

Circular RNA_0025843 Alleviated Cigarette Smoke Extract Induced Bronchoalveolar Epithelial Cells Ferroptosis

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Purpose: Circular RNA (circRNA) plays an important role in various biological processes. However, their functions in cigarette smoke extract (CSE) induced human normal lung epithelial cells (BEAS-2B) injury remain vague. The study aimed to explore circRNA expression profiles and reveal their potential roles in CSE-treated BEAS-2B cells.

Methods: 5% CSE exposure for 24 hours were used to build the BEAS-2B cells ferroptosis model. Differentially expressed circRNAs (DECs) were identified by next-generation RNA sequencing. Six randomly selected DECs were validated via quantitative reverse transcription polymerase chain reaction (qRT-PCR). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis and Gene Ontology (GO) analysis were conducted to clarify the potential functions of the DECs. Furthermore, the role of hsa_circ_0025843 in CSE-related BEAS-2B cells ferroptosis was confirmed.

Results: 5% CSE exposure induced BEAS-2B cells ferroptosis. Fifty-one up-regulated circRNAs and 80 down-regulated circRNAs were revealed in CSE-treated BEAS-2B cells. Hsa_circ_0003461, hsa_circ_0007548, hsa_circ_0025843, hsa_circ_0068896, hsa_circ_0005832, and hsa_circ_0053378 were selected randomly to validate the reliability of next-generation RNA sequencing by qRT-PCR. After KEGG pathway analysis, DECs were found to participate in the process of EGFR tyrosine kinase inhibitor resistance and glycerophospholipid metabolism. The knockdown of hsa_circ_0025843 significantly alleviated CSE-induced BEAS-2B cells ferroptosis.

Conclusion: The study indicated the circRNA expression profiles in CSE-treated BEAS-2B cells. Hsa_circ_0025843 alleviated CSE induced BEAS-2B cells ferroptosis, which might be a potential therapeutic target of CSE related lung injury.

Keywords: cigarette smoke extract, Circular RNA, next-generation RNA sequencing, lung injury, ferroptosis

Introduction

Nearly 1 million people died from smoking-related diseases in China each year, accounting for about 12% of all deaths. Tobacco abuse is an important risk factor for various pulmonary diseases, such as lung cancer, chronic obstructive pulmonary disease (COPD), and interstitial lung disease.^{1,2} Tobacco inhalation directly injured the human normal lung epithelial cells (BEAS-2B).³ However, no treatment can completely reverse tobacco-related lung injury.^{2,4} Therefore, studying the potential pathophysiology of cigarette smoke extract (CSE) induced BEAS-2B cells injury and revealing the exact underlying mechanisms are quite necessary.

Previous research revealed that cigarette smoke exposure induced BEAS-2B cells ferroptosis.⁵ Ferroptosis, a recently discovered form of cell death, involves iron-dependent lipid peroxidation, oxidative stress, mitochondrial disorder, excessive Fe²⁺ level, and an overabundance of reactive oxygen species (ROS). This process was first identified by Dixon in 2012.⁶ Glutathione peroxidase 4(GPX4), an enzyme containing both selenium and cysteine, acts to diminish the levels of peroxide,

resulting in keeping cellular redox homeostasis. This action effectively prevents the chain oxidation of cell membranes.⁷ Additionally, Acyl-CoA synthetase long-chain family member 4 (ACSL4) plays a critical role in the biosynthesis and remodeling of cell membrane-specific polyunsaturated fatty acid-phosphatidylethanolamine (PUFA-PE). Activation of ACSL4 facilitates the conversion of free polyunsaturated fatty acid (PUFA) into PUFA-PE.⁸ Consequently, GPX4 and ACSL4 are recognized as indicative biomarkers of ferroptosis.

Circular RNA (circRNA) is a special class of newly discovered non-coding RNA. The closed ring structure of circRNA makes it impervious to RNA exonuclease, so the expression levels are stable. It is one of the research hotspots recently. The function of circRNA covers almost all levels of gene expression regulation, such as histone modification, transcription regulation, post-transcriptional processing, protein modification, localization, and chromatin remodeling. Accumulating evidence confirmed that circRNAs affected numerous biological processes of various diseases, such as cell apoptosis, ferroptosis, epithelial-mesenchymal transformation, etc.^{9–11} However, a few studies focused on the circRNA expression profiles in CSE-treated BEAS-2B cells.

Last decade, the development of second-generation sequencing technology benefited in exploring the mechanism of disease occurrence and development.¹² High-throughput data have the advantage of fully understanding the alters in the expression profile of non-coding RNA. However, this technology was used to investigate the issue of CSE-related lung injury in a few studies.

The present study was designed to perform circRNA sequencing in CSE-treated BEAS-2B cells and normal BEAS-2B cells. The potential functions of differentially expressed circRNAs were predicted by bioinformatics methods. This study was aimed to explore the function of circRNAs in CSE-related lung injury.

Materials and Methods

Cell Culture

BEAS-2B cells were purchased from Biyuntian Biological Technology Co., Ltd (Shanghai, China). BEAS-2B cells were cultured in RPMI-1640 (HyClone) with 1% Streptomycin penicillin mixture (HyClone) and 10% fetal bovine serum (Gibco). The incubator was set at 37°C with 5% CO₂ (Thermo Fisher Scientific, Waltham, MA, USA).

CSE Preparation and the Cell Model Establishment

Prepare 100% CSE as following procedures: Combusted a cigarette (Septwolves, Longyan Cigarette Factory, Fujian, China) in a 500 mL glass bottle with the suction device.¹³ Dissolved the smoke in the 20 mL culture medium of RPMI-1640. Filtered the solution through a filter of 0.22 µm pore size and moved out the bacteria and large particles. Adjusted the pH value to 7.4. The obtained solution was 100% CSE. The 5% CSE was the mixture of RPMI-1640 complete medium and 100% CSE. The 5% CSE was used for subsequent experiments within 30 min of preparation. In the CSE group, the BEAS-2B cells were exposed in 5% CSE for 24 hours when BEAS-2B cells reached 70–80% confluency. The experimental methods of cell apoptosis rate were reviewed in the previous study.⁵

Isolation of RNA

TRIzol Reagent (Invitrogen, USA) was used for RNA extraction. The quantity and quality of the total RNA were detected by DNA/RNA concentration assay (Pharmathea INC., China). At least 3 µg of total RNA was isolated from BEAS-2B cells.

