

RESEARCH ARTICLE

Noncanonical role for Ku70/80 in the prevention of allergic airway inflammation via maintenance of airway epithelial cell organelle homeostasis

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Rehman R, Vijayakumar VE, Jaiswal A, Jain V, Mukherjee S, Vellarikkal SK, Dieffenbach PB, Fredenburgh LE, Prakash YS, Ghosh B, Agrawal A, Mabalirajan U. Noncanonical role for Ku70/80 in the prevention of allergic airway inflammation via maintenance of airway epithelial cell organelle homeostasis. *Am J Physiol Lung Cell Mol Physiol* 319: L728–L741, 2020. First published September 2, 2020; doi:10.1152/ajplung.00522.2019.—Airway epithelial homeostasis is under constant threat due to continuous exposure to the external environment, and abnormally robust sensitivity to external stimuli is critical to the development of airway diseases, including asthma. Ku is a key nonhomologous end-joining DNA repair protein with diverse cellular functions such as VDJ recombination and telomere length maintenance. Here, we show a novel function of Ku in alleviating features of allergic airway inflammation via the regulation of mitochondrial and endoplasmic reticulum (ER) stress. We first determined that airway epithelial cells derived from both asthmatic lungs and murine asthma models demonstrate increased expression of 8-hydroxy-deoxyguanosine (8-OHdG), a marker of oxidative DNA damage. Ku protein expression was dramatically reduced in the bronchial epithelium of patients with asthma as well as in human bronchial epithelial cells exposed to oxidative stress. Knockdown of Ku70 or Ku80 in naïve mice elicited mitochondrial collapse or ER stress, leading to bronchial epithelial cell apoptosis and spontaneous development of asthma-like features, including airway hyperresponsiveness, airway inflammation, and subepithelial fibrosis. These findings demonstrate an essential noncanonical role for Ku proteins in asthma pathogenesis, likely via maintenance of organelle homeostasis. This novel function of Ku proteins may also be important in other disease processes associated with organelle stress.

airway epithelium; asthma; Ku

INTRODUCTION

Asthma is a chronic inflammatory disease of airways affecting one in six people in developed countries, and its incidence is increasing worldwide. Pathologically, asthma is character-

ized by profound allergic airway inflammation resulting in increased mucus production, airway remodeling, and bronchospasm. Recently, multiple studies have demonstrated the role of primary airway epithelial dysfunction as a key etiologic factor driving airway inflammation in asthma (17, 19, 33) rather than inflammation-induced airway remodeling. Indeed, innate immune responses of airway epithelia lead to downstream effects on adaptive immunity, and insufficiency in these responses have been linked to acute exacerbations in asthma (19). Airway epithelia exposed to various insults such as airborne allergens, foreign particles, and oxidants demonstrate increased stress response and accentuate asthma-like responses. In this context, our laboratory and others have demonstrated that mitochondrial dysfunction and endoplasmic reticulum (ER) stress in airway epithelia are prominent features of the stress response in human asthma (27, 30). In addition, downregulation of epithelial stress responses in vivo has been shown to attenuate asthma-like features in mouse models of allergic airway inflammation via mesenchymal stem cell-mediated mitochondrial donation (2). These findings suggest that maintenance of organelle homeostasis in airway epithelial cells may be protective against the allergic inflammatory response that develops in asthmatic patients. This accords with a large body of recent work that has highlighted the importance of organelles such as mitochondria in the pathogenesis of airway diseases (1).

There is a tight correlation between intracellular organelles and nuclear DNA repair mechanisms, as organelles can send stress signals to the nucleus that upregulate checkpoint and repair proteins to modulate cell death (43). Ku, a heterodimer of Ku70 and Ku86 (Ku80 in mice; for the sake of clarity we have used Ku80 for human as well in this paper), is a key nonhomologous end-joining repair protein that binds to double-stranded DNA breaks. It participates in diverse cellular functions including V(D)J recombination (where V is variable, D is diversity, and J is joining) and telomere length maintenance (11). Ku80 knockout mice show enhanced radiation sensitivity and premature senescence (25), mimicking features

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of cells exposed to high oxidative stress environments. In accordance with this phenotype, we and others have recently found that Ku70 is expressed in normal lungs of healthy individuals but is decreased upon oxidative stress and caspase-3 activation (35, 37). Earlier work on Ku has demonstrated reduced expression of Ku80 in airway epithelia of smokers or smoke-exposed mice (8); however, Ku has not been well studied in asthma. Notably, one study showed increased DNA damage and elevated Ku70 expression in whole lung (9) of house dust mite-induced asthmatic mice, but did not further explore the role of Ku70 in asthma pathogenesis in detail.

In this study, we found increased DNA damage associated with a pronounced reduction in the expression of Ku70 and Ku80 in the bronchial epithelia of asthmatic lungs. Mimicking these reductions in naïve mice led to mitochondrial dysfunction and ER stress along with spontaneous development of asthma-like features. These results indicate that Ku proteins are critical in maintaining airway epithelial health and preventing allergic airway inflammation, possibly by controlling organelle homeostasis and reducing cell death. Our findings suggest that reduced Ku expression likely contributes to mitochondrial dysfunction and ER stress in asthmatic airway epithelia, and interventions to restore Ku function may represent a novel therapeutic target in asthma.

MATERIALS AND METHODS

Mouse studies. Male BALB/c mice (6–8 wk, 18–25 g) were obtained from Central Drug Research Institute (Lucknow, India) and acclimatized for 7–10 days before experiments. All study protocols were approved by the Institutional Animal Ethics Committee at the Institute of Genomics and Integrative Biology (IGIB), Delhi, India. There were four groups of mice: Naïve/VEH (vehicle), Naïve/Scrambled siRNA, Naïve/Ku80 siRNA, and Naïve/Ku70 siRNA. Naïve mice were given either PBS or 75 μ g of scrambled/Ku70/Ku80 siRNA intranasally with brief isoflurane anesthesia (28, 29) on days 1 and 3 followed by euthanasia on day 5 with airway hyperresponsiveness (AHR) measurement. For the ovalbumin (OVA)-induced bronchial hyperresponsiveness model, mice were immunized with four intraperitoneal injections of 50 μ g of OVA in 4 mg of aluminum hydroxide or 4 mg of aluminum hydroxide alone on days 0, 7, 14, and 21 and further challenged from day 28 to day 34 (30 min/day) with 3% OVA in PBS or PBS alone (28, 29). To evaluate the clinical relevance of this finding, we also used a mouse model of cockroach extract allergen-induced allergic airway inflammation, as described previously (2).

Human studies. After ethical committee clearances from IGIB and collaborating hospitals were obtained, normal control subjects and patients with asthma were recruited for serum studies of asthma and written informed consent was obtained from all study participants. Asthma was diagnosed clinically by physicians of the collaborating hospitals. We performed skin prick testing for common antigens, IgE testing, and health questionnaires in both groups as described earlier (23, 36). Only nonsmokers without a cancer history were recruited. Subjects without asthma or family history of asthma/atopy and with negative skin prick tests were assigned to the control group. Patients diagnosed clinically with asthma who had both positive skin prick testing and bronchial reversibility [15% improvement in forced expiratory volume in one second (FEV1) with salbutamol] were assigned to the asthma group. The skin prick test was performed as described earlier (23, 36) with 15 common region specific allergens (house dust mites, *Amaranthus spinosus*, *Brassica campestris*, *Azadirachta indica*, *Parthenium hysterophorus*, *Prosopis juliflora*, *Ricinus communis*, *Adhatoda vasica*, *Aspergillus fumigatus*, cockroach males, moong dal, mosquitoes, moths, grain dust rice, *Eucalyptus tereticornis*,

Table 1. Demographics and clinical characteristics of asthmatic patients and controls

	Controls	Asthmatic Patients
Birthplace	North/northwest India	North/northwest India
Age, yr (mean $\pm$ SE)	32.1 $\pm$ 2.7	28.1 $\pm$ 2.4
Sex (M:F)	9:5	25:23
Family history of atopy	0/14	48/48
Total IgE, IU/mL (mean $\pm$ SE)*	118.6 $\pm$ 17.89	759.8 $\pm$ 104.1
Skin prick test positivity*	0/14	48/48
Bronchial reversibility (>15% improvement in FEV1 postbronchodilator)	Not performed	48/48
Current tobacco use	0/14	0/48

M, male; F, female; FEV1, forced expiratory volume in one second. *The details of skin prick test and individual IgE levels are included in Supplemental Table S1.

House dust, and *Albizia lebbek*; histamine and normal saline were taken as positive and negative controls, respectively). Wheal response of more than 3 mm diameter for at least one allergen was considered positive. Clinical details of this cohort are summarized in Table 1 and Supplemental Table S1 (all Supplemental material is available at <https://doi.org/10.6084/m9.figshare.12736604>). We subsequently performed 8-hydroxy-deoxyguanosine (8-OHdG) immunohistochemistry (IHC) from the sera of this cohort of asthmatic patients ($n = 48$) and matched normal controls ($n = 14$).

8-OHdG immunohistochemistry was performed on postsurgical lung samples from a small convenience sample of asthmatic patients ($n = 5$) and nonasthmatic controls ($n = 5$) from the Mayo Clinic Rochester (St. Mary's Hospital). The indications for thoracic surgery were focal, noninfectious causes, including early-stage cancers and nonmalignant nodules. Only uninvolved tissue was used for immunohistochemical staining. Asthma had been previously diagnosed by clinical evaluation/spirometry/bronchodilator responses; patients who received systemic steroids chronically were excluded. Demographic details and comorbid conditions are detailed in Supplemental Table S2.

In vitro studies. Human bronchial epithelial cells (BEAS-2B, ATCC, Manassas, VA) were maintained in serum free LHC-9 medium (GIBCO). Upon near confluence, these cells were transfected with scrambled/Ku70/Ku80 (25–50 nM) siRNA for 48 h using jetPRIME reagent (Polypus Transfection, France) per the manufacturer's instructions. To determine the effects of various oxidants on the expressions of Ku70 or Ku80, BEAS-2B cells were induced with different concentrations of H₂O₂ for 24 h, followed by harvesting for Western blot analysis.

