Large T-antigen, EBV nuclear antigen, and HPV E6 protein, result in loss of cell cycle control and subsequent unchecked proliferation (1–3). Additionally, oncogenic viruses upregulate cell survival pathways, such as the phosphatidylinositol 3-kinase (PI3K), mitogen-activated protein kinase (MAPK), and nuclear factor kappa B (NF κ B) cascades, that allow unregulated proliferation, even in the absence of sufficient metabolic supply (4). Some viruses, such as HPV, must counteract oxidative stress occurring during viral infection in order to continue the replication cycle (5). Others, such as EBV, HTLV-1, and HCV, can significantly influence tumor development and aggressiveness through modulation of the cellular oxidant load (6, 7). In the case of HCV, this oxidative stress leads to cyclooxygenase-2 (COX-2) activation, which can also feed back to enhance HCV RNA

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replication (7). Thus, the seizure of cell cycle control, proliferation, and oxidative stress pathways by oncogenic viral proteins promotes viral infection, viral protein expression, and in some cases, subsequent transformation.

Though much is known about the molecular signaling involved in virus-induced proliferation and cell survival, little work has been done to investigate the involvement of oncogenic viruses in the regulation of metabolic activities in relation to cellular transformation. Both the initiating metabolic events that contribute to transformation as well as the activated metabolic pathways that are responsible for tumor propagation remain unclear. Furthermore, and perhaps more interestingly, the impact of viruses on metabolic processes that govern tumor expansion and the feedback of these processes on viral protein expression has remained understudied. A more thorough exploration of these processes could lead to better targeted therapies for virus-induced malignancies and may significantly benefit patients diagnosed with these cancers.

Oncogenic viruses modulate glucose metabolic signaling through control of cellular growth, DNA damage, and stress signals

The process of cellular transformation is closely linked to dysregulated proliferation, which can lead to a similar type of metabolic transformation, marked by a unique metabolomic profile (8). Both of these processes can be stimulated by potent oncogenic proteins, which fuel tumor growth and adapt metabolic pathways to this accelerated proliferation. Though the specific mechanisms of transformation may vary, the net effect is often the same – DNA damaging agents that induce cellular mutations and viral oncoproteins that induce expression of their target proteins can both cause constitutive activation or stabilization of oncogenes or inactivation of tumor suppressor genes, thereby facilitating cellular transformation. As a result, many of the metabolic outcomes of transformation occur independently from the manner of tumorigenesis. For example, AMP-activated protein kinase (AMPK), a critical energy sensor of the cell that regulates glucose utilization in tumors, is activated by the AMP:ATP ratio in cancer cells, but in the context of viral transformation, can be induced through targeted oncogenic proteins independent of the energy status of the cell (9). Alternatively, deficiency of certain cellular processes that occurs as a result of genetic mutations and that is not necessarily directly affected by viral transformation, such as nucleotide excision repair mediated by xeroderma pigmentosa C (XPC), can induce a glycolytic phenotype and promote tumorigenesis (10). Nonetheless, oncogenic viruses play a critical role in the metabolic effects of cellular transformation, and viral oncogenes interact with and regulate a variety of growth stimulatory pathways and promote metabolic alterations during cellular transformation. For example, EBV latent membrane protein 2A, HPV E7 protein, and SV40 small t-antigen all activate the PI3K/Akt pathways to facilitate proliferation and enhanced migration and invasion (11–13). Interestingly, the PI3K/Akt pathway is overactive in many types of tumor cells, inhibits p53-dependent apoptosis, and also exhibits significant impact on glycolysis by stimulating expression of key glycolytic enzymes (14, 15). Additionally, the MAPK cascade is activated by tumorigenic viruses (16, 17), and MAPKs function in oncogene-driven and oxidative stress-promoted tumorigenesis as well as the induction of glucose transporter expression (18). Therefore, virus-mediated transformation through this pathway may lead to concomitant increases in glucose uptake and glycolytic flux, further strengthening the tumor's growth potential. In fact, this symbiotic relationship involving oncogenic viruses and transformed cells is similar to the relationship of fibroblasts and tumor stroma in mediating the “Reverse Warburg Effect” in cancer, whereby transformed cells utilize oxidative stress to promote autophagy in surrounding stromal cells, thereby providing metabolic precursors that can be shuttled back to cancer cells to promote accelerated growth (19). Similar to virus-enhanced glucose uptake, the development of tumor-driven oxidative

stress can induce upregulation of lactate transporters in fibroblasts, which then supply neighboring tumor cells with bioenergetic substrates (20). While oncogenic viruses provide the initial cues necessary for transformation, their metabolic role can be viewed as analogous to stromal cells in facilitating the supply of the necessary precursors for rapid glycolytic metabolism and tumor expansion.