Screening for Differentially Expressed circRNA and Bioinformatics Analysis

The differentially expressed circRNA was screened by next-generation RNA sequencing. First, removing Ribosomal RNA and digesting the linear RNAs with the manufacturer's instructions. After that, it will amplify suitable fragments and construct the cDNA library via polymerase chain reaction (PCR). Then, assess the cDNA library's quantity and quality via qPCR quantification methods and Agilent 2100 Bioanalyzer (Agilent, USA). Last, sequencing the libraries by NovaSeq 6000 (Illumina, USA). The trimmed data were compared to the reference transcriptome to obtain Backsplice Junction. The circRNA was annotated and quantified by analyzing the comparison results. The circRNA with CSE vs

control fold change >1.50 (or $<1/1.5$), and p -value < 0.05 were defined as differentially expressed circRNA. The scatter diagram, volcano diagram, and cluster diagram were drawn to obtain visual data.

Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were carried out to clarify the functions and interactions among these dysregulated expressed circRNAs by GO database (<http://geneontology.org>) and KEGG database (<https://www.genome.jp/kegg/>). The enrichment GO terms and KEGG pathways of the differentially expressed circRNAs were selected and ranked by enrichment score $[-\log_{10}(p\text{-value})]$.

Real-Time Quantitative PCR (qRT-PCR)

The cDNA was obtained by reverse transcription of the total RNA of BEAS-2B cells via the PrimeScript RT reagent Kit (Takara Bio Inc., Japan). Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed to detect the expression levels of GPX4, ACSL4, hsa_circ_0003461, hsa_circ_0005832, hsa_circ_0007548, hsa_circ_0025843, hsa_circ_0053378 and hsa_circ_0068896 on an ABI 7500 thermocycler (Applied Biosystems, Foster City, CA, USA) with the SYBR Green PCR Master Mix (Takara Bio Inc., Japan). The sequences of primers were listed in Table 1. $2^{-\Delta\Delta CT}$ methods were used to calculate the Fold changes.

Cell Transfection

Hsa_circ_0025843 small interfering RNA (Si-circ_0025843), and the corresponding control (Si-nc-circ_0025843) were synthesized by Hanbio Biotechnology Co. (Shanghai, China). The transfection of Si-circ_0025843 was performed according to the manufacturer's instructions of Lipofectamine 3000 (Invitrogen, USA).

Detection of ROS Levels

The fluorescence probe DCFH-DA was used to detect intracellular ROS levels. BEAS-2B cells were seeded in 6-well plates and subjected to different treatments. After 24 h of CSE exposure, cells were collected in the 1.5 mL ep tubes and then suspended in serum-free RPMI-1640 with 10 μ M DCFH-DA (Beyotime, China). After incubating in a cell incubator at 37°C for 20 minutes, the cells were washed three times with serum-free RPMI-1640. When the samples were ready, a C6 flow cytometer was used to detect the ROS levels (Becton Dickinson, USA).

Table 1 List of Primer Sequence

Genes	Primer Sequence
hsa_β-actin	F: 5' GTGGCCGAGGACTTTGATTG 3' R: 5' CCTGTAACAACGCATCTCATATT 3'
hsa_circ_0003461	F: 5' GAGATCTCCACTACAAGATTAAGGA 3' R: 5' CTCTGCTCCTTCACATGGTACAG 3'
hsa_circ_0025843	F: 5' AATAAAAGAAGGACAGATCCTCA 3' R: 5' GTGAAGTTTTTGCTCATTAGTCT 3'
hsa_circ_0007548	F: 5' ACAACACTATTAAACTGACCAGG 3' R: 5' GCTGTAAATTCTGACTTTGATGT 3'
hsa_circ_0068896	F: 5' GCAGCATCCCAGTTTTTGGT 3' R: 5' TCCTTGGCTACTTGAGGGTTGA 3'
hsa_circ_0053378	F: 5' GTGGTAGACCGTCTAAGAGAAGG 3' R: 5' AGGAGTTGAAGTGGTTCGTGA 3'
hsa_circ_0005832	F: 5' TGTATTGAACCACAAAAACCTA 3' R: 5' GCCATGCTATTCTTCGTCTCT 3'
hsa_GPX4	F: 5' CCGCTGTGGAAGTGGATGAAGATC 3' R: 5' CTTGTCGATGAGGAAGTGTGGAG 3'
hsa_ACSL4	F: 5' TCTGCTTCTGCTGCCCAATT 3' R: 5' CGCCTTCTTGCCAGTCTTTT 3'

Detection of Fe²⁺ Levels

To measure the Fe²⁺ levels in different groups, FerroOrange (F-374, Dojindo, China) was employed. Cells were collected into 1.5 mL ep tubes, washed with non-serum RPMI-1640, and then incubated at 37°C in an incubator with 1 μ M FerroOrange for 30 minutes before being tested by a C6 flow cytometry (Becton Dickinson, USA).

Detection of Mitochondrial Membrane Potential

Mitochondrial membrane potential (MMP) was assessed using the JC-1 assay (Beyotime, C2006, China). Following the instructions, the JC-1 working solution was prepared. After the cell collection from each group, they were thoroughly mixed with 500 μ L of the JC-1 working solution, and incubated at 37°C for 20 minutes. Then, the cells were washed twice with precooled 1 \times JC-1 Assay Buffer and then tested by a C6 flow cytometry (Becton Dickinson, USA).

