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Effects of circRNA_103993 on the proliferation and apoptosis of NSCLC cells through miR-1271/ERG signaling pathway

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Abstract. – OBJECTIVE: Non-small cell lung cancer (NSCLC) accounts for more than 80% of lung cancer. CircRNA is a new type of non-coding RNA. CircRNA was found to be deeply involved in the regulation of NSCLC cells. However, the principle of CircRNA regulating NSCLC cells needs to be further explored.

MATERIALS AND METHODS: The relative mRNA expression levels of CircRNA_103993, miR-1271 and ERG were detected by quantificational real-time polymerase chain reaction (qRT-PCR) in NSCLC cells and human bronchial epithelial cell line HBE. Cell proliferation was detected by the Cell Counting Kit (CCK8) when NSCLC cells were transfected with si-CircRNA_103993, si-NC, miR-1271 mimics, miR-NC, LV-ERG, respectively. The apoptotic rate of NSCLC cells was measured by apoptotic assay and flow Cytometry. The relative mRNA and protein expression levels of PCNA and caspase-3 were detected by Western blot and qRT-PCR.

RESULTS: CircRNA_103993 and ERG were significantly up-regulated while miR-1271 was significantly down-regulated in NSCLC cells. Knockdown of CircRNA_103993 and high expression of miR-1271 significantly inhibited NSCLC cell proliferation and promoted apoptosis. The double luciferin report showed that CircRNA_103993 served as a sponge of miR-1271 and miR-1271 could directly target ERG in NSCLC cells. More importantly, low expression of CircRNA_103993 increased the expression level of miR-1271, and high expression of miR-1271 decreased the expression level of ERG. Further experiments showed that miR-1271 inhibitors reversed the effect of si-CircRNA_103993 on proliferation and apoptosis of NSCLC cells. However, miR-1271 mimics significantly promoted the effects of si-CircRNA_103993 on the proliferation and apoptosis. Moreover, LV-ERG reversed the effect of miR-1271 mimics on proliferation and apoptosis of NSCLC cells. However, si-ERG significantly promoted the effects of miR-1271 mimics on the proliferation and apoptosis.

CONCLUSIONS: CircRNA_103993 was highly expressed in NSCLC cells. CircRNA_103993 regulated proliferation and apoptosis of NSCLC cells by acting as a sponge of miR-1271. The CircRNA_103993 /miR-1271/ ERG axis had an important effect on the proliferation and apoptosis of NSCLC cells. Therefore, CircRNA_103993 may be a target for treating lung cancer.

Key Words:
CircRNA_103993, Proliferation, Apoptosis, NSCLC, MiR-1271.

Introduction

Lung cancer is one of the most common and highest incidence cancers in the world which seriously affects people's physical and mental health[1]. About 85% of lung cancers are non-small cell lung cancer (NSCLC)[2]. Cancer cells in most patients' body transferred at the time of consultation because of the lack of biomarkers for early diagnosis, treatment targets and prognostic markers[3-5]. So, lung cancer remains the leading cause of cancer-related deaths.

Circular RNA (CircRNA) is a special endogenous non-coding RNA[6]. With the rapid development of high-throughput sequencing, growing number of evidence indicated that the abnormal expression of circRNA was related to the occurrence and development of cancer. CircRNA was involved in many biological processes such as tumor cell proliferation, invasion and differentiation[7-9]. CircFBLIMl[3] was found to serve as an endogenous competitive RNA which could regulate the expression of FBLIMl in liver cancer by sponging miR-346. Cao et al[10] also pointed out that circRNA100876 was up-regulated in ESCC tissues and was associated with poor tumor prognosis. The circRNA

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may increase tumor metastasis by promoting ESCC cell migration, invasion and epithelial-mesenchymal transition. Ouyang et al[6] found that circPDSSl promoted GC cell cycle and inhibited apoptosis through sponge action with miR-186-5p. While miR-186-5p inhibited cell cycle and promoted apoptosis by targeting NEK2. Li et al[7] found that the expression of circ-IARS was up-regulated in pancreatic cancer tissues and plasma exosomes. Circ-IARS entered human microvascular endothelial cells through exosomes to promote tumor invasion and metastasis. The expression level of circMYLK in prostate cancer (PCa) tissues and cells was significantly higher than that in normal tissues and prostate cells. CircMYLK could promote the proliferation, invasion and migration of PCa cells by down-regulating the expression of miR-29a[9]. However, the research on the regulation of CircRNA in NSCLC has just begun and its specific mechanism remained to be studied.

The purpose of this study was to investigate the effect of CircRNA_103993/miR-1271/ ERG axis on proliferation and apoptosis of NSCLC cells. The regulatory mechanism of CircRNA_103993 on proliferation and apoptosis will provide more theoretical possibilities for the treatment of lung cancer.

Materials and Methods

Cell Culture

We purchased human NSCLC cell lines including H292, A549, H1299 and H23 from American Type Culture Collection (ATCC; Manassas, VA, USA). The 16HBE (normal bronchial epithelial cells) was preserved in our laboratory. All cell lines were maintained in Roswell Park Memorial Institute-1640 (RPMI-1640) media (HyClone, South-Logan, UT, USA) and supplemented with 10% fetal bovine serum (FBS; HyClone, GE Healthcare Life Science, South-Logan, UT, USA). Moreover, we maintained all cells in a humidified incubator with 5% CO_2 at 37°C.