As with oncogenic cascades, viruses also influence tumor suppressor pathways and impair their function. The potent tumor suppressor, p53, exerts pleiotropic effects to counteract tumor proliferation yet is a critical regulator of glycolysis as well. p53 downregulates the expression of glucose transporters, decreases glycolytic metabolism, and promotes oxidative phosphorylation, implicating p53 as an anti-Warburg molecule (21, 22). p53 is also a downstream target of AMPK, which is responsible for upregulating glycolysis in response to metabolic stressors within the tumor microenvironment. Many viral oncogenes, including mouse polyomavirus Large T-antigen protein, HPV E6 protein, and EBV nuclear antigen (EBNA) 3C (23–25), regulate the p53 pathway, either through direct interaction or indirect cooperation with downstream effectors. Therefore, virus-mediated p53 inactivation may enhance glucose uptake and accelerate glycolysis in transformed cells, thereby providing sufficient growth substrates in these cells.

In response to nutrient deprivation, malignant cells can induce cytotoxic processes and force cells into apoptotic or necrotic cell death or promote protective autophagy. Apoptotic and necrotic processes are also associated with DNA damage, and it is the net result of these cytotoxic signals which predict cell survival. DNA damage checkpoint pathways are often impaired in tumor cells, allowing these cells to adapt during starvation conditions. In addition, in terms of viral transformation, virus-derived oncogenes can overcome cell death signals induced by DNA damage proteins. Anti-glycolytic therapy with 2-deoxyglucose (2-DG) induces oxidative stress and reduces the constitutive activation of ataxia telangiectasia mutated (ATM) protein (26), thereby promoting cell death in the absence of DNA repair. In contrast, virus-driven oxidant production and simultaneous ATM suppression may induce chemoresistance in tumors treated with alkylating chemotherapeutic agents by propagating DNA damage-inducing mutations and promoting chromosomal instability. Therefore, virus-triggered ATM inactivation (27) may represent a significant route of metabolic transformation and may also signify a potential mechanism of chemoresistance in virus-derived malignancies. In addition, this loss of ATM activation driven by many viruses leads to an increase in the stability of hypoxia-inducible factor-1 α (HIF-1 α), which promotes elevated glycolytic flux (28). ATM suppression in the context of viral oncogenesis may also prevent stress-induced AMPK-mediated induction of tumor suppressors, such as p53, that may limit tumor growth (29). In this sense, transforming viruses may effectively adapt the cellular environment to manage bombardment with cell stress signals and, in their presence, may overpower cellular controls to maintain rapid expansion.

Hypoxia represents a significant metabolic stressor that impacts tumor proliferation. One of the critical mediators of the response to physiological perturbations and hypoxia within the tumor mass is HIF-1 α , which acts as a molecular switch by turning on pro-survival pathways and adapting tumor cells to a low oxygen environment. Through stabilization of HIF-1 α and import into the nucleus, HIF-1 α upregulates genes involved in angiogenesis, glucose transport, and glycolysis, such as hexokinase, phosphofructokinase-2, and pyruvate kinase (30). This gene induction links HIF-1 α with the metabolic phenotype and allows tumor cells with stable HIF-1 α expression to alter their growth pattern based on changing signals within the surrounding parenchyma. EBV and HPV have been shown to induce HIF-1 α expression and to utilize HIF-1 α to regulate viral infection (31, 32). In addition, oncogenic viruses may depend on HIF-1 α to alter the metabolic rate and fulfill the demands of viral oncogene-driven proliferation. In this way, HIF-1 α may be a necessary intermediate

between viral infection and subsequent transformation, and during key oncogenic steps, may allow virus-derived tumors to survive hypoxic signals from the surrounding tissue. Therefore, it is evident that oncogenic viruses utilize physiological stressors, as well as cellular pathways controlling growth and DNA damage, to enhance transformation and promote tumor progression (Figure 1).

