Regular Article

Astragalus Polysaccharide Suppresses Cell Proliferation and Invasion by Up-Regulation of miR-195-5p in Non-small Cell Lung Cancer

Xingkui Tao, Xingtao Zhang, and Fan Feng*

School of Biological and Food Engineering, Suzhou University; 49 Bianhe Middle Road, Suzhou, Anhui Province 234000, China.

Received July 28, 2021; accepted February 1, 2022; advance publication released online March 18, 2022

Non-small cell lung cancer (NSCLC) accounts for approximately 85% of lung cancer-related mortality, and it has a high risk of early recurrence and distant metastasis. The prerequisite for the deterioration of NSCLC is the malignant proliferation and migration of cancer cells, and in this study *Astragalus membranaceus* polysaccharides (APS) was firstly showed that it could decrease the cell proliferation of A549 and NCI-H1299. Through bioinformatics analysis, the up-regulation of miR-195-5p was positively correlated with the survival rate of lung cancer patients. Real-time PCR indicated APS could increase the expression level of miR-195-5p, and the miR-195-5p inhibitor was used to verify that it could reverse the inhibitory effect of *Astragalus* polysaccharide on lung cancer cell migration and invasion. Therefore, we believe that APS could inhibit the proliferation and migration of NSCLC cells by regulating miR-195-5p, which laid the foundation for further research on the functional mechanism of miR-195-5p in NSCLC.

Key words *Astragalus* polysaccharide; non-small cell lung cancer (NSCLC); cell proliferation; miR-195-5p

INTRODUCTION

Traditional Chinese medicine substances come from herbal plants have been widely used to treat various diseases.[1,2] A large number of studies have showed that the herb polysaccharides have important biological function such as anti-diabetic, anti-cancer, anti-oxidant, antiviral, immunomodulatory, and other activities with lower toxicity and side effects.[3–5] Therefore, the separation, characterization and biological activity detection of polysaccharides in Chinese herbal medicines have become a research hotspot in China.[6] *Astragalus membranaceus* polysaccharides (APS) are found it possesses many biological function such as immune regulation, anti-oxidant stress, as well as the anti-apoptosis.[7,8] The recent valuable studies about the effects of APS rides on cancers treatment have been attracted widespread attention.

Non-small cell lung cancer (NSCLC) accounts for approximately 85% of lung cancer-related mortality,[9] and early metastasis is the main cause of deaths from the disease.[10,11] Combined with a plastic and responsive microenvironment, random epigenetic and genetic mutations in cancer support the metastatic evolution of tumors.[12] Currently among tested molecular factors in lung cancer patients, differential expression and regulation of microRNA (miRNA) molecules seem to be the most attractive.[13] These small non-coding RNA molecules do not carry information about the structure of the protein but could regulate the gene expression by targeting selected mRNA that belong to the epigenetic mechanism.[14,15] MiRNAs, binding to untranslated region located in 3′ tail (3′-UTR) of mRNA, modulate its function through the impact on translation process, therefore miRNAs regulate gene expression post-transcriptionally.[16] In mammals, many researches have proved that miRNAs have important regulation functions in multidrug resistance (MDR) such as miR-125b, miR-491, miR-365, and miR-133a,[17–20] and several other miRNAs have been proved to be differentially expressed in sensitive and resistant lung cancer cells through next-generation sequencing in the miRNA database.[21] However, the anti-cancer role of miRNA regulated by APS has rarely reported in lung tumor.

In the present research, the anti-cancer effects of APS was investigated in lung cancer cell. Combined with the miRNA database screening, the miR-195-5p was found it could be up-regulated by the APS in lung cancer cell, and the experiments proved that miR-195-5p inhibitor could obviously promote the cell proliferation and invasion of NSCLC. These results supplied important insights into the regulation mechanism by which APS regulates miR-195-5p expression and affects the malignant phenotypic characteristics of lung cancer cells.

MATERIALS AND METHODS

Experimental Reagent *Astragalus* polysaccharides were purchased from Macklin (Shanghai Macklin Biochemical, CAS No. 89250-26-0). Unless otherwise stated, chemicals purchased from Sigma (MO, U.S.A.) or domestic suppliers in China.

Cell Lines and Cell Culture BEAS-2B, A549 and NCI-H1299 cell lines were maintained in our laboratory. Cells were cultured in minimum essential medium (MEM) (Gibco, U.S.A.) supplemented with 1% streptomycin and penicillin (Invitrogen, China) and 10% calf bovine serum (Gibco) in a humidifed atmosphere with 5% CO_2 at 37 °C. All the cells were adapted to culture in the culture medium for 2 weeks before being used in the experiment.

Cell Proliferation Detection The Cell Counting Kit 8 (CCK-8) was conducted for cell proliferation assay as follows. Briefly, the culture plate was placed in 5% CO_2 conditioned incubator at 37 °C. After the cells adhere to the wall, they are replaced with serum-free medium. After culturing for 24 h, the cells were added with corresponding drugs and divided into groups of 0, 5, 10, and 20 μg/mL, with 3 wells in

* To whom correspondence should be addressed. e-mail: fengfan_514@163.com

Table 1. miRNA Primers Used for RT-PCR

miRNAs	Primers
miR-195-5p: uagcagcacagaaauauuggc	miR-195-5p-F: 5′-ACACTCCAGCTGGGTAGCAG CACAGAAAT-3′
	miR-195-5p-R: 5′-CTCAACTGGTGTCGTGGA-3′
miR-30a-3p: cuuucagucggauguuugcagc	miR-30a-3p-F: 5′-ACACTCCAGCTGGGCTTTCAG TCGGATGT-3′
	miR-30a-3p-R: 5′-CTCAACTGGTGTCGTGGA-3′
miR-338-3p: aacaauauccuggugcugagug	miR-338-3p-F: 5′-ACACTCCAGCTGGGAACAA TATCCTGGTG-3′
	miR-338-3p-R: 5′-CTCAACTGGTGTCGTGGA-3′
miR-30b-5p: uguaaacauccuacacucagcu	miR-30b-5p-F: 5′- ACACTCCAGCTGGGtGTAAACAT CCTAC-3′
	miR-30b-5p-R: 5′-CTCAACTGGTGTCGTGGA-3′

each group. After drug treatment for 24, 48, and 72 h, 10 μL solution of CCK-8 was supplemented to every well, and the culture plate was incubated in an incubator for 4 h. The absorbance at 450 nm measured with a microplate reader.

