



Phosphatidylinositol-3-kinase-Akt pathway in negative-stranded RNA virus infection: a minireview

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Abstract

The PI3K/Akt signalling pathway is a crucial signalling cascade that regulates transcription, protein translation, cell growth, proliferation, cell survival, and metabolism. During viral infection, viruses exploit a variety of cellular pathways, including the well-known PI3K/Akt signalling pathway. Conversely, cells rely on this pathway to stimulate an antiviral response. The PI3K/Akt pathway is manipulated by a number of viruses, including DNA and RNA viruses and retroviruses. The aim of this review is to provide up-to-date information about the role of the PI3K-Akt pathway in infection with members of five different families of negative-sense ssRNA viruses. This pathway is hijacked for viral entry, regulation of endocytosis, suppression of premature apoptosis, viral protein expression, and replication. Although less common, the PI3K/Akt pathway can be downregulated as an immunomodulatory strategy or as a mechanism for inducing autophagy. Moreover, the cell activates this pathway as an antiviral strategy for interferon and cytokine production, among other strategies. Here, we present new data concerning the role of this pathway in infection with the paramyxovirus Newcastle disease virus (NDV). Our data seem to indicate that NDV uses the PI3K/Akt pathway to delay cell death and increase cell survival as a means of improving its replication. The interference of negative-sense ssRNA viruses with this essential pathway might have implications for the development of antiviral therapies.

Abbreviations

BAD	Bcl-2-associated death promoter	PHLPP	PH domain leucine-rich repeat protein phosphatase
FOXO	Forkhead box O1 protein	PI3K	Phosphoinositide 3-kinase
GSK3	Glycogen synthase kinase 3	PI(3,4)P2=PIP2	Phosphatidylinositol 3,4-bisphosphate
INPP4B	Inositol polyphosphate 4-phosphatase type II	PI(3,4,5)P3=PIP3	Phosphatidylinositol 3,4,5-trisphosphate
mTORC2	Mammalian target of rapamycin complex 2	PKB	Protein kinase B
PDK-1	Phosphoinositide-dependent kinase 1	PP2A	Protein phosphatase 2A
PH	Pleckstrin homology	PTEN	Phosphatase and tensin homologue deleted on chromosome 10
		SHIP	Src homology 2 (SH2)-containing inositol 5-phosphatase
		ssRNA	Single-stranded RNA

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The PI3K/Akt signalling pathway

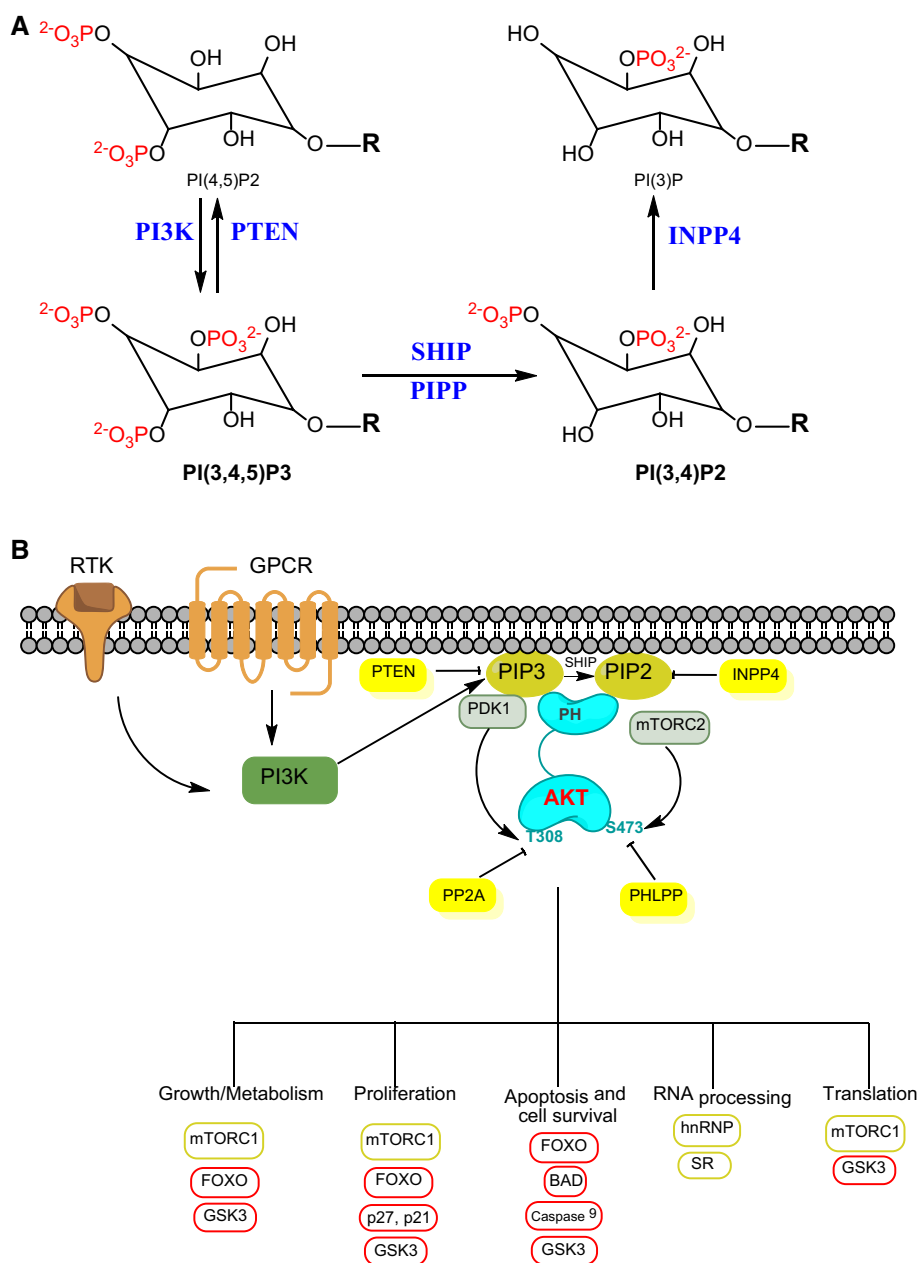
The PI3K/Akt signalling pathway is a ubiquitous pathway involved in many cellular processes, such as cell survival and apoptosis, autophagy, cell growth, cellular metabolism, RNA processing, and translation. The PI3K-Akt signalling cascade is triggered by activation of phosphoinositide 3-kinases (PI3Ks). Class I PI3Ks are the best-studied of all PI3Ks. They are heterodimeric proteins comprising a

