Tobacco nicotine promotes TRAIL resistance in lung cancer through SNHG5

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Abstract. Tobacco nicotine use is carcinogenic and a well-known risk factor for lung cancer. However, whether tobacco nicotine can induce drug resistance in lung cancer is not clear. The objective of the present study was to identify the TNF-related apoptosis-inducing ligand (TRAIL) resistance of long noncoding RNAs (lncRNAs) that are differentially expressed in smokers and nonsmokers with lung cancer. The results suggested that the nicotine upregulated small nucleolar RNA host gene 5 (SNHG5) and markedly decreased the levels of cleaved caspase-3. The present study found that cytoplasm lncRNA SNHG5 overexpression was associated with TRAIL resistance in lung cancer and that SNHG5 can interact with X-linked inhibitor of apoptosis protein to promote TRAIL resistance. Therefore, nicotine promoted TRAIL resistance in lung cancer through SNHG5/X-linked inhibitor of apoptosis protein.

Introduction

Lung cancer is a major cause of cancer-related deaths worldwide and has limited treatment options. The numbers of patients with lung cancer has been increased by 51% in the world since 1985 (1). Recent advances in the treatment of lung cancer have increased our understanding of tumor progression and disease biology. However, its cure and survival rates remain poor, especially in advanced lung cancer. Therefore, strategies to overcome drug resistance and develop new drugs are required to increase the applicability of current treatments to a larger lung cancer population (2,3). TNF-related apoptosis-inducing ligand (TRAIL) induces apoptosis via interaction with the death receptors TRAILR1/death receptor (DR)4 and TRAILR2/DR5 in several types of cancer, but not in normal cells. Despite the significant potential for use in cancer treatment, the translation of TRAIL into clinical use has been limited because of TRAIL resistance (4).

Oncogenic drivers in lung cancer are directed against a large proportion of targeted therapies, which are more prevalent in patients with only light exposure to tobacco smoke (5). A large proportion of glioblastoma patients continue to smoke or use e-cigarettes during treatment, which may affect the efficacy of treatment (6). Nicotine is the primary component of tobacco. Tobacco nicotine can lead to drug resistance during glioblastoma chemotherapy (6). Tobacco smoke induces LKB1/AMPK pathway deficiency by reducing epithelial growth factor receptor-tyrosine kinase inhibitor sensitivity in lung cancer (7).

Some studies show that lncRNAs might be useful drug targets (8). A number of lncRNAs play pivotal roles in lung cancer drug resistance (9). The lncRNA SNHGS is also involved in cancers (10). To study whether nicotine influences TRAIL resistance, the dysregulation of lncRNA SNHGs in lung cancer tissues from smokers and nonsmokers was screened. It was discovered that lncRNA SNHG5 was upregulated by nicotine in lung cancer cells and that SNHG5 overexpression could promote TRAIL resistance in lung cancer. The recruitment of X-linked inhibitor of apoptosis protein (XIAP) by overabundant cytoplasm SNHG5 regulates cleaved caspase-3 expression to influence cell apoptosis. The results showed that SNHG5/XIAP can promote TRAIL resistance in lung cancer by altering cleaved caspase-3 expression, which is pivotal for lung cancer treatment.

Materials and methods

Patients and samples. Lung cancer tissues were collected from the Chaohu Hospital affiliated to Anhui Medical University. All patients signed informed consent for using samples. Inclusion criteria were primary patients with lung cancer receiving surgery as initial treatment and all clinicopathologic data were collected. Smoking patients had been smoking for at least 20 years and smoked 20 cigarettes a day before surgical operation. The human ethics and research ethics committees of Anhui Medical University approved the study (approval no. 202104001).

Cells culture and transfection. Lung cancer cell lines of A549, HCC827 were obtained from Cell Culture Center, Chinese Academy of Medical Sciences. The characteristics of A549

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and HCC827 cell lines were identified by STR analysis in 2020 and 2021 respectively. All experiments were performed with mycoplasma-free cells. A549 and HCC827 cell lines were cultured in RPMI 1640 (Invitrogen; Thermo Fisher Scientific, Inc.) with 10% fetal calf serum (HyClone; Cytiva), streptomycin (100 μg/ml) and penicillin (100 U/ml). SNHG5 siRNAs, XIAP siRNAs and control siRNAs were purchased from Shanghai GenePharma Co., Ltd. The sequences of siRNA are in Table SI. A549 and HCC827 cells were cultured in 6-well plates with $1x10^6$ cells in 2 ml complete medium for 24 h until they were 90% confluent. Cells were transiently transfected with 50 nM SNHG5 siRNAs, XIAP siRNAs and control siRNAs, respectively, using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) following the manufacturer's instructions. Following ~6 h of transfection at room temperature, the medium was refreshed. The subsequent experiments were performed after 48 h. Nicotine was obtained from Sigma-Aldrich (cat. no. N3876). Nicotine was added to lung cancer cells with the concentration 5 μg/ml or 10 μg/ml for an additional 24 h.

RNA isolation and reverse transcription-quantitative (RT-q) PCR. SNHG5 was identified by RT-qPCR. Cells were seeded at a density of 1 million cells per ml for each subculture and a minimum of 2 million cells were used for each RNA extraction. As following the manufacturer's protocols, total cells or tissues RNAs were extracted with TRIzol® (Thermo Fisher Scientific, Inc.). The quantity and purity of RNA were measured with a Thermo Scientific NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc.). Regarding the cDNA synthesis, 1 μg total RNA was reverse transcribed with the oligo(dT)18 primer and Reverse Transcriptase M-MLV (Takara Bio Inc.) following the manufacturer's instructions. qPCR was conducted using QuantiTect SYBR Green PCR kit (Takara Bio, Inc.) according to the manufacturer's protocol on a StepOne Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.). The reaction conditions were as follows for Cq values: 95˚C for 30 sec, followed with 40 cycles of 95˚C for 5 sec, 60˚C for 34 sec; and for melting curves: 95˚C for 15 sec, 60˚C for 1 min and 95˚C for 15 sec. All primers were designed using Primer Premier 5.0 (Premier, Inc.). The primers were obtained from Sangon Biotech Co., Ltd. (Table SII). RNA expression was normalized to GAPDH or U6. RNA relative expression levels were calculated by using the $2^{-\Delta\Delta Cq}$ method (Bio-Rad CFX manager software 3.1; Bio-Rad Laboratories, Inc.) (11). The whole experiment was repeated with two different sets of biological samples.

