Article

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Effects of Maternal Smoke Exposure on Endoplasmic Reticulum Stress and Autophagy in the Lungs of Offspring Mice

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Background: Cigarette smoke (CS) induces autophagy and endoplasmic reticulum (ER) stress in the lungs. Research suggests that maternal exposure to CS during pregnancy leads to decreased lung function in offspring. However, the effects of maternal CS exposure on lung autophagy and ER stress in offspring during pregnancy remain unclear.
Methods: C57BL/6J female mice were divided into the AA (air treatment during both pre-pregnancy and pregnancy), AS (air treatment during pre-pregnancy and CS treatment during pregnancy), SA (CS treatment during pre-pregnancy and air treatment during pregnancy), and SS (CS treatment during both pre-pregnancy and pregnancy) groups. The male offspring mice were selected to the study and euthanized 49 days after birth for the study. Hematoxylin and eosin (HE) staining was employed to observe pathological alterations, while transmission electron microscopy (TEM) was utilized to examine ultrastructure and autophagic vesicles. Additionally, the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) method was applied to identify apoptosis in lung tissues. Immunofluorescence, Real-Time PCR, and Western Blot analyses were conducted to assess the expression of ER stress and autophagy-related markers in lung tissues.
Results: The findings revealed that exposure to CS heightened the extent of pathological damage and the abundance of autophagosomes in the lungs of offspring mice. TUNEL results indicated an increased fluorescence intensity in the AS, SA and SS groups, with the most significant in AS and SS groups. Furthermore, CS exposure augmented the fluorescence intensity and expression of ER stress and autophagy-related proteins. The expression of C/EBP-homologous protein (CHOP) exhibited no discernible difference between the SA and SS groups but showed a significant increase in the AS group. Conversely, the expression levels of glucose-regulated protein 78 (GRP78), Caspase-12, Beclin-1, and microtubule-associated protein 1 light chain 3 (LC3) exhibited no significant difference between the AS and SA groups, whereas they were significantly upregulated in the SS group.
Conclusions: Preconceptional and gestational exposure to CS heightened ER stress and autophagy in the lungs of mouse offspring. However, in mothers who smoked, withdrawal from CS during pregnancy led to a reduction in ER stress and autophagy in the lungs of their offspring.

Keywords: pregnant; cigarette smoke; lungs of offspring; endoplasmic reticulum stress; autophagy

Introduction

Exposure to cigarette smoke (CS) during pregnancy stands as the most significant modifiable risk factor for offspring developing lung diseases [1]. Extensive research indicates that maternal nutrition, infections, stress induced by toxins like CS, and environmental exposures can lead to enduring health implications for offspring [2]. Presently, there is widespread acknowledgment that maternal smoking during pregnancy leaves lifelong impacts on offspring's lung function, heightening the risk of childhood wheezing and asthma [3]. Consequently, quitting smoking within the initial three months of pregnancy can notably mitigate the risks linked to tobacco use [4].

Endoplasmic reticulum (ER) stress, stemming from an imbalance of protein misfolding within the ER, is implicated in the progression of various lung diseases [5]. Stress responses in turn trigger protective autophagy in epithelial barrier cells. Autophagy is the process whereby cells encase damaged organelles or unwanted proteins in a double membrane structures, directing them to the lysosome for degradation [6]. Intrinsic autophagy serves as an important regulator of lung development and morphogenesis, with its involvement extended to a spectrum of lung diseases, including pulmonary hypertension, acute lung injury, asthma, and lung cancer [7,8]. These findings suggest a close interrelation between autophagy and ER stress in maintaining lung function.

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CS exposure induces ER stress and autophagy in the lung. For instance, CS exposure impairs protein folding, leading to ER stress in the lung [9]. Additionally, CS extract triggers ER stress, thereby facilitating the differentiation of fibroblasts into myofibroblasts in pulmonary fibrosis [10]. Autophagy levels are heightened in mouse lungs subjected to chronic CS exposure and in lung epithelial cells exposed to cigarette smoke extract [11]. Moreover, CS exposure induces lung fibrosis by stimulating autophagy in alveolar epithelial type II cells [12]. Furthermore, in human bronchial epithelial cells, ER stress and autophagy collaborate to promote apoptosis induced by cigarette smoke extract (CSE) [13]. These findings suggest that CS exposure induces ER stress and autophagy in the lung, leading to lung injury. However, the impacts of CS exposure and smoking cessation during pregnancy on autophagy and ER stress in the lungs of offspring have been rarely investigated.

Therefore, this study aimed to assess the effects of CS exposure on ER stress and autophagy in the lungs of male offspring mice during both preconception and gestation stages. The objective was to evaluate the risks associated with long-term exposure to a CS environment and CS exposure during pregnancy on infant lung development, thereby shedding light on the detrimental impact of CS exposure during pregnancy on offspring lungs.

