

## 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 that result from chronic or spontaneous viral infection and transformation. Although many of the molecular signaling pathways that underlie 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 that regulate 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 $\alpha$  and AMPK, viruses exert control over critical metabolic signaling cascades. Additionally, oncogenic viruses modulate the tumor metabolomic profile through direct and indirect interactions 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 and energy-use methods of transformed cells; therefore, it may be possible to develop novel antiglycolytic therapies to target these dysregulated pathways in virus-derived malignancies. *Mol Cancer Ther*; 11(1); 14–23. ©2012 AACR.

### Introduction

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, Epstein-Barr virus (EBV) nuclear antigen, and human papilloma virus (HPV) E6 protein, results 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 $\kappa$ B) cascades, that allow unregulated proliferation even in the absence of sufficient metabolic supply (4). Some viruses, such as HPV, must counteract the oxidative stress that occurs during viral infection to continue the replication cycle (5). Others, such as EBV, human T-lymphotropic virus 1 (HTLV-1), and hepatitis C virus (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 activation, which can also feed back to enhance HCV RNA 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.

Although 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 and the activated metabolic pathways that are responsible for tumor propagation remain unclear. Furthermore, and perhaps of more interest, the impact of viruses on the metabolic processes that govern tumor expansion and the feedback of these processes on viral protein expression remain 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.

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### 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 that fuel tumor growth and adapt metabolic pathways to this accelerated proliferation. Although 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 of the manner of tumorigenesis. For example, AMP-activated protein kinase (AMPK), a critical energy sensor of the cell that regulates glucose use in tumors, is activated by the AMP/ATP ratio in cancer cells, but in the context of viral transformation, it can be induced through targeted oncogenic proteins independently of the energy status of the cell (9). Alternatively, the deficiency of certain cellular processes that occurs as a result of genetic mutations and is not necessarily directly affected by viral transformation, such as nucleotide excision repair mediated by xeroderma pigmentosa C, 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 pathway to facilitate proliferation and enhanced migration and invasion (11–13). Of interest, the PI3K/Akt pathway is overactive in many types of tumor cells, inhibits p53-dependent apoptosis, and substantially affects glycolysis by stimulating the 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 in 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 use 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). Although oncogenic viruses provide the initial cues necessary for transformation, their metabolic role can be viewed as analogous to that of 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. Although the potent tumor suppressor p53 exerts pleiotropic effects to counteract tumor proliferation, it is also a critical regulator of glycolysis. 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 3C, regulate the p53 pathway, either through direct interaction or indirect cooperation with downstream effectors (23–25). 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 they can promote protective autophagy. Apoptotic and necrotic processes are also associated with DNA damage, and it is the net result of these cytotoxic signals that predicts 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. Antiglycolytic 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 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 $\alpha$  (HIF-1 $\alpha$ ), 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 is a significant metabolic stressor that affects tumor proliferation. One of the critical mediators of the response to physiological perturbations and hypoxia within the tumor mass is HIF-1 $\alpha$ , which acts as a molecular switch by turning on prosurvival pathways and adapting tumor cells to a low-oxygen environment. Through stabilization of HIF-1 $\alpha$  and import into the nucleus, HIF-1 $\alpha$  upregulates genes involved in angiogenesis, glucose transport, and glycolysis, such as hexokinase, phosphofructokinase-2 (PFK-2), and pyruvate kinase (30). This gene induction links HIF-1 $\alpha$  with the metabolic phenotype and allows tumor cells with stable HIF-1 $\alpha$  expression to alter their growth pattern based on changing signals within the surrounding parenchyma. EBV and HPV have been shown to induce HIF-1 $\alpha$  expression and to use HIF-1 $\alpha$  to regulate viral infection (31, 32). In addition, oncogenic viruses may depend on HIF-1 $\alpha$  to alter the metabolic rate and fulfill the demands of viral oncogene-driven proliferation. In this way, HIF-1 $\alpha$  may be a necessary intermediate between viral infection and subsequent transformation, and during key oncogenic steps, it may allow virus-derived tumors to survive hypoxic signals from the surrounding tissue. Therefore, it is evident that oncogenic viruses use physiological stressors, as well as cellular pathways that control growth and DNA damage, to enhance transformation and promote tumor progression (Fig. 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 $\alpha$  that upregulate 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 Fujinama 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 exhibit not only transcriptional regulation of glucose transport but also alterations in transporter trafficking during transformation (34). In fact, transport of glucose may account for the upregulation of glycolysis seen in some transformed cells, such as those transformed by Rous sarcoma virus (RSV; ref. 35). Of importance, the increase in glucose