Identification of Differentially Expressed miRNAs The differentially expressed miRNAs was analyzed in lung cancer by using the OncomiR website (http://www.oncomir.org/), the gene expression omnibus (GEO) database used NSCLC and miRNA to obtain the data set GSE135918, and then GEO2R analysis was conducted to obtain the differential genes. Genes were respectively corresponding to miRNAs for intersection, and finally miRNAs differentially expressed in NSCLC were obtained. Kaplan–Meier method was used to calculate survival curves, conducting with the R Bioconductor 'survival' package. Kaplan–Meier curves were generated using a database of public microarray datasets (http://kmplot.com) *via* website interface 2015.

Small RNA Transfection The miRNAs (miR-195-5p inhibitor: 5-GCCAAUAUUUCUGU GCUGCUA-3′, NC inhibitor: 5′-CUAACGCAUGCACAGUCGUACG-3′) were supplied from Genepharma company (Shanghai, China). The cells were seeded into a 6-well plate at a density of 1×10^5 cells/well. Twenty four hours later, according to the manufacturer's protocol, 80 nM miRNA and its negative control were transfected into the cells by using Lipofectamine 2000 reagent (Invitrogen). The transfected cells were then harvested for research after 48 h in culture. Three independent experiments were performed.

Quantitative Real-Time RT-PCR (qRT-PCR) According to the manufacturer's instructions, small RNAs were extracted from lung cancer cells by using RISO RNA ISOlation Reagent (Biomics, U.S.A.). Then mature miRNAs were collected with a stem-loop kit, and expression levels of miRNAs were conducted by using TaqMan Universal PCR Master Mix as described by kit instruction. The U6 miRNA was employed as an endogenous control for data standardization. All reactions were repeated three times. The expression level of miRNA was tested by using the $2^{-\Delta\Delta Ct}$ method, where $\Delta\Delta Ct = (Ct_{miRNA} - Ct_{internal\ reference})$-experiment $- (Ct_{miRNA} - Ct_{internal\ reference})$control. All the qRT-PCR primers used in qRT-PCR experiments were listed in Table 1.

Cell Invasion Detection Seventy two hours after transfection of miRNA or control RNA, cells were harvested and resuspended in culture medium. Then the cells were seeded at a density about 2.0×10^6 cells/mL. A total of 0.2 mL cells solution were loaded into the upper chamber of the transwell chamber (24-well insert, 8 μm pore size; Millipore, U.S.A.), and 0.6 mL medium was added to the lower chamber containing 10% calf bovine serum as a chemical attractant.

Western Blot Analysis Approximately 3×10^6 to 5×10^6 liver cancer cells were collected by centrifugation at 1000 rpm for 5 min at 4 °C, and lysed in Radio-Immunoprecipitation Assay (RIPA) buffer (Beyotime, China) for 30 min at 4 °C.

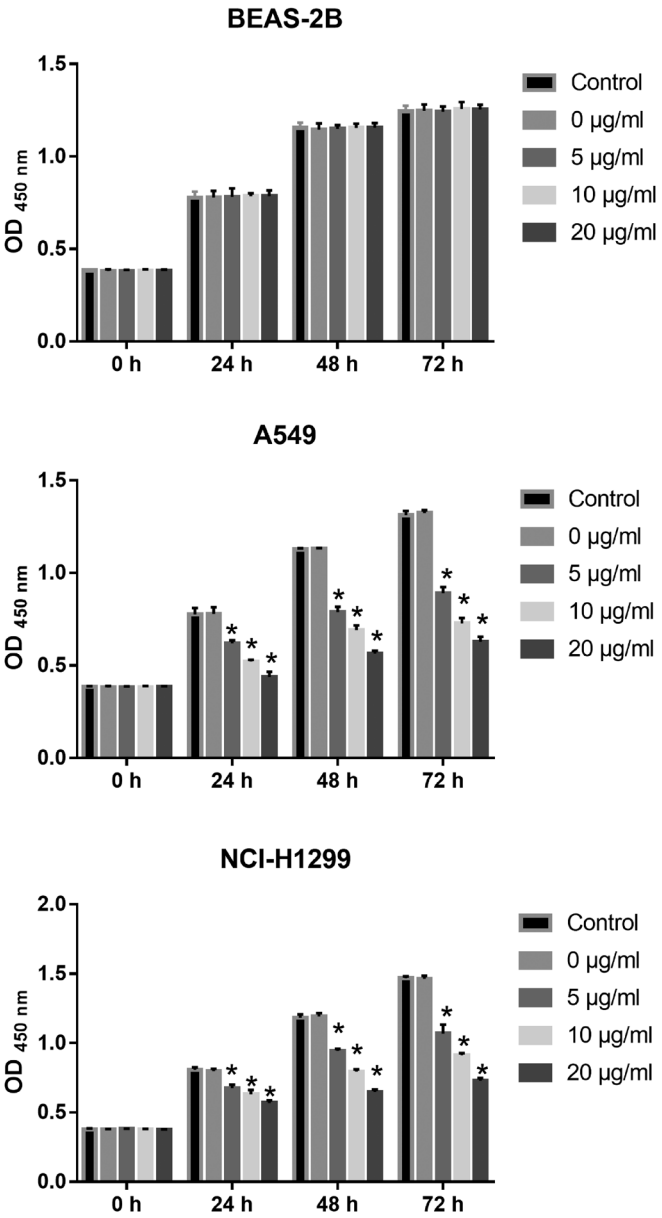

Fig. 1. *Astragalus* Polysaccharides Inhibited the Proliferation of Non-small Cell Lung Cancer Cells

Cells treated with different concentration APS at different time showed that APS could obviously inhibited lung cancer cell proliferation of A549 and H1299, but the APS has no effect to the human lung epithelial cell BEAS-2B. The data are shown as means ± S.D., and * means $p < 0.05$.