Transforming viruses trigger alterations in glucose uptake by tumor cells

Enhanced glucose uptake is a hallmark feature of transformed cells, which depend on glucose for glycolytic metabolism, maintenance of biosynthetic processes, and control of cell stress signaling. Cellular processes that aid tumor expansion also favor glucose uptake, and many of these processes are controlled by molecules, such as HIF-1 α , which upregulates membrane glucose transporters in response to low oxygen tension. By hijacking glucose transport, oncogenic viruses are similarly able to deliver increased amounts of glucose to proliferating cells, which enhances their tumorigenic capacity. Early studies in this area involved the Fujinami sarcoma virus (FSV), which was shown to upregulate glucose transporter 1 (GLUT1) mRNA in fibroblasts transfected with a temperature-sensitive FSV mutant when incubated at permissive temperatures (33). Alterations in glucose transport may also involve trafficking, rather than regulated expression, of glucose transporters to the plasma membrane. For example, SV40-transformed cells exhibit redistribution of hexose transporters from microsomal membranes to the plasma membrane, indicating that oncogenic viruses not only exhibit transcriptional regulation of glucose transport but also alterations in transporter trafficking during transformation (34). In fact, transport of glucose may account for upregulation of glycolysis seen in some transformed cells, such as those transformed by RSV (35). Importantly, the increase in glucose transport in transformed cells is likely not due to de novo synthesis of a new transport system itself but rather to modification of existing glucose transport (36). Therefore, viruses themselves may be able to effect changes in the cell membrane that would facilitate increased glucose transport, such as unmasking of glucose transport sites or alterations in the rates of glucose uptake reactions, which would greatly enhance cellular glucose stores. Enhanced glucose uptake is also a notable feature of other tumor including HPV and polyoma viruses (37). As with studies in other oncogenic viruses, these effects are not always limited to elevated levels of GLUT1 expression but can also involve altered GLUT1 trafficking in transformed cells (38). These studies indicate that functional changes in the membrane transport system may be critical to virus-induced oncogenesis.

The involvement of glucose transporters in oncogenic virus infection also presents an interesting perspective on their role in glucose metabolism. Initial work demonstrating that GLUT1 is the putative receptor for the human T-lymphotropic virus type 1 (HTLV-1) expanded on the function of membrane transporters as retroviral receptors but also indicated that these receptors could involve transporters implicated in glucose signaling pathways (39). A general effect of HTLV-1 infection is to reduce lactate production and glucose consumption, and cells starved of glucose are more readily infected by HTLV-1 as well. However, in certain cell types, HTLV-1 infection results in enhanced glucose uptake and lactate production, which indicate that the metabolic disturbances induced by HTLV-1 are cell type-specific (40). These effects may result from enhanced GLUT1 expression in certain cells, which would be beneficial for HTLV-1 infection. In addition, since immature cells express elevated levels of GLUT1, these cells may display a higher propensity for infection. As with other viruses, the particular tropism of HTLV-1 for relative undifferentiated cells may aid in its oncogenic potential. For example, these cells may possess both a greater capability for infection and a propensity for transformation, depending on the active status of the GLUT1 transporter. In this sense, glucose uptake processes may impact both infection

and transformation, thus validating this family of membrane transporters as potential therapeutic targets for oncogenic viruses and resultant tumor masses (see Figure 2).

Oncogenic viruses enhance the activity of key glycolytic enzymes

Unlike their normal counterparts, tumor cells preferentially utilize enhanced aerobic glycolysis for energy metabolism, a phenomenon first described by Otto Warburg in 1925 known as the Warburg effect (41). This shift towards increased glycolytic flux allows tumor cells to produce sufficient ATP to fulfill metabolic demands and leads to increased glucose consumption, decreased oxidative phosphorylation, and increased lactate production. Though glycolysis produces much less energy in the form of ATP than oxidative phosphorylation, several glycolytic enzymes are highly active in tumor cells and support enhanced anaerobic glycolysis and energy production. Specifically, the rate-limiting enzyme that catalyzes the final step in glycolysis, pyruvate kinase, is upregulated in tumor cells. Interestingly, tumor cells exclusively express the M2 isoform of this enzyme (M2PK) (42), which is typically expressed only during embryonic development, rather than the M1 isoform, expressed in most normal adult tissues.