regulatory subunit that contains a Src homology domain SH2, p85, and the catalytic subunit p110. PI3K is activated by the stimulation of receptor tyrosine kinases, G-protein-coupled receptors, or Ras [1, 2]. After stimulation by certain effectors (cytokines, hormones, grow factors, viruses), the activation of PI3K leads to an increase in the membrane phosphoinositides phosphatidylinositol-3,4,5-trisphosphate (PI(3,4,5)P₃) and phosphatidylinositol-3,4-bisphosphate (PI(3,4)P₂) [3] (Fig. 1A). PI(3,4,5)P₃ is directly synthesized by the catalytic action of PI3K on the substrate PI(4,5)P₂, whereas PI(3,4)P₂ is mainly generated after dephosphorylation of PI(3,4,5)P₃ by SHIP (Src homology 2 [SH2]-containing inositol 5-phosphatase) and other phosphatases [4].

Both phosphoinositides, PI(3,4,5)P₃ and PI(3,4)P₂, recruit different proteins to the plasma membrane by interacting with the pleckstrin homology (PH) domains of their targets [5, 6]. Akt [7, 8] and PDK-1 (phosphoinositide-dependent kinase 1) [9] are among these target proteins (Fig. 1B). Although the different functions of these phosphoinositides in PI3K/Akt signalling still need to be determined [4, 10], it has been shown that PI(3,4)P₂ can exert an inhibitory effect on PI3K [11].

Akt, also known as protein kinase B PKB, is a serine/threonine kinase present in all mammalian cell types. The protein contains three different domains: a PH domain at its N-terminus, a catalytic domain, and a regulatory

Fig. 1 (A) Chemical structure of phosphoinositides. Phosphoinositides are synthesized by PI3K and controlled by the lipid phosphatases PTEN, SHIP, PIPP and INPP4. (B) The PI3K/Akt signalling pathway. Stimulation of RTKs or GPCRs leads to activation of PI3K, resulting in PIP₃ production at the plasma membrane. Akt is recruited to the membrane by interacting with the phosphoinositides PIP₃ and PIP₂ through its PH domain. Signal termination is achieved by the action of SHIP, INPP4, PP2A and PHLPP phosphatases. Upon activation, Akt phosphorylates many downstream targets to control different cellular processes



domain [12, 13]. After activation of PI3K, Akt is recruited to the membrane and activated by double phosphorylation at Thr308 and Ser473 (Fig. 1B). PDK-1 is also recruited and phosphorylates Thr308, which is located in a segment at the entrance to the active site known as the activation loop [9]. In turn, mTORC2 (mammalian target of rapamycin complex 2) phosphorylates Ser473 in the hydrophobic motif [14]; reviewed in references 3 and 15.

The levels of PI(3,4,5)P3 and PI(4,5)P2 are regulated by phosphoinositide phosphatases such as PTEN and SHIP (Fig. 1A). PTEN (phosphatase and tensin homologue deleted on chromosome 10) is the major controller of PI(3,4,5)P3 levels and interrupts PI3K/Akt signalling by PI(3,4,5)P3 dephosphorylation at the 3-position of the inositol head [16]. PTEN is a tumour suppressor that is altered in many cancers, leading to PI3K activation. PI(3,4,5)P3 is also dephosphorylated at the 5-position of the inositol ring by inositol 5-phosphatases such as SHIP and PIPP (proline-rich inositol polyphosphate 5-phosphatase) [reviewed in reference 17]. Moreover, the INPP4 phosphatases (inositol polyphosphate 4-phosphatase type II, INPP4A, and INPP4B) are highly specific for PI(3,4)P2, which is degraded to PI(3)P (Fig. 1A) [10, 18]. Additionally, the activity of Akt signalling can be decreased through direct dephosphorylation of Akt by phosphatases such as PHLPP (PH domain leucine-rich repeat protein phosphatase), which dephosphorylates Ser473 [19], and PP2A (protein phosphatase 2A), which dephosphorylates Thr308 [20].

