CALL FOR PAPERS | Translational Research in Acute Lung Injury and Pulmonary Fibrosis

Role of GADD45a in murine models of radiation- and bleomycin-induced lung injury

Biji Mathew,1* Daisuke Takekoshi,1,2* Saad Sammani,1 Yulia Epshtein,1 Rajesh Sharma,1 Brett D. Smith,3 Sumegha Mitra,1 Ankit A. Desai,1 Ralph R. Weichselbaum,3 Joe G. N. Garcia,4 and Jeffrey R. Jacobson1

1Division of Pulmonary, Critical Care, Sleep and Allergy, University of Illinois at Chicago, Chicago, Illinois; 2Department of Respiratory Medicine, Tohoku University Hospital, Miyagi, Japan; 3Department of Radiation Oncology, University of Chicago, Chicago, Illinois; and 4Arizona Health Sciences Center, University of Arizona, Tucson, Arizona

Submitted 3 June 2014; accepted in final form 25 August 2015

Mathew B, Takekoshi D, Sammani S, Epshtein Y, Sharma R, Smith BD, Mitra S, Desai AA, Weichselbaum RR, Garcia JG, Jacobson JR. Role of GADD45a in murine models of radiation- and bleomycin-induced lung injury. Am J Physiol Lung Cell Mol Physiol 309: L1420–L1429, 2015. First published October 23, 2015; doi:10.1152/ajplung.00146.2014.—We previously reported protective effects of GADD45a (growth arrest and DNA damage-inducible gene 45 alpha) in murine ventilator-induced lung injury (VILI) via effects on Akt-mediated endothelial cell signaling. In the present study we investigated the role of GADD45a in separate murine models of radiation- and bleomycin-induced lung injury. Initial studies of wild-type mice subjected to single-dose thoracic radiation (10 Gy) confirmed a significant increase in lung GADD45a expression within 24 h and persistent at 6 wk. Mice deficient in GADD45a (GADD45a−/−) demonstrated increased susceptibility to radiation-induced lung injury (RILI, 10 Gy) evidenced by increased bronchoalveolar lavage (BAL) fluid total cell counts, protein and albumin levels, and levels of inflammatory cytokines compared with RILI-challenged wild-type animals at 2 and 4 wk. Furthermore, GADD45a−/− mice had decreased total and phosphorylated lung Akt levels both at baseline and 6 wk after RILI challenge relative to wild-type mice while increased RILI susceptibility was observed in both Akt1−/− mice and mice treated with an Akt inhibitor beginning 1 wk prior to irradiation. Additionally, overexpression of a constitutively active Akt1 transgene reversed RILI-susceptibility in GADD45a−/− mice. In separate studies, lung fibrotic changes 2 wk after treatment with bleomycin (0.25 U/kg IT) was significantly increased in GADD45a−/− mice compared with wild-type mice assessed by lung collagen content and histology. These data implicate GADD45a as an important modulator of lung inflammatory responses across different injury models and highlight GADD45a-mediated signaling as a novel target in inflammatory lung injury clinically.

GOOGL (growth arrest and DNA damage-inducible 45 alpha) is a stress-induced gene that we previously reported is significantly upregulated in multispecies preclinical models of ventilator-induced lung injury (VILI) (9). GADD45a-silenced human lung endothelial cells are found to have decreased expression of Akt, a serine/threonine (Ser/Thr) protein kinase, whereas increased susceptibility to VILI is abrogated in GADD45a−/− mice overexpressing a constitutively active Akt1 transgene (23),(25). GADD45a encodes an 18-kDa protein that is constitutively expressed in low abundance but is transcriptionally activated by a variety of stress stimuli, including ultraviolet and ionizing radiation, and interacts with a number of intracellular signaling molecules involved in cell cycle regulation, apoptosis, maintenance of genomic stability, DNA methylation excision/repair, and immune responses (12). Nuclear localization of GADD45a and interaction with nuclear functional proteins associated with cell cycle arrest and cell apoptosis mediates repair of damaged DNA and inhibits the stress-response-induced segregation of damaged chromosomes (34), effects relevant to lung injury associated with both thoracic radiation and the administration of bleomycin.