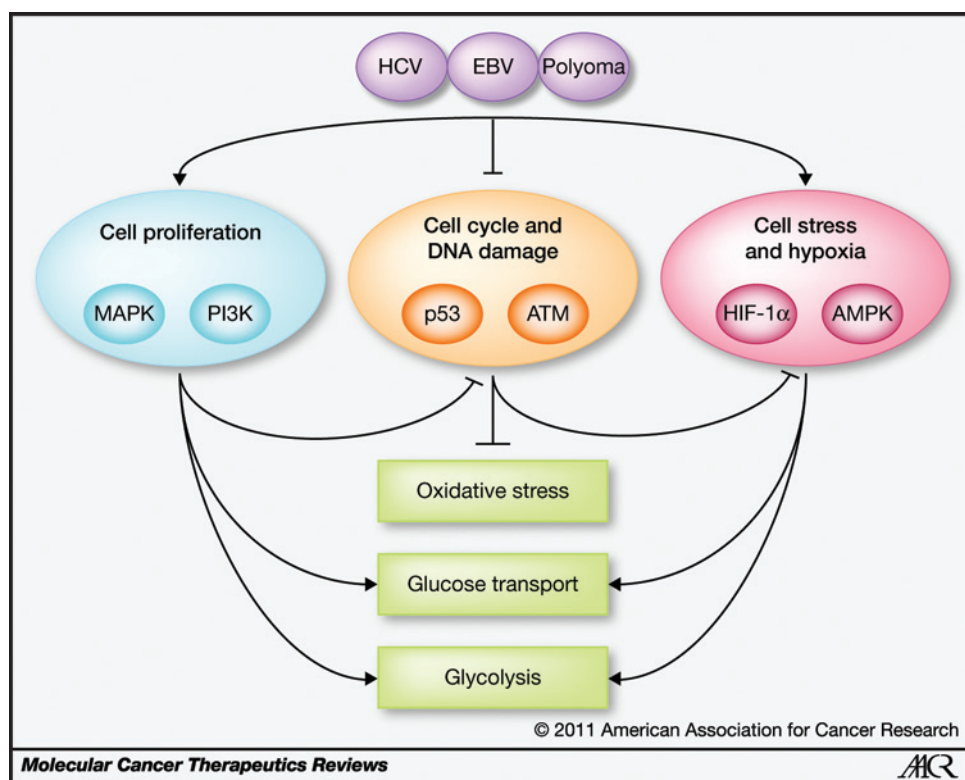


Figure 1. Oncogenic viruses exert metabolic control through the regulation of cellular growth, cell cycle, and cell stress signals. Oncogenic viruses, including HCV, EBV, and polyoma viruses, induce cellular proliferation through the PI3K and MAPK pathways and inhibit cell-cycle arrest and DNA-damage responses by inhibiting p53 and ATM. On the other hand, these viruses induce HIF-1 $\alpha$  expression as well as AMPK-dependent signaling. As a result, these pathways activate oxidative stress, glucose transport, and glycolytic metabolism.