Upon activation, Akt can phosphorylate many downstream targets that control different cellular functions [for a detailed review, see references 3 and 15]. This suggests that the PI3K/Akt pathway plays a role in several different cellular functions (Fig. 1B). Upon Akt phosphorylation, the substrates can either be activated or, more commonly, inactivated. Some examples of Akt downstream substrates and associated functions are the forkhead box O (FOXO) transcription factors (inhibition of apoptosis and induction of cell survival), the pro-apoptotic factor BAD, p27 and p21 (proliferation), mTORC1 (protein translation, cell growth, proliferation), and GSK3 (glycogen synthase kinase 3; glucose and lipid metabolism, cell survival, proliferation). The PI3K/Akt signalling pathway is also involved in the regulation of the cellular splicing machinery [see references in reference 21].

It is also reported that PI3K signalling interacts with other cellular pathways. For example, Akt signalling can activate the transcription factor NF- κ B or block both Erk signalling and the MAPK/JNK and p38 proapoptotic pathways [see references in reference 15].

Modulation of the PI3K/Akt pathway by viruses

During viral infection, viruses exploit a variety of cellular pathways for their own benefit, such as the PI3K/Akt signalling pathway. The PI3K/Akt pathway is manipulated by a number of viruses, including DNA and RNA viruses and retroviruses [reviewed in references 21–25]. A growing body of evidence has revealed that the PI3K/Akt signalling pathway can be used for many functions during viral infection, including the suppression of apoptosis, synthesis of RNA, alternative splicing, endocytosis, and remodelling of actin. Moreover, the cell triggers this pathway in response to viral infections.

This review summarizes the many different strategies that negative-stranded ssRNA viruses use to hijack the PI3K-Akt pathway to ensure their replication. In particular, we focus on the viral families *Orthomyxoviridae*, *Paramyxoviridae*, *Pneumoviridae*, *Rhabdoviridae* and *Filoviridae* (Table 1).

Orthomyxoviruses

Members of the family *Orthomyxoviridae* have a negative-sense, single-stranded, segmented RNA genome, and they enter the host cell through endocytosis [26]. Among these, influenza A viruses comprise a major class of human respiratory pathogens. There is a large volume of research highlighting the different roles of the PI3K/Akt signalling pathway in influenza virus A infection. In a complex scenario, influenza A viruses interact with the PI3K/Akt signalling pathway at different stages of their viral life cycle with pro- or antiviral consequences depending on the stage [reviewed in references 25 and 27].

Early on in the viral cycle, the PI3K/Akt signalling pathway is hijacked by influenza virus to promote virus entry [28]. It has been suggested that this virus binds to the cell surface leading to the activation of Akt [29] through the clustering of different receptor tyrosine kinases, RTKs, at the cell surface. Accordingly, it has been suggested that peptides inhibiting Akt would block influenza virus entry in a virus-subtype-specific manner [30, 31]. More specifically, the PI3K/Akt signalling pathway could be hijacked by the virus to stimulate viral entry through clathrin-independent endocytosis [32]. As a result, a peptide derived from PI3K has been described as having the ability to inhibit endocytic uptake of the virus and infection [33]. In addition to the role of the PI3K/Akt signalling pathway in early internalization events and endocytosis, PI3K signalling together with the ERK pathway seem to

Table 1 Interplay between negative-strand ssRNA viruses and the PI3K/Akt signalling pathway

Family of viruses	General effect of PI3K/Akt Signalling on viral cycle	Viral signalling inducer	PI3K/Akt Signalling and targets	Viral target	Relevance to virus infection	References
<i>Orthomyxoviridae</i> Influenza A virus	Supportive	Binding to cell surface; clustering of RTKs	+ Akt		Virus entry	[28, 29]
	Supportive		+ Akt		Endocytosis; endosome acidification	[33, 34]
	Supportive	NS1	+ PI3K p85 Akt		Suppression of premature apoptosis	[27, 31, 36–40, 42]
	Supportive	HA	+ Akt/mTORC1		Viral protein synthesis and replication	[35]
	Supportive	NP, M2	+ Akt-mTOR		Autophagy	[45, 46]
	Antiviral	vRNA	+ PI3K/Akt		Production of IFN, IL-8 and RANTES cytokines	[28, 47, 48]
<i>Paramyxoviridae</i> Sendai virus, PIV3, NDV PIV5, Mumps virus Measles virus Sendai virus	Supportive		+ XIAP stabilization		Suppression of premature apoptosis	[50–53]
	Supportive			RNA P polymerase; V protein	Viral RNA synthesis	[54]
	Supportive	Contact of envelope viral proteins with lipid rafts	- AKT		Immunoregulation	[58–60]
	Antiviral	L		F protein	IFN β and NF κ B production Fusion inhibition	[55–57]
<i>Pneumoviridae</i> RSV	Supportive	NS1, NS2	+ Shingosine kinase, p85 regulatory subunit of PI3K; Akt, GS3K, NF κ B (+), p53 (-)		Suppression of premature apoptosis	[61–64, 66, 67]
	Antiviral				NETosis	[68]
<i>Rhabdoviridae</i> VSV Rabies BEFV	Supportive	M	- Akt downregulation		IFN-downregulation	[73]
	Antiviral	G	- Akt downregulation		Autophagy upregulation	[75]
	Antiviral		+ Akt		IFN-upregulation	[74]
	Supportive	P	+ PI3K/Akt/mTOR (+)		Incomplete autophagy	[76]
	Supportive		+ PI3K/ Akt, clathrin and dynamin 2		Endocytosis	[72]
	Supportive	GP	+ PI3K/Akt		Macropinocytosis; vesicular trafficking	[71, 78]

