

Distinct roles and molecular mechanisms of nicotine and benzo(a)pyrene in ferroptosis of lung adenocarcinoma and lung squamous cell carcinoma

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ABSTRACT

INTRODUCTION The essence of ferroptosis is the accumulation of membrane lipid peroxides caused by increased iron, which disrupts the redox balance within cells and triggers cell death. Abnormal metabolism of iron significantly increases the risk of lung cancer and induces treatment resistance. However, the roles and mechanisms of smoking in ferroptosis in patients with lung cancer are still unclear.

METHODS Our study was a secondary bioinformatics analysis followed by an experimental cell culture analysis. In this study, we identified the different ferroptosis-related genes and established the signature in lung squamous cell carcinoma (LUSC) and lung adenocarcinoma (LUAD) patients with different smoking status, based on The Cancer Genome Atlas (TCGA) database. Fanyl diphosphate fanyl transferase 1 (FDFT1) in LUSC patients and solute carrier one family member 5 (SLC1A5) in LUAD patients were confirmed to be related to ferroptosis. Next, we checked the roles of two main components of smoke, nicotine, and benzo(a)pyrene (BaP), in ferroptosis of non-small-cell lung cancer (NSCLC) cells.

RESULTS We confirmed that nicotine inhibited reactive oxygen species (ROS) levels and induced glutathione peroxidase (GPX4) expression, while the opposite roles of BaP were observed in NSCLC cells. Mechanically, nicotine protected NSCLC cells from ferroptosis through upregulation of epidermal growth factor receptor (EGFR) and SLC1A5 expression. BaP-induced ferroptosis in NSCLC cells depends on FDFT1 expression.

CONCLUSIONS In this study, the ferroptosis-associated gene signature was identified in LUAD and LUSC patients with different smoking status. We confirmed nicotine-protected LUAD and LUSC cells from ferroptosis by upregulating EGFR and SLC1A5 expression. BaP-induced ferroptosis in these cells depends on FDFT1 expression.

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INTRODUCTION

Primary bronchogenic carcinoma mainly occurs in bronchial mucosal epithelial cells, with high malignancy and mortality rate¹. Non-small cell lung cancer (NSCLC) is the most common histological type of lung cancer, including squamous cell carcinoma (LUSC), adenocarcinoma (LUAD), and large cell carcinoma². Although the pathogenesis of lung cancer is still unclear, smoking, environmental pollution, and diet are all related to the occurrence of lung cancer³. Smoking

is the most important risk factor for lung cancer, especially with a direct impact on the occurrence of NSCLC³. Although previous studies have shown that ferroptosis-related genes were upregulated in chronic obstructive pulmonary disease patients who smoke compared to non-smoking healthy individuals⁴, to our knowledge, no study has shown the roles and mechanisms of smoking in ferroptosis in patients with lung cancer.

Nicotine, as the main alkaloid in cigarettes, is not carcinogenic but can promote the development of lung cancer⁵. Benzo(a)pyrene (BaP) is the main carcinogen in smoke and is closely related to the occurrence of lung cancer⁶. Both nicotine and BaP promote tumor cell proliferation, invasion, epithelial-mesenchymal transition (EMT), and angiogenesis⁵⁻⁷. Previous studies have demonstrated that benzo(a)pyrene-7,8-dihydrodiol-9,10-epoxide induces ferroptosis in neuroblastoma cells⁸. However, no study has shown the roles of nicotine in ferroptosis. Ferroptosis is an iron-dependent new type of programmed cell death, which differs from apoptosis, necrosis, and autophagy⁹. The main mechanism of ferroptosis is that divalent iron or ester oxygenase catalyzes the peroxidation of unsaturated fat acid, leading to cell death⁹. The depletion of intracellular glutathione leads to a decrease in glutathione peroxidase 4 (GPX4) activity, and lipid peroxides cannot be metabolized through the reduction reaction catalyzed by GPX4, leading to the accumulation of a large amount of lipid peroxidation and inducing ferroptosis¹⁰. Ferroptosis has great potential in cancer treatment by inhibiting tumor growth and killing tumor cells¹⁰.

In this study, we identified the expression profiles of ferroptosis-related genes in the smoking and non-smoking patients with LUSC and LUAD, based on The Cancer Genome Atlas (TCGA) database. In addition, we analyzed the influence of nicotine and BaP on ferroptosis in NSCLC cells. According to these hints, the distinct molecular mechanisms of nicotine and BaP in ferroptosis of LUSC and LUAD cells were found.

METHODS

Study design

This study was a secondary bioinformatics analysis followed by an experimental cell culture analysis.