Methods and Materials

Animal

Adult C57BL/6J mice (5 weeks old) were procured from Beijing Viton Lihua Laboratory Animal Technology Co., Ltd. (SCXK (Beijing) 2016-0006, Beijing, China). The mice were housed in a specific pathogen-free (SPF) environment with 55% ± 15% relative humidity, maintained at 22 ± 2 °C, and subjected to a 24-hour light-dark cycle. Experiments commenced following one week of acclimatization and adaptive feeding for the mice. This study received approval from the Ethics Committee of the First Hospital of Hebei Medical University [20210909] and was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Reagents and Materials

The glucose-regulated protein 78 (GRP78, also known as Bip) antibody (111587-1-AP) and Beclin-1 antibody (11306-1-AP) were sourced from San Ying Biotechnology Co., Ltd. (Wuhan, China), while the microtubule-associated protein 1 light chain 3 alpha/microtubule-associated protein 1 light chain 3 beta (LC3A/B) antibody (AF5402) and C/EBP-homologous protein (CHOP) antibody (DF6025) were obtained from Affinity Biosciences (Jiangsu, China). The Caspase-12 antibody (A0217) was procured from ABclonal Technology (Wuhan, China), and the β-actin (AP0060) antibody was acquired from Biogot Technology Co., Ltd. (Nanjing, China). The goat anti-rabbit IgG (A23920) was obtained from Abbkine (Wuhan, China). The 20× TBST buffer (T1082) was obtained from Solarbio Technology Co., Ltd. (Beijing, China), and the 12% TGX Stain-Free FastCast Kit (#1610185) was purchased from BIO-RAD (Hercules, CA, USA). The Prestained Protein Marker (26616) was sourced from Thermo Fisher Scientific (Waltham, MA, USA). The FastKing RT Kit (With gDNase) (KR116) and Total RNA Extraction Kit (DP419) were procured from Tiangen Biochemical Technology Co., Ltd. (Beijing, China), while the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) Apoptosis Detection Kit (ab66110) was purchased from Abcam (Cambridge, MA, USA). GoTaq® qPCR Master Mix 2× (A600A) was obtained from Promega Corporation (Madison, WI, USA). The Vortex Oscillator (QL-902) was sourced from Haimen Qilinbel Instrument Manufacturing Co., Ltd. (Jiangsu, China), the Fluorescence Quantitative PCR instrument (ABI7500) was purchased from Applied Biosystems (Carlsbad, CA, USA), and the dual-color infrared laser imaging analyzer (CLx) was acquired from Gene Company Limited (Hong Kong, China).

Grouping and Modeling

After one week of adaptive feeding, the mice were categorized into four groups: AA (air treatment during both pre-pregnancy and pregnancy), AS (air treatment during pre-pregnancy and CS treatment during pregnancy), SA (CS treatment during pre-pregnancy and air treatment during pregnancy), and SS (CS treatment during both pre-pregnancy and pregnancy), as illustrated in Fig. 1. Following two weeks of rearing under either air or smoke conditions, adult male and female mice were mated and continued to be reared under the same conditions for three weeks. Male offspring mice in each group were born without any smoke exposure until reaching week 7.

During cigarette smoke exposure, the concentration of CO was monitored to be between 800–1300 ppm, while the O_2 concentration was maintained at 18% or higher. The cigarettes utilized were Diamond cigarettes from Hebei China Tobacco Company (Heibei, China), containing 13 mg of tar, 1.2 mg of nicotine, and 14 mg of carbon monoxide. After seven weeks, male offspring from each group were selected (n = 5). At the conclusion of the experiment, the mice were euthanized using cervical dislocation, and their lungs were collected for further analysis.

Hematoxylin and Eosin (HE) Staining

The lung tissues were fixed in a 4% paraformaldehyde solution, followed by dehydration and embedding in paraffin. Sections of 4 µm thickness were then prepared and stained with hematoxylin and eosin solution. Subsequently, the sections were examined for pathological changes under a light microscope (ECLPSE 80i, Nikon, Tokyo, Japan).