Immunohistochemistry. Immunohistochemistry (28, 29) was performed in formalin-fixed lung tissue sections with primary antibodies for 8-OHdG (Abcam), ATM (Abcam), DNA-PKcs (Novus Biologicals), γ -H2AX (γ -H2AX (phosphorylated form of H2AX, a member of the H2A protein family; Abcam), Ku80 (Abcam), and Ku70 (Abcam) followed by incubation with respective horseradish peroxidase-conjugated secondary antibodies (Sigma). Quantification of each protein was performed by experimentally blinded experts, who counted a minimum of 200 bronchial epithelia per lung section.

Real-time PCR. Approximately 30 mg of lung was immersed in RNAlater solution (Invitrogen) for 2–4 h at 4°C and stored at -80°C , and then RNA isolation was performed (Qiagen RNeasy isolation kit); 1–2 μ g of isolated RNA was converted into cDNA (Applied Biosystems), and the relative expressions of ATM (ataxia telangiectasia mutated), ATR (ataxia telangiectasia and Rad3-related protein), DNAPKcs (DNA-dependent protein kinase, catalytic subunit), Ku70, and Ku80 were determined using Kappa SYBR Green on a LightCycler 480 (Roche) and normalized to 18S RNA and β -actin. Primer details were as

follows: ATM (reverse primer: GGGTTTCCTGAGAACTCCGC, forward primer: GACCTGGGAGTGGCTTTTGA), ATR (reverse primer: GATGCTGCAGGACTTGCTTT, forward primer: TCCTGATCTTA-ATCGTTTCCTGA), DNA-PKcs (reverse primer: TTCCAATCACA CTGCCTGTC, forward primer: TTGGAGACAGACACCTGAACA), KU70 (reverse primer: GCCAGGGAACCCAGTCTTTT, forward primer: TCCGCTTCACATAGAGGAGC, KU80 (reverse primer: CTG-GAGACTCGCTTCCTCAA, forward primer: AAGTGACTGCT-CAGGACGTT).

Single-cell gel electrophoresis assay. DNA damage was assessed by single-cell gel electrophoresis (comet) assay (Trevigen). In brief, ~30 mg of human or mouse lung tissue was made into single-cell suspensions via cell strainer and centrifugation in a 20 mM EDTA buffer. Approximately 50,000 cells were then mixed into 0.75% low-melting agarose (LMA) and placed on 0.1% LMA precoated slides until the agarose was solidified. The neutral/alkaline comet assay was performed per manufacturer's protocol, and propidium iodide-stained slides were observed (Lieca DMI6000 B). Images were captured and analyzed using OpenComet software (15), which is freely available. There were at least 400–500 comets per sample used for analysis. The graphs were plotted using the R package.

Enzyme-linked immunosorbent assay. Levels of 8-OHdG [mouse total lung protein, human serum, human bronchoalveolar lavage (BAL) fluid] and 8-isoprostane (mouse total lung protein) were determined per the manufacturer's instructions (Cayman Chemicals). IL-13 (BD Biosciences), Ku70, and Ku80 (USCN Life Science Inc.), and cytochrome *c* (R&D Systems) were estimated in mouse total lung protein/cytosols.

AHR, BAL, and lung histopathology. Airway hyperresponsiveness (AHR) was determined via dosing with increasing concentrations of methacholine using a FlexiVent (SciReq Canada) in tracheotomized mice under anesthesia. After AHR estimation, BAL was performed (28, 29) to obtain BAL fluid cells that were stained with Leishman's stain to determine the differential and total cell counts. Lungs were then removed, fixed in formalin, embedded in paraffin, and sectioned (5 μ m) for hematoxylin and eosin (H&E), Periodic acid-Schiff (PAS), and Masson's trichrome stainings to assess airway inflammation, goblet cell metaplasia, and subepithelial fibrosis, respectively (28, 29). All microphotographic images were captured using a bright-field microscope (Nikon YS100). The inflammation score was determined in H&E-stained lung sections.

Western blotting. Western blot analysis was performed as described previously (27, 28). Briefly, mouse lung proteins were separated using SDS gel (8–15%) electrophoresis, transferred to PVDF membranes, blocked (with 3–5% BSA in TBS-Tween), probed with primary antibodies [γ -H2AX, Ku70, C/EBP-homologous protein (CHOP), glucose-regulated protein of 94 kDa (Grp94; Santa Cruz Biotechnology), Ku80, binding immunoglobulin protein (BIP; Cell Signaling Technology), cytochrome *c* (Abcam), and respective secondary antibodies (Banglore Genei, India) followed by DAB (Sigma) development. The quantitative densitometry for the Western blots was performed utilizing ImageJ software in grayscale images. Additionally, respective band intensities were plotted after normalization with loading control intensities from the same sample. The uncropped Western blot images are found in the Supplemental material (<https://doi.org/10.6084/m9.figshare.12736604>).

Flow cytometry. To determine the expression of Ku-positive cells that also express either cytokeratin or CD19, 50 mg of lung tissue was used for making single-cell suspensions and incubated with either CD19 (BD) or pan-cytokeratin (Cell Signaling Technology) antibodies followed by permeabilization and further incubation with anti-Ku (Abcam). This was followed by treatment with or without fluorescence-labeled respective secondary antibodies (BD) and analyzed via FACS (BD FACSCalibur).

Mitochondrial stress and apoptosis assays. MitoSOX Red (mitochondrial superoxide indicator) and tetramethylrhodamine methyl ester (TMRM) dyes (Invitrogen) were used to determine mitochon-

drial oxidative stress and mitochondrial membrane potential, respectively. Ku70 or Ku80 siRNA-transfected BEAS-2B cells were incubated with MitoSOX Red (5 μ M) and TMRM (10 nM) dyes at 37°C for 15 min followed by washing and final resuspension in PBS before acquisition (BD FACSCalibur).

For apoptosis assays, cells were labeled with annexin V (PE-conjugated, BD Biosciences) in combination with 7-aminoactinomycin D (7-AAD; Invitrogen). Briefly, cells were first washed with PBS and then with annexin buffer and were finally suspended in a 100- μ L volume of annexin buffer followed by addition of annexin V (0.125 μ L) and 0.125 μ g 7-AAD for 20 min and were acquired using the BD FACSCalibur machine.

Estimation of inner mitochondrial membrane integrity and mitochondrial enzyme activities. The inner mitochondrial membrane (IMM) integrity was estimated using 2 μ g of isolated lung mitochondria (MITO-ISO1, Sigma Chemical Co.) as described previously (28, 29). In addition, complex I (MitoSciences) and IV (Sigma) activities of mitochondria were measured with 45 μ g and 10 μ g of the isolated lung mitochondria, respectively.

Activity assays of calpain and various caspases. Activities for cytosolic calpain (20 μ g lung cytosols) and caspases-3, -8, -9, and -12 (10 μ g lung cytosol) were performed according to the manufacturer's instructions (Biovision).

Statistical analysis. An unpaired Student's *t* test and one-way ANOVA tests were performed to compare two groups and more than two groups, respectively, using Prism and JMP software packages. $P \leq 0.05$ was considered statistically significant.

RESULTS

Increased DNA damage and reduced expression of key DNA repair proteins in asthmatic lungs. Before examining the status and role of Ku proteins in asthma pathogenesis, we first wanted to determine the functional activity of Ku in asthma by assessing DNA damage in asthmatic lungs. We therefore performed neutral and alkaline comet assays in lung biopsies obtained from nonasthmatics and asthmatics. Single-cell suspensions from lungs of asthmatics showed an increase in the percentage DNA in tails, an index of DNA damage, compared with those of non-asthmatics (Fig. 1, *A1*, *A2*, *B1*, and *B2*). Although both neutral and alkaline-based assays demonstrated a large increase in tail formation in cells derived from asthmatic lungs, the alkaline assay was somewhat more sensitive. This expected finding is likely due to detection of both single-strand breaks and other alkali-labile sites, such as base adducts, alkylated bases, and pyrimidine dimers that are formed in the process of repairing initial DNA damage.

To determine whether our findings could be replicated in murine models, we assessed DNA damage in lungs derived from ovalbumin (OVA)-induced and cockroach extract (CE)-induced mouse models of asthma (2, 27, 30), which show many asthma-like features including increased airway hyperresponsiveness, infiltration of inflammatory cells around the bronchovascular regions, and goblet cell metaplasia (2) (data not shown). Similar to human asthmatic lungs, lungs from OVA-challenged and CE-exposed mice (Figs. 1, *C1*, *C2*, *D1*, *D2*, *E1*, and *E2*) demonstrated an increase in DNA damage compared with SHAM control mice.

To determine the predominant cell type exhibiting DNA damage in asthma, we evaluated 8-hydroxy-deoxyguanosine (8-OHdG), a reliable in vivo oxidative DNA damage marker, in both human lungs and murine models. Human samples demonstrated significantly higher levels of 8-OHdG in the serum and bronchoalveolar lavage (BAL) fluid of asthmatic patients compared with

