Cancer Management and Research

ORIGINAL RESEARCH

RETRACTED ARTICLE: Matrine Regulates Proliferation, Apoptosis, Cell Cycle, Migration, and Invasion of Non-Small Cell Lung Cancer Cells Through the circFUT8/miR-944/YES1 Axis



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Correspondence: Liuyang Yu Department of Oncology, Jingmen No. 2 People's Hospital, No. 39 Xiangshan Dadao, Jingmen, Hubei, 448000, People's Republic of China Tel/Fax +86 724-2633000 Email yuliuyang2005@163.com **Background:** Non-small cell lung carcine to (NSC) to is the proor histological subtype of cancer cases. In the present study, we introduce the astronometer between Matrine, an active component of Chinese medicine, an circle of the NSCLC cells.

Methods: The proliferation ability of NSCL wells was assessed by MTT and colonyforming assays. Flow cytomeny assay was performed to show the apoptosis and cell cycle distribution in NSCLC cell. The protein expression levels of Bcl-2, Cleaved Caspase-3 (C-Caspase3), and YES proteoncogene 1 M (ES1) were measured by Western blot assay. Migration and invasion of NSCC cells are determined by transwell assay. The expression levels of circFUT and 944 and YES1 were quantified by real-time quantitative polymerase chain reaction (h ($PqPC_{1}$) as μ). The interaction relationship between miR-944 and circFUT (YES1) we confirmed by dual-luciferase reporter assay. The anti-tumor role of Mattine in v to was colored by a xenograft experiment.

In tults: Marine functioned as a carcinoma inhibitor by repressing proliferation, cell cycle procure nigration, and invasion while inducing apoptosis in NSCLC cells. Importantly, overexpression of circFUT8 counteracted Matrine-induced effects on NSCLC cells. MiR-944, interacted with YES1, was a target of circFUT8. Under Matrine condition, overexpression of circFUT8 increased proliferation, migration, and invasion while inhibited apoptosis, while was abolished by the upregulation of miR-944. Whereas the silencing of YES1 counteracted miR-944 inhibitor-induced effects on NSCLC cells. Eventually, we also confirmed that Matrine impeded NSCLC tumor growth in vivo.

Conclusion: Matrine regulated proliferation, apoptosis, cell cycle, migration, and invasion of NSCLC cells through the circFUT8/miR-944/YES1 axis, which provided novel information for Matrine in NSCLC.

Keywords: Matrine, circFUT8, miR-944, YES1, NSCLC

Background

Non-small cell lung cancer (NSCLC) is a universal histological subtype in all lung cancers, occupying nearly 85% proportion of all lung cancers.¹ What is more, the clinical outcomes of NSCLC patients remain worse, and the 5-year survival rate of NSCLC patients is only 15%.² In the recent study, it was reported that circular RNAs (circRNAs) were identified as vital regulators in NSCLC.³

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Micro RNAs (miRNAs) are small RNAs without coding protein abilities that modulate target mRNA expression involved in tumor progression by targeting the 3'untranslated region (UTR) of mRNAs, resulting in and inhibiting translation.⁶ Previous degradation researches found that miR-944 was frequently dysregulated in a variety of human cancers.^{7,8} Furthermore, the high-expression of miR-944 was a reliable prognostic index for pancreatic cancer patients.9 Consistently, the diagnostic value of miR-944 was found in operable squamous lung cell carcinoma.¹⁰ Nevertheless, the role of miR-944 has not been investigated thoroughly in NSCLC.

YES proto-oncogene 1 (YES1) is a member of Src family tyrosine kinases (SFKs) family, which w involved in the development of human cancers.¹¹ Fo example, Liu et al revealed that activating YES ngthls.¹² ened the malignant phenotype of mela ma c Moreover, YES1 has been demonstrated contracted tumorigeneses such as pancreatic cap I_{1}^{13} gas c cancer.-¹⁴ and thyroid cancer.¹⁵ Suppression SYES1 cou inhibit rhabdomyosarcoma cell growth in vitro ad in vivo, identifying YES1 as a critice signal for the pr growth.¹⁶ Interestingly, YES1 also induce chemotherapeutic resistance to several cancely including NSCLC.¹⁷ Therefore, YES, yas in stigated in NSCLC. the functional r In the current research, we estigated the function of Matrine tree nent C cells and association with

circFUT8/miR-___4/YES1 axis.

Methods

Cell Lines and Cell Culture

The normal human lung epithelial cell line (BEAS-2B) and NSCLC cell lines (H522 and H1975) were purchased from the American Type Culture Collection (Rockville, MD, USA). All cells were maintained in RPMI 1640 medium (Biochrom KG, Berlin, Germany) supplemented with 10% fetal bovine serum (Sigma, San Francisco, CA,

USA), 1% penicillin/streptomycin (Sigma) at the condition of 37°C and 5% CO₂. In addition, Matrine (SM8130-20mg; Solarbio, Beijing, China) was dissolved in ddH₂O (40 mg/mL) and placed at 4°C as stock solution.

