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The Role of Phosphoinositide 3-Kinase-Akt Signaling in Virus Infection

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Summary

Successful virus infection of host cells requires efficient viral replication, production of virus progeny and spread of newly synthesized virus particles. This success, however also depends on the evasion of a multitude of antiviral signaling mechanisms. Many viruses are capable of averting antiviral signals through modulation of host cell signaling pathways. Apoptotic inhibition, for example, is a universal intracellular antiviral response, which prolongs cellular survival and allows viruses to complete their life cycle. Ongoing apoptotic inhibition contributes to the establishment of latent and chronic infections, and has been implicated in viral oncogenesis. The phosphoinositide 3-kinase (PI3K)-Akt pathway has become recognized as being pivotal to the inhibition of apoptosis and cellular survival. Thus, modulation of this pathway provides viruses with a mechanism whereby they can increase their survival, in addition to other established mechanisms such as expression of viral oncogenes and direct inhibition of proapoptotic proteins. Recent research has revealed that this pathway is up-regulated by a number of viruses during both short-term acute infections and long-term latent or chronic infections. During acute infections PI3K-Akt signaling helps to create an environment favorable for virus replication and virion assembly. In the case of long-term infections, modulation of PI3K-Akt signaling by specific viral products is believed to help create a favorable environment for virus persistence, and contribute to virus-mediated cellular transformation.

Key Words: Phosphoinositide 3-kinase; Akt; virus; transformation; signaling; survival.

1. Introduction

Efficient virus replication and production of virus progeny is dependent on the ability of viruses to survive in a hostile host environment. In order to survive inside cells, viruses have evolved mechanisms by which they can modulate cellular events, particularly those governing apoptosis and cellular survival. Virus-mediated apoptotic inhibition is a well-established survival mechanism. During acute infections, such as those caused by respiratory viruses like influenza A and respiratory syncitial virus (RSV), apoptotic inhibition plays a role in maintenance of cell viability during virus replication and growth. During long-term infections such as latent herpesviruses infections, or chronic hepatitis B and C virus infections, apoptotic inhibition plays a role in prolonged survival of infected cells. In latently infected cells apoptotic mechanisms are often held in check by specific viral proteins and the cell cycle can also be modulated, creating an environment favorable for cellular transformation and tumor development. During chronic infection, it is believed that multiple biochemical changes occur within the host cell, resulting from both virus-dependent and -independent mechanisms, which can lead to cellular transformation. The molecular mechanisms that result in cellular transformation

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in vivo as a result of long-term virus infections are not well defined, and are likely to depend also on the virus species, the cell type infected, and the genetics of the host.

Viruses block apoptosis through inhibition of classical apoptotic pathway proteins such as death receptors, caspases, and p53, and expression of viral homologues to anti-apoptotic proteins such as Bcl-2 *(1)*. However, numerous mitogenic signaling pathways within the eukaryotic cell also regulate the balance between apoptosis and cell survival. Phosphoinositide 3-kinases (PI3Ks) are pivotal to several signal transduction pathways and act on a number of downstream signaling molecules to regulate cellular events such as cellular survival, differentiation and proliferation *(2)*. Akt kinase is one such molecule, and PI3K-Akt signaling has been demonstrated to be extremely important in cell survival *(3)*. Constitutive up-regulation of PI3K-Akt cell survival signaling has also been implicated in oncogenesis, as it averts apoptotic cell death during uncontrolled cellular proliferation *(4)*.

Activation of the PI3K-Akt signaling pathway during virus infection is emerging as a common mechanism for virus survival during early replication, the establishment of latent and chronic infections, and virus-mediated cellular transformation. This chapter will describe the PI3K-Akt pathway in detail and discuss the viral modulation of this pathway as a means for survival in different types of virus infection.

2. PI3K-Akt Signaling

2.1. PI3Ks

PI3Ks are a family of enzymes that phosphorylate the 3′ hydroxyl group of the inositol ring of phosphatidylinositol (PtdIns) and related inositol phospholipids, generating 3′-phosphoinositide products (*see* Fig. 1) *(5)*. These phosphoinositide products act as second messengers, which aid the recruitment of numerous proteins into signaling complexes at the plasma membrane, and in this way can activate numerous downstream signaling events.

There are three classes of PI3Ks (I, II, and II), which differ in their substrate specificity and regulation. Class I PI3Ks, however, are by far the best studied, as they function to regulate downstream signaling events in response to external mitogenic stimuli. In addition, it is only the effect of signaling downstream class I PI3Ks that has been studied in the context of virus infections, therefore only this class will be discussed. Further information on class II and III PI3Ks can be found in a number of recent reviews *(2,6)*.

Class I PI3Ks are heterodimeric proteins consisting of a catalytic subunit (110 kDa, p110) and a regulatory (or adaptor) subunit. This class of PI3Ks has been further subdivided into subgroups I_A and I_B which are activated downstream of tyrosine kinases and G-protein-coupled receptors (GPCRs) respectively *(6)*. In mammals the p110 catalytic subunit of class I_A PI3Ks has 3 isoforms (α, β,δ), each encoded by a separate gene. They also have seven adaptor subunits, generated by expression and alternative splicing of three different genes (p85α, p85β, and p55γ). The prototype p85α subunit has an Src-homology 3 (SH3) domain, a proline-rich domain and two Src-homology 2 (SH2) domains that mediate protein-protein interactions (Table 1). The p110 catalytic subunits form functional complexes with the adaptor subunits by binding to a region between their SH2 domains (the inter-SH2 region) (Table 1) *(7)*. Forms of class I_A PI3Ks have also been identified in *Drosophila melanogaster*, *Caenohabiditis elegans*, and the slime mold *Dictyostelum discoideum (6)*.

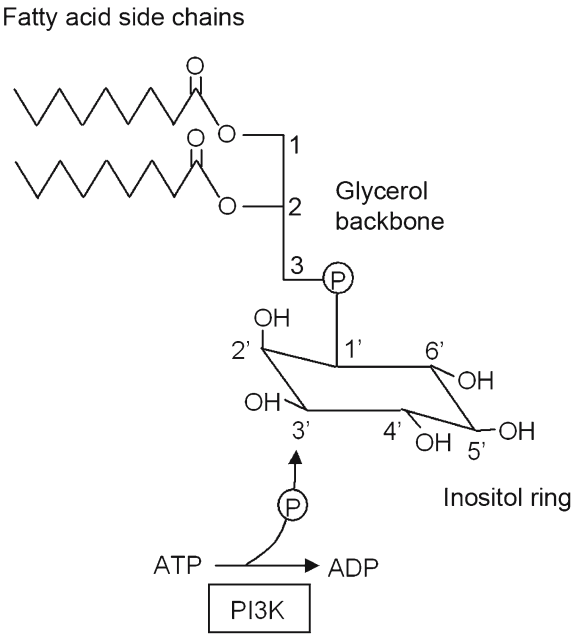
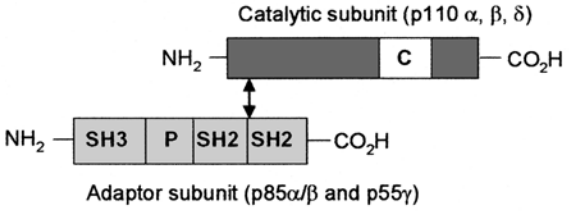
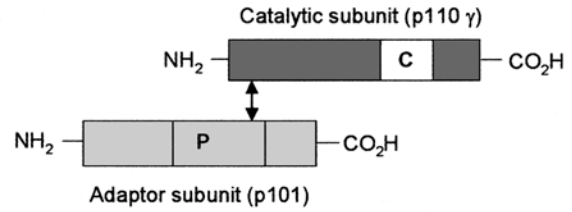