RT-qPCR Assays and Cell Transfection

We used TRIzol reagent (Invitrogen, Carlsbad, CA, USA) to extract the total RNA from NSCLC cells and tissues according to the manufacturer's instructions. RiboBio reverse transcription kit (Applied Biosystems, Foster City, CA, USA) was used to reverse total RNAs for miRNA. The expression levels of CircRNA_103993 mRNA and miRNA were measured by SYBR Green PCR Kit (TaKaRa, Otsu, Shiga, Japan). Genery (Guangzhou, China) was used to design and synthesize all primer sequences. The levels of mRNA and CircRNA_103993 expression were normalized by the GAPDH and U6. The $2^{-\Delta\Delta Ct}$ methods were used to measure the results. All primers are described as follows: CircRNA_103993, forward, 5'-GGAAACTGTGAGCCAGCCAA-3'; reverse, 5'-TACTGTAGCCGCAGGTTCTGA-3'; miR-1271, forward, 5'-CTAGACGTCCAGATTGAATAGAC-3'; reverse, 5'-GTCCGAGCTTGGTCAGAATG-3'; ERG, forward, 5'- GTGGGTTATGACGCTGTCAG -3'; reverse, 5'-CTAACTGCGCTCTCTGCTC-3'; U6, forward, 5'-CGCTTCGGCAGCACATATAC-3' and reverse, 5'-AAATATGGAACGCTTCACGA-3'; GAPDH, forward, 5'-ATGTCGTGGAGTCTACTGGC-3' and reverse, 5'-TGACCTTGCCCACAGCCTTG-3'.

CCK-8 Assay

We firstly seeded H1299 and H23 cells in 96-wells plate with 4000 cells per well. 10 **µl** of CCK-8 solution was added to the incubator overnight and the temperature was set at 37°C. CCK-8 (CCK-8, CK04, Dojindo Molecular Technologies, Shanghai, China) was used to measure cell proliferation of H1299 and H23 cells under different transfection condition. The absorbance of cells was analyzed using a microplate reader (Synergy4, BioTek, Winooski, VT, USA) for incubation of H1299 and H23 cells at every 24 hours.

Cell Apoptosis Assay

The apoptosis of H1299 and H23 cells was analyzed by PE Annexin V apoptosis detection kits (BD Pharmingen, Franklin Lakes, NJ, USA). CellQuest analysis software (Becton, Dickinson, Franklin Lakes, NJ, USA) was used to analyze the experiment data. All experiments were performed in triplicate.

Luciferase Reporter Assays

The wild type (WT) or mutant (MUT) circRNA_103993 was cloned into the pmirGLO plasmid receptor (Realgene, Nanjing, China). Then, miR-1271 mimics or miR-NC were introduced into H1299 cells, respectively. The Dual-Luciferase receptors system (Promega, Madison, WI, USA) was used to detect the dual lucif-

erin activity after co-culture for 48 hours. Finally, ERG-WT and ERG-MUT were introduced into H1299 cells. The dual luciferase receptors system was used to measure Dual-Luciferase activity after co-culture for 48 hours.

Western Blot

The total protein was extracted, and the solubility of the protein was measured by the BCA kit (Pierce, Rockford, IL, USA) after 24 h transfection of H1299 and H23 cells. Under 110V electrophoresis and 250 mA electroporation to polyvinylidene fluoride (PVDF) membrane, 40 μg of the protein was added to each well of the sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel (Millipore, Billerica, MA, USA). Then we washed the membrane with tris buffered saline-tween (TBST) for 30 minutes, incubated the secondary antibody at 37°C for 1 h and washed the membrane with TBST for 3×30 min. β-actin (Abcam, Cambridge, UK) was used as an internal reference. Quantity one software was used to analyze the gray value of protein bands.

Statistical Analysis

All post-processing data of experimental was expressed as mean ± standard deviation. SPSS 21.0 Statistical software (SPSS Inc., Chicago, IL, USA) was used to statistically analyze the data. $p<0.05$ was considered statistically significant.

Results

CircRNA_103993 Was Overexpressed in NSCLC Cells and Knockdown of CircRNA_103993 Significantly Inhibited Proliferation of NSCLC Cell

The expression levels of circRNA_103993 in NSCLC cells (H292, A549, H1299 and H23) were measured by qRT-PCR. The results revealed that the expression levels of circRNA_103993 in NSCLC cells (H292, A549, H1299 and H23) were highly expressed in comparison with human bronchial epithelial cell line (HBE) (Figure 1A). The expression level of circRNA_103993 was highest in H23 cells, followed by H1299 cells. Compared to the si-NC group, the expression levels of CircRNA_103993 in both H1299 and H23 cells were much lower when they were transfected with si-CircRNA_103993 (Figure 1B). When H1299 and H23 cells were transfected with si-NC and si-CircRNA_103993 and cultured for three days, the results showed that the proliferation ability of H1299 and H23 cells after transfection with si-CircRNA_103993 was significantly lower than that in the transfected si-NC group (Figure 1C and 1D). After transfection with si-CircRNA_103993, the mRNA and protein expression levels of PCNA in H1299 and H23 cells were significantly lower than those in transfected si-NC group (Figure 1E and 1F).