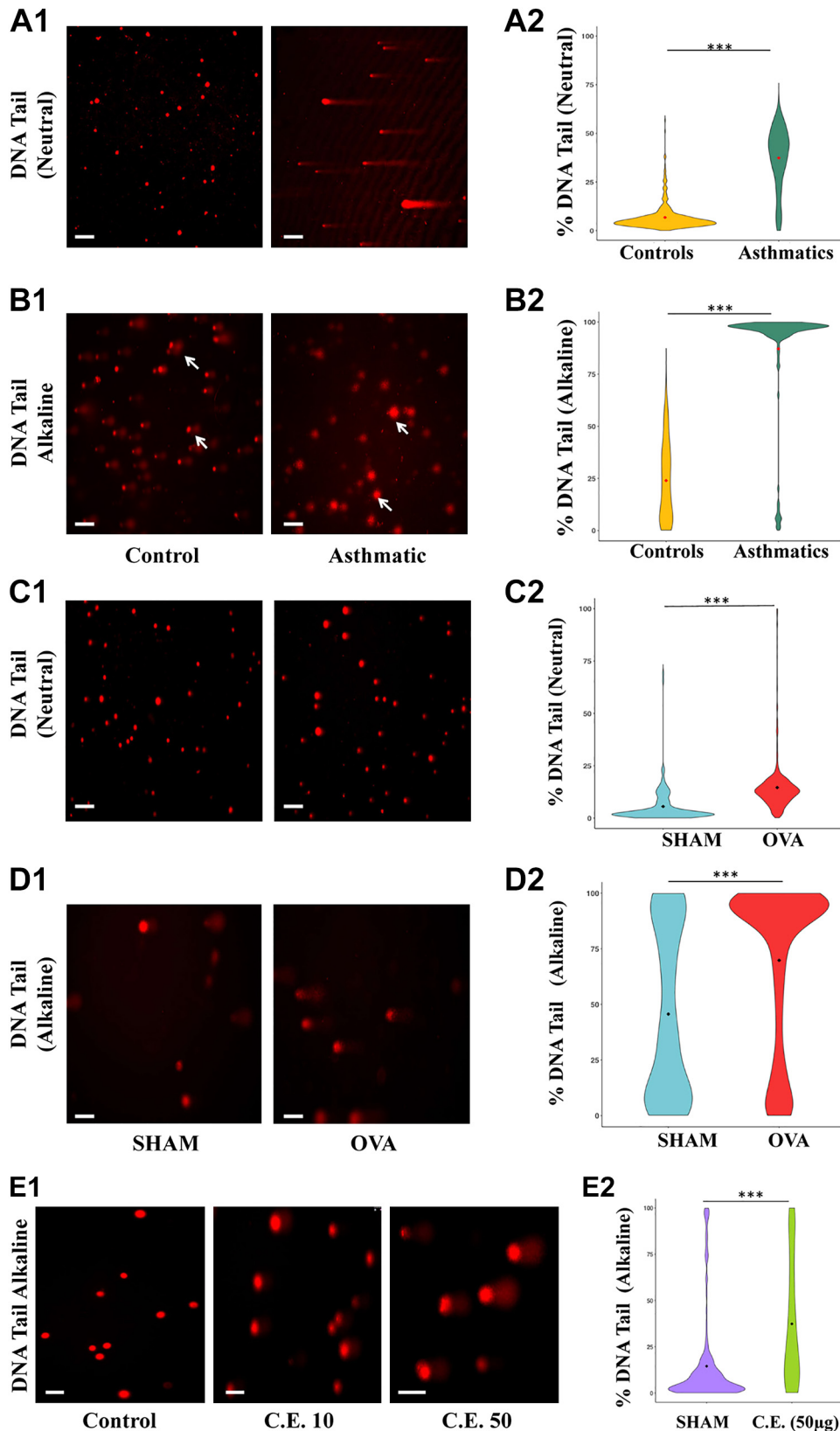


Fig. 1. Asthmatic lungs show increased DNA damage. Single-cell suspensions were made from murine or human lung tissue to perform neutral or alkaline comet assays as described in MATERIALS AND METHODS. Representative images of neutral (A1 and C1) and alkaline (B1, D1, and E1) comet assays in normal controls/asthmatics or SHAM (normal mice)/OVA (ovalbumin-induced mice with allergic airway inflammation) or SHAM/CE (different concentrations of cockroach extract-induced mice with allergic airway inflammation) mice. The percentage of DNA tail was determined by neutral (A2 and C2) and alkaline (B2, D2, and E2) comet assays in lung single-cell suspensions of PBS-sensitized and challenged (SHAM) and OVA allergen-sensitized and challenged (OVA) mice or cockroach extract allergen (CE)-exposed mice or normal controls and asthmatic individuals. Images were analyzed through OpenComet software using default parameters published previously and $n \geq 400$ comets in each sample. *** $P < 0.0001$.

normal controls (Fig. 2, A and B). Lung airway immunohistochemistry (IHC) demonstrated increased staining of 8-OHdG in asthmatic airway epithelia (Fig. 2, C–E), implicating the lung epithelium as a likely site of oxidative stress.

Based on these results, we next assessed expression of the major DNA damage sensors including ATM (ataxia telangiectasia mutated), ATR (ataxia telangiectasia and Rad3-related protein), DNAPkcs (DNA-dependent protein kinase, catalytic

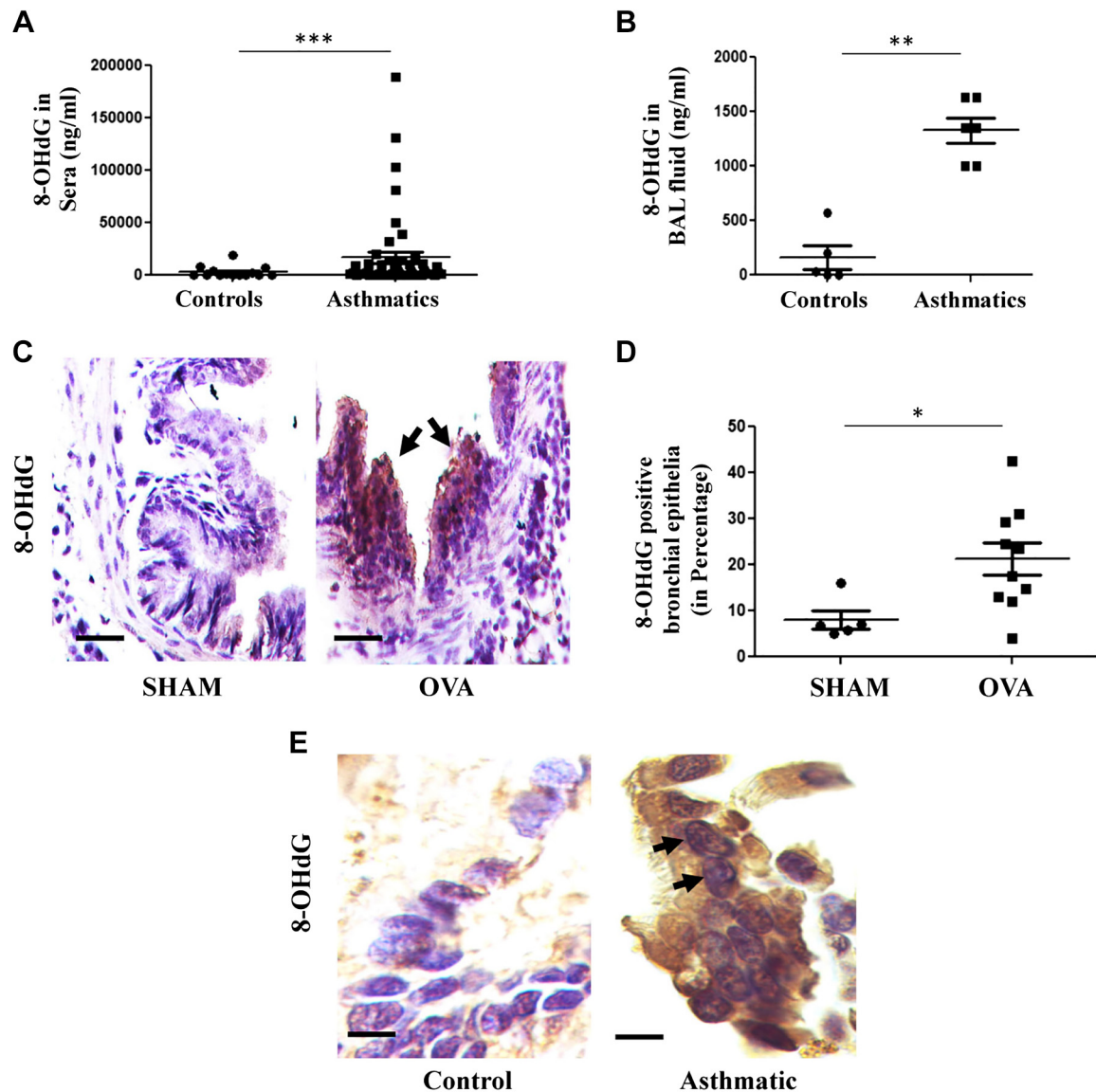


Fig. 2. Asthmatic lungs show increased 8-hydroxy-deoxyguanosine (8-OHdG), a marker of oxidative DNA damage. 8-OHdG levels were determined in sera (A, $n = 14$ controls and 48 asthmatics) and bronchoalveolar lavage (BAL) fluid (B, $n = 5$ controls and 6 asthmatics) of normal control and asthmatic individuals. Immunohistochemistry (IHC) analysis of 8-OHdG in mouse lungs (C, $n = 6$) and patient lung biopsies (E, $n = 3$). Brown color indicates positive expression; arrows indicate the expression in bronchial epithelium. Images are at $\times 40$ magnification. D: percentage of bronchial epithelia stained positive for 8-OHdG in lung tissue sections of SHAM and ovalbumin (OVA) micemice ($n = 6$ in each group). Data represent means $\pm$ SE; $n = 6$ in each group. * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$.

subunit), and γ -H2AX (phosphorylated form of H2AX that is a member in H2A protein family), along with the Ku proteins. In whole lung lysate from OVA-challenged mice, we found significantly reduced gene expression of only ATM and Ku80 in asthmatic mouse lungs (Fig. 3A). However, immunohistochemistry revealed decreased protein expression of ATM, DNA-PK, and γ -H2AX in the bronchial epithelium of allergen-exposed asthmatic mouse lungs compared with SHAM control lungs. (Fig. 3, B–G). These findings suggest a broad reduction in expression of key DNA repair proteins in the bronchial epithelium of OVA-challenged mice and may explain the susceptibility of this compartment to DNA damage in asthma. To further investigate the mechanism and implications of impaired DNA damage sensing in asthma, we focused on

the alterations in Ku expression and their downstream consequences.