Statistical Analysis

After the normality test, the data were described by the mean \pm standard or the interquartile range deviation. An independent *t*-test or the Mann–Whitney test was used to compare the mean of continuous variables. A *p*-value less than 0.05 was defined as a statistically significant difference. Statistical analysis and the figures were performed by GraphPad Prism 7.0 (GraphPad Software Inc., USA).

Results

CSE Induced BEAS-2B Cells Injury

After being exposed in 5% CSE for 24 hours, the apoptosis rate of BEAS-2B cells in the CSE group increased (Figure 1a), the ROS levels and the Fe²⁺ levels of BEAS-2B cells in the CSE group increased (Figure 1b and d), while the MMP levels of BEAS-2B cells in the CSE group decreased (Figure 1c), all *p*<0.05. The mRNA and protein expression levels of GPX4 decreased (Figure 1e and f); The mRNA and protein expression levels of ACSL4 increased (both *p*<0.05) (Figure 1e and f). The original WB images were shown in Figure S1.

The Expressions of circRNAs

After 24-hour exposure to 5% CSE, differentially expressed circRNAs were explored by RNA sequence. Fifty-one upregulated circRNAs and 80 downregulated circRNAs were screened out when the differential multiple was more than 1.5 times and the *p*-value was less than 0.05. Among them, the circRNAs with the top ten differential expression multiples were shown in Tables 2 and 3.

To visualize the differentially expressed genes, the scatter plots and volcano figures of differentially expressed circRNAs were shown in Figure 2. Hierarchical clustering showed the distinguishable expression profiles of differentially expressed circRNAs. The color legend of the small squares of the heat map illustrated the corresponding relationship between color and expression, with red indicating high expression and green indicating low expression.

GO Analysis and KEGG Pathway Analysis

GO analysis was performed to determine the biological process (BP), cellular component (CC), and molecular function (MF) of the differentially expressed circRNAs. GO analysis for the upregulated circRNAs were enriched in organelle organization (Figure 3a; BP), nucleoplasm, nuclear lumen (Figure 3a; CC), protein binding, and enzyme binding (Figure 3a; MF). GO analysis for the downregulated circRNAs were enriched in the regulation of the cellular process, regulation of signal transduction, (Figure 3b; BP), cytosol, cytoplasm (Figure 3b; CC), and protein binding, GTPase regulator activity, and GTPase activator activity (Figure 3b; MF). Additionally, 7 pathways for upregulated circRNAs and 20 pathways for downregulated circRNAs were involved in CSE-induced BEAS-2B cells injury via KEGG pathway analysis. We selected all upregulated pathways and the top 10 downregulated pathways based on the values of enrichment score and showed them in Figure 4. The significantly upregulated circRNAs were associated with glycerophospholipid metabolism, Phospholipase-D signaling pathway; The significantly downregulated circRNAs were

