HHS Public Access

Author manuscript

J Neurovirol. Author manuscript; available in PMC 2017 March 14.

Published in final edited form as:

J Neurovirol. 2015 October ; 21(5): 568–575. doi:10.1007/s13365-015-0361-z.

The herpes simplex virus type 1 (HSV-1) latency-associated transcript (LAT) protects cells against cold-shock-induced apoptosis by maintaining phosphorylation of protein kinase B (AKT)

Dale Carpenter[1], **Chinhui Hsiang**[1], **Xianzhi Jiang**[1], **Nelson Osorio**[1], **Lbachir BenMohamed**[1,2,3,4], **Clinton Jones**[5], and **Steven L. Wechsler**[1,6,7,8]

[1]Virology Research, Gavin Herbert Eye Institute and Department of Ophthalmology, School of Medicine, University of California, Irvine, Irvine, CA 92697, USA

[2]Laboratory of Cellular and Molecular Immunology, Gavin Herbert Eye Institute and Department of Ophthalmology, School of Medicine, University of California, Irvine, Irvine, CA 92697, USA

[3]Chao Family Comprehensive Cancer Center, University of California Irvine Medical Center, Irvine, CA 92868-32013, USA

[4]Institute for Immunology, School of Medicine, University of California Irvine, Irvine, CA 92697, USA

[5]School of Veterinary Medicine and Biomedical Sciences, Nebraska Center for Virology, University of Nebraska, Lincoln, NE 68503, USA

[6]Department of Microbiology and Molecular Genetics, School of Medicine, University of California, Irvine, Irvine, CA 92697, USA

[7]Center for Virus Research, University of California, Irvine, Irvine, CA 92697, USA

[8]Ophthalmology Research, University of California Irvine, 843 Health Sciences Road, Hewitt Hall (Building 843), Room 2012, Irvine, CA 92697, USA

Abstract

The herpes simplex virus type 1 (HSV-1) latency-associated transcript (LAT) blocks apoptosis and inhibits caspase-3 activation. We previously showed that serum starvation (removal of serum from tissue culture media), which takes several days to induce apoptosis, results in decreased levels of both AKT (protein kinase B) and phosphorylated AKT (pAKT) in cells not expressing LAT. In contrast in mouse neuroblastoma cells expressing LAT, AKT, and pAKT levels remained high. AKT is a serine/threonine protein kinase that promotes cell survival. To examine the effect of LAT on AKT-pAKT using a different and more rapid method of inducing apoptosis, a stable cell line expressing LAT was compared to non-LAT expressing cells as soon as 15 min following recovery from cold-shock-induced apoptosis. Expression of LAT appeared to inhibit dephosphorylation of pAKT. This protection correlated with blocking numerous pro-apoptotic events that are inhibited

Correspondence to: Steven L. Wechsler.

Conflict of interest All of the authors declare that they have no conflict of interest.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript

by pAKT. These results support the hypothesis that inhibiting dephosphorylation of pAKT may be one of the pathways by which LAT protects cells against apoptosis.

Keywords

Cold-shock-induced apoptosis; Herpes simplex virus type 1; Latency-associated transcript; AKT

Introduction

Over 50 % of adults in the USA harbor a herpes simplex virus type 1 (HSV-1) latent infection (reviewed in references (Jones 1998; Jones 2003)). Following primary infection of HSV-1 at the periphery, the virus establishes a lifelong latent infection in sensory neurons in the ganglia that innervate the peripheral site. Sporadic virus reactivations throughout the life of the infected individual result in virus returning to the periphery and being shed. In some instances, the reactivated virus triggers an episode of recurrent herpetic disease. Although usually thought of as a facial virus, recurrent HSV-1 can cause genital disease as well as cold sores at the mouth and corneal disease. When the initial site of infection is the eye, the virus establishes a latent infection in the sensory neurons of the trigeminal ganglia and virus reactivation results in shedding of virus in tears and less often potentially blinding episodes of recurrent ocular disease. During neuronal latency, the latency-associated transcript (LAT) is the only viral gene that is abundantly and consistently detected in the ganglia of humans, mice, and rabbits (Jones 1998; Rock et al. 1987).

The HSV-1 latency-associated transcript (LAT) plays a key role in the virus' reactivation phenotype since $LAT^{(-)}$ viruses have a significantly reduced reactivation phenotype in mice and rabbits (Leib et al. 1989; Hill et al. 1990; Perng et al. 1994). Following primary HSV-1 infection of the eye, the virus travels up axons of sensory neurons to the trigeminal ganglia (TG) where lifelong latency is established. We previously showed that when rabbits are ocularly infected with a $LAT^{(-)}$ mutant virus, apoptotic neurons can be detected in the TG. In contrast, when wt $LAT^{(+)}$ virus is used, fewer apoptotic neurons are detected (Perng et al. 2000). Thus, LAT reduces the amount of apoptosis during the time at which latency is being established. In addition, plasmids expressing LAT block apoptosis induced by a variety of methods in tissue culture experiments (Perng et al. 2000; Inman et al. 2001; Henderson et al. 2002). LAT's anti-apoptosis activity has been confirmed by others (Ahmed et al. 2002). The ability to block apoptosis appears to be a crucial mechanism by which LAT enhances the reactivation phenotype since we have shown that substituting various anti-apoptotic genes in place of LAT restores the wild-type reactivation phenotype to an otherwise $LAT^{(-)}$ mutant (Perng et al. 2002; Jin et al. 2005, 2007, 2008).

Although LAT's anti-apoptosis activity is critical for LAT's ability to enhance the reactivation phenotype, whether the main impact is at the time of establishment of latency, the maintenance of latency, or at the time of reactivation from latency, or a combination of the three, remains to be fully elucidated. The mechanism(s) by which LAT impacts apoptosis also remains to be determined. We previously reported that LAT can block both caspase-8- and caspase-9- induced apoptosis (Inman et al. 2001; Henderson et al. 2002). Thus, LAT can

Author Manuscript

block both the extrinsic and the intrinsic apoptotic pathways. LAT also efficiently blocks apoptosis induced during recovery from cold shock (Carpenter et al. 2007), commonly called cold-shock-induced apoptosis. LAT reduces caspase-3 cleavage (activation) a very late step in both the extrinsic and the intrinsic pathways. However, blocking caspase-3 cleavage does not account for how LAT blocks both major apoptotic pathways, since LAT has also been shown to block upstream events such as caspase-8 and caspase-9 cleavage. Thus, LAT either blocks multiple steps in the apoptosis cascades or blocks a more upstream step common to both major pathways. To investigate this, we recently determined the impact of LAT on AKT and phosphorylated AKT (pAKT) (Li et al. 2010). AKT or protein kinase B is a serine/threonine protein kinase that promotes cell survival. Its active form is phosphorylated at threonine 308 and serine 473 (Scheid and Woodgett 2003) and can regulate and phosphorylate anti-apoptotic and pro-apoptotic proteins (Cooray 2004).