Bioinformatics analysis

Lung squamous cell carcinoma (LUSC) and lung adenocarcinoma (LUAD) mRNA data (HTSeq FPKM) and corresponding clinical data, were downloaded from the TCGA database (<http://cancergenome.nih.gov/>). R programming *ggplot2* and *pheatmap* packages were used to show the different expression levels of ferroptosis-related genes between the patients with LUSC and LUAD. R programming *Survival* package was used to evaluate the association of ferroptosis-related genes with the prognosis of the patients.

Cell culture

Lung adenocarcinoma cells, A549, and lung squamous cell carcinoma cells, H1869, were purchased from the American Type Culture Collection (ATCC, Bethesda, MD) and grown in DMEM medium (Hyclone, Logan, UT) supplemented with 10% fetal bovine serum and antibiotics (100 U/mL penicillin and 100 µL/mL streptomycin).

shRNA transfection and nicotine/BaP treatment

A549 cells and H1869 cells were transfected with shFDF1 plasmid (sc-61610-SH, Santa Cruz Biotechnology, Shanghai, China) or shSLC1A5 plasmid (sc-60210-SH, Santa Cruz) using Lipofectamine 2000 (Invitrogen, Shanghai, China). Cells with or without shRNA transfection were treated with 0.5 µM nicotine (613223, Sigma-Aldrich, Shanghai, China) or BaP (B1760, Sigma-Aldrich) to keep a constant concentration for 48 h before experiments.

Real-time reverse transcription PCR

Total RNA was isolated from cells using an RNeasy Mini Kit (Beyotime Biotechnology, Shanghai, China). FDF1 primers were:

5'-TGTGACCTCTGAACAGGAGTGG-3' (sense) and 5'-GCCCATAGAGTTGGCACGTTCT-3' (antisense).

SLC1A5 primers were:

5'-TCCTCTTCACCCGCAAAAACCC-3' (sense) and 5'-CCACGCCATTATTCTCCTCCAC-3' (antisense).

GAPDH was used as an internal control for normalization of the results. GAPDH primers were: 5'-AATGGACAACTGGTTCGTGGAC-3' (sense) and

5'-CCCTCCAGGGGATCTGTTTG-3' (antisense).

MTT assay

Cytotoxicity assay kit was purchased from Beyotime. After cells (1×10^3 cells/well) were attached on 96-well plates for 48 h, 20 μ L of MTT solution was added to each well. Absorbance density values were checked at 570 nm to determine the cell viability after 4 h using a TECAN microplate reader (Tecan Trading AG, Switzerland).

Detection of reactive oxygen species (ROS)

Concentrations of ROS in cells were measured using the ROS detection kit (Beyotime) according to the manufacturer's instructions. Cells were washed with PBS and incubated with DCFH-DA (1:1000) at 37°C. After incubation, cells were analyzed by flow cytometry (Becton, Dickinson and Company, Shanghai, China).

Detection of malondialdehyde (MDA)

Concentrations of MDA were determined using the lipid peroxidation MDA assay kit (Beyotime) according to the manufacturer's instructions. Absorbance was measured at 532 nm using a TECAN microplate reader (Tecan Trading AG).

Glutamine uptake assay

Cells were incubated with [3H]-L-Gln (200 nmol/L) in Gln-free medium at 37°C for 20 min and then were harvested for Gln measurements using a liquid scintillation counter (PerkinElmer, Shanghai, China).

Western blot

Cellular protein (30 μ g) was separated with 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and transferred to transferred to nitrocellulose (NC) filter membranes (Beyotime). The membranes were then incubated in 5% milk for 2 h at room temperature and with the first antibody overnight at 4°C. Primary antibodies were FDFT1 (sc-271602, Santa Cruz), SLC1A5 (8057, Cell Signaling Technology, Shanghai, China), GPX4 (sc-166570, Santa Cruz) and GAPDH (sc-74512, Santa Cruz). After 24 h, the membranes were incubated with secondary antibodies for 2 h at room temperature. The signals were detected using an

enhanced chemiluminescence kit (Beyotime).

Statistical analysis

Data are expressed as means \pm SD of three independent experiments performed in triplicate. Unpaired, two-tailed Student's t-test assessed differences between groups; $p < 0.05$ was considered significant.