Cell Proliferation Assay

In brief, 200 μ L of H522 and H1975 cells (1.5×10⁴ cells/ mL) were seeded into 96-well plates. After 24 h, 20 µL of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazol-3ium bromide (MTT; Sigma) was added into cells and then incubated for 4 h. Dimethyl sulfoxide (DMSO) reagents were added to dissolve formazan stals. The cell viability was measured under a mic plate reade (BioTek, Winooski, VT, USA). Furthermore, Jony-for ing assay was performed as descrifed by Ge et a. ⁸ / short, H522 and H1975 cells were sperse into a single cell suspension. Approximate 600 see were ded into a 12-well plate preheater 37°C. Folk in incubation at the condition of 37 C and 16 CO₂ for 14 days, the clones were observertinder a moscope (40×, Leica, Wetzlar, Ger any).

Flot Cytometry Assay

Apoptote and were monitored by the annexin V-1 scein isothiocyanate (FITC) Apoptosis Detection at (Sigma) in compliance with the references. In brief, H522 and H1975 cells were diluted into the density of 10⁶ cells/mL and then incubated with the staining buffer supplemented with FITC and propidium iodine (PI). After 10 min of incubation at 4°C for 30 min in dark condition, mixtures were analyzed under a flow cytometry (Applied Biosystems, Foster City, CA, USA). Cell cycle detection CycleTEST PLUS DNA Reagent Kit (BD Biosciences, San Jose, CA, USA) was used to show cell cycle distribution in compliance with the instructions of manufacturer.

Western Blot Assay

Briefly, H522 and H1975 cells were lysed by RIPA lysis (Cell Signaling Technology, Danvers, MA, USA) on ice for 30 min. After centrifuging and quantifying, 40 µg of total protein was segregated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis and then electrophoretically transferred nitrocellulose filter membranes (GE Healthcare, Piscataway, NJ, USA). After blocking with 3% Albumin Bovine V (Amyjet scientific, Wuhan, China), the membranes were incubated with anti-Cleaved Caspase-3 (C-Caspase3; ab2302; 1:1500 dilution; Abcam, Cambridge, MA, USA), anti-B-cell lymphoma-2 (Bcl-2;

ab59348; 1:1500 dilution; Abcam), or anti-YES1 (ab109744; 1:1500 dilution; Abcam) overnight at 4°C, with anti-GAPDH (ab181602; 1:1500 dilution; Abcam) as control. After that, secondary antibody (ab1500771; 1:3000 dilution; Abcam) was added into membranes and incubated for 2 h at room temperature. The immunoreactive bands were shown on Alpha Innotech Imaging System (Protein Simple, Santa Clara, CA, USA) with ECL Western Blotting Detection Kit (Solarbio).

Migration and Invasion Assay

The migration assay of H522 and H1975 cells was conducted using 24-well transwell chambers (Millipore, Billerica, MA, USA) with 8-um polycarbonate membrane. In brief, H522 or H1975 cells (5×10^5 cells/well) were seeded into the upper chamber with 200 µL of serum free-medium while complete medium was injected into the bottom chamber as a chemoattractant. 24 h later, cells that migrated to the underside of the membrane were fixed by 95% ethanol for 30 min, stained with crystal violet (Beyotime, Shanghai, China), and then imaged under a microscope (Leica). Image Lab software 5.2 (Bio-Rad, Hercules, CA, USA) was used for data analysis. 24-well transwell was covered with Matrigel Biosciences) in advance for invasion assay, while her procedures were not changed.

Real-Time Quantitative Polyner se Cham Reaction (RT-qPCR) Array

Trizol reagent (Sigma) was pplied isolate the otal RNA following the manufact rer's protoco. The complementary DNA was accorded using a reverse transcription kit Dalian, Chir, or microRNA Reverse (Takara, Transcription Wit (Qia, o, Hilde, Germany). To compare revels etween to os, RT-qPCR was performed expression with a vantiTe SYBR Green RT-PCR Kit (Qiagen) on thermal CFX6 System (Bio-Rad). The RT-qPCR results were value by $2^{-\Delta\Delta Ct}$ method, with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or endogenous small nuclear RNA U6 as reference. Furthermore, PARIS™ Kit was purchased from Abcam for subcellular localization of circFUT8 according to the manufacturer's instructions. The RNase R was purchased from Epicentre Technologies (Madison, WI, USA) for RNase R treatment assay. Total RNA was treated with or without 3U/mg RNase R for 15 min at 37°C, following by RT-qPCR assay.

The sequences of primers:

circFUT8 (sense-5'-CACTCTAGCCGAGAACTGT CC-3'; antisense-5'-TTGTCCTGTACTTCATGCGCT-3');

linearFUT8 (sense-5'-AACTGGTTCAGCGGAGAAT AAC-3'; antisense-5'-TGAGATTCCAAGATGAGTGTT CG-3');

miR-944 (sense-5'-GCCGAGAAATTATTGTACAT-3'; antisense-5'-CTCAACTGGTGTCGTGGA-3');

GAPDH (sense-5'-CATCCATGACAACTTTGGTA-3'; antisense-5'-CGTTGGCAGTGGGGACACGG-3');

U6 (sense-5'-CTCGCTTCGGCAGCACA-3'; antisense-5'-AACGCTTCACGAATTTCCGT-3').