Fig. 1. The site of action of PI3Ks. Phosphatidylinositol (PtdIns) is composed of a glycerol backbone with fatty acid side chains attached at positions 1 and 2 and an inositol ring attached at position 3. The fatty acid side chains lie within the inner leaflet of the plasma membrane, with the inositol ring in the cytoplasm. PI3Ks transfer a phosphate group (P) from adenosine triphosphate (ATP) to the 3′ hydroxyl of the inositol ring of phosphatidylinositol. This results in the production of adenosine diphosphate (ADP) and phosphoinositide-3-phosphate (PtdIns-3-P). PI3Ks can also transfer a phosphate group to phosphoinositides already phosphorylated at other inositol hydroxyl positions (1′, 2′, 4′, and 5′) to produce 3′ phosphoinositides.

Activation of class I_A PI3Ks is mediated by the adaptor subunits. In quiescent cells the p85α regulatory subunit has been shown to inhibit the catalytic activity of the p110 *(8)*. Upon mitogenic stimulation p85α mediates the translocation of p110 to the plasma membrane through binding of its SH2 domains to autophosphorylated receptor tyrosine kinases (RTKs) (*see* Fig. 2) *(9)*. This binding event is also believed to result in a conformational change which releases the p110 subunit from the inhibitory binding of p85α, allowing it to be free to phosphorylate its lipid substrates *(10–12)*. Class I_A PI3Ks can also be activated by tyrosine kinases in the cytoplasm downstream of other types of receptor. Src-family kinases, for example, have been shown to bind constitutively to class I_A PI3Ks and increase activity through phosphorylation of the p85 subunit at Tyr-688 *(13,14)*.

Class I_B PI3Ks appear only to exist in mammals and consist of a single p110γ catalytic subunit, associated with a single 101 kDa (p101) adaptor subunit (Table 1) *(2,5,15)*. Unlike the class I_A adaptor subunits, p101 does not contain any Src homology domains and interacts with p110γ instead via its proline-rich domain (Table 1) *(5)*. Class I_B PI3Ks are activated by binding of the catalytic subunit to the $G_{\beta\gamma}$ subunits of heterotrimeric GTP-binding proteins *(16)*. It has been observed that class I_A PI3Ks can also be activated by G-proteins, and both class I_A and I_B PI3Ks can bind Ras. In addition,

Table 1
The Properties of Class I_A and I_B PI3Ks

PI3K Class	Regulated by	*In vitro* substrates
IA 	Tyrosine Kinases Ras GPCRs?	PtdIns PtdIns-4-P PtdIns-4,5-P_2
IB 	GPCRs Ras	PtdIns PtdIns-4-P PtdIns-4,5-P_2

Key
C – catalytic domain
SH2 – Src homology 2 domain
SH3 – Src homology 3 domain
P – proline-rich domain(s)

the HA-Ras isoform is associated with activation of PI3K and Akt rather than the classical mitogen activated Raf-MEK-ERK signaling cascade (*see* Fig. 2) *(17)*. However, the regulation and activation of PI3K-Akt signaling events by Ras has not been well studied, and the influence this has on downstream signaling events remains to be determined *(16,18,19)*.

The primary target for class I PI3Ks, both in vitro and in vivo, is phosphatidylinositol-4,5-diphosphate (PtdIns-4,5-P_2), and hence the primary product is phosphatidylinositol-3,4,5-triphosphate (PtdIns-3,4,5-P_3) (*see* Fig. 2) *(20)*. The production of PtdIns-3,4,5-P_3 is regulated by the phosphatase, PTEN, which catalyses the dephosphorylation of PtdIns-3,4,5-P_3 to PtdIns-4,5-P_2 (*see* Fig. 2) *(21,22)*.

2.2. Activation and Antiapoptotic Function of Akt

The production of PtdIns-3,4,5-P_3 by class I PI3Ks results in the recruitment of a wide variety of signal transduction proteins to the plasma membrane, which is facilitated by their lipid-binding pleckstrin homology (PH) domains (*see* Fig. 2) *(23)*. Once at the plasma membrane these proteins are most commonly activated by secondary phosphorylation events. One such protein, considered largely responsible for the antiapoptotic and cell survival mechanisms downstream of PI3K, is Akt (*see* Fig. 2).

Akt was discovered as a cellular homologue (c-*Akt*) of the viral oncogene (v-*Akt*) from the acutely transforming retrovirus AKT8, isolated from a murine T-cell lymphoma

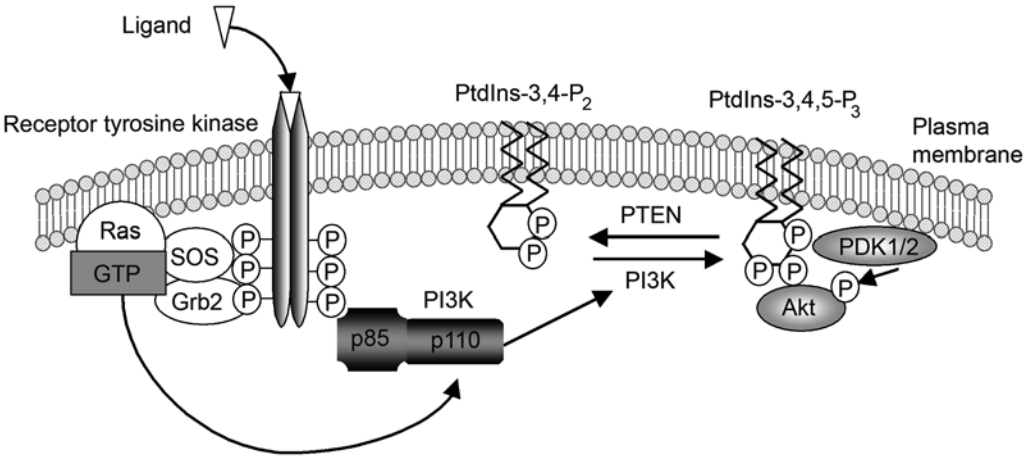


Fig. 2. Activation of class I_A PI3Ks. RTKs bind to extracellular mitogens, which trigger their activation through dimerization and autophosphorylation of their cytoplasmic domains. The p85 (or p55) adaptor subunits of class I_A PI3Ks recruit the p110 catalytic subunits to the plasma membrane through binding of their SH2 domains to the phosphorylated tyrosines on the RTK. This binding also causes a conformational change resulting in activation of p110. p110 is also known to be activated by G-proteins such as Ras. Activated PI3Ks then phosphorylate plasma membrane PtdIns such as PtdIns-4,5-P_2 to produce Ptd-3,4,5-P_3. Various signaling proteins such as Akt are recruited to the plasma membrane via binding of their PH domains to phosphorylated PtdIns. Once there, they are subsequently phosphorylated and activated by kinases such as PDK1/2.

