Int J Clin Exp Med 2020;13(5):2956-2965
www.ijcem.com /ISSN:1940-5901/IJCEM0108031

Review Article

miR-128-3p inhibits EMT and invasion of lung carcinoma cells by targeting SIRT1

Wei Chen[1], Kai Lou[2], Kunyu Wang[1], Jichen Zhang[1]

[1]Thoracic and Cardiovascular Surgery, [2]Emergency of Taizhou First People's Hospital, Taizhou 318020, Zhejiang Province, China

Received January 17, 2020; Accepted March 3, 2020; Epub May 15, 2020; Published May 30, 2020

Abstract: Objective: To figure out the function and mechanism of miR-128-3p in the progression of lung carcinoma. Methods: miR-128-3p and SIRT1 expression in lung carcinoma cells A549, H460, H1650, SK-MES-1 and normal lung epithelial cell BEAS-2B were measured with the help of qRT-PCR and Western blot. miR-128-3p over-expression and SIRT1 inhibitory vector were transfected into A549 and H1650 cells, respectively. CCK-8, transwell and flow cytometry were applied to observe cell proliferation, invasion and apoptosis. Changes of epithelial-mesenchymal transition (EMT) markers N-cadherin, Vimentin and E-cadherin were detected. The relationship between miR-128-3p and SIRT1 was determined by means of dual luciferase reporter. Results: miR-128-3p was reduced in the four types of lung carcinoma cells, while SIRT1 was up-regulated. After A549 and H1650 were treated with miR-128-3p over-expression or SIRT1 inhibition, both proliferation and invasion ability of the two were inhibited and their apoptosis increased, and E-cadherin was elevated, while N-cadherin and Vimentin were reduced in cells. The dual luciferase reporter identified a targeted regulatory correlation between miR-128-3p and SIRT1. Conclusion: miR-128-3p can inhibit EMT and invasion of lung carcinoma cells via targeting SIRT1.

Keywords: miR-128-3p, SIRT1, EMT, lung carcinoma

Introduction

Lung carcinoma is the most common cause responsible for carcinoma-related deaths worldwide [1]. Although the treatment of lung carcinoma has been improved with the development of targeted therapy and immunotherapy, its overall prognosis is still unsatisfactory [2]. It is reported that lung carcinoma kills about 1.6 million people every year, and these numbers will continue to grow [3]. Lung carcinoma is characterized by high invasion and metastasis, which is also the main cause of failed treatment and poor prognosis [4]. The potential mechanism of lung carcinoma progression has not been fully elucidated [5]. Therefore, it is urgent to find anti-metastasis drugs with a clear mechanism and explore new targets for lung carcinoma.

microRNA (miR) is a non-coding small RNA that is widely distributed in eukaryotic cells and plays a pivotal role in a variety of cellular biological events [6, 7]. Mis-expression or mutation of miR is believed to be responsible for the occurrence and development of many human diseases [8]. miR-128-3p is located on chromosome 2 and is approximately 81 bp in length. miR-128-3p plays a vital role as a tumor suppressor in a variety of carcinomas such as esophageal squamous cell carcinoma, liver carcinoma and breast carcinoma [9-11]. In lung carcinoma, down-regulated miR-128-3p implies a poor prognosis [12]. The mechanism of action of miR-128-3p in lung carcinoma has not been fully elucidated. We found targeted binding sites between miR-128-3p and SIRT1 through an online biological prediction website Targetscan. SIRT1 is a class III histone deacetylase that regulates basic biological functions such as cell survival, growth, and aging [13]. SIRT1 can be targeted and regulated by multiple miRs to participate in tumor development; for example, SIRT1 can be targeted and regulated by miR-30a to prevent the progression of lung carcinoma [14]. SIRT1 can also be targeted by miR-34a to suppress the develop-

ment of prostate cancer [15]. Epithelial-mesenchymal transition (EMT) is a basic biological event that is closely associated with tumor metastasis [16].