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transport in transformed cells is likely not due to *de novo* synthesis of a new transport system itself but to a modification of existing glucose transport (36). Therefore, the viruses themselves may be able to effect changes in the cell membrane that would facilitate increased glucose transport, such as unmasking 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 tumors, such as those induced by HPV and polyoma viruses (37, 38). As observed in studies of other oncogenic viruses, these effects are not always limited to elevated levels of GLUT1 expression and can also involve altered GLUT1 trafficking in transformed cells (39). These observations indicate that functional changes in the membrane transport system may be critical for 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 showing that GLUT1 is the putative receptor for HTLV-1 expanded on the function of membrane transporters as retroviral receptors and also indicated that these receptors could involve transporters implicated in glucose signaling pathways (40). 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, indicating that the metabolic disturbances induced by HTLV-1 are cell-type-specific (41). These effects may result from enhanced GLUT1 expression in certain cells, which would be beneficial for HTLV-1 infection. In addition, because 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 influence both infection and transformation, thus validating this family of membrane transporters as potential therapeutic targets for oncogenic viruses and resultant tumor masses (see Fig. 2).

### Oncogenic Viruses Enhance the Activity of Key Glycolytic Enzymes

Unlike their normal counterparts, tumor cells preferentially use enhanced aerobic glycolysis for energy metabolism, a phenomenon first described by Otto Warburg in 1925 and known as the Warburg effect (42). This shift toward 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 aerobic glycolysis and energy production. Specifically, the rate-limiting enzyme that catalyzes the final step in glycolysis, pyruvate kinase, is upregulated in tumor cells. Of interest, tumor cells exclusively express the M2 isoform of this enzyme (M2PK; refs. 38, 43), which is typically expressed only during embryonic development, rather than the M1 isoform, which is expressed in most normal adult tissues.

Because pyruvate kinase is a key enzyme that was shown to be involved in tumor progression, researchers 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), Zwerschke and colleagues (44) 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. Of interest, Mazurek and colleagues (45) reported that this switch in structure is controlled by fructose 1,6-bisphosphate and regulates the glycolytic flux in tumor cells. They 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 (46). These cells also produced high levels of alanine, which is associated with the metastasizing capabilities of a growing tumor (47). Because alanine is produced by this step in the glutaminolytic pathway and because 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.

A further indication that oncogenic viruses have a role in the activity of M2PK comes from studies that showed targeting of M2PK by RSV. Chicken embryonic fibroblasts (CEF) transformed by RSV show 3-fold higher M2PK activity, which is dependent on the oncogenic protein Src (48). In fact, cellular M2PK may represent a target of pp60<sup>src</sup>, the protein product of Src that may initially inhibit and then activate M2PK through the accumulation of glycolytic intermediates (48) such as fructose 1,6-bisphosphate that accumulate in RSV-transformed cells and potentially relieve 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 phosphoserine and phosphothreonine, only