(24–26). It was simultaneously identified as a novel kinase with many similarities to protein kinase A (PKA) and protein kinase C (PKC), and therefore was also named protein kinase B (PKB) *(27)*. In mammals, there are three isoforms of Akt (Akt 1, 2, and 3 or PKB α, β, and γ) that have a broad tissue distribution. All three isoforms are composed of an N-terminal PH domain, a central catalytic domain, and a C-terminal hydrophobic domain. As mentioned above, binding of the PH domain of Akt to the phosphoinositide products of PI3K results in its recruitment to the plasma membrane. Once there, Akt is activated by phosphorylation at Thr-308 of the catalytic domain by phosphoinositide-dependent kinase 1 (PDK-1), and at Ser-473 of the C-terminal hydrophobic region (Akt1/PKBα) by another kinase, termed phosphoinositide-dependent kinase 2 (PDK-2), which is yet to be identified *(28)*.

Upon activation, Akt phosphorylates a wide variety of targets at Ser/Thr residues, which are involved in the regulation of cell differentiation, proliferation and survival /apoptotic inhibition (*see* Fig. 3). A number of Bad proapoptotic proteins are inactivated by Akt phosphorylation. These include the Bcl-2 family member Bad (Ser-136), the cell death effector protease caspase-9 (Ser-196) and glycogen synthase kinase-3 beta (GSK-3β) (Ser-9) (*see* Fig. 3) *(29)*. Akt-phosphorylated BAD binds to 14-3-3 proteins and is sequestered in the cytosol. This interaction prevents it from heterodimerizing with and inactivating anti-apoptotic Bcl_2-family members, such as Bcl-2 and Bcl-xL, at the mitochondrial membrane *(15,30)*. Akt-phosphorylation and inactivation of human caspase-9, and several other caspases, blocks activation of the proteolytic apoptotic caspase cascade *(31)*. GSK-3β has been shown to induce apoptosis and this is blocked by Akt phosphorylation *(32)*. GSK-3β

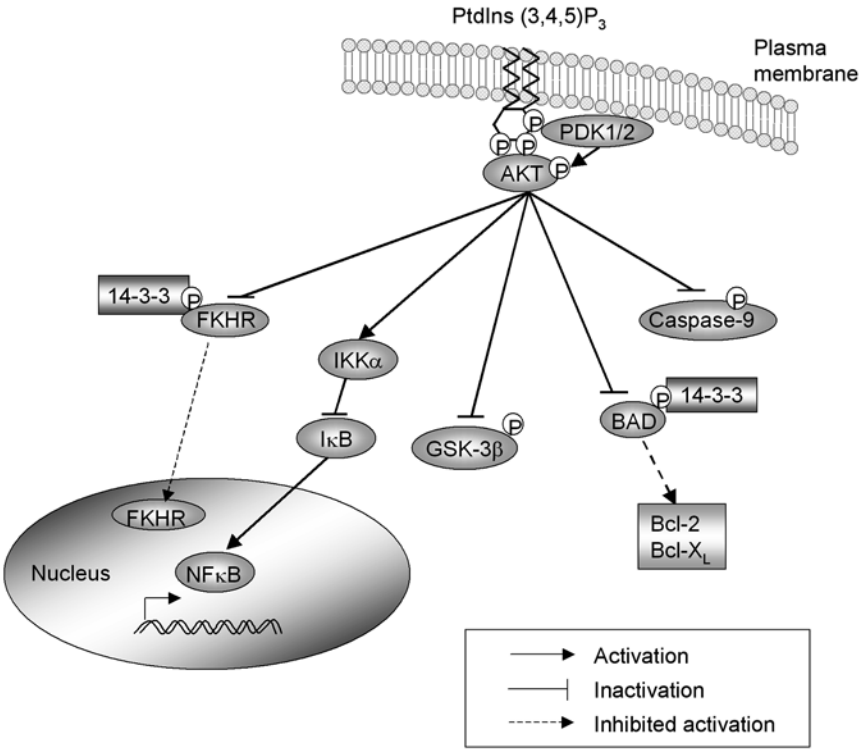


Fig. 3. Akt-mediated survival. Activated Akt mediates cell survival by phosphorylating and inhibiting a number of Bad proapoptotic proteins, including, caspase-9, Bad and GSK-3β. Akt phosphorylates and inhibits forkhead transcription factors like FKHR, preventing them from migrating to the nucleus and up-regulating the expression of Bad proapoptotic genes. Akt can also activate transcription of Bad prosurvival genes by activation IKKα, which degrades IκB and releases the transcription factor NF-κB. The inhibition of Bad and FKHR is aided by 14-3-3 proteins, which bind and sequester their phosphorylated forms in the cytoplasm.

normally phosphorylates and inhibits glycogen synthase, therefore it has been suggested that Akt inactivation of GSK-3β may also increase glycogen synthesis *(33)*.

In addition, Akt inhibits the transcription of proapoptotic genes by phosphorylating members of the forkhead family of transcription factors such as FKHR. FKHR predominantly resides in the nucleus, where it regulates the transcription of a number of genes crucial to apoptosis, for example FasL and Bim *(34)*. Akt-phosphorylation promotes the export of FKHR from the nucleus into the cytosol, where it is bound and inhibited by 14-3-3 proteins *(34,35)*. Akt can also activate the transcription of antiapoptotic genes (e.g., inhibitors of apoptosis [AIFs]) through phosphorylation of IκB kinase α (IKKα). IKKα phosphorylates the NF-κB inhibitor IκB and facilitates its ubiquitin-mediated degradation. This permits NF-κB to translocate to the nucleus where it can up-regulate gene expression *(36–40)*.

It is considered that the ability of Akt to simultaneously block apoptotic factors and increase survival factors correlates with protection against apoptotic stimuli in a variety of cell types in which activated Akt has been constitutively over-expressed.

3. PI3K-Akt Signaling in Virus Infection

3.1. PI3K-Akt Mediated Cell Survival During the Early Stages of Acute Virus Infections

Activation of Akt downstream of PI3K has been shown to be important in cell survival and apoptotic inhibition during different types of virus infection. Recent research on viruses that cause acute infections, for example, suggests that activation of PI3K-Akt signaling may contribute to survival during the early stages of infection, when virus replication and protein synthesis are taking place. However, activation of such survival responses may also be induced by the infected cells themselves to permit sufficient time for the activation of antiviral cellular defense mechanisms and viral clearance by the host immune system.