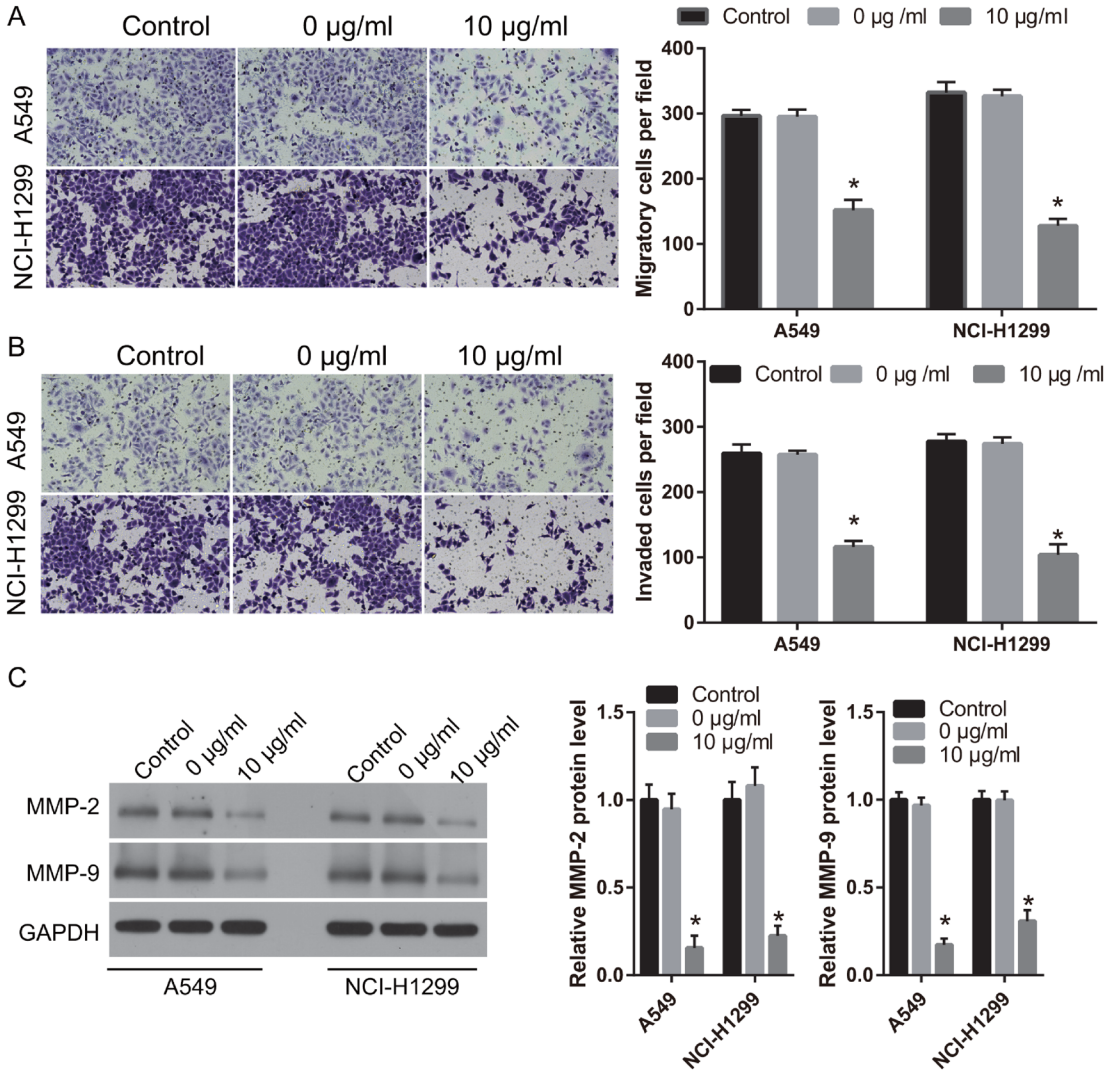

Fig. 2. *Astragalus* Polysaccharides Inhibited Cell Migration and Invasion in NSCLC Cells

A, the number of migratory cells in APS group were much less than those in the control group, and figures of transwell experimental plate were obtained by digital camera, *represents $p < 0.05$; B, the number of invaded cells in APS group were much less than those in the control group, and figures of transwell experimental plate were obtained by digital camera, *represents $p < 0.05$. C, Western blotting experiments indicated the APS could obviously inhibit the expression of MMP-2 and MMP-9 proteins in lung cancer cells. The magnification of the microscope is 100×.

According to the reagent kit protocol, the supernatants containing extraction protein were collected by centrifuge at 12000 rpm for 10 min at 4 °C. The concentration of the proteins was detected by the bicinchoninic acid (BCA) kit (KGPBCA, KeyGEN, China) based on the manufacturer's instruction. Western blot analysis was performed as following described. Briefly, protein samples of the same quality were separated by 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes. After blocking with phosphate buffered saline (PBS) Tween-20 (PBST) solution containing 3% skimmed milk powder overnight at 4 °C, the membranes were incubated with the primary indicated antibodies (MMP-2, MMP-9 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Invitrogen) (1 : 2000) for 1.5 h at room temperature. Following washing with PBST solution five times, the membranes were incubated with HRP-conjugated secondary antibodies (Sigma-Aldrich, St. Louis, MO, U.S.A.) (1 : 5000) at room temperature for 1.0 h. After washing with PBS solution for three times, the blots were visualized usingthe enhanced chemoluminescence (ECL) method (Thermo Fisher, Shanghai, China).

Statistical Analysis The data are shown as mean ± standard deviation (S.D.). SPSS PASW Statistics version 18 Multilingual (SPSS Inc., U.S.A.) used Student's *t*-test to evaluate statistical significance between groups. A *p*-value less than 0.05 is considered statistically significant.

RESULTS

***Astragalus* Polysaccharides Inhibited Cell Proliferation in NSCLC Cells** To identify the role of *Astragalus* polysaccharides in lung cancer, the control group of human lung epithelial cells BEAS-2B and the experimental group of A549 and NIC-H1299 cell lines were incubated with different concentration APS. Compared with the control BEAS-2B cells, the APS obviously suppressed cell proliferation of A549 and NCI-H1299 (Fig. 1). Therefore, APS was confirmed that it could obviously inhibit the cell proliferation of NSCLC.