RESULTS

Identification of the different genes related to ferroptosis in LUAD and LUSC tissues

In order to identify the influence of smoking on ferroptosis, we compared the genes related to ferroptosis in the LUAD and LUSC patients with different smoking habits based on the TCGA database. There was a statistical difference in FDFT1 expression in the smoking and non-smoking LUSC patients and SLC1A5, ALT1, LPCAT3, DPP4, and RPL8 in the smoking and non-smoking LUAD patients (Figures 1A and 2A, $p < 0.05$). FDFT1 was expressed lower in the non-smoking LUSC patients than in the smoking LUSC patients (Supplementary file Figure 1A, $p < 0.05$). Lower SLC1A5 and RPL8 expression were observed in the non-smoking LUAD patients than in the smoking LUAD patients, while ALT1, LPCAT3, and DPP4 were opposite (Supplementary file Figure 1B, $p < 0.05$). In addition, we checked the correlation among these genes in each tissue. A positive correlation was found between FDFT1 and GPX4 in smoking LUSC patients but not in non-smoking LUSC patients (Figures 1B and 1C). SLC1A5 was positively correlated with GPX4 in the smoking LUAD patients, while no correlation was found between them in the non-smoking LUAD patients (Figures 2B and 2C).

The function and molecular mechanisms of smoking in ferroptosis of LUAD and LUSC cells

Based on the bioinformatic results, we checked the influence of two main chemical components of smoking, nicotine and benzo(a)pyrene (BaP), on ferroptosis in LUAD and LUSC cells. The proliferation of A549 and H1869 cells was inhibited after BaP treatment, compared with the parental cells (Figure 3A, $p < 0.05$). After FDFT1 knockdown, the proliferation of A549 and H1869 cells was higher than the parental cells (Figure 3A, $p < 0.05$). Compared with

Figure 1. Differentially ferroptosis-related genes in the LUSC patients with different smoking status: A) Heatmap of differentially ferroptosis-related genes in smoking and non-smoking LUSC patients; B) G1: non-smoking patients; G2: smoking patients. Circles represent the ferroptosis-related mRNA, line represents the relationship between genes in non-smoking LUSC patients; and C) Smoking LUSC patients

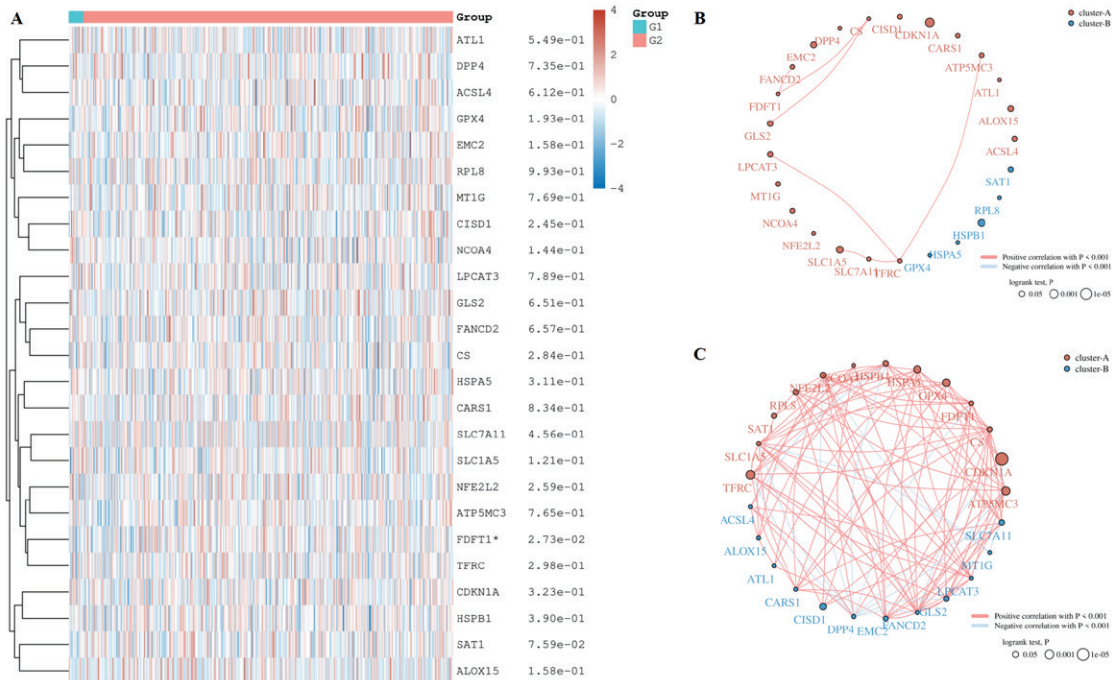


Figure 2. Differentially ferroptosis-related genes in the LUAD patients with different smoking status: A) Heatmap of differentially ferroptosis-related genes in smoking and non-smoking LUAD patients; B) G1: non-smoking patients; G2: smoking patients. Circles represent the ferroptosis-related mRNA, and lines represent the relationship between genes in non-smoking LUAD patients; and C) Smoking LUAD patients