Early activation of PI3K-Akt survival signaling has been most commonly observed in vitro with RNA viruses that cause acute infections such as human RSV, severe acute respiratory syndrome (SARS) coronavirus, and rubella virus (RV). Infections with these viruses usually results in the induction of apoptosis, which is considered, for nonlytic viruses, to facilitate spread of progeny *(1)*. As these viruses cannot evade immune system detection, they have to replicate and spread rapidly in order to survive, which may be aided by initial activation of survival responses followed by induction of apoptosis *(1)*.

RSV is an important cause of serious respiratory tract illness in children and immunocompromised adults *(41)*. This virus preferentially infects airway epithelial cells, leading to a severe inflammatory response characterized by the up-regulation of inflammatory cytokines and chemokines, as well as signal transducers and activators of transcription (STATS) *(42–44)*. This response has been shown in vitro to be dependent on an increase in the transcriptional activity of NF-κB *(42,43)*. The induction of NF-κB activity during RSV infection in A549 airway epithelial cells has been shown to result from activation of PI3K and Akt *(45)*. Interestingly, although RSV infection ultimately leads to cell death, a large proportion of RSV infected cells remain viable well into the infection. This maintenance of cell viability is also dependent on the activation of PI3K, Akt, and NF-κB, as inhibition of PI3K with the drug LY294002 has been shown to cause a rapid increase in the speed and magnitude of RSV-induced apoptosis *(45)*. Further studies have demonstrated that RSV infection of A549 cells and primary tracheobronchial epithelial cells leads to the activation of ceramidase and sphingosine kinase resulting in an up-regulation of the production of the prosurvival molecule sphingosine 1-phosphate (S1P) *(46)*. S1P subsequently mediates the downstream activation of PI3K-Akt (as well as extracellular regulated kinase [ERK]) leading to cell survival and apoptotic inhibition in the initial stages of RSV infection *(46)*. This data suggests that PI3K-Akt signaling contributes to cell survival to preserve host cells until the life cycle of RSV is complete.

Similar signaling events have been observed during RV infection in vitro. RV infection has been shown to increase the phosphorylation of Akt and GSK-3β, and like RSV, inhibition of PI3K with LY294002 increases the speed and magnitude of RV-induced caspase-dependent apoptosis *(47)*. However, in contrast to RSV, these survival signals occur concomitantly with, and no prior to, apoptotic signals that occur early in RV infection *(48–52)*. However extensive apoptosis does occur at later stages of the virus life cycle, implying that the cell survival signals are eventually overridden following the production and release of large amounts of virus progeny. This suggests that in RV

infection, the signals regulating downstream Akt survival events as well as apoptosis, be they viral or cellular, probably differ from those of RSV. The significance of cell survival and apoptosis during RV associated disease such as acute lymphadenopathy, rash and congenital rubella syndrome, is unknown.

Like RV, infection with SARS-associated coronavirus (CoV) in Vero E6 cells has been shown to increase phosphorylation of Akt and GSK-3β as well as a PKCζ, another downstream mediator of PI3K survival signaling *(53,54)*. However, in contrast, the survival response resulting from PI3K-Akt signaling was deemed to be weak, as LY294002 treatment did not result in an increase in apoptotic DNA laddering *(54)*. The authors conclude that the weak activation of Akt and GSK-3β in SARS-CoV infected cells is not sufficient to prevent virus-induced apoptosis at any stage of infection. The inability of cells to mount an adequate survival response may contribute to the pathology of SARS in vivo, however further studies need to be done to investigate this.

Coxsackievirus B3 (CVB3) is the causative agent of acute myocarditis, although infection with CVB3 can also lead to chronic cardiomyopathy *(55,56)*. CVB3 infection of cardiac myocytes results in caspase-dependent apoptotic cell death, the extent of which is considered to influence not only the fate of infected cells, but also the severity of the disease. Esfandiarei and colleagues *(57)* have demonstrated that CVB3 infection of human lung epithelial (HeLa) cells results in a gradual increase in both Akt and GSK-3β phosphorylation, and akin to RV and RSV, inhibition of PI3K with LY294002 increases CVB3-induced apoptosis. An increase in apoptosis was also detected when a dominant negative mutant of Akt1 was transfected into cells prior to CVB3 infection *(57)*. LY29002 and dominant negative Akt were also used to show that PI3K-Akt signaling was necessary for viral RNA synthesis and viral protein expression. Interestingly activation of Akt was not dependent on the caspase cascade, suggesting that PI3K-Akt signaling and caspase-dependent apoptosis work independently of each other. However, unlike studies with the viruses mentioned above, the time course over which the apoptotic and survival signals were activated were not analyzed. Therefore it is difficult to say whether or not PI3K-Akt signaling is activated for cell survival early in infection, although the dependence of such signals for RNA synthesis is certainly suggestive of this. The activation and involvement of PI3K-Akt signaling during CVB3-induced chronic cardiomyopathy has not been investigated.

It is tempting to speculate that infection of cells with viruses such as RV, RSV, SARS-CoV, and CVB3 all result in the initial activation of PI3K-Akt and other survival signals to support their replication, followed thereafter by induction apoptosis to facilitate virus spread. However, as the viral products that could potentially mediate such effects have not been identified, one cannot conclude that the effects are virus- rather than cell-mediated.

3.2. PI3K-Akt Mediated Survival in Lytic/Latent Viral Infections

PI3K-Akt mediated survival has also been found to be important during both the lytic and latent stages of lytic/latent virus infections. The lytic stage of a lytic/latent infection is similar to an acute infection in that virus replication and production of virus progeny results in death of infected cells, which can also correlate with the appearance of disease symptoms. However such viruses can avoid host immune system detection and clearance by establishing a latent infection. During viral latency, a limited set of viral

proteins is expressed and infectious viral progeny are not produced *(58)*. Viruses that are able to establish latent infections such as human papillomavirus (HPV), and herpesviruses such as human cytomegalovirus (HCMV), Epstein-Barr virus (EBV), and Kaposi's sarcoma-associated herpesvirus (KSHV) maintain their genomes as extrachromosomal episomes during latency *(59)*. Lytic replication can be re-initiated at any time and this is often accompanied by the reappearance of disease symptoms.

Activation of PI3K-Akt signaling is believed to contribute to the maintenance of the latent state by suppressing apoptosis, and hence the elimination of virus-infected cells. Long periods of latency, or reactivation from latent to lytic state, can also lead to transformation of infected cells. Signaling downstream of PI3K and Akt has been shown to contribute to both reactivation from latency, and virus-mediated transformation. Viral proteins expressed during the lytic or latent stages of infection have been shown to activate PI3K either through direct interaction with the catalytic or adaptor subunits, or by facilitating the association of PI3K with receptor or non-receptor tyrosine kinases.

Although a leading cause of congenital defects worldwide *(60)*, HCMV infection in healthy individuals in usually asymptomatic. However like other herpesviruses, HCMV is capable of establishing life-long latent infections *(61)*. Establishment of HCMV latent infection in vitro has been demonstrated to result in cellular transformation. However, the molecular mechanisms and viral proteins involved in the establishment of HCMV latency and transformation have not been well characterized. Studies looking at the activation of signaling pathways such as PI3K-Akt during HCMV infection have focused on virus entry and replication during the initial stages of primary lytic infections in vitro.