***Astragalus* Polysaccharides Inhibited Cell Migration**

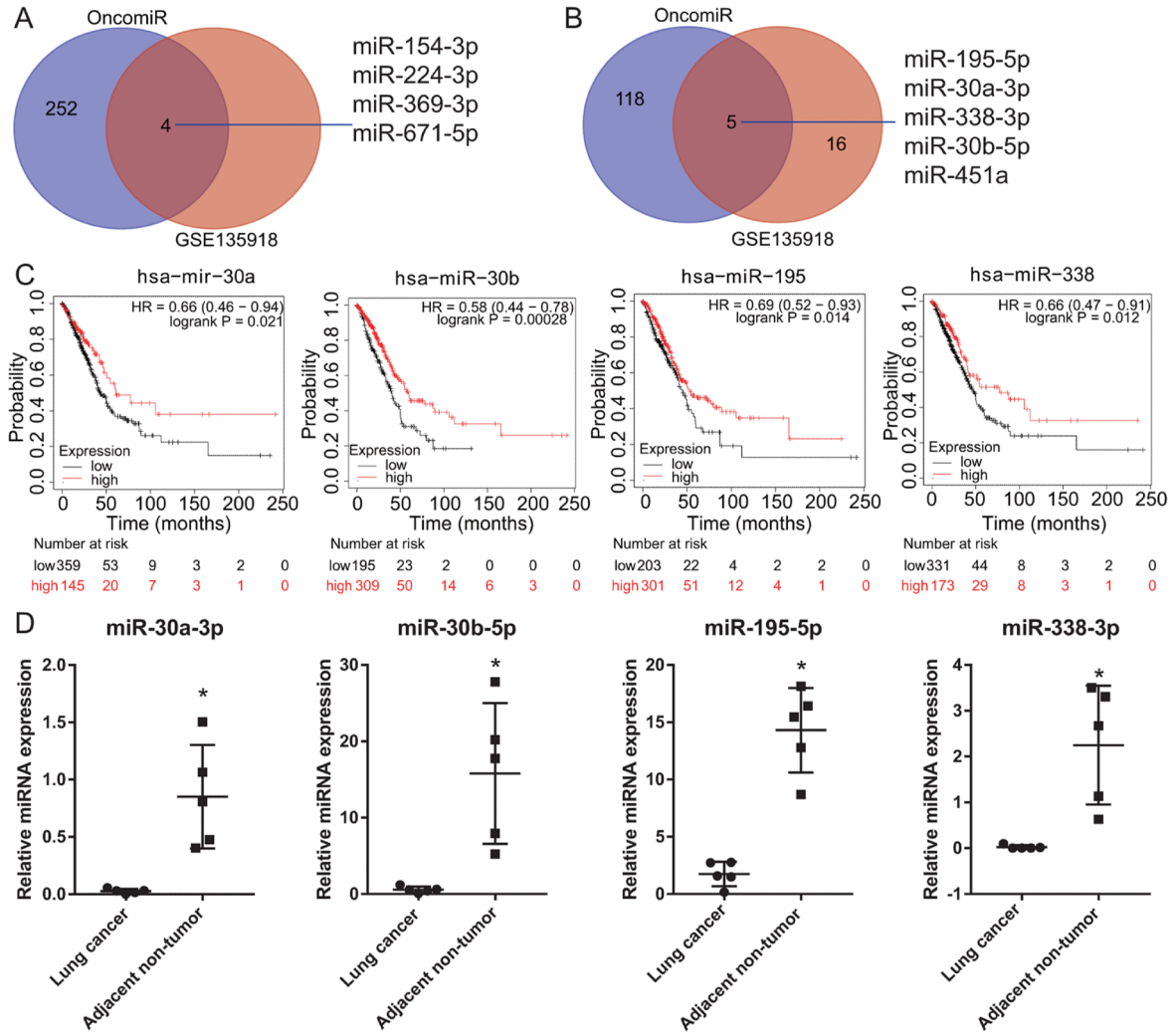

Fig. 3. Screening of Differentially Expressed miRNAs of Lung Cancer and Their Effects on Patient Survival

A, the up-regulated expressed miRNAs in lung cancer screened by the database OncomiR and GSE135918; B, the down-regulated expressed miRNAs in lung cancer screened by the database OncomiR and GSE135918; C, Kaplan–Meier plots of recurrence-free survival (RFS) from data in the dataset GSE135918. The p value was calculated using a log-rank test; D, the down-regulated expression miRNAs in lung cancer tissue verified by relative miRNA expression in dataset GSE135918, *represents $p<0.05$.

and Invasion in NSCLC Cells To investigate whether the *Astragalus* polysaccharides could affect the cell migration and invasion of lung cancer, the human lung cancer cells A549 and NIC-H1299 were incubated with different concentration APS. Compared with the control group cells, the APS obviously inhibited cell migration and invasion of A549 and NCI-H1299 through transwell assays (Fig. 2). As a result, APS was confirmed that it could obviously suppress the cell migration and invasion of NSCLC.

Screening of Differentially Expressed miRNAs of Lung Cancer and Their Effects on Patient Survival According to the methods mentioned above, the up-regulated miRNAs (miR-154-3p, miR-224-3p, miR-369-3p and miR-671-5p) and down-regulated miRNAs (miR-195-5p, miR-30a-3p, miR-338-3p, miR-30b-5p and miR-451a) were obtained by the intersection set base on the OncomiR and GSE135918 (Figs. 3A, B). In order to further determine the relationship between these differentially expressed miRNAs and the clinical prognosis of lung cancer patients, we evaluated the prognostic value of miRNAs in the public clinical microarray database of lung cancer cases. The expression level of miRNAs were closely related to recurrence-free survival rate (RFS) in the dataset GSE135918. As shown in Fig. 3C, Kaplan–Meier analysis of 504 lung cancer patients showed that highly expressed miRNAs (miR-195-5p, miR-30a-3p, miR-338-3p, miR-30b-5p) were significantly related to the high RFS rate compared with low expressed miRNAs ($p<0.05$). And these miRNAs have lower expression in the tumor tissue than that in the adjacent non-tumor tissue of lung cancer patients in the GSE135918 (Fig. 3D).

***Astragalus* Polysaccharides Increased the Expression of miR-195-5p in NSCLC Cells** The miR-195-5p, miR-30a-3p, miR-30b-5p and miR-338-3p were downregulated in the lung tumor patients according to the database screening. To investigate the APS whether influence these miRNAs expressed in lung tumor cells, BEAS-2B, A549 and NIC-H1299 cells were incubated with different concentration APS, and real-time PCR results indicated these miRNAs expression were increased, but only the miR-195-5p showed the concentration-dependent difference (Fig. 4), this indicated the APS mainly regulated miR-195-5p to inhibit the proliferation and invasion of lung tumor cells.