be involved in regulating the endosome acidification that triggers fusion between the viral and endosomal membranes [34], specifically through interaction of PI3K with the vacuolar ATPase.

Midway through the viral life cycle, the mTOR complex 1 (mTORC1) is activated to promote optimal viral protein expression and replication, using a mechanism that relies on Akt activation and the influenza virus HA protein [35].

Moreover, activation of the PI3K/Akt signalling pathway leads to suppression of premature apoptosis. Treatment of influenza-virus-infected cells with the classical PI3K inhibitor LY294002 accelerates apoptosis and impairs viral replication [31, 36, 37]. The viral non-structural protein 1 (NS1) has been implicated in the regulation of apoptosis [27, 36, 38, 39] through its interaction with the p85 regulatory subunit of PI3K, which results in activation of Akt [39–41]. Akt seems to be differentially phosphorylated by influenza virus to yield different outcomes related to viral protein expression and cell death at T308 and S473, respectively [35]. Direct interaction of NS1 with phosphorylated Akt has also been reported, suggesting that NS1 can enhance Akt kinase activity [42]. Conversely, using a panel of NS1-mutant viruses, it has been shown that the activation of the PI3K/Akt signalling pathway is not required to prevent apoptosis [43]. NS1 activation of the PI3K signalling pathway seems to be strain dependent [44].

Autophagy also may be induced in influenza-A-virus-infected cells at different stages of viral infection in which the PI3K-Akt pathway has been suggested to be crucial [45, 46]. It has been proposed that some influenza virus strains such as H5N1 may induce autophagic cell death by inhibiting the Akt-mTOR pathway [45] in a mechanism in which NP and M2 proteins would be involved [46].

In addition to the supporting functions of the PI3K/Akt signalling pathway summarized above, this pathway is also known to have an antiviral function in influenza virus infection. Accumulated viral RNA in the cytoplasm of infected cells activates PI3K signalling at late stages of the infection cycle to induce a type I interferon IFN response [28, 47]. Moreover, production of the cytokines IL-8 and RANTES in influenza-virus-infected cells is dependent on the PI3K/Akt signalling pathway [48].

Paramyxoviruses

Members of the family *Paramyxoviridae* are closely related to members of the family *Orthomyxoviridae*. This family comprises enveloped negative-stranded RNA viruses with a non-segmented genome and includes a large number of pathogens that infect humans and animals, such as measles virus (one of the most infectious viruses known), parainfluenza viruses, mumps virus, Sendai virus, and Newcastle disease virus (NDV, one of the biggest threats to the poultry industry) [49].

Inhibition of the PI3K/Akt signalling by LY294002 accelerates apoptosis in cells infected with Sendai virus (mouse parainfluenza virus type 1, SeV) and human parainfluenza virus type 3 (hPIV3) [50], suggesting that the PI3K/Akt pathway plays a role in preventing early apoptosis in infected cells. It has been shown that the

activation of the PI3K-Akt pathway in SeV-infected cells leads to the stabilization of the antiapoptotic protein XIAP (X-linked inhibitor of apoptosis protein), which in turn blocks caspase 9 activation [51]. For NDV, which is an avian paramyxovirus that has oncolytic properties, we have seen that treatment of HeLa cells with the PI3K inhibitor LY294002 accelerates cell death after NDV infection and enhances the DNA “ladder” pattern typical of apoptotic fragmentation (Fig. 2). These results are in agreement with those of a recent report that shows an increase in apoptosis in chicken cells infected with NDV after preincubation with LY294002 [52], indicating that NDV activates the PI3K/Akt pathway early on during infection. Moreover, NDV infection enhances cancer cell apoptosis by suppressing Akt signalling [53]. Nevertheless, pre-treatment of HeLa cells with LY294002 does not impair infectivity or the expression of viral proteins (Fig. 3). These data seem to indicate that NDV can manipulate the PI3K/Akt pathway to suppress premature apoptosis, although the pathway might be dispensable for virus replication.