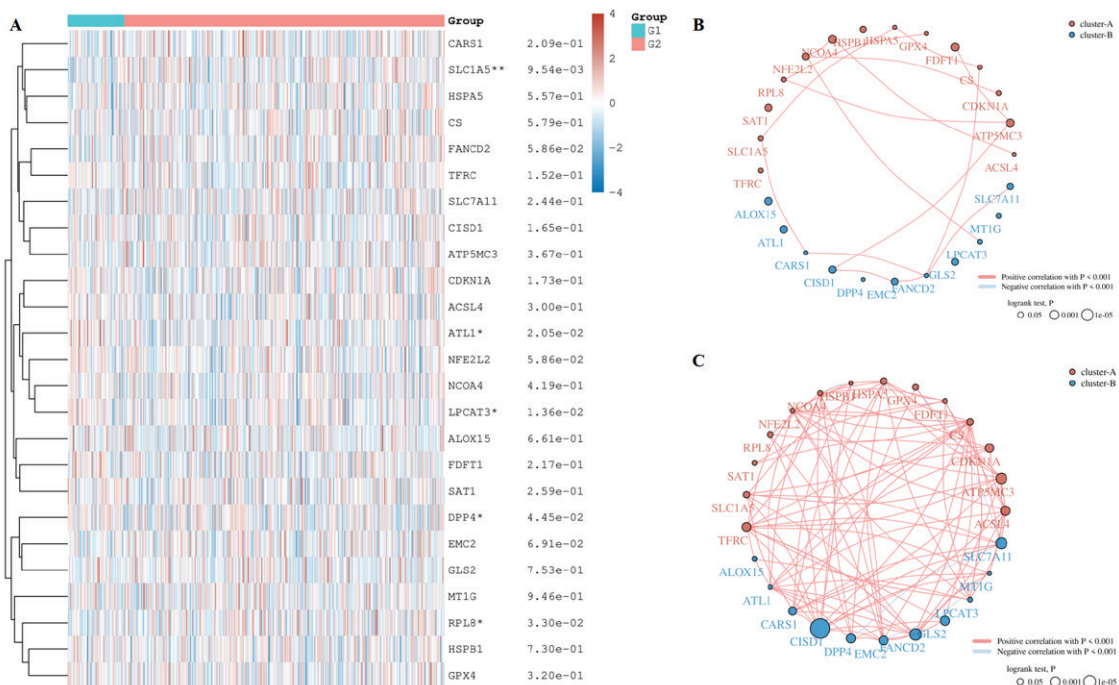


Figure 3. The roles of *FDFT1* and BaP in ferroptosis of NCSLC cells: A) Cell viability was assayed by MTT assays of A549 cells and H1869 cells after *FDFT1* knockdown and/or BaP treatment and their parental cells; B) Intracellular ROS; C) MDA formation; and D) Gln uptake activities of A549 cells and H1869 cells after *FDFT1* knockdown and/or BaP treatment and their parental cells