Using a C1300 mouse neuroblastoma cell line that stably expresses LAT (DC-LAT6 cells (Carpenter et al. 2007)) and serum starvation to induce apoptosis, we showed that compared to LAT$^{(-)}$ cells, cells expressing LAT had increased intracellular levels of both AKT and pAKT (Li et al. 2010). This strongly suggests that LAT can either directly or indirectly regulate AKT and pAKT levels in apoptotically stressed cells. However, serum starvation is a very slow method of inducing apoptosis, usually taking days for significant effects to be seen. To confirm and extend our previous results using a different and much more rapid method of inducing apoptosis, in this report, we studied pAKT and other apoptotic landmarks following cold-shock-induced apoptosis.

We report here that within 15 min after cold shock, LAT inhibited dephosphorylation of pAKT at both threonine 308 and serine 473 residues. This correlated with protection against cleavage of caspase-3, a late stage in the apoptosis pathways, and cleavage of caspase-2, an early step in the apoptosis cascade. Stabilization of pAKT by LAT also correlated with protection against caspase-9 cleavage, loss of protective Bcl2 and increase of pro-apoptotic BCL-Xs, and pro-apoptotic tBid. In addition, in the presence of AKT VIII, an AKT inhibitor that blocks AKT phosphorylation to pAKT, LAT was unable to maintain pAKT during recovery from cold-shock and this correlated with increased caspase-3 cleavage within 30 min of the start of recovery from cold-shock treatment.

Materials and methods

Cell lines

The C1300-derived mouse neuroblastoma cell line DC-LAT6 is stably transfected with a plasmid containing a NotI-NotI LAT restriction fragment that contains the LAT promoter and expresses LAT nts +1 to 3224. DC- LAT311 cells are stably transfected with the same plasmid, but lacking the LAT promoter so no LAT is made. Both cell lines were previously described (Carpenter et al. 2007). Cells were grown as monolayers in MEM with 10 % FBS in a 37 °C incubator with 5 % CO_2. They do not have a neuronal morphology when grown in this manner, and neither retinoic acid (RA) nor any other agent was used to induce neuronal morphology.

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

Cold shock

Cell culture flasks or plates were sealed with Parafilm and placed on ice for 2 h. The Parafilm was then removed, and the cells returned to a 37 °C incubator with 5 % CO_2 for various times as indicated for each study.

Western blots

Proteins were extracted in protein extraction buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1 % NP-40, 0.25 % sodium deoxycholate, 1× protease inhibitor cocktail [P8340, Sigma-Aldrich, St. Louis, MO], 10 mM NaF, and 1 mM sodium orthovanadate). Proteins were separated on 4–20 % precast gels (Invitrogen) by SDS-PAGE and then transferred to polyvinylidene fluoride (PVDF) membrane (Millipore Corporation, Bedford, MA). Antibodies specific to cleaved caspase-3 (Asp175), caspase-3, cleaved caspase-9 (Asp353), caspase-9, pAKT (Ser473), pAKT (Thr308), and AKT were purchased from Cell Signaling Technology (Danvers, MA). Bcl-2 (N-19), Bcl-Xs, BID, tBID, and caspase-2_L antibodies were purchased from Santa Cruz Biotechnology, Inc (Dallas, TX). All primary antibodies were used at a dilution of 1:1000 in 5 % BSA. Primary antibodies were detected with goat anti-rabbit or goat anti-mouse HRP-conjugated secondary antibody and visualized using either SuperSignal West Pico Chemiluminescent Substrate or SuperSignal West Femto Chemiluminescent Substrate (Pierce, Rockford, IL) and X-ray film. Membranes were stripped using Restore Plus Western blot Stripping buffer (Thermo Scientific, cat #46430) according to the manufacturer's instructions. They were re-probed with different primary antibodies and detected as described immediately above. This allowed multiple specific proteins to be visualized on the same Western blot.

AKT VIII

The AKT inhibitor AKT VIII was purchased from Calbiochem. A stock solution of 10 mM was made by dissolving AKT VIII in DMSO.

Results

LAT maintains AKT phosphorylation during the first 2 to 6 h of recovery from cold shock

We have previously shown that a stable cell line expressing LAT can prevent cells from cold-shock-induced apoptosis (Carpenter et al. 2007). We have also shown that AKT is involved in protecting LAT expressing cells from serum starvation-induced apoptosis (Li et al. 2010). Here, we study the role of AKT in LAT's anti-apoptosis activity using an alternative method of inducing apoptosis, namely recovery from cold shock. In addition to being a different trigger for apoptosis, cold shock also induces apoptosis rapidly (minutes) compared to serum starvation (days).

C1300 cells are a mouse neuroblastoma cell line that has been used as a model of mouse neuronal cells (Jiang et al. 2011). DC-LAT6 cells, C1300 cells that stably express LAT nts 1–3224 of the 8.3 Kb primary LAT transcript were compared to C1300 cells containing the same LAT region but without the LAT promoter (DC- LAT311 cells) (Carpenter et al. 2007). Monolayers of cells were cold shocked by placing them on ice for 2 h and then recovering them at 37 °C for 2, 4 or 6 h as described in "Materials and methods." Attached

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

and unattached cells were collected. Whole cell lysate was prepared and Western blots performed using antibody specific to phosphorylated AKT (pAKT S473) and cleaved caspase-3 as described in “Materials and methods” (Fig. 1). The phosphorylated form of AKT almost completely disappeared as a result of 2 h on ice (Fig. 1; lanes 0 °C) regardless of the presence or absence of LAT. Within 2 h of recovery from the cold at 37 °C, the amount of pAKT had returned to normal levels in DC-LAT6 cells. In contrast, only a small amount of recovery was seen in the absence of LAT at this time in DC- LAT311 cells. Furthermore, the levels of pAKT were higher in DC-LAT6 cells than in DC- LAT311 cells throughout the 6 h recovery time. This suggested that LAT directly or indirectly either increased the amount of AKT that got phosphorylated or protected pAKT from being dephosphorylated. The amount of AKT appeared higher in DC-LAT6 cells than in DC- LAT311 cells throughout the 6-h recovery time. Interestingly, pAKT levels in DC- LAT311 cells appeared to increase between 2 to 6 h of recovery from cold shock. This may be because by 6 h, more than 90 % of the DC- LAT311 cells were detached from the surface and lysed. The proteins on the Western blot are therefore mostly from a subset of DC- LAT311 cells that for whatever reason survived the cold-shock-induced apoptosis. This is also likely the reason that some markers of apoptosis decreased over time in these LAT$^{(-)}$ cells. In summary, these studies suggested that LAT directly or indirectly either increased the amount of AKT that got phosphorylated or protected pAKT from being dephosphorylated.