Entry of HCMV into host cells is facilitated by binding of its envelope glycoproteins gB (UL55) and gH (UL75) to receptors on the surface of the host cell. This binding has been demonstrated to result in the activation of several downstream intracellular signaling molecules which are important for viral DNA replication *(62–64)*. However, exactly how these molecules are activated downstream of the host cell receptors is not well understood *(64–66)*. Recently it was demonstrated that following HCMV infection of human embryonic lung fibroblasts (HELs), PI3K was strongly activated via phosphorylation of its p85 adaptor subunit. This resulted in the subsequent activation of Akt, p70 S6 kinase, and NF-κB *(67)*. p70 S6 kinase is another downstream target of Akt, which is associated with cellular proliferation rather than survival, as it phosphorylates ribosomal protein S6 to elevate protein production *(68,69)*. Activation of PI3K-Akt signaling did not produce a cell survival response in HCMV-infected cells, as inhibition of PI3K with LY294002 did not induce apoptosis. This is in contrast to RSV, where inhibition PI3K, Akt, and NF-κB results in increased apoptosis during the initial stages of infection. Inhibition of PI3K did, however, block DNA replication, and inhibited expression of viral proteins IE1-72, IE2-86, UL44, and UL84 *(67)*. Up-regulation of transcription factor NF-κB and p70 S6 kinase downstream of PI3K and Akt may result in cellular proliferation rather than survival, and such signaling appears to be important for transcription and translation of immediate early genes and completion of the lytic cyle *(67)*. The HCMV proteins involved in the up-regulation of PI3K-Akt signaling remain to be determined, and the involvement of PI3K-Akt signaling during HCMV latency and cell mediated transformation in vitro has not yet been investigated.

EBV (human herpesvirus 4) is the causative agent of infectious mononucleosis *(59)* and is implicated in the development of a variety of B-cell and epithelial-cell malignancies including Burkitt's lymphoma, Hodgkin's disease, and nasopharyngeal carcinoma *(70,71)*. Like HCMV, EBV can establish latent infections leading to the transformation of cells in vitro. EBV infection of primary human B-cells results in their transformation to lymphoblastoid cell lines (LCLs). During latent infection, EBV constitutively expresses a restricted set of proteins, which are also detected both in vitro in EBV-transformed B-cells and in a number of the EBV-associated malignancies *(72)*. Two of these latently encoded proteins, the integral membrane proteins LMP1 and LMP2A, interfere with PI3K-Akt signaling and upregulate PI3K-Akt mediated cell survival.

LMP1 behaves like a constitutively active tumor necrosis factor receptor (TNFR), and facilitates the recruitment of TNFR-associated death domain proteins (TRADD and RIP), and TNFR-associated factors (TRAFs) to the plasma membrane *(73–75)*. In this way LMP1 is able to regulate a number of mitogenic signaling cascades and has been shown to be essential for in vitro B-cell transformation *(76–78)*. The C-terminal cytoplasmic domain of LMP1 has been shown to bind to the p85 adaptor subunit of PI3K, leading to the activation of Akt (*see* Fig. 4). LMP1 activation of the PI3K-Akt pathway is thought to significantly contribute to cell survival and the morphological changes observed in B-cell transformation, as inhibition of PI3K with LY2940092 reverses the transformed phenotype *(79)*.

LMP2A, like LMP1, has also been shown to activate PI3K and Akt in B-cells, although a direct interaction has not been demonstrated *(80)*. However, it is possible that direct binding occurs via the C-terminal cytoplasmic tail of LMP2A, which is phosphorylated, providing binding sites for the SH2 domains of Src protein tyrosine kinases (PTKs) like Syk, as well as Lyn, both important mediators of B-cell signal transduction. Phosphorylation and subsequent activation of such tyrosine kinases in B-cells, is required for activation of PI3K by LMP2A. This is presumably because although LMP2A can recruit molecules via is phosphorylated cytoplasmic tail, it probably lacks the tyrosine kinase activity of autocatalytic RTKs (*see* Fig. 4) *(80)*. LMP2A activation of PI3K-Akt signaling does not appear to contribute to B-cell survival in vitro *(80)*. However, in primary B-cells from LMP2A transgenic mice, Ras is constitutively activated, as is PI3K and Akt *(81)*. These cells also show constitutive and up-regulation of antiapoptotic Bcl-2 family protein Bcl-xL, but no activation of mitogen activated kinases Raf, MEK1/2 and ERK1/2, which are traditionally associated with Ras *(81)*. Thus the Ras isoform involved is likely to be HA-Ras, which is associated with activation of PI3K rather than Raf. In addition, inhibitors of Ras, PI3K, and Akt but not Raf resulted in an increase in apoptosis in these cells. These findings suggest that during B-cell development LMP2A mimics a B-cell receptor (BCR) through constitutive activation of Ras, PI3K, Akt, and Bcl-xL proteins. This allows excess BCR negative B-cells, which would normally be eliminated by apoptosis, to survive in peripheral lymphoid organs and this may be involved in the development of EBV-associated B-cell lymphomas such as Hodgkin's disease *(81)*.

Expression of LMP2A in the human epithelial keratinocytes, also activates PI3K-Akt cell survival signals, leading to cellular transformation *(82)*. In Ramos Burkitt's lymphoma and HSC-39 epithelial gastric carcinoma cell lines, LMP2A expression protects against transforming growth factor (TGF)-β induced caspase-dependent