Therefore, we hypothesized that miR-128-3p inhibits EMT and invasion of lung carcinoma cells by targeting SIRT1. In order to demonstrate this hypothesis, the following experiments were conducted.

Materials and methods

Cells and treatments

Lung carcinoma cells A549, H460, H1650, SK-MES-1 and normal lung epithelial cells BEAS-2B were from ATCC in the United States. The above five cell lines were placed in RPMI1640 medium (Gibco, USA) with 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin-streptomycin (100 ×, Solarbio, USA) separately, and cultured in an incubator at 37°C and 5% CO_2. When the density of adherent growth of cells reached to 80-90%, it was digested with trypsin, and passaged every 2 d. Cell transfection: Cells were transfected using Lipofectamine™ 2000 kit (Thermo Scientific, USA). miR-128-3p over-expression plasmids (miR-128-3p-mimics), SIRT1 inhibitory plasmids (si-SIRT1), and blank controls (miR-NC, si-NC) were established using pcDNA 3.1 plasmid as a vector and then transfected into cells. After 6 h, the infected cells were placed in new medium and cultured for 48 h.

qRT-PCR detection (qPCR)

TRIzol kit (Wuhan Chundu Biotechnology Co., Ltd.) was applied to extract the total RNA of target cells. UV spectrophotometer and agglutinate gel electrophoresis were applied to detect the purity, concentration and integrity of extracted total RNA. Five μL total RNA was taken and reverse-transcribed. The operation was conducted in accordance with the instructions of Reverse Transcription kit (Invitrogen, USA) to obtain cDNA for subsequent research. PCR amplification was conducted by means of the PrimeScript RT Master Mix kit (Takara Bio, Japan). The amplification system was as follows: 10 μL SYBR qPCR Mix, 0.8 μL of upstream primers, 0.8 μL downstream primers, 2 μL cDNA product, 0.4 μL 50 × ROX reference dye, and finally RNase-free water was added to supplement to 20 μL. PCR reaction conditions were as follows: pre-denaturation at 95°C for 60 s, denaturation at 95°C for 30 s, annealing extension at 60°C for 40 s, with a total of 40 cycles. This data was analyzed using $2^{-\Delta\Delta ct}$ [17]. Primers were designed and synthesized by Shanghai Generay Biotech Co., Ltd. miR-128-3p upstream: 5'-CGAGAGCTGGTGTTGTGAAT-3', downstream: 5'-GTGCAGGGTCCGAGGTAT-3'. U6 upstream: 5'-GTAGCGTCGTGAAGCGTTC-3', downstream: 5'-GTGCAGGGTCCGAGGTAT-3'.

Western blot assay (WB)

RIPA lysis (Thermo Scientific, USA) was put into the cells in each group after culture to extract total protein. Centrifugation of lysate was performed in a 4°C centrifuge at 12000 × g for 20 min. The supernatant was taken and the concentration of protein was detected by BCA (Thermo Scientific, USA). Twelve% SDS-PAGE was applied for protein electrophoretic separation and then transferred to a PVDF membrane (Life Technologies, USA). It was soaked in PBST for 5 min for washing, and blocked with 5% skim milk powder for 4 h at indoor temperature. SIRT1, E-cadherin, N-cadherin, Vimentin, and β-actin (1:1000) primary antibodies (Abcam, USA) were put incubated with membrane and sealed at 4°C for one night. The primary antibody was removed through washing the membrane, and goat anti-rabbit secondary antibody (Abcam, USA) was added (1:2000), incubated at 37°C for 1 h, then rinsed with TBST for 3 times, with 5 min each. ECL was used to illuminate and develop in a dark room. Quantity One software was utilized for statistical analysis of the bands after film scanning. Relative expression of the protein = target protein band grayscale value/β-actin protein band.