Radiation is an important treatment modality for the management of a variety of malignancies. However, radiation-induced lung injury (RILI) characterized early by pneumonitis (6–24 wk) and later by fibrosis, is the dominant dose-limiting normal tissue side effect of thoracic radiotherapy and occurs in up to 30% of treated patients (36). Similarly, bleomycin, an antibiotic used to treat a variety of cancers, can also induce lung injury characterized initially by interstitial pneumonitis that may ultimately progress to pulmonary fibrosis. The incidence of bleomycin lung toxicity is dependent on the cumulative drug dose and may be as high as 20% in patients administered doses of 360 units or more (4–6, 18, 20). Although mechanisms underlying disease pathogenesis in both cases are poorly understood, both radiation and bleomycin can cause stress-induced generation of reactive oxygen and nitrogen species leading to cellular damage with apoptosis, inflammation, and dysregulated repair processes (8, 27, 35).

Given the role of direct cell damage in radiation- and bleomycin-induced lung injuries, the role of GADD45a as a cellular repair molecule and our previous findings of GADD45a as an important modulator of injury in a murine VILI model, we hypothesized that GADD45a is also a critical modulator of lung injury induced by either radiation or bleomycin via Akt1 induction. We utilized GADD45a−/− mice in previously established models of RILI (22) and bleomycin-induced lung injury (14) and assessed lung vascular permea-
bility and inflammation by bronchoalveolar lavage (BAL) fluid analyses. In addition, we investigated the role of Akt signaling in these settings. Our findings support GADD45α as an important determinant of injury in both models and further implicate a significant functional role for GADD45α in inflammatory lung injury in general.

MATERIALS AND METHODS

Reagents. Antibodies against p-Akt Ser/Thr (Cell Signaling Technology, Danvers, MA), total Akt (Santa Cruz Biotechnology, Santa Cruz, CA), GADD45α (Santa Cruz Biotechnology), β-actin (Sigma, St. Louis, MO), and horseradish peroxidase-conjugated anti-mouse and anti-rabbit secondary antibodies (Cell Signaling Technology) were commercially purchased. Akt Inhibitor XIII, Isozyme-Selective, Akt1-2/ and anti-rabbit secondary antibodies (Cell Signaling Technology) were purchased from EMD Millipore (Billerica, MA), and horseradish peroxidase-conjugated anti-mouse and -actin (Sigma, St. Louis, MO), and bl omycin was purchased from APP Pharmaceuticals (Skokie, IL).

Animals. All experiments and animal care procedures were approved by the University of Illinois at Chicago Animal Care and Use Committee. Female C57BL/6 and Akt−/− mice, 8–10 wk old (20–25 g), were purchased from Jackson Laboratory (Bar Harbor, ME) and housed in cages with free access to food and water in a temperature-controlled room with a 12:12-h dark-light cycle. GADD45α−/− and GADD45α−/− mice (129/Ola background) were generously provided by Dr. Michael O’Reilly (University of Rochester, Rochester, NY) and Dr. Albert Fornace (Brigham and Women’s Hospital, Boston, MA), respectively, and were backcrossed onto the C57BL/6 background for eight generations (13). These animals were similarly housed according to approved university protocols.

Preliminary model of RILI and bleomycin-induced lung injury. For the RILI model, anesthetized mice were subjected to thoracic irradiation as we have previously described (14). Each experimental group consisted of 10 mice subjected to a single dose of thoracic irradiation (10 or 20 Gy). The variation of the dose delivered within the lung estimated to be within ±5% of the prescribed dose with thermoluminescence dosimeters. For specified experiments, mice were treated with an Akt inhibitor, 1 mg/kg via intraperitoneal (IP) injection, 3×/wk beginning 1 wk prior to irradiation and continuing for up to 6 wk afterward. The dosing of the Akt inhibitor was based on our previous experience with these agents and was extrapolated from the existing literature (23). Mice were euthanized and BAL fluid protein and albumin content, total cell counts, and measurements of inflammatory cytokines were assessed as previously described (23). Lungs were harvested and stored at −80°C for Western blotting and histological evaluation.

