The pivotal role of phosphatidylinositol 3-kinase–Akt signal transduction in virus survival

Samantha Cooray

1Enteric, Neurological and Respiratory Virus Laboratory, Health Protection Agency, 61 Colindale Avenue, London NW9 5HT, UK
2Department of Infection, Virology Section, Guy’s, King’s and St Thomas’ School of Medicine, St Thomas’ Hospital, Lambeth Palace Road, London SE1 7EH, UK

Over the course of evolution, viruses have developed the ability to modulate a variety of host cell signalling pathways. Inhibition of apoptosis, in particular, has become recognized as an important contributory factor in virus survival. Apoptotic inhibition contributes to the establishment of latent and chronic infections and has been implicated in viral oncogenesis. The phosphatidylinositol 3-kinase (PI3K)–Akt pathway is utilized by many cell types for inhibition of apoptosis and cellular survival. Virus modulation of this pathway provides an alternative to the expression of viral oncogenes or the direct inhibition of pro-apoptotic proteins. It has become evident that many viruses require up-regulation of this pathway to sustain long-term infections and it is modulated, in some cases, by specific viral products to create an environment favourable for cellular transformation. In other cases, PI3K–Akt signalling simply helps to create an environment favourable for virus replication and virion assembly. This review details the modulation and function of PI3K–Akt signalling for virus survival.

INTRODUCTION

Successful virus survival relies upon the evolution of strategies that modulate host cell signalling pathways, in particular those governing apoptosis and cell survival. Apoptotic inhibition is a well-studied mechanism of virus survival and often plays a role in maintenance of short-term cell viability during acute infection. Sustained apoptotic inhibition is utilized during latency and the development of chronic infections. During latent infection, this inhibition may be actively mediated by specific viral proteins, which, along with modulation of the cell cycle, can create an environment favourable for cellular transformation. During chronic infection, it is suggested that both virus-dependent and independent mechanisms contribute to multiple biochemical changes in the host cell, including apoptotic inhibition, which can ultimately lead to transformation. However, the molecular mechanisms governing oncogenic transformation in vivo due to both latent and chronic virus infection are not well defined and are likely to differ from one virus to another.

Virus inhibition of apoptosis can be achieved through inhibition of pro-apoptotic proteins such as caspases and p53 or expression of viral counterparts to anti-apoptotic proteins such as Bcl-2 (Roulston et al., 1999). However, numerous other signalling pathways exist within the eukaryotic cell, which regulate the balance between apoptosis and cell survival. The enzyme phosphatidylinositol 3-kinase (PI3K) is central to many cell signal transduction pathways and acts on several downstream effectors to regulate a diverse range of cellular events (Cantrell, 2001). The serine–threonine kinase, Akt, is one such effector, and the PI3K–Akt pathway has proven to be vitally important in cell survival (Chan et al., 1999). Constitutive up-regulation of PI3K–Akt cell survival signalling has also been implicated in oncogenic transformation and tumour development, as it prevents apoptotic cell death during uncontrolled proliferation (Chang et al., 2003).

Virus modulation of PI3K–Akt signalling is emerging as an important mechanism of apoptotic inhibition during acute infection, long-term virus survival and virus transformation. This review outlines the important components of the PI3K–Akt pathway and discusses viral modulation of this pathway as a means of survival during acute, chronic and latent infections.

An overview of the signal transduction molecules PI3K and Akt

Phosphatidylinositol 3-kinases. The phosphatidylinositol 3-kinases (PI3Ks) are a family of enzymes (Classes I, II and III) that produce lipid second messengers by phosphorylation of plasma membrane phosphoinositides at the 3’OH of the inositol ring (for a detailed review, see Vanhaesebroeck et al., 2001). Class I PI3Ks are heterodimeric proteins consisting of a catalytic subunit (110 kDa, p110) and an adaptor/regulatory subunit and have been subgrouped into Class IA and Class IB PI3Ks. In Class IA PI3Ks, the catalytic subunit has three isoforms (α, β and...


δ), each encoded by a separate gene, and seven adaptor proteins generated by expression and alternative splicing of three different genes (p85α, p85β and p55γ). Class Iα PI3Ks consist of a p110γ catalytic subunit, associated with a 101 kDa (p101) adaptor subunit (Vanhaesebroeck & Alessi, 2000; Cantrell, 2001; Vanhaesebroeck et al, 2001). Due to the limited space available, the other classes of PI3Ks (reviewed by Vanhaesebroeck & Waterfield, 1999) will not be discussed here.

