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ORIGINAL RESEARCH

A Peptide Encoded by lncRNA HOXB-AS3 Promotes Cigarette Smoke-Induced Inflammation in Bronchial Epithelial Cells via EZH2-Mediated H3K27me3 Modification

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Background: Chronic obstructive pulmonary disease (COPD) primarily results from cigarette smoke (CS)-induced chronic inflammation. Although numerous long non-coding ribonucleic acids (lncRNAs) have been extensively studied for their crucial roles in COPD, the peptides encoded by these lncRNAs have garnered limited attention. This study aimed to investigate the role of a peptide encoded by lncRNA HOXB-AS3 in cigarette smoke extract (CSE)-induced inflammation and in 16HBE cells.

Methods: Open reading frames (ORF) Find software was utilized to predict the encoding potential of HOXB-AS3. Quantitative real-time polymerase chain reaction (qRT-PCR) was employed to detect the levels of peptide HOXB-AS3-32aa in peripheral blood mononuclear cells (PBMCs) from both healthy controls and COPD patients and in 16HBE cells exposed to different CSE. To establish an in vitro inflammatory cell model of COPD, 16HBE cells were treated with 2% CSE. Enzyme-Linked Immunosorbent Assay (ELISA) measured inflammatory cytokines, while CCK-8 assay assessed cell viability. Flow cytometry was employed to assess cell apoptosis. Western blot analysis was performed to measure the expression of HOXB-AS3-32aa, EZH2, and H3K27me3 proteins. Co-Immunoprecipitation (Co-IP) was conducted to verify the interaction between EZH2 and HOXB-AS3-32aa.

Results: Our findings revealed elevated expression of HOXB-AS3-32aa in PBMCs of COPD patients compared to controls. CSE treatment dose-dependently increased HOXB-AS3-32aa expression. Overexpression of HOXB-AS3-32aa exacerbated CS-induced inflammation in bronchial epithelial cells, leading to inhibited cell proliferation and increased cell apoptosis. Furthermore, HOXB-AS3 -32aa suppressed EZH2 and H3k27me3 protein levels in 16HBE cells. Co-IP results confirmed the interaction between HOXB-AS3 -32aa and EZH2 protein.

Conclusion: Our results demonstrate that the novel peptide HOXB-AS3-32aa encoded by lncRNA HOXB-AS3 promotes CS-induced inflammation and apoptosis in 16HBE cells via EZH2-mediated H3K27me3 modification.

Keywords: HOXB-AS3-32aa, EZH2, H3K27me3 modification, inflammation, chronic obstructive pulmonary disease

Introduction

Chronic obstructive pulmonary disease (COPD) is a common and serious chronic respiratory condition that significantly contributes to global morbidity and mortality.[1] Cigarette smoke (CS) is the main cause of COPD,[2] with over 7000 chemicals present in cigarettes.[3] These chemicals cause inflammation and oxidative stress in the respiratory system.[4,5] However, COPD has multiple origins. Besides CS, other risk factors include environmental pollutants (eg, particulate matter, biomass fuel combustion),[6–8] occupational hazards (eg, silica, cadmium),[9] genetic predisposition (eg, alpha-1 antitrypsin deficiency),[10] respiratory infections,[11] and aging-related cellular senescence.[12] These factors may work

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together with CS or alone to drive disease progression through pathways like chronic inflammation and oxidative stress. Although COPD and bronchial asthma both involve chronic airway inflammation, but CS-induced COPD has distinct features such as neutrophilic infiltration, alveolar destruction (emphysema), and small airway fibrosis, which are different from the eosinophilic inflammation and bronchial hyperresponsiveness in asthma.[13,14] The precise mechanisms underlying the transition from CS exposure to COPD development remain incompletely understood. Consequently, there is a need to further elucidate the pathogenesis of CS-induced COPD, as this is important for developing preventive and therapeutic strategies.

Long non-coding ribonucleic acids(lncRNAs), defined as transcripts exceeding 200 nucleotides in length, play pivotal roles in both transcriptional and post-transcriptional regulation. Their significance in the pathogenesis of COPD has garnered considerable attention in recent literature.[15–17] While lncRNAs were initially believed to be non-coding transcripts devoid of protein-coding potential, recent evidence has revealed that lncRNAs harbor small open reading frames (smORFs) encoding peptides, thus imparting coding activity and engaging in diverse biological processes.[18–20] One such lncRNA, HOXB-AS3 (HOXB Cluster Antisense RNA 3), situated on human chromosome 17q21.32, exhibits dual functionality as both a lncRNA and a putative coding peptide in tumorigenesis and progression.[21] For instance, HOXB-AS3 has been implicated in lung cancer tumor growth through modulation of the PI3K/AKT pathway,[22] while the 53-amino acid (aa) peptide encoded by HOXB-AS3 suppresses colon cancer growth.[23] However, the precise role of peptides encoded by HOXB-AS3 in CS-induced inflammation within bronchial epithelial cells remains enigmatic. Therefore, a thorough investigation of the mechanistic actions of lncRNA-encoded peptides in COPD development holds the promise of revealing novel drug targets and treatment strategies for this debilitating respiratory condition.

