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ORIGINAL RESEARCH

Xeroderma Pigmentosum Group C Deficiency Alters Cigarette Smoke DNA Damage Cell Fate and Accelerates Emphysema Development

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Abstract

Cigarette smoke (CS) exposure is a major risk factor for the development of emphysema, a common disease characterized by loss of cells comprising the lung parenchyma. The mechanisms of cell injury leading to emphysema are not completely understood but are thought to involve persistent cytotoxic or mutagenic DNA damage induced by CS. Using complementary cell culture and mouse models of CS exposure, we investigated the role of the DNA repair protein, xeroderma pigmentosum group C (XPC), on CS-induced DNA damage repair and emphysema. Expression of XPC was decreased in mouse lungs after chronic CS exposure and XPC knockdown in cultured human lung epithelial cells decreased their survival after CS exposure due to activation of the intrinsic apoptosis pathway. Similarly, cell autophagy and apoptosis were increased in XPC-deficient mouse lungs and were further increased by CS exposure. XPC deficiency was associated with structural and functional changes characteristic of emphysema, which were worsened by age, similar to levels observed with chronic CS exposure. Taken together, these findings suggest that repair of DNA damage by XPC plays an important and previously unrecognized role in the maintenance of alveolar structures. These findings support that loss of XPC, possibly due to chronic CS exposure, promotes emphysema development and further supports a link between DNA damage, impaired DNA repair, and development of emphysema.

Keywords: chronic obstructive lung disease; xeroderma pigmentosum; apoptosis; autophagy

Clinical Relevance

These findings support a novel role of xeroderma pigmentosum group C (XPC) in emphysema development and more globally expounds on the poorly understood link between cigarette smoke and DNA damage repair on emphysema development and susceptibility to lung cancer. Furthermore, our findings are of considerable interest to the DNA repair field, as they expand upon a rather new mechanism for XPC in repair of DNA oxidative damage caused by cigarette smoke.

Exposure to chronic cigarette smoke (CS) is the major risk factor for chronic obstructive pulmonary disease (COPD), such as emphysema, which is the third leading cause of death in the United States, and predicted to be the third-highest cause of death globally by 2020 (1). Emphysema is characterized by progressive loss of alveolar and lung parenchymal structures involved in gas exchange and decreased lung elastance. The pathogenesis of emphysema involves inflammation, increased oxidative stress, excessive structural cell apoptosis, altered autophagy, and premature senescence (2–4). Increased DNA damage, as observed in smokers with and without COPD (5–7), activates DNA damage responses, which, in turn, regulate

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apoptosis, autophagy, and senescence (2, 5–7). Because only certain individuals are susceptible to developing emphysema, individual differences in DNA repair and damage response are likely to be important in the resilience of the lung to the cytotoxic effects of CS. We postulate that a deficient function of proteins involved in DNA damage repair may increase susceptibility to CS-induced emphysema.

Given their direct exposure, the lungs are particularly vulnerable to CS effects. CS causes one DNA-damaging lesion for every 10^6–10^7 DNA bases, well above the level of DNA damage associated with biological relevance (8). Much of the DNA damage induced by CS is direct formation of DNA–chemical adducts. However, indirect mechanisms are also involved in inducing DNA damage by altering chemical metabolism and by inducing local CS-induced inflammation, causing oxidative stress and the formation of reactive oxygen species and reactive nitrogen species. Many DNA lesions caused by CS are large, bulky, and structurally distorting, requiring the nucleotide excision repair (NER) pathway for repair. CS also increases oxidative stress directly and indirectly, by increasing local inflammation, reactive oxygen species, and reactive nitrogen species. Oxidative DNA lesions include oxidized DNA bases (i8-oxo-7,8-dihydroguanine) and abasic sites, both of which are repaired via the base excision repair (BER) pathway. CS exposure has been associated with DNA single-strand breaks and double-strand breaks (DSBs), which often require other DNA repair pathways, such as nonhomologous end-joining or homologous recombination repair for resolution. Furthermore, CS exposure is associated with altered DNA promoter methylation, silenced gene expression, mutations, and allelic loss, all of which may further impact the DNA damage response and, ultimately, cell fate (8). Although CS-induced DNA damage is thought to be a dominant factor in development of CS-induced lung disease, how DNA damage and its repair impacts COPD is largely uncharacterized.

The DNA repair protein xeroderma pigmentosum group C (XPC), is essential to global genomic (GG) NER (GG-NER). Along with its complexing proteins HR23B and Centrin2, XPC identifies bulky, strand-distorting DNA lesions throughout the genome and initiates downstream damage repair by NER (9). However, more recently, XPC has also been implicated in BER by a mechanism that has not been fully elucidated. Decreased repair of the oxidized DNA base, i8-oxo-7,8-dihydroguanine, has been observed *in vitro* in both mouse and human cells (10). Furthermore, mice deficient in XPC accumulate oxidative DNA damage and XPC deficiency is associated with hypersensitivity to DNA oxidizing agents (11). XPC has been implicated in decreased BER activity *in vitro* (12, 13), by binding to oxidatively damaged DNA (14) and possibly augmenting the activity of the BER glycosylases, including 8-oxoguanine DNA glycosylase (15).

We hypothesized that XPC protects the lung from CS-induced emphysema-like changes through increased DNA repair and decreased apoptotic cell death. Using complementary cell culture and mouse models of CS exposure, we show that loss of XPC increases susceptibility to CS-induced apoptosis, and that this effect can be abrogated by the use of the antioxidant chemical, N-acetyl cysteine (NAC). XPC expression is reduced in response to exposure to chronic CS, and the absence of XPC causes age-dependent functional and morphologic changes consistent with emphysema, similar to those observed with chronic CS exposure. These data support a role for XPC-dependent DNA repair of oxidative DNA damage to protect against CS-induced emphysema.

Some of the results of these findings have been previously reported in the form of abstracts (16, 17).

Methods

Compounds and reagents purchased from Thermo-Fisher Scientific, unless otherwise stated.

In Vivo Studies

Animal studies were approved by the Indiana University Institutional Animal Care and Use Committee. Mice (C57BL/6;129) heterozygous for XPC (Jackson Laboratory) were bred to provide $XPC^{-/-}$ (knockout [KO]) and $XPC^{+/+}$ (wild-type [WT]) mice with genotype confirmed by PCR (*see* the supplemental Methods). Littermate male and female XPC-KO and WT mice (aged 6–8 wk) were exposed to air control/ambient air (AC) or CS for indicated durations (18). Briefly, mice were exposed to reference cigarettes (3R4F; Tobacco Research Institute) 5 h/d, 5 d/wk via TE-10 smoking device (Teague Enterprises). Lung compliance was determined by flexiVent device (SCIREQ Inc.). Lung harvest and BAL were performed as previously described (19). Mean linear intercept and volume-to-surface area were determined as previously published (18, 20) and detailed in the supplemental Methods. Results were collected and analyzed in a blinded fashion by separate investigators.

Cell Culture and Treatment

Beas2B cells (SV40-transformed human bronchial epithelial cells; ATCC) were maintained as previously described (19). SV40-transformed skin fibroblast cell lines derived from patients who were $XPC^{+/+}$ (GM637), $XPC^{-/-}$ (GM15983), and established from GM15983 by stable transfection with XPC cDNA using pXPC3 plasmid (GM16248), were obtained from Coriell Cell Biorepositories and maintained according to recommended conditions. Transduction of shRNA lentiviral particles and cell selection was performed using manufacturer instructions (Sigma-Aldrich). NER function was determined using dual luciferase activity as published (21) with modifications (supplemental Methods). CS extract (100%) was prepared by bubbling smoke from two 3R4F cigarettes or ambient air into 20 ml PBS, adjusting pH to 7.4, and passing through a 0.2-μm filter (22). Incubation of cells with the indicated concentrations (v:v) occurred after 16 hours of serum starvation for the durations indicated. Clonogenic survival assays were performed as previously described (23).