Additional apoptosis-related proteins were analyzed on the same Western blot by stripping the blot and re-probing it with different antibodies. Cleaved (activated) caspase-3 levels were decreased in DC-LAT6 cells compared to LAT$^{(-)}$ DC- LAT311 cells. The amount of cleaved caspase-3 was inversely correlated with pAKT levels. Cleaved caspase-2 levels were higher in the LAT$^{(-)}$ cells compared to the LAT$^{(+)}$ cells and were again inversely correlated with pAKT levels. The results for caspase-9 were similar to those for caspase-3. More cleaved caspase-9 was seen in LAT$^{(-)}$ cells compared to LAT$^{(+)}$ cells within 2 h of recovery from cold shock. With both caspase-3 and caspase-9, there is so much cleavage in the LAT$^{(-)}$ cells that the uncleaved versions can be seen to have decreased. The anti-apoptosis protein Bcl-2 was decreased during cold shock prior to recovery at 37 °C (lanes 0 °C) but rapidly rebounded to pre-cold-shock levels by 2 h at 37 °C in both cell lines. However, in LAT$^{(-)}$ DC-LAT 311 cells, Bcl-2 decreased from 2 to 4 h and from 4 to 6 h after recovery at 37 °C. In contrast, in LAT$^{(+)}$ DC-LAT6 cells, the level of Bcl-2 remained high at these times. The pro-apoptotic Bcl-Xs protein was significantly elevated in the LAT$^{(-)}$ cells at 4 and 6 h after being switched to 37 °C. No such accumulation of this pro-apoptotic factor was seen in the LAT$^{(+)}$ cells at any time. In summary, these studies provided evidence of a correlation between LAT expression in C1300 cells, reduced levels of caspase activation, reduced Bcl-Xs, and increased expression of Bcl-2.

Examination of pAKT from 15 to 60 min after recovery from cold shock

To further understand the expression of the above regulatory proteins after cold shock, shorter recovery time periods were studied. DC-LAT6 and DC- LAT311 cells were cold shocked on ice for 2 h as above and then rewarmed at 37 °C for 15, 30, 45, or 60 min instead of the 2, 4, or 6 h time period used in the study shown in Fig. 1 above. Figure 2 shows a

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

typical Western blot analysis for pAKT and AKT. As in Fig. 1, prior to recover from cold shock, very little pAKT was detected in either DC-LAT6 or DC- LAT311 cells (Fig. 2, lanes 0 °C). Within 15 min at 37 °C, both cell lines appeared to contain normal (pre-cold shock) levels of pAKT (compare the 37 °C lanes of each cell to their corresponding 0 °C→37 °C 15 min lanes). In the LAT$^{(+)}$ DC-LAT6 cells, the level of pAKT remained constant during the 15- to 60-min recovery period. In contrast, in LAT$^{(-)}$ DC- LAT311 cells, the amount of pAKT decreased by 30 min and was essentially reduced to the very low level seen at 0 °C prior to recovery. This result suggests that re-phosphorylation of AKT to pAKT occurred very rapidly in a LAT independent manner upon shifting the cells to 37 °C following cold shock. It also suggests that in the absence of LAT, phosphorylation of pAKT is rapidly lost during recovery from cold shock. These results suggest that LAT either directly or indirectly helps maintain phosphorylation (i.e., activation) of pAKT rather than affecting the phosphorylation process.

LAT and pAKT phosphorylation at amino acids threonine 308 and serine 473 following cold shock

The AKT-pAKT analyses in Figs. 1 and 2 were performed using an antibody that recognizes AKT and a different antibody that recognizes pAKT phosphorylated at S473. Since activated pAKT requires that T308 and S473 are both phosphorylated, it was of interest to confirm that the pAKT seen in the previous studies was in fact phosphorylated at both residues. DC-LAT6 and DC- LAT311 cells were cold shocked and recovered at 37 °C as in Fig. 2. Western blots using an antibody specific for pAKT-T308 and an antibody specific for pAKT-S473 were used to identify the sites of phosphorylation. Cold shock without any recovery period resulted in almost complete loss of phosphorylation at both T308 and S473 (Fig. 3). In both DC-LAT6 and DC- LAT311 cells, phosphorylation at both sites was rapidly restored within 15 min of recovery at 37 °C. In DC-LAT6 cells, phosphorylation levels at both sites were maintained through the 15 to 60 min recovery period. In contrast, in DC- LAT311 cells, T308 and S473 were rapidly dephosphorylated. These results suggest that LAT directly or indirectly protects phosphorylation of pAKT at both T308 and S473, thus maintaining pAKT activity and protecting these cells from apoptosis.

Changes in additional apoptotic factors affected by LAT shortly after recovery from cold shock

The Western blot shown in Fig. 2 was stripped and re-probed multiple times to analyze the effect of LAT on various pro- and anti-apoptosis factors within 15–60 min of recovery from cold shock (Fig. 4). In DC-LAT6 cells, only small amounts of cleaved caspase-2 accumulated during the 60 min recovery. In contrast, higher levels of cleaved caspase-2 were detected in DC- LAT311 cells, consistent with more apoptosis in these cells. Caspase-3 and caspase-9 were also cleaved at much higher levels in DC- LAT311 cells than in DC-LAT6 cells, with the activated cleavage products increasing from 15 to 60 min of recovery. In fact, so much cleavage appears to have occurred that a decrease in uncleaved caspase-3 and caspase-9 during this 15 to 60 min period is apparent.