For the intratracheal (IT) instillation of bleomycin, mice were anesthetized by IP injection of a mixture of ~100 mg/kg of ketamine and 10 mg/kg of xylazine. Isoflurane inhalation was added as needed to optimize the level of anesthesia. The trachea was then exposed by surgical cutdown and PBS or 40–60 μl of bleomycin solution (0.25–1.50 U/kg) was injected IT. Mice were euthanized at 1 or 2 wk and analysis was performed as described above.

Of note, we used a range of dosing for both the radiation and bleomycin experiments. In both cases dosing was based on the previous experience and published work of our laboratory (15, 22). In general, low-dose injury models (10 Gy radiation or 0.25 U/kg bleomycin) were used for experiments investigating relative susceptibility to injury. Conversely, we used high-dose injury models (20 Gy radiation or 1.5 U/kg bleomycin) for experiments designed to study conditions associated with protection from injury.

Delivery of Akt1 expression vector in vivo. Akt was overexpressed in the lungs of GADD45α−/− and wild-type mice by using a constitutively active Akt1 mammalian expression vector (Millipore) in PBS (50 μg/kg) or a control plasmid delivered IT as we have previously described (25). For RILI experiments, the Akt transgene or control plasmid was administered IT to mice once every week during the course of experiments. Mice were then subjected to irradiation (20 Gy) 3 days after the initial transfection and were then euthanized 6 wk.

---

**Fig. 1.** Radiation-induced increased GADD45α expression in murine lungs. A: GADD45α protein levels detected by Western blotting of lung homogenates from wild-type (WT) mice subjected to single-dose thoracic irradiation (IR, 10 Gy) are shown at various time points (n = 5 animals/condition, *P < 0.05 compared with control). Representative blot shown. Note: image was spliced to show relevant time points. B: immunohistochemical staining for GADD45α (brown) is shown in representative control and irradiated mouse lungs (10 Gy) at the time points shown. Areas of increased staining in bronchiolar epithelium and intra-alveolar septae consistent with increased GADD45α expression are indicated by small and large arrows, respectively (n = 3 mice sampled/condition with a minimum of 3 fields sampled from each animal, representative images from individual animals are shown; scale bar = 100 μm).
postirradiation. For experiments using the bleomycin-induced lung injury model, Akt transgene or control plasmid was administered IT 2×/wk beginning 1 wk prior to bleomycin and continuing until 3 wk postbleomycin. At the end of experiments, BAL fluid analyses were performed as described above and lung were harvested for histology.

**Lung histology and immunohistochemistry.** Lungs from animals in each experimental group were fixed in 10% formalin, embedded in paraffin, cut into 10-μm sections, and stained with either hematoxylin and eosin or Sirius red for collagen as we have previously described (14, 15). To evaluate the expression of GADD45a and Akt, lung sections were used for immunohistochemistry and stained with polyclonal antiserum to respective antibodies by the avidin-biotin-peroxidase method. Histology slides were scanned and evaluated with ImageScope (Aperio, Vista, CA).

**Western blotting.** Lung tissue was homogenized by use of a tissue homogenizer (TissueRuptor, Qiagen) with RIPA buffer (Cell Signaling Technology) containing protease and phosphatase inhibitors. The protein concentrations were measured with a BCA protein assay kit (Pierce, Rockford, IL). Western blotting was performed according to standard protocols by loading equal amounts of protein (30 μg) onto SDS/PAGE gels and band densities were determined with ImageJ.