Apoptosis Measures

Lung tissue, frozen and embedded in optimum cutting temperature compound, was evaluated for TUNEL staining (Roche Diagnostics). Images were obtained at 200× magnification (DAPI and FITC filters) using a Nikon Eclipse 90i microscope, captured by digital camera using NIS Elements (Nikon Instruments, Inc.). A total of 10 pictures per deidentified specimen were analyzed by a blinded investigator for DAPI- and FITC-positive nuclei. Detection of annexin V–propidium iodide (PI) was

performed as previously described (21) by flow cytometry and quantified by CellQuest Pro (FACSCalibur; BD Biosciences).

Western Blot Analysis

Immunoblotting was performed on mouse lung homogenates and whole cell extracts as previously described (23) using validated antibodies (supplemental Methods). Densitometry was quantified using Image Lab Software (Bio-Rad).

qRT-PCR for mRNA Analysis

RNA was isolated by TRIzol separation and cDNA generated (High Capacity RNA-to-cDNA kit; Thermo Fisher). Validated 6-carboxyfluorescein–labeled gene primer/probe sets (supplemental Methods) were used for qRT-PCR, per the manufacturer's instructions (Taqman). Gene expression was quantified by ΔΔCT Relative Quantification (ABI 7,500; Applied Biosystems). Each assay was performed in triplicate using 3 replicates and the means compared using ΔCt (DataAssist Software).

Statistical Analysis

Unless otherwise noted, statistical analysis was by ANOVA using SigmaPlot (Systat Software, Inc.) with statistical significance defined as P less than 0.05.

Results

Chronic CS Decreases XPC Expression in Mouse Lungs

Chronic CS exposure in mice alters the expression of several genes involved in DNA repair (24). We first evaluated whether XPC expression was altered by chronic CS exposure in a widely used mouse model, where mice of C57Bl/6 background strain are exposed to CS for 6 months to develop emphysema-like lung disease (18, 24). Expression of XPC and other DNA repair proteins was measured by qRT-PCR and determined by ΔΔCt. There was a statistically significant decrease in XPC expression in mouse lung homogenates from C57Bl/6 mice exposed to 6 months of CS compared with those exposed to ambient air (Figure 1A). This change in XPC expression was unique, as the expression of other genes involved in DNA repair via GG-NER (Figures 1B and 1C), transcriptionally coupled NER (TC-NER) (Figure 1E), GG- and TC-NER (Figure 1D), and BER (Figure 1F) showed no significant change in this model of chronic CS exposure.

XPC Deficiency Increases Cell Susceptibility to Cell Death through Apoptosis during CS Exposure

The DNA repair protein, XPC, is involved in repair of bulky, strand-distorting DNA lesions, such as 4-aminobiphenyl and benzo(a)pyrene diol epoxide, through the NER pathway (8, 9) and has more recently been implicated in repair of oxidative DNA damage, such as 8-oxoG, through BER (10). We next investigated the mechanism by which XPC deficiency may impact DNA repair after cytotoxic CS exposure. We developed clonally expanded, stable XPC knockdown (KD) (shXPC) and nontargeted control (shCtrl) cell lines using lentiviral transfection of shRNA in Beas2B cells (an immortalized human lung epithelial cell line). Using Western blot analysis and qRT-PCR we determined that compared with control cells, both XPC KD cell lines generated (shXPC 119B-1 and shXPC 119B-3) exhibited significantly decreased XPC protein and RNA expression, respectively (Figures 2A and 2B). Cells deficient in XPC were also deficient in NER activity catalyzed repair of a ultraviole-damaged plasmid, as indicated by decreased firefly luciferase expression after transient transfection (Figure 2C).

To determine the impact of XPC on cell response of lung epithelial cells to CS, we exposed Beas2B cells with WT XPC (shCtrl) or XPC KD (Beas2B clones 119B-1 and 199B-3) to an aqueous extract of CS or ambient air control. Compared with controls, cells with XPC KD had significantly decreased clonogenic survival when exposed to increasing concentrations of CS. (Figures 3A and 3B). To determine whether this effect is associated with increased cell death rather than impaired clonal proliferation, we measured apoptosis using flow cytometry–based staining for phosphatidylserine with annexin V and for membrane permeability with PI. Compared with controls, XPC-deficient cells had significantly higher percentages of annexin V–positive and annexin V–PI dually positive cells in a dose-dependent CS exposure study (Figure 3C). XPC-deficient cells exhibited between 4.5- to 14-fold increased apoptosis after exposure to CS (10%; v:v, $P < 0.05$; Figure 3D). CS-induced apoptosis in XPC-deficient cells was associated with activation of the intrinsic

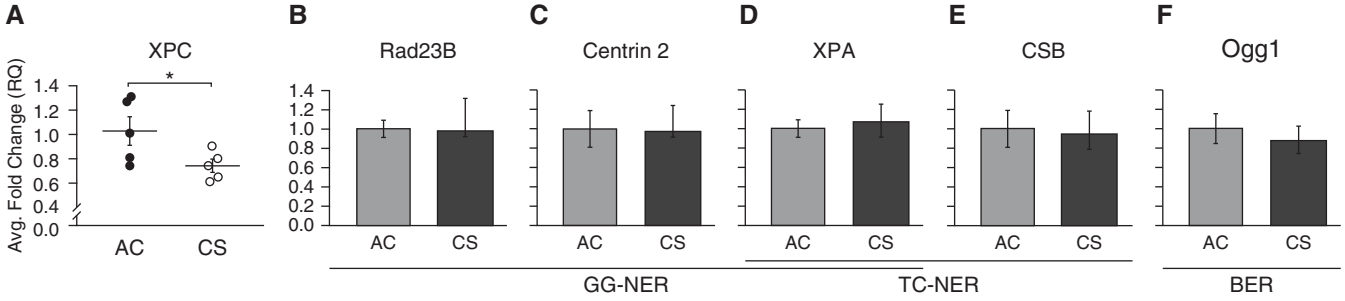

Figure 1. Effect of chronic cigarette smoke (CS) on lung xeroderma pigmentosum group C (XPC) expression. XPC RNA expression by qRT-PCR from whole-lung homogenates of mice exposed to 6 months of CS compared with lungs from mice exposed to ambient air (*A*). RNA expression by qRT-PCR of other DNA repair proteins involved in global genomic (GG)–nucleotide excision repair (NER) (*B* and *C*), GG- and transcriptionally coupled (TC)-NER (*D*), TC-NER (*E*), and base excision repair (BER) (*F*) from whole-lung homogenates of mice exposed to 6 months of CS compared with lungs from mice exposed to ambient air ($n = 5$ per group, three replicates performed in triplicate). *$P = 0.027$; statistical analysis performed by one-way ANOVA using ΔCt values. AC = air control/ambient air; Avg. = average; CSB = Cockayne syndrome B; Ogg1 = 8-oxoguanine DNA glycosylase; Rad23B = ultraviolet excision repair protein RAD23 homolog B; RQ = relative quantification; XPA = xeroderma pigmentosum group A.