SNHG5 expression vector construction and transfection. According to the instructions of the manufacturer, total RNA in the cells was extracted by using Rneasy Mini kit (Invitrogen; Thermo Fisher Scientific, Inc.). The first-strand complementary DNA (cDNA) of SNHG5 was obtained by using Maxima TM First Strand cDNA Synthesis kit (Fermentas; Thermo Fisher Scientific, Inc.) and DNA was amplified using the primers (Table SII). RT-qPCR products and pcDNA3.1 vector were digested. The full-length sequence of SNHG5 was inserted into pcDNA3.1 vector to obtain pcDNA3.1-SNHG5. pcDNA3.1-SNHG5 and pcDNA3.1 empty vector were transfected in A549 and HCC827 cells as the overexpression group and control group, respectively, using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) following the manufacturer's instructions. Cells were plated at a density of 500,000 cells/10-cm plate 2 days prior to transfection. A total of 1 μg SNHG5-overexpression plasmid and vector were transfected into the 6 well-plate cells, respectively. Following ~6 h of transfection, the medium was refreshed. After 48 h, the cells were used for the subsequent experiments.

Cellular fractionation. Norgen's cytoplasmic and nuclear RNA purification kits (Norgen Biotek Corp.) were used to extract nuclear and cytoplasmic RNA.

Apoptosis detection. The cells were washed three times using phosphate-buffered saline (PBS). The cells were stained using the Annexin V/fluorescein isothiocyanate (FITC). The single cell suspension was first incubated with 500 μl of 1X buffer, 5 μl of Annexin V and 5 μl of Propidium Iodide (PI; Beyotime Institute of Biotechnology) at 37˚C for 35 min in the dark. The cells were detected by a FACSAria III flow cytometer (BD Biosciences) and analyzed by Win MDI. 2.9 software (TSRI Flow Cytometry Core Facility). The rate of apoptosis was defined as the total percentage of early and late apoptotic cells.

Western blotting. Using RIPA lysis buffer (RIPA; Beyotime Institute of Biotechnology) total protein was extracted from the cells. Using bicinchoninic acid (BCA) method (Pierce; Thermo Fisher Scientific, Inc.) the protein was quantified, and each sample (2 μg) was loaded on 10% gels for SDS-PAGE. The protein was transferred on a polyvinylidene difluoride membrane (Millipore Sigma). Then the membranes were blocked with 5% skimmed milk for ~2 h at room temperature and the membrane was soaked in primary antibodies against pro Caspase-3 (1:1,000; cat. no. ab32150; Abcam), cleaved Caspase-3 (1:500; cat. no. ab32042; Abcam) and GAPDH (1:1,000; cat. no. bs-0755R; BIOSS) at 4˚C for 12 h and secondary antibodies (goat anti-rabbit IgG H&L; 1:1,000; cat. no. bs-0295G; BIOSS) for 2 h at room temperature. Bands were visualized by enhanced chemiluminescence (Amersham ECL; cat. no. RPN3243; Cytiva) and analyzed by ImageJ Software v1.53 (National Institutes of Health).

RNA pull-down assay. SNHG5 and SNHG5-antisense were transcribed using the templates of pcDNA3.1-SNHG5 and pcDNA3.1-SNHG5-antisense plasmids. The plasmids were amplified. The primers are shown in the Table SI. Using Biotin-RNA Labeling Mix (Roche Diagnostics) biotin-labeled RNAs were transcribed in vitro. Then biotin-labeled RNAs were treated with RNase-free DNase I (Takara Bio, Inc.) and purified with an RNeasy Mini kit (Roche Diagnostics). RNA structure buffer [10 mM Tris (pH 7), 0.1 M KCl, and 10 mM $MgCl_2$] included biotinylated RNA was in 98˚C for ~2 min, on ice for 5 min, and then kept at room temperature for ~35 min. Total protein lysates of A549 cells were mixed with biotinylated RNA and incubated at room temperature. Streptavidin agarose beads (5 μl; Cytiva; Thermo Fisher Scientific, Inc.) were added to each binding reaction and further incubated at room temperature