Low Expression of CircRNA_103993 Facilitated Apoptosis of NSCLC Cells

In order to investigate the effect of CircRNA_103993 on the apoptosis of NSCLC cells, H1299 and H23 cells were transfected with si-NC and si-CircRNA_103993. The results showed that cell apoptosis in H1299 and H23 cells after transfection with si-CircRNA_103993 was obviously higher than si-NC group (Figure 2A-2E). RT-qPCR and Western blot assays were used to detect the relative expression levels of caspase-3 mRNA and caspase-3 protein. The data showed that caspase-3 mRNA and caspase-3 protein were all highly expressed after transfection with si-CircRNA_103993 when compared to si-NC group (Figure 2F-2H).

CircRNA_103993 Served as a Sponge of miR-1271 in NSCLC cells

To further explore the influence of CircRNA_103993 on the progress of NSCLC cells, Starbase and TargetScan (bioinformatics analysis software) were used to predict the potential targeted miRNAs. As a result, the binding sites between CircRNA_103993 and miR-1271 were shown in Figure 3A. When CircRNA_103993-WT group was transfected with miR-541 mimics and mi-NC in H23 cells, the dual-fluorescein activity of miR-541 mimics was much lower than mi-NC group (Figure 3B). The relative expression of miR-1271 after transfection with miR-1271 mimics was significantly higher than miR-NC group. However, the relative expression of miR-1271 after transfection with miR-1271 mimics was much lower than miR-NC group (Figure 3C). We detected the relative expression of miR-1271 in NSCLC cells (H292, A549, H1299 and H23); the results showed that the expression levels of miR-1271 in NSCLC cells (H292, A549, H1299 and H23) were lowly expressed when compared to human bronchial epithelial (HBE) cell line

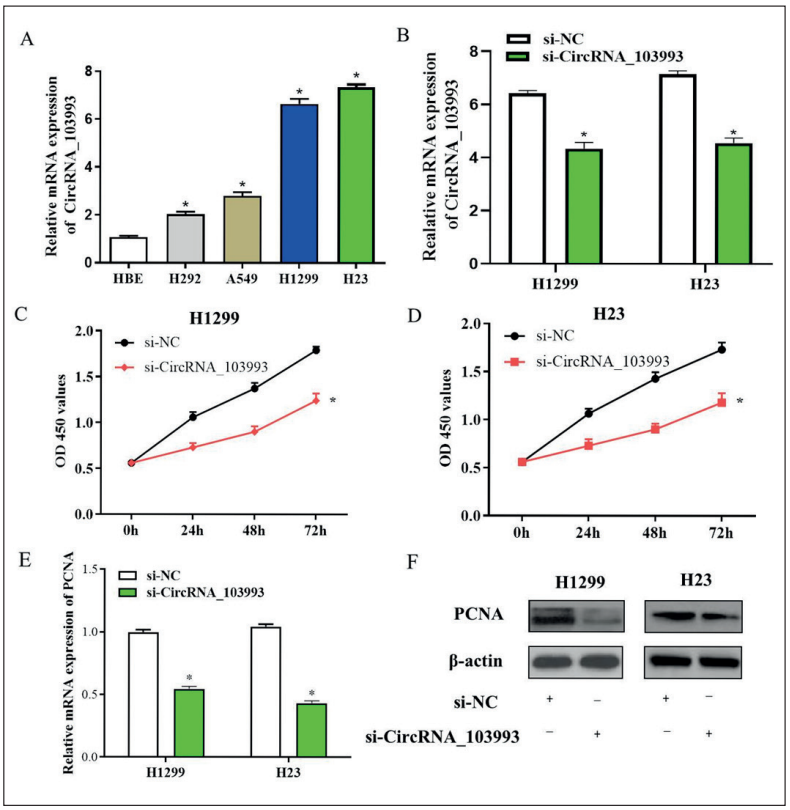

Figure 1. CircRNA_103993 was significantly overexpressed in NSCLC cells and knockdown of CircRNA_103993 significantly inhibited proliferation of NSCLC cell. **A,** QRT-PCR was used to measure the relative mRNA expression levels of CircRNA_103993 in NSCLC cells and human bronchial epithelial cell lines (HBE). **B,** the relative mRNA expression levels of CircRNA_103993 after transfection with si-CircRNA_103993 in H1299 and H23 cells**. C-D,** CCK-8 assay was used to detect cell proliferation of H1299 and H23 cells after transfection with si-NC and si-CircRNA_103993, respectively. **E,** The relative mRNA expression of PCNA was detected by qRT-PCR in H1299 and H23 cells after transfection with si-NC and si-CircRNA_103993. **F,** Western blot was used to measure protein level of PCNA in H1299 and H23 cells after transfection with si-NC and si-CircRNA_103993.

(Figure 3D). The expression level of miR-1271 was lowest in H23 cells, followed by H1299 cells. Besides, the relative expression of miR-1271 in H1299 and H23 cells transfected with si-CircRNA_103993 was significantly lower than si-NC group (Figure 3E).

