Adenovirus vectors activate survival pathways in lung epithelial cells

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It is well documented that first-generation recombinant adenovirus vectors induce potent host immune responses that promote inflammation and activation of a number of cell signaling pathways. Adenovirus vectors have been shown to modulate the MAP kinase signal transduction pathway, affecting intracellular signaling. In A549 cells, adenoviral vectors induce ERK, JNK, and p38 MAP kinase phosphorylation within minutes of addition of virus, resulting in downstream NF-κB activation with induction of ICAM mRNA (54). P38 and ERK are activated in kidney-derived epithelial cells in response to adenovirus infection with subsequent increases in expression of the CXC chemokine interferon-γ-inducible protein 10 (IP-10) (56). Li and colleagues (34) have shown that adenovirus serotype 2 infection in a colon adenocarcinoma cell line, SW480, results in phosphorylation of the integrin-associated focal adhesion complex protein, p125FAK and p130CAS, with downstream activation of phosphatidylinositol 3-kinase (PI3-kinase) (5, 56). The activation of PI3-kinase appears to be essential for adenovirus internalization and promotes reorganization of the actin cytoskeleton, a requirement for efficient entry of virus into the cell (32). These are early immune responses to the virus and do not appear to be dependent on viral gene expression (19, 23, 54).

Adenovirus vectors also trigger an adaptive immune response, making the cells more susceptible to host-mediated elimination that involves cytotoxic T-cell killing of the virus-infected cells (8, 27, 62, 63). This, in fact, has been one major limitation in the development of these vectors for in vivo gene therapy since rapid host elimination occurs, making persistent expression of the desired gene product difficult. Although wild-type adenovirus is well documented to have ant apoptotic effects on the host cell (17, 37, 47, 57), the only studies that have shown adenoviral vector-mediated enhancement in cell survival and inhibition of apoptosis have been of the endothelium (21, 50, 65). In these studies, human umbilical vein endothelial cells were infected with replication-deficient adenovirus vectors deleted in the E1 and E3 viral genes but expressing the E4 genes and were found to have increased viability in culture. These investigators also found that apoptosis was suppressed in these cells and that this enhanced survival phenotype was dependent on the expression of the viral E4 genes.

Because airway epithelial cells are an important site of adenovirus vector infection, we sought to determine the effects of adenovirus vectors on cell survival and resistance to apoptosis in human bronchial epithelial (HBE) and A549 cells. In the following studies, we show that adenovirus vectors are protective against TNF-α-induced apoptosis. We also show that this effect of the vectors is due to the activation of the prosurvival pathways, ERK and Akt.

MATERIALS AND METHODS

Reagents. LY-294002 and U-0126 were purchased from Calbiochem. Ser473-phospho-Akt, phospho-ERK, and cleaved poly(ADP-ribose) polymerase (PARP, Asp214) antibodies were purchased from Cell Signaling Laboratories (Beverly, MA). Antibodies against total ERK and total Akt were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The active caspase-3 antibody was purchased from Cell Signaling Laboratories (Beverly, MA). Phosphatidylinositol 3-kinase (PI3-kinase) was purchased from Cell Signaling Laboratories (Beverly, MA). Recombinant human TNF-α was purchased from R & D Systems (Minneapolis, MN), and actinomycin D was from Calbiochem (San Diego, CA).

Cell culture. A549 cells, a tumor cell line with properties of normal airway epithelial cells (30, 41), were obtained from American Type Culture Collection (Manassas, VA) and incubated at 37°C in 5% CO₂.
The cells were cultured in Eagle’s minimum essential medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (HyClone, Logan, UT) and 40 mg/ml gentamicin and were subcultured by harvesting in 0.12% trypsin no more than 20 times from stock originally designated at passage 70. HBE cells, kindly provided by Dr. Dwight Look at our institution, were obtained from human donor lungs and cultured as previously described (22). All protocols were approved by the University of Iowa Institutional Review Board.