MiR-195-5p Inhibitor Reversed the Inhibitory Effect of *Astragalus* Polysaccharides on Lung Cancer Cell Migration

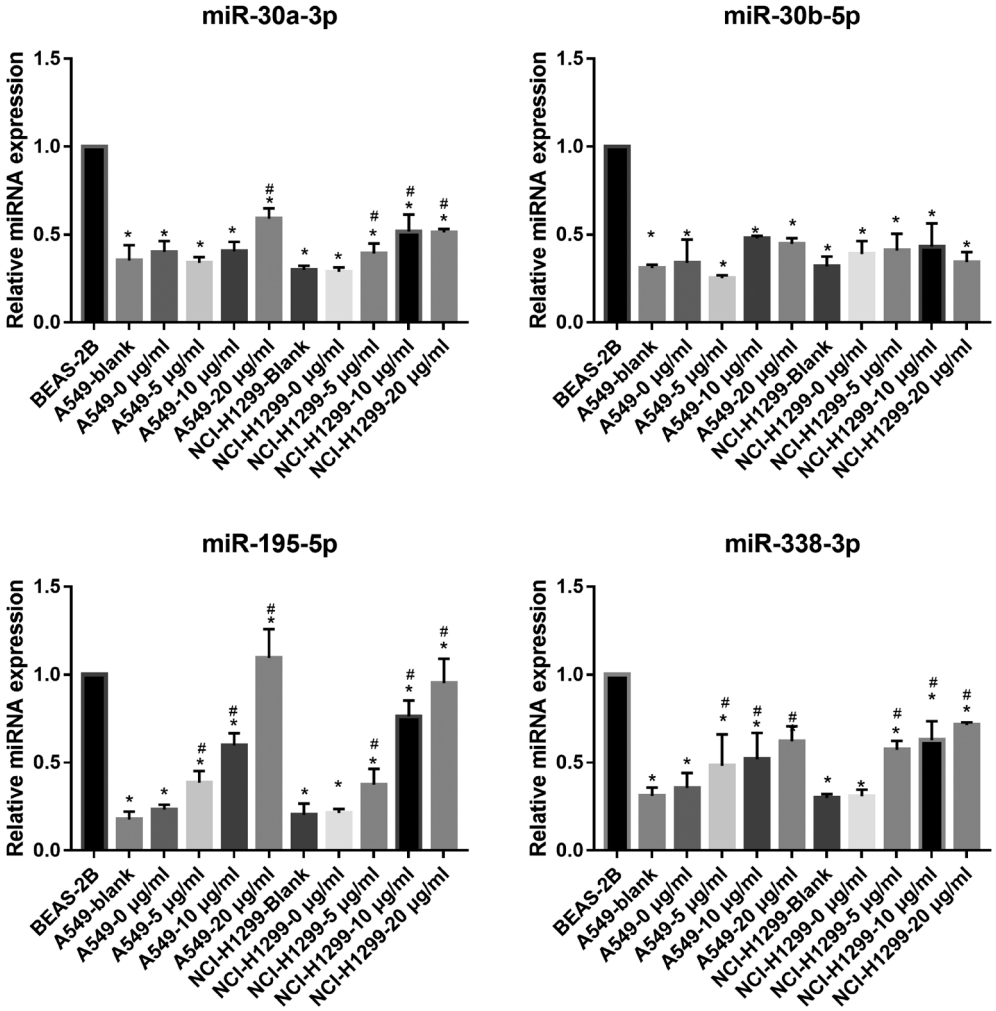

Fig. 4. *Astragalus* Polysaccharides Increased the Expression of miR-195-5p in NSCLC Cells

Real-time PCR was used to detect the expression of miR-30a-3p, miR-30b-5p, miR-195-5p, and miR-338-3p in BEAS-2B, A549, NCI-H1299 cells treated with APS at different concentration, and the results indicated that only miR-195-5p was concentration-dependent on APS treatment in lung cancer cells A549 and NCI-H1299. *represents $p < 0.05$, and #represents $p < 0.01$.

and Invasion According to the above experiments result, *Astragalus* polysaccharides could obviously suppress cell migration and invasion of A549 and NCI-H1299, in which the miR-195-5p could be up-regulated by the APS. To investigate whether miR-195-5p influenced the NSCLC cell migration and invasion, the miR-195-5p inhibitor was employed for transfection after NSCLC cells incubated with APS for 24 h. As shown in Fig. 5, the miR-195-5p inhibitor could obviously decrease the expression level of miR-195-5p. And miR-195-5p inhibitor could reverse the inhibitory effect of *Astragalus* polysaccharides on lung cancer cell migration and invasion, which indicated the downregulation of miR-195-5p could promote the cell migration and invasion of A549 and NCI-H1299.

DISCUSSION

For thousands of years, Chinese medicine has been regarded as an effective treatment method, which shows that Chinese herbal medicine can be used as a suitable candidate drug for drug development. More and more researches have begun to pay attention to the specific mechanism of action of Chinese medicine components on diseases.[22–24] It is reported that APS has a variety of biological function such as antioxidant, anti-inflammatory, anti-aging and mitochondrial protection.[25,26] This is also the reason why *Astragalus* is most commonly used in Chinese herbal medicine. APS can enhance innate immunity by increasing the expression of Toll-like receptor 4 during mucosal bacterial infection *in vivo* and *in vitro*.[27] It was also used as an adjuvant for avian infectious bronchitis virus vaccine.[28] However, APS treatment could reduce the cisplatin-induced apoptosis in mouse kidney cells and HK-2 cells,[29] and combination treatment with vinorelbine and cisplatin in patients with advanced NSCLC significantly improved the QOL.[30] Based on these characteristics of APS in many pathological processes, we separately explored its inhibitory effect on lung cancer cells in this research.