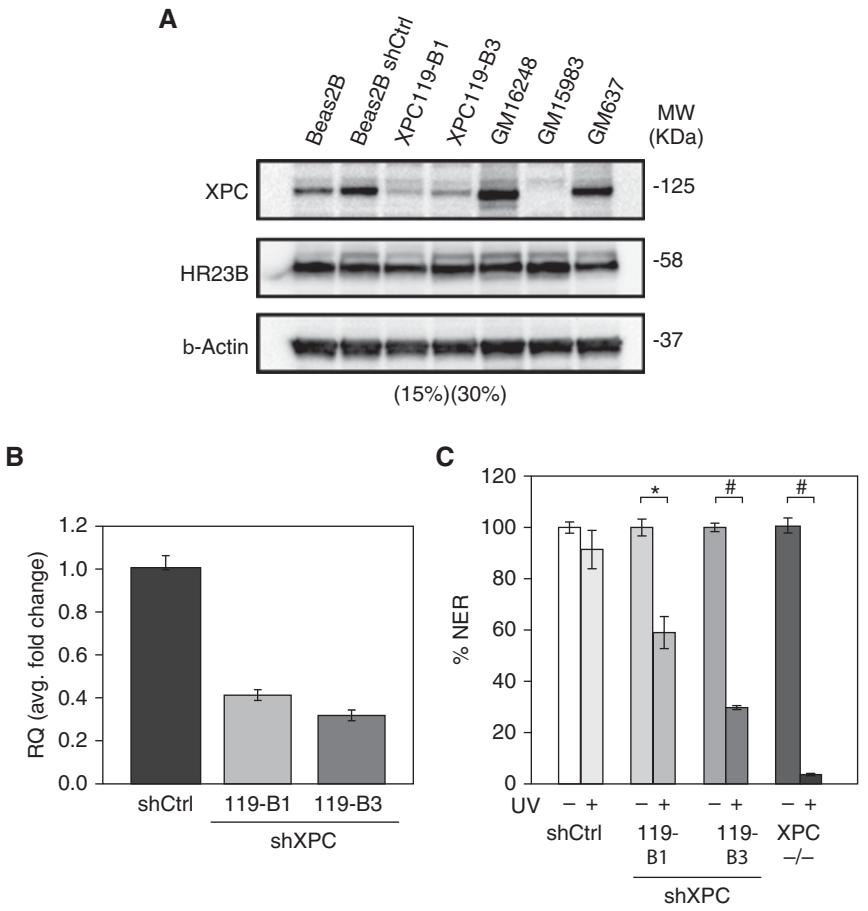

Figure 2. Stable lentiviral XPC knockdown (KD) cell culture models. XPC expression levels are measured by Western blot analysis in Beas2B cells after stable KD of XPC by lentiviral shRNA and compared with loading control (β-actin), with specificity confirmed by expression levels of the XPC complexing protein, HR23B (*A*). Two XPC KD clones (Beas2B: 119-B1 and 119-B3) are shown with the parent cell line (Beas2B), nontargeting shRNA control (shCtrl) and control fibroblast cell lines from an unaffected human (GM637), and XPC-deficient proband unmodified (GM15983) and reconstituted with XPC cDNA (GM16248). Calculated XPC expression levels normalized to loading control and compared with shCtrl are shown below a representative immunoblot. XPC RNA expression levels by qRT-PCR are shown compared with shCtrl (*B*). An *in situ* repair assay is used to confirm decreased repair capacity (% NER) in Beas2B XPC KD clones (*shXPC*) compared with shCtrl and in the XPC-deficient fibroblast cell line, GM15983 (*C*). The relative ratio of firefly:renilla luciferase activity is measured after cotransfection with either an unmodified (*C*) or ultraviolet (UV)-modified plasmid encoding firefly luciferase and a plasmid encoding for renilla luciferase as a transfection control (mean ± SD; $n = 3$; $^{*}P < 0.05$, $^{\#}P < 0.001$). MW = molecular weight.

apoptosis pathway, with increased cleaved caspase-3 (four- to fivefold), cleaved caspase-9 (1.3- to 1.7-fold), and cleaved poly(ADP-ribose) polymerase (seven- to ninefold), noted at 24 hours after CS (5%) (Figure 3E). CS-exposed cells treated with NAC (5 mM) had significantly less apoptosis (Figure 3F), suggesting that CS-induced apoptosis was due at least in part to increased oxidative stress. Consistent with prior studies (25), exposure to CS for 24 hours led to activation of the extrinsic apoptosis pathway, as measured by cleaved caspase-8 (Figure 3G). However, the abundance of cleaved caspase-8 did not vary with XPC expression (Figure 3G), suggesting little effect of XPC on the extrinsic apoptosis pathway.

XPC Deficiency Increases Apoptosis in Mouse Lungs Exposed to AC and CS Associated with Altered Autophagy Markers

To determine if XPC deficiency increases susceptibility to CS-induced lung injury *in vivo*, littermate mice (C57Bl/6;129, male and female) either WT or deficient in XPC (WT or KO), aged 6–8 weeks, were exposed to chronic CS (TE-10 smoking device, 5 h/d, 5 d/wk) or ambient air for 1–9 months. Apoptosis was measured by Western blot analysis of cleaved caspases-9, -3, and -7 in lung homogenates of XPC KO and WT mice after exposure to 1 month of CS (mice aged 2.5–3 mo; Figure 4A). CS exposure led to increased cleaved caspase-9 in the lungs of both WT and XPC-deficient mice and, compared with WT animals, XPC-deficient mice showed increased cleaved caspase-9 abundance in their lung homogenates, after either AC or CS exposures ($P < 0.001$, Figure 4B). XPC-deficient mice had increased cleaved caspase-3 abundance in their lung homogenates, after either ambient air or in CS exposures ($P < 0.001$; Figure 4C), as well as increased cleaved caspase-7 lung levels ($P = 0.002$; Figure 4D). As a complementary assay for apoptosis, that also allowed us to focus on lung parenchyma, rather than large airways, frozen lung sections from XPC KO and WT mice were evaluated for apoptotic cell death, using TUNEL staining. Quantification was performed in a blinded fashion, using automated image analysis of lung parenchyma only. This showed a genotype-specific increase in TUNEL-positive cells, with staining highest in XPC KO mice exposed to 9 months of CS (Figure 4E). XPC deficiency alone was sufficient to increase TUNEL staining, independent of CS exposure ($P = 0.03$).

Because apoptosis may be preceded by unmitigated or unsuccessful autophagy during CS-induced lung injury (3, 26, 27), we investigated whether XPC deficiency increased signs of autophagy in mouse lungs after chronic CS exposure. Measurements of autophagy marker abundance by Western blot and densitometry showed significant increases in both the microtubule-associated protein light chain 3B (LC3B)-II (Figures 4E and 4F) and p62 (Figures 4G and 4H) levels in XPC-deficient mouse lungs, independent of CS exposure. The increase in LC3B-II may reflect either increased autophagosome formation or decreased degradation in the lysosome, whereas increased p62 typically reflects decreased lysosomal