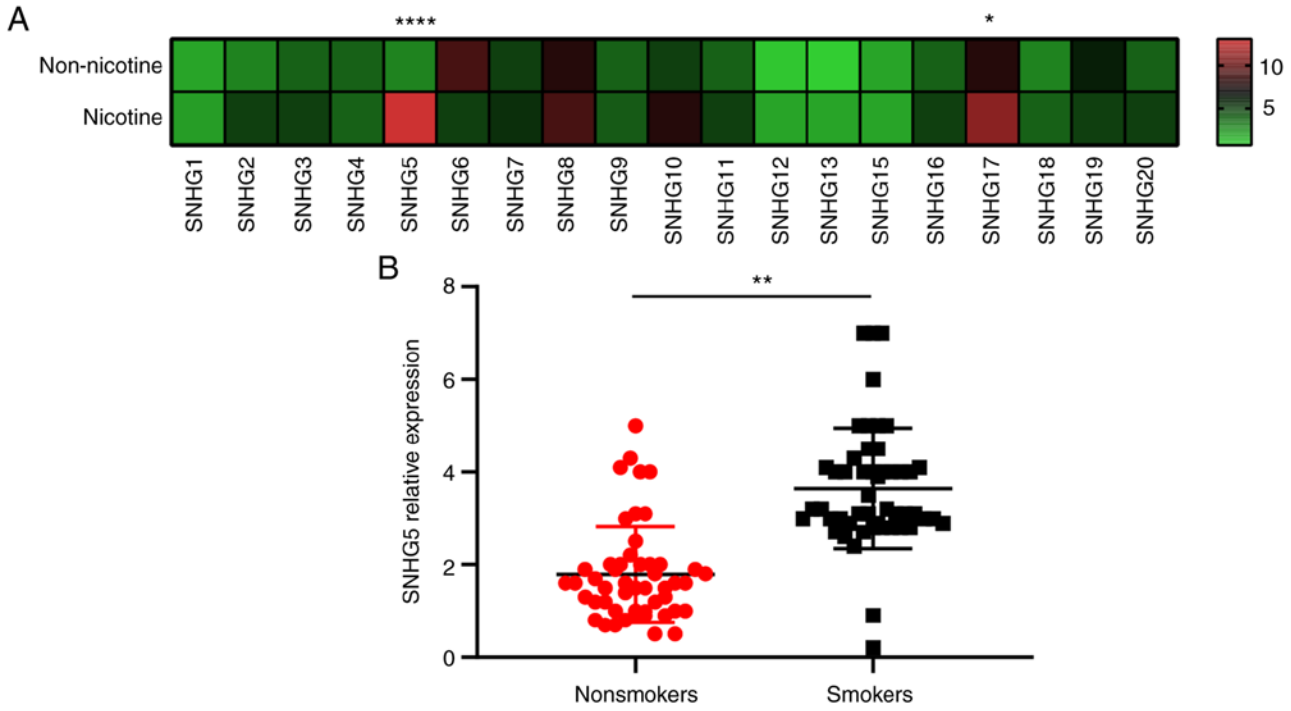

Figure 1. SNHG5 expression is increased in lung cancer tissues from smokers. (A) Reverse transcription-quantitative PCR analysis of SNHG5 expression in A549 cells with and without nicotine treatment. (B) Comparison of SNHG5 expression between tissues from nonsmokers (n=50) and smokers (n=50). SNHG5, small nucleolar RNA host gene 5. *P<0.05; **P<0.01; ****P<0.001.

with rotation. The RNA-protein-beads compound were then centrifuged at 1,000 x g for 1 min at room temperature and washed three times with Wash buffer I and dissolved into RNase-free water. After washes, the pull-down complexes were eluted by denaturation in 1X protein loading buffer for 10 min at 100˚C. The samples were then assessed by western blotting or proceeded to mass spectrometry analysis. The captures were given electrophoresis with 12% sodium dodecyl sulfate polyacrylamide gel and silver stained. Mass spectrometry (LC-MS/MS, A TripleTOF; AB Sciex Pte. Ltd.) identified the protein bands after in-gel trypsin digestion.

RNA binding protein immunoprecipitation (RIP) assay. Magna RIP RNA-binding protein immunoprecipitation kit (Millipore Sigma) was used to perform RIP according to the manufacturer's instructions in A549 cells induced by nicotine for 36 h. Antibody against XIAP obtained from Cell Signaling Technology, Inc. The coprecipitated RNAs were adsorbed with magnetic beads and detected by qPCR.

UV-RIP. A549 cells induced by nicotine for 36 h applied 100 mM 4-thiouridine (4-SU) were cultured for ~12 h. Then cells were cross-linked by 365 nm UV light at a dose of 400 mJ/cm^2 in UV-RIP assay. The other UV-RIP protocols were the same as RIP.

Statistical analysis. The statistical analysis was performed with SPSS software (version 15.0; SPSS, Inc.). The results were analyzed by t-test or ANOVA followed by Bonferroni's test. Categorical variables were analyzed by χ^2 test. Data are presented as the mean ± SEM. P<0.05 was considered to indicate a statistically significant difference.

Results

SNHG5 expression is increased in lung cancer tissues from smokers. RT-qPCR was performed to identify the panel of SNHGs in A549 cells. One group of cells was induced by nicotine and the other group of cells was not induced. Among all the SNHGs tested, nicotine induced increased expression of SNHG5 and SNHG17 in A549 cells, indicating that they might be important in lung cancer (Fig. 1A). RT-qPCR was performed on lung cancer samples from smokers (n=50) and nonsmokers (n=50). A comparison between tissues from nonsmokers and smokers showed a marked enhancement of SNHG5 expression and no increase in SNHG17 expression (Fig. 1B). There were no significant correlations of SNHG5 expression with age, sex, or the lung cancer tissue differentiation level of patients. However, SNHG5 expression level was correlated with tumor node metastasis stage (P=0.039), distant metastasis (P=0.040), lymph node metastasis (P=0.002), and smoking (P=0.0004; Table SIII). These results suggested that SNHG5 might play an important role in smokers with lung cancer.

SNHG5 is associated with TRAIL resistance in lung cancer. To further determine whether nicotine alters the SNHG5 expression in vitro, the present study measured the SNHG5 expression following nicotine (10 μg/ml) induction in A549 cells at different times. The cells were in good condition after nicotine was applied. The results showed that SNHG5 expression increased with nicotine induction in a time-dependent manner (Fig. 2A). The A549 cells were exposed to different doses of nicotine (5 and 10 μg/ml) for 48 h. The SNHG5 expression increased in a dose-dependent manner (Fig. 2B).