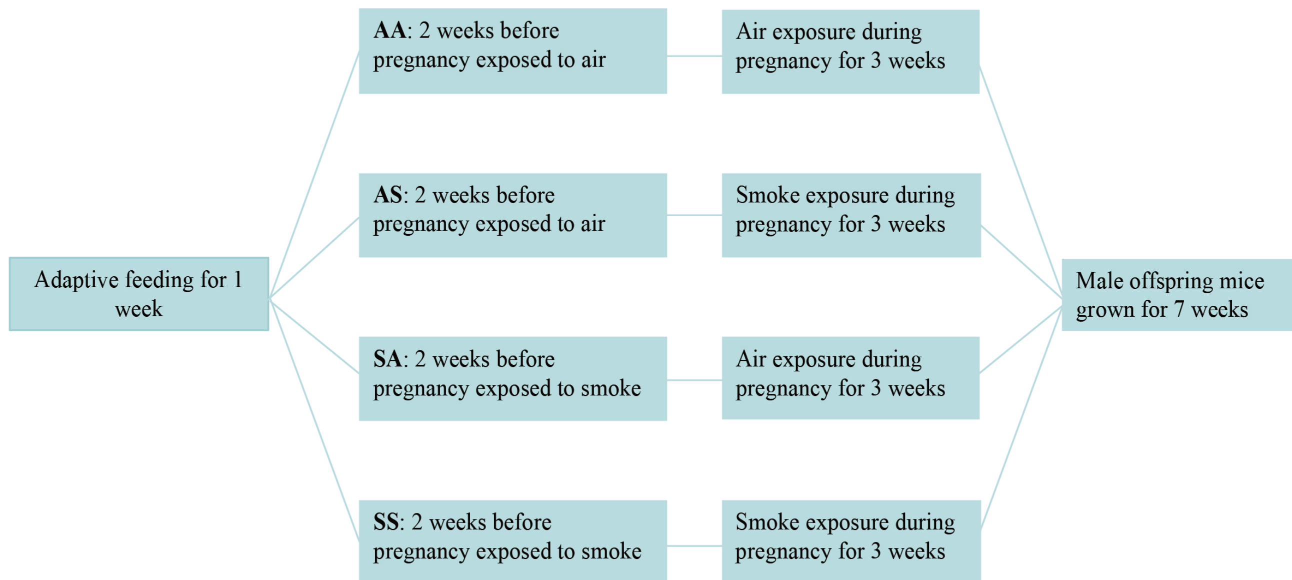

Fig. 1. Experimental grouping and cigarette smoke (CS) exposure treatments. Female mice were divided into the AA (air treatment during both pre-pregnancy and pregnancy), the AS (air treatment during pre-pregnancy and CS treatment during pregnancy), the SA (CS treatment during pre-pregnancy and air treatment during pregnancy), and the SS (CS treatment during both pre-pregnancy and pregnancy) groups randomly. The mice in the SA and SS groups were kept under CS conditions for 2 weeks, and the AA and AS groups were kept under air conditions for 2 weeks. After mating with male mice and determining pregnancy, the AA and SA groups were kept in air for 3 weeks, and the AS and SS groups were kept in CS for 3 weeks. The offspring of each group were born without CS exposure until reaching week 7.

Transmission Electron Microscopy

The lung tissues were initially cut into 1 mm^3 pieces. These pieces were then fixed in electron microscope fixative/2.5% glutaraldehyde (BL911A, Biosharp, Hefei, China) for 4 hours and subsequently post-fixed in 1% osmium tetroxide for 2 hours. After fixation, the tissues were dehydrated in a series of ethyl alcohol concentrations (ranging from 50% to 100%) and finally embedded in epoxy resin. Sections of the lung tissues were then cut at a thickness of 60–80 nm and observed under a transmission electron microscope (HT7700, Hitachi, Tokyo, Japan). Images were captured and subjected to analysis.

TUNEL Staining

Paraffin sections were conventional dewaxing to water, washed in PBS, co-incubated with protein kinase K for 5 minutes. Slides were covered with 50 µL of DNA labeling solution, and the sections were incubated at 37 °C for 1 hour, shielding from light. Afterward, they were washed and covered with 100 µL of antibody solution and incubated for 30 minutes, shielding from light. They were then washed with ddH_2O for 5 minutes. Next, 100 µL of 7-AAD/RNase A Staining Buffer was added and incubated for 30 minutes in the dark. The slides were washed again with ddH_2O for 5 minutes. Finally, the sections were blocked with an anti-fluorescent attenuator and visualized using fluorescence microscopy (Nikon, Tokyo, Japan).

Immunofluorescence

The paraffin sections underwent conventional dewaxing in water followed by antigen retrieval using Citric Acid Antigen Retrieval Solution (BL619A, Biosharp, Hefei, China). A histochemical pen was utilized to outline the water barrier (BC006, Biosharp, Hefei, China), which was then sealed by incubating with 5% BSA dropwise for 30 minutes. Upon removal of the sealing solution, 40–50 µL of primary antibodies (CHOP 1:200; Caspase-12 1:50; Beclin-1 1:200; LC3A/B 1:300) were added and incubated flatly in a humid chamber at 4 °C overnight. Subsequently, the corresponding fluorescent secondary antibody was added dropwise to cover the tissues within the outlined circle, and incubated at room temperature away from light for 1 hour. The section was then washed three times with PBS, stained with DAPI adding dropwise to the circle, and incubated at room temperature away from light for 3–5 minutes. After washing the section with PBS, it was sealed with a drop of anti-fluorescent attenuator resin, and observed under a fluorescence microscope (ECLPSE 80i, Nikon, Tokyo, Japan) to capture the image.

Western Blot (WB) Assay

The RIPA lysate was added to the lung tissues and incubated at 4 °C for 30 minutes to facilitate lysis. Following centrifugation, the supernatant was collected to obtain the total protein. Protein concentration was quantified using

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Table 1. Primer sequence.

Gene	Primer	Primer sequences (5′-3′)
β-actin	Forward	5′-ATGCCATCCTGCGTCTG-3′
	Reverse	5′-CTGGAAGGTGGACAGTGAG-3′
CHOP	Forward	5′-CTCGCTCTCCAGATTCCAGTC-3′
	Reverse	5′-CTTCATGCGTTGCTTCCCA-3′
GRP78	Forward	5′-GCATCACGCCGTCGTATGT-3′
	Reverse	5′-ATTCCAAGTGCGTCCGATGAG-3′
Caspase-12	Forward	5′-ATGCTGGATTGGCCCATGAAT-3′
	Reverse	5′-AGACGTGTTCGTCCCTCCTT-3′

CHOP, C/EBP-homologous protein; *GRP78*, glucose-regulated protein 78.

the BCA Protein Assay Kit (P0012, Beyotime, Shanghai, China). Subsequently, 15 µg of proteins were loaded onto a 10% SDS-PAGE gel and transferred to a PVDF membrane. The membranes were then incubated overnight at 4 °C with primary antibodies (*β*-actin 1:2000, GRP78/Bip 1:1000, CHOP 1:1000, Caspase-12 1:800, Beclin-1 1:1000, LC3A/B 1:1000). Following the primary antibody incubation, the membranes were exposed to a secondary antibody (goat anti-rabbit IgG; 1:3000) for an additional 2 hours at 37 °C. Finally, the membrane was scanned and analyzed using a dual-color infrared laser imaging analyzer. Densitometry analysis was performed using Image J 1.6 software (NIH, Bethesda, MD, USA).