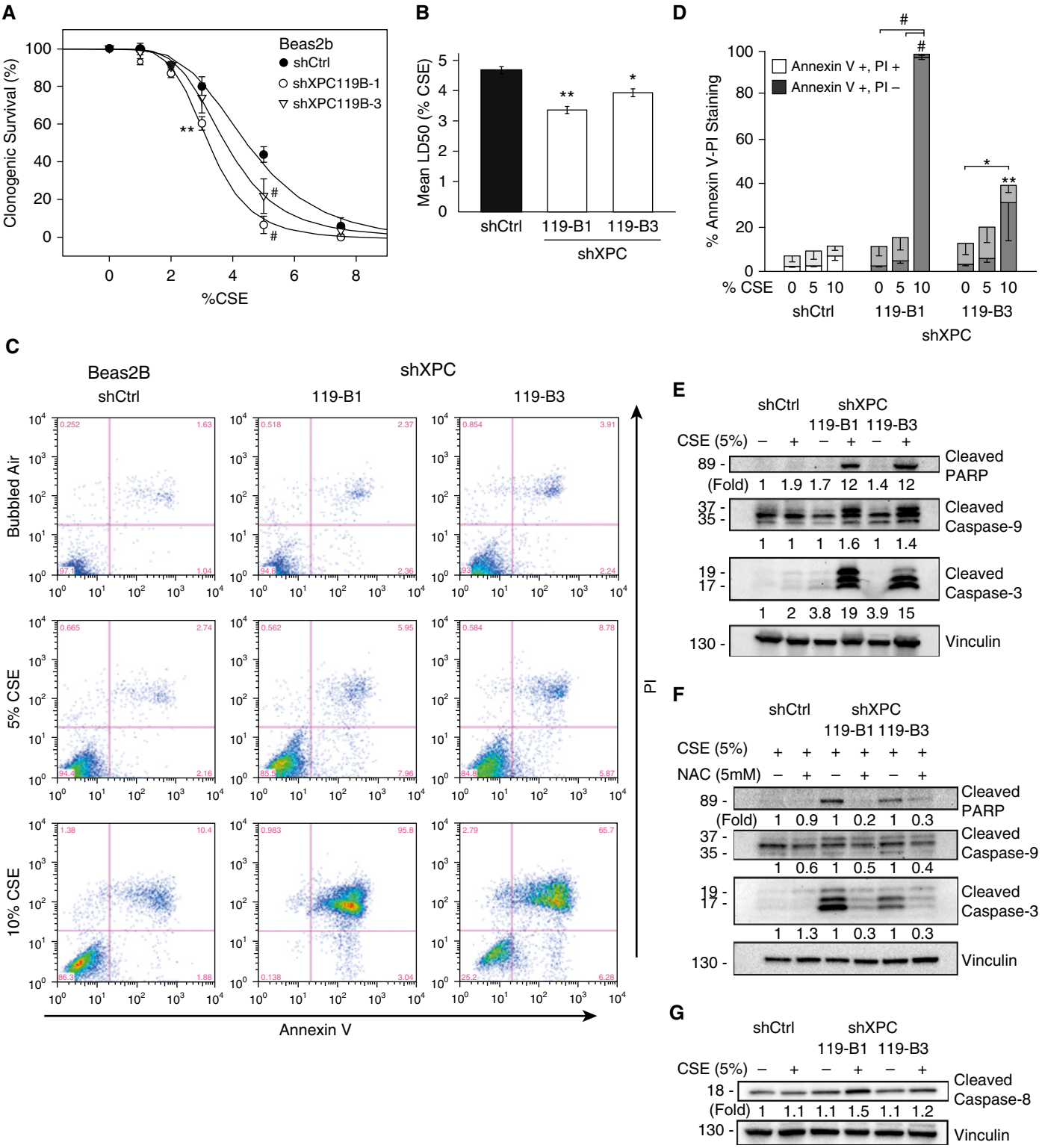

Figure 3. Impact of XPC deficiency on survival and apoptosis in Beas2B cells treated with CS. Clonogenic survival assays of XPC knockdown (shXPC) Beas2B cells compared with control (shCtrl) with increasing concentrations of CS extract (CSE) (*A*). Survival is shown as individual points (±SEM) by a best-fit four-parameter logistic curve. Statistical difference is observed between the CSE survival of each shXPC cell line compared with shCtrl by two-way ANOVA with repeated measures (*A*) and by comparison of LD50s (lethal dose, 50%) (*B*) ($n = 3$ independent experiments performed in duplicate [±SEM]). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{\#}P < 0.001$. Early (annexin V) and late (propidium iodide [PI]) apoptosis were measured by flow cytometry in Beas2B cells with XPC KD (119-B1 and 119-B3) compared with shCtrl exposed to 5% and 10% CS for 24 hours. Results measured at 48 hours. A representative dot

degradation of autophagosome. Together, our results demonstrate that XPC-deficient mice possess impaired autophagic flux and aggresome accumulation, which have been recently described in the lungs of mice and humans with emphysema (26).

XPC Deficiency Increases Age-Dependent Lung Compliance and Exacerbates Lung Dysfunction Similar to that Caused by Chronic CS

Because increased apoptosis and impaired autophagy have been implicated in the development of emphysema associated with CS, we investigated the impact of XPC deficiency on lung alveolar structure and function after chronic CS exposure. CS caused an expected decrease in weight gain and increase in lung inflammation measured by cellularity of the BAL fluid, independent of genotype (*see* Figure E1 in the data supplement). Compared with AC, exposure to CS in this mouse strain caused an increase in lung compliance, independent of XPC expression, after 1 month ($P = 0.035$) and 6 months ($P = 0.008$) of chronic CS exposure (Figure 5A). The increased lung compliance observed with chronic CS reached a plateau by 9 months of chronic CS exposure (Figure 5A) and persisted in mice exposed to 5 months of CS followed by 4 months of removal from CS exposure ("5 + 4," Figure 5B), consistent with fixed airway disease. Subgroup analysis showed increased lung compliance in male compared with female mice after 9 months of CS exposure in both XPC KO and WT mice, consistent with recently published observations (28) (Figure 5A and Figure E2). Interestingly, XPC-deficient mice aged 10.5–11 months (XPC KO AC that were exposed to 9 mo of ambient air) showed a CS-independent increase in lung compliance (Figures 5A and 5B), consistent with an aging effect on lung function. Consistent with this effect, age-dependent progressive airspace enlargement was observed in XPC KO mouse lungs by hematoxylin and eosin (H&E) stain, which was further increased in XPC KO mice exposed to 9 months of chronic CS and persisted in XPC KO mice exposed to 5 months of CS followed by 4 months of AC (Figure 5C). Stereological quantification performed on H&E-stained sections derived from multiple planes throughout the lung showed an increase in lung volume to surface area in XPC KO mice exposed to 5 months of CS followed by 4 months of AC compared with WT AC mice (Figure 5D), and showed a trend to have an increased mean linear intercept (Figure 5E). There were no changes indicative of lung fibrosis by H&E (Figure 5C) and trichrome staining (data not shown). The increased lung compliance and alveolar rarefaction observed in XPC KO mice supports a role for XPC in age-related increases in lung compliance due to alveolar rarefaction, which may be further exacerbated by chronic CS and similar to changes caused by chronic CS exposure.

Discussion

In this study, we show that XPC plays an important role in the response of lung epithelial cells to CS-induced DNA damage and response, ultimately determining cell fate and development of lung disease. Using a mouse model of chronic CS, we are the first to show that the DNA repair protein, XPC, plays a role in protecting the lungs from age-induced emphysema changes, and may play a role in CS-induced emphysema.

The development of oxidative and bulky DNA adducts after CS exposure has been well established. These DNA adducts are believed to be the primary drivers of a number of smoking-related diseases, particularly lung cancer, in which this connection has perhaps been best studied. Although the link between CS exposure and emphysema has been well established, low penetrance has been observed, with only 20%–25% of smokers developing chronic obstructive lung disease, suggesting a possible role for gene–environment interactions in its development (29). Our findings suggest that chronic CS exposure itself may be sufficient to suppress DNA repair through decreased XPC expression, possibly through promoter hypermethylation, as has been previously described as modulating XPC expression (30). Decreased XPC expression was previously observed 24 hours after *in vitro* exposure to CS condensate due to proteasomal degradation (31). This suggests that XPC expression may be altered both early after CS exposure, by post-translational modification, as well as later through transcriptional regulation, leading to decreased DNA damage repair, causing increased pulmonary cell death, altered autophagic function, and development of emphysema. However, genetic susceptibility may also play a role, as altered expression of DNA repair genes have been noted in mouse models of chronic CS (24), and a recent study observed a correlation between the XPC polymorphism (rs2228001) in Russian ethnic Tatar smokers with COPD (32).