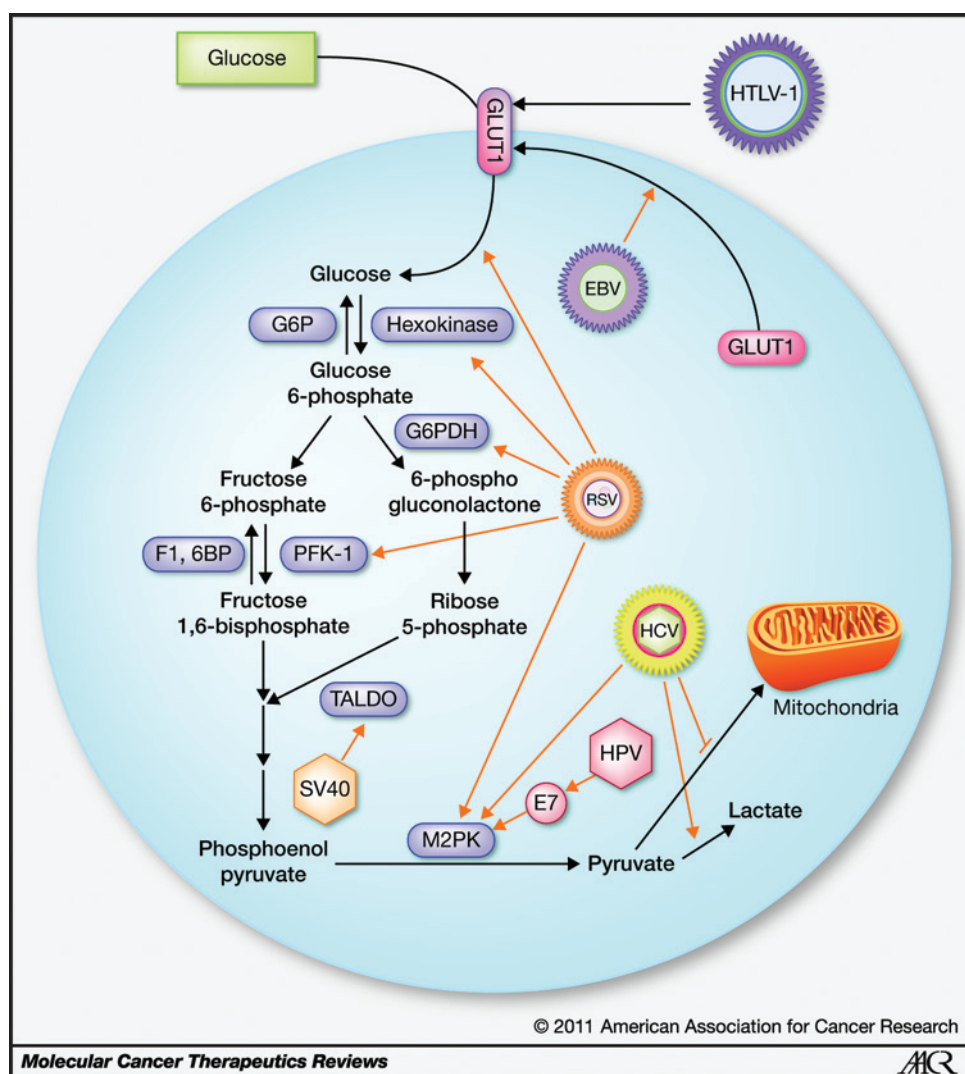


Figure 2. Oncogenic viruses regulate glucose uptake and glycolytic flux in transformed cells. Glucose uptake processes are enhanced by RSV, and EBV induces trafficking of the GLUT1 transporter to the plasma membrane. HTLV-1 uses the GLUT1 transporter to enter and infect cells, in which it may then induce subsequent tumor formation. RSV enhances the activities of key glycolytic enzymes, including hexokinase, G6PDH, and PFK-1, all of which contribute to enhanced glycolytic ATP production. SV40 enhances TALDO activity, providing nucleotide precursors and sufficient NADPH-reducing equivalents for cellular demands. M2PK is affected by multiple viruses, such as HPV, RSV, and HCV, that activate and induce changes in M2PK structure. In addition, HCV enhances lactate production while inhibiting mitochondrial oxidative phosphorylation, thus promoting glycolytic flux.

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transformed CEFs contain phosphotyrosine (49). The amount of phosphotyrosine may correlate with the kinetic activity of M2PK, which shows correspondingly less affinity for its substrate, phosphoenolpyruvate, in transformed CEFs. The extent of a causal relationship between phosphotyrosine and virus-induced transformation may involve an additional negative charge on M2PK or altered partitioning of this enzyme from the sarcolemmal or endoplasmic reticulum (50), both of which could drastically modify functional enzyme activity. Of interest, it was shown that alterations in M2PK activity make it necessary for the transforming protein to have tyrosine phosphorylation capabilities, similar to what has been observed for other glycolytic enzymes in RSV-transformed cells (51).