The enhancer of zeste homolog 2 (EZH2), a critical catalytic subunit within the Polycomb Repressive Complex 2 (PRC2), executes epigenetic repression by catalyzing the methylation of lysine 27 on histone H3 (H3K27me3).[24] Recent bioinformatics analyses have implied a pivotal role for EZH2 in the pathogenesis of COPD and lung adenocarcinoma (LUAD).[25] Notably, elevated expression levels of both EZH2 and H3K27me3 have been observed in bronchial epithelium cells isolated from COPD patients and smokers compared to healthy controls. Furthermore, cigarette smoke extract (CSE) has been shown to exacerbate the expression of both EZH2 and H3K27me3. Of particular interest, the metaplastic epithelium of COPD patients exhibits heightened immunoreactivity scores for EZH2 and H3K27me3.[26] Contrary to these observations, however, EZH2 expression is reduced in COPD patients and CSE-treated Beas-2B cells.[27] Our previous research has demonstrated that the peptide encoded by HOXB-AS3 (designated as HOXB-AS3-aa) is significantly upregulated in the COPD group compared to controls and exerts an inhibitory effect on EZH2 expression. Building upon these findings, we hypothesize that the peptide HOXB-AS3-32aa can modulate CSE-induced inflammation in human bronchial epithelial (16HBE) cells through EZH2-mediated modifications of H3K27me3. This proposed mechanism offers a novel avenue for exploring potential therapeutic targets and strategies in the treatment of COPD.

The primary aim of the present study was to elucidate the precise cellular mechanisms underlying the modulation of CSE-induced inflammation by the peptide HOXB-AS3-32aa, employing an in vitro model of human bronchial epithelial (16HBE) cells exposed to CSE. The detailed process of this study is shown in Figure 1. The ultimate goal of this research is to facilitate the translation of preclinical findings into clinically relevant applications, ultimately aiming to develop innovative therapeutic strategies for the effective treatment of COPD.

Materials and Methods

Subjects Enrollment

A total of 40 participants were recruited for the study from the Hainan General Hospital, including 20 patients with COPD and 20 healthy controls. The COPD patients were diagnosed based on the criteria established by the Global Initiative for Chronic Obstructive Lung Disease (GOLD).[28] The inclusion criteria for COPD patients were adults aged 18 years or older, a confirmed diagnosis of COPD according to the GOLD criteria, at least one spirometry in the last year with a post-bronchodilator forced expiratory volume in 1 second/forced vital capacity (FEV_1/FVC) < 0.70, and stable disease (no exacerbations in the past 4 weeks). Healthy controls were required to have normal lung function ($FEV_1/FVC \geq 0.70$), be matched with the COPD group in terms of age and gender, and have no active infections or systemic

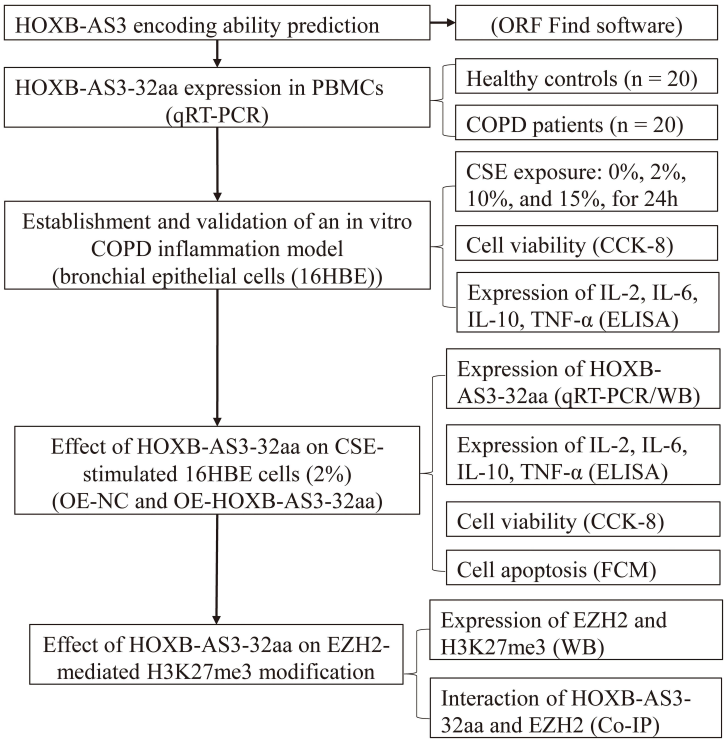

Figure 1 A flowchart showing all the steps involved in our analysis.

inflammation. The exclusion criteria for all participants comprised asthma, lung cancer, major cardiovascular diseases, or other significant comorbidities. Additionally, participants with poor compliance, refusal to complete tests, or unwillingness to participate in the study, as well as those with multiple quality control failures of samples resulting in unsuccessful determination after repeated submissions, were excluded. Before the commencement of the study, written informed consent was obtained from all the participants.