CCK-8 proliferation detection

Cell proliferation was measured using CCK-8 (Beyotime Biological Co., Ltd., China). The procedure was conducted in accordance with the kit instructions, and the specifics were as follows: after 24 h of transfection, the cells were cultured in a 96-well plate with a cell density of 2.5×10^3 per well. CCK-8 (10 μl) was put into the well at 24 h, 48 h, 72 h and 96 h, and the plate was incubated at room temperature for 2 hours. Then the absorbance of each well was detected by microplate reader (Molecular

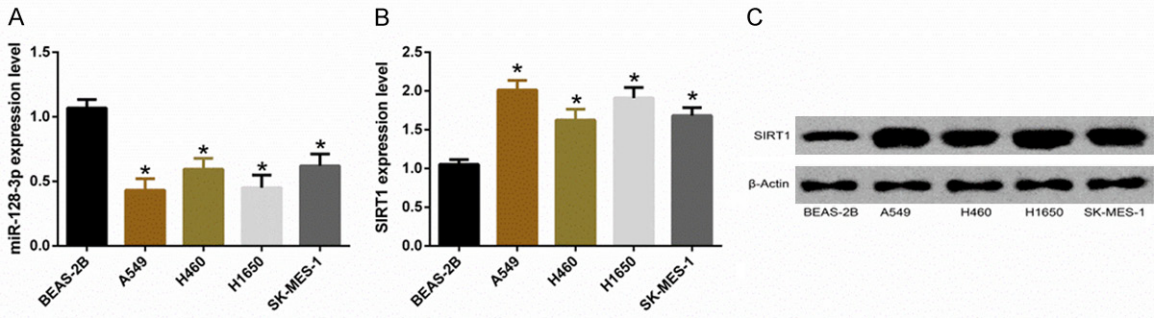

Figure 1. Expression of miR-128-3p and SIRT1 in lung carcinoma. A. miR-128-3p decreased in lung carcinoma cells. B. SIRT1 increased in lung carcinoma cells. C. WB image. *denotes P<0.05.

Devices, USA) at 490 nm, and the corresponding growth curve was drawn.

Cell invasion detection

Transwell kit (Gibco, USA) was applied for invasion detection and separated to an upper and lower compartment. The lower compartment had 500 μL of medium (comprising 10% FBS), and the upper compartment had serum-free medium containing 5 * 10^4 cells. After 48 h of incubation at 37°C, cells in the upper compartment that did not penetrate the membrane surface were removed, and fixed with paraformaldehyde for 10 min. Then excess paraformaldehyde was washed with double distilled water. After drying, the cells were stained with 0.5% crystal violet, and the cells that did not penetrate the membrane were counted.

Cell apoptosis detection

Transfected cells were collected and digested with 0.25% trypsin. After digestion, they were configured into 1 * 10^6/mL suspension. Annexinv-fitc/PI (Invitrogen, USA) was successively added. Incubation of cells was conducted at indoor temperature in the dark for 5 min, and FCM system FC500MCL was applied for detection.

Dual luciferase reporter

Lipofectamine™ 2000 kit was used to clone SIRT1 into pmirGLO dual luciferase target expression vector. SIRT1-3' UTR wild type (Wt) and SIRT1-3' UTR mutant (Mut) were established and transferred to downstream of the luciferase reporter gene to identify the sequencing of constructed plasmids. Subsequently, the correctly sequenced plasmids were co-transfected into HEK293T cells, and the luciferase activity was measured 48 h later with the help of dual luciferase reporter assay kit (Promega, USA).

Statistical analysis

Sample data of this study were obtained from three repeated independent experiments. SPSS 18.0 software package was used for data analysis and GraphPad 7 software package was used to draw create the required images. Data were expressed as mean ± standard deviation (Meas ± SD). Independent sample t test was utilized for intragroup comparison, one-way ANOVA was used for intergroup comparison. LSD-t test was used for pair-wise comparison afterwards. Repeated measurement ANOVA was used for expression of multiple time points. Bonferroni was used for back testing. When P<0.05, statistical difference was indicated.