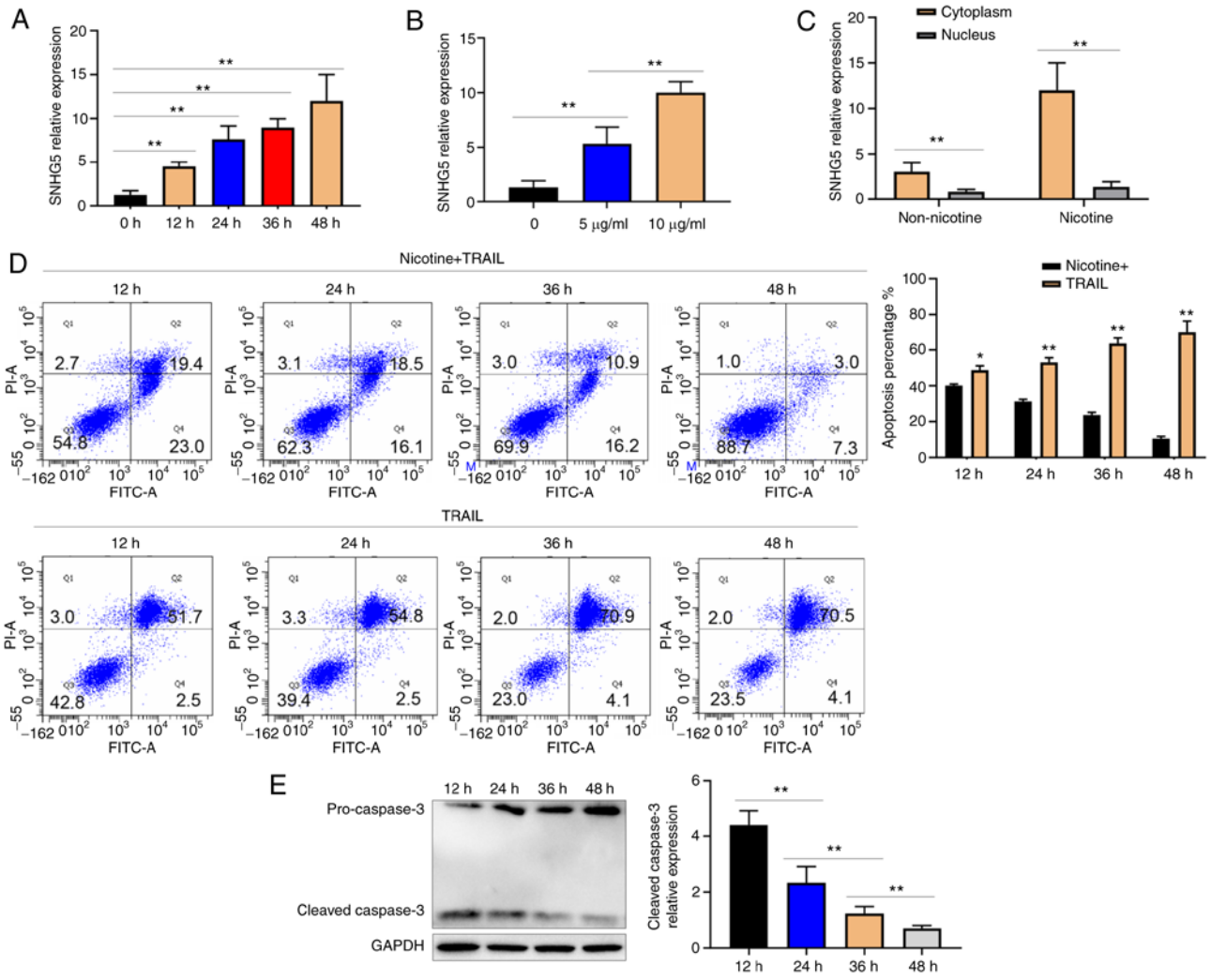

Figure 2. SNHG5 is associated with TRAIL resistance in lung cancer. (A) RT-qPCR analysis of SNHG5 expression in A549 cells treated with nicotine (10 μg/ml) at different times. (B) RT-qPCR analysis of SNHG5 expression in A549 cells treated with nicotine (5 and 10 μg/ml) for 48 h. (C) The SNHG5 levels in the nuclear and cytoplasmic compartments of A549 cells were determined using RT-qPCR. GAPDH was used as the cytoplasmic control, and U6 snoRNA was used as the nuclear control. (D) The apoptosis rate of A549 cells with and without nicotine treatment for different times by flow cytometry. Samples were assayed three times. (E) Western blot analysis of cleaved caspase-3 in A549 cells treated with nicotine at different times. Samples were assayed three times. SNHG5, small nucleolar RNA host gene 5; TRAIL, TNF-related apoptosis-inducing ligand; RT-qPCR, reverse transcription-quantitative PCR; snoRNA, small nucleolar RNAs. *P<0.05; **P<0.01.

Next, the SNHG5 expression in the cytoplasmic and nuclear fractions of RNA of A549 cells exposed to nicotine (10 μg/ml) for 48 h was quantified. It was found that the SNHG5 upregulation was greater among cytoplasmic RNA than nuclear RNA (Fig. 2C). Nicotine (10 μg/ml) and TRAIL (1.6 μg/ml) were then added to A549 cells, and flow cytometry was performed. Flow cytometry showed that the apoptosis rate gradually decreased with increasing SNHG5 expression. The addition of TRAIL (1.6 μg/ml) alone to A549 cells did not decrease the apoptosis rate (Fig. 2D). It was also found that the expression of cleaved caspase-3 decreased with increasing SNHG5 expression, as detected by western blotting (Fig. 2E). The results showed that nicotine promoted the expression of SNHG5 and SNHG5-induced TRAIL resistance in lung cancer.

Knockdown of SNHG5 expression decreases the TRAIL resistance in A549 and HCC827 cells. A549 and HCC827 cells were exposed to nicotine (10 μg/ml) and TRAIL (1.6 μg/ml). As shown in Fig. 3A and B, SNHG5 expression was significantly knocked down in A549 and HCC827 cells by RT-qPCR of siRNA. The siRNA sequences are presented in Table SI. SNHG5 knockdown resulted in a markedly increased apoptosis rate in A549 and HCC827 cells as measured by flow cytometry (Fig. 3C and D). Moreover, the expression of cleaved caspase-3 was significantly increased with decreasing SNHG5 expression (Fig. 3E and F). Collectively, these data revealed a functional role of SNHG5 in TRAIL resistance.