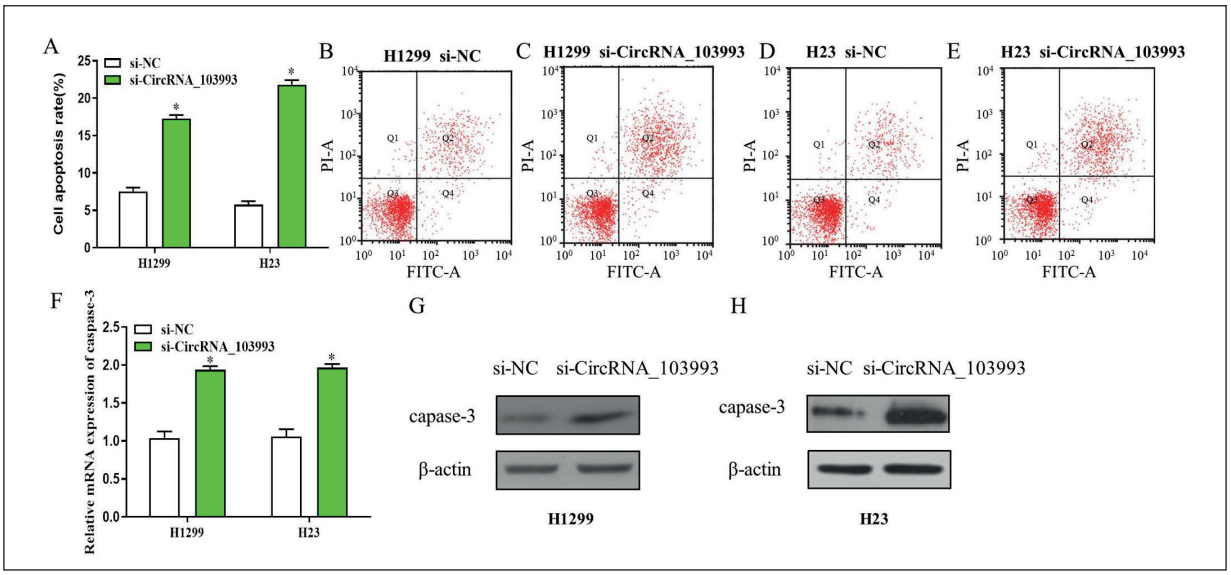

Figure 2. Low expression of CircRNA_103993 facilitated apoptosis of NSCLC cells. **A-E,** Flow cytometry was used to measure the cell apoptosis rate in H1299 and H23 cells after transfection with si-NC and si-CircRNA_103993. **F-H,** RT-qPCR and Western blot assays were used to detect the relative expression levels of caspase-3 mRNA and caspase-3 protein in H1299 and H23 cells after transfection with si-NC and si-CircRNA_103993.

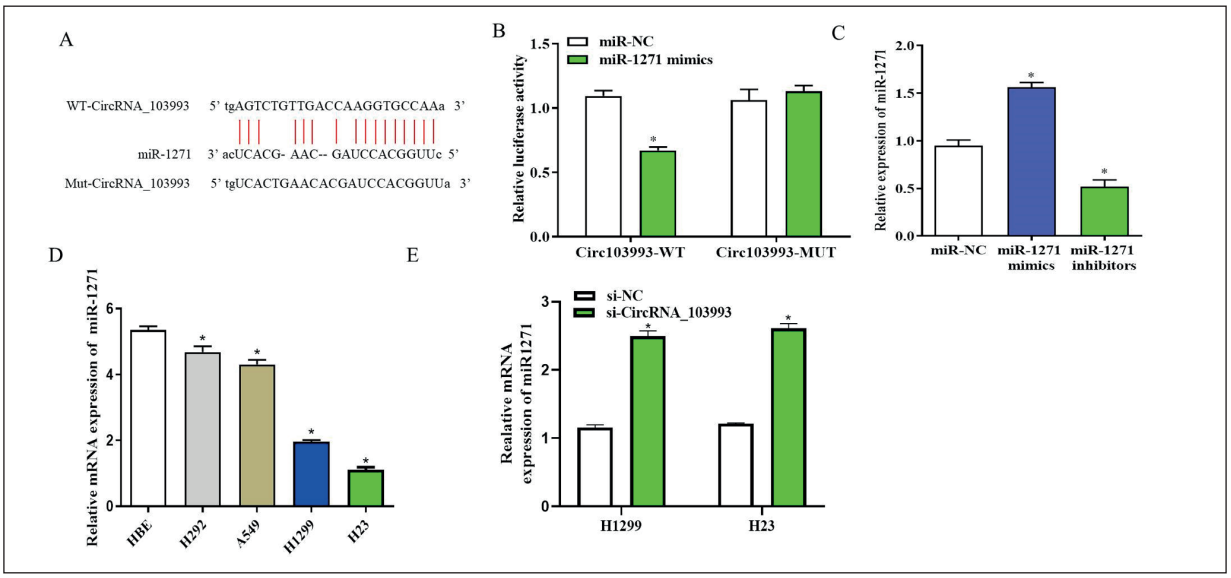

Figure 3. CircRNA_103993 served as a sponge of miR-1271 in NSCLC cells. **A,** The predicted miR-1271 binging sites in CircRNA_103993 mRNA 3'-UTR. **B,** Luciferase assays were used to measure double luciferase activity of CircRNA_103993 WT and CircRNA_103993 MUT after transfection with miR-NC and miR-1271 mimics, respectively. **C,** QRT-PCR was used to detect the relative expression levels of miR-1271 after transfection with miR-NC and miR-1271 mimics and miR-1271 inhibitors. **D,** QRT-PCR was used to detect the relative mRNA expression levels of miR-1271 in NSCLC cells and human bronchial epithelial cell lines (HBE). **E,** The relative expression levels of miR-1271 after transfection with si-NC and si-CircRNA_103993 was detected by qRT-PCR.