Class Iα PI3Ks can be activated through binding of the Src homology (SH2) domain in the adaptor subunit to auto-phosphorylated tyrosine kinase receptors (Fig. 1) or alternatively to non-receptor tyrosine kinases in the cytoplasm, such as the Src family kinases or JAK kinases (not shown). Class Iα kinases are activated by binding of the catalytic subunit to heterotrimeric guanosine 5’-triphosphate-binding proteins or ‘G proteins’ (not shown). Class Iα PI3Ks can also be activated by G proteins. Activated PI3Ks preferentially phosphorylate phosphotyrosine-9,4,5-diphosphate (PtdIns(4,5)P2) in vivo, to produce phosphotyrosine-9,4,5-triphosphate (PtdIns(4,5,5)P3) (Cantrell, 2001). In turn, the production of PtdIns(3,4,5)P3 is regulated by the phosphatase PTEN, which catalyses the dephosphorylation of PtdIns(3,4,5)P3 to PtdIns(4,5)P2 (Maehama & Dixon, 1999; Leslie et al, 2002) (Fig. 1). A wide variety of signal transduction proteins, including Akt, interact with PI3K-generated phosphorylated phosphoinositides via lipid-binding pleckstrin homology (PH) domains (Bottomley et al, 1998). This binding facilitates the recruitment of these proteins to the plasma membrane and their subsequent activation.

Akt/protein kinase B. 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 (Staal et al, 1977; Bellacosa et al, 1991; Jones et al, 1991). At the same time, it was identified as a novel kinase similar in many respects to protein kinase A (PKA) and protein kinase C (PKC) and hence is also referred to as protein kinase B (PKB) (Coffer & Woodgett, 1991). In mammals, there are three isoforms of Akt (Akt1, -2 and -3, or PKBα, -β and -γ), which 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 Thr308 of the catalytic domain by phosphoinositide-dependent kinase 1 (PDK-1) and at Ser473 of the C-terminal hydrophobic region (Akt1/PKBα) by an as yet uncharacterized kinase, termed phosphoinositide-dependent kinase 2 (PDK-2) (Scheid & Woodgett, 2003).

Following its activation, Akt phosphorylates a host of cell signalling proteins at Ser/Thr residues, which transduces signals downstream of PI3K regulating cell growth, cell cycle entry and cell survival (Fig. 1). A number of pro-apoptotic proteins have been identified as targets for Akt phosphorylation, including the Bcl2 family member BAD, caspase-9 and glycogen synthase kinase-3 beta (GSK-3β) (Fig. 1) These proteins are usually inactivated by the phosphorylation event and in this way Akt can delay apoptosis in response to pro-apoptotic stimuli (Kulik et al, 1997; Chen et al, 1998; Eves et al, 1998). 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 (Bcl-2 and Bcl-X) at the mitochondrial membrane (Datta et al, 1997; Vanhaesebroeck & Alessi, 2000). Akt phosphorylation and inactivation of human caspase-9 and several other caspases blocks activation of the proteolytic apoptotic caspase cascade (Cardone et al, 1998). GSK-3β has been shown to induce apoptosis and this is blocked by Akt phosphorylation (Pap & Cooper, 1998). GSK-3β normally phosphorylates and inhibits glycogen synthase; therefore Akt inactivation of GSK-3β may also lead to an increase in glycogen synthesis (Cross et al, 1995).

Akt has also been found to regulate apoptosis through transcriptional control of pro- and anti-apoptotic genes. Akt can phosphorylate members of the forkhead (FH) transcription factor family such as FKHR. FKHR predominantly resides in the nucleus, where it regulates the transcription of a number of genes crucial to apoptosis and cellular proliferation (Burgering & Medema, 2003). Akt phosphorylation promotes the export of FKHR from the nucleus into the cytosol, where it is bound and inhibited by 14-3-3 proteins (Brunet et al, 1999; Rena et al, 1999). Akt can mediate survival through activation of NF-κB downstream of interferons and growth factors, leading to the transcription of inhibitors of apoptosis proteins (Khwaja, 1999; Ozes et al, 1999; Romashkova & Makarov, 1999; Hatano & Brenner, 2001; Yang et al, 2001).