BID, a substrate of caspase-2, was also examined. Cleavage of BID to activated truncated BID (tBID) is a major apoptotic switch of ER stress and heat shock-induced death (Upton et

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

al. 2008; Bonzon et al. 2006). A very small amount of tBID can be seen at 30 min after recovering from cold shock in DC- LAT311 cells, and this increased by 45 and 60 min concurrent with an apparent loss of full length BID at 60 min. The timing of BID cleavage appears to be delayed and less obvious when compared to caspase-9 and caspase-3. This may seem paradoxical because cleavage of BID by caspase-2 triggers mitochondria cytochrome c release and activates executioner caspases. However, minuscule amounts of tBID are sufficient to induce robust cytochrome c release in cells (Korsmeyer et al. 2000).

Inhibition of AKT phosphorylation prevents LAT from blocking cold-shock-induced apoptosis

The above studies suggest that one of the early steps in how LAT blocks apoptosis following cold shock is by directly or indirectly protecting pAKT (the active anti-apoptotic form) from being dephosphorylated (the inactivated form). To investigate this further, DC-LAT6 cells were subjected to cold shock and then incubated at 37 °C as described above in the presence or absence of 20 uM AKT VIII, a cell-permeable and reversible quinoxaline compound that potently and selectively inactivates AKT. At 30 and 60 min after recovery from cold shock, AKT phosphorylation was completely absent in the presence of the AKT inhibitor (Fig. 5; lanes “+”). At the same times, in the presence of the AKT inhibitor, caspase-3 cleavage was dramatically increased even though the DC-LAT6 cells express LAT. This result suggests that when AKT phosphorylation is inhibited, LAT no longer prevents cells from cold-shock-induced apoptosis. This supports the above studies suggesting that LAT blocks apoptosis by maintaining high levels of pAKT.

Discussion

Apoptosis is triggered through two main signaling pathways, the intrinsic or mitochondrial pathway and the extrinsic or Fas ligand-mediated pathway. In the extrinsic pathway, caspase-8 is important in directly activating other caspase family members to execute apoptosis. However, it is common for neuroblastoma cells to have lost their caspase-8 (Takita et al. 2000; Teitz et al. 2000), and like most neuroblastoma cells, C1300 cells, the parental cell line for the DC-LAT6 and DC- LAT311 cells used here expresses little or no caspase-8. Therefore, in this report, we investigated proteins involved in the mitochondrial pathway.

pAKT, the phosphorylated activated form of AKT, has anti-apoptosis properties and can act as a major early inhibitor of the intrinsic apoptosis pathway. We found here that at early times following recovery from cold shock, LAT directly or indirectly protects against dephosphorylation of pAKT to AKT. In this system, the presence of LAT and pAKT correlated with less activation of various pro-apoptotic proteins involved in the intrinsic apoptosis pathway including cleaved caspase-2, tBID, Bcl-Xs, cleaved caspase-9, and cleaved caspase-3. Production of each of these pro-apoptotic factors can be directly or indirectly decreased by pAKT. Since AKT is linked to caspase-2 activation (Lin et al. 2007; Troy et al. 2001), one potential scenario is that in the absence of sufficient pAKT, caspase-2 is activated resulting in cleavage of BID to tBID. This disrupts the mitochondrial membrane, thus activating caspase-9, which activates caspase-3 and results in apoptosis. LAT’s ability

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

to maintain high levels of pAKT blocks this pathway by pAKT directly or indirectly inhibiting activation of caspase-2. In the presence of the pAKT inhibitor AKT VIII, LAT no longer maintained pAKT levels and this correlated with increased apoptosis as measured by cleavage of caspase-3. Although it is not certain that similar results would be obtained using primary neuron culture, these results nevertheless strongly support the hypothesis that one of the mechanisms by which LAT protects cells against apoptosis is by directly or indirectly helping to maintain phosphorylation of pAKT.

AKT/pAKT activation requires the binding of phospholipid and phosphorylation of threonine 308 (T308) and Serine 473 (S473). We found that in DC-LAT6 cells recovering from cold shock, phosphorylation at both T308 and S473 were protected, while in DC- LAT311 cells dephosphorylation occurred at both locations. Interestingly and unexpectedly, in both DC-LAT6 and DC- LAT311 cell lines, cold shock resulted in dephosphorylation of pAKT to AKT. The reason for this is not known, but it is possible that phosphorylation of pAKT requires one or more continuous kinase activities that do not occur at 0 °C. Regardless, within 15 min of recovery from the cold at 37 °C, normal levels of pAKT (i.e., levels similar to those seen in cells never subjected to cold shock) were present in both DC-LAT6 and DC- LAT311 cells. This suggests that LAT does not enhance the phosphorylation process of pAKT. In contrast, after this initial 15-min recovery period, in DC- LAT311 cells, the level of pAKT significantly decreased and was almost completely gone by 60 min, while in DC-LAT6 cells, the level of pAKT was maintained. Taken together, these findings suggest that following the apoptosis, inducing stress of recovery from cold-shock LAT helps protects against loss of pAKT but does not increase conversion of AKT to pAKT.

In addition to inhibiting apoptosis, the ability of LAT to stabilize AKT phosphorylation may influence viral gene expression, as discussed below. When primary superior cervical ganglion sympathetic neurons are infected with HSV-1, a quiescent infection resembling latency can be established if cultures are treated with aphidicolin, a DNA polymerase inhibitor (Kim et al. 2012; Camarena et al. 2010). In this in vitro latency model, LAT is readily detected and a quiescent infection can be maintained for weeks if nerve growth factor (NGF) signaling is not interrupted. When NGF is removed from these cultures, lytic cycle viral gene expression is induced and infectious virus produced, thus resembling reactivation from latency in vivo. Like other receptor tyrosine kinases, downstream targets of the NGF receptor include PI3-kinase and AKT, reviewed in (Lemmon and Schlessinger 2010). When these latently infected neuronal cultures containing NGF are treated with the same AKT inhibitor used in this study, reactivation from latency occurs with similar efficiency as when NGF is removed (Camarena et al. 2010). Although withdrawal of NGF leads to apoptosis of superior cervical ganglion neurons (Deckwerth and Johnson 1993), it is not apoptosis alone that results in HSV-1 lytic cycle gene expression following disruption of NGF signaling, since lytic cycle gene expression occurs even if apoptosis is blocked (Camarena et al. 2010). Thus, the ability of LAT to stabilize AKT phosphorylation may promote the maintenance of latency by both interfering with lytic cycle viral gene expression and inhibiting apoptosis. Downregulation of LAT or the bovine herpesvirus-1 LAT homolog, LR-RNA, both of which occur when reactivation is induced (Du et al. 2011, 2012; Rock et

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

al. 1992), may therefore be a trigger for reactivation via dephosphorylation of pAKT which leads to removal of inhibition of HSV-1 lytic gene expression.