Overexpression of MiR-1271 Suppressed Proliferation While Promoted Apoptosis of NSCLC Cells

To study the effect of miR-1271 on the proliferation and apoptosis of NSCLC cells, H1299 and H23 cells were transfected with miR-NC and miR-1271 mimic, respectively, and cultured for three days. Apoptosis levels were detected by flow cytometry and apoptosis assay. The results showed that the apoptosis rate of H1299 and H23 cells after miR-1271 mimic transfection was significantly higher than that of miR-NC group (Figure 4A-4E). Moreover, the expression of caspase-3 in H1299 and H23 cells was significantly higher than that in miR-NC group after transfection with miR-1271 mimic (Figure 4F and 4G). When H1299 and H23 were transfected with miR-1271 mimics, the cell proliferation ability was significantly lower than that in the transfected miR-NC group (Figure 4H and 4I). Besides, the mRNA and protein expression levels of PCNA were significantly lower than those in the transfected miR-NC group (Figure 4J and 4K).

MiR-1271 Directly Targeted ERG in NSCLC Cells

In order to figure out the effect of miR-1271 on the development of NSCLC cells, the binding sites of miR-1271 and ERG were shown in Figure 5A. The Dual-Luciferase report showed that the Dual-Luciferase activity of the ERG WT group after transfection with miR-1271 mimics was significantly lower than that of miR-NC (Figure 5B). Besides, the expression levels of ERG in NSCLC cells (H292, A549, H1299 and H23) were highly expressed when compared to human bronchial epithelial cell line (HBE) (Figure 5C). The expression level of ERG was highest in H23 cells, followed by H1299 cells. The relative expression of miR-1271 in H1299 and H23 cells transfected with miR-1271 mimics was significantly lower than miR-NC group (Figure 5D). Moreover, the relative expression of ERG after transfection with LV-ERG was significantly higher than LV-NC group in H1299 and H23 cells (Figure 5E).

CircRNA_103993/MiR-1271/ERG Axis Affected Proliferation and Apoptosis of NSCLC Cells

To further verify the effect of CircRNA_103993/miR-1271/ERG axis on NSCLC cell proliferation and apoptosis, H1299 and H23 cells were transfected with si-NC, si-CircRNA_103993, si-CircRNA_103993 and miR-1271 inhibitors, si-CircRNA_103993 and miR-1271 mimics, respectively. The results showed