Akt seems to be critical for the replication of other prototypical paramyxovirus such as parainfluenza virus type 5 (PIV5, formerly known as simian virus 5), mumps virus, and measles virus [54]. Inhibition of Akt by either siRNA or specific drug treatments results in a significant inhibition of PIV5 protein expression and replication. Akt might regulate viral RNA synthesis through phosphorylation of a component of the viral RNA polymerase P protein. Moreover, the effect of Akt on PIV5 might be exerted through interaction with the V protein, a non-structural protein that regulates viral RNA synthesis [54].

Like in the case of influenza virus, the PI3K/Akt signalling pathway may be activated in paramyxovirus-infected cells to promote antiviral immune responses. The production of IFN- $\kappa\beta$ in SeV-infected cells relies on PI3K and Akt activation [55]. Likewise, the PIV5 L protein (the catalytic component of the viral RNA polymerase) activates the expression of NF- κB in an Akt-dependent manner [56]. The findings of a study using reconstituted SeV envelopes bearing the viral fusion (F) protein suggest that Akt negatively regulates membrane fusion through phosphorylation and inactivation of the F protein [57].

In contrast to many paramyxoviruses, measles virus infecting T cells targets the PI3K/Akt pathway and down-regulates Akt as a part of the viral immunomodulatory strategy [58, 59]. Disruption of the Akt pathway seems to be triggered when envelope viral proteins are in contact with lipid raft domains in the membrane before virus internalization [58, 60]. This prevents membrane recruitment of the PI3K regulatory subunit p85 and, consequently, the activation of Akt [60]. This effect seems to be cell-type dependent, as an Akt inhibitor impairs virus release, and to a lesser extent, virus production, in the Vero fibroblastoid cell line [54, 59].

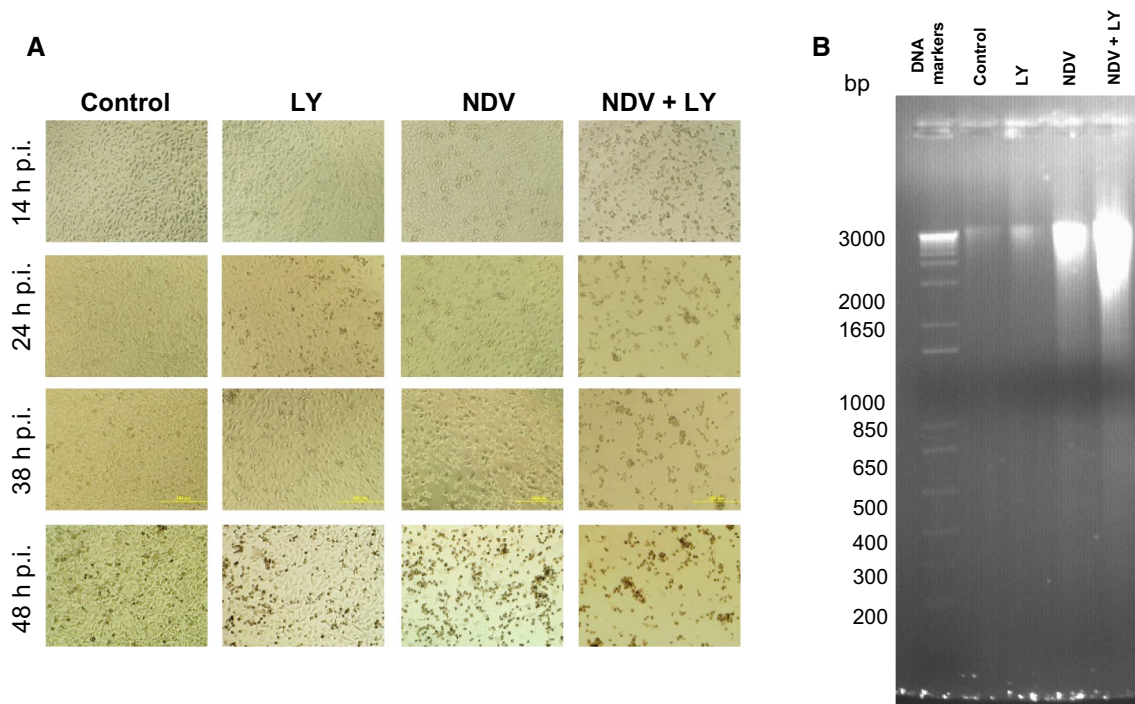


Fig. 2 LY294002 treatment prior to NDV infection increases virus-induced cell death. (A) Light microscopy photographs taken at different times after infection of HeLa cell monolayers that had been incubated with 20 μ M LY294002 (LY) for 1 h and then infected with NDV at an MOI of 1. At 14 h postinfection (pi), a cytopathic effect started to be seen in cells that were infected and pretreated with the drug, whereas the cytopathic effect induced by viral infection was evident at 38 h pi. Magnification bars (500 μ m) are shown in pictures taken at 38 h pi. (B) DNA fragmentation analysis of NDV-infected cells. At 12 h pi, total cellular DNA was extracted from HeLa cells

infected with NDV at an MOI of 1 in the presence or absence of LY294002. DNA was resolved by electrophoresis in a 1.5% agarose gel, stained with ethidium bromide, and visualized using UV transillumination. A molecular size marker was run in the left lane. Treatment with LY294002 did not induce apoptosis, as evidenced by the lack of DNA fragmentation (lane LY). NDV infection resulted in smearing of high-molecular-weight DNA. Preincubation with LY294002 increased the rate of DNA smearing in NDV-infected cells (lane NDV + LY). Experimental details are given in Online Resource 1

By contrast, the number of virus progeny released is not affected by overexpression of Akt [59].