SNHG5 overexpression increases TRAIL resistance in A549 and HCC827 cells. SNHG5 was overexpressed in A549 (Fig. 4A) and HCC827 (Fig. 4B) cells as revealed by RT-qPCR. The cells were exposed to nicotine (10 μg/ml) and TRAIL (1.6 μg/ml) for 48 h. SNHG5 overexpression resulted in a significantly decreased apoptosis rate in A549 and HCC827 cells by flow cytometry (Fig. 4C and D). The expression of cleaved caspase-3 decreased with increasing SNHG5 expression by western blotting (Fig. 4E and F). Therefore, the results showed that nicotine might promote SNHG5 overexpression,

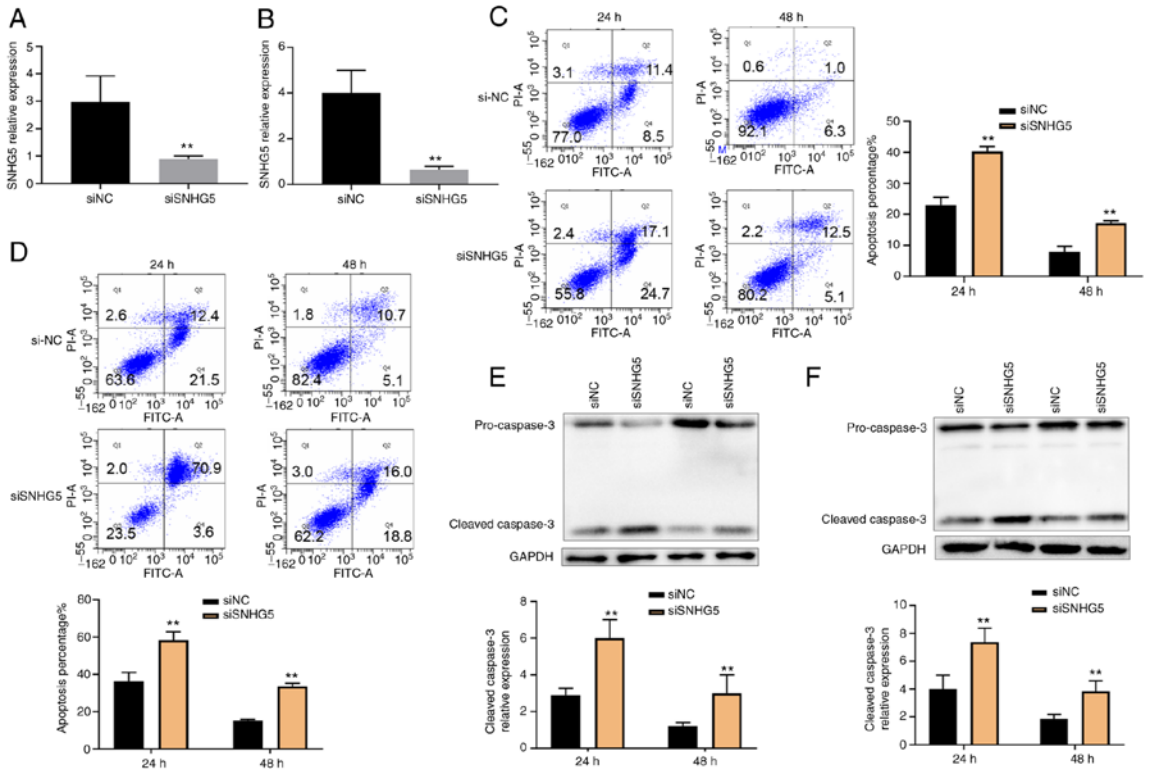

Figure 3. Knockdown of SNHG5 expression decreases TRAIL resistance in A549 and HCC827 cells. Knockdown of SNHG5 expression in (A) A549 and (B) HCC827 cells. Knockdown (C) A549 and (D) HCC827 cells were treated with nicotine for 48 h. Flow cytometry of apoptosis was performed. Samples were assayed three times. Knockdown (E) A549 and (F) HCC827 cells were treated with nicotine for 48 h. Western blotting analysis of cleaved caspase-3 was performed. Samples were assayed three times. SNHG5, small nucleolar RNA host gene 5; TRAIL, TNF-related apoptosis-inducing ligand. **P<0.01.