Since pyruvate kinase represents a key enzyme shown to be involved in tumor progression, studies began to investigate the potential interaction between oncogenic viruses and M2PK activity and expression. In normal rat kidney cells with constitutive expression of Ras as well as the E7 protein of human papilloma virus (HPV), Zwierschke et al., 1999 found that E7 can both bind to M2PK in the cytoplasm and induce a change in its quaternary structure from the more active tetrameric to the less active dimeric form (43). Interestingly, this switch in structure is controlled by fructose 1,6-bisphosphate and regulates the glycolytic flux in tumor cells (44). In this study, it was also noted that E7 overexpression results in increased conversion of glucose to lactate, a phenomenon indicative of enhanced glycolytic flux to fulfill increased requirements for nucleotide precursors and to reduce the demand for oxygen. Additional studies showed that E7 transformation of highly glycolytically active cells resulted in a high glutaminolytic rate with a concomitant low glycolytic rate (45). These cells also produced high levels of alanine, which is associated with the metastasizing capabilities of a growing tumor (46). As alanine is produced by this step in the glutaminolytic pathway, and since enhanced glutaminolytic rates often occur during initial tumor formation, E7 may alter these pathways to enhance malignant transformation. Thus, it is evident that cytoplasmic E7, in addition to its nuclear transforming counterpart that interacts with members of the retinoblastoma family of proteins, can possess oncogenic characteristics within glycolytic pathways that may indicate a role for HPV in the progression of established tumors.

Further indication for a role of oncogenic viruses in the activity of M2PK comes from studies demonstrating targeting of M2PK by the Rous sarcoma virus (RSV). Chicken embryonic fibroblasts (CEFs) transformed by RSV demonstrate 3-fold higher M2PK activity, which is dependent on the oncogenic protein, Src (47). In fact, cellular M2PK may represent a target of the protein product of Src, pp60^{src}, which may initially inhibit and then activate M2PK through the accumulation of glycolytic intermediates (47), such as fructose 1,6-bisphosphate, which accumulates in RSV-transformed cells and potentially relieves the glycolytic block induced by cellular transformation. Modifications in the phosphorylation of M2PK during viral transformation also implicate this enzyme in virus-controlled metabolism. Whereas M2PK from RSV-transformed CEFs and normal CEFs contain phospho-serine and phospho-threonine, only transformed CEFs contain phospho-tyrosine (48). The amount of phospho-tyrosine may correlate with the kinetic activity of M2PK, which demonstrates correspondingly less affinity for its substrate, phosphoenol pyruvate (PEP), in transformed CEFs. The extent of a causal relationship between phospho-tyrosine

and virus-induced transformation may involve additional negative charge on M2PK or altered partitioning of this enzyme from the sarcolemmal or endoplasmic reticulum (49), both of which could drastically modify functional enzyme activity. Interestingly, it has been demonstrated that alterations in M2PK activity necessitate that the transforming protein have tyrosine phosphorylation capabilities, similar to what has been observed in other glycolytic enzymes in RSV-transformed cells (50).

Not only does RSV affect the M2PK pathway, but this virus also causes enhanced activities of the other rate-limiting enzymes in glycolysis, hexokinase and phosphofructokinase, in transformed cells, alterations that are specific to cells transformed but not infected by RSV (51). Aside from enhanced glycolytic flux, RSV transformation leads to enhanced flow through the pentose phosphate pathway, presumably to maintain reductive capacity within the cell, a phenomenon independent of the growth rate of transformed cells (52). Other studies found that there was a significant increase in phosphofructokinase-2 (PFK-2) activity as well as increased levels of fructose 2,6-bisphosphate in RSV-transformed CEFs as compared to normal CEFs, both of which were specific to transformation-capable virus forms (53). However, this oncogenic increase in PFK-2 activity seems not to be a direct effect but rather dependent on protein kinase C (PKC) (54). During RSV infection, the rate of glucose uptake and lactic acid production is significantly increased and allows for enhanced production of glycolytic anions and pyruvate, intermediates that fuel the energy demands of growing tumor cells (55). Importantly, levels of glucose, phosphoglycerate, and phosphoenol pyruvate all decrease in RSV-transformed cells, emphasizing that increased glucose uptake alone is not responsible for the observed alterations on the glycolytic phenotype. Interestingly, there does not seem to be a significant change in the steady-state concentration of ATP in transformed cells, indicating that enhanced glycolytic flux may in fact serve other purposes than the mere production of energy for tumor progression.