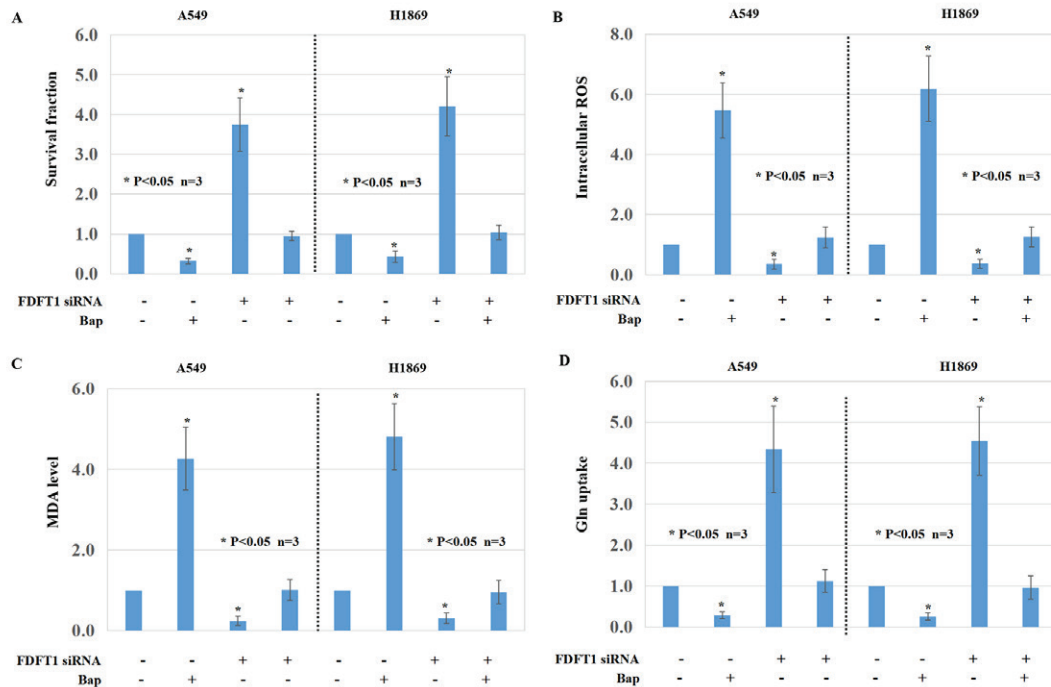
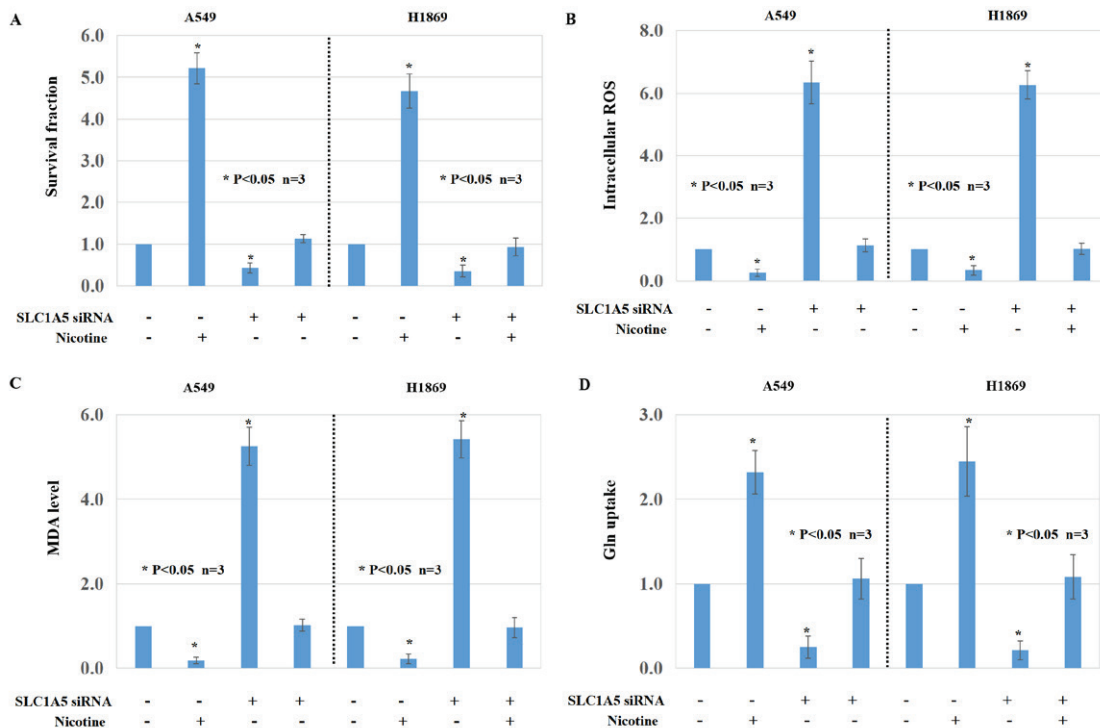


Figure 4. The roles of *SLC1A5* and nicotine in ferroptosis of NCSLC cells: A) Cell viability was assayed by MTT assays of A549 cells and H1869 cells after *SLC1A5* knockdown and/or nicotine treatment and their parental cells; B) Intracellular ROS; C) MDA formation; and D) Gln uptake activities of A549 cells and H1869 cells after *SLC1A5* knockdown and/or nicotine treatment and their parental cells



matched cells, ROS and MDA levels were upregulated in the cells with BaP treatment (Figures 3B and 3C, $p < 0.05$). Lower glutamine (Gln) consumption was observed in the cells with BaP treatment compared with the parental cells (Figure 3D, $p < 0.05$). However, BaP-induced ferroptosis in LUAD and LUSC cells was inhibited by FDFT1 knockdown (Figure 3, $p < 0.05$). Nicotine induced the proliferation of A549 and H1869 cells (Figure 4A, $p < 0.05$) and downregulated the ROS level and the MDA levels in these two cells (Figures 4B and 4C, $p < 0.05$). Nicotine upregulated Gln consumption in A549 and H1869 cells (Figure 4D, $p < 0.05$). Interestingly, SLC1A5 knockdown can counteract the effect of nicotine on ferroptosis of A549 and H1869 cells (Figure 4, $p < 0.05$).