Pneumoviruses

Pneumoviridae is a family of large enveloped negative-strand RNA viruses. It was formerly included as a subfamily of the family *Paramyxoviridae*, but in 2016 it was reclassified (ICTV International Committee on Taxonomy of Viruses website: <http://www.ictvonline.org>). Human respiratory syncytial virus (RSV) is the most relevant member of this family. RSV is the main cause of severe lower respiratory tract infections in infants, in the elderly, and in immunocompromised adults. To date, no effective anti-RSV vaccines are available; however, the monoclonal antibody palivizumab is being used for prophylaxis in high-risk infants.

RSV activates the PI3K/Akt signalling pathway at early stages of infection to block premature apoptosis [61–63]. Inhibition of PI3K by LY294002 reduces virus growth and accelerated apoptosis in RSV-infected airway epithelial

infected cells [61, 64] and in infected human granulocytes [63]. Conversely, it has been reported that treatment of a fibroblast cell line (BHK-21) with LY294002 does not impair RSV protein expression [65]. The antiapoptotic effect of RSV seems to be dependent on both PI3K activity and NF- κ B activation [61, 63, 66]. Many efforts have been made to identify the mechanism involved in the prosurvival effect in RSV-infected cells at early stages of viral infection and have revealed different upstream targets, such as sphingosine kinase [62], and downstream targets, such as NF κ B [61, 63, 64], GS3K [61, 64] and p53 [66, 67]. The viral non-structural proteins NS1 and NS2 seem to be essential for the activation of the PI3K/Akt and NF- κ B pathways [64, 66]. However, the contribution of the PI3K/Akt and NF- κ B in the regulation of the expression of several proinflammatory cytokines and chemokines in RSV-infected cells will need further investigation [66]. In a recent report, Muraro et al. showed that preincubation of neutrophils with LY294002 abolished neutrophil extracellular trap (NET) formation induced by the virus. As a result, it was concluded that NETosis stimulation by

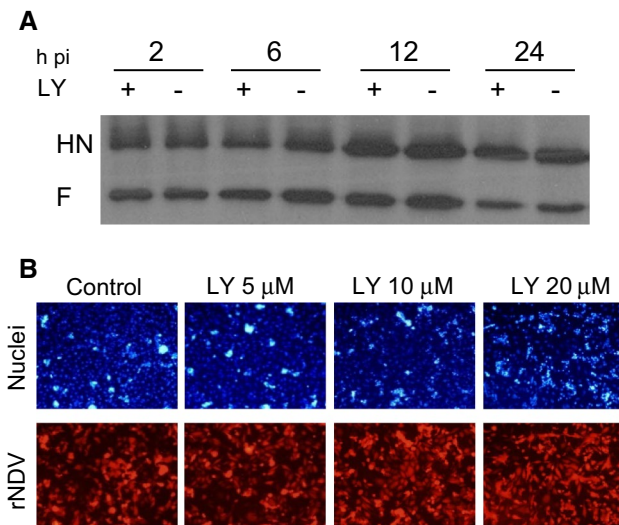


Fig. 3 (A) Pretreatment of HeLa cells with LY294002 does not impair the expression of viral proteins. At different times postinfection, the expression of the enveloped proteins HN and F in NDV-infected cells (control and LY294002-treated cells) was analysed by western blot using polyclonal anti-NDV antibodies as the probe. The expression of the HN and F proteins was similar in untreated and treated infected cells. (B) LY294002 does not inhibit viral infection. Monolayers of HeLa cells pre-treated with LY294002 (LY) at different concentrations were infected with recombinant NDVrNDV-F3aa-mRFP to express a monomeric red fluorescent protein. After 24 h at 37 °C, the cells were observed under an Olympus IX51 inverted fluorescence microscope with a 10x objective. Experimental details are given in Online Resource 1

RSV is dependent on the PI3K/Akt signalling pathway, among others [68].