Molecular regulation of glycolytic and pentose phosphate pathways by oncogenic viruses

Though the Warburg Effect is clearly elicited by several viruses during cellular transformation, other oncogenic viruses exhibit glycolytic and pentose phosphate pathway modulation in transformed cells through the interaction of viral oncoproteins and cellular signaling molecules. Kaposi's sarcoma-associated herpesvirus (KSHV), the causative agent of Kaposi's sarcoma, upregulates glucose uptake and sensitizes infected cells to glycolytic inhibition (56). This effect may be mediated at least in part through transcriptional activation of key glycolytic enzymes by HIF-1 α in response to alterations in oxygen tension within the tumor microenvironment (57). Glycolytic inhibition can also block transcription of HPV E6/E7, indicating that flux through this pathway may be responsible for the expression of HPV-targeted transcription factors (58). While there are many potential pathways that regulate HPV transcription, the insulin-like growth factor (IGF) family of proteins may play a role in this process, given their necessity for HPV-mediated transformation and importance for glucose uptake capabilities. Cells transformed by the simian virus, SV40, also exhibit alterations in the levels of glycolytic intermediates through pentose phosphate pathway regulation. SV40-mediated increases in the activity of the transaldolase (TALDO) enzyme, a key toggle switch between the pentose phosphate shunt and glycolysis, indicate that SV40 is capable of regulating glycolytic precursors and the redox status maintained by pentose phosphate NADPH production (59). While elevated TALDO activity shifts metabolic precursors from the pentose phosphate pathway towards glycolysis to maintain high levels of ATP production, this activity also favors production of nucleotide precursors by SV40-transformed cells, which allows for rapid cell division. The precise role of glycolytic regulation during infection, replication, and oncogenic transformation remains another

intriguing area in this field. While viruses require increased synthesis of nucleotides, fatty acids, and lipid materials for production of progeny, the fact that many oncogenic viruses enter a latent state either prior to or subsequent to cellular transformation indicates that initial products of glycolysis may later be diverted to bolster accelerated metabolism and to complete cellular transformation. In other words, virus-mediated glycolytic regulation may proceed through a metered course that culminates in ultimate transformation and a metabolic phenotype that facilitates undisturbed propagation.

Hepatitis viruses also modulate glycolytic regulation to enhance tumorigenic potential. Cells infected with the woodchuck hepatitis virus (WHV) exhibit enhanced hexokinase activity, which yields greater phosphorylated glucose to be utilized in glycolysis (60). Long-term HCV protein expression also induces enhanced ATP production, a process evidently specific to cells using glucose as their main energy source, as well as enhanced lactate production (61). Additionally, HCV inhibits oxidative phosphorylation and allows a concomitant accumulation of glycolytic intermediates (61). This process may depend on the HIF-1 α signaling pathway, since these intermediates, such as pyruvate, stabilize HIF-1 α , possibly through inhibition of prolyl hydroxylases (PHDs). Furthermore, stabilized HIF-1 α would have the potential for nuclear translocation and subsequent regulation of the expression of glycolytic enzymes. It has been proposed that, contrary to cellular transformation occurring as a result of step-wise genetic mutations that gradually confer a tumorigenic phenotype to transformed cells, HCV may have the capacity to transform cells through core protein activity, with fewer initiating mutations (62). Since HCV exerts broad effects on mitochondrial, glycolytic, and cell proliferation pathways, this virus may have facilitated oncogenic capabilities, which may help to explain the development of HCC in such a large percentage of HCV carriers. A recent study also demonstrates that HCV NS5B, the HCV RNA-dependent RNA polymerase, interacts with M2PK, but not the L type PK, and that downregulation of M2PK also reduces HCV replication (63). These findings further depict metabolism-dependent viral replication and also ensnare HCV in the process of cellular transformation.