Transfection Assay

CircFUT8-overexpression vector bircFUT9, and negative control (vector), small interfering RCA (aRNA) objecting to YES1 (si-YES1) and si-NC were acquired from RiboBio (Grangzhou, China). anR-944 mimic (miR-944), miPacifi miR-944 behavior (anti-miR-944), antimiR-NC were designed by GeneCopoeia (Rockville, MPACIFA). Transport transfection with the aforemenoned oligonucleotides or plasmids into H522 and 11975 cells was conducted with Lipofectamine 2000 (witrogen, carlsbad, CA, USA) under the producer's instructions.

Dual-Luciferase Reporter Assay

The possible target of circFUT8 and miR-944 were shown by starBase v2.0 (http://starbase.sysu.edu.cn/). Partial sequences of circFUT8 or 3' UTR of YES1 mRNA containing interacted sites with miR-944 were synthesized and cloned into pGL3-basic vectors (Realgene, Nanjing, China), named as circFUT8 WT and YES1 3' UTR WT, with circFUT8 MUT and YES1 3' UTR MUT as controls. When reached 60–70% confluence in 24-well plates, H522 and H1975 cells were co-transfected with 0.4 µg of vectors and 20 pmol of miR-944 mimic or miR-NC. After 48 h, H522 and H1975 cells were collected for luciferase activity assay under the VICTOR2 fluorometry (PerkinElmer, Waltham, MA, USA), with Renilla luciferase activity as reference.

In vivo Experiment

All animal experiments were performed with the instruction of the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023), which was permitted by the Institutional Animal Care and Use Committee of Jingmen NO.1 people's hospital. In animal experiments, 6 nude mice per group were used and nude mice were randomly assigned to two groups before injection of H522 cells. H522 cells (5×10^6) in 100 µL of phosphate buffer saline were implanted subcutaneously into the right flank of nude mice (Vital River Laboratory, Beijing, China) under specific pathogen-free conditions. The mice were treated with or without 5 g/kg of Matrine in saline once daily by intraperitoneal injection. Tumor growth was monitored based on volume=1/2 (length×width²). 35 d after injection, mice were killed by cervical dislocation after deep anesthesia with 2% isoflurane for subsequent experiments. Immunohistochemical assay was performed as previous description.¹⁹

Statistical Analysis

The statistical analyses were conducted with statistical software GraphPad Prism 7 (GraphPad, La Jolla, CA, USA). Statistically significant of two treatment groups or multiple groups was assessed using Student's *t*-test or one-way analysis of variance, respectively. All data were shown as mean \pm standard deviation from three independent experiments.

Results

Matrine Inhibited Proliferation, Cell Cycle Process, Migration, and Invasion While Increased the Apoptotic Response in NSCLC Cells

To assess the effects of Matrine on NSC C c. 1522 and H1975 cells were cultured with nor media sup. mented Matrine at different concentrations (0, 0, 1, and 2 n/mL)for 24 h. As shown in Figure 1A and B, wiability was reduced in H522 and H1255 cells exposed to Matrine, especially in 2 mg/mL of trine roup (over 50% decrease). Likewise, colony, probers for reduce, after treatment with Matrine, suggesting lony-fe ping inhibition of NSCLC cells (Figure 1C ar D) Furthermore, the treatment of nearly 4-told increase in apoptosis rate Matrine induc in H522 and H19 cells compared with control, having a dose-dependent method (Figure 1E and F). As Bcl-2 and C-Caspase3 were important apoptosis-related proteins, their expression was assessed by Western blot assay. Bcl-2 was downregulated by 50% while C-Caspase3 was upregulated by 3-fold in H522 and H1975 cells after treatment with 2 mg/ mL of Matrine (Figure 1G and H). Matrine inhibited cell cycle process by increasing G0/G1 phase and decreasing S phase cells (Figure 1I and J). The results of the transwell assay suggested that migration and invasion of H522 and

H1975 cells were downregulated by 50% in 2 mg/mL of Matrine compared with control (Figure 1K and L). Together, these results revealed that Matrine played a key role in NSCLC cells.

Matrine Inhibited circFUT8 Expression in NSCLC Cells

As presented in Figure 2A, GEO accession (https://www.ncbi. nlm.nih.gov/geo/geo2r/?acc=GSE112214) suggested that circFUT8 was upregulated in NSCLC tissues when compared with normal tissues. We also confirm un pircFUT8 was increased by 2 to 3-fold in H522 are H1975 cells than that in BEAS-2B (Figure 2B). After perform, RNase R eperiments, linearFUT8 was obviously ownregular over 0%, while circFUT8 was not change, implying that changes, T8 was resistant to RNase R (Figtre 2, net). As desribed by Figure 2E and F, the results a RT-qPC, ssay sowed the predominant cytoplasmic d'arbien of circFee. What is more, we found that circFUT8 expressive was decreased by as much as 50% in La Fr 975 cells after reatment with Matrine (Figure 2G H522 H). Therefore, the association between Matrine and and circle T8 was investigated in the next experiments.