**Adenoviral vectors and wild-type adenovirus.** First-generation recombinant adenovirus was generated by the University of Iowa Gene Transfer Vector Core (2). The particle titer of adenoviral stocks were typically 10^{12} DNA particles/ml; functional titers were ~4 × 10^{11} plaque-forming units/ml. Adenovirus vectors expressing the transgene for green fluorescent protein (AdGFP) driven by the cytomegalovirus promoter or an empty vector containing no transgene (AdEV) were used to transduce the cells at a multiplicity of infection (MOI) of 100. These vectors were free of wild-type virus contamination as determined by plaque assay and PCR (64). Wild-type adenovirus serotype 5 was obtained from Advanced Technologies (Columbia, MD). The virus particle titers were 7.9 × 10^9, and cells were infected at an MOI of 10. Cells were plated and grown overnight and then virus was added for 2 h in serum-free media. In the A549 cells, fetal bovine serum was added back to the cultures to a final concentration of 10%. HBE cells were grown in specially supplemented media as previously described (22). The cells were incubated at 37°C for 24 h and harvested for total cellular protein. In some studies, the cells were treated for 1 h with 20 μM LY-294002 (an inhibitor of PI3-kinase), 10 μM U0126 (an inhibitor of ERK), or both inhibitors before viral infection. Efficiency of transduction was determined in each experiment by examining green fluorescence of the AdGFP-infected cells under a Leica DMIRB inverted fluorescence microscope (Wetzlar, Germany). Similar transduction efficiencies were assumed with the AdEV virus. For UV inactivation, recombinant adenovirus particles (AdGFP or AdEV at 1 × 10^{12} particles/ml were placed in 250 μl of serum-free medium per well in a 24-well tissue culture plate and exposed to varying amounts of UV irradiation with a Stratalinker 1800 (Stratagene, La Jolla, CA). A dose-response curve was created, and an energy amount was selected (0.94 J) that resulted in <1/25,000 infected cells per well, as detected by green fluorescence (data not shown). For these experiments, cells were plated and treated as described above, and freshly UV-inactivated virus was added to the cells for the 24-h infection time. Lack of GFP expression in the UV-inactivated AdGFP-infected cells was confirmed by fluorescence microscopy (data not shown). To confirm that the UV-inactivated virus remained intact, we examined cells infected with untreated and UV-inactivated virus using transmission and scanning electron microscopy.

**Determination of apoptosis.** Terminal deoxynucleotidyltransferase deUTP nick end labeling (TUNEL) analysis for DNA fragmentation was carried out using the In Situ Cell Death Kit (Roche Diagnostics, Indianapolis, IN). Briefly, either HBE or A549 cells were plated on glass chamber slides at 5 × 10^4 cells/well and grown overnight. The following day, they were infected with the adenovirus vectors (AdGFP or AdEV) at 100 MOI as described above. After 20–24 h of infection, cells were treated with TNF-α (1 ng/ml) plus actinomycin D (2.5 μg/ml) for 12–18 h. After treatment, cells were fixed, washed, permeabilized, and then stained with the TUNEL reaction mix according to the manufacturer’s protocol. Red fluorescence cells were counted in a blinded fashion under a fluorescent microscope. Cells (200–400) were counted for each sample, and percent apoptotic cells was determined. A second method used to examine apoptosis was Western analysis with a polyclonal antibody directed against cleaved PARP.

**Western analysis.** After experimental exposure, we washed cells in sterile PBS and harvested them by scraping them into lysis buffer (0.05 M Tris, pH 7.4, 0.15 M NaCl, and 1% Nonidet P-40) with added protease (EDTA-free mini-tab, Roche Diagnostics) and phosphatase (Calbiochem) inhibitors. The cell material was sonicated for 20 s on ice, allowed to sit for 20 min, and then centrifuged at 15,000 g for 10 min. The protein concentration in the lysate supernatant was measured by the Bradford assay normalized to bovine serum albumin. Equal amounts of protein (30–50 μg) were mixed 1:1 with 2× sample buffer (20% glycerol, 4% SDS, 10% 2-mercaptoethanol, 0.05% bromphenol blue, and 1.25 M Tris, pH 6.8; all chemicals from Sigma Chemical), loaded onto a 10% SDS-PAGE gel, and run at 100 V for 2 h. Cell proteins were transferred to nitrocellulose (ECL; Amersham, Arlington Heights, IL), blocked with 5% milk in Tris-buffered saline with 0.1% Tween 20 for 1 h, washed, and then incubated with the primary antibody (1:1,000 for phospho-ERK, 1:200 for phospho-Akt) for 1 h. The blots were washed and incubated with a horseradish peroxidase-conjugated secondary antibody and developed with a chemiluminescent substrate, ECL Plus (Amersham). After development of phosho-Akt and phospho-ERK, we removed bound immunoglobulins from the membranes by washing them twice at 30 min each at room temperature in ImmunoPure IgG Elution Buffer (Pierce), and the membranes were reprobed for total Akt and ERK.