Ethical Statement

The study protocol was approved by the ethics committee of Hainan General Hospital (No. Med-Eth-Re [2020]10), in accordance with the Declaration of Helsinki.

Human Peripheral Blood Mononuclear Cells (PBMCs) Isolation

PBMCs were isolated from peripheral venous blood of COPD patients and healthy controls using the Ficoll-Paque method. Peripheral blood (5 mL) was carefully layered onto a tube containing Ficoll. Then, the tube was centrifuged at 400 g for 30 minutes at room temperature, resulting in the formation of three distinct layers. The middle layer, containing mononuclear cells known as PBMCs, was carefully aspirated and transferred to a new tube. The PBMCs were washed twice with PBS to create a single-cell suspension and subsequently frozen at −80°C for future analysis.

Cell Culture and Treatment

Human bronchial epithelial cells (16HBE) were purchased from the American Type Culture Collection (ATCC) Cell Bank (China) and were cultured in Dulbecco's Modified Eagle Medium (DMEM, Hyclone), supplemented with 10% fetal bovine serum (FBS, Gibco) and 1% penicillin/streptomycin (Genview), in an incubator containing 5% CO_2 at 37°C. For the cigarette smoke extract (CSE) exposure experiment, the human 16HBE cells were treated with different concentrations of CSE: 0%, 2%, 10%, and 15%, for 24 h. To facilitate detection through Western blot, we designed a peptide fused 3FLAG protein tag to obtain ORF-3FLAG (HOXB-AS3). To explore the coding ability of lncRNA itself, we retained the original 5'UTR end of lncRNA and successfully constructed 5UTR-ORF-3FLAG (HOXB-AS3). In

addition, to investigate the effect of the start codon on encoding, we mutated the start codon ATG of ORF to ATT, resulting in 5UTR-ORF-3FLAG mut (HOXB-AS3). Subsequently, we transfected these three vectors with empty control into 16HBE cells and detected FLAG tags through Western blot. These overexpression plasmids of HOXB-AS3-32aa or negative controls (OE-NC) were transfected using Lipofectamine (Invitrogen) according to the manufacturer's instructions.

Cell Viability Assay

The cell viability was assessed using the Cell Counting Kit-8 (CCK-8, TransGen Biotech, China) as per the manufacturer's guidelines. Briefly, following a 24 h treatment with CSE or transfection, log-phase 16HBE cells were seeded into 96-well plates at a density of 5×10^4 cells per well. At the indicated time points (0, 24, 48, 72, 96, and 120 h), the medium in each well was replaced with fresh medium containing 10% CCK-8 solution. The plates were then incubated at 37°C in a 5% CO_2 incubator for 3 h. Subsequently, the absorption values were measured at a wavelength of 450 nm using a microplate reader (BioTek, USA).

Enzyme-Linked Immunosorbent Assay (ELISA)

To assess the levels of inflammatory factors in human 16-HBE cell supernatants, we employed ELISA kits (Jiangsu Jingmei Biotechnology Co., Ltd., China) specifically designed for the detection of interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-10 (IL-10), and tumor necrosis factor-alpha (TNF-α). The assays were performed strictly adhering to the manufacturer's instructions to ensure accuracy and reproducibility. After the reaction was terminated, optical density (OD) values were measured at a wavelength of 450 nm using a microplate reader (Multiskan FC, Thermo Fisher Scientific, USA). These OD values were then plotted against the concentrations of the standards to generate a standard curve. This curve served as a reference for calculating the levels of inflammatory factors in the samples.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted from each group of 16HBE cells using TRIzol reagent (TIANGEN, China) following the manufacturer's protocol. The concentration of extracted RNA was determined using a Nanodrop® 2000 spectrophotometer (Thermo Fisher Scientific, USA). Subsequently, 3 μg RNA was reverse-transcribed into complementary DNA (cDNA) by a PrimeScript RT reagent kit (Takara Bio Inc., Japan). Afterward, the resulting cDNA was then used as a template for qRT-PCR analysis, which was conducted on an ABI Q1 (Applied Biosystems, Thermo Fisher Scientific, USA) RT-PCR system using SYBR Green Master Mix (Thermo Fisher Scientific, USA). The thermocycling conditions for the qRT-PCR were as follows: 94°C for 10 min, 95°C for 5 sec, followed by 40 cycles at 95°C for 5 sec, 60°C for 15 sec, and 72°C for 10 sec. Relative expression levels of the target gene were analyzed using the $2^{-\Delta\Delta Ct}$ method, with GAPDH serving as the internal control for expression normalization. The specific primer sequences used in this study are presented in Table 1.