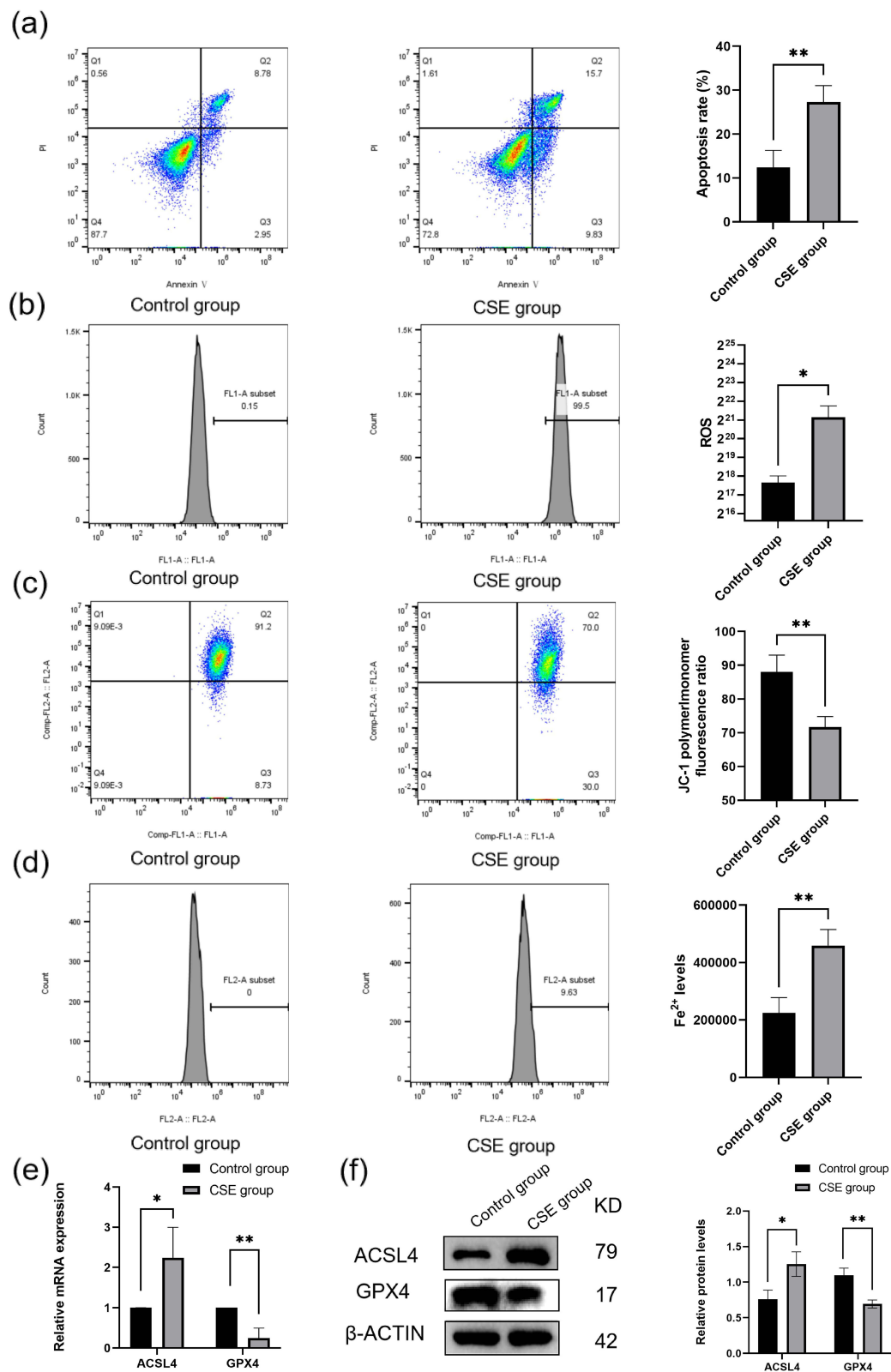


Figure 1 Cigarette smoke extract induced bronchoalveolar epithelial cell injury. (a) The apoptosis rate of BEAS-2B cells in the CSE group and control group; (b) The ROS levels of BEAS-2B cells in the CSE group and control group; (c) The MMP levels of BEAS-2B cells in the CSE group and control group; (d) The Fe²⁺ levels of BEAS-2B cells in the CSE group and control group; (e) The mRNA levels of GPX4 and ACSL4 in the CSE group and control group; (f) The protein levels of GPX4 and ACSL4 in the CSE group and control group. **p*<0.05; ***p*<0.01.

Abbreviations: BEAS-2B, human normal lung epithelial cells; CSE, Cigarette smoke extract; ROS, reactive oxygen species; MMP, mitochondrial membrane potential. ACSL4: Acyl-CoA synthetase long-chain family member 4; GPX4: glutathione peroxidase 4.

Table 2 List of Top Upregulated CircRNAs in CSE Group Compared to Control Group

circRNA_ID	Chromosome	Gene_Name	log2FC	p value
hsa_circ_0075320	chr5:179,249,957–179,260,782:+	SQSTM1	5.66	0.001
hsa_circ_0003461	chr4:184,129,105–184,130,178:+	WWC2	5.35	0.003
hsa_circ_0129428	chr5:68,396,633–68,400,543:+	SLC30A5	5.34	0.002
hsa_circ_0060122	chr20:34,243,123–34,246,904:-	CPNE1	5.25	0.003
hsa_circ_0025843	chr12:32,751,430–32,764,217:+	FGD4	4.33	0.007
hsa_circ_0001183	chr21:33,750,753–33,756,773:-	URBI	3.62	0.013
hsa_circ_0005081	chr19:38,609,945–38,610,522:+	SIPA1L3	3.62	0.009
hsa_circ_0108941	chr18:9,117,835–9,124,981:+	NDUFV2	3.58	0.010
hsa_circ_0007674	chr12:104,712,690–104,721,449:+	TXNRD1	3.55	0.013
hsa_circ_0115202	chr20:3,891,223–3,893,281:+	PANK2	3.48	0.013

Abbreviation: CSE, Cigarette smoke extract.

Table 3 List of Top Downregulated CircRNAs in CSE Group Compared to Control Group

circRNA_ID	Chromosome	Gene_Name	log2FC	p value
hsa_circ_0132059	chr6:64,389,900–64,395,812:+	PHF3	–5.33	0.004
hsa_circ_0053378	chr2:30,785,011–30,791,042:+	LCLAT1	–5.33	0.002
hsa_circ_0016866	chr1:230,798,886–230,800,333:+	COG2	–5.30	0.005
hsa_circ_0008975	chr4:148,800,382–148,803,083:+	ARHGAP10	–4.72	0.022
hsa_circ_0032826	chr14:81,329,100–81,366,373:-	CEP128	–4.59	0.021
hsa_circ_0007714	chr3:141,087,462–141,122,873:+	ZBTB38	–4.11	0.002
hsa_circ_0013171	chr1:93,159,358–93,170,301:-	EVI5	–3.90	0.003
hsa_circ_0001308	chr3:52,771,601–52,773,650:-	NEK4	–3.79	0.006
hsa_circ_0008773	chr11:85,692,787–85,695,016:-	PICALM	–3.60	0.009
hsa_circ_0129195	chr5:56,152,426–56,155,742:+	MAP3K1	–3.59	0.011

Abbreviation: CSE, Cigarette smoke extract.

associated with EGFR tyrosine kinase inhibitor resistance, Ubiquitin mediated proteolysis, Platinum drug resistance, ErbB signaling pathway.

Verification of Differentially Expressed circRNAs

To validate the results of the RNA sequence, 6 circRNAs were selected randomly and performed qRT-PCR. The results of qRT-PCR had similar trends to the results of RNA-sequence. The expression of hsa_circ_0003461, hsa_circ_0007548, hsa_circ_0025843 and hsa_circ_0068896 upregulated. The expression of hsa_circ_0005832 and hsa_circ_0053378 was downregulated, as shown in [Figure 5](#).

Knockdown of hsa_circ_0025843 Alleviated CSE Induced BEAS-2B Cells Injury

After transfected with Si-circ_0025843 for 48 hours, the transfection efficiency was verified. The Fold change of hsa_circ_0025843 mRNA was 0.18 ± 0.11 (p < 0.01) after transfection ([Figure 6e](#)).

Compared with the CSE group, hsa_circ_0025843 knockdown alleviated CSE induced BEAS-2B cells injury. After hsa_circ_0025843 knockdown, the apoptosis rate, ROS levels, and Fe²⁺ levels decreased, MMP levels increased ([Figure 6a–d](#)), the mRNA and protein levels of GPX4 increased, the mRNA and protein levels of ACSL4 decreased ([Figure 6f and g](#)), all p < 0.05. The original WB images were shown in [Figure S2](#).