Expression of Ku proteins in non-asthmatic and asthmatic lungs. Evaluation of Ku expression in non-diseased human lung biopsies and control murine lungs revealed nuclear and cytoplasmic expression of Ku throughout the airway epithelium (Fig. 4, A and B) consistent with earlier reports (8, 11). In contrast, bronchial epithelial expression of Ku in human asthmatic samples and OVA-induced asthmatic mice was dramatically reduced (Fig. 4, A and B top). In addition, there was significant Ku expression in inflammatory cells in lungs of OVA mice (Fig. 4B, bottom), possibly due to its known role in V(D)J recombination in B cells (11). Using single-cell suspensions of lungs, we found decreased numbers of Ku⁺/cytoker-

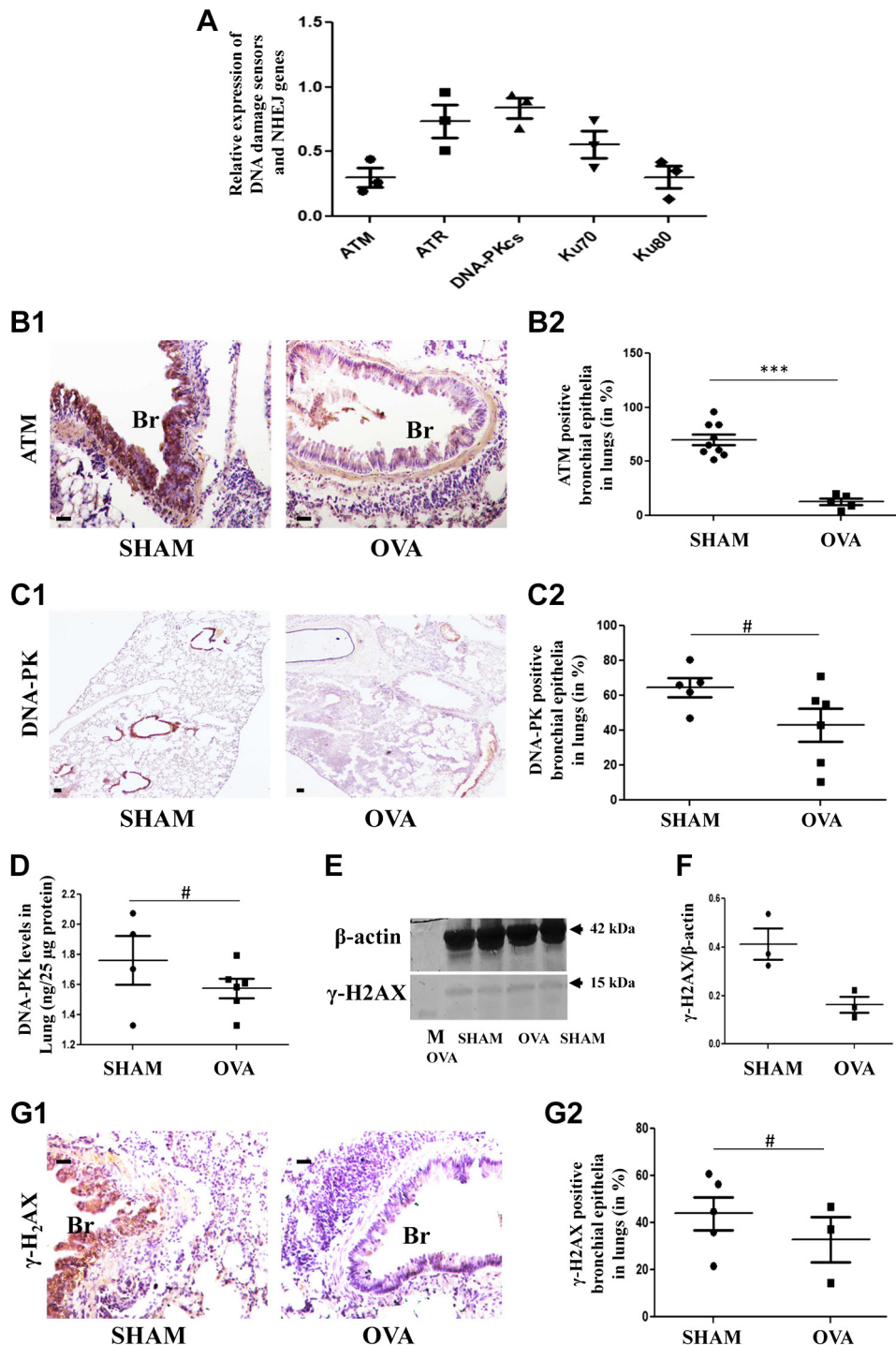
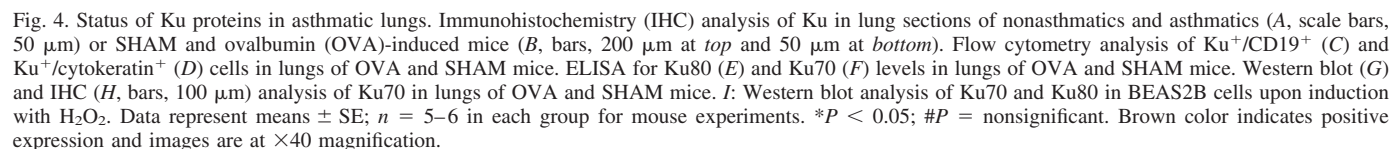


Fig. 3. Status of DNA damage sensors in murine asthmatic lungs. **A**: gene expression of key DNA damage sensors Ku70 and Ku80 in lungs of SHAM and ovalbumin (OVA) mice. **B**: representative immunohistochemistry (IHC) images of ataxia telangiectasia mutated (ATM), DNA-dependent protein kinase (DNA-PK), and γ -H2AX (**B1**, **C1**, and **G1**, $n = 3$ in each group) in lung sections of SHAM/OVA mice. ATR, ataxia telangiectasia and Rad3-related protein. Percentage of bronchial (Br) epithelial cells positive for these markers was calculated from ATM, DNA-PK, and γ -H2AX (**B2**, **C2**, and **G2**, $n = 3$ in each group) IHC images. **D**: DNA-PK levels in total lung protein from SHAM and OVA mice ($n = 6$ in each group). **E** and **F**: Western blot analysis of γ -H2AX in mouse lungs. Data represent means $\pm$ SE. * $P < 0.05$, *** $P < 0.0001$; # $P =$ nonsignificant.

atin⁺ cells and increased Ku⁺/CD19⁺ B cells in OVA-induced asthmatic mouse lungs compared with controls (Fig. 4, **C** and **D**). Quantification of Ku70 and Ku80 individually from whole lung revealed a decrease in Ku80 but no reduction in Ku70 levels in OVA-treated mice (Fig. 4, **E–G**); nevertheless, Ku70 expression in the bronchial epithelium was markedly decreased after ovalbumin sensitization (Fig. 4H). Taken together, these findings demonstrate 1) Ku induction in inflammatory B cells and 2) significant reduction in Ku proteins in bronchial airway epithelia and whole lung of ovalbumin-sensitized mice.

Given the important role of oxidative stress in asthma pathogenesis, we examined whether oxidative stress could induce these alterations in Ku expression. Indeed, human bronchial epithelial cells (BEAS-2B) treated with H₂O₂ demonstrated a dose-dependent reduction in Ku70 and Ku80 expression (Fig. 4I).

Knockdown of Ku70 or Ku80 in naïve mice yields spontaneous asthma-like features. To investigate the effects of Ku70 or Ku80 deficiency in asthma pathogenesis, we individually knocked down expression of these two proteins in naïve mice



Histological analysis revealed that both Ku70- and Ku80-deficient mice developed spontaneous airway inflammation with higher inflammation scores compared with vehicle and scrambled siRNA control mice (Fig. 6A, 6C). Furthermore, Ku80 knockdown mice demonstrated spontaneous goblet cell metaplasia (Fig. 6D), likely related to elevated production of

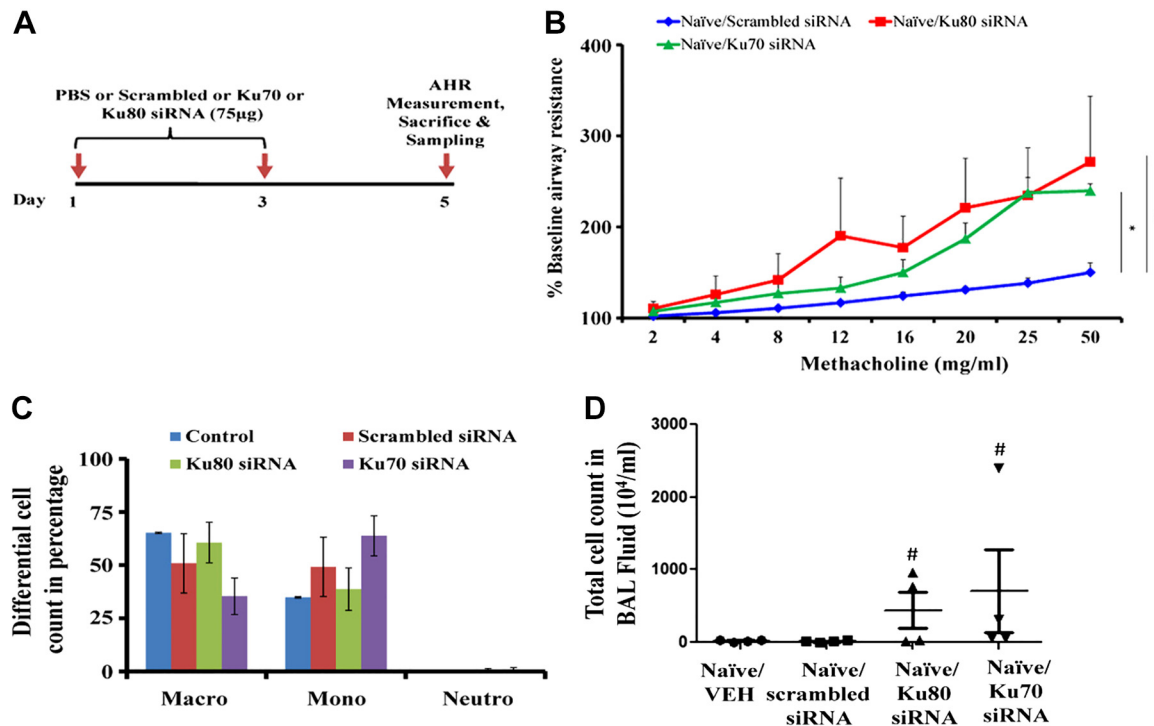


Fig. 5. siRNA-mediated knockdown of Ku80 or Ku70 leads to spontaneous airway hyperresponsiveness (AHR) and airway inflammation in naïve mice. *A*: study design to knock down Ku70 or Ku80 expression in naïve mice. *B*: AHR was determined with increasing concentrations of methacholine and the results are shown as percentage of baseline airway resistance, considering PBS values as 100% for siRNA-treated mice. Differential cell counts from bronchoalveolar lavage (BAL) fluid (*C*) and total cell count in BAL fluid (*D*) from vehicle control (VEH), scrambled siRNA, Ku80 siRNA, and Ku70 siRNA mice. Data represents means $\pm$ SE; $n = 5-6$ in each group. * $P < 0.05$, ** $P < 0.001$; # $P =$ nonsignificant.