**Statistical analysis.** One-way analysis of variance with multiple comparisons and paired t-tests were performed for all statistical parameter calculations.

**RESULTS**

**Adenovirus vectors protect airway epithelial cells from TNF-α-mediated apoptosis.** TNF-α binding to its receptor can induce apoptosis by a signaling pathway that involves activation of caspases, cleavage of downstream substrates, and characteristic changes in cells, resulting in eventual cell death (44). To address the question of whether adenovirus vectors could affect the cellular response to apoptosis, we examined the extent of apoptosis in A549 and HBE cells using a known inducer of apoptosis, TNF-α plus actinomycin D. We first showed that TNF-α/actinomycin D induced apoptosis by evaluating active caspase-3 by Western blot analysis. Both exposures caused a clear upregulation of active caspase-3 (data not shown), as previously reported (38, 39, 58, 59). Using TUNEL staining and the detection of cleaved PARP, we also showed that TNF-α/actinomycin D induces apoptosis in both the A549 cell line and primary HBE cells. In Fig. 1, we show that TNF-α plus actinomycin D induces apoptosis in >20% of the cells (Fig. 1, A and B, A549 and HBE cells, respectively). When these cells are infected with a first-generation adenovirus vector containing the nonspecific transgene AdGFP or an empty vector, AdEV, 24 h before addition of the TNF-α and actinomycin D, apoptosis is reduced by >70% (P < 0.01). Another well-established method for the determination of apoptosis is detection of cleaved PARP. PARP is a nuclear poly(ADP-ribose) polymerase that is involved in DNA repair in response to environmental stress. It is one of the main targets for cleavage by caspases, and the presence of the cleaved PARP protein serves as a marker for apoptosis (48). Using this method for detection of apoptosis, we also showed that cells infected with adenovirus vectors before treatment with the proapoptotic stimuli had less cleaved PARP than cells treated with TNF/actinomycin alone. Although the vectors inhibited apoptosis induced by TNF-α/actinomycin D, the vectors themselves induced apoptosis in a small percentage of the cells.

**Adenovirus vectors activate ERK at late time points after infection.** Replication-deficient adenoviral vectors have been described to activate the ERK MAP kinase signal transduction pathway (3, 6, 54). These prior studies examined the host
response to adenovirus vector infection and showed early activation of this pathway that was independent of viral gene transcription and was accompanied by the activation of downstream proinflammatory cytokines. Because signaling through the ERK MAPK pathway has an important role in cell survival, we asked whether activation of ERK might be involved in the protection against apoptosis observed in the adenovirus-infected cells. We treated A549 and HBE cells with and without a specific MEK1/MEK2 inhibitor (U0126) followed by transduction with a first-generation adenoviral vector (AdGFP or AdEV) and examined activation of ERK using a phosphospecific ERK antibody after 24 h of infection. Figure 2A shows that A549 cells are transduced with 100% efficiency by the AdGFP vector at an MOI of 100. In Fig. 2B, we show that both the viral vectors significantly activated ERK above control levels (P < 0.005), and MEK1/2 inhibition blocks this effect without a change in levels of total ERK. When we performed this experiment using HBE cells, we observed similar results (Fig. 2C) using either the adenovirus vectors or the wild-type virus. In time course studies of viral infection, we observed a biphasic activation of both ERK and Akt at 1 h postinfection that quickly returned to control levels, increasing again at 12 h, with persistent activation out to 24 h (data not shown). Results were similar regardless of serum conditions (10 vs. 0.5%).