Our study provides a direct, mechanistic link between decreased DNA repair and morphologic and histologic measures of accelerated emphysema development. Others have postulated a link between impaired DNA repair, particularly of oxidant DNA damage, and COPD development. Increased DNA damage is observed in humans and mice exposed to CS, and increased levels of DNA damage have been observed in the lungs of patients with COPD, largely ascribed to increased oxidative stress causing oxidized bases and DNA DSBs (5, 6, 33, 34). Downregulation of DNA repair gene expression has been implicated in mice exposed to chronic CS (24), and one group showed decreased expression of Ku80 (X-ray repair cross-complementing protein 5), a required protein for DNA DSB repair by nonhomologous end joining, both in mice exposed to chronic CS and in human

Figure 3. (Continued). plot is shown (*C*), as well as quantification of flow cytometry results, represented by percent cells staining with annexin V and PI (shCtrl, white; shXPC, gray) and annexin V alone (shCtrl, white striped; shXPC, gray striped) (*D*) ($n = 3$ independent experiments, mean [±SEM]). $^{\#}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ by two-way ANOVA. Activation of the intrinsic apoptosis pathway, including initiator (cleaved [poly(ADP-ribose) polymerase (PARP)] and cleaved caspase-9) and the effector proteins (cleaved caspase-3) in Beas2B cells exposed to 5% CS, with or without KD of XPC (*E*). Fold changes compared with shCtrl exposed to AC are shown below the respective blots. Activation of the intrinsic apoptosis pathway (cleaved PARP, cleaved caspase-9, cleaved caspase-3) in cells (shCtrl and shXPC) treated with 5% CSE with and without addition of N-acetylcysteine (NAC; 5 mM) (*F*). Fold changes compared with AC for each cell type shown below the respective blots. Activation of the extrinsic apoptosis pathway (cleaved caspase-8) in Beas2B cells treated with 5% CSE, with or without KD of XPC (*G*). Fold changes compared with AC for each time point shown below the respective blots. Immunoblots are representative of three independent experiments.

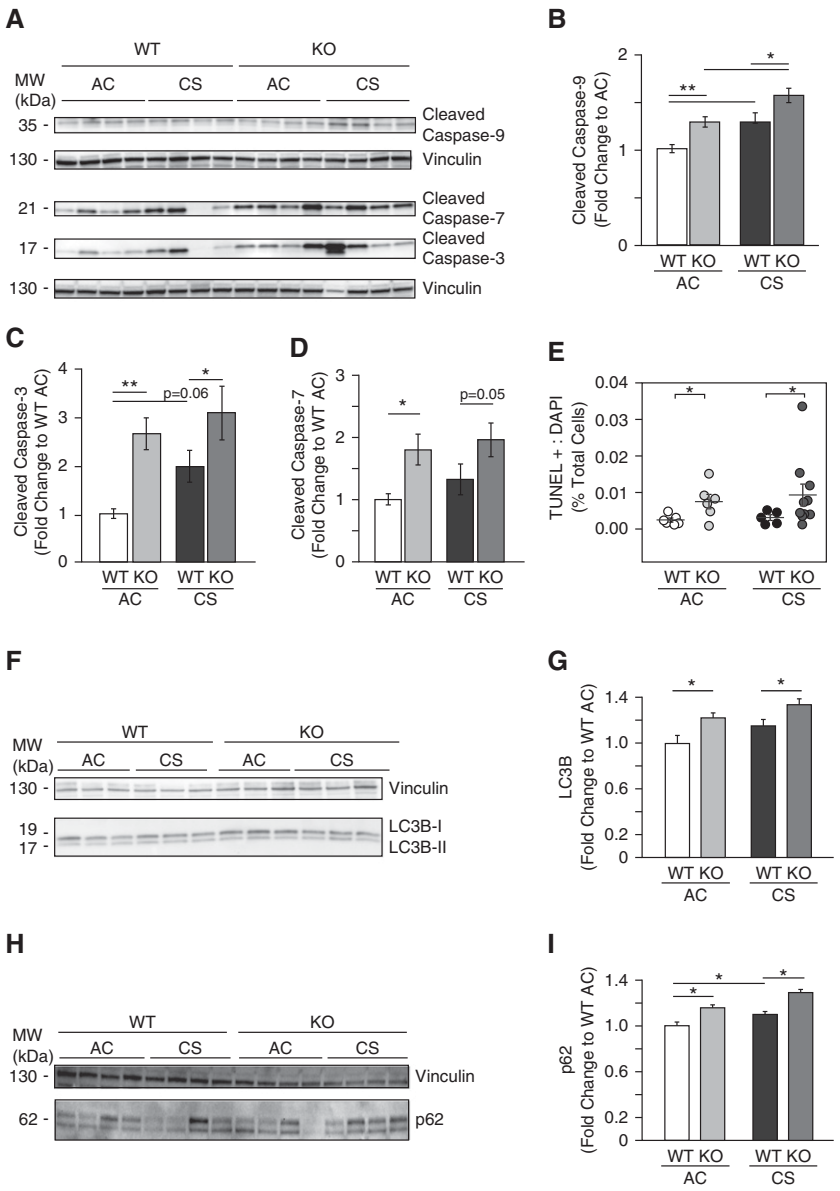

Figure 4. Impact of XPC on autophagy and apoptosis in CS and AC-exposed mouse lungs. (*A*) Representative Western blots of cleaved caspase-9, cleaved caspase-7, and cleaved caspase-3 in mouse lung homogenates from XPC knockout (KO) and wild-type (WT) mice exposed to 1 month of AC or CS with loading control (vinculin). (*B*) Quantification of cleaved caspase-9 in mouse lung homogenates relative to WT AC (mean ± SEM). XPC WT versus KO, $P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$. (*C*) Quantification of cleaved caspase-3 expression relative to WT AC mouse lung homogenates (mean ± SEM). XPC WT versus KO, $P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$. (*D*) Quantification of cleaved caspase-7 expression relative to WT AC mouse lung homogenates (mean ± SEM). XPC WT versus KO, $P = 0.002$; $^{*}P < 0.05$. Means of eight mouse lung homogenates per group, each averaged from five independent Western blots. (*E*) Quantification of immunofluorescence staining by TUNEL in the lung parenchyma of XPC KO and WT mice exposed to 9 months of AC or CS (10 pictures per stain; mean ± SEM; $^{*}P < 0.05$). (*F*) Representative Western blot showing microtubule-associated proteins 1A/1B light chain 3B (LC3B) expression (LC3B-I and LC3B-II) in the lungs of XPC KO and WT mice exposed to 9 months of AC or CS along with loading control (vinculin). (*G*) Relative LC3B expression in lung homogenates from XPC WT (white, AC; black, CS) and XPC KO (light gray, AC; dark gray, CS) mice (mean of five blots [±SEM]; $^{*}P < 0.05$). (*H*) Representative Western blot showing p62 expression in the lungs of XPC KO and WT mice exposed to 9 months of AC or CS along with loading control (vinculin). (*I*) Relative p62 expression by Western blot of lung homogenates from XPC WT (white, AC; black, CS) and XPC KO (light gray, AC; dark gray, CS) mice (mean of three blots [±SEM]; $P < 0.05$). Statistical comparisons by two-way ANOVA.

patients with COPD (5). In addition, oxidative stress alters DNA repair function by epigenetic modifications, and is also associated with premature aging from telomere shortening caused by impaired DNA repair; telomere shortening has been linked to cigarette smoking, COPD, and lung cancer development (35, 36). Our model provides a role for XPC in prevention of CS-induced COPD. However, given wide interindividual variations in DNA damage in smokers, the lack of a single gene mutation to define an at-risk population, and evidence of alterations in other DNA repair proteins, it is possible that a number of factors, including acquired gene mutations and epigenetic modifications, are involved in a phenotype of decreased DNA repair capacity, which, in smokers, leads to acceleration of CS-induced lung diseases, including COPD.