Western blot results showed that nicotine-induced EGFR, SLC1A5, and GPX4 expression in A549 and H1869 cells (Supplementary file Figure 3A, $p < 0.05$). In SLC1A5 knockdown cells, nicotine can still induce GPX4 expression slightly (Supplementary file Figure 3B, $p < 0.05$). BaP induced FDFT1 expression and inhibited GPX4 expression in A549 and H1869 cells (Supplementary file Figure 3C, $p < 0.05$). FDFT1 knockdown can restore GPX4 protein levels in A549 and H1869 cells with BaP treatment (Supplementary file Figure 3D, $p < 0.05$).

DISCUSSION

Smoking is the most important cause of chronic obstructive pulmonary disease (COPD) and an important trigger for lung cancer, especially lung squamous cell carcinoma³. Cigarette smoke induces lipid peroxidation, ROS accumulation, and reduced mitochondrial volume in human bronchial epithelial cells⁴. Iron metabolism disorders are closely related to the occurrence and development of lung cancer¹¹. High expression of GPX4 indicates poor prognosis in lung cancer patients¹². In this study, we screened the distinct ferroptosis-related genes in LUAD and LUSC patients with different smoking habits based on the TCGA database for the first time. We confirmed high FDFT1 expression in smoking LUSC patients and high SLC1A5 expression in smoking LUSC patients. Their expressions were correlated with GPX4 expression. FDFT1 and SLC1A5 have been proven to be regulatory factors of ferroptosis in physiological and pathological conditions¹³⁻¹⁵. Consistent with previous

studies¹³⁻¹⁵, we also found that FDFT1 knockdown will inhibit ROS accumulation and MDA elevation in both LUAD and LUSC cells, while SLC1A5 knockdown was the opposite. All the evidence showed us that smoking will influence the ferroptosis in LUAD and LUSC patients by changing the expression of FDFT1 and SLC1A5.

Cigarette smoke contains over 4000 known chemicals, of which over 400 are toxic to the human body⁵. Nicotine is the highest content of alkaloids in smoke and is the main chemical substance that makes people dependent on cigarettes⁵. A previous study showed nicotine-induced blood testis barrier (BTB) damage by causing ferroptosis in the testis¹⁶. Nicotine-induced epidermal growth factor receptor (EGFR) expression promotes the progression of non-small-cell lung cancer¹⁷. Downregulation of the EGFR-SLC1A5 complex sensitizes human head and neck squamous cell carcinoma cells to ROS-induced apoptosis¹⁸. This study found nicotine-induced EGFR expression, followed by increased SLC1A5, in LUAD and LUSC cells. SLC1A5 takes up glutamine, which is metabolized into glutamate through mitochondrial glutaminase GLS2, to protect cells from ferroptosis¹⁹. BaP is a representative carcinogen of polycyclic aromatic hydrocarbons, mainly produced by high-temperature decomposition and incomplete combustion of carbon-containing substances⁵. The function of BaP for pulmonary diseases has been studied widely and comprehensively, including COPD, pulmonary fibrosis, and lung cancer²⁰. It can induce lung toxicity and inflammation and convert COPD into lung cancer via epithelial-mesenchymal transition (EMT)²¹. The roles and mechanisms of BaP in ferroptosis are relatively complex. BaP has been used to construct lung cancer animal models²². Reduced GSH and MDA levels and increased oxidative stress were observed in BaP-induced lung cancer in rat models²². Benzo(a)pyrene diol epoxide (BPDE), an active metabolite of BaP, downregulated GPX4 and SLC7A11 and induced ferroptosis in neuroblastoma cells⁸. It seems that BaP has a dual effect on lung cancer.

On the one hand, it induces tumorigenesis; on the other hand, it can induce ferroptosis. This study found BaP-induced ferroptosis in LUAD and LUSC cells through upregulation of FDFT1. After FDFT1

knockdown, BaP cannot induce GPX4 expression and ferroptosis in these cells. FDFT1 has been identified as a ferroptosis-associated gene in colorectal cancer and renal cell carcinoma^{13,23}. To our knowledge, this is the first study to show the roles of FDFT1 in the ferroptosis of lung cancer cells with BaP treatment.