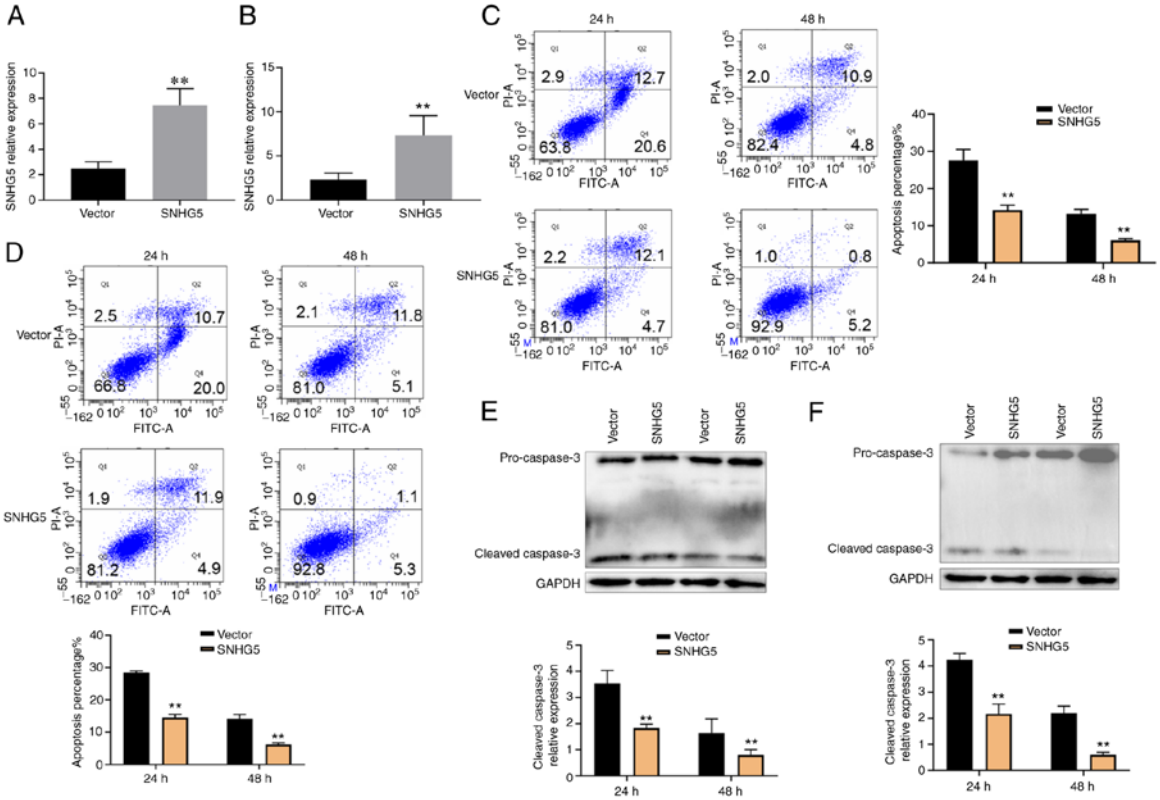

Figure 4. SNHG5 overexpression increased TRAIL resistance in A549 and HCC827 cells. SNHG5 overexpression in (A) A549 and (B) HCC827 cells. (C) A549 and (D) HCC827 cells that overexpressed SNHG5 were treated with nicotine for 48 h. Flow cytometry analysis of apoptosis was performed. Samples were assayed three times. (E) A549 and (F) HCC827 cells that overexpressed SNHG5 were treated with nicotine for 48 h. Western blotting analysis of cleaved caspase-3 was performed. Samples were assayed three times. SNHG5, small nucleolar RNA host gene 5; TRAIL, TNF-related apoptosis-inducing ligand. **P<0.01 vs. siNC.