In summary, cells expressing LAT are resistant to apoptosis induced by recovery from cold shock. One mechanism by which LAT protects against apoptosis thus increasing the HSV-1 reactivation phenotype appears to be by directly or indirectly maintaining high levels of pAKT which in turn acts to decrease multiple pro-apoptotic factors. LAT's ability to stabilize pAKT may also help maintain latency by preventing lytic viral gene expression.

Acknowledgments

This work was supported by Public Health Service research grants from the NIH EY013191, 1R56AI098985, and 1R56AI093133 to SLW, EY14900, EY019896, and EY024618 to LB, Agriculture and Food Research Initiative Competitive Grants Program (USDA National Institute of Food and Agriculture) to CJ (13–01041), The Discovery Center for Eye Research, and a Research to Prevent Blindness Challenge grant.

References

Ahmed M, Lock M, Miller CG, Fraser NW. Regions of the herpes simplex virus type 1 latency-associated transcript that protect cells from apoptosis in vitro and protect neuronal cells in vivo. J Virol. 2002; 76(2):717–729. [PubMed: 11752162]

Bonzon C, Bouchier-Hayes L, Pagliari LJ, Green DR, Newmeyer DD. Caspase-2-induced apoptosis requires bid cleavage: a physiological role for bid in heat shock-induced death. Mol Biol Cell. 2006; 17(5):2150–2157. [PubMed: 16495337]

Camarena V, Kobayashi M, Kim JK, Roehm P, Perez R, Gardner J, Wilson AC, Mohr I, Chao MV. Nature and duration of growth factor signaling through receptor tyrosine kinases regulates HSV-1 latency in neurons. Cell Host Microbe. 2010; 8:320–330. [PubMed: 20951966]

Carpenter D, Hsiang C, Brown DJ, Jin L, Osorio N, BenMohamed L, et al. Stable cell lines expressing high levels of the herpes simplex virus type 1 LAT are refractory to caspase 3 activation and DNA laddering following cold shock induced apoptosis. Virology. 2007; 369(1):12–18. [PubMed: 17727910]

Cooray S. The pivotal role of phosphatidylinositol 3-kinase-Akt signal transduction in virus survival. J Gen Virol. 2004; 85(Pt 5):1065–1076. [PubMed: 15105524]

Deckwerth TL, Johnson EM Jr. Temporal analysis of events associated with programmed cell death (apoptosis) of sympathetic neurons deprived of nerve growth factor. J Cell Biol. 1993; 123:1207–1222. [PubMed: 7503996]

Du T, Zhou G, Roizman B. HSV-1 gene expression from reactivated ganglia is disordered and concurrent with suppression of latency-associated transcript and miRNAs. Proc Natl Acad Sci U S A. 2011; 108(46):18820–18824. [PubMed: 22065742]

Du T, Zhou G, Roizman B. Induction of apoptosis accelerates reactivation of latent HSV-1 in ganglionic organ cultures and replication in cell cultures. Proc Natl Acad Sci U S A. 2012; 109(36): 14616–14621. [PubMed: 22908263]

Henderson G, Peng W, Jin L, Perng GC, Nesburn AB, Wechsler SL, et al. Regulation of caspase 8- and caspase 9-induced apoptosis by the herpes simplex virus type 1 latency-associated transcript. J Neurovirol. 2002; 8(Suppl 2):103–111. [PubMed: 12491160]

Hill JM, Sedarati F, Javier RT, Wagner EK, Stevens JG. Herpes simplex virus latent phase transcription facilitates in vivo reactivation. Virology. 1990; 174(1):117–125. [PubMed: 2152989]

Inman M, Perng GC, Henderson G, Ghiasi H, Nesburn AB, Wechsler SL, et al. Region of herpes simplex virus type 1 latency-associated transcript sufficient for wild-type spontaneous reactivation promotes cell survival in tissue culture. J Virol. 2001; 75(8):3636–3646. [PubMed: 11264353]

Jiang X, Chentoufi AA, Hsiang C, Carpenter D, Osorio N, BenMohamed L, et al. The herpes simplex virus type 1 latency-associated transcript can protect neuron-derived C1300 and Neuro2A cells

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

from granzyme B-induced apoptosis and CD8 T-cell killing. J Virol. 2011; 85(5):2325–2332. [PubMed: 21177822]

Jin L, Perng GC, Mott KR, Osorio N, Naito J, Brick DJ, et al. A herpes simplex virus type 1 mutant expressing a baculovirus inhibitor of apoptosis gene in place of latency-associated transcript has a wild-type reactivation phenotype in the mouse. J Virol. 2005; 79(19):12286–12295. [PubMed: 16160155]

Jin L, Perng GC, Carpenter D, Mott KR, Osorio N, Naito J, et al. Reactivation phenotype in rabbits of a herpes simplex virus type 1 mutant containing an unrelated antiapoptosis gene in place of latency-associated transcript. J Neurovirol. 2007; 13(1):78–84. [PubMed: 17454452]

Jin L, Carpenter D, Moerdyk-Schauwecker M, Vanarsdall AL, Osorio N, Hsiang C, et al. Cellular FLIP can substitute for the herpes simplex virus type 1 latency-associated transcript gene to support a wild-type virus reactivation phenotype in mice. J Neurovirol. 2008; 14(5):389–400. [PubMed: 18989818]

Jones C. Alphaherpesvirus latency: its role in disease and survival of the virus in nature. Adv Virus Res. 1998; 51:81–133. [PubMed: 9891586]

Jones C. Herpes simplex virus type 1 and bovine herpesvirus 1 latency. Clin Microbiol Rev. 2003; 16(1):79–95. [PubMed: 12525426]

Kim JY, Mandarino A, Chao MV, Mohr I, Wilson AC. Transient reversal of episome silencing precedes VP16-dependent transcription during reactivation of HSV-1 in neurons. PLoS Pathog. 2012; 8:e1002540. [PubMed: 22383875]

Korsmeyer SJ, Wei MC, Saito M, Weiler S, Oh KJ, Schlesinger PH. Pro-apoptotic cascade activates BID, which oligomerizes BAK or BAX into pores that result in the release of cytochrome c. Cell Death Differ. 2000; 7(12):1166–1173. [PubMed: 11175253]