Discussion

The present study used the method of next-generation sequencing to provide a comprehensive and quantitative analysis of circRNA in CSE treated BEAS-2B cells. The results revealed that the expression profiles of circRNA were

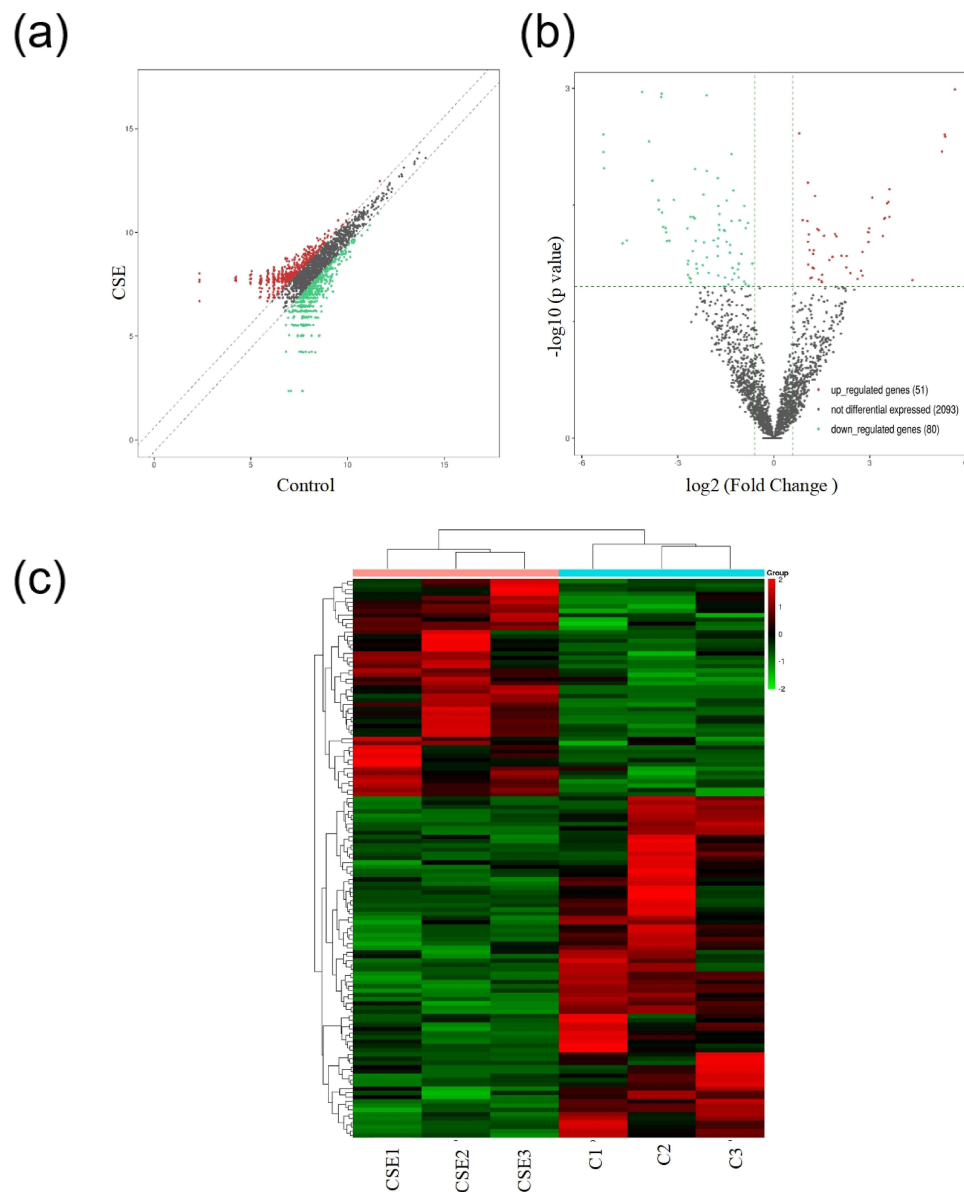


Figure 2 The differentially expressed circRNAs of the BEAS-2B cells in the CSE group and control group. (a) Scatter plot of differentially expressed circRNAs; (b) Volcano plot of differentially expressed circRNA; (c) Heat map of differentially expressed circRNAs. The color legend of the small squares of the heat map illustrated the corresponding relationship between color and expression, with red indicating high expression and green indicating low expression.

Abbreviations: circRNAs, circular RNAs; BEAS-2B, human normal lung epithelial cells; CSE, Cigarette smoke extract.

significantly different in CSE-treated BEAS-2B cells. Hsa_circ_0025843 knockdown relieved CSE-related BEAS-2B cells ferroptosis.

Cigarette smoking is one of the important risk factors for COPD and various cell types of lung cancer. Tobacco-induced airway epithelial cell injury, as an important pathogenesis of COPD and lung cancer, has received widespread attention.^{14–18} Cigarette exposure is related to the biological process of apoptosis, ferroptosis, inflammatory response, oxidative stress and et al.^{5,19} In the study, after 24 h exposure to CSE, the cell apoptosis rate increased, the mRNA and protein expression levels of GPX4 and ACSL41 changed. GPX4 and ACSL4 were the typical genes in ferroptosis. These changes suggested that 5% CSE treatment for 24 hours successfully induced the BEAS-2B cells ferroptosis. Altered levels of Fe^{2+} and mitochondrial membrane potential also support the occurrence of Ferroptosis.