Results

miR-128-3p is down-regulated and SIRT1 is increased in four types of lung carcinoma cells

In this study, qPCR and WB were applied to measure miR-128-3p and SIRT1 expression in lung carcinoma cell lines H460, SK-MES-1, H1650, A549 and BEAS-2B in normal lung epithelial cells. It was seen that the miR-128-3p expression decreased in the four types of lung carcinoma cells, while SIRT1 expression increased. Shown in **Figure 1**.

miR-128-3p can inhibit the progression of lung carcinoma

For the purpose of determining the effects of miR-128-3p in lung carcinoma, A549 and

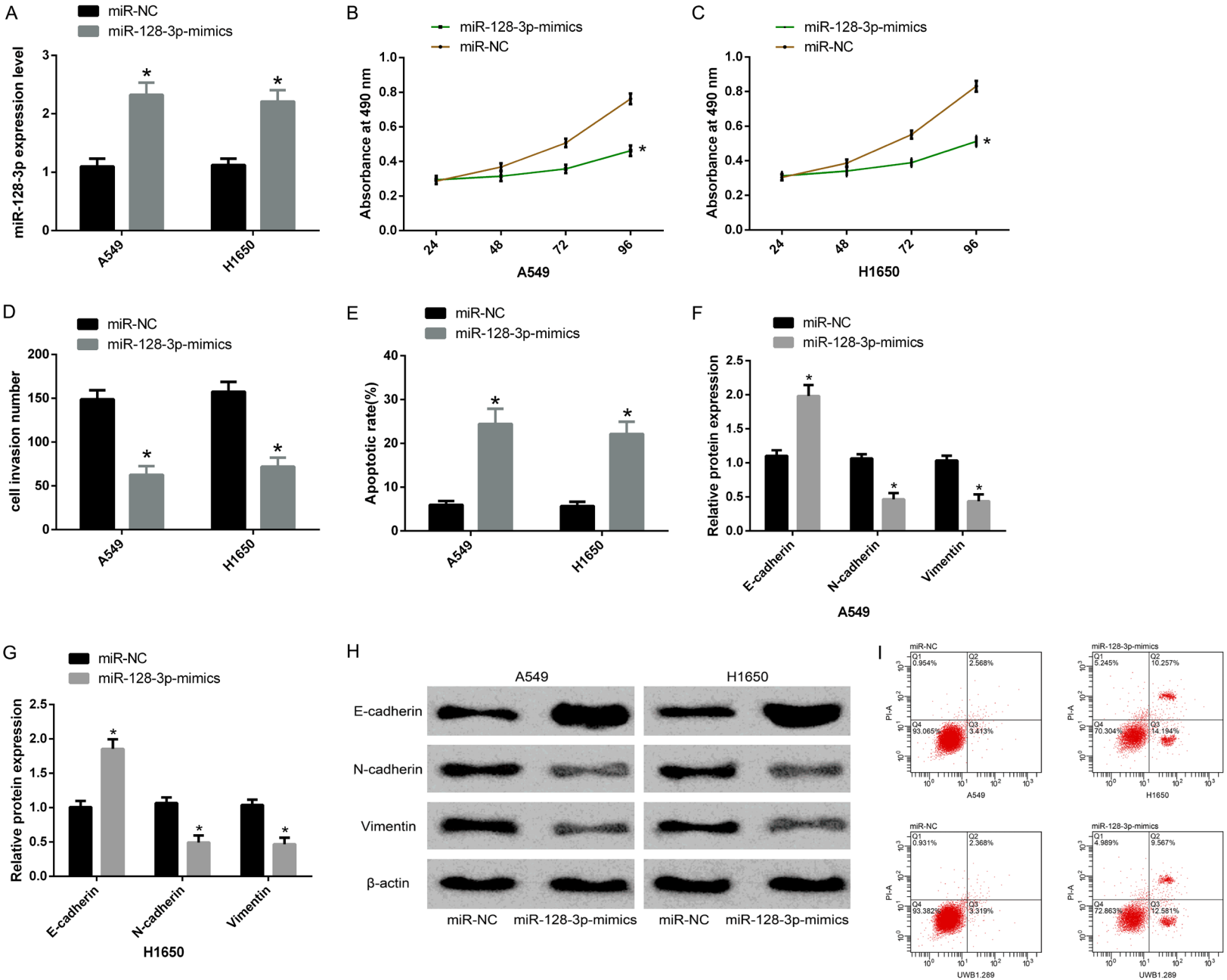
A
miR-NC
miR-128-3p-mimics
miR-128-3p expression level
A549
H1650
B
miR-128-3p-mimics
miR-NC
Absorbance at 490 nm
A549
C
miR-128-3p-mimics
miR-NC
Absorbance at 490 nm
H1650
D
miR-NC
miR-128-3p-mimics
cell invasion number
A549
H1650
E
miR-NC
miR-128-3p-mimics
Apoptotic rate(%)
A549
H1650
F
miR-NC
miR-128-3p-mimics
Relative protein expression
E-cadherin
N-cadherin
Vimentin
A549
G
miR-NC
miR-128-3p-mimics
Relative protein expression
E-cadherin
N-cadherin
Vimentin
H1650
H
A549
H1650
E-cadherin
N-cadherin
Vimentin
β-actin
miR-NC
miR-128-3p-mimics
miR-NC
miR-128-3p-mimics
I
miR-NC
Q1 0.954%
Q2 2.568%
Q4 93.065%
Q3 3.413%
A549
miR-128-3p-mimics
Q1 5.245%
Q2 10.257%
Q4 70.304%
Q3 14.194%
H1650
miR-NC
Q1 0.931%
Q2 2.368%
Q4 93.382%
Q3 3.319%
UWB1.289
miR-128-3p-mimics
Q1 4.989%
Q2 9.567%
Q4 72.863%
Q3 12.581%
UWB1.289