**Viruses modulation of PI3K–Akt signalling**

**Latent infection and cellular transformation.** A number of viruses have developed strategies to survive for long periods in the host. One such strategy is the establishment of latency, during which few viral proteins are expressed and infectious viral progeny are not produced (Tyler & Nathanson, 2001). Some viruses, such as *Human papillomavirus* (HPV) and the gammaherpesviruses *Human herpesvirus 4* (Epstein–Barr virus; EBV) and *Human herpesvirus 8* (Kaposi’s sarcoma-associated herpesvirus; KSHV), maintain their genome as an extrachromosomal episome that is preserved during latent infection (Kieff & Rickinson, 2001). Latent viruses retain the ability to reinitiate lytic replication, which is often accompanied by the reappearance of disease symptoms. The precise molecular mechanisms that lead to reactivation from the latent state are poorly understood. Long periods of latency, with periodic cycles of reactivation can, in some cases, result in immortalization of the infected cell. Activation of PI3K–Akt signalling is believed to contribute to the maintenance of the latent state by suppressing apoptosis and hence the elimination of virus-infected cells. PI3K–Akt signalling has
also been shown to be required for disruption of latency and for virus-mediated transformation both during latency and following reactivation to lytic replication (Darr et al., 2001). Viral products can 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. The viral and cellular proteins involved include both transmembrane and cytosolic proteins.

EBV expresses several proteins that modulate PI3K–Akt
signalling at different stages of its life cycle. During latent infection, EBV constitutively expresses a restricted set of latently encoded viral proteins, which include the integral membrane proteins LMP1 and LMP2A (Rickinson & Kieff, 2001). These proteins are detected both in vitro in EBV-transformed B cells and in vivo in a number of EBV-associated B cell and epithelial cell malignancies (Rickinson & Kieff, 2001). LMP1 acts like a constitutively active tumour necrosis factor receptor (TNFR) and recruits TNFR-associated death domain proteins, TRADD and RIP, and TNFR-associated factors (TRAFs) to the plasma membrane (Mosialos et al., 1995; Gires et al., 1997; Izumi et al., 1999). LMP1 can thus regulate a number of mitogenic signalling pathways from the plasma membrane and has been shown to be essential for in vitro B cell transformation (Eliopoulos & Young, 1998; Roberts & Cooper, 1998; Gires et al., 1999).

The cytoplasmic C-terminal domain of LMP1 binds to the p85 adaptor subunit of PI3K, which stimulates its activation and the downstream phosphorylation of Akt (Fig. 2) (Dawson et al., 2003). LMP1 activation of the PI3K–Akt pathway is thought to contribute significantly to cell survival and the morphological changes observed in B cell transformation, as inhibition of PI3K reverses the transformed phenotype (Dawson et al., 2003). LMP2A has also been shown to activate PI3K and Akt in B cells, although a direct interaction has not been demonstrated (Swart et al., 2000). However, direct binding seems likely as the C-terminal cytoplasmic tail of LMP2A is phosphorylated and provides binding sites for the SH2 domain of Src protein tyrosine kinases (Src PTKs). Activation of Src PTK Lyn, and another non-receptor tyrosine kinase Syk, is required for activation of PI3K by LMP2A (Fig. 2) (Swart et al., 2000). LMP2A activation of PI3K–Akt signalling does not contribute to B cell survival in vitro and may alternatively regulate cell cycle progression (Longnecker et al., 1992; Speck et al., 1999; Swart et al., 2000). Expression of LMP2A in epithelial keratinocytes, in contrast, activates PI3K–Akt cell survival signals, which are likely to be required for anchorage-independent cell growth. These LMP2A-expressing cells also induce tumours when injected into nude mice (Scholle et al., 2000). Although EBV establishes latent infection in B cells, the oropharyngeal epithelium is the primary site of infection. This indicates that LMP2A activation of PI3K may play an important role in the establishment of EBV epithelial neoplasias such as nasopharyngeal carcinoma, but not in B cell lymphomas. However, further work is required to define the role of PI3K signalling downstream of both LMP1 and LMP2A in different cell types in vitro and in vivo.

The latent form of EBV is periodically converted to the lytic form by expression of transcriptional co-activators BRLF1 and BZLF1, which can also activate each other (Flemington & Speck, 1990; Zalani et al., 1996). PI3K–Akt signalling is required for reactivation from latency as BRLF1 activates PI3K and Akt, and inhibition of PI3K abrogates BRLF1 transcriptional activity and ability to disrupt virus latency (Darr et al., 2001). BZLF1, however, does not activate PI3K, but PI3K–Akt signalling may be required for the synergistic action of BRLF1 and BZLF1.