Traditional Chinese medicine has attracted widespread attention due to its synergy and low side effects in treating lung cancer.[31] Jinfukang (JFK), a Chinese herbal formula consisted of 12 herbs, has been used clinically in China for the treatment of NSCLC for decades.[32] However, the mechanism of TCM treatment of tumors is very complicated. Among the molecular markers of patients currently tested, microRNAs molecules seem to be the most attractive in lung cancer patients. MiRNAs, binding to untranslated region located in 3′ tail (3′-UTR) of mRNA, modulate its function through the im-

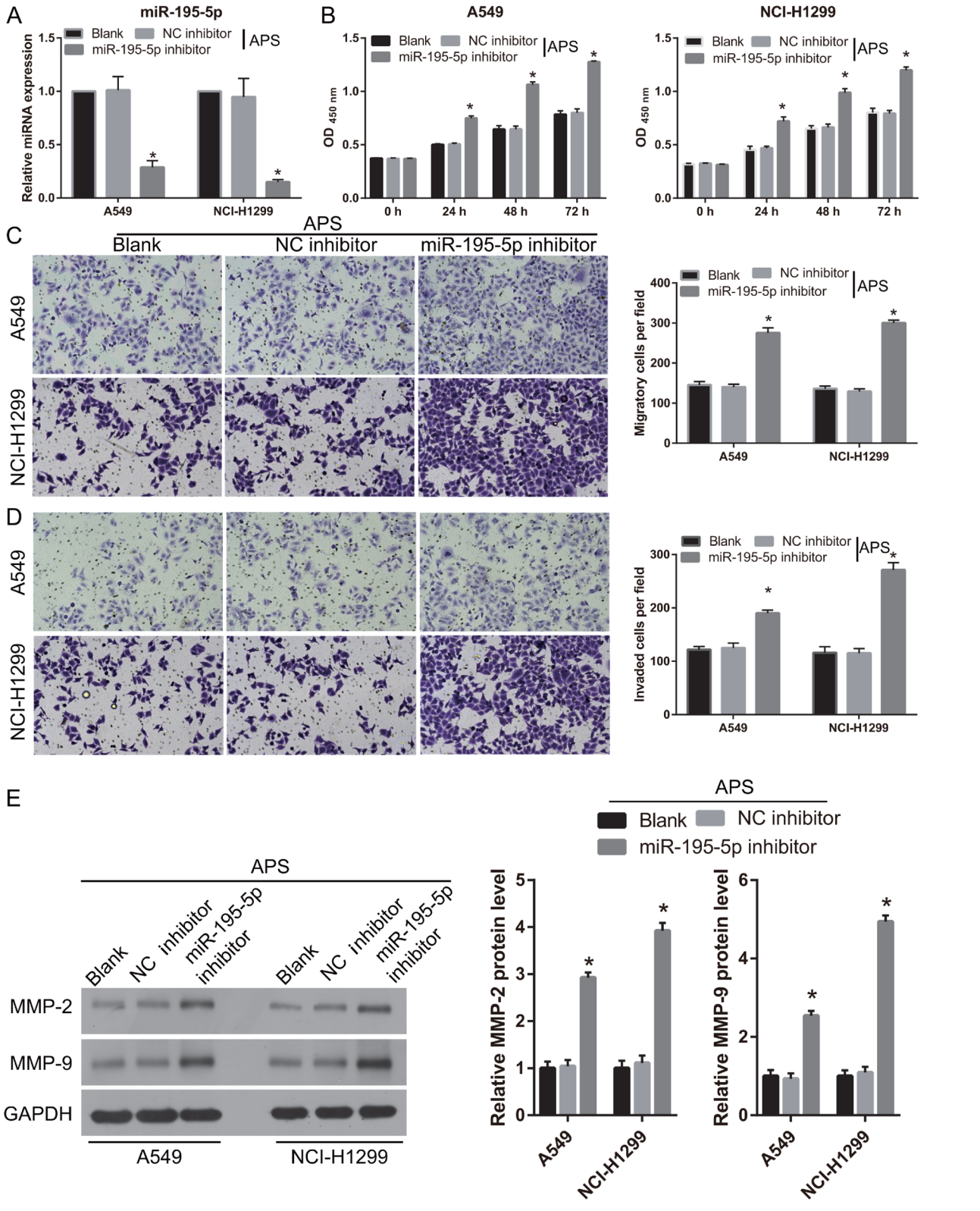

Fig. 5. MiR-195-5p Inhibitor Reversed the Inhibitory Effect of *Astragalus* Polysaccharides on Lung Cancer Cell Migration and Invasion

A, miR-195-5p inhibitor decreased the miR-195-5p expression level in A549 and NCI-H1299 cells; B, miR-195-5p inhibitor increased the proliferation of A549 and NCI-H1299 cells; C, miR-195-5p inhibitor reversed the inhibitory effect of *Astragalus* polysaccharides on lung cancer cell migration; D, miR-195-5p inhibitor reversed the inhibitory effect of *Astragalus* polysaccharides on lung cancer cell invasion. E, Western blotting experiments indicated the miR-195-5p inhibitor reversed the inhibitory effect of *Astragalus polysaccharides* on lung cancer cell migration and invasion related proteins such as MMP-2 and MMP-9. *represents $p < 0.05$, the magnification of the microscope is 100×.

pact on translation process, therefore miRNAs regulate gene expression post-transcriptionally.[33,34] The expression of glycolytic enzyme Hexokinase 2 (HK2) was targeted regulated by miR-143-5p, which has been proved to be downregulated in lung cancer through excessive activation of mammalian target of rapamycin (mTOR) signalling pathway.[35] Similarly, the

expression of miR-124-5p in NSCLC cells exerted its cancer suppressor function by decreasing lung cancer cell proliferation and migration.[36] In addition, it has been demonstrated that the expression of miR-210-3p is elevated in advanced lung cancer cells with hypoxic characteristics.[37] Therefore, several miRNAs have been proved to be involved in regulating the progression and deterioration of lung cancer, and in our experiment, we proved the APS could increase expression level of the miR-195-5p for inhibiting the lung cancer cell invasion and migration.

In this paper, bioinformatic analysis indicated that the high expression of miR-195-5p in lung cancer patients was closely related to the RFS rate. Experiments indicate microRNA-195-5p may regulate lung cancer growth and metastasis through the regulation of Forkhead box k1.[38] Moreover, our experiments showed the APS could obviously increase the miR-195-5p expression level in lung cancer inhibition experiment. MiR-195 has been demonstrated that it could regulate the immune evasion of breast cancer cells *via* targeting CD274,[39] and study have indicated that miR-195-5p promoted cardiac hypertrophy *via* targeting MFN2 and FBXW7.[40] Notably, miR-195-5p inhibitor used in our experiments has been demonstrated it could reverse the inhibitory effect of *Astragalus* polysaccharides on lung cancer cell migration and invasion, and this may provide promising therapeutic strategies for interfering lung cancer.