Figure 2. Effects of elevated miR-128-3p on the biological function of lung carcinoma cells. A. miR-128-3p was increased in A549 and H1650 cells after transfection with miR-128-3p-mimics. B, C. Invasion was inhibited after transfection of miR-128-3p-mimics in A549 and H1650 cells. D. Proliferation was inhibited after transfection of miR-128-3p-mimics in A549 and H1650 cells. E. Apoptosis rate was increased after transfection of miR-128-3p-mimics in A549 and H1650 cells. F, G. E-cadherin increased while N-cadherin and Vimentin decreased after transfection of miR-128-3p-mimics in A549 and H1650 cells. H. WB image. I. Flow cytometry. *denotes P<0.05.

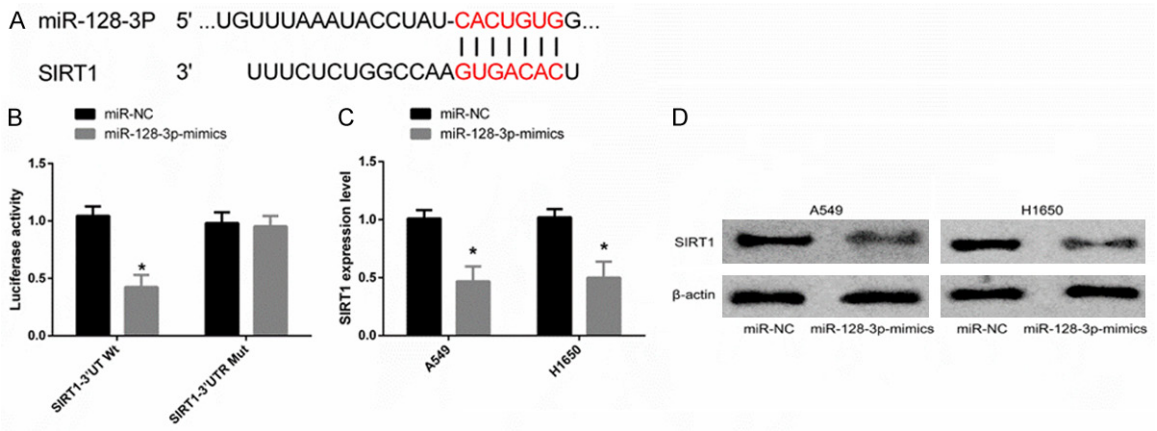

Figure 3. Exploration of relationship between SIRT1 and miR-128-3p. A. Binding sites existed between SIRT1 and miR-128-3p. B. Transfection of miR-128-3p-mimics could inhibit luciferase activity of SIRT1-3' UT Wt in HEK293T without affecting luciferase activity of SIRT1-3' UTR Mut. C. SIRT1 was inhibited after transfection of miR-128-3p-mimics in A549 and H1650 cells. D. WB image. *denotes P<0.05.

H1650 cells (miR-128-3p was the least expressed in these two) were transfected with miR-128-3p-mimics, and the proliferation, invasion and apoptosis of transfected cells were observed with the help of CCK-8, transwell and flow cytometry. The results exhibited that miR-128-3p was increased in A549 and H1650 cells after transfection of miR-128-3p-mimics, and the proliferation and invasion ability of A549 and H1650 were inhibited after transfection, with increased apoptosis rate. Later, we detected the impact of miR-128-3p-mimics transfection on EMT markers in A549 and H1650 cells by WB, and found that E-cadherin reduced, N-cadherin and Vimentin decreased. As shown in **Figure 2**.

SIRT1 is the downstream target of miR-128-3p

For the purpose of understanding the mechanism by which miR-128-3p inhibits the progression of lung carcinoma, we predicted through the Targetscan website that there was a binding site between SIRT1 and miR-128-3p. Therefore, in this paper, dual luciferase activity was detected to verify the correlation between them. The report showed that transfection of miR-128-3p-mimics could inhibit SIRT1-3' UT Wt luciferase activity in HEK293T cells without affecting SIRT1-3' UTR Mut luciferase activity; suggesting that SIRT1 can be used as the target gene for miR-128-3p. Subsequently, it was found through WB that SIRT1 was inhibited after A549 and H1650 cells were transfected with miR-128-3p-mimics; further proving that SIRT1 could be targeted by miR-128-3p. As shown in **Figure 3**.

SIRT1 can promote the progression of lung carcinoma

SIRT1 was previously found to be decreased in the four types of lung carcinoma cells purchased, suggesting that it might have the ability to inhibit the progression of lung carcinoma. To investigate the role of SIRT1 in lung carcinoma, si-SIRT1 was transfected into A549 and H1650 cells to observe the changes in cell biological functions. The results showed that si-SIRT1 transfection could reduce SIRT1 in A549 and H1650 cells. Also proliferation and invasion of A549 and H1650 cells after transfection were inhibited, and their apoptosis rate increased. WB detected that si-SIRT1 transfection could also increase E-cadherin in A549