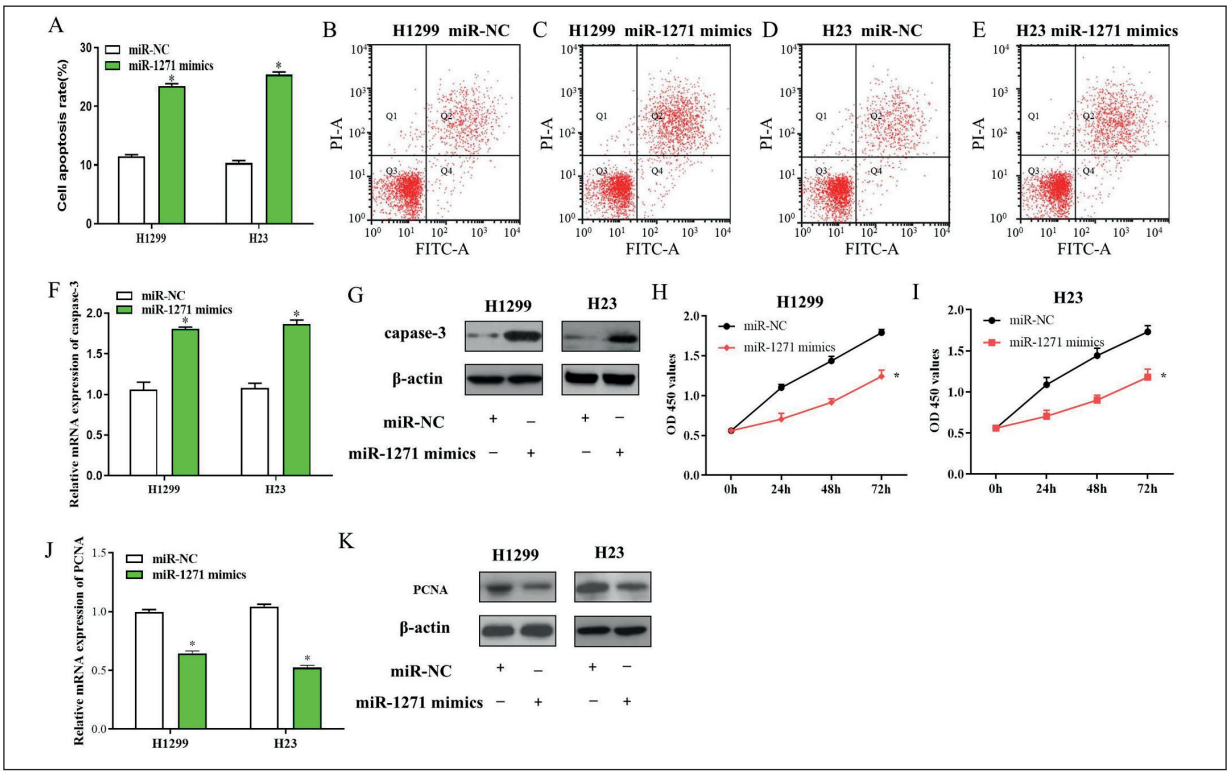

Figure 4. Overexpression of miR-1271 suppressed proliferation while promoted apoptosis of NSCLC cells. **A-E,** Flow cytometry was used to measure the cell apoptosis rate in H1299 and H23 cells after transfection with miR-NC and miR-1271 mimics. **F-G,** RT-qPCR and Western blot assays were used to detect the relative expression levels of caspase-3 mRNA and caspase-3 protein in H1299 and H23 cells after transfection with miR-NC and miR-1271 mimics. **H-I,** CCK-8 assay was used to detect cell proliferation of H1299 and H23 cells after transfection with miR-NC and miR-1271 mimics, respectively. **J-K,** QRT-PCR and Western blot were used to detect the relative mRNA and protein expression of PCNA in H1299 and H23 cells after transfection with miR-NC and miR-1271 mimics, respectively.

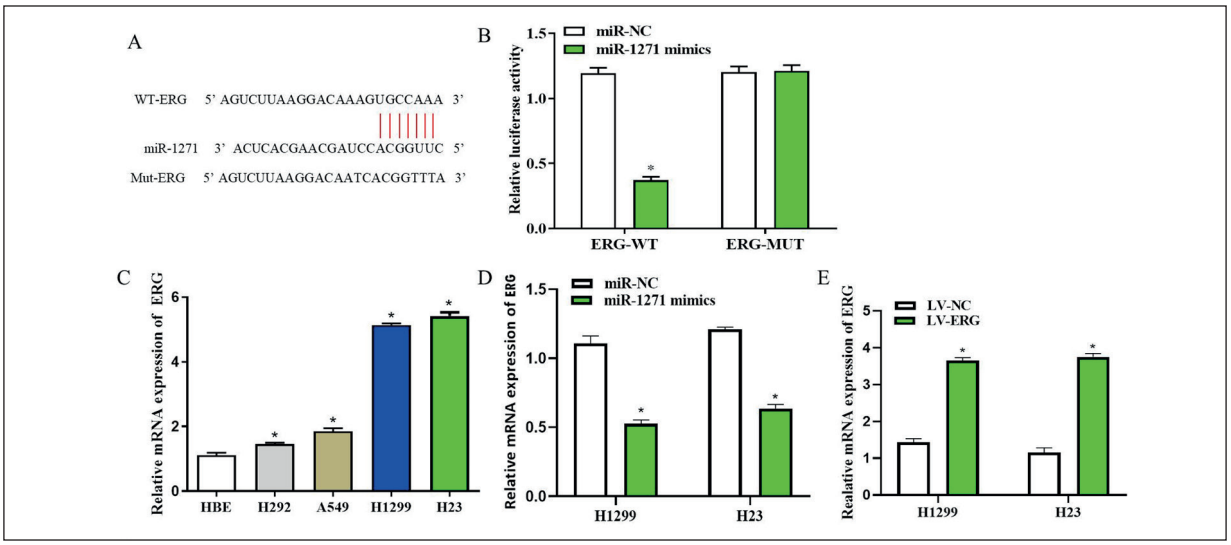

Figure 5. MiR-1271 directly targeted ERG in NSCLC cells. **A,** The predicted miR-1271 binging sites in ERG mRNA 3'-UTR. **B,** Luciferase assays were used to measure double luciferase activity of ERG WT and ERG MUT after transfection with miR-NC and miR-1271 mimics, respectively. **C,** The relative mRNA expression levels of ERG in NSCLC cells and human bronchial epithelial cell lines (HBE) were detected by qRT-PCR. **D,** QRT-PCR was used to detect the relative expression levels of ERG after transfection with miR-NC and miR-1271 mimics. **E,** The relative expression levels of ERG after transfection with LV-NC and LV-ERG were detected by qRT-PCR.

that the proliferation ability of H1299 and H23 cells after transfection of si-CircRNA_103993 and miR-1271 inhibitors was significantly higher than that of si-CircRNA_103993 group. However, the proliferation ability of H1299 and H23 cells after transfection of si-CircRNA_103993 and miR-1271 mimics was significantly lower than that of si-CircRNA_103993 group (Figure 6A and 6C). The results showed that the apoptosis rate of H1299 and H23 cells after transfection of si-CircRNA_103993 and miR-1271 inhibitors was significantly lower than that of si-CircRNA_103993 group (Figure 6B). However, the apoptosis rate of H1299 and H23 cells after transfection of si-CircRNA_103993 and miR-1271 mimics at the same time was significantly higher than that of si-CircRNA_103993 group (Figure 6D). The proliferation of H1299 and H23 cells after transfection of miR-1271 mimics and si-ERG simultaneously was significantly lower than that of miR-1271 mimics group. However, the proliferative capacity of H1299 and H23 cells after transfection of miR-1271 mimics and LV-ERG simultaneously was significantly higher than that of miR-1271 mimics group (Figure 6E and 6G). The apoptosis rate of H1299 and H23 cells after transfection of miR-1271 mimics and si-ERG simultaneously was significantly higher than that of miR-1271 mimics group (Figure 6F). However, the apoptosis rate of H1299 and H23 cells after transfection of miR-1271 mimics and LV-ERG simultaneously was significantly lower than that of the miR-1271 mimics group (Figure 6H).