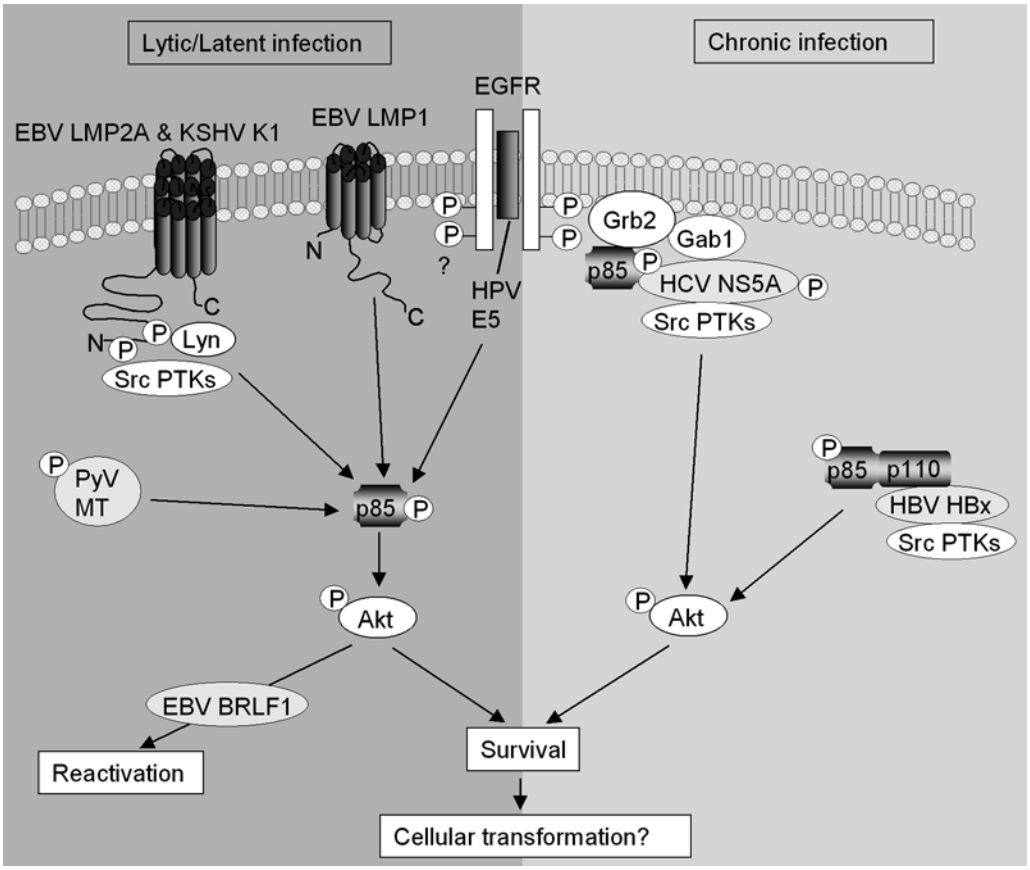


Fig. 4. Viral proteins that mediate PI3K-Akt survival during lytic/latent infections, chronic infections and cellular transformation. Viruses that cause lytic/latent infections express proteins that activate PI3K-Akt mediated survival to maintain the latent state such as EBV LMP1 and LMP2A, and KSHV K1. This is believed to contribute to cellular transformation following prolonged periods of latency, or as in the case of HPV E5, upon reactivation from latency. PI3K-Akt has also been shown to be required for the reactivation to the lytic state mediated by EBV protein BRLF1. PI3K-Akt signaling also facilitates PyV-mediated transformation in non-permissive cells. Viruses that cause chronic infections also express proteins that activate PI3K-Akt survival signals such as HCV NS5A and HBV HBx protein. However, whether constitutive up-regulation of PI3K and Akt contributes to cellular transformation after long periods of chronic infection is unknown. Activation of PI3K by viral proteins requires viral-protein mediated translocation to plasma membrane and binding to activated RTKs and/or the recruitment of cytoplasmic Src PTKs.

apoptosis via activation of PI3K and Akt *(83)*. Inhibition of PI3K with LY294002 was shown to inhibit Akt phosphorylation and block the antiapoptotic effect of LMP2A. These findings demonstrate that LMP2A can protect both B-cells and epithelial cells from apoptosis, perhaps providing a clonal selective advantage to such cells resulting in their immortilization. The role of LMP1 and LMP2A mediated apoptotic protection via PI3K and Akt has yet to be demonstrated for epithelial cells in vivo, and the involvement of PI3K-Akt signaling in EBV-epithelial cell malignancies is less well understood.

The latent form of EBV is periodically converted to the lytic form by expression of two proteins which work in conjunction to activate transcription, BRLF1 and BZLF1 *(84,85)*. PI3K-Akt signaling has been shown to be necessary for EBV reactivation from latency as PI3K and Akt are activated by BRLF1, and inhibition of PI3K abrogates BRLF1 transcriptional activity and ability to disrupt viral latency *(86)*. BZLF1, however, does not activate PI3K, but PI3K-Akt signaling may be required for the synergistic action of BRLF1 and BZLF1.

KSHV or human herpesvirus 8 has been identified as the etiologic agent of Kaposi's sarcoma (KS), of which there are various types including transplant-KS, endemic-KS, classical-KS, and acquired immunodeficiency syndrome (AIDS)-associated KS *(87–89)*. All forms of KS are histologically identical, and are angiogenic multicellular tumours *(88)*. However AIDS-associated KS is the most aggressive, and is the most common tumor to arise in human immunodeficiency virus (HIV)-infected individuals *(90)*. KSHV encodes an array of "pirated" regulatory proteins, which control cell growth, and are thought to contribute to KSHV latency and development of KS, although the molecular mechanisms involved are not well understood *(91–93)*. One such protein is K1 a transforming BCR-like transmembrane protein, which is similar to EBV LMP2A, and can also recruit tyrosine kinases like Syk to the plasma membrane via its cytoplasmic domain *(94,95)*. The cytoplasmic domain of K1 can also induce phosphorylation of a number of signaling molecules, perhaps via Src PTKs, including the p85 subunit of PI3K (*see* Fig. 4) *(94)*. This phosphorylation has been shown to correspond to the up-regulation of PI3K activity in B-cells over-expressing K1 *(96)*. Increased PI3K activity leads to the phosphorylation and activation of Akt, and inhibition of PTEN phosphatase and forkhead transcription factors (*see* Fig. 4) *(96)*. In addition K1 expression can protect cells from both FKHR- and Fas-mediated apoptosis *(96)*. This suggests that K1 may protect KHSV-infected cells early in the virus life cycle and contribute to the survival of tumorigenic cells during the development of KS.

HPV can also result in immortilization of infected cells, however, unlike the herpesviruses, HPV-mediated transformation occurs upon reactivation to the lytic state rather than after long periods of latency. HPV causes benign epithelial warts and is associated with the development of cervical and urogenital cancers *(97)*. The high-risk HPV type 16 (HPV 16), which is regularly detected in cervical cancers, encodes a putative transmembrane membrane protein E5, that can activate the PI3K-Akt pathway (*see* Fig. 4) *(98,99)*. HPV 16 E5 has been shown to interact with the epidermal growth factor receptor (EGFR) in human epithelial keratinocytes, stimulating activation through facilitating dimerization and autophosphorylation *(100,101)*. EGFR activation by HPV16 E5 up-regulates PI3K-Akt survival signaling, which can protect cells against ultraviolet (UV) induced apoptosis *(99)*. Whether PI3K and Akt activation also contributes to HPV reactivation from latency is unknown. However, E5 is necessary for full activation of the HPV transforming protein E7, therefore induction of PI3K-Akt dependent apoptotic inhibition by E5 may contribute to E7-mediated oncogenesis *(99,102–104)*. E5-mediated activation of PI3K, like EBV LMP1 and LMP2A, probably occurs at the plasma membrane through association of the phosphorylated cytoplasmic domain of the EGFR with p85 adaptor subunit of PI3K (*see* Fig. 4).

The *Polyomaviridae* differ from the herpesviruses and HPV, in that they only persist and stimulate cellular proliferation and transformation in non-permissive host cells that

do not support their replication *(105)*. During the early stages of polyomavirus infection, the "tumor," or T-antigens, are produced which are able to stimulate resting cells to re-enter the cell cycle, and have transforming capability. Primate polyomaviruses encode two T-antigens, large T (LT) and small T (ST), whose transforming capability result, in part, from inhibition of apoptosis by blocking the activity of the p53 tumor suppressor. The LT antigen from mouse polyomavirus (PyV) does not have a binding site for p53. However PyV does encode a novel middle T (MT) antigen, a cytosolic phosphoprotein that interacts with a number of SH2 containing proteins, including PI3K, phospholipase C γ (PLCγ) and Shc *(106–108)*. The SH2 domain of the PI3K p85 subunit associates with the phosphorylated Tyr-315 of MT, which leads to its activation subsequent activation of Akt *(108–110)*. Recent studies suggest that MT may utilize the PI3K-Akt pathway to block apoptosis during viral transformation, independently of p53 *(109)*.