Rhabdoviruses

The family *Rhabdoviridae* includes plant and animal pathogens that are similar to members of the family *Paramyxoviridae* in the organization and expression of their non-segmented genomes [49]. Among these viruses, vesicular stomatitis virus (VSV) has been widely studied as a prototype of nonsegmented, negative-strand RNA viruses [69]. The role of the PI3K/Akt signalling pathway in VSV infection is controversial. Due to the inhibitory effect of the Akt inhibitor AKT-IV on VSV replication and the reduction of the viral cytopathic effect, it has been suggested that VSV exploits Akt signalling for replication [54]. However, the results of a different study established that AKT-IV does not alter Akt phosphorylation, suggesting that AKT-IV inhibits viral replication by an Akt-independent mechanism [65]. Moreover, it has been shown that constitutively active Akt enhances VSV replication in a rat fibroblast cell line through activation of the mTOR pathway [70]. Nevertheless, research involving the treatment of target cells with several

inhibitors of the pathway, including direct inhibition of Akt phosphorylation by Akt-V (triciribine) and Akt-VIII, suggests that VSV does not rely on the PI3K/Akt pathway [65, 71]. The participation of PI3K/Akt signalling in rhabdovirus entry has been demonstrated for bovine ephemeral fever virus (BEFV), a rhabdovirus that enters the cell through clathrin-mediated endocytosis. BEFV simultaneously activates PI3K-Akt-NF- κ B and the Src-JNK-AP1 signalling pathways to induce expression of clathrin and dynamin 2, which leads to an enhancement of viral entry [72].

On the other hand, infection of cultured cells by VSV results in the early inactivation of the signalling pathway by inhibiting Akt phosphorylation in a mechanism dependent on the viral matrix protein M [73]. It has been suggested that Akt inactivation is a viral mechanism to reduce the IFN response [73]. Conversely, Akt phosphorylation is stimulated in VSV-infected macrophages as an antiviral mechanism that leads to the synthesis of the IFN I [74]. Additionally, making the relationships even more complex, some reports have shown that the PI3K/Akt signalling pathway is downregulated in VSV-infected cells as a cellular defence against viral infection. This is the case with VSV infection of *Drosophila melanogaster* cells, which results in the inhibition of the PI3K/Akt signalling pathway, which, in turn, activates autophagy and inhibits VSV replication [75]. For this to occur, the viral G protein seems to be sufficient for inducing autophagy. Likewise, it has been shown in a recent report that another rhabdovirus, rabies virus, induces the upregulation of the PI3K catalytic subunit and Akt and mTOR phosphorylation, leading to autophagosome accumulation and incomplete autophagy pathways in order to favour viral replication [76].

Filoviruses

The family *Filoviridae* of the order *Mononegavirales* includes emerging non-segmented, negative-strand RNA viruses such as Marburg virus and Ebola virus [77]. These viruses cause severe haemorrhagic fever in humans and nonhuman primates, with mortality rates of up to 90% [78]. Unlike other negative-strand RNA viruses, not much research has been carried out addressing the role of the PI3K pathway in the replication cycle of filoviruses. By contrast, several studies have shown that Ebola virus infection leads to an increase in Akt phosphorylation early after infection. Also, treatment of cells with LY294002, or with an Akt inhibitor, significantly reduces viral infection [71, 78] and causes virus particles to accumulate aberrantly within the cell cytoplasm without leading to membrane fusion [71]. These data indicate that the PI3K/Akt signalling pathway plays a role in the entry of Ebola virus by receptor-mediated

micropinocytosis [78] and trafficking by the endosomal route [71].

The PI3K/Akt signalling pathway as a target for antiviral therapies

The development of viral inhibitors targeting host factors such as cellular signalling pathways constitutes a promising novel strategy for controlling viral infections. In particular, targeting the PI3K/Akt pathway may represent a viable strategy for the development of broad antiviral agents against different negative-stranded RNA viruses. Moreover, inhibitors of the PI3K/Akt pathway are under investigation as anti-cancer and anti-inflammatory therapies [79]. Therefore, the use of already-approved cancer drugs would facilitate the approval of these new antiviral therapies. For influenza virus, a growing body of research has revealed that this strategy might be applied successfully using several promising anti-influenza candidate drugs. Inhibition of the PI3K catalytic subunit p110 by PI-103, a compound that has also been studied in relation to cancer therapy, has been shown to be effective against influenza virus infection [80]. IC87114, a selective inhibitor of the δ isoform of class I PI3K, has been shown to reduce the replication of human metapneumovirus, a pneumovirus related to RSV, in cell culture assays [81]. Additionally, Fujioka et al. [33] have identified a PI3K sequence that seems to be essential for endocytosis. The overexpression or the application of a peptide derived from this sequence has been shown to inhibit influenza virus infection, suggesting that a peptide-based therapy could be applied against infections with influenza virus and other viruses that enter the cell through endocytosis [33].

Antiviral drugs targeting viral proteins involved in modulating PI3K/Akt signalling have also shown promising results. This is the case of the flavonoid baicalin, whose anti-influenza activity has been assayed *in vitro* and *in vivo* [82]. Baicalin seems to interfere with the viral activation of PI3K/Akt signalling by inhibiting the interaction of viral NS1 protein with the p85 regulatory subunit of PI3K [39–41]. Moreover, the development of viral strains with mutations in the proteins that are essential for upregulating the pathway may facilitate the production of efficient vaccines, as suggested for the influenza virus [23].