Apprine Functioned as an Anti-Carcinogenesis Role Through Regulation of circFUT8 in NSCLC Cells

s Matrine can inhibit circFUT8 expression in H522 and H1975 cells, it is necessary to assess the relationship between Matrine and circFUT8 in NSCLC cells. The downregulation of circFUT8 (over 50%) induced by Matrine was overturned in H522 and H1975 cells by transfection with circFUT8 (Figure 3A and B). Matrine could repress the proliferation ability of H522 and H1975 cells by over 50% decrease, whereas co-transfected with circFUT8 counteracted the effects of Matrine (Figure 3C-F). The flow cytometry assay uncovered that overexpression of circFUT8 could partly neutralize the enhancement effects of Matrine on cell apoptosis (Figure 3G and H). As verified in Figure 3I and J. Matrine inhibited Bcl-2 while enhanced C-Caspase3 expression in H522 and H1975 cells, which were overturned by the upregulation of circFUT8. Furthermore, overexpression of circFUT8 abolished the suppressive function of Matrine on cell cycle progress (Figure 3K and L). The analysis results of transwell assay indicated that migration and invasion were restored by circFUT8 in H522 and H1975 cells



Figure I continued.

exposed to Matrine (Figure 3M and N). Considering space, the representative pictures of colony-forming, flow cytometry, and transwell assays were presented in <u>Supplementary Figure S1</u>. Conclusively, Matrine functioned as a carcinoma inhibitor by decreasing circFUT8 expression in H522 and H1975 cells.

CircFUT8 Regulated miR-944 Expression in NSCLC Cells

By bioinformatics analysis, we identified miR-944 as a potential target miRNA for circFUT8 (Figure 4A). The predicted results were confirmed by dual-luciferase reporter assay; miR-944 mimic lead a 50% decrease in



Figure I The effects of Matrine on proliferation, apoptosis, cell cycle, migration, and invasion in NSCLC cells. (A-L) H522 and H1975 cells were treated with Matrine with different concentrations (0, 0.5, 1, and 2 mg/mL) for 24 h. (A-D) The proliferation ability of H522 and H1975 cells were measured by MTT and colony-forming assays. (E and F) The flow cytometry assay was performed to monitor apoptotic cells. (G and H) The protein expression levels of Bcl-2 and C-Caspase3 were detected by Western blot assay. (I and J) The cell cycle distribution was shown by flow cytometry assay. (K and L) The migration and invasion of H522 and H1975 cells were determined by transwell assay. *P < 0.05.



Figure 2 The expression level of circFUT8 in NSCLC tissues and cells. (A) The expression level of circFUT8 was shown from G maccession. (B) CircFUT8 level was measured by RT-qPCR assay in NSCLC cells and control group. (C and D) RT-qPCR assay was conducted to show expression of circ T8 and line rFUT8 in H522 and H1975 cells after treatment with RNase R. (E and F) The abundant of circFUT8 in the cytoplasm and nucleus was maintified by RT-qPC massay (G and H) After being treated by Matrine, the level of circFUT8 was shown by RT-qPCR assay in H522 and H1975 cells. *P < 0.05.



Figure 3 Effects of circFUT8 overexpression on proliferation, apoptosis, cell cycle, migration, and invasion in NSCLC cells treated with Matrine. (A–N) H522 and H1975 cells were divided into four groups: Control, Matrine, Matrine+vector, and Matrine+circFUT8. (A and B) The expression level of circFUT8 was examined by RT-qPCR assay after transfection. (C–F) MTT and colony-forming assays were used to measuring cell proliferation. (G and H) The apoptosis rate of H522 and H1975 cells was shown by flow cytometry assay. (I and J) The Western blot assay was performed to assess the expression of Bcl-2 and C-Caspase3 in H522 and H1975 cells. (K and L) The cell cycle distribution was shown by flow cytometry assay. (M and N) The transwell assay was conducted in H522 and H1975 cells post-transfection. *P < 0.05.

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luciferase activity of cells transfected with circFUT8 WT, while there was no change in circFUT8 MUT group (Figure 4B and C). Moreover, overexpression of circFUT8 suppressed miR-944 expression in NSCLC cells by 50% decrease (Figure 4D and E). After treatment of Matrine, H522 and H1975 cells showed a 2-fold increase in miR-944 level compared with control (Figure 4F and G). In summary, miR-944 was a direct target of circFUT8 and was negatively regulated by circFUT8 in NSCLC cells.

CircFUT8/miR-944 Regulated Proliferation, Apoptosis, Cell Cycle, Migration, and Invasion of NSCLC Cells Treated with Matrine

It had been confirmed that miR-944 was a target of circFUT8 in NSCLC cells, and the effects of miR-944 overexpression were explored in NSCLC cells. Under Matrine stimulus conditions, transfection of miR-944 mimic reversed the low-expression of miR-944 caused by circFUT8 (Figure 5A and B). The upregulation of

miR-944 obviously restrained circFUT8-induced enhancement effects on cell proliferation in H522 and H1975 cells treated with Matrine (Figure 5C-F). Additionally, miR-944 mimic increased the apoptosis of H522 and H1975 cells by almost 4 times in the presence of circFUT8 and Matrine (Figure 5G and H). The results of Western blot assay suggested that the upregulation of Bcl-2 and downregulation of C-Caspase3 induced by circFUT8 were overturned by miR-944 mimic in H522 and H1975 cells exposed to Matrine (Figure 5I and J). Interestingly, the enhancement effects on cell cycle **provis** in Matrineinduced H522 and H1975 cells careed by ch. UT8 were abolished by overexpression of R-944 (Figu 5K and L). Co-transfection of circE 18 and R-944 uld counteract the circFUT8-indy ed upre-ulation Augration and invasion of H522 and 1977 cells exposed to Matrine N). Nadditic, Supplementary (Figure 5M ar Figure S2 show the repres ve pictures of colonyforming, flow cytonery, and transwell assays. Therefore, Matrin gulated pro eration, apoptosis, cell cycle, tion, and invasion of NSCLC cells by regulating mig circ UT8/miR-94 axis.