Leib DA, Bogard CL, Kosz-Vnenchak M, Hicks KA, Coen DM, Knipe DM, et al. A deletion mutant of the latency-associated transcript of herpes simplex virus type 1 reactivates from the latent state with reduced frequency. J Virol. 1989; 63(7):2893–2900. [PubMed: 2542601]

Lemmon MA, Schlessinger J. Cell signaling by receptor tyrosine kinases. Cell. 2010; 141:1117–1134. [PubMed: 20602996]

Li S, Carpenter D, Hsiang C, Wechsler SL, Jones C. Herpes simplex virus type 1 latency-associated transcript inhibits apoptosis and promotes neurite sprouting in neuroblastoma cells following serum starvation by maintaining protein kinase B (AKT) levels. J Gen Virol. 2010; 91(Pt 4):858–866. [PubMed: 19955563]

Lin CF, Chen CL, Chiang CW, Jan MS, Huang WC, Lin YS. GSK-3beta acts downstream of PP2A and the PI 3-kinase-Akt pathway, and upstream of caspase-2 in ceramide-induced mitochondrial apoptosis. J Cell Sci. 2007; 120(Pt 16):2935–2943. [PubMed: 17666435]

Perng GC, Dunkel EC, Geary PA, Slanina SM, Ghiasi H, Kaiwar R, et al. The latency-associated transcript gene of herpes simplex virus type 1 (HSV-1) is required for efficient in vivo spontaneous reactivation of HSV-1 from latency. J Virol. 1994; 68(12):8045–8055. [PubMed: 7966594]

Perng G, Jones C, Ciacci-Zanella H, Henderson G, Yukht A, Slanina S, et al. Virus induced neuronal apoptosis blocked by the herpes simplex virus latency associated transcript (LAT). Science. 2000; 287:1500–1503. [PubMed: 10688801]

Perng GC, Maguen B, Jin L, Mott KR, Osorio N, Slanina SM, et al. A gene capable of blocking apoptosis can substitute for the herpes simplex virus type 1 latency-associated transcript gene and restore wild-type reactivation levels. J Virol. 2002; 76(3):1224–1235. [PubMed: 11773398]

Rock DL, Nesburn AB, Ghiasi H, Ong J, Lewis TL, Lokensgard JR, et al. Detection of latency-related viral RNAs in trigeminal ganglia of rabbits latently infected with herpes simplex virus type 1. J Virol. 1987; 61(12):3820–3826. [PubMed: 2824816]

Rock D, Lokensgard J, Lewis T, Kutish G. Characterization of dexamethasone-induced reactivation of latent bovine herpesvirus 1. J Virol. 1992; 66(4):2484–2490. [PubMed: 1312639]

Scheid MP, Woodgett JR. Unravelling the activation mechanisms of protein kinase B/Akt. FEBS Lett. 2003; 546(1):108–112. [PubMed: 12829245]

Takita J, Yang HW, Bessho F, Hanada R, Yamamoto K, Kidd V, et al. Absent or reduced expression of the caspase 8 gene occurs frequently in neuroblastoma, but not commonly in Ewing sarcoma or rhabdomyosarcoma. Med Pediatr Oncol. 2000; 35(6):541–543. [PubMed: 11107112]

Teitz T, Wei T, Valentine MB, Vanin EF, Grenet J, Valentine VA, et al. Caspase 8 is deleted or silenced preferentially in childhood neuroblastomas with amplification of MYCN. Nat Med. 2000; 6(5): 529–535. [PubMed: 10802708]

Troy CM, Rabacchi SA, Hohl JB, Angelastro JM, Greene LA, Shelanski ML. Death in the balance: alternative participation of the caspase-2 and -9 pathways in neuronal death induced by nerve growth factor deprivation. J Neurosci. 2001; 21(14):5007–5016. [PubMed: 11438576]

Upton JP, Austgen K, Nishino M, Coakley KM, Hagen A, Han D, et al. Caspase-2 cleavage of BID is a critical apoptotic signal downstream of endoplasmic reticulum stress. Mol Cell Biol. 2008; 28(12): 3943–3951. [PubMed: 18426910]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