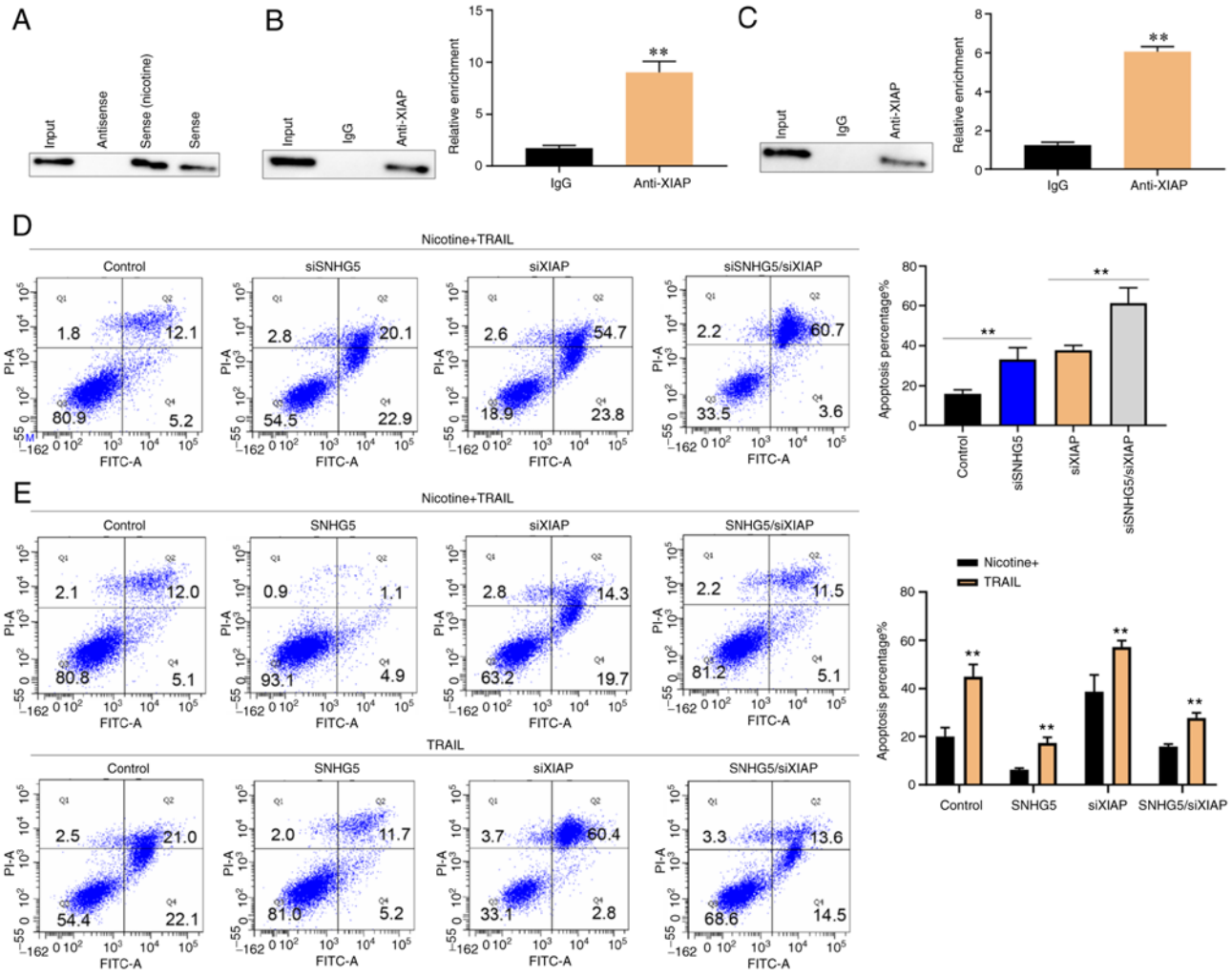

Figure 5. Cytoplasm SNHG5 directly interacted with XIAP to regulate TRAIL resistance. (A) The binding of SNHG5 with XIAP in total protein extracted from A549 cells was analyzed by immunoblot analysis. The cells were treated with nicotine. Samples were assayed three times. (B) XIAP UV-RIP or (C) native RIP was performed in A549 cells with nicotine induction. Then qPCR analysis was followed to analyzed copurified RNA in A549 cells treated with nicotine for 36 h. (D) Nicotine and TRAIL (5 and 10 μg/ml) were added to A549 cells for 48 h. XIAP knockdown enhanced the SNHG5 knockdown-mediated trail resistance reduction of A549 cells. (E) Knockdown of XIAP reversed the SNHG5 overexpression-mediated trail resistance in A549 cells with or without nicotine treatment. SNHG5, small nucleolar RNA host gene 5; XIAP, X-linked inhibitor of apoptosis protein; RIP, protein immunoprecipitation. Data are presented as mean ± SEM (n=3). Two-tailed Student's t-test: **P<0.01.

as well as suggested an important role of SNHG5 in TRAIL resistance.

Cytoplasm SNHG5 directly interacts with XIAP to regulate TRAIL resistance. The mechanistic insights into the mechanism and interactions of SNHG5 were explored. As SNHG5 was simultaneously located in the nucleus and cytoplasm, the possibility that SNHG5 functions by physically interacting with proteins was explored. A biotinylated SNHG5 RNA pull-down assay and subsequent mass spectrometry analysis of the differentially displayed bands revealed that XIAP was the main protein bound to SNHG5. A number of studies show that XIAP is involved in the apoptosis pathway (12,13). The relationship between SNHG5 and XIAP was studied using an immunoblot assay (Fig. 5A). RNA immunoprecipitation (UV-RIP) assays with an XIAP-specific antibody confirmed a direct interaction between SNHG5 and XIAP (Fig. 5B). Then, native RIP was performed to confirm the binding between SHNG5 and XIAP (Fig. 5C). The interactive effects of SNHG5 and XIAP on the TRAIL resistance were evaluated using XIAP knockdown in A549 cells. The cells were exposed to nicotine (10 μg/ml) and TRAIL (1.6 μg/ml) for 48 h. As shown in Fig. 5D, XIAP knockdown promoted A549 cell apoptosis. In addition, apoptosis of SNHG5-knockdown A549 cells was enhanced by XIAP knockdown. The TRAIL resistance in SNHG5-overexpression A549 cells was rescued by XIAP siRNA with or without nicotine treatment (Fig. 5E).

Discussion

In humans and other mammals, most small nucleolar RNAs (snoRNAs) are located in the introns of protein-coding and non-coding genes. Some previous studies assume that the host snoRNA genes have no function and only carried the snoRNA-encoding sequences in their introns. However, a number of SNHGs have been found to play important roles in cancers (13-15). The present study detected the SNHG expression in A549 cells exposed to nicotine and found that

SNHG5 expression was significantly increased by nicotine treatment. The alterations suggested that SNHG5 overexpression might be associated with nicotine. The present study also found that SNHG5 expression was significantly higher in lung cancer tissues from smokers compared with those from nonsmokers. The results suggested that SNHG5 expression was related to important cell behaviors in lung cancer. The present study also found that SNHG5 overexpression due to nicotine treatment was associated with TRAIL resistance in lung cancer. Tobacco nicotine plays a role in cancer drug resistance and can inhibit cancer cell apoptosis induced by chemotherapeutic drugs via increasing XIAP expression (16). Nicotine can suppress apoptosis by inducing multi-site Bad phosphorylation (17); it can also activate Akt in a dose- and time-dependent manner. Akt inhibitors can lead to a mild reduction in chemotherapy-induced apoptosis, and the effects from nicotine are blocked in A549 cells (18).

In the present study, SNHG5 directly interacted with XIAP, which promoted TRAIL resistance by inhibiting the cleaved caspase-3 expression in A549 and HCC827 cells. XIAP can bind caspase-3 to suppress apoptosis (19-21). The activity of processed caspases can be inhibited by XIAP, which increases the threshold of apoptosis activation. Proapoptotic factors, which are released from mitochondria, can inhibit XIAP. The proapoptotic factors SMAC/DIABLO can bind and prevent XIAP activity (22). High levels of XIAP can explain inducible melanoma TRAIL resistance. SMAC is released from the mitochondria and inhibits apoptosis by XIAP (23). XIAP inhibitors promote TRAIL-induced apoptosis and counter tumors in pancreatic cancer (24,25). The present study showed that SNHG5 promoted TRAIL resistance, which explains the association with XIAP in lung cancer. The results have significant implications regarding our understanding of the roles of SNHG5.

SNHG5 was evidently increased in lung cancer cells treated with nicotine. Tobacco nicotine induced SNHG5 expression and promoted TRAIL resistance through SNHG5/XIAP. How SNHG5 and XIAP to combine needs further detection. The present study provided a new strategy to allow the use of TRAIL as a cancer treatment. A small clinic sample size is the limitation of the present study. Animal experiments are also required.

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Availability of data and materials

All data generated and/or analyzed in the present study are included in this published article.