Figure 4 MiR-944 was a direct target of circFUT8 in NSCLC cells. (A) Binding regions between miR-944 and circFUT8 were shown. (B and C) The relative luciferase activity was analyzed in H522 and H1975 cells co-transfected with reports and miR-944 mimic or miR-NC. (D and E) RT-qPCR assay was conducted to evaluate miR-944 level in H522 and H1975 cells transfected with vector or circFUT8. (F and G) The expression level of miR-944 was assessed by RT-qPCR assay in H522 and H1975 cells exposed to Matrine with different concentrations (0, 0.5, 1, and 2 mg/mL) for 24 h. *P < 0.05.



apoptosis, cell cycle, migration, and invasion of NSCLC cells through circFUT8/miR-944 axis. (A–N) H522 and H1975 cells were oroliferati Figure 5 Matrine regulat The, Matring+vector, Matrine+circFUT8, Matrine+circFUT8+miR-NC, and Matrine+circFUT8+miR-944. (A and B) The expression divided into six groups: Co -qPCR assa level of miR-944 was detected -F) The proliferation capability of H522 and H1975 cells was analyzed by MTT and colony-forming assays. (G and H) The optosis rate of H522 and H1975 cells. (I and I) The expression levels of BcI-2 and C-Caspase3 were calculated with Western flow cytometry used to w the d L) The blot assay. (🖌 ell cycle di on was shown by flow cytometry assay. (M and N) The transwell assay was performed to assess migration and invasion of H522 sfection. *P < 0.05. ells post-t and HI97

YESI Wash Target mRNA of miR-944 in NSCLC Cells

The possible target mRNA of miR-944 was predicted by bioinformatics tools. As displayed in Figure 6A, miR-944 had the binding region in 3'UTR of YES1 mRNA. The results of dual-luciferase reporter assay suggested that miR-944 mimic inhibited the luciferase activity of the YES1 3' UTR WT group by 50% compared with miR-NC group, and this reduction was disappeared in YES1 3'

UTR MUT group (Figure 6B and C). The expression level of YES1 was increased over 2 times in H522 and H1975 cells after the silencing of miR-944 (Figure 6D and E). In addition, Matrine inhibited YES1 expression in H522 and H1975 cells with a dose-dependent method (Figure 6F and G). Interestingly, the upregulation of YES1 in H522 and H1975 cells (over 2-fold) induced by circFUT8 was abolished by overexpression of miR-944 (Figure 6H and I). These data suggest that circFUT8/



Figure 6 CircFUT8/miR-944 axis regulated YESI expression in NSCLC wils. (A) Mix 244 had the binding region in 3'UTR of YESI mRNA. (B and C) The luciferase activities of the wild-type YESI 3' UTR WT and mutant YESi. UTR M⁴ to the proceed. (D and E) Western blot assay was performed to assess YESI level in H522 and H1975 cells transfected with anti-miR-NC or anti-miR-94. (Figure 4 After treatment with Matrine with different concentrations (0, 0.5, 1, and 2 mg/mL) for 24 h, the expression of YESI was shown by Western blot at P_{1} . (H and I) protein expression level of YESI was determined by Western blot assay in H522 and H1975 cells transfected with vector, circFUT8, circFUT8+m⁴ and I) protein expression level of YESI was determined by Western blot assay in H522 and H1975 cells transfected with vector, circFUT8, circFUT8+m⁴ and I) protein expression level of YESI was determined by Western blot assay in H522 and H1975 cells transfected with vector, circFUT8, circFUT8+m⁴ and I) protein expression level of YESI was determined by Western blot assay in H522 and H1975 cells transfected with vector, circFUT8, circFUT8+m⁴ and I) protein expression level of YESI was determined by Western blot assay in H522 and H1975 cells transfected with vector, circFUT8, circFUT8+m⁴ and I) protein expression level of YESI was determined by Western blot assay in H522 and H1975 cells transfected with vector, circFUT8, circFUT8+m⁴ and I) protein expression level of YESI was determined by Western blot assay in H522 and H1975 cells transfected with vector, circFUT8, circFUT8+m⁴ and I) protein expression level of YESI was determined by Western blot assay in H522 and H1975 cells transfected with vector, circFUT8, circFUT8+m⁴ and I) protein expression level of YESI was determined by Western blot assay in H522 and H1975 cells transfected with vector.

miR-944 axis regulated (ES1 expression in NSCLC cells.