IL-13 in this group (Fig. 6B), whereas Ku70 knockdown mice did not show this feature (Fig. 6D). In addition, both Ku70- and Ku80-deficient mice developed spontaneous subepithelial fibrosis (Fig. 6E). These findings indicate that suppression of Ku proteins alone is sufficient to spontaneously lead to many asthma-like features in the lungs of otherwise naïve mice.

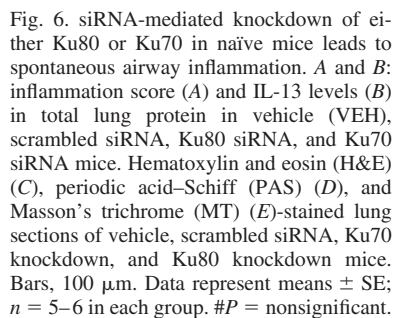
Knockdown of Ku70 or Ku80 leads to oxidative stress, DNA damage, and bronchial epithelial cell apoptosis. To determine the possible mechanisms underlying the spontaneous development of asthma-like features in the setting of Ku70/Ku80 deficiency, we first assessed whether there was significant

oxidative stress under these conditions. Both Ku70 and Ku80 knockdown mice showed a dramatic (27- to 160-fold) increase in pulmonary 8-isoprostane, a reliable *in vivo* marker of oxidative stress (Table 2). We subsequently performed DNA damage assays in lung epithelial cells isolated from Ku70 and Ku80 knockdown mice. As expected based on the high level of oxidative stress, these mice showed increased DNA tail percentage in neutral comet assays (Fig. 7A). This finding was further verified by a direct examination of oxidative DNA damage via 8-OHdG ELISA, which demonstrated a modest (3- to 5-fold) increase in oxidative damage after Ku70/Ku80 siRNA treatment (Fig. 7B). These results suggest that Ku

Table 2. Mitochondrial and ER stress parameters in lungs of Ku80 and Ku70 knockdown mice

Marker	Naïve/Scrambled siRNA	Naïve/Ku80 siRNA	Naïve/Ku70 siRNA	P Value (Scrambled siRNA versus Ku80 siRNA)	P Value (Scrambled siRNA versus Ku70 siRNA)
Ku80 levels, ng/30 µg TLP	18.4 $\pm$ 0.65	13.92 $\pm$ 1.1	15.10 $\pm$ 2.03	0.01	0.14
Ku70 levels, ng/30 µg TLP	8.12 $\pm$ 0.22	6.16 $\pm$ 0.48	5.73 $\pm$ 0.23	0.01	0.0007
8-Isoprostane levels, pg/0.1 µg TLP	0.15 $\pm$ 0.08	7.49 $\pm$ 3.49	19.07 $\pm$ 5.42	0.10	0.03
IMM integrity, AU/2 µg lung Mito	2,848.4 $\pm$ 224.2	1,387.4 $\pm$ 141.7	2,202.9 $\pm$ 115.8	0.004	0.0430
Cytochrome c, ng/15 µg lung Cyto	3.50 $\pm$ 0.80	18.11 $\pm$ 3.96	7.95 $\pm$ 1.11	0.02	0.03
Complex I activity, mOD·min ⁻¹ ·45 µg lung Mito	0.96 $\pm$ 0.09	1.20 $\pm$ 0.18	1.01 $\pm$ 0.05	0.27	0.63
Complex IV activity, AU/10 µg lung Mito	1.79 $\pm$ 0.19	1.73 $\pm$ 0.18	1.40 $\pm$ 0.10	0.83	0.13
Cytosolic calpain activity, RFU/20 µg P	21,519 $\pm$ 1,486	36,311 $\pm$ 7,184	21,048 $\pm$ 4,985	0.06	0.93
Caspase-8 activity, AU/20 µg lung Cyto	8,743 $\pm$ 1,071	13,305 $\pm$ 1,290	10,988 $\pm$ 995	0.05	0.36
Caspase-12 activity, AU/20 µg lung Cyto	33,469.3 $\pm$ 1,814	40,251 $\pm$ 163.4	39,233 $\pm$ 1,730	0.0068	0.07
Caspase-9 activity, AU/20 µg lung Cyto	7,921 $\pm$ 396.4	11,411 $\pm$ 1,305	8,547 $\pm$ 639.1	0.03	0.43
Caspase-3 activity, AU/20 µg lung TLP	197.8 $\pm$ 4.73	287.0 $\pm$ 33.96	2,23.0 $\pm$ 7.76	0.02	0.03

Values are means $\pm$ SE. AU, arbitrary units; ER, endoplasmic reticulum; RFU, relative fluorescence units; TLP, total lung protein; IMM, inner mitochondrial membrane; Cyto, cytosol; Mito, mitochondria; mOD, milli-optical density units.



ER stress responses. Mitochondrial stress is a frequent precursor to apoptotic cell death, and Ku70 has been shown to

To confirm mitochondrial-mediated cell death, we measured cytochrome *c* in lung cytosol from Ku-deficient mice. Deficiency of either Ku protein showed significant increases in cytochrome *c* compared with control mice, although once again the phenotype was more robust with Ku80 knockdown (Fig. 8, A–C, and Table 2). Similarly, Ku80 and Ku70 deficiency in vivo demonstrated reduced inner mitochondrial membrane integrity in isolated lung mitochondria, with stron-

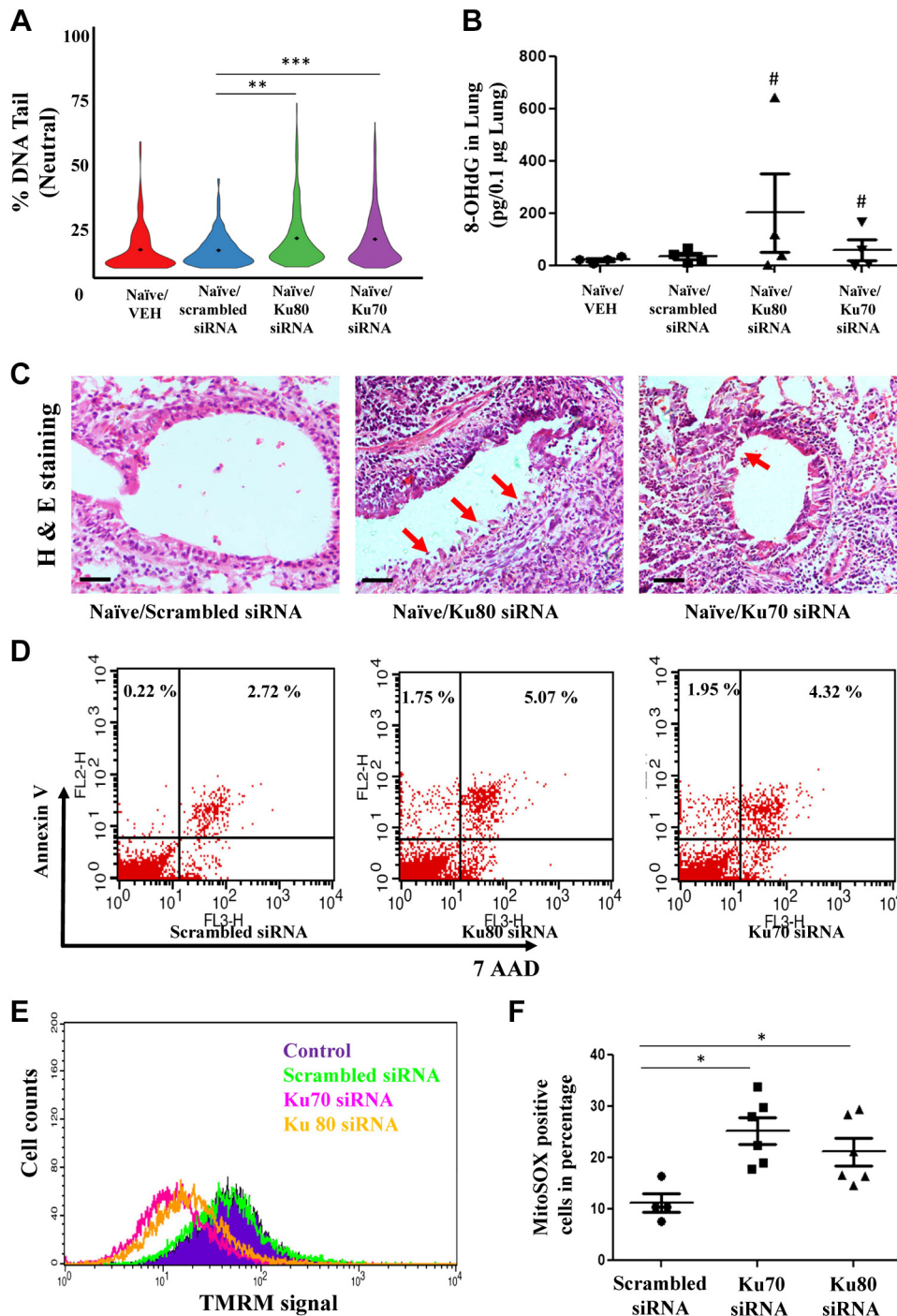


Fig. 7. siRNA-mediated knockdown of Ku80 and Ku70 in naïve mice induces DNA damage, cell death, and mitochondrial stress. Neutral comet assay in lungs (A) and 8-hydroxy-deoxyguanosine (8-OHdG) levels (B) in lungs from Ku70/ Ku80 knockdown mice. C: H&E-stained lung sections of Ku70/ Ku80 knockdown mice showing epithelial denudation. VEH, vehicle. Bars, 100 µm; arrows indicate the denudation of bronchial epithelium. D and E: flow cytometry analysis of 7-aminoactinomycin D (7-AAD) or annexin V (D) and tetramethylrhodamine methyl ester (TMRM) (E) in BEAS-2B cells transfected with Ku70 siRNA or Ku80 siRNA. F: flow cytometry analysis of MitoSOX-positive cells (percentage) in human bronchial epithelial cells transfected with Ku70 siRNA or Ku80 siRNA. Data represent means $\pm$ SE; $n = 5-6$ in each group for mouse experiments and $n = 3-4$ for in vitro cell experiments. * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$; # $P =$ nonsignificant versus naïve/scrambled siRNA.

ger suppression after Ku80 siRNA treatment (Table 2). Despite these findings, however, neither Ku70 nor Ku80 knockdown mice showed a significant reduction in complex I and IV activities (Table 2).