Real-Time Fluorescent Quantitative Polymerase Chain Reaction (RT-qPCR) Assay

Total RNA was extracted utilizing an RNA isolation kit, following the manufacturer's instructions. Subsequently, cDNA was reversely transcribed using the FastKing RT Kit (with gDNase, KR116, Tiangen, Beijing, China), and the resulting cDNA samples underwent quantitative PCR using the Real-Time PCR system (ABI7500, Applied Biosystems, Carlsbad, CA, USA). RT-qPCR reaction conditions were as follows: 95 °C for 2 minutes, followed by 40 cycles of 95 °C for 15 seconds and 60 °C for 1 minute. Relative mRNA expression levels were determined using the $2^{-\Delta\Delta CT}$ method. The primer sequences for all reactions are listed in Table 1.

Statistical Analysis

The data were analyzed using IBM SPSS 26.0 software (IBM SPSS, Watson, NY, USA) and reported in the format of mean ± SD. One-way ANOVA followed by Tukey's post hoc test was employed to evaluate differences between experimental groups. Statistical significance was determined when the p value was less than 0.05.

Results

The effects of CS exposure on lung histopathology and ultrastructure in offspring mice are depicted in Fig. 2A. Mice in the AS and SS groups exhibited slightly lower body weight compared to those in the AA and SA groups, particularly evident at 3 weeks of age. HE staining results revealed that the alveolar structure of the lungs in the AA group remained intact with uniform size. The SA group showed a lesser increase in alveolar space and alveolar wall thickening, accompanied by a small amount of exudate. Conversely, the AS and SS groups displayed variations in alveolar size, thickening of the alveolar wall, and fusion of some alveoli, forming large cystic cavities. Alveoli around the trachea were collapsed, and distal alveoli were dilated and fused, indicative of pulmonary emphysema, with the SS group exhibiting more severe symptoms and significant exudate accumulation (Fig. 2B). Transmission electron microscopy (TEM) results indicated that type II epithelial cells in the lung tissues of all groups exhibited intact cell membranes without apparent damage or signs of autophagy. In the AA group, cell nuclei appeared irregularly shaped, with slight expansion of rough endoplasmic reticulum (RER) and partial loss of lamellar body (LB) structure. The SA group displayed cell nuclei with regular shape and no apparent RER expansion, with partial loss of LB structure. In the AS group, cell nuclei were irregularly shaped, with slight RER expansion, some membrane damage, and most LB structures appearing loose or absent. Finally, in the SS group, cell nuclei showed regular shape, mild RER expansion, membrane damage, sparse LB structure, significant loss of lamellar structure, and occasional vacuolization (Fig. 2C).

Effects of CS Exposure on Apoptosis in the Lungs of Offspring Mice

The study subsequently investigated apoptosis in the lungs of offspring mice in each group, considering that ER stress and autophagy can induce apoptosis. Compared with the AA group, the fluorescence intensity was significantly increased in the AS, SA and SS groups, with the increase being greater in the AS and SS groups exposed to CS during pregnancy (Fig. 3).

Effects of CS Exposure on ER Stress in the Lungs of Offspring Mice

In this study, the expression of ER stress-related proteins was investigated. Immunofluorescence results revealed no significant difference in the fluorescence intensity of CHOP among the AA, SA, and SS groups, while it showed an increase in the AS group (Fig. 4A). Similarly, the fluorescence intensity of Caspase-12 exhibited a slight increase in the SA and AS groups, but was notably elevated in the SS group compared to the AA group (Fig. 4B). Consistent with these findings, the expression of GRP78, Caspase-12, and CHOP proteins (Fig. 4C) and mRNA levels (Fig. 4D) were increased after CS exposure compared to the AA group. However, the expression of GRP78 and Caspase-12 did not significantly differ between the AS and