Discussion

NSCLC accounts for about 80% of all pathological types of lung cancer and is mainly composed of adenocarcinoma, squamous cell carcinoma and large cell carcinoma[11]. The incidence of NSCLC is extremely high and the cure rate is extremely low[12]. In recent years, with the continuous maturity of high-throughput sequencing technology and the development of bioinformatics, studies have found that the abnormal expression of CircRNA is closely related to the occurrence and development of NSCLC[13-15]. Yao et al[16] found that the expression of circular RNA 100876 in NSCLC tissues was up-regulated by 1.23 times compared to normal tissues. The data showed that the expression of circular RNA 100876 was statistically correlated with lymph node metastasis and tumor stage in NSCLC. The result had profound effects on tumor invasion and metastasis. Circ0000064 significantly reduced cell proliferation, blocked cell cycle progression, promoted apoptosis and reduced the migration and invasion of A549 and H1229 cells through the expression of matrix metalloproteinase-2 and matrix metalloproteinase-9[17]. Zhu et al[18] showed that hsa-circ 0013958 promoted cell proliferation and invasion and inhibited apoptosis of adenocarcinoma cells. In our study, we found that CircRNA_103993 was significantly up-regulated in NSCLC cells compared to HBE cells, especially in H1299 and H23 cells. More importantly, after transfection with si-CircRNA_103993, the proliferation ability of H1299 and H23 cells was obviously lower than that of transfected si-NC group, and the expression levels of mRNA and protein of PCNA were also significantly lower than those of transfection si-NC group. The apoptosis rate of H1299 and H23 cells and the mRNA and protein expression levels of caspase-3 were significantly increased after CircRNA_103993 was silenced. This indicated that silencing CircRNA_103993 significantly inhibited proliferation and promoted apoptosis of NSCLC cell, but the specific principle is unclear.

MiRNA is a single-stranded non-coding RNA containing around 19-25 nucleotide molecules[19]. MiRNA can inhibit targeting gene expression through base complementary pairing, thereby participating in processes such as cell proliferation, differentiation, migration, and apoptosis or other progress of tumor cell[20,21]. MiRNA was also involved in the occurrence and development of NSCLC[17,18,22-24]. Gao et al[25] analyzed the expression levels of miR-143, miR-181a and miR-21 in lung cancer tissues and studied the relationship between their expression levels and parameters such as poor survival rate and clinical covariates. It provides theoretical basis as a new diagnostic or prognostic biomarker for NSCLC. MiR-1287 was found to participate in the invasion and apoptosis of NSCLC by directly targeting GAGE1[26]. In our study, we discovered that miR-1271 performed a high expression in NSCLC cells, especially in H1299 and H23 cells. The double luciferin report showed that CircRNA_103993 served as a sponge for miR-1271. The double luciferase activity of the CircRNA_103993-WT group after transfection with miR-1271 mimics was significantly lower than miR-NC group. Silencing CircRNA_103993 could significantly increase the expression level