Western Blot

Total protein from each group of 16HBE cells was extracted using the radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime Biotechnology, China). The concentration of the extracted protein was determined using the bicinchoninic acid (BCA) protein determination kit (Beyotime Biotechnology, China). A total of 20 μg protein per lane was then separated via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto the polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA). Subsequently, the PVDF membranes were blocked

Table 1 Primer Sequences for Quantitative Real-Time Polymerase Chain Reaction

Gene-ID	Forward (5' to 3')	Reverse (5' to 3')
HOXB-AS3-32aa	GAAGTTGGGCCAAGCTGGAA	CTAAGGGACGTCCTGGTTTC
GAPDH	CTGACTTCAACAGCGACACC	GTGGTCCAGGGGTCTTACTC

with phosphate-buffered saline (PBS) containing 5% bovine serum albumin (BSA) powder for 1 h at room temperature. Then, the membranes were incubated with specific primary antibodies: anti-HOXB-AS3-32aa (Proteintech, 17168-1-AP, 1: 2000), anti-EZH2 (Abcam, ab186006, 1: 5000), anti-H3k27me3 (Abcam, ab6147, 1: 5000) or reference gene anti-GAPDH (Proteintech, 60004-1-Ig, 1: 5000) at 4°C overnight. This was followed by incubation with a Goat Anti-Rabbit IgG horseradish peroxidase-labeled (HRP) secondary antibody (KPL, 074–1506, 1:5000) for 1 h at room temperature. The protein bands were visualized using an enhanced chemiluminescent (ECL) kit (Beyotime Biotechnology, China), and the intensity of each band was analyzed using Image J 1.8.0 software.

Apoptosis Assay

To detect cell apoptosis, we employed the Annexin V-FITC Detection Kit (Biolegend, USA) in conjunction with flow cytometry. Initially, 16HBE cells were washed twice with cold PBS and resuspended in 500 μL of binding buffer, adjusting the cell concentration to 1×10^6 cells/mL. Subsequently, 100 μL of this cell suspension was mixed with 5 μL of Annexin V-FITC and 10 μL of propidium iodide (PI). This mixture was gently agitated and incubated in the dark for 15 min at room temperature. Following incubation, the stained cells were analyzed using a flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA) to detect and calculate the percentage of apoptotic cells.

Co-Immunoprecipitation (Co-IP)

Human 16HBE cells were washed twice with cold PBS to eliminate any residual culture media. Following this, the cells were lysed on ice for 30 minutes using RIPA lysis (Beyotime Biotechnology, China). Cell lysates were collected and centrifuged at 13,000 rpm for 15 minutes at 4 °C. The supernatant containing the cytosolic proteins was transferred to a new tube. The cell lysate was incubated with protein A/G beads (Beyotime Biotechnology, China) at 4°C for 2 h. The beads were then pelleted by centrifugation, and the supernatant was collected. The precleared lysate was incubated with either an anti-EZH2 antibody (Abcam, ab186006, 1:5000) or control rabbit anti-IgG (KPL, 074–1506, 1:5000) overnight at 4 °C. The beads were washed extensively with lysis buffer to remove unbound proteins and nonspecific interactions. Subsequently, the beads were boiled in an SDS loading buffer to elute the bound proteins. Western blot analysis was then performed to study the immunoprecipitated proteins, allowing for the detection of interacting protein complexes.

Statistical Analysis

To ensure reproducibility and reliability, all experiments were independently replicated three times. The collected experimental data were analyzed using GraphPad Prism 8.0 software (GraphPad Software Inc., San Diego, CA, USA) and presented as means ± standard deviation (SD). For comparisons between the two groups, we employed the unpaired two-tailed *t*-test (Student's *t*-test). For comparisons among multiple groups, we utilized a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. p-value <0.05 was considered statistically significant.