MiR-944/YEST xis contrinpated in the Regulate to Function of Matrine in NSCLC C 12

As presented in Figure 7A and B, YES1 was significantly increased in miR-944-depleted H522 and H1975 cells, while co-transfected with si-YES1 inhibited YES1 expression under Matrine stimulus conditions. Furthermore, the silencing of YES1 significantly abolished anti-miR-944-induced increase in cell proliferation in NSCLC cells exposed to Matrine (Figure 7C–F). In flow cytometry assay, knockdown of YES1 impeded anti-miR-944-induced inhibitory effects on apoptosis in H522 and H1975 cells treated with Matrine

(Figure 7G and H). Moreover, the silencing of miR-944 increased Bcl-2 and decreased C-Caspase3 expression in Matrine-induced H522 and H1975 cells, which were abolished by knockdown of YES1 (Figure 7I and J). Co-transfection of anti-miR-944 and si-YES1 could offset the anti-miR-944-induced effects on cell cycle process in Matrine-induced H522 and H1975 cells (Figure 7K and L). The results of transwell assay implied that the enhancement effects of anti-miR-944 on migration and invasion were eliminated by si-YES1 in H522 and H1975 cells exposed to Matrine (Figure 7M and N). The representative pictures of functional experiments were presented in <u>Supplementary Figure S3</u>. These results suggested that Matrine-induced effects on proliferation, apoptosis, cell cycle, migration, and invasion of NSCLC cells were dependent on miR-944/YES1 axis.



Figure 7 MiR-944/YESI regulated proliferation, apoptosis, cell cycle, migration, and invasion of NSCLC cells exposed to Matrine. (A–N) H522 and H1975 cells were divided into six groups: Control, Matrine, Matrine+anti-miR-944, Matrine+anti-miR-944, Si-NC, or Matrine+anti-miR-944+si-YESI. (A and B) The protein expression level of YESI was evaluated by Western blot assay. (C–F) MTT and colony-forming assays were conducted to assess cell proliferation. (G and H) The apoptosis rate was determined by flow cytometry assay. (I and J) The expression levels of Bcl-2 and C-Caspase3 were quantified with Western blot assay. (K and L) The cell cycle distribution was displayed by flow cytometry assay. (M and N) The migration and invasion were measured by transwell assay in H522 and H1975 cells. *P < 0.05.

Matrine Suppressed Tumor Growth in vivo

The regulatory roles of Matrine were explored in vivo by a xenograft experiment. As shown in Figure 8A and B, when compared with control group, Matrine group showed significant decrease in volume; besides, it also showed a 2-fold decrease xenograft weight compared with control group. CircFUT8 was decreased by 50%, and miR-944 was increased by 3-fold in Matrine group compared with control group (Figure 8C and D). The results of Western blot assay revealed that YES1 was downregulated by 50% in Matrine group (Figure 8E). Additionally, immunohistochemical assay revealed the increased C-Caspase 3-positive cells and decreased ki-67-positive cells in Matrine group (Figure 8F). Therefore, Matrine could impede tumor growth by regulation of circFUT8/miR-944/YES1 axis partially.

including anti-cancer.²⁰ Functionally, Matrine suppressed proliferation, while induced apoptosis in breast carcinoma cells through Bcl-2 signal path.²¹ Similarly, Tan et al also confirmed that Matrine induced-apoptosis was contributed to the regulation of caspase activity in NSCLC cells.²² Not surprisingly, Matrine promoted apoptosis via affecting apoptosis-related protein expression, including Bcl-2 and Caspase3; importantly, Matrine could inhibit drug-resistance of NSCLC cells for chemotherapeutic drugs with a dosage-dependent manner, implying the anti-cancer potentials of Matrine in NSCLC.²³ Analogous tumor inhibition impacts hereine in NSCLC were also confirmed by other resear ners.^{24,25}

As we all know, it was widely aported that ircRNAs exhibited their effects though functioning as miRNA sponges through company end genous N-As network.²⁶ A previous report a year of that circFUS8 was a differentially expressed arcRNA in fiver oncer, and performed function by arcRNA miRNA-micNA network.²⁷ Not surprisingly the significance of circFUT8/miR-570-3p/ Krüppe-like Factor 10 network was reported in bladder

Discussion

Matrine, an active component isolated from the roots of Sophora species, has various pharmacological activities,



Figure 8 Matrine repressed NSCLC tumor growth in vivo. (A and B) The volume and weight of xenograft tumors were shown. (C and D) The expression levels of circFUT8 and miR-944 were estimated with RT-qPCR assay. (E) Western blot assay was used to show the expression level of YES1 in tumor tissues, with GAPDH as control. (F) Immunohistochemistry for ki-67 and C-Caspase3 was performed in tumor tissues. *P < 0.05.

cancer.²⁸ We confirmed that miR-944 was a target of circFUT8 in NSCLC. Additionally, it has been reported that deregulation of miR-944 play either tumor suppressive or oncogenic function in human malignancies,^{8,29} which may contribute to the different regulatory patterns in different diverse of tumors. In NSCLC, Liu et al revealed that the upregulation of miR-944 could suppress NSCLC cell proliferation in vitro.³⁰ The tumor-suppressive function of miR-944 was dependent on inhibiting expression of oncogenes, such as transcription 1.³¹

The silencing of YES1 triggered the inhibitory effects on proliferation and cell cycle process in ovarian cancer cells.³² The target gene array analyses by Lee et al revealed that miR-203 enhanced apoptosis of human oral cancer cells by binding to YES1.33 Similar to previous results.^{34,35} the cancerogenic function of YES1 was confirmed in NSCLC. Although previous researches had reported that YES1 was targeted by miRNAs in NSCLC cells, we investigated other miRNAs involved in YES1 in NSCLC. YES1 was involved in the pathogenesis of NSCLC by acting as a target of miR-133a, overexpression of YES1 eliminated the suppressive effect of miR-133a on NSCLC cell growth.³⁶ In this paper, YES1 was predicted as a 15 of miR-944 in NSCLC cells, and the cancerogenic nction of YES1 was inhibited by miR-944 clusiv our findings illuminated how Matrine ays a rcinon inhibitor in NSCLC by interaction w h cir 944/YES1 axis, which might ffer not therapeutic strategies for NSCLC in the rule

Conclusion

Matrine suppresses circFUC a expression in NSCLC cells. The anti-cancer effects a Matrix in NSCLC showed a dosage-derivated manner. In admmary, we investigated the control of Matrine in NSCLC and its interaction with circe VTC anR-2 to TES1 axis, which indicated a new perspective with roles of Matrine in NSCLC.