A number of studies have highlighted the role of CS-induced oxidative stress in emphysema development. Oxidative stress, caused either directly from CS exposure or indirectly from infiltrating inflammatory cells or damaged/dying cells within the lungs, has been linked to alveolar epithelial cell death through apoptosis and matrix proteolysis (2, 4). These findings have been further supported by models that perturb the oxidant response. For instance, mice lacking the antioxidant transcription factor, Nrf-2, show accelerated emphysema development (37), and glutathione depletion increases inflammation and emphysema development in a mouse model of chronic CS (38). Inflammation, apoptosis, and increased emphysema development are observed in mice with inactivation of superoxide dismutase in several different emphysema models (CS, ceramide, and elastase), with these effects attenuated with the addition of superoxide dismutase (39–41). However, countering this effect may be more complicated than simply limiting oxidative stress, as antioxidant diet was associated with decreased emphysema, but increased mortality in a mouse model of chronic CS exposure (42). It should be noted that the impact of CS and oxidative stress is not limited to alveolar epithelial cells, and similar effects have been observed in lung endothelial cells (43), which play a significant role in emphysema development as well. In addition, considering the changing patterns of cigarette smoking, it is important to note that similar effects have

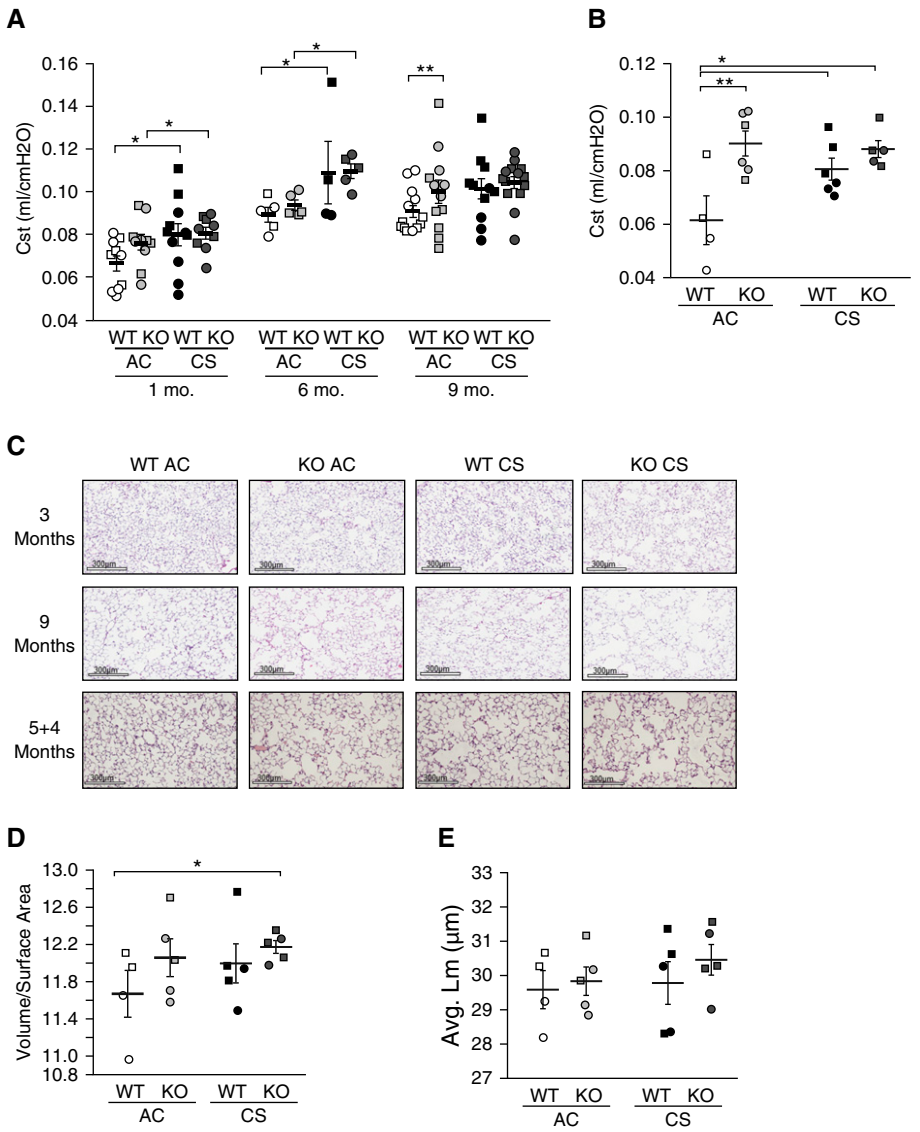

Figure 5. Lung compliance and morphometry in CS–exposed XPC–deficient and WT mouse lungs. (*A*) Levels of lung compliance (Cst) in XPC-deficient (KO) and WT mice exposed to 1 month, 6 months, and 9 months of CS or AC (B6;129 mice; age 6–8 wk before exposure; mean ± SEM; male, squares; females, circles; $^{*}P < 0.05$, $^{**}P < 0.001$ by two-way ANOVA). (*B*) Lung compliance in XPC KO and WT mice exposed to 5 months of CS followed by 4 months of AC (mean ± SEM; male, squares; females, circles; $^{*}P < 0.05$). (*C*) Representative images of hematoxylin and eosin–stained lung parenchyma in XPC KO and WT mice after 3 months or 9 months of CS or AC and 5 months of CS followed by 4 months of AC ("5 + 4 months"). Scale bars: 300 μm. Measures of (*D*) volume/surface area and (*E*) mean linear intercept (cord length [Lm]) in XPC KO and WT mouse alveoli in "5 + 4" mice (mean ± SEM; male, squares; females, circles; $^{*}P < 0.05$).

been observed recently after exposure to nicotine-containing e-cigarettes (22).

Emphysema is characterized by increased apoptosis, and our findings support a protective role of XPC in decreasing CS-induced apoptosis *in vitro* and *in vivo*. Emphysema is characterized by inflammation and oxidative stress, both of which cause DNA damage, including oxidized bases and abasic sites. In addition to its canonical role as the initiating and rate-limiting step in GG-NER, XPC has been more recently implicated in repair of oxidized bases through BER (11, 12, 14, 15). In this study, we find that XPC protects against CS-induced cell death through decreased activation of the intrinsic apoptosis pathway, similar to the protective mechanism proposed for XPC in response to other DNA-damaging agents (44). The increase in CS-induced apoptosis is further reduced with the addition of the antioxidant NAC *in vitro*, further supporting a likely role of XPC in augmentation of base-excision repair of oxidatively modified DNA bases. Although a number of studies have shown that CS increases apoptosis *in vitro* and *in vivo* (2, 3, 39, 43), one group previously published data showing increased DNA damage without induction of apoptosis in CS extract–treated Beas2B cells (45). However, this more likely reflects differences in techniques and assay sensitivity, and further supports the heterogeneity observed in development of emphysema in smokers. Our *in vitro* data show mild activation of the extrinsic apoptosis pathway by CS extract exposure, as previously observed (25), but this effect was independent of XPC, indicating specificity for XPC in protecting against CS-induced activation of the intrinsic apoptosis pathway. We also saw evidence of a role for XPC in regulating autophagy, which has been shown to play a role in CS-induced emphysema (27, 29), and can culminate in apoptosis when unmitigated or impaired by lack of a proper lysosomal degradation step, as described after CS exposure (3). In this model, we observed a modest increase in both LC3B and p62 by both XPC deficiency and CS exposure. These findings are more consistent with impaired autophagic flux causing aggresome accumulation, as has been recently implicated in the development of CS-induced emphysema in mice and in humans (26). This might represents a cellular adaptation to DNA damage, which, in XPC deficiency, overwhelms the DNA repair capacity activating apoptosis, as observed in our mouse and cell models. The relatively modest changes in lung apoptosis and autophagy *in vivo* may be due to the mild nature of the emphysema in this exposure model, the cell-specific nature of the effect, which may be diluted when evaluating the whole organ, or the temporal heterogeneity of cell injury events, limitations that may be in part addressed in the future by using inbred mouse strains and more potent CS exposure regimens.