Limitations

This study has certain limitations. Although we have provided evidence on the association between nicotine/BaP and ferroptosis in NSCLC cells, the exact molecular mechanisms were still unclear. In addition, the results need to be validated *in vivo*.

CONCLUSIONS

In this study, the ferroptosis-associated gene signature was identified in LUAD and LUSC patients with different smoking status. We confirmed nicotine-protected LUAD and LUSC cells from ferroptosis by upregulating EGFR and SLC1A5 expression. BaP-induced ferroptosis in these cells depends on FDFT1 expression. Interestingly, our research shows that cigarette smoke also contains chemical components that can induce tumor cell death.

REFERENCES

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA Cancer J Clin.* 2022;72(1):7-33. doi:[10.3322/caac.21708](https://doi.org/10.3322/caac.21708)
2. Molina JR, Yang P, Cassivi SD, Schild SE, Adjei AA. Non-small cell lung cancer: epidemiology, risk factors, treatment, and survivorship. *Mayo Clin Proc.* 2008;83(5):584-594. doi:[10.4065/83.5.584](https://doi.org/10.4065/83.5.584)
3. Yoshida K, Gowers KHC, Lee-Six H, et al. Tobacco smoking and somatic mutations in human bronchial epithelium. *Nature.* 2020;578(7794):266-272. doi:[10.1038/s41586-020-1961-1](https://doi.org/10.1038/s41586-020-1961-1)
4. Lian N, Zhang Q, Chen J, Chen M, Huang J, Lin Q. The role of ferroptosis in bronchoalveolar epithelial cell injury induced by cigarette smoke extract. *Front Physiol.* 2021;12:751206. doi:[10.3389/fphys.2021.751206](https://doi.org/10.3389/fphys.2021.751206)
5. Li Y, Hecht SS. Carcinogenic components of tobacco and tobacco smoke: a 2022 update. *Food Chem Toxicol.* 2022;165:113179. doi:[10.1016/j.fct.2022.113179](https://doi.org/10.1016/j.fct.2022.113179)
6. Dasgupta P, Rizwani W, Pillai S, et al. Nicotine induces cell proliferation, invasion and epithelial-mesenchymal transition in a variety of human cancer cell lines. *Int J Cancer.* 2009;124(1):36-45. doi:[10.1002/ijc.23894](https://doi.org/10.1002/ijc.23894)
7. Kasala ER, Bodduluru LN, Barua CC, Sriram CS, Gogoi R. Benzo(a)pyrene induced lung cancer: role of dietary phytochemicals in chemoprevention. *Pharmacol Rep.* 2015;67(5):996-1009. doi:[10.1016/j.pharep.2015.03.004](https://doi.org/10.1016/j.pharep.2015.03.004)
8. Lyu Y, Ren XK, Guo CC, Li ZF, Zheng JP. Benzo(a)pyrene-7,8-dihydrodiol-9,10-epoxide induces ferroptosis in neuroblastoma cells through redox imbalance. *J Toxicol Sci.* 2022;47(12):519-529. doi:[10.2131/jts.47.519](https://doi.org/10.2131/jts.47.519)
9. Dixon SJ, Lemberg KM, Lamprecht MR, et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell.* 2012;149(5):1060-1072. doi:[10.1016/j.cell.2012.03.042](https://doi.org/10.1016/j.cell.2012.03.042)
10. Wu S, Zhu C, Tang D, Dou QP, Shen J, Chen X. The role of ferroptosis in lung cancer. *Biomark Res.* 2021;9(1):82. doi:[10.1186/s40364-021-00338-0](https://doi.org/10.1186/s40364-021-00338-0)
11. Zhang Y, Guo R, Li J, Zhu L. Research progress on the occurrence and therapeutic mechanism of ferroptosis in NSCLC. *Naunyn Schmiedebergs Arch Pharmacol.* 2022;395(1):1-12. doi:[10.1007/s00210-021-02178-z](https://doi.org/10.1007/s00210-021-02178-z)
12. Liu CY, Liu CC, Li AF, et al. Glutathione peroxidase 4 expression predicts poor overall survival in patients with resected lung adenocarcinoma. *Sci Rep.* 2022;12(1):20462. doi:[10.1038/s41598-022-25019-2](https://doi.org/10.1038/s41598-022-25019-2)
13. Huang R, Zhang C, Wang X, et al. Identification of FDFT1 as a potential biomarker associated with ferroptosis in ccRCC. *Cancer Med.* 2022;11(21):3993-4004. doi:[10.1002/cam4.4716](https://doi.org/10.1002/cam4.4716)
14. Han L, Zhou J, Li L, et al. SLC1A5 enhances malignant phenotypes through modulating ferroptosis status and immune microenvironment in glioma. *Cell Death Dis.* 2022;13(12):1071. doi:[10.1038/s41419-022-05526-w](https://doi.org/10.1038/s41419-022-05526-w)
15. Yuan Z, Liu T, Huo X, Wang H, Wang J, Xue L. Glutamine transporter SLC1A5 regulates ionizing radiation-derived oxidative damage and ferroptosis. *Oxid Med Cell Longev.* 2022;2022:3403009. doi:[10.1155/2022/3403009](https://doi.org/10.1155/2022/3403009)
16. Zhang Z, Cheng J, Yang L, et al. The role of ferroptosis mediated by Bmal1/Nrf2 in nicotine -induce injury of BTB integrity. *Free Radic Biol Med.* 2023;200:26-35. doi:[10.1016/j.freeradbiomed.2023.02.024](https://doi.org/10.1016/j.freeradbiomed.2023.02.024)
17. Li H, Wang S, Takayama K, et al. Nicotine induces resistance to erlotinib via cross-talk between $\alpha 1$ nAChR and EGFR in the non-small cell lung cancer xenograft model. *Lung Cancer.* 2015;88(1):1-8. doi:[10.1016/j.jungcan.2015.01.017](https://doi.org/10.1016/j.jungcan.2015.01.017)
18. Lu H, Li X, Lu Y, Qiu S, Fan Z. ASCT2 (SLC1A5) is an EGFR-associated protein that can be co-targeted by cetuximab to sensitize cancer cells to ROS-induced apoptosis. *Cancer Lett.* 2016;381(1):23-30. doi:[10.1016/j.canlet.2016.07.020](https://doi.org/10.1016/j.canlet.2016.07.020)
19. Gao M, Monian P, Quadri N, Ramasamy R, Jiang X. Glutaminolysis and transferrin regulate ferroptosis. *Mol Cell.* 2015;59(2):298-308. doi:[10.1016/j.molcel.2015.06.011](https://doi.org/10.1016/j.molcel.2015.06.011)
20. Wang L, Chen Q, Liu T, et al. Role and mechanism of benzo(a)pyrene in the transformation of chronic obstructive pulmonary disease into lung adenocarcinoma. *J Cancer Res Clin Oncol.* 2023;149(8):4741-4760. doi:[10.1007/s00432-022-04353-y](https://doi.org/10.1007/s00432-022-04353-y)
21. Hýždálová M, Procházková J, Strapáčová S, et al. A prolonged exposure of human lung carcinoma epithelial cells to benzo(a)pyrene induces p21-dependent epithelial-