Authors' contributions

XX, JX and XS collected and analyzed the data and drafted the manuscript. XX and XS designed the project. CW, JD and XS revised the manuscript. XX, JX, XS, CW and JD confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The research protocol was reviewed and approved by the Ethics Committee of Anhui Medical University (approval no. 202104001).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Akhtar N and Bansal JG: Risk factors of lung cancer in nonsmoker. Curr Probl Cancer 41: 328-339, 2017.
2. Herbst RS, Morgensztern D and Boshoff C: The biology and management of non-small cell lung cancer. Nature 553: 446-454, 2018.
3. Hirsch FR, Scagliotti GV, Mulshine JL, Kwon R, Curran WJ Jr, Wu YL and Paz-Ares L: Lung cancer: Current therapies and new targeted treatments. Lancet 389: 299-311, 2017.
4. Deng D and Shah K: TRAIL of hope meeting resistance in cancer. Trends Cancer 6: 989-1001, 2020.
5. Middleton G, Fletcher P, Popat S, Savage J, Summers Y, Greystoke A, Gilligan D, Cave J, O'Rourke N, Brewster A, *et al*: The National lung matrix trial of personalized therapy in lung cancer. Nature 583: 807-812, 2020.
6. McConnell DD, Carr SB and Litofsky NS: Potential effects of nicotine on glioblastoma and chemoradiotherapy: A review. Expert Rev Neurother 19: 545-555, 2019.
7. Cheng FJ, Chen CH, Tsai WC, Wang BW, Yu MC, Hsia TC, Wei YL, Hsiao YC, Hu DW, Ho CY, *et al*: Cigarette smoke-induced LKB1/AMPK pathway deficiency reduces EGFR TKI sensitivity in NSCLC. Oncogene 40: 1162-1175, 2021.
8. Liang WC, Ren JL, Wong CW, Chan SO, Waye MM, Fu WM and Zhang JF: LncRNA-NEF antagonized epithelial to mesenchymal transition and cancer metastasis via cis-regulating FOXA2 and inactivating Wnt/β-catenin signaling. Oncogene 37: 1445-1456, 2018.
9. Liu MY, Li XQ, Gao TH, Cui Y, Ma N, Zhou Y and Zhang GJ: Elevated HOTAIR expression associated with cisplatin resistance in non-small cell lung cancer patients. J Thorac Dis 8: 3314-3322, 2016.
10. Yang Z, Li Q, Zheng X and Xie L: Long Noncoding RNA small nucleolar host gene: A potential therapeutic target in urological cancers. Front Oncol 11: 638721, 2021.
11. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25: 402-408, 2001.
12. Tu H and Costa M: XIAP's profile in human cancer. Biomolecules 10: 1493, 2020.
13. Zimta AA, Tigu AB, Braicu C, Stefan C, Ionescu C and Berindan-Neagoe I: An emerging class of long Non-coding RNA with oncogenic role arises from the snoRNA host genes. Front Oncol 10: 389, 2020.
14. Qin Y, Sun W, Wang Z, Dong W, He L, Zhang T and Zhang H: Long Non-Coding small nucleolar RNA host genes (SNHGs) in endocrine-related cancers. Onco Targets Ther 13: 7699-7717, 2020.
15. Yang T and Shen J: Small nucleolar RNAs and SNHGs in the intestinal mucosal barrier: Emerging insights and current roles. J Adv Res: Jun 11, 2022 (Epub ahead of print).
16. Dasgupta P, Kinkade R, Joshi B, Decook C, Haura E and Chellappan S: Nicotine inhibits apoptosis induced by chemotherapeutic drugs by up-regulating XIAP and survivin. Proc Natl Acad Sci USA 103: 6332-6337, 2006.

17. Jin Z, Gao F, Flagg T and Deng X: Nicotine induces multi-site phosphorylation of Bad in association with suppression of apoptosis. J Biol Chem 279: 23837-23844, 2004.
18. Zhang J, Kamdar O, Le W, Rosen GD and Upadhyay D: Nicotine induces resistance to chemotherapy by modulating mitochondrial signaling in lung cancer. Am J Respir Cell Mol Biol 40: 135-146, 2009.
19. Riedl SJ, Renatus M, Schwarzenbacher R, Zhou Q, Sun C, Fesik SW, Liddington RC and Salvesen GS: Structural basis for the inhibition of caspase-3 by XIAP. Cell 104: 791-800, 2001.
20. Von Karstedt S, Montinaro A and Walczak H: Exploring the TRAILs less travelled: TRAIL in cancer biology and therapy. Nat Rev Cancer 17: 352-366, 2017.
21. Jost PJ, Grabow S, Gray D, McKenzie MD, Nachbur U, Huang DC, Bouillet P, Thomas HE, Borner C, Silke J, *et al*: XIAP discriminates between type I and type II FAS-induced apoptosis. Nature 460: 1035-1039, 2009.
22. Jost PJ and Vucic D: Regulation of cell death and immunity by XIAP. Cold Spring Harb Perspect Biol 12: a036426, 2020.
23. Eberle J: Countering TRAIL resistance in melanoma. Cancers (Basel) 11: 656, 2019.
24. Vogler M, Walczak H, Stadel D, Haas TL, Genze F, Jovanovic M, Bhanot U, Hasel C, Möller P, Gschwend JE, *et al*: Small molecule XIAP inhibitors enhance TRAIL-induced apoptosis and antitumor activity in preclinical models of pancreatic carcinoma. Cancer Res 69: 2425-2434, 2009.
25. Stadel D, Mohr A, Ref C, MacFarlane M, Zhou S, Humphreys R, Bachem M, Cohen G, Möller P, Zwacka RM, *et al*: TRAIL-induced apoptosis is preferentially mediated via TRAIL receptor 1 in pancreatic carcinoma cells and profoundly enhanced by XIAP inhibitors. Clin Cancer Res 16: 5734-5749, 2010.