Decreased DNA repair has been investigated in the lung, most notably for its role in genomic instability and lung cancer development. A number of studies have

demonstrated that decreased DNA repair, including decreased NER capacity, is linked to higher odds of lung cancer development (46), and gene expression analysis showed decreased expression of a number of DNA repair proteins, including XPC, in lung adenocarcinoma tumors compared with surrounding noncancerous lung (47). Common polymorphisms of XPC have been linked to development of lung cancer in humans (48), and mouse models of XPC are noted to develop spontaneous adenomas (49). Our findings suggest that, in addition to its potential role in cancer development, XPC deficiency accelerates emphysema-like changes in the lungs similar to those observed with CS. Impaired DNA repair through decreased XPC expression and function may explain the long-established link between COPD and lung cancer (50).

Our study has some limitations that are related to well-established CS exposure models, which are not exactly reproducing the human experience of intermittent exposure to CS over decades. Although the use of an aqueous extract of CS has been well established to investigate the impact of CS on cells *in vitro*, it is possible that results may differ if cells were exposed to aerosolized CS in an air–liquid interface model using primary cells obtained from lung biopsies. However, such cells are difficult to stably transfect to reduce XPC expression in a consistent manner, which is why we have chosen the present model, complemented with *in vivo* experiments for relevance. Although our study was not sufficiently powered, we, similar to others, noted an effect of sex on the magnitude of emphysema development in response to CS (28), even in this genetically heterogeneous mouse strain, with male mice being more susceptible to CS than female counterparts, regardless of XPC levels.

In summary, our findings support a role for impaired DNA repair due to decreased XPC expression in loss of lung structure and function, consistent with emphysema development. They also suggest that XPC function not only in GG-NER, but also in repair of oxidative lesions through BER, is important in protecting the lung from cell death and dysregulated autophagy. Given the previously established link between XPC and lung cancer development, this is intriguing as a possible role for XPC in development of both emphysema and lung cancer. Our findings would support a model in which decreased repair of oxidative DNA lesions, caused by a CS-induced decrease in XPC expression and function, causes early alveolar loss due to apoptosis, leading to emphysema. However, we hypothesize that unrepaired DNA lesions in cells that survive are either tolerated or repaired through alternative, lower-fidelity pathways, leading to genetic instability and a predisposition to cancer. Further investigations into the role of DNA repair, XPC, and CS in the development of these diseases are necessary to discover predictive biomarkers and novel targets to prevent progression. ■

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References

1. Lopez AD, Shibuya K, Rao C, Mathers CD, Hansell AL, Held LS, *et al*. Chronic obstructive pulmonary disease: current burden and future projections. *Eur Respir J* 2006;27:397–412.
2. Rangasamy T, Misra V, Zhen L, Tankersley CG, Tuder RM, Biswal S. Cigarette smoke–induced emphysema in A/J mice is associated with pulmonary oxidative stress, apoptosis of lung cells, and global alterations in gene expression. *Am J Physiol Lung Cell Mol Physiol* 2009;296:L888–L900.
3. Chen Z-H, Lam HC, Jin Y, Kim H-P, Cao J, Lee S-J, *et al*. Autophagy protein microtubule-associated protein 1 light chain-3B (LC3B) activates extrinsic apoptosis during cigarette smoke–induced emphysema. *Proc Natl Acad Sci USA* 2010;107:18880–18885.
4. van der Vaart H, Postma DS, Timens W, ten Hacken NH. Acute effects of cigarette smoke on inflammation and oxidative stress: a review. *Thorax* 2004;59:713–721.
5. Caramori G, Adcock IM, Casolari P, Ito K, Jazrawi E, Tsaprouni L, *et al*. Unbalanced oxidant-induced DNA damage and repair in COPD: a link towards lung cancer. *Thorax* 2011;66:521–527.
6. Deslee G, Woods JC, Moore C, Conradi SH, Gierada DS, Atkinson JJ, *et al*. Oxidative damage to nucleic acids in severe emphysema. *Chest* 2009;135:965–974.
7. Tzortzaki EG, Dimakou K, Neofytou E, Tsikritsaki K, Samara K, Avgousti M, *et al*. Oxidative DNA damage and somatic mutations: a link to the molecular pathogenesis of chronic inflammatory airway diseases. *Chest* 2012;141:1243–1250.
8. Hang B. Formation and repair of tobacco carcinogen–derived bulky DNA adducts. *J Nucleic Acids*. 2010;2010:709521.
9. Renaud E, Miccoli L, Zacal N, Biard DS, Craescu CT, Rainbow AJ, *et al*. Differential contribution of XPC, RAD23A, RAD23B and CENTRIN 2 to the UV-response in human cells. *DNA Repair (Amst)* 2011;10:835–847.
10. Parlanti E, D'Errico M, Degan P, Calcagnile A, Zijno A, van der Pluijm I, *et al*. The cross talk between pathways in the repair of 8-oxo-7,8-dihydroguanine in mouse and human cells. *Free Radic Biol Med* 2012;53:2171–2177.
11. Melis JP, Kuiper RV, Zwart E, Robinson J, Pennings JL, van Oostrom CT, *et al*. Slow accumulation of mutations in Xpc$^{-/-}$ mice upon induction of oxidative stress. *DNA Repair (Amst)* 2013;12:1081–1086.
12. Kassam SN, Rainbow AJ. Deficient base excision repair of oxidative DNA damage induced by methylene blue plus visible light in xeroderma pigmentosum group C fibroblasts. *Biochem Biophys Res Commun* 2007;359:1004–1009.
13. Liu SY, Wen CY, Lee YJ, Lee TC. XPC silencing sensitizes glioma cells to arsenic trioxide via increased oxidative damage. *Toxicol Sci* 2010;116:183–193.
14. Menoni H, Hoeijmakers JH, Vermeulen W. Nucleotide excision repair-initiating proteins bind to oxidative DNA lesions *in vivo*. *J Cell Biol* 2012;199:1037–1046.
15. D'Errico M, Parlanti E, Teson M, de Jesus BMB, Degan P, Calcagnile A, *et al*. New functions of XPC in the protection of human skin cells from oxidative damage. *EMBO J* 2006;25:4305–4315.
16. Sears CR, Sandusky GE, Albrecht M, Poirier C, Justice MJ, Petrache I. The role of XPC in DNA damage, oxidative stress and metaplasia during chronic cigarette smoke exposure [abstract]. *Am J Respir Crit Care Med* 2015;191:A5092.
17. Sears CR, Zhou H, Fisher AJ, Justice MJ, Van Demark M, Saliba J, *et al*. DNA repair protein XPC alters pulmonary cell fate following cigarette smoke exposure and may play a role in emphysema and lung cancer development [abstract]. *Am J Respir Crit Care Med* 2017;195:A4957.
18. Clauss M, Voswinckel R, Rajashekhar G, Sigua NL, Fehrenbach H, Rush NI, *et al*. Lung endothelial monocyte-activating protein 2 is a mediator of cigarette smoke–induced emphysema in mice. *J Clin Invest* 2011;121:2470–2479.