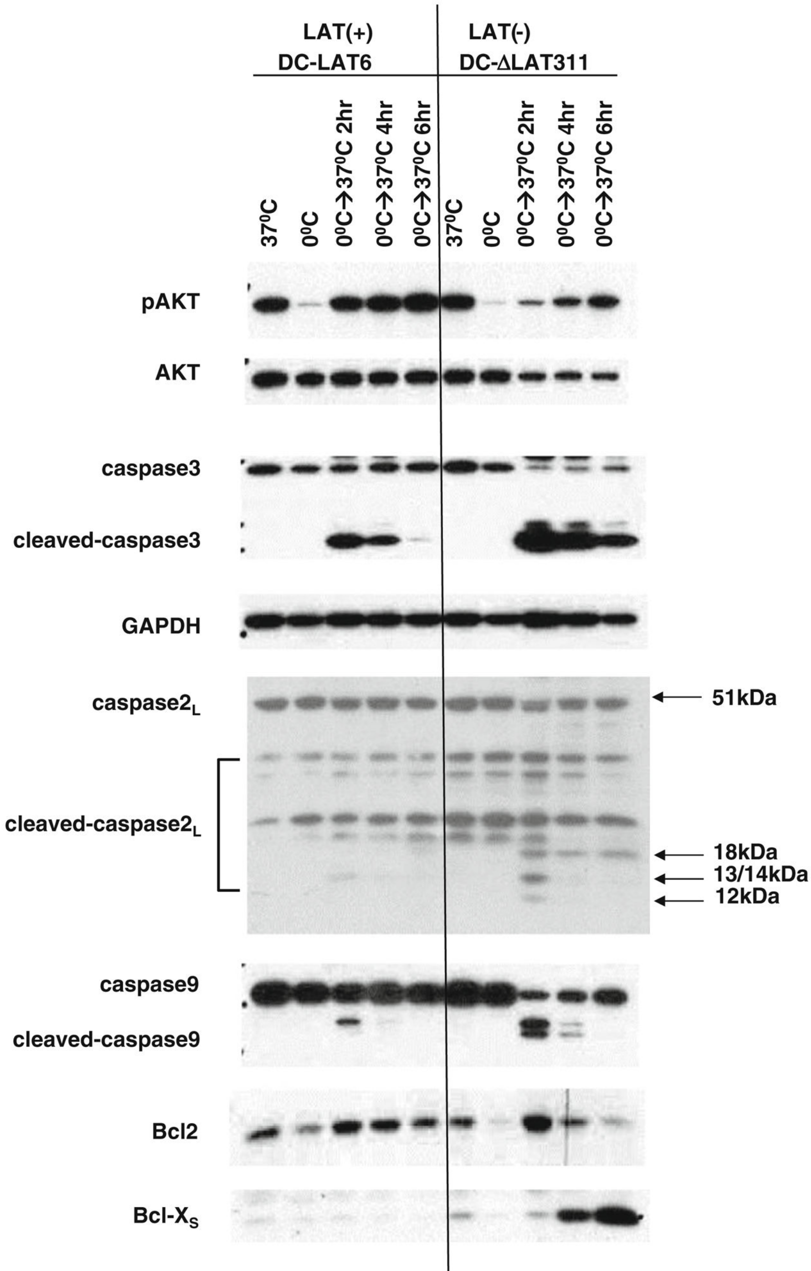

Fig. 1.

LAT maintains AKT phosphorylation during the first 2 to 6 h of recovery from cold shock. Monolayers of 60–70 % confluent DC-LAT6 or DC- LAT311 cells were cold shocked for 2 h and then returned to 37 % for 0, 2, 4, or 6 h as described in "Materials and methods." Cells were harvested and Western blots performed as described in "Materials and methods" at the indicated times. The images shown are from one blot that was striped and re-probed multiple times with different antibodies and are representative of at least two independent experiments. *Lanes*: 37 °C=control cells, no cold shock; 0 °C=cells harvested after cold shock, no 37 °C recovery; 0 °C→37 °C 2, 4, or 6 h=cells cold shocked then recovered for either 2, 4, or 6 h at 37 °C. GAPDH is a loading control

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

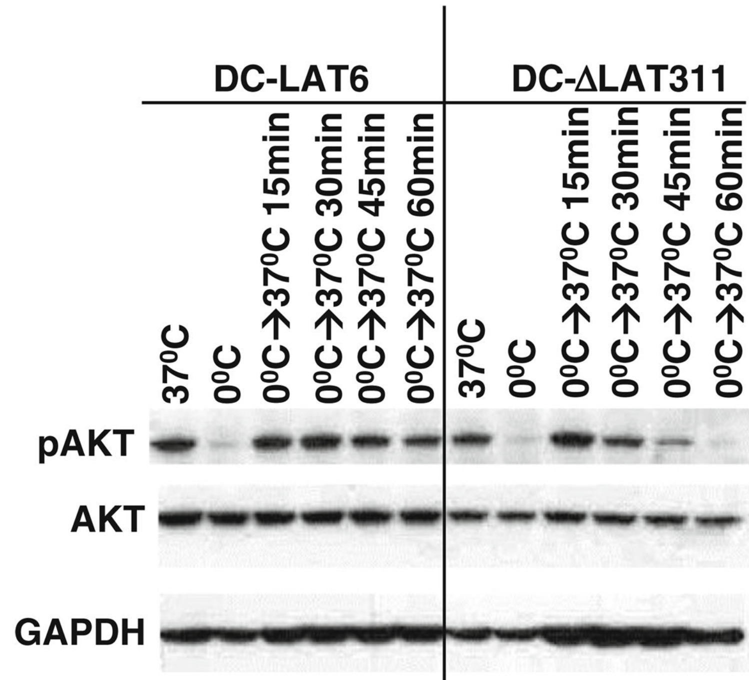

Fig. 2.

LAT maintains AKT phosphorylation during 15 to 60 min of recovery from cold shock. DC-LAT6 and DC- LAT311 cells were cold shocked, recovered, and analyzed by Western blot as in the legend to Fig. 1, except that recovery times at 37 °C were 15, 30, 45, or 60 min as indicated *above each lane*

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

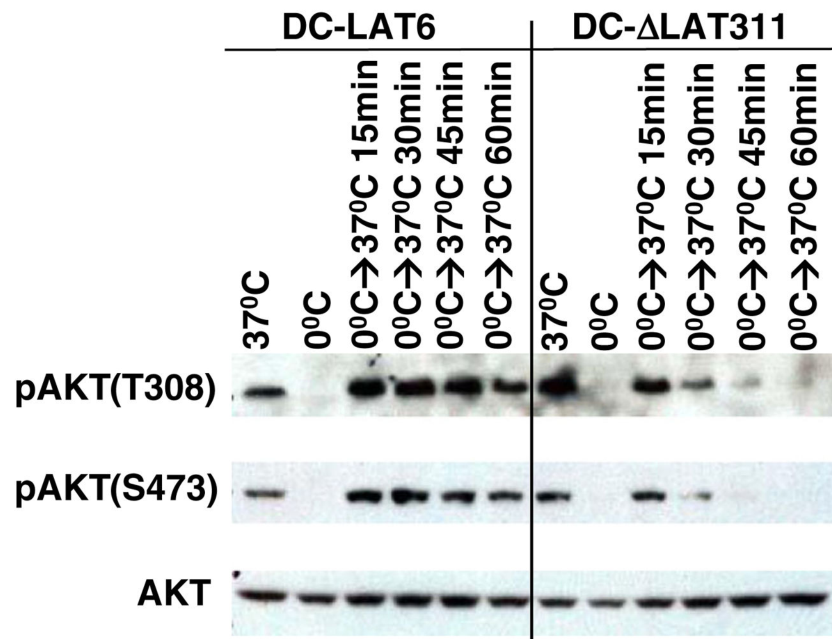

Fig. 3.