The involvement of PI3K–Akt signalling in cellular transformation following reactivation from latency is exemplified by HPV. HPV causes benign epithelial lesions (warts) and has been associated with cervical and urogenital cancers (zur Hausen & de Villiers, 1994). The high-risk HPV type 16 (HPV-16), which is regularly detected in cervical cancers, encodes a putative integral membrane protein, E5, that can activate PI3K–Akt signalling (Zhang et al., 2002; zur Hausen, 2002). In human epithelial keratinocytes, HPV-16 E5 is a transmembrane protein, which interacts with the epidermal growth factor receptor (EGFR) and stimulates its activation, through dimerization and autophosphorylation (Hwang et al., 1995; Crussius et al., 1998). This subsequently results in the up-regulation of PI3K–Akt survival signalling, which protects HPV-16 E5-expressing cells from apoptosis induced by ultraviolet irradiation (Zhang et al., 2002). The major transforming capability of HPV is dependent on E6 and E7 (Hawley-Nelson et al., 1989; Kaur et al., 1989; Munger et al., 1989). However, E5 is necessary for full activation of E7, and it has been suggested that the induction of PI3K–Akt-dependent apoptotic inhibition by E5 contributes to E7-mediated oncogenesis (Zhang et al., 2002). Like EBV LMP1 and LMP2A, E5-mediated activation of PI3K during HPV infection is likely to occur through a binding event at the plasma membrane, probably in association with the phosphorylated cytoplasmic domain of the EGFR (Fig. 2).

KSHV is unique among the other transforming viruses in that its genome encodes an array of ‘pirated’ regulatory proteins, which control cell growth and immunoregulation (Chang & Moore, 1996; Moore et al., 1996; Cheng et al., 1997; Bais et al., 1998). It is the up-regulation of cellular proliferation and inhibition of cell death by these KSHV products that is thought to contribute to the development of Kaposi’s sarcoma (KS). There are various types of KS (transplant KS, endemic KS, classical KS and AIDS-associated KS), which are histologically identical. AIDS-associated KS, however, is the most aggressive as, unlike the other forms, it disseminates throughout the body (Ensoli et al., 1990; Gallo, 1998; Antman & Chang, 2000). The regulation of PI3K–Akt signalling by KHSV proteins has so far not been studied. However, the aggressive nature of AIDS-associated KS has been attributed to the ability of Human immunodeficiency virus 1 (HIV-1) (see below) Tat protein to stimulate a variety of biological effects, including activation of PI3K (Ensoli et al., 1990, 1993; Albini et al., 1996). In KS cells, Tat inhibits apoptosis and increases cell viability via phosphorylation of Akt and BAD, which is down-regulated by the chemotherapeutic agent vincristine (Cantaluppi et al., 2001; Deregibus et al., 2002). Inhibition of PI3K abrogated Tat-induced Akt activity, BAD phosphorylation and the overall anti-apoptotic effect (Deregibus et al., 2002). Therefore, Tat-mediated apoptotic protection via PI3K–Akt signalling during KSHV oncogenic transformation is likely to
contribute to tumour cell survival and the particularly aggressive nature of AIDS-associated KS.

The Polyomaviridae differ from the herpesviruses and HPV in that they persist and stimulate cellular proliferation and transformation in non-permissive host cells (Gottlieb & Villarreal, 2001). During the early stages of infection, the ‘tumour’ or T antigens are produce, which stimulate cellular

**Fig. 2.** Virus activation of PI3K–Akt signalling and its role in virus infection. A schematic representation is shown of the association of different viral proteins with the adaptor or enzyme subunits of PI3K and the proposed requirement of PI3K signalling in different types of infection.
DNA replication to prepare the cell for replication of viral DNA. These T antigens also stimulate resting cells to reenter the cell cycle and have transforming capability. Primate polyomaviruses encode two T antigens, large T (LT) and small T (ST), whose transforming capability is partly due to inhibition of apoptosis by negative regulation of the tumour suppressor p53. Murine polyomavirus LT antigen lacks a binding site for p53 but encodes an additional middle T (MT) antigen. MT is a cytosolic phosphoprotein that interacts with a number of SH2-containing proteins, including PI3K, phospholipase C gamma (PLCγ) and Shc (Whitman et al., 1985; Campbell et al., 1994; Su et al., 1995). The SH2 domain of the PI3K p85 regulatory subunit associates with the phosphorylated Tyr315 of MT, which leads to its subsequent activation of Akt (Whitman et al., 1985; Dahl et al., 1998; Summers et al., 1998). Recent studies suggest that MT utilizes the PI3K–Akt pathway to block apoptosis during transformation, independently of p53 (Dahl et al., 1998).