Increasing evidence revealed that circRNAs play crucial roles in various diseases. Many studies have focused on the different expression profiles of circRNAs in COPD. CircRNA0001859 might act as a new diagnostic and prognostic

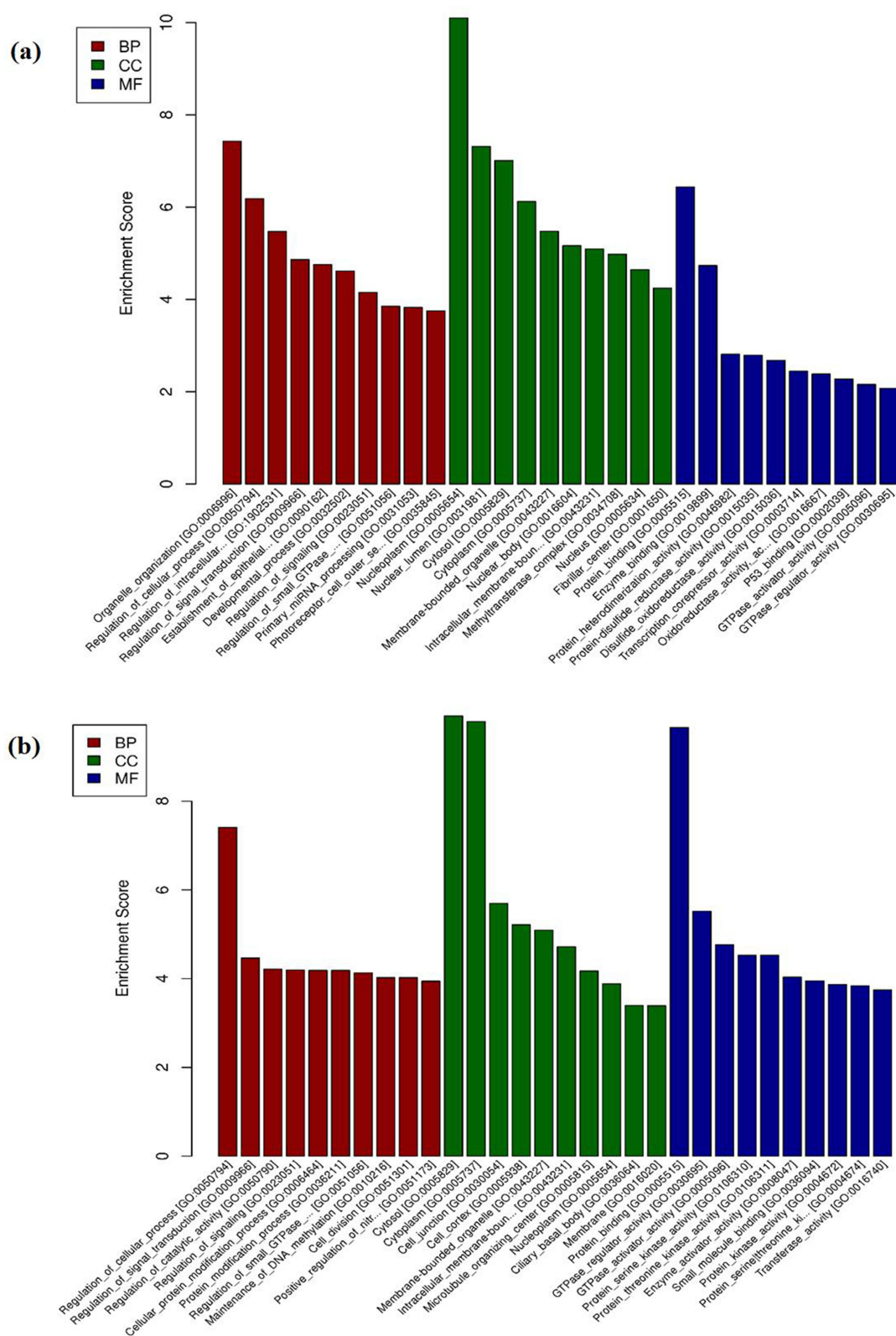


Figure 3 GO analysis of the differentially expressed circRNAs in the CSE group and control group. (a) GO analysis for the upregulated circRNAs; (b) GO analysis for the downregulated circRNAs.

Abbreviations: circRNAs, circular RNAs; CSE, Cigarette smoke extract; GO, Gene Ontology.