Not only does RSV affect the M2PK pathway, but it also causes enhanced activities of the other rate-limiting enzymes in glycolysis (i.e., hexokinase and PFK) in transformed cells, alterations that are specific to cells that have been transformed but not infected by RSV (52). 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 that is independent of the growth rate of transformed cells (53). Other studies showed a significant increase in PFK-2 activity, as well as increased levels of fructose 2,6-bisphosphate, in RSV-transformed CEFs compared with normal CEFs, both of which were specific to transformation-capable virus forms (54). However, it appears that this oncogenic increase in PFK-2 activity is not a direct effect but, instead, depends on protein kinase C (PKC; ref. 55). During RSV infection, the rates of glucose uptake and lactic acid production are significantly increased and allow for enhanced production of glycolytic anions and pyruvate, intermediates that fuel the energy demands of growing tumor cells (56). Of importance, the levels of glucose, phosphoglycerate, and phosphoenolpyruvate all decrease in RSV-transformed cells, emphasizing that increased glucose uptake alone is not responsible for the observed alterations of the glycolytic phenotype. Of interest, there does not appear 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

Although 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 herpes virus (KSHV), the causative agent of Kaposi's sarcoma, upregulates glucose uptake and sensitizes infected cells to glycolytic inhibition (57). This effect may be mediated, at least in part, through transcriptional activation of key glycolytic enzymes by HIF-1 $\alpha$  in response to alterations in oxygen tension within the tumor microenvironment (58). 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 (59). Although there are many potential pathways that regulate HPV transcription, the insulin-like growth factor 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 (60). Although elevated TALDO activity shifts metabolic precursors from the pentose phosphate pathway toward 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. Although 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 before or after cellular transformation indicates that the initial products of glycolysis may be diverted later 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 exhibit enhanced hexokinase

activity, which yields more phosphorylated glucose for use in glycolysis (61). Long-term HCV protein expression also induces enhanced ATP production, a process that evidently is specific to cells that use glucose as their main energy source, as well as enhanced lactate production (62). Additionally, HCV inhibits oxidative phosphorylation and allows a concomitant accumulation of glycolytic intermediates (62). This process may depend on the HIF-1 $\alpha$  signaling pathway, because these intermediates (e.g., pyruvate) stabilize HIF-1 $\alpha$ , possibly through inhibition of prolyl hydroxylases. Furthermore, stabilized HIF-1 $\alpha$  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 stepwise 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 (63). Because 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 showed that HCV NS5B, the HCV RNA-dependent RNA polymerase, interacts with M2PK but not with the L-type PK and that downregulation of M2PK also reduces HCV replication (64). These findings further depict metabolism-dependent viral replication, and they ensnare HCV in the process of cellular transformation.

The pentose phosphate pathway plays a key role in producing the reducing equivalents that are needed to counteract excessive reactive oxygen species (ROS) development within rapidly expanding tumors. Although 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 appears 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 (65). 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 TALDO 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 5-carbon sugars, depletion of NADPH, and hyperosmolarity-driven JNK activation, which inhibits apoptosis (66). Several viruses, such as HCV and EBV, may influence this process through interaction with JNK signaling pathways. Additionally, TALDO deficiency leads to activation of the

redox-sensitive  $\beta$ -catenin transcription factor, which is known to be elevated in >90% of human HCC cases. Therefore, HCV may induce deficiencies in the TALDO enzyme to produce a transformed phenotype. This phenotype also yields a prooxidant environment that is counterbalanced by HCV-mediated activation of antioxidant molecules such as cyclooxygenase-2 and STAT-3 (7, 67). In the case of HCV and other viruses, the ability to neutralize deleterious ROS products that accumulate 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

Given the importance of virus-encoded oncogenes for metabolic dysregulation observed in multiple malignancies, it appears plausible to conclude that the development of antiglycolytic therapies might represent a worthwhile avenue of exploration in the treatment of virus-based cancers. Therapeutic agents such as 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 against nutrient deprivation and the resultant loss of growth factors (8). Because tumor cells experience fluctuating periods of decreased nutrient availability, 2-DG, through glycolytic inhibition, may sensitize these cells to death. Additionally, because tumors that are highly reliant on glycolysis are typically more aggressive, it is likely that the use of 2-DG would greatly reduce the viability of such 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.