Results

Prediction HOXB-AS3 Encoding Ability and Expression

We used ORF Find software (https://www.detaibio.com/sms2/orf_find.html) to predict the encoding ability of HOXB-AS3, and the results showed that HOXB-AS3 can encode three peptides (Supplementary Table 1). The qRT-PCR results showed that the relative expression of the peptide (HOXB-AS3-32aa) in the COPD group was significantly higher than that in the control group ($p < 0.001$, Figure 2A). To further verify the ability of HOXB-AS3 to encode peptides, we designed three expression vectors based on the coding information mentioned above. The results showed that the expression bands of ORF-3FLAG and 5'UTR-ORF-3FLAG groups were approximately 6kD, while the original 3FLAG size was 2.73kD (Figure 2B). This result is consistent with expectations, that is, the encoded peptide is composed of 32 amino acids, with a molecular weight of approximately 32 * 110 Dalton = 3.52 kD. This discovery suggests that HOXB-AS3 can indeed encode a peptide composed of 32 amino acids, and its encoding ability is consistent with the expected size. The ORF-3FLAG group showed a decrease in EZH2 expression compared to the NC group, suggesting that ORF regulation of EZH2 may be one of its mechanisms ($p < 0.001$, Figure 2B).

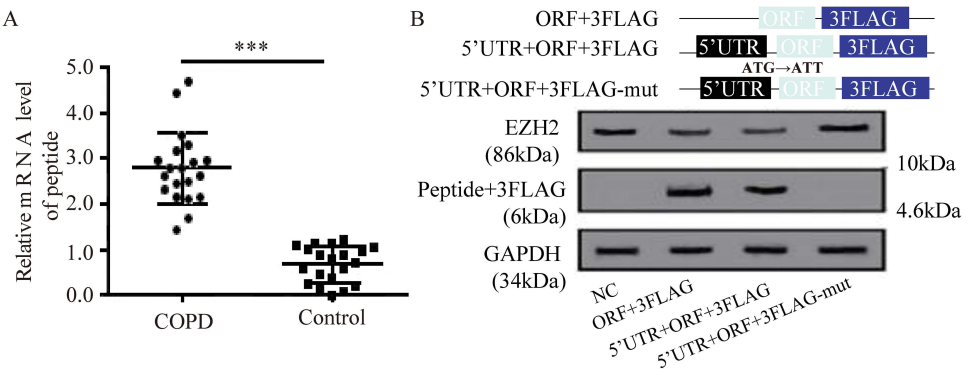

Figure 2 HOXB-AS3-32aa expression analysis in COPD and validation of peptide encoding. (**A**) The relative expression of the peptide (HOXB-AS3-32aa) in the COPD and control groups (qRT-PCR). (**B**) The expression bands of ORF-3FLAG and 5'UTR-ORF-3FLAG groups (Western blot). ***$p < 0.001$.

Establishment and Validation of an in vitro COPD Inflammation Model

Bronchial epithelial cells treated with CSE serve as a reliable in vitro model for studying COPD. In our study, the 16HBE cells were exposed with different concentrations of CSE (0, 2, 10, 15%) for 24 h. The results of the CCK-8 assay revealed a concentration-dependent decrease in cell viability of 16HBE cells upon CSE exposure (Figure 3A). This observation indicates that CSE is cytotoxic to 16HBE cells, simulating the toxic effects of cigarette smoke on bronchial epithelium in vivo. To further investigate the inflammatory response, we employed ELISA to measure the levels of key inflammatory cytokines. The results demonstrated a concentration-dependent increase in the levels of IL-2, IL-6, and TNF-α upon CSE exposure, whereas a concentration-dependent increase was observed in the levels of IL-10 (Figure 3B). These findings suggest that CSE induces inflammatory responses by promoting the release of inflammatory cytokines, mimicking the inflammatory milieu observed in COPD patients.

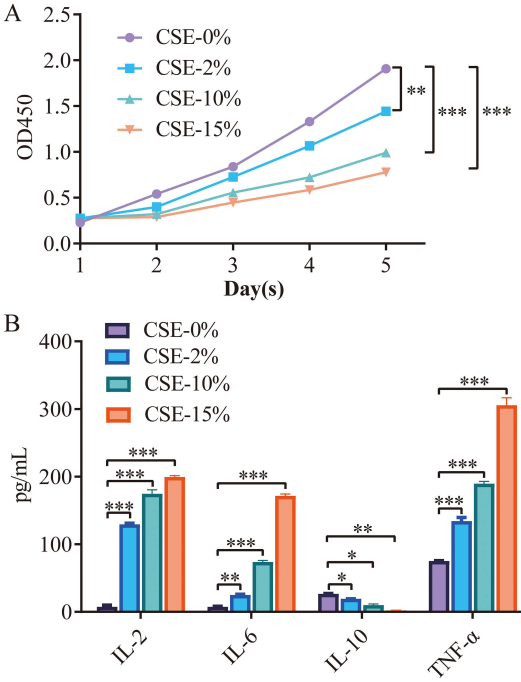

Figure 3 COPD inflammation model establishment and validation. (**A**) The cell viability of 16HBE cells (CCK-8). (**B**) The levels of inflammatory cytokines (ELISA). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Effect of HOXB-AS3-32aa on CSE-Stimulated 16HBE Cells