**Quantitative RT-PCR.** Quantification of selected transcripts was performed by relative quantitative real-time RT-PCR with SYBR Green real-time RT-PCR assays and CFX384 Real-Time PCR System (Bio-Rad, Hercules, CA). cDNAs were generated from 1 μg of total RNA by using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). The resulting cDNA was subjected to 40-cycle PCR amplification according to the manufacturer’s protocol. Three replicates were run for each gene for each sample in a 384-well plate. TATA binding protein was used as the endogenous reference gene. The relative quantitation method (ΔΔCt) was used, with the ratio of the mRNA level for the gene of interest normalized to the level of internal control and the average of the no-template controls as the calibrator value. SYBR Green data were considered significant for \( P < 0.05 \).

**Collagen assay.** Lung collagen was measured with SirCol Collagen Assay from Biocolor (Carrickfergus, UK) according to the manufacturer’s instruction. Briefly, whole lungs with the exception of the right middle lobe used for histology were frozen in liquid nitrogen and pulverized. Acid extracts were prepared by adding 0.5 M acetic acid and protease inhibitor cocktail to the pulverized lung, which was then rotated under 4°C overnight. Sircol dye reagent was added to make an insoluble collagen-dye complex, which was then precipitated by centrifugation and dissolved in alkali reagent. The optic absorbance of samples was measured by a microplate reader at a wavelength of 560 nm.

**Statistical analysis.** Two-way ANOVA was used to compare the means of data from two or more experimental groups. If significant difference was present by ANOVA \( (P < 0.05) \), a least significant differences test was performed post hoc. Subsequently, differences between groups were considered statistically significant for \( P \) values less than 0.05. Results are expressed as means ± SE.

## RESULTS

**Radiation-induced lung GADD45a expression.** In initial studies, Western blotting of lung homogenates form wild-type mice subjected to low-dose thoracic radiation (10 Gy) revealed a significant time-dependent upregulation of GADD45a mRNA evident at 24 h and further increased at 6 wk (Fig. 1A). Immunohistochemical staining further confirmed significantly increased lung GADD45a expression 6 wk postirradiation compared with nonradiated controls (Fig. 1B).

**RILI susceptibility of GADD45a−/− mice.** To evaluate the role of GADD45a in the elaboration of RILI, wild-type and...
GADD45a−/− mice were exposed to single-fraction, low-dose thoracic radiation (10 Gy) and lung vascular permeability and inflammation were assessed at 2 and 4 wk by BAL fluid protein and albumin content and total cell counts. Compared with GADD45a−/− controls, RILI-challenged GADD45a−/− mice were found to have significantly increased BAL fluid protein and albumin content at 2 wk that was further increased at 4 wk; similar results were observed with respect to BAL fluid total cell counts (Fig. 2, A–C). In contrast, low-dose irradiation (10 Gy) did not have a significant effect in wild-type animals by BAL fluid protein or albumin measurements or by BAL fluid total cell counts at either 2 or 4 wk. Measurements of inflammatory cytokines in the BAL fluid, which we previously reported to be increased in our RILI model (22), confirmed a significant increase in IL-1β and TNF-α at 2 and 4 wk in GADD45a−/− mice compared with GADD45a−/− control mice and RILI-challenged wild-type mice at the same time points.

To assess the relative effects of gene dose on RILI susceptibility, we next subjected wild-type, GADD45a+/−, and GADD45a−/− mice to thoracic radiation. We chose to use high-dose (20 Gy) radiation in these experiments to elicit a more robust injury response that would allow us to detect potentially small differences between heterozygous and ho-
mozygous animals. BAL fluid collected at 6 wk again demonstrated protein levels and total cell counts in GADD45α−/− animals that were significantly increased compared with wild-type mice. However, GADD45α+/− mice were found to have an intermediate phenotype since BAL protein levels in these animals were not significantly different from either wild-type or GADD45α−/− mice (Fig. 3A) whereas BAL total cell counts were significantly increased compared with wild-type mice but not GADD45α−/− animals (Fig. 3B). This intermediate susceptibility to RILI in GADD45α+/− mice was further corroborated by relative changes in interstitial edema and inflammatory cell infiltration evident by lung histology (Fig. 3C).