3.3. PI3K-Akt Mediated Survival During Chronic Viral Infections

Another strategy by which viruses can persist in the infected host is through establishment of a chronic infection. In contrast to viruses which persist in a latent state, viruses that cause chronic infections continuously replicate and produce infectious progeny *(58)*. Chronic infections result from failure of the host immune system to clear the initial infection, and thus disease symptoms are ongoing. In some circumstances malignant transformation can result from chronic infection of a specific cell type, although unlike latent infections, expression of particular viral proteins are usually not involved. Instead chronic infection is believed to lead to a series of biochemical events that are thought to bring about a cellular environment favorable for tumor development. PI3K-Akt signaling has been proposed to be involved in the survival of the host cell during chronic infection and contribute to cellular transformation.

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infect hepatocytes and cause acute liver disease. A small percentage of HBV and a large percentage of HCV infections become chronic, and after many years can lead to hepatocellular carcinoma (HCC). During chronic HBV infection and HBV-associated HCC, the viral DNA becomes integrated at random into the host cell genome, which causes gene duplications, deletions, and chromosomal translocations. The core and polymerase regions of the genome are often destroyed but interestingly the gene encoding the hepatitis B X protein (HBx) remains intact. The HBx protein transcriptionally transactivates a variety of viral and cellular promoter and enhancer elements *(111)*. HBx also indirectly activates transcription factors through up-regulation of several mitogenic signaling pathways, including the Ras-Raf-MEK-ERK and JNK pathways *(112,113)*. In hepatoma cells, transcriptionally active HBx has been shown to associate with the catalytic subunit of PI3K (*see* Fig. 4), leading to increased phosphorylation of the p85 adaptor subunit and activation of PI3K *(114)*. This is in contrast to the other viral proteins discussed herein, which interact with the p85 adaptor subunit, suggesting that HBx may be novel in its mechanism of PI3K activation.

HBx-induced PI3K activation was further demonstrated to block TGF-β-induced apoptosis through downstream activation of Akt, phosphorylation Bad, and subsequent inhibition of caspase-3 *(114,115)*. This inhibition of TGF-β-induced apoptosis via PI3K and Akt, is similar to that mediated by EBV LMP2A, and also requires Src PTKs.

Src PTK activity is elevated following HBx expression and Src kinase inhibitors block PI3K protection against TGF-β-induced apoptosis *(116)*. This suggests that like EBV LMP2A and KSHV K1, HBx probably facilitates the activation of PI3K by bringing it into close proximity with the Src PTKs, although this event would not require recruitment to the plasma membrane.

HBx-induced apoptotic inhibition via PI3K-Akt signaling may provide HBV-infected hepatocytes with a selective growth advantage. This could be important during the initial stages of HCC development, however the situation in vivo is likely to be more complex and further studies are required to unravel the complexities of tumor development following chronic HBV infection *(114,115)*.

The molecular effects of HCV infection in hepatocytes that contribute to chronic infection and HCC are less well defined. However, many studies have focused on the HCV nonstructural protein NS5A following the discovery that mutations in this protein correlate to resistance to interferon treatment *(117)*. NS5A is an HCV nonstructural protein thought to play a role in virus replication, although its exact function is unknown. Like polyomavirus MT, NS5A is a cytosolic phosphoprotein that can interact with and regulate a number of signaling molecules *(118–121)*. The C-terminus of NS5A contains a highly conserved polyproline motif which can interact with the Src homology 3 (SH3) domains of the adaptor protein Grb2 and the PI3K p85 subunit and form a complex with the EGFR substrate Grb2-associated binder 1 (Gab1) (*see* Fig. 4) *(122–124)*. In human lung fibroblasts and hepatoma cells stably expressing NS5A, or harboring subgenomic HCV replicons the NS5A-PI3K p85 complex has been found to increase p85 phosphorylation, PI3K kinase activity and downstream phosphorylation of Akt and Bad *(122,124)*. This results in an increased protection against apoptotic stimuli *(124)*. Thus in a mechanism similar to that of HPV E5, the complex that NS5A forms with the PI3K p85 subunit as well as Grb2 and Gab1 may facilitate binding of p85 to the phosphorylated cytoplasmic tail of EGFR and subsequent activation of PI3K. These findings suggest that NS5A is important for survival during HCV infection. However whether NS5A up-regulates survival signals during chronic liver disease in vivo and the development of HCC requires further investigation.

3.4. HIV: A Law Unto Itself

In terms of virus infection, human immunodeficiency virus type 1 (HIV-1) is characteristically unique. HIV-1 is able to cause acute cytopathic infection but at the same time can evade the host immune system by establishing a latent infection in target $CD4^+$ cells through integration of proviral DNA into the host genome. However unlike retroviruses such as human T-cell leukemia virus (HTLV), and viruses with lytic/latent infectious cycles, persistence of HIV is not known to directly lead to malignant transformation. Therefore, studies on PI3K-Akt signaling during HIV-1 infection have focused on viral replication and acute pathogenicity, and HIV involvement in the development of KS. Several HIV-1 proteins have been shown to interact with PI3K, either directly or indirectly in association with other proteins.

HIV-1 entry is facilitated by binding of the HIV-1 glycoprotein gp120 to the CD4 surface molecule on T-cells and macrophages, and also requires the presence of chemokine coreceptors *(125–127)*. The interaction of gp120 with CD4 in primary $CD4^+$ T-cells and macrophages has been shown to result in rapid phosphorylation of the p85 adaptor subunit.

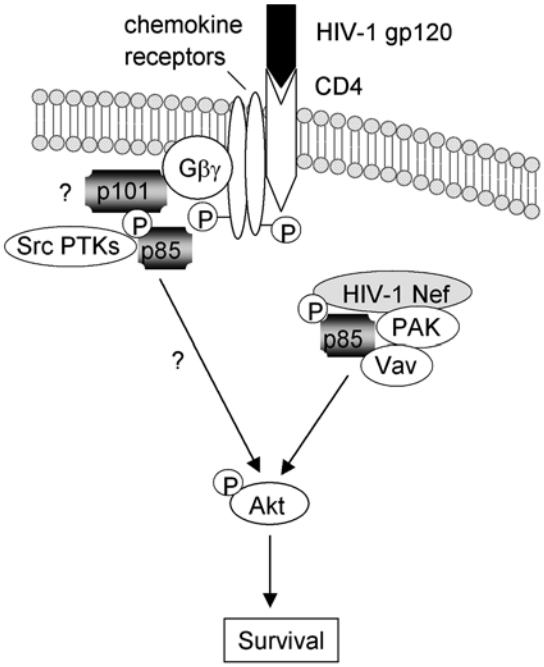


Fig. 5. HIV proteins regulating PI3K-Akt signaling. Binding of HIV-1 gp120 to CD4+ T-cells and macrophages, and subsequent virus entry causes the recruitment and activation of PI3K, which is required for viral replication and reverse transcription. At a later stage of HIV-1 infection the proline-rich protein Nef recruits PI3K, PAK and Vav into a signaling complex, which causes the activation of PI3K and Akt, and inhibition of caspase-dependent apoptosis. This probably protects cells from premature apoptosis and allows HIV-1 to complete its life cycle.