The pentose phosphate pathway plays a key role in producing reducing equivalents needed to counteract excessive ROS development within rapidly expanding tumors. Though this pathway, and its rate-limiting enzyme, glucose 6-phosphate dehydrogenase (G6PDH), are often activated to protect cells from oxidative damage, differential regulation of this pathway seems to underlie notable features of virus-induced malignant tumors. In some situations, the stepwise progression of cellular transformation leads to gradual changes in metabolic phenotypic characteristics. For example, some transformed cells can initially exhibit enhanced oxidative phosphorylation, presumably as a reaction to oncogene-driven glucose metabolism (64). However, it is likely that as cells equilibrate to elevated metabolic activity, they slowly switch to a more glycolytic phenotype to maintain high levels of ATP production and to prevent excessive ROS production derived from the mitochondrial electron transport chain. In addition, activation of the transaldolase enzyme from the pentose phosphate pathway can lead to enhanced levels of free radicals, which are detrimental to a tumor's continued proliferation. Therefore, in some cases, downregulation of TALDO expression induces accumulation of five-carbon sugars, depletion of NADPH, and hyperosmolarity-driven JNK activation, which inhibits apoptosis (65). Several viruses, such as HCV and EBV, may influence this process through interaction with JNK signaling pathways. Additionally, TALDO deficiency also leads to activation of the redox-sensitive β -catenin transcription factor, which is known to be elevated in over 90% of human HCC cases. Therefore, HCV may induce deficiencies in the TALDO enzyme in order to produce a transformed phenotype. This phenotype also yields a pro-oxidant environment, which is counterbalanced through HCV-mediated activation of anti-oxidant molecules, such as cyclooxygenase-2 and STAT-3 (7, 66). In the case of HCV and with other viruses, the

ability to neutralize deleterious ROS products accumulated during viral infection and transformation can act as a harbinger of tumor aggressiveness and growth potential.

Treatments for oncogenic virus-induced cancers based on glycolysis

Based on the importance of virus-encoded oncogenes for metabolic dysregulation observed in multiple malignancies, it seems plausible that the development of anti-glycolytic therapies might represent a worthwhile avenue of exploration in the treatment of virus-based cancers. Therapeutic agents, such as 2-deoxyglucose (2-DG), have a multitude of effects on glycolysis in cancer cells, and in the field of viral oncology, this compound may likewise prove beneficial for the treatment of virus-derived malignancies. 2-DG inhibits glycolysis by interfering with the action of glucose after initial phosphorylation by hexokinase. In tumor cells, glycolysis protects from nutrient deprivation and resultant loss of growth factors (8). Since tumor cells experience fluctuating periods of decreased nutrient availability, 2-DG, through glycolytic inhibition, may sensitize these cells to death. Additionally, since tumors that are highly reliant on glycolysis are typically more aggressive, it is likely that the use of 2-DG would greatly reduce viability of these cells. Highly malignant tumor cells can result from transformation by the majority of oncogenic viruses, and thus, 2-DG may serve a therapeutic role in these tumors as well.

Through interference with intracellular pathways, 2-DG would also prove to be protective against virus-mediated transformation. Because many of these tumors, such as those induced by EBV, HPV, HTLV-1, RSV, and polyoma viruses, exhibit enhanced glucose uptake, these tumors would preferentially take up this non-metabolizable drug and would suffer from glycolytic shutdown. Though agents, such as 2-DG, are not themselves overly toxic to tumor cells given alternative means of glucose utilization in the presence of glycolytic inhibition, these drugs enhance the efficacy of concomitant chemotherapeutic agents and radiation regimens and could be used in this context for virus-mediated malignancy. The use of 2-deoxyglucose with adjuvant radiotherapy has already been indicated for the treatment of glioblastoma (67), and similar efficacy with concomitant chemotherapeutic regimens is currently in clinical trial for patients with advanced solid malignancies. Importantly, compounds used to treat virus-derived cancers, such as carboplatin and paclitaxel, have already shown synergism with 2-DG in ovarian carcinoma, osteosarcoma, and non-small cell lung cancers (8). Furthermore, 2-deoxy-2-(^{18}F)fluoro-D-glucose positron emission tomography (^{18}F -FDG-PET), which is used to image and diagnose metabolically active tumors, may demonstrate higher efficacy in virus-derived cancers that have elevated metabolic rates.