19. Petrache I, Kamocki K, Poirier C, Pewzner-Jung Y, Laviad EL, Schweitzer KS, *et al*. Ceramide synthases expression and role of ceramide synthase-2 in the lung: insight from human lung cells and mouse models. *PLoS One* 2013;8:e62968.
20. Tuder RM, Zhen L, Cho CY, Taraseviciene-Stewart L, Kasahara Y, Salvemini D, *et al*. Oxidative stress and apoptosis interact and cause emphysema due to vascular endothelial growth factor receptor blockade. *Am J Respir Cell Mol Biol* 2003;29:88–97.
21. Sears CR, Turchi JJ. Complex cisplatin-double strand break (DSB) lesions directly impair cellular non-homologous end-joining (NHEJ) independent of downstream damage response (DDR) pathways. *J Biol Chem* 2012;287:24263–24272.
22. Schweitzer KS, Chen SX, Law S, Van Demark M, Poirier C, Justice MJ, *et al*. Endothelial disruptive proinflammatory effects of nicotine and e-cigarette vapor exposures. *Am J Physiol Lung Cell Mol Physiol* 2015;309:L175–L187.
23. Sears CR, Cooney SA, Chin-Sinex H, Mendonca MS, Turchi JJ. DNA damage response (DDR) pathway engagement in cisplatin radiosensitization of non–small cell lung cancer. *DNA Repair (Amst)* 2016;40:35–46.
24. Miller MA, Danhorn T, Cruickshank-Quinn CI, Leach SM, Jacobson S, Strand MJ, *et al*. Gene and metabolite time-course response to cigarette smoking in mouse lung and plasma. *PLoS One* 2017;12:e0178281.
25. Park JW, Kim HP, Lee SJ, Wang X, Wang Y, Ifedigbo E, *et al*. Protein kinase C alpha and zeta differentially regulate death-inducing signaling complex formation in cigarette smoke extract-induced apoptosis. *J Immunol* 2008;180:4668–4678.
26. Tran I, Ji C, Ni I, Min T, Tang D, Vij N. Role of cigarette smoke–induced aggresome formation in chronic obstructive pulmonary disease–emphysema pathogenesis. *Am J Respir Cell Mol Biol* 2015;53:159–173.
27. Aggarwal S, Mannam P, Zhang J. Differential regulation of autophagy and mitophagy in pulmonary diseases. *Am J Physiol Lung Cell Mol Physiol* 2016;311:L433–L452.
28. Awji EG, Seagrave JC, Tesfaigzi Y. Correlation of cigarette smoke–induced pulmonary inflammation and emphysema in C3H and C57Bl/6 mice. *Toxicol Sci* 2015;147:75–83.
29. Tuder RM, Petrache I. Pathogenesis of chronic obstructive pulmonary disease. *J Clin Invest* 2012;122:2749–2755.
30. Wu YH, Tsai Chang JH, Cheng YW, Wu TC, Chen CY, Lee H. Xeroderma pigmentosum group C gene expression is predominantly regulated by promoter hypermethylation and contributes to p53 mutation in lung cancers. *Oncogene* 2007;26:4761–4773.
31. Holcomb N, Goswami M, Han SG, Clark S, Orren DK, Gairola CG, *et al*. Exposure of human lung cells to tobacco smoke condensate inhibits the nucleotide excision repair pathway. *PLoS One* 2016;11:e0158858.
32. Korytina GF, Akhmadishina LZ, Kochetova OV, Aznabaeva YG, Zagidullin ShZ, Victorova TV. Inflammatory and immune response genes polymorphisms are associated with susceptibility to chronic obstructive pulmonary disease in Tatars population from Russia. *Biochem Genet* 2016;54:388–412.
33. Aoshiba K, Zhou F, Tsuji T, Nagai A. DNA damage as a molecular link in the pathogenesis of COPD in smokers. *Eur Respir J* 2012;39:1368–1376.
34. Pastukh VM, Zhang L, Ruchko MV, Gorodnya O, Bardwell GC, Tuder RM, *et al*. Oxidative DNA damage in lung tissue from patients with COPD is clustered in functionally significant sequences. *Int J Chron Obstruct Pulmon Dis* 2011;6:209–217.
35. Durham AL, Adcock IM. The relationship between COPD and lung cancer. *Lung Cancer* 2015;90:121–127.
36. Mercado N, Ito K, Barnes PJ. Accelerated ageing of the lung in COPD: new concepts. *Thorax* 2015;70:482–489.
37. Rangasamy T, Cho CY, Thimmulappa RK, Zhen L, Srisuma SS, Kensler TW, *et al*. Genetic ablation of Nrf2 enhances susceptibility to cigarette smoke–induced emphysema in mice. *J Clin Invest* 2004;114:1248–1259.
38. Gould NS, Min E, Huang J, Chu HW, Good J, Martin RJ, *et al*. Glutathione depletion accelerates cigarette smoke–induced inflammation and airspace enlargement. *Toxicol Sci* 2015;147:466–474.
39. Petrache I, Medler TR, Richter AT, Kamocki K, Chukwueke U, Zhen L, *et al*. Superoxide dismutase protects against apoptosis and alveolar enlargement induced by ceramide. *Am J Physiol Lung Cell Mol Physiol* 2008;295:L44–L53.
40. Foronjy RF, Mirochnitchenko O, Propokenko O, Lemaitre V, Jia Y, Inouye M, *et al*. Superoxide dismutase expression attenuates cigarette smoke– or elastase-generated emphysema in mice. *Am J Respir Crit Care Med* 2006;173:623–631.
41. Yao H, Arunachalam G, Hwang JW, Chung S, Sundar IK, Kinnula VL, *et al*. Extracellular superoxide dismutase protects against pulmonary emphysema by attenuating oxidative fragmentation of ECM. *Proc Natl Acad Sci USA* 2010;107:15571–15576.
42. Nyunoya T, March TH, Tesfaigzi Y, Seagrave J. Antioxidant diet protects against emphysema, but increases mortality in cigarette smoke–exposed mice. *COPD* 2011;8:362–368.
43. Petrusca DN, Van Demark M, Gu Y, Justice MJ, Rogozea A, Hubbard WC, *et al*. Smoking exposure induces human lung endothelial cell adaptation to apoptotic stress. *Am J Respir Cell Mol Biol* 2014;50:513–525.
44. Wang QE, Han C, Zhang B, Sabapathy K, Wani AA. Nucleotide excision repair factor XPC enhances DNA damage–induced apoptosis by downregulating the antiapoptotic short isoform of caspase-2. *Cancer Res* 2012;72:666–675.
45. Liu X, Conner H, Kobayashi T, Kim H, Wen F, Abe S, *et al*. Cigarette smoke extract induces DNA damage but not apoptosis in human bronchial epithelial cells. *Am J Respir Cell Mol Biol* 2005;33:121–129.
46. Shen H, Spitz MR, Qiao Y, Guo Z, Wang LE, Bosken CH, *et al*. Smoking, DNA repair capacity and risk of nonsmall cell lung cancer. *Int J Cancer* 2003;107:84–88.
47. Stearman RS, Dwyer-Nield L, Grady MC, Malkinson AM, Geraci MW. A macrophage gene expression signature defines a field effect in the lung tumor microenvironment. *Cancer Res* 2008;68:34–43.
48. Jin B, Dong Y, Zhang X, Wang H, Han B. Association of XPC polymorphisms and lung cancer risk: a meta-analysis. *PLoS One* 2014;9:e93937.
49. Hollander MC, Philburn RT, Patterson AD, Velasco-Miguel S, Friedberg EC, Linnoila RI, *et al*. Deletion of XPC leads to lung tumors in mice and is associated with early events in human lung carcinogenesis. *Proc Natl Acad Sci USA* 2005;102:13200–13205.
50. Hopkins RJ, Duan F, Chiles C, Greco EM, Gamble GD, Aberle D, *et al*. Reduced expiratory flow rate among heavy smokers increases lung cancer risk: results from the National Lung Screening Trial–American College of Radiology Imaging Network cohort. *Ann Am Thorac Soc* 2017;14:392–402.