LAT maintains AKT phosphorylation at both Threonine 308 and Serine 472 residues. DC-LAT6 and DC- LAT311 cells were cold shocked, recovered, and analyzed by Western blot as in the legend to Fig. 2. Antibody specific for pAKT at residue T308, or specific for pAKT at residue S473, were used as indicated. GAPDH levels show equal loading over the gel

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

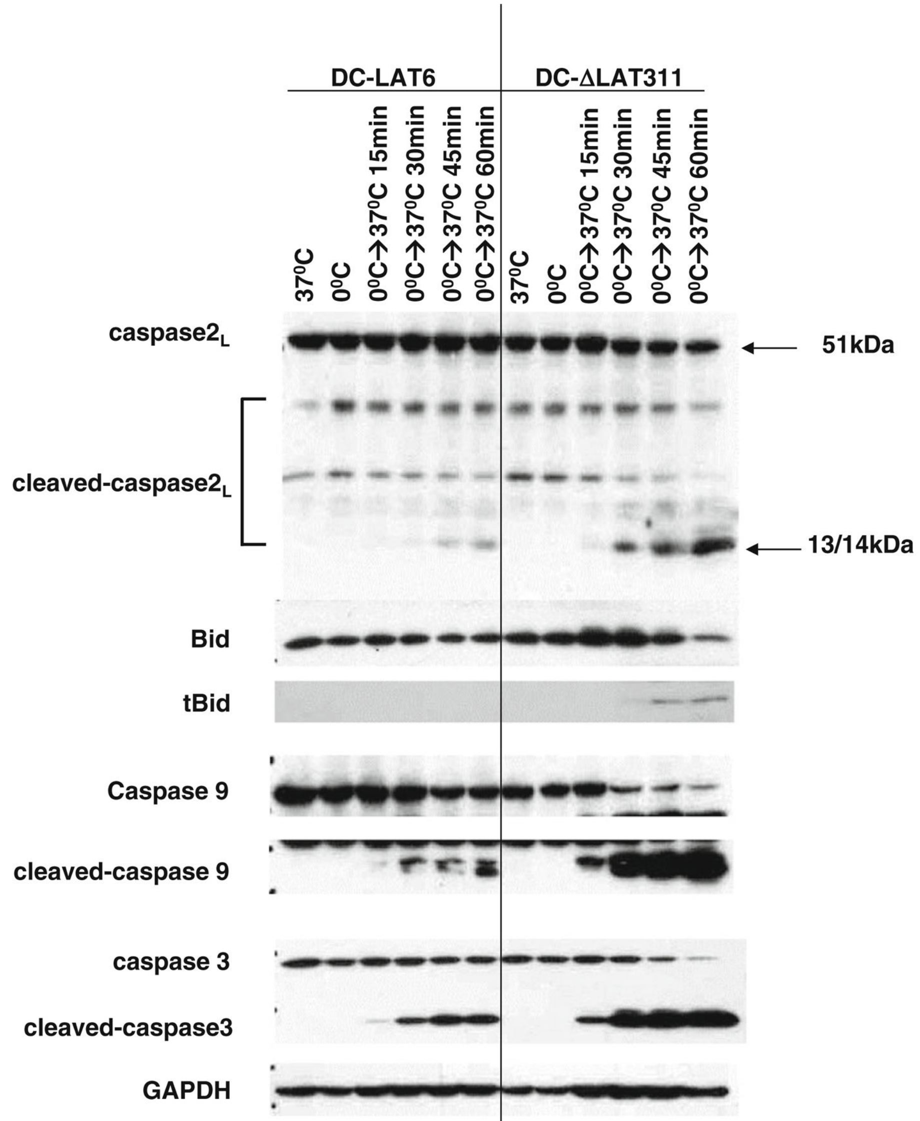

Fig. 4.

Changes in additional apoptotic factors affected by LAT shortly after recovery from cold shock. DC-LAT6 and DC- LAT311 cells were cold shocked, recovered at 37 °C for the indicated times, and analyzed by Western blot as described in the legend to Fig. 1. Each result is representative of at least 2 independent experiments. The *blots* shown were processed by multiple cycles of stripping and re-probing of the Western blot shown in Fig. 2

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

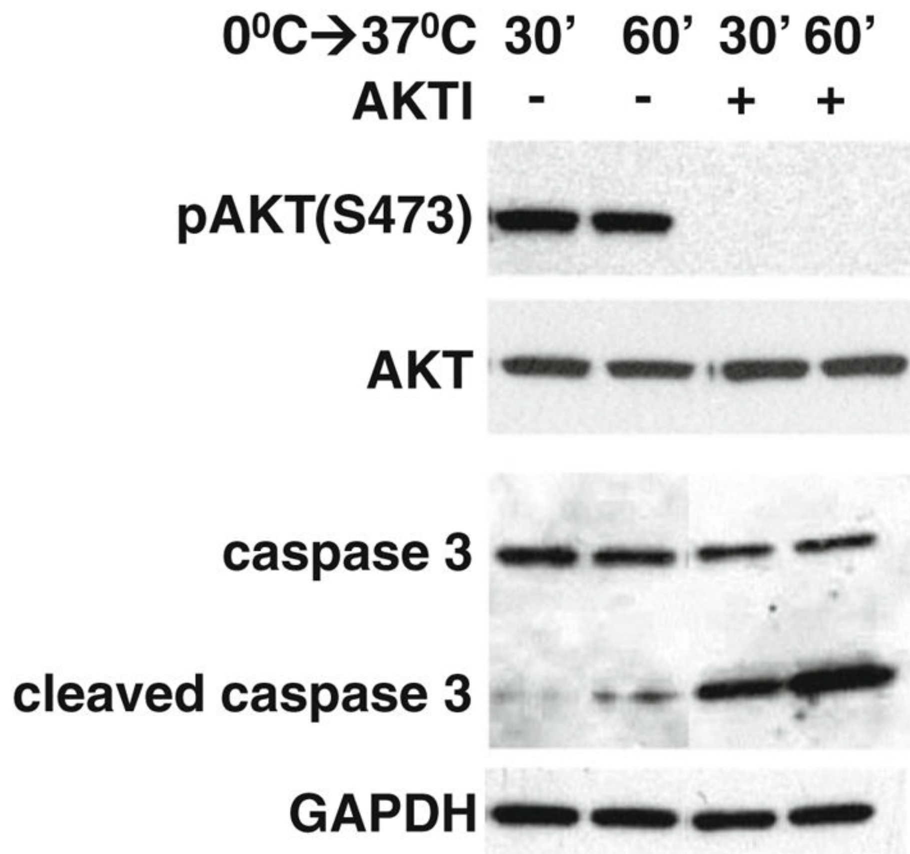

Fig. 5.
Inhibition of AKT phosphorylation reduces cold-shock-induced apoptosis in DC-LAT6 cells. DC-LAT6 cells were cold shocked in media containing 20 μM AKTI (AKT VIII, an inhibitor of AKT phosphorylation) recovered for either 30 or 60 min at 37 °C and analyzed by Western blot as described in "Materials and methods"