In summary, we found that APS could inhibit cell proliferation and migration of lung cancer. MiR-195-5p was found to be diferentially expressed in lung cancer tissues and to be closely correlated with prognosis of patients, which could be up-regulated by the APS in lung cancer cells. Our results firstly demonstrated the relationship between miR-195-5p expression level with APS used in lung cancer cell. Therefore, our experimental research results provide new mechanistic insights into the functions of miRNAs as potential therapeutic targets for lung cancer treatment.

Acknowledgments This research was funded by the University Natural Science Research Project of the Department of Education of Anhui Province (KJ2019A0665, KJ2017ZD36), the Suzhou University Professor (PhD) Research Startup Fund (2019jb22), and Suzhou University Scientific Research Platform Open Project (2020ykf20). Funders are not involved in research design, data collection and analysis, publication decisions, or manuscript preparation. Funders are not involved in research design, data collection and analysis, publication decisions, or manuscript preparation.

The research funded by the Provincial Natural Science Research Project of Anhui Colleges (KJ2019A0665, KJ2017ZD36), the Suzhou University Professor (PhD) Research Startup Fund (2019jb22), and Suzhou University Scientific Research Platform Open Project (2020ykf20).

Author Contributions Study concept and design: XKT, FF. Acquisition of data: XKT, XTZ. Analysis and interpretation of data: XKT, FF. First draft of the manuscript: XKT, FF. Statistical analysis: XKT, FF. All authors have read and approved the final version of this manuscript.

Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials This article contains supplementary materials.

REFERENCES

1) Wang YS, Zhang QF, Chen YC, Liang CL, Liu HZ, Qiu FF, Dai ZH. Antitumor effects of immunity-enhancing traditional Chinese medicine. *Biomed. Pharmacother.*, **121**, 109570 (2020).
2) Zheng Y, Ren W, Zhang L, Zhang Y, Liu D, Liu Y. A review of the pharmacological action of *Astragalus* polysaccharide. *Front. Pharmacol.*, **11**, 349 (2020).
3) Chen W, Sun QL, Ju J, Chen WJ, Zhao XL, Zhang Y, Yang YH. Effect of *Astragalus* polysaccharides on cardiac dysfunction in db/db mice with respect to oxidant stress. *Biomed Res. Int.*, **2018**, 8359013 (2018).
4) Wei W, Li ZP, Bian ZX, Han QB. *Astragalus* polysaccharide RAP induces macrophage phenotype polarization to M1 *via* the notch signaling pathway. *Molecules*, **24**, 2016 (2019).
5) Graziani V, Esposito A, Scognamiglio M, Chambery A, Russo R, Ciardiello F, Troiani T, Potenza N, Fiorentino A, D'Abrosca B. Spectroscopic characterization and cytotoxicity assessment towards human colon cancer cell lines of acylated cycloartane glycosides from Astragalus boeticus L. *Molecules*, **24**, 1725 (2019).
6) Wang YM, Liu L, Ma YK, Guo LN, Sun Y, Liu Q, Liu JC. Chemical discrimination of *Astragalus* mongholicus and *Astragalus* membranaceus based on metabolomics using UHPLC-ESI-Q-TOF-MS/MS approach. *Molecules*, **24**, 4064 (2019).
7) Guo MF, Dai YJ, Gao JR, Chen PJ. Uncovering the mechanism of *Astragalus* membranaceus in the treatment of diabetic nephropathy based on network pharmacology. *J. Diabetes Res.*, **2020**, 5947304 (2020).
8) Liu J, Nile SH, Xu G, Wang Y, Kai G. Systematic exploration of *Astragalus membranaceus* and *Panax ginseng* as immune regulators: insights from the comparative biological and computational analysis. *Phytomedicine*, **86**, 153077 (2021).
9) Zhou Y, Hong T, Tong L, Liu W, Yang XT, Luo JH, Wang FL, Li J, Yan LJ. *Astragalus* polysaccharide combined with 10-hydroxycamptothecin inhibits metastasis in non-small cell lung carcinoma cell lines *via* the MAP4K3/mTOR signaling pathway. *Int. J. Mol. Med.*, **42**, 3093–3104 (2018).
10) Wu Y, Han C, Wang Z, Gong L, Liu J, Chong Y, Liu X, Liang N, Li S. An externally-validated dynamic nomogram based on clinicopathological characteristics for evaluating the risk of lymph node metastasis in small-size non-small cell lung cancer. *Front. Oncol.*, **10**, 1322 (2020).
11) Uchida M, Yamaguchi Y, Hosomi S, Ikesue H, Mori Y, Maegawa N, Takano A, Sato Y, Hosohata K, Muroi N, Tomii K, Hashida T, Nakamura T. Risk factors for febrile neutropenia induced by docetaxel chemotherapy in patients with non-small cell lung cancer. *Biol. Pharm. Bull.*, **43**, 1235–1240 (2020).
12) Chatterjee A, Rodger EJ, Eccles MR. Epigenetic drivers of tumourigenesis and cancer metastasis. *Semin. Cancer Biol.*, **51**, 149–159 (2018).
13) Shimoda M, Moroishi T. The emerging link between the Hippo pathway and non-coding RNA. *Biol. Pharm. Bull.*, **43**, 1–10 (2020).
14) Lelli D, Pedone C, Majeed M, Sahebkar A. Curcumin and lung cancer: the role of microRNAs. *Curr. Pharm. Des.*, **23**, 3440–3444 (2017).
15) Liu M, Meng Y, He K, Luan C. Hsa_circ_0002060 Knockdown ameliorates osteoporosis by targeting MiR-198-5p. *Biol. Pharm. Bull.*, **44**, 88–95 (2021).
16) Chen LM, Gibbons DL, Goswami S, *et al.* Metastasis is regulated *via* microRNA-200/ZEB1 axis control of tumour cell PD-L1 expression and intratumoral immunosuppression. *Nat. Commun.*, **5**, 5241 (2014).
17) Huang WC, Jang TH, Tung SL, Yen TC, Chan SH, Wang LH. A