By interfering 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, they would preferentially take up this nonmetabolizable drug and would suffer from glycolytic shutdown. Although agents such as 2-DG are not themselves overly toxic to tumor cells given alternative means of glucose usage in the presence of glycolytic inhibition, they enhance the efficacy of concomitant chemotherapeutic agents and radiation regimens, and they could be used in that context for virus-mediated malignancy. The use of 2-DG with adjuvant radiotherapy has already been indicated for the treatment of glioblastoma (68), and a similar application with concomitant chemotherapeutic regimens is currently in clinical trial for patients with advanced solid malignancies. Of importance, 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-( $^{18}\text{F}$ )fluoro-D-glucose positron emission tomography ( $^{18}\text{F}$ -FDG-PET), which is used to image and diagnose metabolically active tumors, may show higher efficacy in virus-derived cancers that have elevated metabolic rates.

Because 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. DHEA, a well-known inhibitor of G6PDH, can inhibit EBV replication and prevent accelerated biosynthetic processes during EBV infection (69). Additionally, inhibition of the nonoxidative branches of the pentose phosphate cycle by the transketolase inhibitor oxythiamine results in cell-cycle arrest and decreased tumor growth, effects that may be recapitulated in virus-derived cancers (70). Oncogenic signaling cascades are induced by the oxidative environment; thus, pentose phosphate pathway activity and the 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 (71), and EBV latent membrane protein 1 (LMP1)-mediated oncogenic transformation and proliferation are dependent on the JNK pathway (72). 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 (73). In the case of HCV-infected cells, JNK inhibition would relieve aberrant glucose uptake and subsequent glucose-dependent signaling, and it may thwart tumor growth. Given that HCV replication induces oxidative stress in a JNK-dependent manner, it is possible that by preventing ROS production, JNK inhibition would also halt viral replication (74). In terms of HCV-driven tumorigenesis, inhibition of JNK signaling has been shown to prevent HCV nonstructural protein 3 (NS3)-mediated cell proliferation, indicating that inhibition of this pathway would halt HCV infection on multiple levels (75). Given the 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 (76) 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 TGF- $\beta$ . 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 it has been shown to be activated by nutrient status in EBV-transformed cells (77). Rapamycin, the prototype inhibitor of mTOR, also inhibits tumor development in EBV-transformed cells (78), 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 (79, 80). Similar to the synergistic effects of alkylating and antiglycolytic 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 at depleting cellular ATP and disrupting glucose uptake, leading to increased cytotoxicity in leukemia cells (81). Sirolimus and everolimus also inhibit cellular lactate production and cytosolic glycolysis, which suggests that these inhibitors may provide an additional benefit to antiglycolytic therapies for virus-derived cancers (82).

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, ranging from glucose uptake to glycolytic enzyme activity, oxidative stress, and pentose phosphate pathway-mediated neutralization. Although many of these compounds have shown efficacy in the deceleration of tumor growth, it is likely that they will show the greatest use 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 antiglycolytic agents could significantly improve the outcomes of chemotherapeutic schedules. Although systemic antiglycolytic agents have shown 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 advan-

tage of the expression of unique viral oncoproteins in these cancer cells, we may be able to develop approaches to target these therapies to cancer cells that harbor these oncoproteins (e.g., by using 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 being developed. The regulation of growth-signaling pathways by oncogenic viruses has been well 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 the 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 antiglycolytic 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 antiglycolytic therapy for virus-mediated malignancy.

## Disclosure of Potential Conflicts of Interest

K. Khalili is a consultant for or serves on the PML Expert Panel of Genentech. E. Noch declares no potential conflicts of interest.

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