Data Sharing Statement

Please contact the corresponding author for the data request.

Ethics Approval

This study was reviewed and approved by the Ethics Committee of Jingmen NO.1 people's hospital.

Consent to Publish

Not applicable.

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Disclosure

The authors declare that they have no financial or nonfinancial conflicts of interest for this work.

References

- 1. Bray F, Ferlay J, Soerjong aram J, Siegel RL, Ture LA, Jemal A. Global cancer statistics of 18: GLOBAL AN estimates of incidence and mortality worlds are for 36 meets in the countries. *CA Cancer J Clin.* 2018;68(6), 1–424 m:10.3322/caac.21492
- 2. Hirsch FR, Schrioth Verulshine Vert al. Lung cancer: current therapies and new meted dataments. *Lancet*. 2017;389 (10066) 2011. doi:10.10.10.10.10.406-6736(16)30958-8
- 3. Xu N, Chen S, biu Y, et al. Profiles and bioinformatics analysis of differentially expressed circrnas in taxol-resistant non-small cell lung cancer ouls. *Cell Physic Biochem*. 2018;48(5):2046–2060. doi:10.1159/000492543
- Li J, Yang J, Lou P, et al. Circular RNAs in cancer: novel insights into origins, properties, functions and implications. *Am J Cancer Res.* 2015;5(2):472.

Vuan W, Pres S, Wang J, et al. Identification and characterization of as competing endogenous RNAs for miRNA-mRNA in colorectal cancer. *PeerJ*. 2019;7:e7602. doi:10.7717/peerj.7602

- bros V. The functions of animal microRNAs. *Nature*. 2004;431 (7006):350–355. doi:10.1038/nature02871
- Kim YJ, Lee JH, Jin S, Kim JH, Kim SH. Primate-specific miR-944 activates p53-dependent tumor suppression in human colorectal cancers. *Cancer Lett.* 2019;440–441:168–179. doi:10.1016/j.canlet.2018.10.029
- He H, Tian W, Chen H, Jiang K. MiR-944 functions as a novel oncogene and regulates the chemoresistance in breast cancer. *Tumour Biol.* 2016;37(2):1599–1607. doi:10.1007/s13277-015-3844-x
- Schultz NA, Andersen KK, Roslind A, Willenbrock H, Wojdemann M, Johansen JS. Prognostic microRNAs in cancer tissue from patients operated for pancreatic cancer – five microRNAs in a prognostic index. *World J Surg.* 2012;36(11):2699–2707. doi:10.1007/ s00268-012-1705-y
- Powrozek T, Krawczyk P, Kowalski DM, Winiarczyk K, Olszyna-Serementa M, Milanowski J. Plasma circulating microRNA-944 and microRNA-3662 as potential histologic type-specific early lung cancer biomarkers. *Transl Res.* 2015;166(4):315–323. doi:10.1016/j. trsl.2015.05.009
- Xiao X, Mruk DD, Lee WM, Cheng CY. c-Yes regulates cell adhesion at the blood-testis barrier and the apical ectoplasmic specialization in the seminiferous epithelium of rat testes. *Int J Biochem Cell Biol.* 2011;43(4):651–665. doi:10.1016/j.biocel.2011.01.008
- Liu W, Monahan KB, Pfefferle AD, et al. LKB1/STK11 inactivation leads to expansion of a prometastatic tumor subpopulation in melanoma. *Cancer Cell*. 2012;21(6):751–764. doi:10.1016/j.ccr.2012.03.048
- Je DW, Ym O, Yg J, Cho Y, Dh L. The inhibition of SRC family kinase suppresses pancreatic cancer cell proliferation, migration, and invasion. *Pancreas*. 2014;43(5):768–776. doi:10.1097/MPA.00000000000103
- 14. Fang Z, Yin S, Sun R, et al. miR-140-5p suppresses the proliferation, migration and invasion of gastric cancer by regulating YES1. *Mol Cancer.* 2017;16(1):139. doi:10.1186/s12943-017-0708-6