Since pentose phosphate pathway activation mediates many of the protective effects against ROS production in virus-transformed cells, inhibition of this pathway may also prevent the deleterious outcomes associated with cellular transformation. Dihydroepiandrosterone (DHEA), a well known inhibitor of G6PDH, can inhibit EBV replication and prevent accelerated biosynthetic processes during EBV infection (68). Additionally, inhibition of the non-oxidative branches of the pentose phosphate cycle using the transketolase inhibitor, oxythiamine, results in cell cycle arrest and decreased tumor growth, effects which may be recapitulated in virus-derived cancers (69). Oncogenic signaling cascades are induced by the oxidative environment, and thus, pentose phosphate pathway activity and resultant NADPH production may need to be balanced with oncogene activation to provide therapeutic benefit.

Therapies aimed at interfering with growth signaling pathways have also shown benefit in preventing oncogenic transformation in virus-infected cells. The EBV lytic gene product, Na, is unable to bring cells out of viral latency during JNK inhibition (70). Furthermore, EBV latent membrane protein 1 (LMP1)-mediated oncogenic transformation and

proliferation is dependent on the JNK pathway (71). Moreover, LMP1 activation of the JNK pathway induces ERK-dependent cellular migration, which allows these cells to survive cell stress signals, such as low oxygen and/or nutrient availability, within the tumor microenvironment (72). In the case of HCV-infected cells, JNK inhibition would relieve aberrant glucose uptake and subsequent glucose-dependent signaling and may thwart tumor growth. Given that HCV replication induces oxidative stress in a JNK-dependent manner, it may be possible that by preventing ROS production, JNK inhibition would also halt viral replication (73). In terms of HCV-driven tumorigenesis, inhibition of JNK signaling has been shown to prevent HCV non-structural protein 3 (NS3)-mediated cell proliferation, indicating that inhibition of this pathway would halt HCV infection on multiple levels (74). Given ubiquitous PI3K and MAPK activation in cancer, these pathways may represent important targets for virus-transformed cells. PI3K inhibition prevents EBV signaling to initiate reactivation (75) and therefore, may represent a useful target to prevent EBV-infected cells from becoming cancerous. Moreover, constitutive MAPK phosphorylation by EBV LMP1 prevents growth arrest induced by transforming growth factor-1 β (TGF-1 β). As a result, constant activation of these pathways yields enhanced proliferation of EBV-transformed cells, indicating that these cascades could provide useful targets in the treatment of virus-induced tumors.

One of the critical pathways necessary for tumor proliferation, mammalian target of rapamycin (mTOR), plays a key role in the regulation of nutrient uptake, energy metabolism, and cellular proliferation, and has been demonstrated to be activated by nutrient status in EBV-transformed cells (76). Rapamycin, the prototype inhibitor of mTOR, also inhibits tumor development in EBV-transformed cells (77), and the mTOR inhibitors, sirolimus and everolimus, inhibit the growth of EBV-positive smooth muscle tumors and HTLV-1-associated T cell leukemias/lymphomas, respectively (78, 79). Similar to the synergistic effects of alkylating and anti-glycolytic therapies in the treatment of virus-derived cancers, rapamycin and the glycolytic inhibitor, 3-bromo-2-oxopropionate-1-propyl ester (3-BrOP), are highly effective in the depletion of cellular ATP and the disruption of glucose uptake, leading to increased cytotoxicity in leukemia cells (80). Additionally, sirolimus and everolimus inhibit cellular lactate production and cytosolic glycolysis, indicating that these inhibitors may also provide added benefit to anti-glycolytic therapies for virus-derived cancers (81).