Role of Akt in GADD45α-mediated RILI responses. Because we previously identified decreased Akt as a critical determinant of increased VILI susceptibility in GADD45α−/− mice (23, 25), we next investigated the role of Akt in our RILI model. Initial studies identified a significant increase in Akt mRNA by RT-PCR in RILI-challenged (20 Gy) wild-type mice at 6 wk (Fig. 4A). Subsequently, compared with wild-type controls, Western blotting of lung homogenates from GADD45α−/− mice showed significantly decreased phosphorylated Akt (Ser 473) relative to total Akt, both at baseline and at 6 wk postirradiation (Fig. 4, B and C), consistent with our previous findings in a VILI model (2, 3).

To characterize the specific role of Akt in our murine RILI model we performed separate experiments in RILI-challenged Akt+/− mice and in wild-type mice administered an Akt inhibitor (Akt Inhibitor XIII, Isozyme-Selective, Akti2-1/2, 1 mg/kg IP, 3×/wk) beginning 1 wk prior to irradiation and continued until 6 wk postirradiation. Because we had observed only a moderate injury in GADD45α+/− mice subjected to high-dose radiation (20 Gy) and anticipated a similar response in both Akt+/− mice and mice treated with the Akt inhibitor, we used high-dose radiation in these experiments as well. Initial studies confirmed increase lung GADD45α expression in wild-type mice in response to radiation (20 Gy) at 6 wk (Fig. 5A). Subsequently, in both Akt+/− mice (Fig. 5, B and C) and wild-type mice treated with the Akt inhibitor (Fig. 5, D and E) BAL fluid protein levels and total cell counts were significantly increased compared with RILI-challenged wild-type animals at 6 wk. Notably, there was no difference between experimental groups that did not receive radiation.

To confirm the role of Akt in the increased RILI susceptibility of GADD45α−/− animals, GADD45α−/− mice were administered a constitutively active Akt1 mammalian expression vector, with overexpression of Akt in the lungs of the animals confirmed by Western blotting, prior to RILI challenge (Fig. 5F). Compared with GADD45α−/− treated with a control vector, overexpression of Akt in GADD45α−/− animals was associated with a marked attenuation of the increased BAL fluid levels and total cell counts induced by radiation at 6 wk (Fig. 5, G and H).

Susceptibility of GADD45α−/− mice to bleomycin-induced lung injury. To determine whether the increased susceptibility of GADD45α−/− mice to both VILI and RILI was unique to these models, we studied the responses of GADD45α−/− mice in a bleomycin-induced lung injury model that we have previously described (14). Wild-type and GADD45α−/− mice were administered low-dose bleomycin (0.25 U/kg IT) and BAL fluid was collected after 1 wk. Although these studies confirmed a significant increase in bleomycin-induced BAL fluid protein and total cell counts, there was no difference between wild-type and GADD45α−/− mice in the early lung inflammatory changes at 1 wk after bleomycin (data not shown). Bleomycin-induced fibrosis (0.25 U/kg IT) assessed by lung collagen levels was also significantly increased in both wild-type and GADD45α−/− mice after 2 wk compared with their respective untreated controls. However, lung collagen levels 2 wk after bleomycin were also significantly increased in GADD45α−/− mice compared with bleomycin-treated wild-type mice. 

![Fig. 4. Decreased Akt phosphorylation in RILI-challenged GADD45α−/− mice.](http://ajplung.physiology.org/)
type animals (Fig. 6A). Notably, changes in lung collagen levels in response to bleomycin were consistent with our previous reports relative to bleomycin dosing (14, 15). Moreover, these findings were corroborated by changes on lung histology at 2 wk indicating increased bleomycin-induced fibrosis in GADD45a−/− mice compared with wild-type controls (Fig. 6B).