The results of qRT-PCR and Western blot showed that the expression of HOXB-AS3-32aa mRNA and protein in 16HBE cells exposed to CSE increased in a concentration-dependent manner ($p < 0.01$, Figure 4A). When the CSE concentration was 2%, the level of HOXB-AS3-32aa increased to 50%, so 2% was selected as the CSE exposure concentration in this study ($p < 0.01$, Figure 4B). Subsequently, the influence of HOXB-AS3-32aa on the inflammatory response induced by CSE was assayed using ELISA. The overexpression of HOXB-AS3-32aa significantly up-regulated the levels of IL-2, IL-6, and TNF-α, whereas it significantly down-regulated the levels of IL-10, thereby promoting the inflammatory response ($p < 0.001$, Figure 4C). The effects of HOXB-AS3-32aa on CSE-induced 16HBE cell viability were detected by CCK-8 assay. As shown in Figure 4D, overexpression of HOXB-AS3-32aa significantly inhibited cell proliferation ($p < 0.001$). Moreover, the effects of HOXB-AS3-32aa on CSE-induced 16HBE cell apoptosis were detected by flow cytometry. The results showed that overexpression of HOXB-AS3-32aa significantly increased cell apoptosis ($p < 0.001$, Figure 4E).

Effect of HOXB-AS3-32aa on EZH2-Mediated H3K27me3 Modification

Western blot results revealed that the HOXB-AS3-32aa overexpression significantly decreased the protein level of EZH2 and H3k27me3 in 16HBE cells ($p < 0.001$, Figure 5A). The results of Co-IP showed that HOXB-AS3-32aa interaction