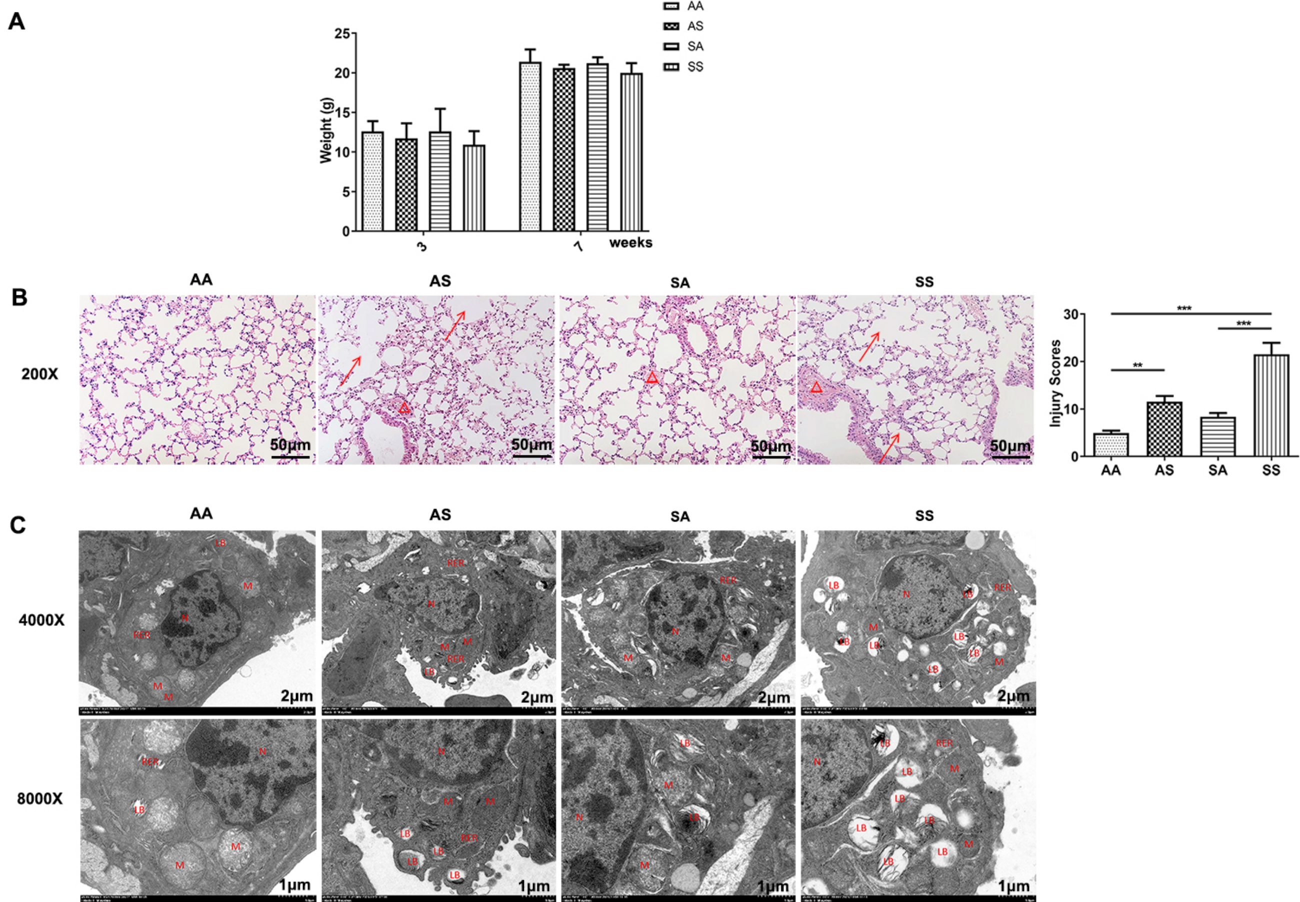

Fig. 2. Effects of CS exposure on lung histopathology and ultrastructure in offspring mice (n = 5). (A) Changes in body weight of offspring mice during different growth cycles. (B) Lung HE staining results, (→) pulmonary emphysema, (△) transudation. (C) Lung transmission electron microscopy results. N, cell nuclei; RER, rough endoplasmic reticulum; LB, lamellar body; M, chondriosome; HE, hematoxylin and eosin. **$p < 0.01$, ***$p < 0.001$.

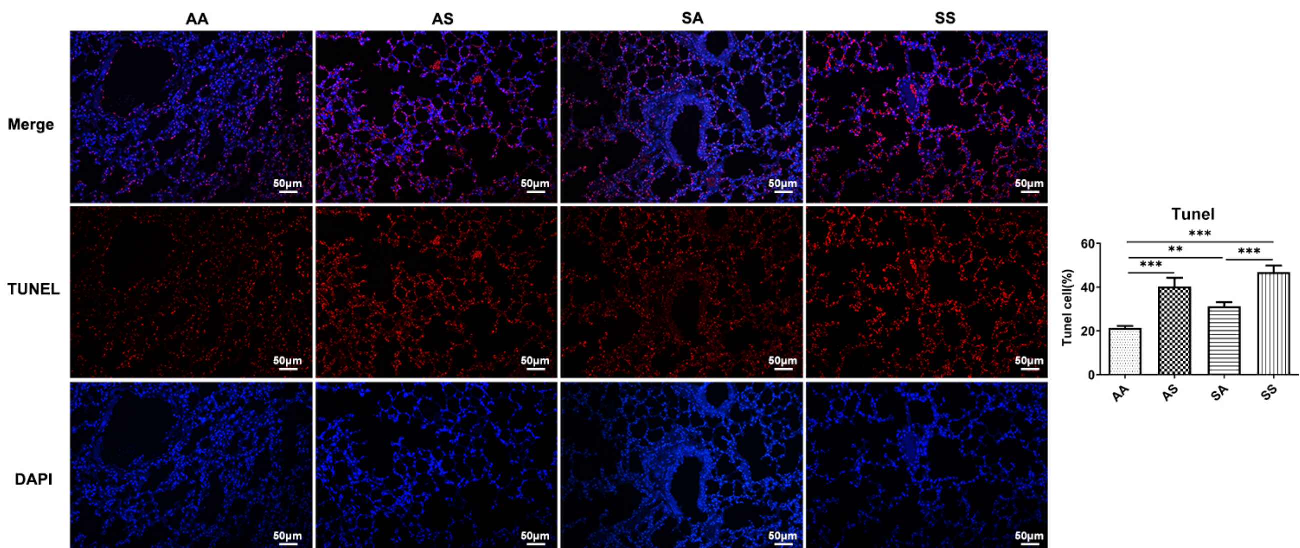

Fig. 3. Effects of CS exposure on apoptosis in the lungs of offspring mice (n = 5). **$p < 0.01$, ***$p < 0.001$. TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling.