**Chronic infection and cellular transformation.** Another strategy used by viruses for long-term survival is the establishment of a chronic infection, characterized by the continuous shed of infectious progeny and recurrent disease symptoms (Tyler & Nathanson, 2001). Chronic infections result from failure of the host immune system to clear the initial infection and in some circumstances chronic infection of a specific cell type may result in malignant transformation. However, unlike latent infections, expression of certain viral proteins is not usually actively involved in transformation following chronic infection. Rather chronic infection may lead to a series of biochemical events that are thought to contribute indirectly to tumour development. PI3K–Akt signalling has been proposed to be involved in the survival of the host cell during chronic infection and the ensuing cellular transformation.

**Hepatitis B virus** (HBV; *Hepadorviridae*) and **Hepatitis C virus** (HCV; *Flaviviridae*) both cause acute liver disease. Approximately 10% of HBV infections and 80% of HCV infections in humans become chronic and after many years can result in the establishment of hepatocellular carcinoma (HCC). During chronic HBV infection and HBV-associated HCC, the viral DNA is randomly integrated into the host, resulting in duplications, deletions and chromosomal translocations. In general, the core and polymerase regions of the genome are destroyed but the S region and the gene encoding the hepatitis B X protein (HBx) remain intact. The HBx protein has been shown to function as a transcriptional transactivator of a variety of viral and cellular promoter and enhancer elements (Murakami, 1999). HBx also activates several signal transduction cascades, including the Ras–Raf–MEK–ERK and JNK signalling pathways, leading to activation of transcription factors (Benn & Schneider, 1994; Benn et al., 1996). Transactivation-proficient HBx blocks transforming growth factor (TGF)-β-induced apoptosis via PI3K in hepatoma cells (Shih et al., 2000). HBx associates with the catalytic subunit of PI3K and elevates phosphorylation of the p85 adaptor subunit and activation of PI3K (Lee et al., 2001) (Fig. 2). HBx-induced PI3K apoptotic inhibition was found to be due to phosphorylation of both Akt and BAD, which led to inhibition of caspase-3 in a p53-independent manner (Lee et al., 2001). Like EBV LMP2A, HBx activation of PI3K appears to require Src PTKs, as Src activity is elevated following HBx expression and Src kinase inhibitors abolished PI3K protection against TGF-β-induced apoptosis (Shih et al., 2003). HBx may function by bringing PI3K into close proximity with the Src PTKs, which are non-receptor tyrosine kinases that could mediate its activation. HBx-induced apoptotic inhibition via PI3K–Akt signalling 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 far more complicated and further studies are required to unravel the complexities of tumour development following chronic HBV infection (Shih et al., 2000; Lee et al., 2001).

The molecular effects of HCV infection in hepatocytes that contribute to chronic infection are less well defined. However, many studies have focused on the HCV non-structural protein NS5A following the discovery that mutations in this protein correlate with resistance to interferon treatment (Enamoto et al., 1996). NS5A is 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 signalling molecules (Tan et al., 1999; Lan et al., 2002; Qadri et al., 2002; Georgopoulou et al., 2003). In vitro, NS5A can form a complex with Grb2-associated binder 1 (Gab1) and PI3K, resulting in phosphorylation of the PI3K p85 adaptor subunit (He et al., 2002) (Fig. 2). Gab1 is a substrate for EGFR; thus in a mechanism similar to that of HPV E5, the NS5A–Gab1–PI3K complex may allow PI3K to bind to the phosphorylated cytoplasmic tail of EGFR and become activated. NS5A-directed activation of PI3K is also accompanied by downstream phosphorylation of Akt and BAD. Thus, NS5A can contribute to cellular survival via PI3K and Akt. However, whether NS5A contributes in any way to the development of chronic liver disease and HCC through up-regulation of PI3K and Akt is unknown.