**Role of Akt in lung injury induced by bleomycin in GADD45a−/− mice.** Finally, to assess the role of decreased Akt as a mechanism of increased susceptibility of GADD45a−/− mice to bleomycin-induced lung injury, GADD45a−/− mice were administered constitutively active Akt expression vector prior to bleomycin (1.5 U/kg IT) followed by assessments of lung inflammation and fibrosis. These studies confirmed a significant decrease in BAL total protein and cell counts in GADD45a−/− that received the Akt transgene compared with GADD45a−/− control animals at 3 wk after bleomycin (Fig. 7, A and B). Similarly, compared with GADD45a−/− controls, lung collagen levels were also significantly decreased at 3 wk in GADD45a−/− animals overexpressing Akt (Fig. 7C). These effects were supported by changes evident on lung histology (Fig. 7D).

**DISCUSSION**

GADD45a is a candidate acute lung injury (ALI)/VILI gene that is upregulated in lung endothelial cells in response to mechanical stress with GADD45a−/− mice demonstrating both increased susceptibility to VILI and reduced levels of Akt, a cell survival and vascular permeability signaling effector (23).
The importance of decreased Akt to the heightened susceptibility of GADD45a−/− mice to VILI is evidenced by the protective effects of overexpression of Akt in these animals prior to VILI challenge (25). In the present study, we sought to determine whether these effects of GADD45a are specific to lung injury induced by excessive mechanical stress or whether GADD45a plays a more comprehensive role in the elaboration of inflammatory lung injury. In support of the latter, we found that GADD45a depletion is associated with increased susceptibility to lung injury induced by either radiation (RILI) or bleomycin. These findings indicate that GADD45a is an important modulator of lung injury in general and suggest that specific GADD45a pathway components may serve as novel therapeutic targets in a broad population of patients with inflammatory lung injury independent of the precipitating cause.

We had previously relied on in vivo and in vitro orthologous gene expression profiling (murine, rat, and canine models as well as human endothelial cells subjected to 18% cyclic stretch) to identify GADD45a as unidirectionally and differentially expressed in response to ALI/VILI across all platforms (9). However, the possibility that GADD45a effects are largely specific to lung injury induced by excessive mechanical stress was suggested by our findings that although GADD45a−/− mice demonstrated increased VILI susceptibility, lung injury was only modestly increased in these animals in an LPS-induced ALI model compared with wild-type mice (23). Nonetheless, since GADD45a is also known to be upregulated in response to a variety of other stress stimuli including ultraviolet or ionizing radiation (11) and hyperoxia (31), we hypothesized that GADD45a is also protective against lung injury induced by variable stress stimuli.

Empiric evidence for the potential protective effects of GADD45a in RILI is abundant. For example, mice deficient in testicular receptor 4, a nuclear receptor superfamily member and a transcriptional regulator of GADD45a, express decreased GADD45a and are more sensitive to ionizing radiation (38). Separately, GADD45a−/− mice are found to have increased DNA damage and impaired apoptosis after ionizing radiation (2). Despite these reports, however, the role of GADD45a in lung injury induced by ionizing radiation has not previously been explored. We not only found that GADD45a is an important modulator of murine RILI but we were able to confirm a gene-dose effect with intermediate susceptibility to
RILI in GADD45a<sup>+/−</sup> mice compared with wild-type (decreased injury) and GADD45a<sup>−/−</sup> mice (increased injury). Interestingly, whereas the 4- and 6-wk time points represent a time frame along a continuous spectrum in which a similar inflammatory response is observed in our RILI model (22), increased GADD45a expression noted at 1 day and 6 wk was not evident at 4 wk. We speculate that this may be indicative of a biphasic response characterized by an acute response followed later by a recovery response at these time points, respectively, although further studies to investigate this are ongoing. In addition, we noted increased leak across the alveolar wall (BAL protein and albumin) that precedes the increase in BAL cell counts, but not the change in early response cytokine levels. This observation further supports the idea that lung injury in response to radiation is a complex process that includes and is a consequence of impaired barrier function. Separately, we also found that decreased Akt expression (Akt<sup>+/−</sup> mice) or pharmacological Akt inhibition in wild-type mice was associated with increased RILI susceptibility whereas RILI could be attenuated in GADD45a<sup>−/−</sup> (which express decreased Akt) via the overexpression of Akt1. Evidence for decreased Akt as a key determinant of RILI severity in GADD45a<sup>−/−</sup> mice is consistent with literature implicating a role for Akt in radiation-induced injury in other models. For example, expression of endothelial plasminogen activator tissue inhibitor type 1 (PAI-1), which is upregulated in response to radiation and has been identified as a mediator of radiation-induced intestinal injury (1, 24), is also negatively regulated by Akt (28). Additionally, simvastatin, an HMG CoA-reductase inhibitor that we have previously reported is protective in murine RILI (22), both induces endothelial Akt phosphorylation and inhibits endothelial PAI-1 expression (28).
We found that GADD45a+/− mice were also more susceptible to bleomycin-induced lung injury assessed by lung collagen levels at 2 wk with corresponding changes evident on lung histology. Consistent with these observations, late-onset fibrotic changes associated with bleomycin have previously been linked to Akt activation (7, 21). Moreover, inhibition of PAI-1 is associated with reduced bleomycin-induced lung fibrosis in a rat model (39) and statin drugs have also been found to be protective in bleomycin-induced murine lung fibrosis (19, 30, 40).