**Adenovirus vector activation of Akt is dependent on PI3-kinase.** Others have shown that adenovirus entry into the host cell is dependent on αv-integrin binding and requires PI3-kinase activation (33). The relationship between activation of PI3-kinase in response to adenovirus infection and enhanced cell survival in epithelial cells, however, has not been previously described. To assess the activation of PI3-kinase by adenovirus, we infected A549 and HBE cells with the adenovirus vector AdGFP or the empty vector AdEV and looked at phosphorylation of the downstream kinase Akt 24 h postinfection. Figure 3 illustrates a significant increase in Akt activation with both adenoviral vectors (P < 0.005) that is blocked with the PI3-kinase inhibitor LY-294002, suggesting that this activation of Akt is PI3-kinase dependent. Figure 3A shows the results obtained in A549 cells, and Fig. 3B shows data using HBE cells. When we performed a similar experiment using the wild-type adenovirus, we observed the same results. These data demonstrate that adenovirus vector infection of lung epithelial cells leads to activation of PI3-kinase and the downstream kinase Akt.

**Adenovirus-mediated ERK and Akt activation occurs via independent pathways.** To test the hypothesis that adenovirus-mediated activation of the two survival pathways, ERK and Akt, occur independently, we treated A549 cells with and
without inhibitors of these pathways and examined activation of ERK and Akt. Similar to the results shown in Figs. 2 and 3, both adenovirus vectors activated ERK and Akt at 24 h after infection. Specific inhibition of the MEK1/2 with U0126 did not affect phosphorylated Akt, nor did inhibition of PI3-kinase with LY-294002 affect ERK phosphorylation (data not shown). This suggests that activation of ERK and Akt by adenovirus occurs independently, via two distinct pathways.

**ERK and Akt activation by adenovirus requires viral gene transcription.** Adenovirus has been shown to stimulate the host inflammatory response, resulting in the production of proinflammatory cytokines and chemokines and the activation of a number of signal transduction pathways including MAP kinases, focal adhesion kinase, and PI3-kinase (6, 19, 33, 34, 56). These responses occur early after virus binding and are independent of viral gene transcription. Because we noted a late
increase in ERK and AKT phosphorylation, 24 h after virus infection, we asked whether or not viral gene transcription is required for this activation. In this set of experiments, the adenovirus vectors AdGFP and AdEV were inactivated by UV irradiation. The exact joules used for inactivation were determined by a dose-response experiment in which varying amounts of energy were used to inactivate the virus followed by the standard infection protocol (see MATERIALS AND METHODS for details). We used the energy level that abolished all but ~0.01% of infectivity as measured by counting fluorescent cells in AdGFP-infected cells. Also, to confirm that the UV irradiation was not damaging the virus particle, we examined cells infected with either control or UV-inactivated AdGFP using scanning and transmission electron microscopy. These studies showed that the virus particles were intact and bound normally after UV inactivation (data not shown). Figure 4 shows that infection with adenovirus vectors increases ERK and Akt activation. In Fig. 4A, transduction with either AdGFP or AdEV results in increased phospho-ERK. This increase is abolished in the UV-inactivated virus-treated cells. Similarly, Fig. 4B shows activation of Akt in response to adenovirus vector transduction that disappears in the UV-inactivated virus groups. Similar results were obtained in separate experiments performed in HBE cells (data not shown). To determine that the lack of GFP fluorescence was not due to decreased amounts of virus from nonspecific binding to the plate, we performed a parallel experiment in which virus was plated but not irradiated and then added to the cells. In these cells, transduction efficiency was 100% as determined by fluorescence microscopy. This supports the hypothesis that late activation of ERK and Akt by adenovirus vectors requires transcriptionally competent virus.

**ERK and Akt activity is required for protection against apoptosis in adenovirus vector-infected cells.** Our data show that adenovirus vector infection is protective against TNF-mediated apoptosis in airway epithelial cells and that, as a result of virus infection, cells upregulate ERK and Akt activation. To assess the role of ERK and Akt in protection against apoptosis in adenovirus vector-infected airway epithelial cells, we examined apoptosis and cell survival after treatment of the cells with specific inhibitors of these pathways. Figure 5A shows the Western analysis for cleaved PARP from cells treated with an inhibitor of PI3-kinase activation (LY-294002) or ERK (U0126) with and without prior adenovirus vector
infection. In cells treated with the inhibitors alone, there is very little apoptosis; however, if ERK or Akt activation is inhibited before adenovirus vector transduction, there is a marked increase in apoptosis. Importantly, the adenovirus vectors cause little or no apoptosis in the absence of other stimuli/inhibitors (see Fig. 1). Figure 5B shows the effect of ERK or Akt inhibition on cell survival and apoptosis in control and virus-infected cells after exposure to the proapoptotic stimulus TNF-α plus actinomycin D. In this experiment, cells were transduced with the adenovirus vector AdGFP for 20–24 h and then treated with TNF/actinomycin or TNF/actinomycin plus inhibitors of ERK or Akt. Cell death measurements were obtained at 12 h posttreatment. As shown, TNF/actinomycin induces marked cell death, and prior viral infection signifi-