**Acute infection.** A number of viruses activate PI3K–Akt signalling not for long-term survival, but for short-term cellular survival during the initial stages of acute infection when virus replication and protein synthesis are taking place. This short-term activation of PI3K–Akt signalling mostly concerns small RNA viruses. Human herpesvirus 5 (human cytomegalovirus; HCMV) is considered here as part of this section as although it can establish latent infections and probably expresses proteins that can modulate pathways such as PI3K to transform mammalian cells *in vitro*, evidence to support this has not yet been demonstrated (Pass, 2001). Also, studies looking at the activation of PI3K–Akt signalling by HCMV have focused on the
initial infection in vitro following virus entry, rather than latent infection and cellular transformation.

HCMV entry is facilitated by binding of its envelope glycoproteins gB (UL55) and gH (UL75) to host cell receptors. This has been shown to activate a number of mitogenic host cell signalling pathways that are important in viral DNA replication (Yurochko et al., 1997a, b; Johnson et al., 2000). The cellular molecules responsible for activation of downstream signalling events from the plasma membrane remain largely uncharacterized (Bresnahan et al., 1997; Johnson et al., 2000, 2001a). Johnson and colleagues (2001b) investigated the role of PI3K as a potential mediator of downstream kinase signalling following HCMV infection in human embryonic lung fibroblasts. PI3K was strongly activated via phosphorylation of its p85 adaptor subunit, which resulted in the subsequent activation of Akt, p70 S6 kinase and NF-κB (Johnson et al., 2001b). p70 S6 kinase is a downstream target of Akt, which is not associated with cellular survival but rather proliferation, as it phosphorylates ribosomal protein S6 to elevate protein production, and NF-κB activates the transcription of a variety of genes. Thus, up-regulation in gene expression and cellular protein synthesis may be required for HCMV to complete its life cycle. Activation of PI3K–Akt signalling did not produce a cell survival response, as blockage of the pathway by LY294002 did not induce apoptosis in the presence of HCMV. However, DNA replication and expression of viral proteins IE1-72, IE2-86, UL44 and UL84 were inhibited, suggesting that PI3K–Akt signalling is important for transcription and translation of immediate-early genes (Johnson et al., 2001b).

HIV-1 causes both acute infection and persistent latent infection through integration of proviral DNA into the host cell genome. Although integration and latency have the potential to disrupt a number of cellular genes, unlike some other retroviruses HIV-1 latency is not known to cause malignant transformation. Therefore, studies on the role of PI3K–Akt signalling in HIV-1 infection have focused on virus replication and acute pathogenicity. HIV-1 is the only virus in this section whose viral proteins have been shown to interact with PI3K, either directly or indirectly in association with other proteins.

Binding of the HIV-1 glycoprotein gp120 to the CD4 molecule on the surface of T cells and macrophages facilitates virus entry and requires the presence of chemokine coreceptors (Dalgleish et al., 1984; Choe et al., 1996; Feng et al., 1996). Recent studies have shown that the gp120–CD4 interaction in vitro results in rapid activation of the p85 adaptor subunit of PI3K (Fig. 2). The chemokine receptors and Src PTK Lck are required for full PI3K activity (Briand et al., 1997; Francois & Klotman, 2003). 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 co-receptors stimulates the activation of class I b PI3Ks rather than class I a. However, it was the class I a p85 adaptor subunit that was shown to be phosphorylated, perhaps through direct association with Lck or the cytoplasmic tails of CD4 and the chemokine co-receptors. PI3K activity is required for several stages of productive infection including post-virus entry, virus replication and reverse transcription, but is dispensable for virus integration and gene expression (Francois & Klotman, 2003).

HIV-1 Nef is a proline-rich protein that enhances virus infectivity through its interaction with Src homology 3 (SH3) domains of a variety of signalling molecules including Src PTKs, T cell receptors, G proteins and p21-activated kinase (PAK) (Baur et al., 1997; Fackler et al., 1999; Simmons et al., 2001; Arora et al., 2002). It has recently been shown that Nef can directly bind to the C-terminal portion of the PI3K p85 subunit and recruit PAK and guanosine 5'-triphosphate (GTP) exchange factor Vav into a signalling complex (Linnemann et al., 2002) (Fig. 2). Inhibition of PI3K in HIV-1-infected Jurkat and Cos cells blocks activation of PAK and decreases production of viral progeny (Linnemann et al., 2002). Nef expression at the plasma membrane in NIH3T3 cells blocks apoptosis, which requires both PAK and PI3K (Wolf et al., 2001). These results suggest that Nef plays an important role in apoptotic inhibition and stimulation of cell survival, via molecules such as PI3K and PAK, during acute HIV infection. The purpose of this is probably to avoid premature host cell death and permit virus replication and production (Roulston et al., 1999; Geleziunas et al., 2001; Wolf et al., 2001).