Given the prominent increase in ER and mitochondrial oxidative stress seen in our previous studies (27, 30), we also examined the role of Ku proteins in buffering ER stress in vivo. We found that Ku80 knockdown mice that demonstrated more robust asthma-like phenotypes in our prior assays also showed increased activity of caspases associated with ER stress, namely caspase-8, caspase-9, and caspase-12 (Table

2). To investigate further, we assessed ER stress markers including binding immunoglobulin protein/78 kDa glucose-regulated protein (BIP/Grp78), 94 kDa glucose-regulated protein (Grp94), and C/EBP homologous protein (CHOP) in the lungs of Ku70 and Ku80 knockdown mice. Interestingly, Ku80 knockdown mice, but not Ku70 knockdown mice, had elevated levels of all tested ER stress markers (Fig. 8, D–F). There was also a trend toward elevated levels (1.7-fold increase) of intracellular calcium based on calpain levels in the Ku80-deficient mice (Table 2). Whether these findings of increased ER stress are truly Ku80 specific or primarily

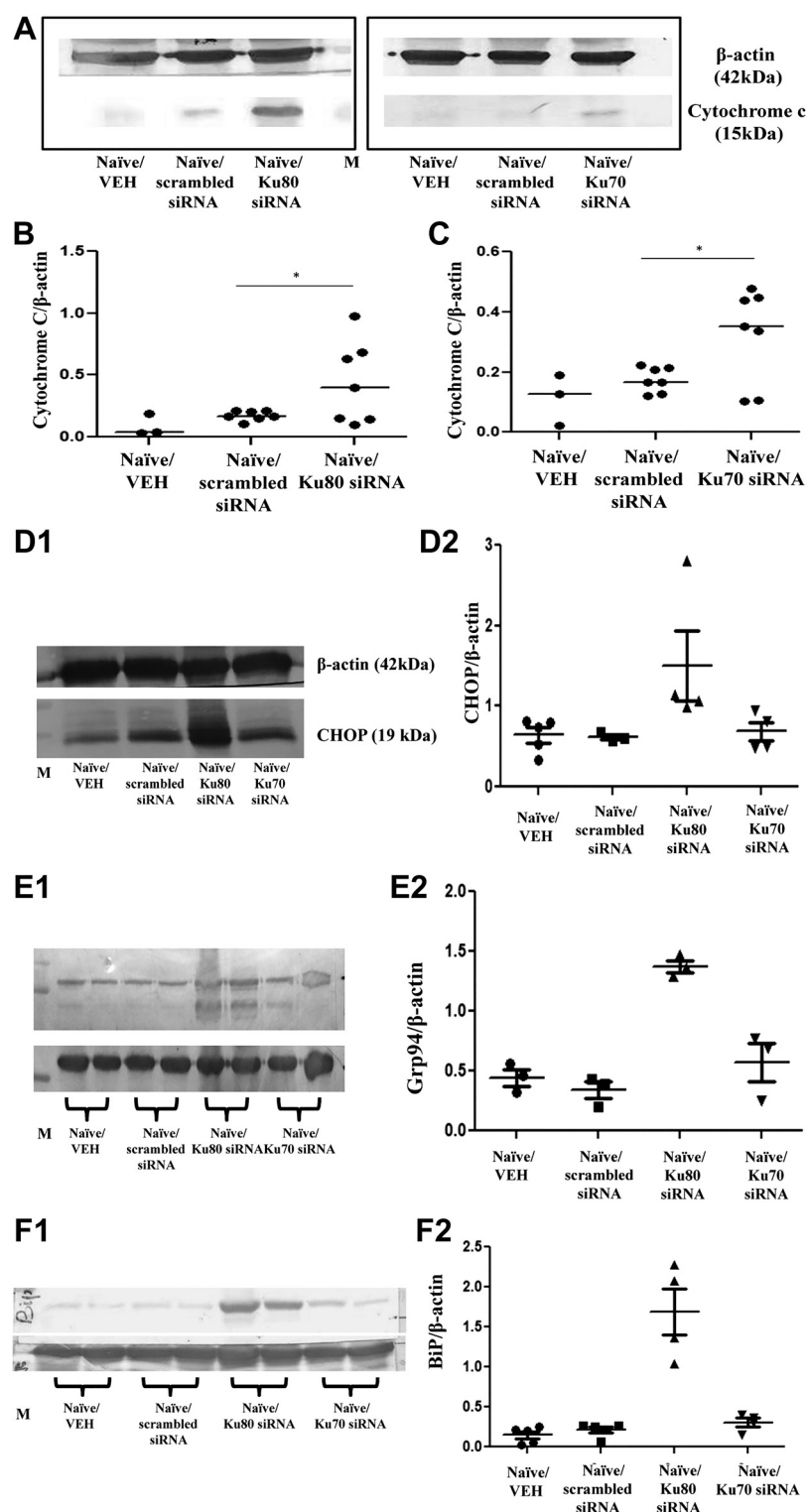


Fig. 8. siRNA-mediated knockdown of Ku80 and Ku70 in naïve mice causes organelle stress. A–C: Western blot analysis of cytochrome *c* in lung cytosol. Western blot analysis of C/EBP-homologous protein (CHOP) (D), glucose-regulated protein 94 kDa (Grp94) (E), and binding immunoglobulin protein (BiP) (F) in lungs of Ku70/ Ku80 knockdown mice. VEH, vehicle. Data represent means $\pm$ SE; $n = 5$ –6 in each group for mice experiments. * $P < 0.05$.

reflect the more robust phenotype seen in these animals remains to be further studied.

Altogether, our findings indicated that Ku protein deficiency leads to increased oxidative stress in both the nucleus and other cellular organelles, leading to DNA damage, mitochondrial dysfunction, and ER stress responses. This profound bronchial epithelial stress likely explains the development of bronchial

airway inflammation and apoptosis, leading to the observed spontaneous asthma-like phenotype.

DISCUSSION

DNA repair proteins are key elements of oxidative stress responses in the nucleus and multiple organelles and likely

have pleiotropic roles in asthma pathogenesis (4, 5, 32). Here, we observed the expression of Ku proteins in the bronchial epithelium and have found strong evidence for a causative role for Ku deficiency in asthma pathogenesis. In particular, Ku protein deficiency is seen in asthmatic patients and ovalbumin-sensitized and challenged (OVA/OVA) mice. Knockdown of either Ku70 or Ku80 exacerbates bronchial hyperresponsiveness, airway subepithelial fibrosis, airway denuding, and other asthma-like features in naïve mice. Examination of mitochondrial and ER stress markers both in vitro and in vivo suggests a pivotal cytosolic role for these proteins in organelle homeostasis that may be mediating these effects.

DNA repair proteins such as Ku, ATM, and γ -H2AX are constitutively expressed in normal bronchial epithelium within the lung (8, 16, 39). This constitutive expression likely indicates either 1) the need for continuous active DNA repair to combat basal metabolism (6) and continuous toxic exposures or 2) another role for these DNA repair proteins in epithelial function and lung homeostasis. Both Ku proteins are reduced in asthmatic bronchial epithelia that we found to be associated with increased DNA damage. The increased oxidative damage identified by 8-OHdG in sera and lungs of asthmatic patients should be carefully interpreted, as 8-OHdG is sensitive to multiple oxidative stress inducers such as exposure to cigarette smoke and malignancies (24). However, we carefully excluded

patients with these comorbidities from our serum study and included only nonsmoking patients with focal tumors in our lung tissue experiments. Furthermore, we evaluated healthy surrounding tissues that have been found to have negligible oxidative stress and 8-OHdG levels in patients with focal malignancies (20, 38). Finally, our murine models were free from confounding factors and still demonstrated increased oxidative stress after induction of asthma-like phenotypes. We therefore believe that our data collectively indicate strong evidence for increased oxidative stress attributable to asthma.

Our studies suggest that both Ku70 and Ku80 play a significant role in preventing mitochondrial stress. Although a role for Ku70 in preventing Bax translocation has previously been described (3), that this effect is recapitulated with Ku80 knockdown has not previously been seen. Whether this is due to Ku80 knockdown itself or the cross-regulation of Ku70 and Ku80 (22, 14, 22) has yet to be determined. Our observation of mitochondrial dysfunction in Ku70- and Ku80-deficient mice may have pathogenetic importance in asthma, as multiple reports have demonstrated dysfunctional mitochondria in asthmatic airway epithelia (28, 29, 34); indeed, the replacement of these dysfunctional mitochondria has been shown to attenuate asthma features (2).