novel miR-365-3p/EHF/keratin 16 axis promotes oral squamous cell carcinoma metastasis, cancer stemness and drug resistance *via* enhancing *β*5-integrin/c-met signaling pathway. *J. Exp. Clin. Cancer Res.*, **38**, 89 (2019).
18) Hu G, Zhao X, Wang J, Lv L, Wang C, Feng L, Shen L, Ren W. miR-125b regulates the drug-resistance of breast cancer cells to doxorubicin by targeting HAX-1. *Oncol. Lett.*, **15**, 1621–1629 (2018).
19) Zhao Y, Qi X, Chen J, Wei W, Yu C, Yan H, Pu M, Li Y, Miao L, Li C, Ren J. The miR-491-3p/Sp3/ABCB1 axis attenuates multidrug resistance of hepatocellular carcinoma. *Cancer Lett.*, **408**, 102–111 (2017).
20) Wang X, Zhu W, Zhao X, Wang P. miR-133a enhances the sensitivity of Hep-2 cells and vincristine-resistant Hep-2v cells to cisplatin by downregulating ATP7B expression. *Int. J. Mol. Med.*, **37**, 1636–1642 (2016).
21) Florczuk M, Szpechcinski A, Chorostowska-Wynimko J. miRNAs as biomarkers and therapeutic targets in non-small cell lung cancer: current perspectives. *Target. Oncol.*, **12**, 179–200 (2017).
22) Fan Y, Ma Z, Zhao L, Wang W, Gao M, Jia X, Ouyang H, He J. Anti-tumor activities and mechanisms of Traditional Chinese medicines formulas: A review. *Biomed. Pharmacother.*, **132**, 110820 (2020).
23) Qiao L, Han M, Gao S, Shao X, Wang X, Sun L, Fu X, Wei Q. Research progress on nanotechnology for delivery of active ingredients from traditional Chinese medicines. *J. Mater. Chem. B*, **8**, 6333–6351 (2020).
24) Howes MR, Fang R, Houghton PJ. Effect of Chinese herbal medicine on Alzheimer's disease. *Int. Rev. Neurobiol.*, **135**, 29–56 (2017).
25) Cassileth BR, Rizvi N, Deng G, Yeung KS, Vickers A, Guillen S, Woo D, Coleton M, Kris MG. Safety and pharmacokinetic trial of docetaxel plus an *Astragalus*-based herbal formula for non-small cell lung cancer patients. *Cancer Chemother. Pharmacol.*, **65**, 67–71 (2009).
26) Song J, Chen Y, He D, Tan W, Lv F, Liang B, Xia T, Li J. *Astragalus* polysaccharide promotes adriamycin-induced apoptosis in gastric cancer cells. *Cancer Manag. Res.*, **12**, 2405–2414 (2020).
27) Yin X, Chen L, Liu Y, Yang J, Ma C, Yao Z, Yang L, Wei L, Li M. Enhancement of the innate immune response of bladder epithelial cells by *Astragalus* polysaccharides through upregulation of TLR4 expression. *Biochem. Biophys. Res. Commun.*, **397**, 232–238 (2010).
28) Zhang P, Wang J, Wang W, Liu X, Liu H, Li X, Wu X. *Astragalus* polysaccharides enhance the immune response to avian infectious bronchitis virus vaccination in chickens. *Microb. Pathog.*, **111**, 81–85 (2017).
29) Ma Q, Xu Y, Tang L, Yang X, Chen Z, Wei Y, Shao X, Shao X, Xin Z, Cai B, Wang Q, Mou S. *Astragalus* polysaccharide attenuates cisplatin-induced acute kidney injury by suppressing oxidative damage and mitochondrial dysfunction. *Biomed Res. Int.*, **2020**, 2851349 (2020).
30) Guo L, Bai SP, Zhao L, Wang XH. *Astragalus* polysaccharide injection integrated with vinorelbine and cisplatin for patients with advanced non-small cell lung cancer: effects on quality of life and survival. *Med. Oncol.*, **29**, 1656–1662 (2012).
31) Li S, Xue X, Yang X, Zhou S, Wang S, Meng J. A network pharmacology approach used to estimate the active ingredients of moutan cortex charcoal and the potential targets in hemorrhagic diseases. *Biol. Pharm. Bull.*, **42**, 432–441 (2019).
32) Que Z, Zhou Z, Luo B, Dong C, Jiang Y, Li H, Tian J. Jingfukang induces anti-cancer activity through oxidative stress-mediated DNA damage in circulating human lung cancer cells. *BMC Complement. Altern. Med.*, **19**, 204 (2019).
33) Saliminejad K, Khorram Khorshid HR, Soleymani Fard S, Ghaffari SH. An overview of microRNAs: biology, functions, therapeutics, and analysis methods. *J. Cell. Physiol.*, **234**, 5451–5465 (2019).
34) Liu Y, Liu X, Yang S. MicroRNA-221 upregulates the expression of P-gp and Bcl-2 by activating the Stat3 pathway to promote doxorubicin resistance in osteosarcoma cells. *Biol. Pharm. Bull.*, **44**, 861–868 (2021).
35) Peschiaroli A, Giacobbe A, Formosa A, Markert EK, Bongiorno-Borbone L, Levine AJ, Candi E, D'Alessandro A, Zolla L, Finazzi Agrò A, Melino G. miR-143 regulates hexokinase 2 expression in cancer cells. *Oncogene*, **32**, 797–802 (2013).
36) Hao C, Xu X, Ma J, Xia J, Dai B, Liu L, Ma Y. MicroRNA-124 regulates the radiosensitivity of non-small cell lung cancer cells by targeting TXNRD1. *Oncol. Lett.*, **13**, 2071–2078 (2017).
37) Ren J, Li X, Dong H, Suo L, Zhang J, Zhang L, Zhang J. miR-210-3p regulates the proliferation and apoptosis of non-small cell lung cancer cells by targeting SIN3A. *Exp. Ther. Med.*, **18**, 2565–2573 (2019).
38) Long Z, Wang Y. miR-195-5p suppresses lung cancer cell proliferation, migration, and invasion Via FOXK1. *Technol. Cancer Res. Treat.*, **19**, 1533033820922587 (2020).
39) Yang L, Cai Y, Zhang D, Sun J, Xu C, Zhao W, Jiang W, Pan C. miR-195/miR-497 regulate CD274 expression of immune regulatory ligands in triple-negative breast cancer. *J. Breast Cancer*, **21**, 371–381 (2018).
40) Wang L, Qin D, Shi H, Zhang Y, Li H, Han Q. MiR-195-5p promotes cardiomyocyte hypertrophy by targeting MFN2 and FBXW7. *Biomed. Res. Int.*, **2019**, 1580982 (2019).