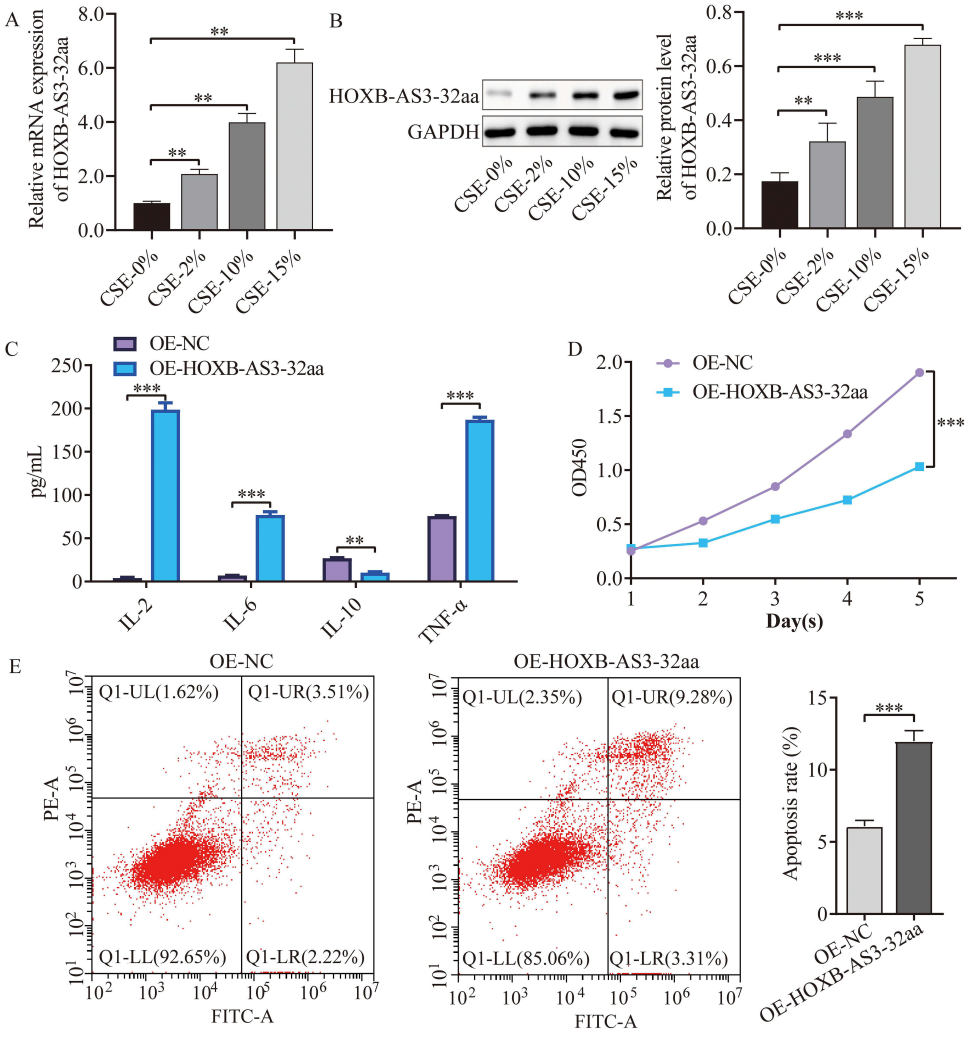

Figure 4 Effect of HOXB-AS3-32aa on CSE-stimulated 16HBE cells. (**A**) The mRNA expression of HOXB-AS3-32aa in CSE-stimulated 16HBE cells (qRT-PCR). (**B**) The mRNA expression of HOXB-AS3-32aa in CSE-stimulated 16HBE cells (Western blot). (**C**) The levels of inflammatory cytokines (ELISA). (**D**) The cell viability of CSE-stimulated 16HBE cells (CCK-8). (**E**) The cell apoptosis of CSE-induced 16HBE cells (flow cytometry). $^{**}p < 0.01$, $^{***}p < 0.001$.
Abbreviations: OE-NC, overexpression-negative control; OE-HOXB-AS3-32aa, overexpression-HOXB-AS3-32aa.

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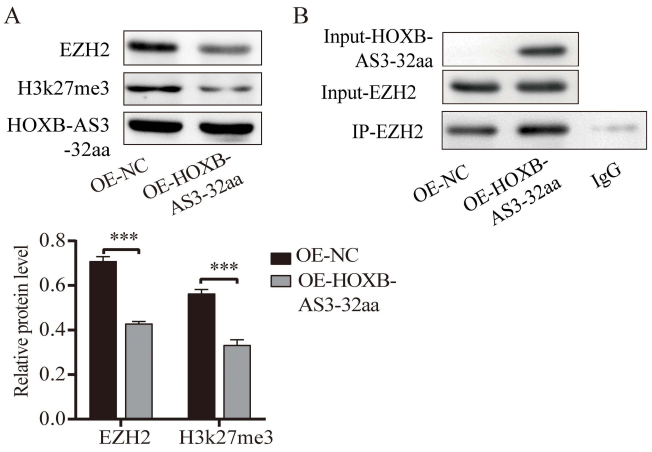

Figure 5 Effect of HOXB-AS3-32aa on EZH2-mediated H3K27me3 modification. (**A**) The expression of EZH2 and H3K27me3 in CSE-stimulated 16HBE cells between OE-NC and OE-HOXB-AS3-32aa groups (Western blot). (**B**) The interaction between HOXB-AS3-32aa and EZH2 (Co-IP). ***$p < 0.001$.
Abbreviations: OE-NC, overexpression-negative control; OE-HOXB-AS3-32aa, overexpression-HOXB-AS3-32aa.

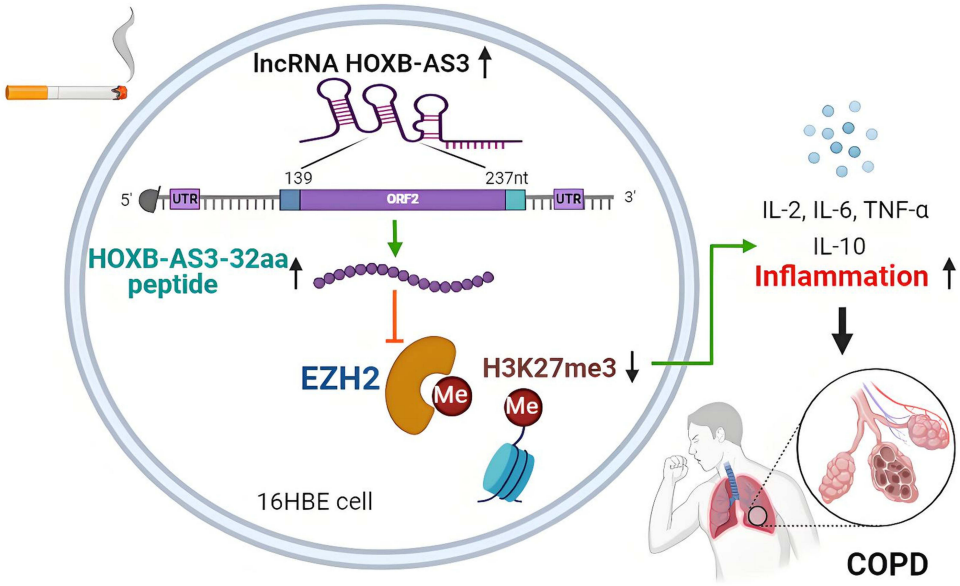

Figure 6 Schematic diagram of this study. The peptide HOXB-AS3-32aa encoded by lncRNA HOXB-AS3 promotes CS-induced inflammation and apoptosis in bronchial epithelial cells through reducing EZH2-mediated H3K27me3 modification.

with EZH2 protein in 16HBE (Figure 5B). It is speculated that the peptide encoded HOXB-AS3-32aa by lncRNA HOXB-AS3 promotes cigarette smoke-induced inflammation in bronchial epithelial cells via EZH2-mediated H3K27me3 modification (Figure 6).