Our findings of increased oxidative stress throughout the cell led us to investigate the role of Ku deficiency in reducing ER

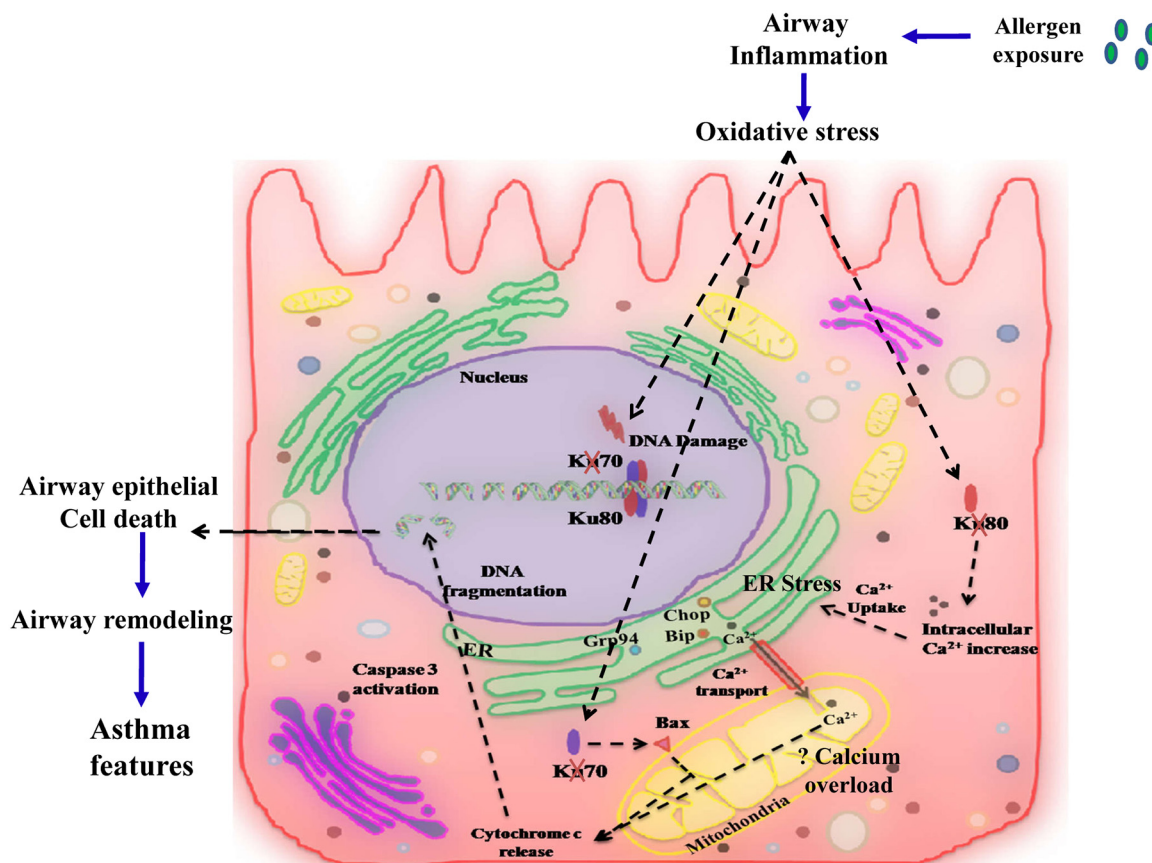


Fig. 9. Schema to illustrate organelle stress caused by Ku deficiency. Oxidative stress due to asthmatic airway inflammation may degrade Ku70 and Ku80 both in the nucleus and in cytoplasm. Nuclear loss of Ku70 or Ku80 leads to elevated DNA damage that promotes eventual cell death. Cytoplasmic loss of Ku70 may lead to more mitochondrial Bax translocation, contributing to cytochrome *c* release, that promotes caspase-mediated apoptosis. Ku80 cytosolic deficiency may lead to accumulation of intracellular calcium, resulting in endoplasmic reticulum (ER) stress and further mitochondrial fragility. The resultant airway epithelial cell death could activate the process of airway remodeling that leads to causing various asthma features like airway hyperresponsiveness.

stress. Intriguingly, Ku80 knockdown mice demonstrated marked increases in numerous ER stress markers. These findings correlate with studies that have highlighted Ku80 deficiency (42) and increased ER stress (21) in humans and mice exposed to cigarette smoke, as well as in our own work showing increased ER stress markers in asthmatic lungs (18, 30, 31). Whether Ku80 deficiency leads to increases in ER stress or vice versa is not fully clear in humans, but our study has shown that Ku80 deficiency in naïve mice is sufficient to predispose to asthma-like phenotypes without an additional stimulus. Although the underlying mechanism requires further study, we suspect that Ku deficiency leads to vulnerability of the epithelium to oxidative stress, inducing ER and mitochondrial dysfunction, DNA damage, and eventually epithelial cell death (Fig. 9).

In terms of the mechanism underlying Ku-mediated protection from oxidative and ER stress, our findings are currently limited. DNA damage is linked to the ER stress response (7, 10) and may represent the common pathway if the Ku80-specific findings are primarily due to the more robust phenotype in this group. However, the minimal change seen in Ku70-deficient mice could also suggest an alternative Ku80-specific mechanism of action. The presence of both Ku proteins in the cytoplasm at significant levels, which has been previously demonstrated for Ku70 (41), is suggestive of possible alternative functional roles. Interestingly, Ku binds with GRP78 and GRP94 promoters (26); however, the exact regulatory role of Ku on these ER resident proteins and further ER stress has not yet been elucidated. This may partially explain the increased GRP78 and GRP94 expression in Ku80 knockdown murine lungs and human bronchial epithelia. Earlier literature also demonstrated possible regulation of heat shock protein70 (hsp70), mouse mammary tumor virus (MMTV), and epithelium-specific ETS transcription factor 1 (ESE-1) (12, 13, 40) by Ku, but the nature of the regulation is not known. The mechanisms responsible for Ku80 deficiency-mediated ER stress response require further investigation.

Limitations of our approach include the possibility of off-target effects from siRNA administration. We believe this is ameliorated in part via supportive work in bronchial epithelial cells in vitro. While certainly an inducible knockout approach would be an elegant system to further investigate these mechanisms in airway epithelia, inducible knockout mice are not currently available.

While we were investigating the role of Ku in asthma pathogenesis, another group has demonstrated evidence for increased DNA damage in asthmatic lungs (9). Although cell death is the converging point for multiple cellular stresses like DNA damage and various organelle stresses, these multiple stress pathways are tightly interconnected. In this context, it is difficult to distinguish the roles of Ku proteins in DNA repair versus organelle maintenance and which result in apoptosis in the setting of Ku70/Ku80 deficiencies. Our study found that deficiency of Ku proteins leads to mitochondrial dysfunction, cytochrome *c* activation, and elevated caspase-3. On the basis of these observations, we hypothesize that Ku proteins play an integral role in maintaining epithelial integrity and lung homeostasis; disruption of these proteins provides a strong stimulus for airway hyperresponsiveness and remodeling. Although additional mechanistic work is needed, this pathway offers an alluring and novel therapeutic target in asthma.

In summary (Fig. 9), we found reduced expression of both Ku70 and Ku80 in asthmatic bronchial epithelia. Knockdown of either protein in naïve mice elicits oxidative stress, DNA damage, and mitochondrial collapse and ultimately results in bronchial epithelial cell apoptosis along with asthma-like features. Although the detailed role of Ku proteins beyond prevention of mitochondrial/ER stress needs to be further investigated, these findings have revealed a previously unknown role for Ku proteins in asthma pathogenesis. This work suggests the potential for Ku regulation as a therapeutic target in asthma and may even have implications in other diseases associated with mitochondrial and ER stress, such as neurodegenerative conditions and diabetes. Future work should examine the mechanism of Ku-mediated homeostasis in more detail and develop methods of upregulating Ku expression in damaged tissue to mitigate the disease.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

R.R. and U.M. conceived and designed research; R.R., V.E.V., A.J., V.J., S.M., and U.M. performed experiments; R.R., V.E.V., A.J., V.J., S.M., S.K.V., Y.S.P., and U.M. analyzed data; R.R., V.E.V., A.J., S.M., S.K.V., and U.M. interpreted results of experiments; R.R., A.J., and U.M. prepared figures; R.R., L.E.F., Y.S.P., B.G., A.A., and U.M. drafted manuscript; R.R., A.J., P.B.D., L.E.F., Y.S.P., B.G., A.A., and U.M. edited and revised manuscript; R.R., V.E.V., A.J., V.J., S.M., S.K.V., P.B.D., L.E.F., Y.S.P., B.G., A.A., and U.M. approved final version of manuscript.

REFERENCES

1. Aghapour M, Remels AHV, Pouwels SD, Bruder D, Hiemstra PS, Cloonan SM, Heijink IH. Mitochondria: at the crossroads of regulating lung epithelial cell function in chronic obstructive pulmonary disease. *Am J Physiol Lung Cell Mol Physiol* 318: L149–L164, 2020. doi:10.1152/ajplung.00329.2019.
2. Ahmad T, Mukherjee S, Pattnaik B, Kumar M, Singh S, Kumar M, Rehman R, Tiwari BK, Jha KA, Barhanpurkar AP, Wani MR, Roy SS, Mabalirajan U, Ghosh B, Agrawal A. Miro1 regulates intercellular mitochondrial transport & enhances mesenchymal stem cell rescue efficacy. *EMBO J* 33: 994–1010, 2014. doi:10.1002/embj.201386030.
3. Amsel AD, Rathaus M, Kronman N, Cohen HY. Regulation of the proapoptotic factor Bax by Ku70-dependent deubiquitylation. *Proc Natl Acad Sci USA* 105: 5117–5122, 2008. doi:10.1073/pnas.0706700105.
4. Ba X, Aguilera-Aguirre L, Sur S, Boldogh I. 8-Oxoguanine DNA glycosylase-1-driven DNA base excision repair: role in asthma pathogenesis. *Curr Opin Allergy Clin Immunol* 15: 89–97, 2015. doi:10.1097/ACI.0000000000000135.
5. Bacsí A, Pan L, Ba X, Boldogh I. Pathophysiology of bronchoconstriction: role of oxidatively damaged DNA repair. *Curr Opin Allergy Clin Immunol* 16: 59–67, 2016. doi:10.1097/ACI.0000000000000232.