- Liu L, Yang J, Zhu X, Li D, Lv Z, Zhang X. Long noncoding RNA H19 competitively binds miR-17-5p to regulate YES1 expression in thyroid cancer. *FEBS J*. 2016;283(12):2326–2339. doi:10.1111/febs.13741
- Yeung CL, Ngo VN, Grohar PJ, et al. Loss-of-function screen in rhabdomyosarcoma identifies CRKL-YES as a critical signal for tumor growth. *Oncogene*. 2013;32(47):5429–5438. doi:10.1038/onc.2012.590
- Iida M, Brand TM, Campbell DA, Li C, Wheeler DL. Correction: yes and Lyn play a role in nuclear translocation of the epidermal growth factor receptor. *Oncogene*. 2019;38(13):2435. doi:10.1038/s41388-018-0572-x
- Ge P, Cao L, Yao YJ, Jing RJ, Wang W, Li HJ. IncRNA FOXD2-AS1 confers cisplatin resistance of non-small-cell lung cancer via regulation of miR185-5p-SIX1 axis. *Onco Targets Ther.* 2019;12:6105– 6117. doi:10.2147/OTT.S197454
- Miyazawa T, Marushima H, Saji H, et al. PD-L1 expression in non-smallcell lung cancer including various adenocarcinoma subtypes. *Ann Thorac Cardiovasc Surg.* 2019;25(1):1–9. doi:10.5761/atcs.oa.18-00163
- 20. Liu Y, Xu Y, Ji W, et al. Anti-tumor activities of matrine and oxymatrine: literature review. *Tumour Biol.* 2014;35(6):5111–5119. doi:10.1007/s13277-014-1680-z
- 21. Li H, Li X, Bai M, Suo Y, Zhang G, Cao X. Matrine inhibited proliferation and increased apoptosis in human breast cancer MCF-7 cells via upregulation of Bax and downregulation of Bel-2. *Int J Clin Exp Pathol.* 2015;8(11):14793–14799.
- 22. Tan C, Qian X, Jia R, Wu M, Liang Z. Matrine induction of reactive oxygen species activates p38 leading to caspase-dependent cell apoptosis in non-small cell lung cancer cells. *Oncol Rep.* 2013;30 (5):2529–2535. doi:10.3892/or.2013.2727
- 23. Pu J, Tang X, Zhuang X, et al. Matrine induces apoptosis via targeting CCR7 and enhances the effect of anticancer drugs in non-small cell lung cancer in vitro. *Innate Immun.* 2018;24(7):394–399. doi:10.1177/1753425918800555
- 24. Chen SF, Zhang ZY, Zhang JL. Matrine increases the inhibit effects of afatinib on H1975 cells via the IL6/JAK1/STAT3 signalin pathway. *Mol Med Rep.* 2017;16(3):2733–2739. doi:10.3892 mmr.2017.6865
- 25. An Q, Han C, Zhou Y, et al. Matrine induces cell parter and apoptosis with recovery of the expression of mill 26 in the A549 non-small cell lung cancer cell line. *Mol Med Re*, 2016;144, 1712, 4048. doi:10.3892/mmr.2016.5753

- 26. Yu T, Wang Y, Fan Y, et al. CircRNAs in cancer metabolism: a review. J Hematol Oncol. 2019;12(1):90. doi:10.1186/s13045-019-0776-8
- Ren S, Xin Z, Xu Y, Xu J, Wang G. Construction and analysis of circular RNA molecular regulatory networks in liver cancer. *Cell Cycle*. 2017;16 (22):2204–2211. doi:10.1080/15384101.2017.1346754
- He Q, Yan D, Dong W, et al. circRNA circFUT8 upregulates krupplelike factor 10 to inhibit the metastasis of bladder cancer via sponging miR-570-3p. *Mol Ther Oncolytics*. 2020;16:172–187. doi:10.1016/j. omto.2019.12.014
- 29. Pan T, Chen W, Yuan X, Shen J, Qin C, Wang L. miR-944 inhibits metastasis of gastric cancer by preventing the epithelial-mesenchymal transition via MACC1/Met/AKT signaling. *FEBS Open Bio*. 2017;7(7):905–914. doi:10.1002/2211-5463.12215
- 30. Liu M, Zhou K, Cao Y. MicroRNA-944 cell growth by targeting EPHA7 in non-small cell log cancer. *J Mol Sci.* 2016;17(10). doi:10.3390/ijms17101.
- 31. An JC, Shi HB, Hao WB, Zhu K, Mu B. miR-944 hibits lung adenocarcinoma tumorigenesit by targene STATe interaction. *Oncol Lett.* 2019;17(4):379–3798. doi:10.38-0012-09.10045
- Li L, He L, Zhao JL, et al. MiR-17-5 up-regulate of ES1 to modulate the cell cycle progression of approxis in overlan cancer cell lines. J Cell Biochem. 2010;16(6):10-11059. doi: 0.1002/jcb.25060
- Lee SA, Kim J. Park SY, et al. niR-155 downregulates Yes-1 and suppresses accogenerativity in human oral cancer cells. *J Biosci Bioeng*. 2015;120(4):2015.02.002
- 34. Garper C. J., Mj P, Herney Prado F, et al. YES1 drives lung cancer growth and progression and predicts sensitivity to dasatinib. *Am J spir Crit Cire Med.* 2019;200(7):888–899. doi:10.1164/ um.201807-129 DC
- 35. Zhe, W, Wang Y Yin D, et al. Effect of miR-140-5p on the regulation exprolify don and apoptosis in NSCLC and its underlying mechanism. *Exp. Ther. Med.* 2019;18(2):1350–1356. doi:10.3892/ 1.019.7701
 - Shen Y, Chen F, Liang Y. MicroRNA-133a inhibits the proliferation of non-small cell lung cancer by targeting YES1. *Oncol Lett.* 2019;18 (6):6759–6765. doi:10.3892/ol.2019.11030

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