Fig. 4. ERK and Akt activation by adenovirus requires viral gene transcription. A549 cells were infected with active or UV-inactivated AdGFP and AdEV, and cells were harvested for total cell protein after 24 h. Western analysis was performed with phospho-specific antibodies to ERK (A) and Akt (B). After development of the immunoreactive bands, IgG was eluted from the membranes, and they were reprobed with antibodies against total ERK and total Akt. These blots are representative of 3 separate experiments. Identical results were obtained in similar experiments with HBE cells (results not shown).

Fig. 5. ERK and Akt are important for cell survival in adenovirus vector-infected cells. Either A549 or HBE cells were infected with the recombinant AdGFP vector for 24 h and then treated with U0126 (20 μM), an inhibitor of ERK activation; LY-294002 (20 μM), an inhibitor of PI3-kinase; or both for 18 h. In some experiments, cells were also treated with TNF-α plus actinomycin D (T/A) to induce apoptosis. A: apoptosis as measured by the presence of cleaved PARP. B: effect of ERK and Akt inhibition on cell survival (graph) and apoptosis (Western analysis) in virus-infected cells. Percent cell death was assessed with trypan blue staining of dead cells. A polyclonal antibody against cleaved PARP was used for the Western blot.
cantly reduces this. If ERK or Akt activation is inhibited in the virus-infected cells, there is a loss of protection from apoptosis. Because the inhibitors are in a DMSO solution, we also performed a DMSO control that showed no differences in apoptosis compared with controls (data not shown).

**DISCUSSION**

In these studies, we show that adenovirus vectors protect epithelial cells in vitro against TNF-α-induced cell death by activating two important prosurvival cell signaling pathways (ERK and PI3-kinase). Because the ERK and PI3-kinase signaling pathways modulate inflammation, as well as apoptosis, the observations of this study are consistent with earlier studies that evaluated the effect of adenovirus on inflammation (5, 6, 8, 11, 35, 43, 45, 46, 54, 56). Activation of inflammatory responses has been well described in utilizing first-generation recombinant adenovirus vectors. Adenovirus has also been shown to modulate a number of cell signaling pathways, including ERK and PI3-kinase, although the exact mechanisms through which the virus affects signal transduction are not well understood. Bruder and Kovessi (6) found that HeLa cells infected with adenovirus vectors exhibited increases in Raf-1 and p42 (ERK) activation after infection. This occurred with a variety of adenovirus vectors and was correlated with increases in IL-8 expression. Tibbles and colleagues (56) also demonstrated activation of the MAP kinase pathway in response to adenovirus vector infection. In a mouse kidney cell line, they found that activation of ERK and p38 occurred early after adenovirus infection and was linked to downstream IP-10 expression. Finally, Tamanini et al. (54) have shown that adenovirus vectors stimulate MAP kinase activation and increases in NF-κB DNA binding after transduction of A549 cells and that this induces ICAM-1 gene expression. Similarly, we observed activation of ERK at early time points after viral infection. Activation occurred at 1 h postinfection, diminished to control levels by 3 h, and peaked again at 12 h with persistent activation to 24 h. This later activation coincides with late viral gene transcription and is abolished by UV inactivation of virus, suggesting that viral gene transcription is involved.

PI3-kinase has also been shown to be activated in response to adenovirus infection. Li and colleagues (34) examined mechanisms of integrin-mediated virus entry into the cell and found that adenovirus internalization and downstream activation of p130Cas were dependent on PI3-kinase. They also showed, in a separate study, that the Rho GTPases Cdc42 and Rac1 could be activated downstream of PI3-kinase by adenovirus infection, promoting endocytosis of the virus (32). Our data showed that Akt, a protein activated downstream of PI3-kinase, was minimally activated at 1 h postinfection and did not show activation again until 12 h after adenovirus transduction. As seen with ERK, activation of Akt by the adenovirus vectors was abolished if cells were infected with UV-treated, transcriptionally inactive virus.