Whether through direct glycolytic inhibition or modification of cell signaling pathways that regulate glycolytic flux, therapies aimed at counteracting glycolytic imbalance in tumor cells may reduce the development of virus-derived malignancies. This barricade to growth can function at a variety of levels – from glucose uptake to glycolytic enzyme activity to oxidative stress and pentose phosphate pathway-mediated neutralization. Though many of these compounds have demonstrated efficacy in the deceleration of tumor growth, it is likely that these compounds will show the greatest utility when combined with existing chemotherapeutic regimens. Synergistic effects on growth signaling, cell cycle control, and metabolic pathways may weaken the tumor cell's ability to survive periods of starvation and stress, such as occurs with traditional therapeutic agents. In addition, the critical dependence of virus-derived malignancies on glucose as a growth substrate and the intimate interaction among viral oncogenes and cellular glucose metabolic machinery indicate that anti-glycolytic agents could significantly improve the outcomes of chemotherapeutic schedules. Though systemic anti-glycolytic agents have demonstrated significant toxicity in patients, combination chemotherapeutic regimens employing these agents along with compounds that target specific growth signaling pathways activated by viruses or their oncogenic proteins may allow decreased chemotherapeutic doses to achieve more significant effects in virus-derived tumor cells. Furthermore, by taking advantage of the expression of unique viral oncoproteins in these cancer cells, it may be possible to develop approaches to target these

therapies to cancer cells harboring these oncoproteins, such as with antibody-drug conjugates that can achieve relatively specific toxicity in tumors.

Conclusions

Virus-derived malignancies represent a significant cancer burden even as new treatments and vaccines are developed. The regulation of growth signaling pathways by oncogenic viruses has been heavily studied, but the metabolic control exerted by these viruses remains less well understood. By subverting glucose metabolism through regulation of glucose uptake, glycolytic flux, and maintenance of ATP and NADPH production, viruses drive expression of oncogenes that potentiate tumor formation and progression. This metabolic phenotype confers a selective advantage to virus-derived tumor cells, which have the capacity to proliferate even under conditions of nutrient and oxygen scarcity. However, these same pathways may be proposed as targets to combat cancers, which are more dependent on glucose and anaerobic glycolysis than normal cells. Therefore, virus-transformed cells may in fact represent a superior cohort for anti-glycolytic therapy, given their wide-ranging metabolic dysregulation. In the future, further investigation into the mechanism of glucose metabolic regulation by oncogenic viruses may lead to the use of anti-glycolytic therapy for virus-mediated malignancy.

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Figure 1. Oncogenic viruses exert metabolic control through the regulation of cellular growth, cell cycle, and cell stress signals

Oncogenic viruses, including Hepatitis C Virus (HCV), Epstein-Barr Virus (EBV), and polyoma viruses, induce cellular proliferation through the PI3K and MAPK pathways and inhibit cell cycle arrest and DNA damage responses through inhibition of p53 and ATM. On the other hand, these viruses induce hypoxia-inducible factor-1 α (HIF-1 α) expression as well as AMP-activated protein kinase (AMPK)-dependent signaling. As a result, these pathways activate oxidative stress, glucose transport, and glycolytic metabolism.

Figure 2.

Oncogenic viruses regulate glucose uptake and glycolytic flux in transformed cells. Glucose uptake processes are enhanced by Rous sarcoma virus (RSV), and Epstein-Barr Virus (EBV) induces trafficking of the GLUT1 transporter to the plasma membrane. HTLV-1 utilizes the GLUT1 transporter to enter and infect cells, where it may then induce subsequent tumor formation. RSV enhances the activities of key glycolytic enzymes, including hexokinase (HK), glucose 6-phosphate dehydrogenase (G6PDH), and phosphofructokinase-1 (PFK-1), all of which contribute to enhanced glycolytic ATP production. SV40 enhances transaldolase (TALDO) activity, providing nucleotide precursors and sufficient NADPH reducing equivalents for cellular demands. The M2 isoform of pyruvate kinase (M2PK) is affected by multiple viruses, such as HPV, RSV, and HCV, which activate and induce changes in M2PK structure. In addition, HCV enhances lactate production while inhibiting mitochondrial oxidative phosphorylation, thus promoting glycolytic flux.