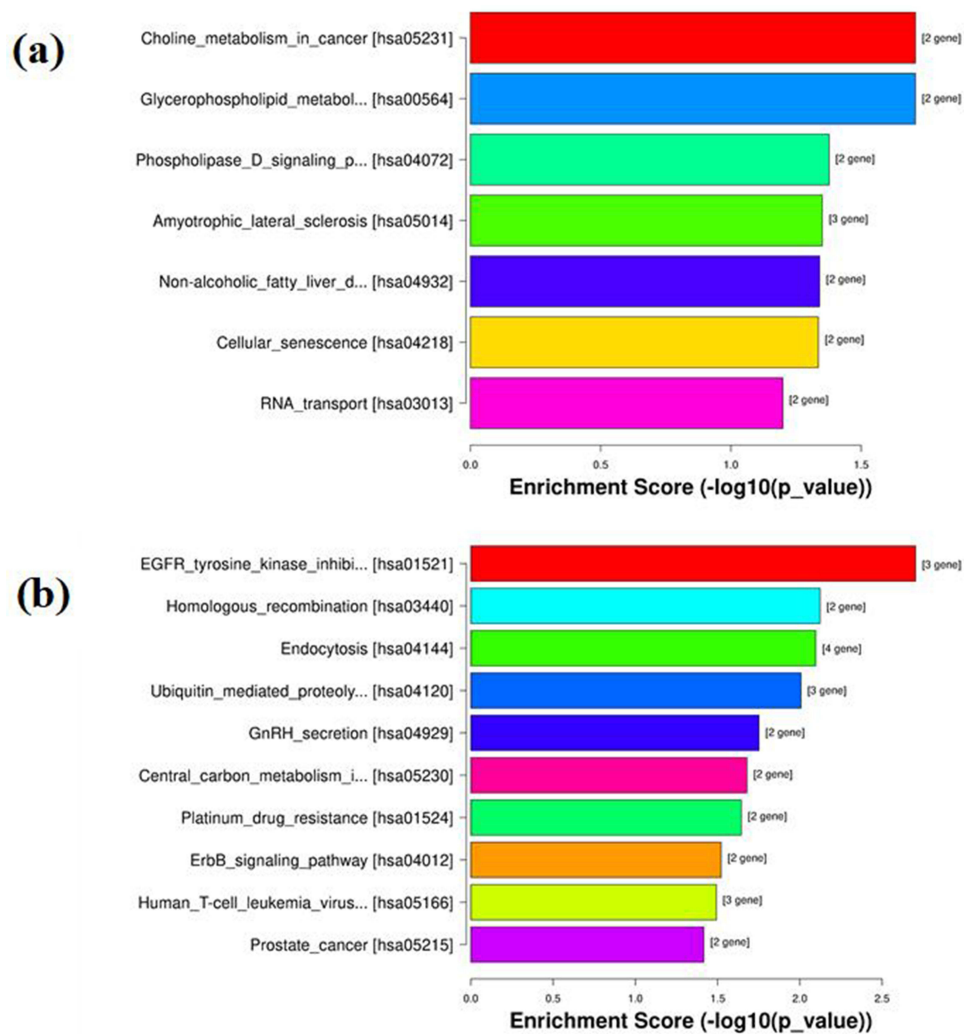


Figure 4 KEGG analysis of the differentially expressed circRNAs in the CSE group and control group. (a) 7 pathways via KEGG analysis for the upregulated circRNAs; (b) Top 10 pathways via KEGG analysis for the downregulated circRNAs.

Abbreviations: circRNAs, circular RNAs; CSE, Cigarette smoke extract; KEGG, Kyoto Encyclopedia of Genes and Genomes.

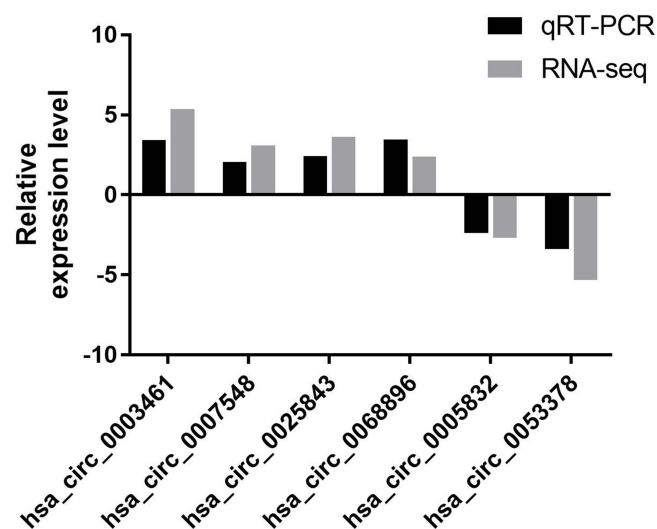


Figure 5 Relative expression levels of 6 randomly selected circRNAs by RNA sequence and qRT-PCR in the CSE group and control group. Upwards, upregulated circRNA; downwards, downregulated circRNAs.

Abbreviations: circRNAs, circular RNAs; CSE, Cigarette smoke extract.