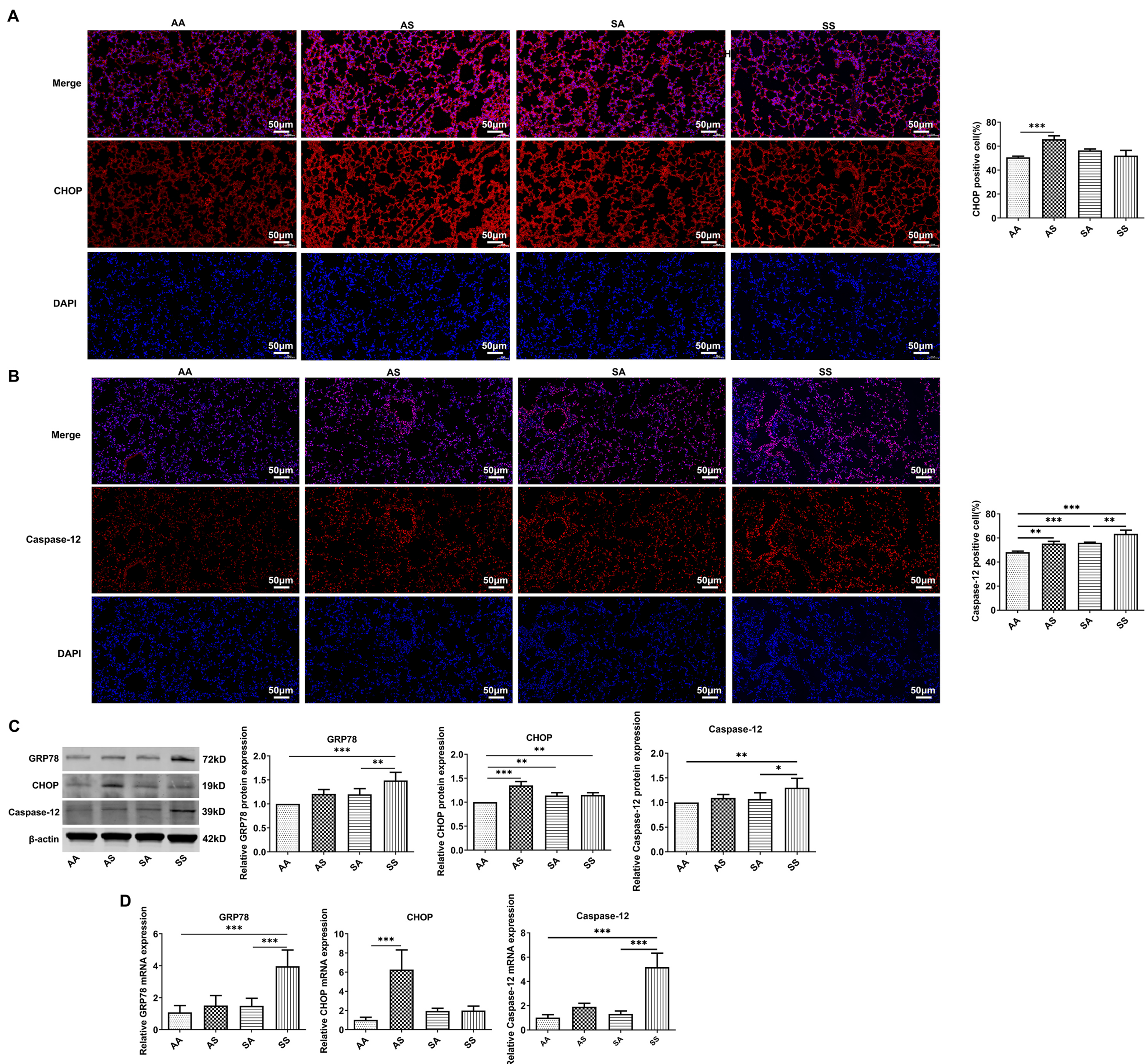

Fig. 4. Effects of CS exposure on endoplasmic reticulum (ER) stress in the lungs of offspring mice (n = 5). The fluorescence intensity of CHOP (A) and Caspase-12 (B). GRP78, Caspase-12, and CHOP proteins expression (C). *GRP78*, *Caspase-12*, and *CHOP* mRNA levels (D). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

SA groups, whereas it was significantly upregulated in the SS group. Additionally, the expression of CHOP was significantly increased in the AS group, while it showed no significant difference between the SA and SS groups.

Effects of CS Exposure on Autophagy in the Lungs of Offspring Mice

Finally, the expression of autophagy-related proteins was examined. The results revealed increased fluorescence intensity of Beclin-1 and LC3 in the AS, SA, and SS groups (Fig. 5A,B), with the most significant increase observed in the SS group. Additionally, staining intensity in the AS and SA groups showed no distinct difference, but the fluorescence intensity was slightly stronger in the AS group compared to the SA group. Similarly, Western Blot results showed that CS exposure increased the expression of Beclin-1 and LC3-II proteins compared to the AA group, with the most pronounced increase observed in the SS group and slightly higher expression in the AS group compared to the SA group (Fig. 5C).