- to-mesenchymal transition (EMT)-like phenotype. *Chemosphere*. 2021;263:128126. doi:[10.1016/j.chemosphere.2020.128126](https://doi.org/10.1016/j.chemosphere.2020.128126)
22. Sikdar S, Mukherjee A, Khuda-Bukhsh AR. Ethanolic extract of *Marsdenia condurango* Ameliorates Benzo[a]pyrene-induced lung cancer of rats. *J Pharmacopuncture*. 2014;17(2):7-17. doi:[10.3831/KPI.2014.17.011](https://doi.org/10.3831/KPI.2014.17.011)
23. Shao Y, Jia H, Huang L, et al. An original ferroptosis-related gene signature effectively predicts the prognosis and clinical status for colorectal cancer patients. *Front Oncol*. 2021;11:711776. doi:[10.3389/fonc.2021.711776](https://doi.org/10.3389/fonc.2021.711776)

CONFLICTS OF INTEREST

The authors have each completed and submitted an ICMJE form for disclosure of potential conflicts of interest. The authors declare that they have no competing interests, financial or otherwise, related to the current work. All the authors report that since the initial planning of the work, this study was funded from the National Natural Scientific Foundation of China (81972784) and from the Liaoning Province Science and Technology Joint Plan (2023JH2/101700234).

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ETHICAL APPROVAL AND INFORMED CONSENT

Ethical approval and informed consent were not required for this study.

DATA AVAILABILITY

The data supporting this research are available from the authors on reasonable request.

AUTHORS' CONTRIBUTIONS

PX: conceived and planned the project. PX: conducted the experiments, supervised the work and wrote the manuscript. PX and YZZ: finished bioinformatic analysis and figure generation. MGW, HXZ and PX: did the experiments, analysis, and discussion of the results. All authors read and approved the final version of the manuscript.

PROVENANCE AND PEER REVIEW

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