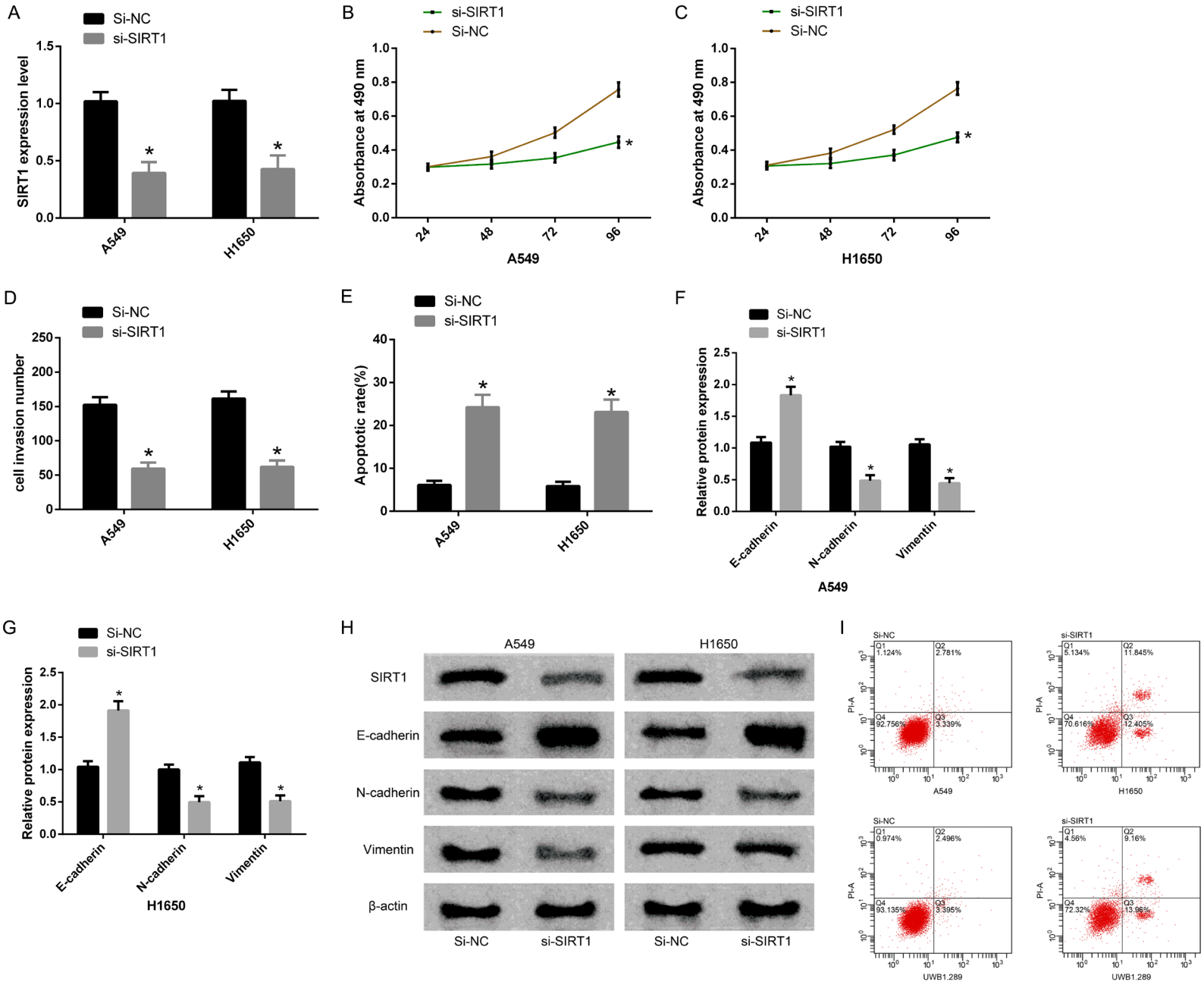
A
Si-NC
si-SIRT1
SIRT1 expression level
A549
H1650
B
si-SIRT1
Si-NC
Absorbance at 490 nm
A549
C
si-SIRT1
Si-NC
Absorbance at 490 nm
H1650
D
Si-NC
si-SIRT1
cell invasion number
A549
H1650
E
Si-NC
si-SIRT1
Apoptotic rate(%)
A549
H1650
F
Si-NC
si-SIRT1
Relative protein expression
E-cadherin
N-cadherin
Vimentin
A549
G
Si-NC
si-SIRT1
Relative protein expression
E-cadherin
N-cadherin
Vimentin
H1650
H
A549
H1650
SIRT1
E-cadherin
N-cadherin
Vimentin
β-actin
Si-NC
si-SIRT1
Si-NC
si-SIRT1
I
Si-NC
si-SIRT1
A549
H1650
UWB1.289