Notably, we did not find a significant difference in lung inflammatory changes after bleomycin between wild-type and GADD45a+/− mice as measured by BAL fluid protein and total cell counts at 2 wk, which raises questions regarding the role of Akt in the early lung inflammatory responses in this model. In this regard, there are other reports that Akt inhibition does not affect bleomycin-induced lung inflammation or fibrosis (26); the literature as a whole is equivocal with respect to the role of Akt in bleomycin-induced lung injury, with several reports implicating Akt activation as a mechanism of injury (14, 32). However, we did find that overexpression of Akt in GADD45a+/− mice significantly attenuated lung inflammation and fibrosis associated with bleomycin treatment after 3 wk. Consistent with our findings, it is likely that other mechanisms in addition to Akt regulation contribute to the GADD45a+/− phenotype in our lung injury models. GADD45a is a participant in cell cycle regulation, DNA repair, and maintenance of genomic stability, as well as in regulation of p53-dependent (and p53-independent) apoptosis (13, 16, 17). Additionally, GADD45α interacts with innate and adaptive immune systems to regulate T cell differentiation and proliferation via p38 MAPK (33, 34). Both radiation and bleomycin are associated with the generation of superoxide radicals that induce DNA double-strand breaks, apoptosis, and increased expression of inflammatory cytokines and chemokines that mediate inflammatory lung injury and subsequent pulmonary fibrosis. Since p53 plays an important role in the early lung response to bleomycin-induced DNA damage (29) it is likely that GADD45a, a downstream target of p53 (3), is a key modulator in this context. Separately, GADD45a is also reported to regulate matrix metalloproteinases, a family of proteins highly upregulated in fibrosis, through p38/MAPK and APC (adenomatous polyposis coli) complex activation (10). This may represent an independent regulatory pathway of GADD45a in bleomycin-induced lung fibrogenesis.

In summary, we have now identified GADD45a as a modulator of lung injury with protective effects across a variety of models including VILI, LPS-induced ALI, as well as RILI and bleomycin-induced fibrosis. Decreased GADD45a expression is associated with decreased Akt and we have found that repletion of Akt reverses lung injury susceptibility in GADD45a+/− mice whereas depletion or inhibition of Akt mimics the GADD45a+/− phenotypic responses. Our findings implicate GADD45a and its signaling axis involving Akt as potentially novel therapeutic targets although further studies are needed that may yield additional insights into the pathophysiology and elaboration of inflammatory lung injury clinically.

GRANTS
This research was supported by National Heart, Lung, and Blood Institute Grant HL 09805.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

REFERENCES