Discussion

In this study, we demonstrate that the lncRNA HOXB-AS3 encodes a conserved 32-aa peptide. This HOXB-AS3 peptide, rather than the lncRNA itself, plays a crucial role in promoting CS-induced inflammation in bronchial epithelial cells. This peptide exerts its inflammatory effects through the modulation of EZH2-mediated H3K27me3 modification. Our findings provide novel insights into the mechanisms underlying lncRNA-encoded peptides in regulating cellular inflammation and offer potential therapeutic targets for the treatment of inflammatory airway diseases.

In recent years, the functional significance of small peptides encoded by non-coding RNAs has garnered significant attention. This interest has been piqued by the discovery that lncRNAs can encode peptides potentially implicated in

tumorigenesis, as highlighted by several studies.[18,29,30] While the noncoding transcripts of lncRNAs have been extensively explored in the context of COPD, particularly about their crucial functions,[17,31,32] the peptides encoded by these transcripts have received limited attention. In this study, we present evidence that the lncRNA HOXB-AS3 encodes a 32-amino acid peptide, designated as HOXB-AS3-32aa. This peptide promotes CS-induced inflammation in bronchial epithelial cells, suppresses cell proliferation, and enhances apoptosis in vitro. Interestingly, previous research has demonstrated that HOXB-AS3 encodes a conserved 53-amino acid polypeptide, which exhibits suppressive effects on colon cancer cell growth, colony formation, invasion, and metastasis.[23] Our findings contribute to the understanding of the diverse and extensive roles of lncRNAs in human COPD and suggest that these peptides may hold potential for the identification of COPD biomarkers in future studies.

Moreover, epigenetic modifications, including histone and DNA modifications, have been implicated in maintaining lung health and the pathogenesis of pulmonary diseases.[33,34] EZH2, a histone methyltransferase, plays a pivotal role in regulating immune responses via H3K27me3 hypermethylation.[35,36] Previous investigations have demonstrated that CS exposure affects EZH2 expression, leading to reduced DAB2IP levels through H3K27me3 hypermethylation in bronchial epithelial cells of COPD patients. This, in turn, triggers apoptotic cell death, and mesenchymal transition, and enhances cellular invasiveness in the bronchial epithelium, thereby favoring the progression of airway inflammation toward lung cancer in COPD patients.[26] Additionally, lncRNA SNHG4 has been shown to promote COPD progression by inhibiting cell viability, promoting apoptosis, and inducing an inflammatory response in bronchial epithelial cells through modulation of the miR-144-3p/EZH2 axis.[27] In our current study, we observed that HOXB-AS3-32aa suppressed the levels of EZH2 and H3K27me3 protein in bronchial epithelial cells. Co-IP results confirmed the interaction between HOXB-AS3 -32aa and EZH2 protein. These findings suggest that the peptide encoded by lncRNA HOXB-AS3 promotes CS-induced inflammation and apoptosis in bronchial epithelial cells through EZH2-mediated H3K27me3 modification, thus contributing to the complex pathogenesis of COPD.

While this study offers valuable insights into the inflammatory mechanisms underlying COPD, it is imperative to acknowledge several limitations. Firstly, the clinical relevance of HOXB-AS3-32aa expression in COPD patients remains unexplored. Secondly, the absence of animal studies limits our understanding of the in vivo expression of HOXB-AS3 -32aa. Additionally, the experimental design lacks a normal control group (16HBE cells) and a group treated solely with 2% CS. Lastly, rescue experiments were not included in the study, which could have provided additional validation. Future research should address these limitations to further advance our understanding of COPD pathogenesis and identify potential therapeutic targets.

Conclusion

This study reveals that the lncRNA HOXB-AS3 encodes a functional 32-aa peptide (HOXB-AS3-32aa) that promotes CSE-induced inflammation and apoptosis in bronchial epithelial cells by suppressing the EZH2/H3K27me3 pathway. These findings highlight the importance of lncRNA-encoded peptides in COPD pathogenesis and suggest HOXB-AS3 -32aa as a potential therapeutic target. Further studies are needed to validate these results in vivo and in clinical settings.

Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Acknowledgments

The authors thank all participants for their support and participation.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article

has been submitted; and agree to be accountable for all aspects of the work. Mei Lin and Xiaoman Zhou are co-first authors.

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Disclosure

The authors declare that they have no competing interests.

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