Human respiratory syncytial virus (RSV) is one of the most important causes of serious respiratory tract disease in children worldwide. It infects airway epithelial cells, resulting in cell death and a severe inflammatory response (Collins et al., 2001). This has been shown to be mediated in vitro by NF-κB and up-regulation of various inflammatory cytokines and chemokines, as well as STATs (signal transducers and activators of transcription) (Bitko et al., 1997; Haeberle et al., 2002; Kong et al., 2003). RSV-induced NF-κB transcriptional activity in A549 airway epithelial cells is dependent on PI3K and Akt (Thomas et al., 2002). In contrast to HCMV, PI3K–Akt signalling and activation of NF-κB also functions to inhibit apoptosis during the initial stages of in vitro RSV infection. However, this inhibition is eventually overcome and the cell succumbs to apoptotic cell death. Inhibition of PI3K–Akt signalling rapidly increases the speed and magnitude of the apoptotic response (Thomas et al., 2002). This suggests that PI3K–Akt signalling increases cell survival to preserve the life of host cells until the virus life-cycle is complete. Following this apoptosis is initiated to facilitate virus spread (Roulston et al., 1999).

A similar pattern is also observed with Rubella virus (RV) infection in vitro. Inhibition of PI3K results in an increase in the speed and magnitude of RV-induced apoptosis. However, the induction of cell survival downstream of PI3K via activation of Akt and up-regulation of GSK-3β phosphorylation occurs concomitantly with activation of the apoptotic caspase cascade (S. Cooray, J. M. Best & L. Jin, unpublished...
observations). This suggests that there is a delicate balance between cell survival and apoptosis during RV infection, although it is unclear whether this is actively modulated by the virus or is simply a cellular response. Apoptosis is also likely to facilitate the spread of RV, as there is a lack of cell lysis.

Human enterovirus B (coxsackievirus B3; CVB3), the causative agent of acute myocarditis and chronic cardiomyopathy, also induces caspase-dependent apoptosis (Carthay et al., 1998; Joo et al., 2003). Yang et al. (1999) demonstrated that CVB3 infection in vitro resulted in up-regulation of a relatively obscure protein, interferon-γ-inducible guanosine triphosphatase (IGTPase). The overexpression of this protein blocked CVB3-induced apoptosis through PI3K–Akt inhibition of caspase activity and up-regulation of GSK-3β phosphorylation (Zhang et al., 2003). PI3K inhibition and transfection of a dominant-negative Akt construct reduced Akt phosphorylation to basal levels and restored caspase activity. Thus akin to acute RV and RSV infection in vitro, the extent of CVB3-induced apoptosis appears to be regulated by PI3K–Akt signalling. However, in contrast to RV, virus replication was reduced during IGTPase activation of PI3K–Akt signalling. This suggests that the up-regulation of PI3K and Akt may serve as a defence mechanism against virus infection.

Conclusions

In the last decade, there has been rapid progress in understanding the mechanisms that regulate host cell death, proliferation and survival. The PI3K–Akt signalling pathway, in particular, has attracted much interest due to its central role in the regulation of apoptotic inhibition. As a