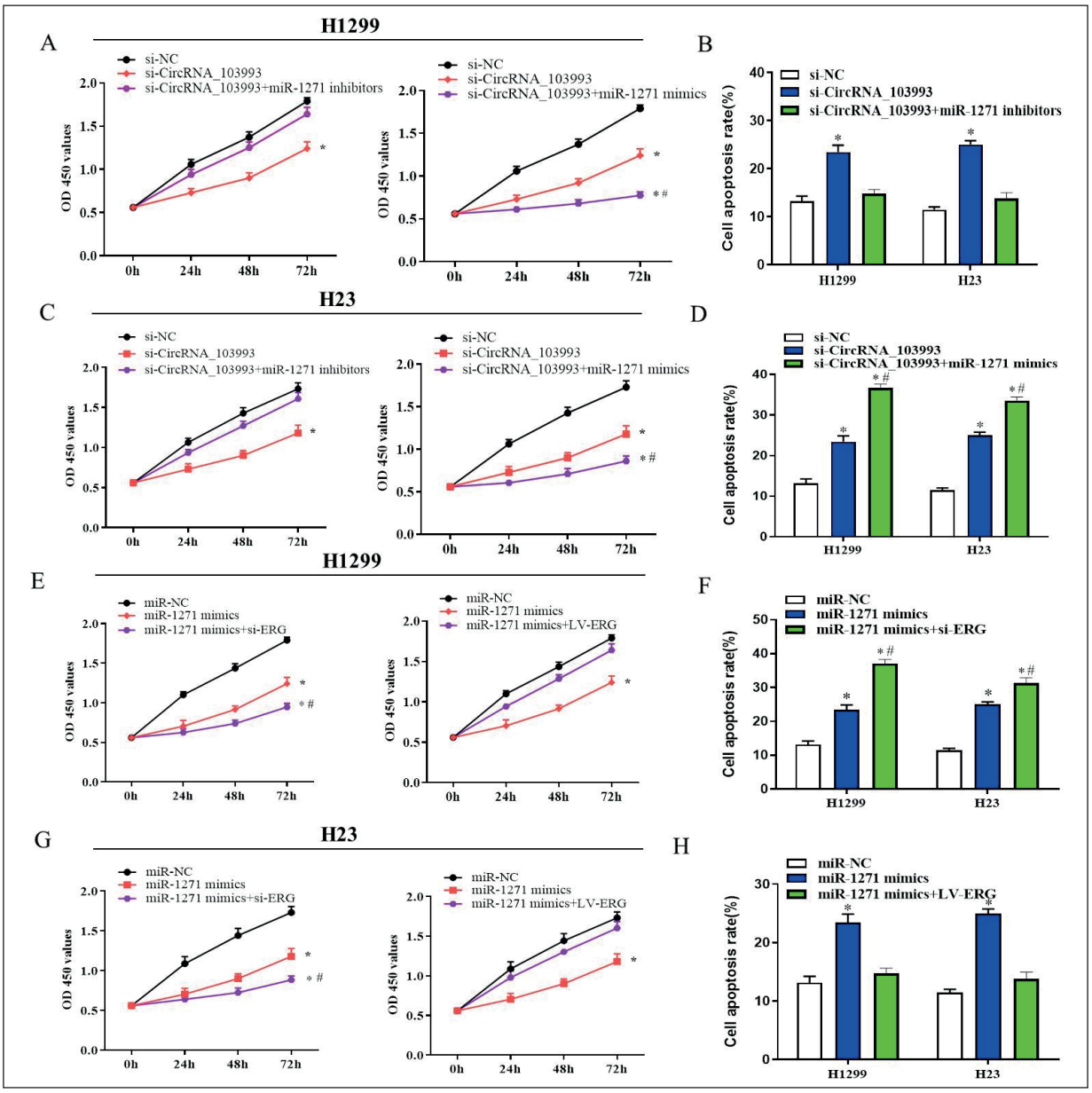

Figure 6. CircRNA_103993/ miR-1271/ ERG axis affected proliferation and apoptosis of NSCLC cells. **A,** CCK-8 assay was used to detect cell proliferation of H1299 cells after transfection with si-NC, si-CircRNA_103993, si-CircRNA_103993 and miR-1271 inhibitors, si-CircRNA_103993 and miR-1271 mimics, respectively. **B,** Flow cytometry was used to measure the cell apoptosis rate after transfection with si-NC, si-CircRNA_103993, si-CircRNA_103993 and miR-1271 inhibitors. **C,** CCK-8 assay was used to detect cell proliferation of H23 cells after transfection with si-NC, si-CircRNA_103993, si-CircRNA_103993 and miR-1271 inhibitors, si-CircRNA_103993 and miR-1271 mimics, respectively. **D,** Flow cytometry was used to measure the cell apoptosis rate after transfection with si-NC, si-CircRNA_103993, si-CircRNA_103993 and miR-1271 mimics. **E,** CCK-8 assay was used to detect cell proliferation of H1299 cells after transfection with miR-NC, miR-1271 mimics, miR-1271 mimics and si-ERG, miR-1271 mimics and LV-ERG, respectively. **F,** Flow cytometry was used to measure the cell apoptosis rate after transfection with miR-NC, miR-1271 mimics, miR-1271 mimics and si-ERG. **G,** CCK-8 assay was used to detect cell proliferation of H23 cells after transfection with miR-NC, miR-1271 mimics, miR-1271 mimics and si-ERG, miR-1271 mimics and LV-ERG, respectively. **H,** Flow cytometry was used to measure the cell apoptosis rate after transfection with miR-NC, miR-1271 mimics, miR-1271 mimics and LV-ERG.

of miR-1271. The expression of miR-1271 was down-regulated in NSCLC cells. The proliferation ability of H1299 and H23 cells after transfection with miR-1271 mimics was significantly lower than that of the transfected miR-NC group. The apoptosis rate and caspase-3 expres-

sion levels of NSCLC were significantly reduced after miR-1271 mimics transfection. This indicated that low expression of CircRNA_103993 inhibited cell proliferation and promoted apoptosis through targeted binding to miR-1271 in NSCLC cells. MiR-1271 has also been shown to target ERG binding. ERG expression was significantly up-regulated in NSCLC cells and overexpression of miR-1271 could significantly inhibit the expression level of ERG. This indicated that miR-1271 participated in the apoptosis process of NSCLC cells through binding to ERG. Further experiments showed that miR-1271 inhibitors significantly reversed the effects of si-CircRNA_103993 on the proliferation and apoptosis of H1299 and H23 cells. However, miR-1271 mimics obviously promoted the effects of si-CircRNA_103993 on the proliferation and apoptosis of H1299 and H23 cells. LV-ERG significantly reversed the proliferation and apoptosis effects of miR-1271 mimics on H1299 and H23 cells. However, si-ERG significantly promoted the proliferation and apoptosis effects of miR-1271 mimics on H1299 and H23 cells. These results indicated that CircRNA_103993 regulated proliferation and apoptosis of NSCLC cells through miR-1271/ ERG signaling pathway.

Conclusions

Our study indicated that CircRNA_103993 was obviously overexpressed in NSCLC cells. CircRNA_103993 was demonstrated to promote proliferation and inhibit the apoptosis process of NSCLC cells by regulate miR-1271/ ERG signal pathway. We for the first time discovered that a novel CircRNA (CircRNA_103993) may be a potential molecular marker in NSCLC. Moreover, CircRNA_103993 acts as a ceRNA to mediate miR-1271/ERG to promote NSCLC progress. Thus, CircRNA_103993 could be a target for lung cancer treatment based on the research.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Foundation

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