Discussion

Currently, it is widely recognized that maternal smoking during pregnancy has a lifelong impact on the lung function of offspring [3]. In this study, female mice were exposed to CS during both pre-pregnancy and pregnancy, and the effects of CS on ER stress and autophagy in the lungs

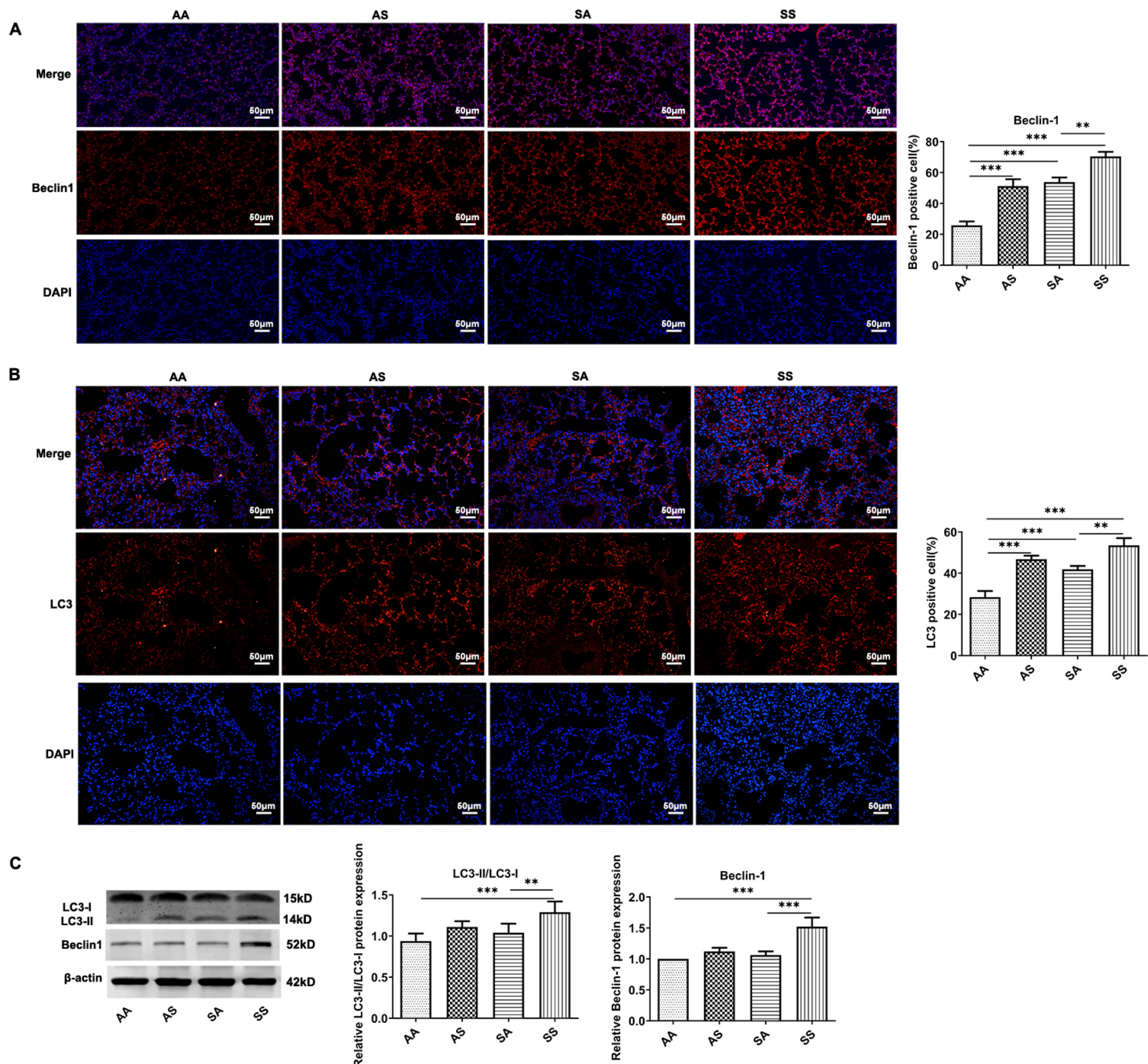

Fig. 5. Effects of CS exposure on autophagy in the lungs of offspring mice (n = 5). (A) The fluorescence intensity of Beclin-1. (B) The fluorescence intensity of microtubule-associated protein 1 light chain 3 (LC3). (C) Beclin-1 and LC3 protein expression detection. $**p < 0.01$, $***p < 0.001$.

of offspring were observed. The results indicated that exposure to CS during both pre-pregnancy and pregnancy increased ER stress and autophagy in the offspring's lungs. The most significant impact was observed when the exposure occurred during both stages.

Exposure to environmental insults, such as tobacco toxins, during lung development, can have lifelong effects on lung growth and function [1]. One study has demonstrated that intrauterine smoke exposure reduces lung function in human newborns [14]. Animal models have further corroborated these findings, revealing that CS exposure in utero leads to significant impairment of lung function in offspring mice up to day 21 [15].

In female mice exposed to CS during pregnancy, offspring exhibited decreased body weight and lung volume [16], a reduced number of pulmonary septal cristae and elastin fibers, increased airway thickness, and elevated oxidative stress levels [17–19]. Moreover, maternal smoking has been associated with defective alveolar formation in offspring [1]. Similarly, in our study, we observed variable alveolar size and thickened alveolar walls in offspring mice exposed to CS. Additionally, the body weight of offspring mice in the AS and SS groups decreased compared to that in the AA and SA groups, with more pronounced effects observed at the third week after birth. These findings suggest that CS exposure adversely affects the growth and alveolar formation of offspring mice.

ER stress and autophagy play crucial roles in the normal development and function of the lungs [7,20]. Stress responses trigger protective autophagy, which promotes the recycling of damaged cell organelles, denatured proteins, and other biomacromolecules essential for the survival of lung cells [21]. Current research has confirmed that exposure to CS can induce ER stress and autophagy in the lungs [13]. However, few studies have reported the effects of CS exposure during pregnancy on ER stress and autophagy in the lungs of offspring. In the present study, immunofluorescence results demonstrated that exposure to CS increased the expression of CHOP, GRP78, Caspase-12, Beclin-1, and LC3 in the lungs of offspring mice. These findings were further validated by Western blotting and PCR, which showed increased expression of CHOP, GRP78, Caspase-12, Beclin-1, and LC3-II, indicating heightened ER stress and autophagy in the lungs of offspring mice from the CS exposure group. Existing evidence suggests that levels of lung autophagy fission marker Drp-1 and autophagic marker LC3-II increase in male offspring exposed to CS [22], which is consistent with the results of autophagy detection in this study.