6. Burcham PC. Internal hazards: baseline DNA damage by endogenous products of normal metabolism. *Mutat Res* 443: 11–36, 1999. doi:10.1016/S1383-5742(99)00008-3.
7. Cao SS, Kaufman RJ. Endoplasmic reticulum stress and oxidative stress in cell fate decision and human disease. *Antioxid Redox Signal* 21: 396–413, 2014. doi:10.1089/ars.2014.5851.
8. Caramori G, Adcock IM, Casolari P, Ito K, Jazrawi E, Tsaprouni L, Villetti G, Civelli M, Carnini C, Chung KF, Barnes PJ, Papi A. Unbalanced oxidant-induced DNA damage and repair in COPD: a link towards lung cancer. *Thorax* 66: 521–527, 2011. doi:10.1136/thx.2010.156448.
9. Chan TK, Loh XY, Peh HY, Tan WNF, Tan WSD, Li N, Tay IJJ, Wong WSF, Engelward BP. House dust mite-induced asthma causes oxidative damage and DNA double-strand breaks in the lungs. *J Allergy Clin Immunol* 138: 84–96.e1, 2016. doi:10.1016/j.jaci.2016.02.017.
10. Dicks N, Gutierrez K, Michalak M, Bordignon V, Agellon LB. Endoplasmic reticulum stress, genome damage, and cancer. *Front Oncol* 5: 11, 2015. doi:10.3389/fonc.2015.00011.
11. Downs JA, Jackson SP. A means to a DNA end: the many roles of Ku. *Nat Rev Mol Cell Biol* 5: 367–378, 2004. doi:10.1038/nrm1367.
12. Featherstone C, Jackson SP. Ku, a DNA repair protein with multiple cellular functions? *Mutat Res* 434: 3–15, 1999. doi:10.1016/S0921-8777(99)00006-3.
13. Giffin W, Torrance H, Rodda DJ, Préfontaine GG, Pope L, Hache RJ. Sequence-specific DNA binding by Ku autoantigen and its effects on transcription. *Nature* 380: 265–268, 1996. doi:10.1038/380265a0.
14. Gu Y, Jin S, Gao Y, Weaver DT, Alt FW. Ku70-deficient embryonic stem cells have increased ionizing radiosensitivity, defective DNA end-binding activity, and inability to support V(D)J recombination. *Proc Natl Acad Sci USA* 94: 8076–8081, 1997. doi:10.1073/pnas.94.15.8076.
15. Gyori BM, Venkatachalam G, Thiagarajan PS, Hsu D, Clement MV. OpenComet: an automated tool for comet assay image analysis. *Redox Biol* 2: 457–465, 2014. doi:10.1016/j.redox.2013.12.020.
16. Halicka HD, Zhao H, Li J, Traganos F, Zhang S, Lee M, Darzynkiewicz Z. Genome protective effect of metformin as revealed by reduced level of constitutive DNA damage signaling. *Aging (Albany NY)* 3: 1028–1038, 2011. doi:10.18632/aging.100397.
17. Heijink IH, Kuchibhotla VNS, Roffel MP, Maes T, Knight DA, Sayers I, Nawijn MC. Epithelial cell dysfunction, a major driver of asthma development. *Allergy* 75: 1898–1913, 2020. doi:10.1111/all.14421.
18. Jeong JS, Kim SR, Cho SH, Lee YC. Endoplasmic reticulum stress and allergic diseases. *Curr Allergy Asthma Rep* 17: 82, 2017. doi:10.1007/s11882-017-0751-9.
19. Kan S, Hariyadi DM, Grainge C, Knight DA, Bartlett NW, Liang M. Airway epithelial-targeted nanoparticles for asthma therapy. *Am J Physiol Lung Cell Mol Physiol* 318: L500–L509, 2020. doi:10.1152/ajplung.00237.2019.
20. Karihtala P, Soini Y. Reactive oxygen species and antioxidant mechanisms in human tissues and their relation to malignancies. *APMIS* 115: 81–103, 2007. doi:10.1111/j.1600-0463.2007.apm_514.x.
21. Kelsen SG. The unfolded protein response in chronic obstructive pulmonary disease. *Ann Am Thorac Soc* 13, Suppl 2: S138–S145, 2016. doi:10.1513/AnnalsATS.201506-320KV.
22. Koike M, Yutoku Y, Koike A. Establishment of ku70-deficient lung epithelial cell lines and their hypersensitivity to low-dose x-irradiation. *J Vet Med Sci* 73: 549–554, 2011. doi:10.1292/jvms.10-0454.
23. Kumar A, Das S, Agrawal A, Mukhopadhyay I, Ghosh B. Genetic association of key Th1/Th2 pathway candidate genes, IRF2, IL6, IFNGR2, STAT4 and IL4RA, with atopic asthma in the Indian population. *J Hum Genet* 60: 443–448, 2015. doi:10.1038/jhg.2015.45.
24. Kytikova OY, Gvozdenko TA, Vitkina TI. Oxidative DNA damage in the pathogenesis of chronic obstructive pulmonary disease in older patients. *Adv Gerontol* 7: 163–165, 2017. doi:10.1134/S2079057017020096.
25. Lim DS, Vogel H, Willerford DM, Sands AT, Platt KA, Hasty P. Analysis of ku80-mutant mice and cells with deficient levels of p53. *Mol Cell Biol* 20: 3772–3780, 2000. doi:10.1128/MCB.20.11.3772-3780.2000.
26. Liu ES, Lee AS. Common sets of nuclear factors binding to the conserved promoter sequence motif of two coordinately regulated ER protein genes, GRP78 and GRP94. *Nucleic Acids Res* 19: 5425–5431, 1991. doi:10.1093/nar/19.19.5425.
27. Mabalirajan U, Dinda AK, Kumar S, Roshan R, Gupta P, Sharma SK, Ghosh B. Mitochondrial structural changes and dysfunction are associated with experimental allergic asthma. *J Immunol* 181: 3540–3548, 2008. doi:10.4049/jimmunol.181.5.3540.
28. Mabalirajan U, Dinda AK, Sharma SK, Ghosh B. Esculetin restores mitochondrial dysfunction and reduces allergic asthma features in experimental murine model. *J Immunol* 183: 2059–2067, 2009. doi:10.4049/jimmunol.0900342.
29. Mabalirajan U, Rehman R, Ahmad T, Kumar S, Singh S, Leishangthem GD, Aich J, Kumar M, Khanna K, Singh VP, Dinda AK, Biswal S, Agrawal A, Ghosh B. Linoleic acid metabolite drives severe asthma by causing airway epithelial injury. *Sci Rep* 3: 1349, 2013. doi:10.1038/srep01349.
30. Makhija L, Krishnan V, Rehman R, Chakraborty S, Maity S, Mabalirajan U, Chakraborty K, Ghosh B, Agrawal A. Chemical chaperones mitigate experimental asthma by attenuating endoplasmic reticulum stress. *Am J Respir Cell Mol Biol* 50: 923–931, 2014. doi:10.1165/rcmb.2013-0320OC.
31. Miller M, Tam AB, Cho JY, Doherty TA, Pham A, Khorram N, Rosenthal P, Mueller JL, Hoffman HM, Suzukawa M, Niwa M, Broide DH. ORMDL3 is an inducible lung epithelial gene regulating metalloproteases, chemokines, OAS, and ATF6. *Proc Natl Acad Sci USA* 109: 16648–16653, 2012. doi:10.1073/pnas.1204151109.
32. Mishra A, Brown AL, Yao X, Yang S, Park SJ, Liu C, Dagur PK, McCoy JP, Keeran KJ, Nugent GZ, Jeffries KR, Qu X, Yu ZX, Levine SJ, Chung JH. Dendritic cells induce Th2-mediated airway inflammatory responses to house dust mite via DNA-dependent protein kinase. *Nat Commun* 6: 6224, 2015. doi:10.1038/ncomms7224.
33. Potaczek DP, Miethe S, Schindler V, Alhamdan F, Garn H. Role of airway epithelial cells in the development of different asthma phenotypes. *Cell Signal* 69: 109523, 2020. doi:10.1016/j.cellsig.2019.109523.
34. Prakash YS, Pabelick CM, Sieck GC. Mitochondrial dysfunction in airway disease. *Chest* 152: 618–626, 2017. doi:10.1016/j.chest.2017.03.020.
35. Rehman R, Mabalirajan U. Proficient DNA repair in house dust mite-induced asthma? *J Allergy Clin Immunol* 139: 713–714, 2017. doi:10.1016/j.jaci.2016.07.045.
36. Sharma M, Batra J, Mabalirajan U, Sharma S, Nagarkatti R, Aich J, Sharma SK, Niphadkar PV, Ghosh B. A genetic variation in inositol polyphosphate 4 phosphatase enhances susceptibility to asthma. *Am J Respir Crit Care Med* 177: 712–719, 2008. doi:10.1164/rccm.200705-781OC.
37. Song JY, Lim JW, Kim H, Morio T, Kim KH. Oxidative stress induces nuclear loss of DNA repair proteins Ku70 and Ku80 and apoptosis in pancreatic acinar AR42J cells. *J Biol Chem* 278: 36676–36687, 2003. doi:10.1074/jbc.M303692200.
38. Sova H, Jukkola-Vuorinen A, Puistola U, Kauppila S, Karihtala P. 8-Hydroxydeoxyguanosine: a new potential independent prognostic factor in breast cancer. *Br J Cancer* 102: 1018–1023, 2010. doi:10.1038/sj.bjc.6605565.
39. Tanaka T, Kajstura M, Halicka HD, Traganos F, Darzynkiewicz Z. Constitutive histone H2AX phosphorylation and ATM activation are strongly amplified during mitogenic stimulation of lymphocytes. *Cell Prolif* 40: 1–13, 2007. doi:10.1111/j.1365-2184.2007.00417.x.
40. Wang H, Fang R, Cho JY, Libermann TA, Oettgen P. Positive and negative modulation of the transcriptional activity of the ETS factor ESE-1 through interaction with p300, CREB-binding protein, and Ku 70/86. *J Biol Chem* 279: 25241–25250, 2004. doi:10.1074/jbc.M401356200.
41. Wang B, Xie M, Li R, Owonikoko TK, Ramalingam SS, Khuri FR, Curran WJ, Wang Y, Deng X. Role of Ku70 in deubiquitination of Mcl-1 and suppression of apoptosis. *Cell Death Differ* 21: 1160–1169, 2014. doi:10.1038/cdd.2014.42.
42. Yao H, Sundar IK, Gorbunova V, Rahman I. P21-PARP-1 pathway is involved in cigarette smoke-induced lung DNA damage and cellular senescence. *PLoS One* 8: e80007, 2013. doi:10.1371/journal.pone.0080007.
43. Zeng H, Nanayakkara GK, Shao Y, Fu H, Sun Y, Cueto R, Yang WY, Yang Q, Sheng H, Wu N, Wang L, Yang W, Chen H, Shao L, Sun J, Qin X, Park JY, Drosatos K, Choi ET, Zhu Q, Wang H, Yang X. DNA checkpoint and repair factors are nuclear sensors for intracellular organelle stresses-inflammations and cancers can have high genomic risks. *Front Physiol* 9: 516, 2018. doi:10.3389/fphys.2018.00516.