Several Akt inhibitors, such as the benzimidazole derivative Akt inhibitor IV, might be used as general antiviral drugs inhibiting the infection of viruses belonging to different families, such as the paramyxovirus PIV5 and the rhabdovirus VSV [54, 83]. Sun et al. [83] have synthesized 21 analogues of Akt-IV and demonstrated their antiviral activity against the paramyxovirus PIV5 in cell culture assays. Nevertheless, the effects of these inhibitors on the PI3K/Akt pathway are not well understood [65, 83]. Two peptides that

inhibit Akt activity efficiently reduced influenza A infection in cultured cells without inhibiting the production of inflammatory cytokines [31]. Moreover, MK2206, an allosteric Akt inhibitor that is in phase I/II clinical trials against cancer, has been shown to inhibit influenza virus infection in a virus-subtype-specific manner [30].

It has also been shown that everolimus, an FDA-approved hydroxyethyl derivative of rapamycin known to inhibit mTOR, is effective against different viruses *in vivo*, including influenza A virus and RSV [84]. Interestingly, the use of the PI3K/mTOR inhibitor BEZ235 to disrupt glucose metabolism results in a reduction in influenza A virus titres in both *in vitro* and *in vivo* assays [85]. The antiviral activity of a coumarin derivative on a fish rhabdovirus, spring viraemia of carp virus, has been evaluated [86]. The coumarin derivative is able to inhibit both virus binding to the cell surface and viral-induced autophagy, the latter by activation of the Akt/mTOR pathway for blocking autophagy and inhibiting viral replication. The polyphenolic compound monoacetylcurcumin, a curcumin analogue, has been shown to inhibit influenza virus infection by reducing the level of Akt phosphorylation [87]. Natural compounds, some of them used in traditional Chinese medicine (TCM), have been shown to inhibit influenza virus infection. For instance, patchouli alcohol, a tricyclic sesquiterpene that is used in TCM for therapy of inflammatory diseases, has been reported to be effective against influenza A virus in both, *in vitro* and *in vivo* assays [88, 89]. The treatment of infected cells with patchouli alcohol significantly reduced the levels of phosphorylated PI3K and Akt, indicating that the PI3K/Akt signalling pathway might be involved in the anti-influenza A mechanisms of this drug [89]. Ko-ken tang (KKT), which is also used in TCM, is made up of seven medicinal herbs and is used in the treatment of several viral infections. It has been shown to inhibit influenza replication *in vitro* by downregulating Akt phosphorylation [90]. Rhein is an anthraquinone compound present in many traditional herbal medicines that inhibits influenza A virus replication *in vitro* by inhibiting the activation of Akt, among other signalling pathways [91].

The results summarized above, in particular those related to influenza virus, show that the PI3K/Akt signalling pathway is a promising target for antiviral therapies. As such, attacking viral infections through this pathway may be a novel therapeutic strategy against influenza virus in particular and negative-strand RNA viruses in general.

Concluding remarks

Negative-sense ssRNA viruses can manipulate the PI3K/Akt signalling pathway by either stimulating or downregulating this pathway (Table 1). Suppression of premature apoptosis by viral activation of the pathway seems to be critical for

the efficient replication of many types of orthomyxoviruses, paramyxoviruses, and pneumoviruses. PI3K/Akt signalling also promotes viral entry in the case of influenza A virus and some rhabdoviruses. The endocytic uptake of influenza virus, rhabdoviruses, and Ebola virus also depends on the activation of this pathway. Viral protein expression and replication exploit the control of PI3K/Akt signalling in the case of influenza virus and the paramyxovirus PIV5. Some viruses, such as measles virus and VSV, downregulate the pathway as a viral immunoregulatory strategy, which may also be manipulated to regulate autophagy. The PI3K/Akt signalling pathway is also activated by the cell as an antiviral mechanism against negative-sense ssRNA viruses for the production of IFN and other cytokines. These examples underline the importance of the PI3K/Akt signalling in the interaction of negative-sense ssRNA virus with cells and its potential role as a target for antiviral treatments. Nevertheless, deciphering the role of viral proteins in the manipulation of this pathway requires additional research, although some progress has been made. Viral envelope proteins, such as influenza virus HA and the VSV G protein, seem to have a function in both viral replication and induction of autophagy. Furthermore, the non-structural proteins NS1 and NS2 of influenza A virus and RSV appear to be involved in the antiapoptotic mechanism triggered by these viruses. Also, the influenza A virus NP and M2 proteins might be involved in the induction of autophagy. In the case of VSV, the M protein might inhibit Akt to reduce an IFN response, but more research is needed to determine the viral targets when this pathway is activated and linked to antiviral activities. The F protein of SeV has been described as being one of these potential targets. Targeting the PI3K/Akt pathway to inhibit viral replication might be a general strategy against negative-sense ssRNA viruses, as is the case with influenza virus, where different anti-Akt peptides block viral entry. Building on what is already known about the mechanisms that underlie viral manipulation or activation of this pathway is important if new targets for developing new antiviral strategies are to be identified.

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