Furthermore, this study observed that compared to the SA group (CS treatment during pre-pregnancy and air treatment during pregnancy), the SS group (CS treatment during both pre-pregnancy and pregnancy) showed a significant increase in ER stress and autophagy levels. This suggests that quitting smoking during pregnancy can effectively reduce the levels of lung ER stress and autophagy in the offspring of smoking women. Moreover, compared to the AA group (air treatment during both pre-pregnancy and pregnancy), the SA group exhibited slightly elevated levels of ER stress and autophagy, although the differences were not statistically significant. This indicates that if the mother smokes long-term before pregnancy or is exposed to CS, residual harmful substances in the body can still affect the lungs of the offspring, posing potential risks. Additionally, compared to the AA group, the AS group (air treatment during pre-pregnancy and CS treatment during pregnancy) exhibited an increasing trend in ER stress and autophagy levels. Between the AS and SA groups, except for significantly higher CHOP expression in the AS group, the other indicators were slightly higher in the AS group than in the SA group. This suggests that even if the mother does not smoke, prolonged exposure to CS during pregnancy can still affect the offspring's lung ER stress and autophagy.

Furthermore, although most indicators related to ER stress and autophagy between the AS group and SA group do not show significant differences, the degree of apoptosis, ER stress, and autophagy is slightly stronger in the AS group compared to the SA group. This suggests that compared to smoking before pregnancy or exposure to secondhand smoke, smoking during pregnancy or exposure to secondhand smoke poses a greater risk to the lung development of offspring. This highlights, once again, the necessity of quitting smoking during pregnancy for the lung health of future generations. Finally, the expression of GRP78, Caspase-12, Beclin-1, and LC3 did not show significant differences between the AS and SA groups, but significantly increased in the SS group. This indicates that exposure to CS before and during pregnancy has an additive effect on inducing ER stress and autophagy in the offspring's lungs, leading to more severe lung damage under the dual action.

While this study provides valuable insights, it is important to acknowledge its limitations. Initially, each group consisted of only 5 male mice for subsequent pathological examination and indicator testing. The inherent individual differences in animal experiments can significantly impact the accuracy of experimental results. Therefore, including a larger number of experimental animals in future studies is essential. Additionally, although we recorded the body weight of offspring mice at the ages of 3 and 7 weeks, we did not account for the food intake of the observed mice. Observing the growth of offspring mice in each group and analyzing the correlation between their growth, ER stress, and autophagy are crucial for a comprehensive understanding of the effects of CS exposure on the lungs of the offspring. Furthermore, the study found that the impact of maternal exposure to CS on the lung function of offspring mice was influenced by the offspring's gender.

Wang *et al.* [22] reported that at 13 weeks, only male offspring mice showed changes in lung inflammation and autophagy levels due to maternal exposure to CS, while female offspring were not affected by maternal CS exposure. One study has shown that prenatal exposure to CS has a greater impact on the susceptibility to asthma in male offspring [23]. It can be seen that the impact of exposure to CS on the lungs of offspring is influenced by the age and gender of the offspring. In this study, male offspring at 7 weeks of age were selected for observing ER stress and autophagy. However, lung development in offspring is a dynamic process. Therefore, it is essential to observe autophagy and ER stress in the lungs of offspring mice at different stages. At the same time, observing the effects of CS exposure on autophagy and ER stress in female offspring mice is an important aspect of understanding the impact of CS exposure on offspring lung function. Additionally, there is mutual influence between ER stress and autophagy, involving interactions of multiple signaling pathways. Therefore, future research should focus on the correlation between ER stress and autophagy, as well as the changes in the signaling pathways involved.

In conclusion, this study modeled the impacts of maternal smoking and smoking cessation on lung ER stress and autophagy in offspring by exposing female mice to CS during two stages: pre-pregnancy and pregnancy. The results indicated that exposure to CS during both pre-pregnancy and pregnancy increased ER stress and autophagy in the lungs of mouse offspring, with the most significant increase observed in female mice exposed to CS during both stages.

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Therefore, irrespective of whether there was a smoking habit before pregnancy, pregnant individuals should avoid CS exposure during pregnancy to mitigate the effects on lung ER stress and autophagy in their offspring.

Conclusions

In conclusion, the results of the present study showed that preconceptional and gestational exposure to CS heightened ER stress and autophagy in the lungs of mouse offspring. For mice exposed to CS during preconceptional, withdrawal from CS during pregnancy partially reduced ER stress and autophagy in the lungs of their offspring.

Availability of Data and Materials

The datasets generated for this study are available on request to the corresponding author.

Author Contributions

HC performed the experimental design and experiments, data statistical analysis, and writing the manuscript. JWS contributed to the experimental design and guided the data statistical analysis. XX conducted the experimental design and experiments. HYZ participated in the experiments and data statistical analysis. WZS provided experimental design and operational guidance, directed data statistical analysis and manuscript writing. All authors contributed significantly to editorial changes of important content. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study received approval from the Ethics Committee of the First Hospital of Hebei Medical University [20210909] and was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Acknowledgment

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This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

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