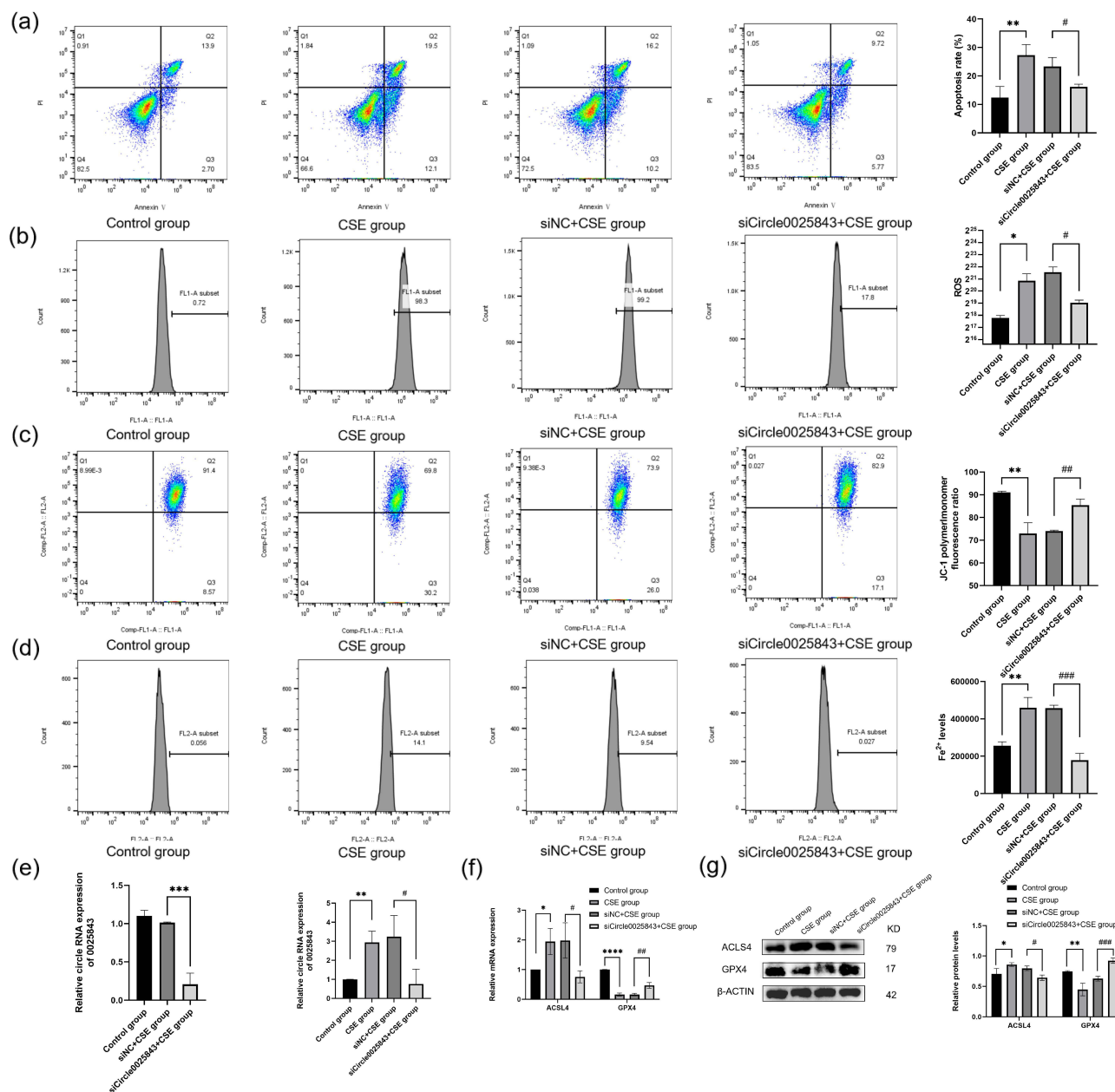


Figure 6 Hsa_circ_0025843 knockdown alleviated cigarette smoke extract induced bronchoalveolar epithelial cell injury. (a) Hsa_circ_0025843 knockdown alleviated CSE-induced apoptosis; (b) Hsa_circ_0025843 knockdown reduced the high ROS level induced by CSE treatment; (c) Hsa_circ_0025843 knockdown reversed the MMP decline induced by CSE treatment; (d) Hsa_circ_0025843 knockdown reversed the Fe²⁺ levels induced by CSE treatment; (e) Verification of Hsa_circ_0025843 knock-down efficiency; (f) Hsa_circ_0025843 knockdown reversed the mRNA levels of GPX4 and ACSL4 in CSE group; (g) Hsa_circ_0025843 knockdown reversed the protein levels of GPX4 and ACSL4 in CSE group. Compared with the control group: *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001, compared with the Sinc+CSE group, #p < 0.05, ##p < 0.01, ###p < 0.001. **Abbreviations:** CSE, Cigarette smoke extract; ROS, reactive oxygen species; MMP, mitochondrial membrane potential. ACSL4, Acyl-CoA synthetase long-chain family member 4; GPX4, glutathione peroxidase 4.

biomarker for COPD and AECOPD.²⁰ Ding Wx. found that CircTMEM30A promotes the malignant progression of COPD with primary lung cancer.²¹ However, the interests in these studies are the diagnostic biomarkers. Furthermore, circRNA regulated the CSE-related lung injury. In animal studies, Lei Zhou. Found knockdown circFOXO3 ameliorated cigarette-related lung injury.²² In this study, a high-throughput approach was used to have a comprehensive description of the circRNAs, which was possible to better understand the role of circRNAs in CSE-related lung injury.

A total of 131 dysregulated circRNAs were identified to reveal the potential roles of circRNAs on CSE induced BEAS-2B cells injury. qRT-PCR verification showed a consistent trend with the RNA sequencing in the randomly

selected 6 differentially expressed circRNAs. The results confirmed the reliability of the RNA second-generation sequencing technology. Furthermore, the results of KEGG pathway analysis showed that dysregulated circRNAs may be involved in tobacco-induced lung injury. Energy metabolism processes, such as glycerophospholipid and phospholipase D, affected the oxidative stress, which participated in the occurrence and development of COPD;^{23–25} EGFR tyrosine kinase inhibitor resistance, Platinum drug resistance, and ErbB signaling pathway are closely related to the occurrence of lung cancer and tumor drug resistance.^{26,27} Therefore, we speculate that regulation of these differentially expressed circRNAs may provide novel clues for the prevention and treatment of tobacco-related lung diseases.

Ferroptosis, as a novel mode of programmed cell death, has been confirmed to occur in CSE-associated bronchoalveolar epithelial cell injury.⁵ Ferroptosis was also involved in the development of COPD.²⁸ The role of circRNA in regulating ferroptosis has been confirmed in previous studies. CircRNA affects the occurrence and development of lung cancer and breast cancer by regulating cellular ferroptosis.^{29,30} In another study, circRNA relieved post-traumatic brain injury by regulating cellular ferroptosis.³¹ Among the differentially expressed circRNA in high-throughput sequencing screening, we selected hsa_circ_0025843 for subsequent functional experiments. The study confirmed that the knockdown of hsa_circ_0025843 alleviated the CSE-induced BEAS-2B cells ferroptosis. Hsa_circ_0025843 may be a potential therapeutic target for CSE-associated lung injury.

There are some limitations in this study. First, the sample size included in the RNA-sequencing experiments was relatively small. Second, we did not conduct an in-depth mechanism study on differentially expressed circRNAs. The treatment effects of hsa_circ_0025843 may be different between in vitro and in vivo. So, we need to further verify the role of hsa_circ_0025843 in lung injury in vivo.

In summary, the study indicated the altered circRNA expression profiles in CSE-treated BEAS-2B cells. Hsa_circ_0025843 alleviated CSE induced BEAS-2B cells ferroptosis. Hsa_circ_0025843 may serve as a potential therapeutic target for CSE-associated lung injury.

Abbreviations

BEAS-2B, human normal lung epithelial cells; CSE, cigarette smoke extract; circRNA, Circular RNA; PCR, polymerase chain reaction; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; ceRNA, competing endogenous RNA; BP, biological process; CC, cellular component; MF, molecular function; COPD, chronic obstructive pulmonary disease; ROS, reactive oxygen species; MMP, mitochondrial membrane potential; ACSL4, Acyl-CoA synthetase long-chain family member 4; GPX4, glutathione peroxidase 4.

Ethics Approval and Consent to Participate

This article does not contain any studies with human participants or animals performed by any of the authors.

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Disclosure

The authors have no relevant financial or non-financial interests to disclose for this work.

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