Full PI3K activation, however, requires the chemokine receptor, and like EBV LPM2A and HBV HBx, also requires Src PTKs (*see* Fig. 5) *(128,129)*. The chemokine receptors are G-protein linked serpentine receptors, and the activation of PI3K by gp120 binding is impaired by pertussis toxin, which is a G-protein inhibitor. This suggests that gp120 binding to CD4 and its coreceptors stimulates the activation of class I_B rather than class I_A PI3Ks. However, it was the class I_A PI3K p85 adaptor subunit that was shown to be phosphorylated, an event that possibly be mediated by Src PTKs, which may also be recruited to the plasma membrane. This suggests that perhaps both class I_A and I_B PI3Ks are activated downstream of HIV-1 binding and entry; however, whether this leads to activation of Akt and downstream survival events, remains unknown. Like PI3K signals triggered downstream of HCMV binding and entry, activation of PI3K is important during the HIV-1 life cycle. Inhibition of PI3K with LY294002 was shown to affect viral replication and reverse transcription, but was not required for viral DNA integration or gene expression *(129)*.

The HIV-1 Nef protein is proline-rich and enhances virus infectivity through its interaction with the SH3 domains of a variety of signaling proteins including Src PTKs, T-cell receptors, G-proteins and p21-activated kinase (PAK) *(130–133)*. Nef is able to directly bind to the C-terminal end of the PI3K p85 subunit and recruit PAK and guanosine 5′ triphosphate (GTP) exchange factor Vav into a signaling complex (*see* Fig. 5)

(134). Inhibition of PI3K with LY294002 in HIV-1 infected Jurkat and Cos-1 cells prevented activation of PAK and decreased the production of viral progeny *(134)*. Nef expression at the plasma membrane in NIH3T3 cells blocks apoptosis, which requires both PAK and PI3K *(135)*. Nef appears to play an important role in apoptotic inhibition and stimulation of cell survival, via molecules such as PI3K and PAK, during acute HIV infection. These data suggest that the regulation of PI3K signaling by different HIV-1 proteins is important during various stages of the virus life cycle. Activation of PI3K during acute HIV-1 infection, in common with other viruses that cause acute infection, is likely to help premature host cell death prior to production of new virus progeny *(1,135,136)*. However, further studies are required to show whether whether Akt-mediated survival is up-regulated downstream of PI3K during HIV-1 infection.

HIV-1 infection leads to a progressive decline in the $CD4^+$ T-cells and macrophages it infects. This results in immunodeficiency and permits infection and development of disease by other opportunistic agents. HIV-1 proteins have been shown to modulate the host cell environment and contribute to the disease symptoms caused by other infectious agents. The HIV-1 Tat protein, for example, is thought to contribute to the aggressiveness of AIDS-associated KS *(88,89,137)*. Tat is able to stimulate a variety of signaling mechanisms in KS cells, including activation of PI3K *(137–139)*. Tat inhibits apoptosis and increases cell viability via phosphorylation of Akt and Bad downstream of PI3K, which is down-regulated by chemotherapeutic agent vincristine, used to treat KS *(140,141)*. Inhibition of PI3K was shown to block Tat-induced Akt activation, Bad phosphorylation, and downstream apoptotic inhibition *(141)*. Therefore, Tat-induced PI3K-Akt survival during KSHV transformed cells, may contribute to tumor cell survival and the aggressive nature of AIDS-associated KS.

4. Conclusion

In the past 20 yr extensive research in the field of cell biology has lead to the discovery and characterization of many molecules and signaling cascades, which regulate cell proliferation, apoptosis, and survival. The PI3K-Akt signaling pathway has received considerable attention, because its importance in cell survival and apoptotic inhibition was realized. As a result, a vast amount of research is emerging into the involvement of this pathway in virus infection. Many of the major breakthroughs in cell biology, leading to the characterization of various signaling molecules and their involvement in disease states, have been made through the identification of unregulated viral counterparts. Thus an understanding of cell signaling in the context of virus infection not only contributes to our understanding of the effects of various signaling proteins, but also to our understanding of virus-host dynamics and virus disease states.

PI3K-Akt signaling appears to be important during the early stages of acute infections, with viruses such as CVB3, RV, RSV, and SARS-CoV. Inhibition of PI3K early in the virus life cycles induces premature apoptotic cell death and has a negative affect on virus replication and production. The induction of survival signals may only be required for virus replication and protein production as virus particle budding and release is often facilitated by apoptosis. However, the host cell itself may initiate induction the of PI3K-Akt survival to allow antiviral mechanisms to get under way.

HIV-1 can also cause acute infection, but at the same time is able to persist for long periods in the host. Activation of PI3K during acute HIV-1 infection in vitro has been

shown to be important for many stages in the virus life cycle. However, unlike the other viruses that cause acute infections it is not know whether activation of PI3K leads to Akt-mediated survival signaling and the effect of such signaling on virus persistence in the host.

A number of viruses including EBV, KSHV, and HPV, have the ability to establish long-term latent infections in the host. After long periods of latency or upon reactivation from latency, such infections can ultimately lead to virus-mediated cellular transformation. It appears that the gene products of latently infecting viruses can constitutively up-regulate PI3K-Akt cell survival signals and therefore continuously block apoptotic signals. This contributes to both virus survival in the latent state and allows proliferative signals to go unchecked resulting in oncogenic transformation. However, activation of this pathway is not only required for viral transformation but also for other stages of the virus life cycle. EBV BZLF1-mediated reactivation from latency, for example, requires the activation of PI3K and Akt. Productive polyomavirus infection requires the up-regulation of PI3K-Akt cell survival and cellular proliferation.

Long-term infections can also be established by chronically infecting viruses such as HBV and HCV, which, after prolonged periods, can also lead to cellular transformation. However, both HBV and HCV viral products have been shown to induce PI3K-Akt survival signals, which blocks apoptosis. Whether this situation occurs in vivo in chronic infection and the cellular transformation that ensues has not been studied, and this is partly due to the lack of efficient cell culture systems for such viruses.

Another limitation to studies examining the effect of virus infection on host cell signaling is the use of continuous tumorigenic cell lines with altered biological properties. The cross-regulation between multiple signaling pathways, which may differ in cell systems in vivo and in vitro, also makes it difficult to obtain results which are conclusive. However, the use of transgenic animals, as in the case of EBV LMP2A and the ongoing development of new techniques such RNA interference (RNAi), will allow for better understanding of the modulation of cell signaling cascades. In future, this may help to identify new cellular and viral proteins, and lead to a more in depth understanding of cellular transformation and other viral and cellular diseases.

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