<table>
<thead>
<tr>
<th>Virus</th>
<th>Protein*</th>
<th>Activation of PI3K–Akt-mediated apoptotic inhibition</th>
<th>Function of PI3K–Akt signalling</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latent infection and transformation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epstein–Barr virus</td>
<td>LMP1</td>
<td>Yes</td>
<td>B cell survival and transformation</td>
<td>Dawson et al. (2003)</td>
</tr>
<tr>
<td></td>
<td>LMP2A</td>
<td>Yes/No</td>
<td>Epithelial cell survival/cell cycle progression</td>
<td>Miller et al. (1994); Gold et al. (2000); Swart et al. (2000); Scholle et al. (2000)</td>
</tr>
<tr>
<td>Papillomavirus</td>
<td>BRLF1</td>
<td>No</td>
<td>Reactivation from latency</td>
<td>Darr et al. (2001)</td>
</tr>
<tr>
<td></td>
<td>E5</td>
<td>Yes</td>
<td>Epithelial cell survival and transformation</td>
<td>Zhang et al. (2002)</td>
</tr>
<tr>
<td>Kaposi’s sarcoma-associated virus</td>
<td>HIV-1 Tat</td>
<td>Yes</td>
<td>Up-regulation of survival in KS cells</td>
<td>Cantaluppi et al. (2001); Deregibus et al. (2002)</td>
</tr>
<tr>
<td>Polyomavirus</td>
<td>MT</td>
<td>Yes</td>
<td>Cellular survival during transformation</td>
<td>Whitman et al. (1985); Kaplan et al. (1986); Webster et al. (1998)</td>
</tr>
<tr>
<td>Chronic infection and transformation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis B virus</td>
<td>HBx</td>
<td>Yes</td>
<td>Inhibition of TGF-β-induced apoptosis in hepatoma cells</td>
<td>Shih et al. (2000, 2003); Lee et al. (2001)</td>
</tr>
<tr>
<td>Hepatitis C virus</td>
<td>NSSA</td>
<td>Yes</td>
<td>Epithelial cell survival</td>
<td>He et al. (2002)</td>
</tr>
<tr>
<td>Acute infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human immunodeficiency virus type 1</td>
<td>gp120</td>
<td>No</td>
<td>Virus replication and reverse transcription</td>
<td>Briand et al. (1997); Francois &amp; Klotman (2003)</td>
</tr>
<tr>
<td></td>
<td>Nef</td>
<td>Yes</td>
<td>Virus replication</td>
<td>Wolf et al. (2001); Linnemann et al. (2002)</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>–</td>
<td>No</td>
<td>Virus replication and protein expression</td>
<td>Zhu et al. (1995); Johnson et al. (2001b); Yu &amp; Alwine (2002)</td>
</tr>
<tr>
<td>Respiratory syncytial virus</td>
<td>–</td>
<td>Yes</td>
<td>Short-term host cell survival prior to virus-induced apoptosis</td>
<td>Thomas et al. (2002)</td>
</tr>
<tr>
<td>Rubella virus</td>
<td>–</td>
<td>Yes</td>
<td>Short-term host cell survival prior to virus-induced apoptosis</td>
<td>S. Cooray and others, unpublished</td>
</tr>
<tr>
<td>Coxsackie B virus</td>
<td>–</td>
<td>Yes</td>
<td>Short-term host cell survival prior to virus-induced apoptosis</td>
<td>Zhang et al. (2003)</td>
</tr>
</tbody>
</table>

*Viral proteins that activate PI3K through binding of its p85 adaptor or p110 catalytic subunit. Unless otherwise stated, these proteins are encoded by the genomes of the viruses listed in the first column.
result, there is now a growing body of research into virus modulation of this pathway, which further contributes to our understanding of the effects, at a molecular level, of virus infection on the host cell.

A number of viruses including EBV, HPV, HBV and HCV have the ability to establish long-term infections in the host, either through the establishment of latent or chronic infections, which can ultimately lead to cellular transformation. It appears that the gene products of these viruses stimulate PI3K–Akt-mediated cell survival and thereby block apoptosis of the cells they infect. This contributes to both virus survival and oncoenic transformation (Fig. 2, Table 1). 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.

In the case of non-transforming viruses, such as CVB3, RV, RSV and HIV-1, PI3K–Akt signalling appears to assist virus replication. Following the production of viral progeny, virus particle budding and release is then facilitated by virus induction of apoptosis (Table 1).

There are, of course, limitations to studies examining the effect of virus infection on host cell signalling, for example the lack of efficient cell culture systems for certain viruses and the use of continuous cell lines with modified biological properties. The cross-regulation between multiple signalling pathways, which may differ in cell systems in vivo and in vitro, also makes it difficult to obtain conclusive results. However, the continuous development of new techniques in molecular and cellular biology, such as RNA interference, will allow a better understanding of the modulation of cell signalling cascades. In future, this may help to identify new cellular and viral proteins for therapeutic targeting.

ACKNOWLEDGEMENTS

I would like to thank Andrew Thomson, Jonathan Clewley, Robin Gopal, John Cason and Jennifer Best for helpful discussions and comments on this manuscript. This work was supported by a grant from the Medical Research Council.

REFERENCES