Both Akt and ERK play important roles in cell survival. Akt, or protein kinase B, is an important effector protein for modulating various cell survival pathways. It is present as a cytoplasmic protein that is activated via phosphorylation by its upstream kinase, PI3-kinase (1). Akt is involved in the regulation of cell survival and protection from apoptosis induced by oxidant injury, Fas, UV irradiation, serum withdrawal, c-Myc, and anoikis (10, 13, 24, 25, 29, 36). It inhibits apoptosis by decreasing activation of caspase-3, caspase-9, Bad, and additional proapoptotic pathways linked to forkhead transcription factors and glycogen synthase kinase 3 (7, 28, 40). In our studies, we observed, consistent with prior studies, that TNF/actinomycin D triggered apoptosis by a process that involved caspase-3, resulting in downstream cleavage of PARP.

ERK activation via the MAP kinase signaling pathway is also important in modulating cell growth and survival (4, 15, 26). Signaling through this pathway usually involves a receptor-mediated event that may involve integrins, receptor tyrosine kinases, or Ca²⁺ influx via ion channels. This results in activation of Ras that allows for recruitment of Raf, downstream phosphorylation of MEK1/2, and subsequent activation of ERK. Activated ERK can then translocate to the nucleus and alter the activity of a number of transcription factors involved in cytokine gene transcription and inhibition of apoptosis (4, 14, 53). Activated ERK has also been shown to inhibit caspase-3 activation, a process that is relevant to these studies (12, 55, 61). We have shown that adenovirus vectors protect epithelial cells from TNF-induced apoptosis and that this effect is, in part, dependent on ERK and Akt activation, since inhibition of these pathways using specific chemical inhibitors results in loss of protection from apoptosis in adenovirus vector-transduced cells. Although wild-type adenovirus is well documented to have antiapoptotic effects on the host cell (17, 37, 47, 57), the only other studies that have shown adenovirus vector-mediated enhancement in cell survival and inhibition of apoptosis have been of the endothelium. Ramalingam and colleagues (51) found that cells infected with adenovirus vectors deleted in the E1 and E3 regions but containing the E4 gene (E1-E4+) exhibited a phenotype different than that of “usual” cultured endothelium and had prolonged viability in culture. They showed that the virus-infected cells had increased Bcl2, an important antiapoptotic protein, and decreased Bax, an initiator of apoptosis, which they hypothesized was involved in the observed changes in cell survival. They subsequently found that this effect was mediated by the adenovirus E4 region (65).

In wild-type adenovirus infections, a number of viral gene products are involved in modulating cellular metabolism, making the host cell more susceptible to viral replication and preventing host cell apoptosis (52). The E1B 19K protein is analogous to Bcl2 and blocks apoptosis by inactivating Bax, a protein involved in the induction of caspases in the death receptor pathway (17, 47). The E1A 12S protein also enables cells to overcome apoptosis and promotes oncopogenesis (49). The E2 gene products are not known to be involved in cell survival, and their primary function is to provide the machinery for viral gene replication (18). E3 genes are involved in the inhibition of Fas-Fas ligand mediated apoptosis (37) and the cellular immune response to viral infection (20, 60). The adenovirus vectors used in these studies do not have fully functional E1 and E3 regions; therefore, the effects of these viral gene products cannot explain the observations of this study. The E4 genes, which are present in the adenovirus vectors, regulate viral DNA replication and downregulate host protein synthesis (16, 31). They may also block apoptosis by interfering with p53 transcriptional activation (9, 42). As suggested by Ramalingam and colleagues (51), any of the E4 gene...
products could also be linked to protection against apoptosis. Our studies show that adenovirus vector infection induces late activation of two important prosurvival pathways, ERK and Akt, a process that is dependent on viral gene transcription, although which viral genes are involved has yet to be defined. Furthermore, activation of these pathways contributes to cell survival in the face of apoptotic signals. The use of adenovirus vectors as a gene delivery system via the airways increases the importance of understanding epithelial biology subsequent to vector infection. This is the first study to demonstrate a role for adenovirus vectors in preventing cell death in lung epithelial cells. It will be interesting to determine whether this observation alters the normal response of the lung epithelium to viral or bacterial infections.

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