Figure 4. Effect of reduced SIRT1 on the biological function of lung carcinoma cells. A. miR-128-3p increased after transfection of si-SIRT1 in A549 and H1650 cells. B, C. Proliferation was inhibited after transfection of si-SIRT1 in A549 and H1650 cells. D. Invasion was inhibited after transfection with si-SIRT1 in A549 and H1650 cells. E. Apoptosis rate increased after transfection of SIRT1 in A549 and H1650 cells. F, G. E-cadherin increased while N-cadherin and Vimentin decreased and after transfection of SIRT1 in A549 and H1650 cells. H. WB image. I. Flow cytometry. *denotes $P<0.05$.

and H1650 cells, and down-regulate N-cadherin and Vimentin. As shown in **Figure 4**.

Rescue experiment

On the basis of above results, it could be preliminarily hypothesized that miR-128-3p could prevent the occurrence and development of lung carcinoma through targeting SIRT1. In order to further understand the connection between the two, we over-expressed miR-128-3p and SIRT1 in A549 and H1650 cells at the same time to observed their biological changes. It was found that the proliferation, invasion, and apoptosis of A549 and H1650 cells after transfection with miR-128-3p-mimics+sh-SIRT1 were not statistically different from those of cells transfected with miR-NC; while the proliferation and invasion ability increased and the apoptosis rate decreased compared with the cells transfected miR-128-3p-mimics. Then, changes of EMT markers of the above three groups were detected using WB. The results of WB exhibited that the expression of EMT markers in A549 and H1650 cells after transfection with miR-128-3p-mimics+sh-SIRT1 was not different from that of those transfected with miR-NC cells, while E-cadherin was reduced and N-cadherin and Vimentin was elevated compared with the cells in miR-128-3p-mimics. As shown in **Figure 5**.

Discussion

High metastasis is the main cause of poor prognosis of lung carcinoma, however, the mechanism of lung carcinoma metastasis remains poorly understood. Therefore, to elucidate the pathogenesis of lung carcinoma is a key step to improve the prognosis of patients. miR plays a role of tumor suppressor and oncogene by regulating its target genes in carcinomas, showing a great application prospect [18]. Previously, studies have found that miR-128-3p down-regulation predicts a poor prognosis for patients with lung carcinoma [12], and this study also revealed that miR-128-3p down-regulation in the four types of lung carcinoma cells, indicating that it is involved with lung carcinoma. Later, cell experiments revealed that elevated miR-128-3p could suppress the proliferation and invasion of A549 and H1650 cells and promote their apoptosis, indicating that miR-128-3p acted as a tumor suppressor in lung carcinoma. However, it remains unclear how miR-128-3p achieves this effect.

miRs participate in cell biology events by binding to the 3' UTR of the mRNA sequence of its target gene to promote transcriptional degradation [19]. A promising binding site between miR-128-3p and SIRT1 was found through an online biological prediction software Targetscan. It was also confirmed that SIRT1 could be used as target gene of miR-128-3p through dual luciferase reporter. SIRT1 exerts its biological role by acetylating histones and non-histones [20]. It has been reported that SIRT1 is elevated in lung carcinoma and promotes the development of the disease [21]. SIRT1 was discovered to be highly expressed in four types of lung carcinoma cells. Cell experiments showed that inhibition of SIRT1 could suppress proliferation and invasion of A549 and H1650 cells, and promote their apoptosis, which was similar to the results of the above studies. In this study, the association between miR-128-3p and SIRT1 was further explored through rescue experiments. The results elaborated that proliferation, invasion and apoptosis of A549 and H1650 cells transfected with miR-128-3p-mimics+sh-SIRT1 appeared no different compared with the those cells transfected with miR-NC, while the proliferation and invasion ability increased and the apoptosis rate decreased compared with those cells transfected with miR-128-3p-mimics. These findings can prove that miR-128-3p can prevent the progression of lung carcinoma through SIRT1.

EMT is an important factor affecting tumor growth, invasion and metastasis, among which miR is an important regulator [22]. Some stud-

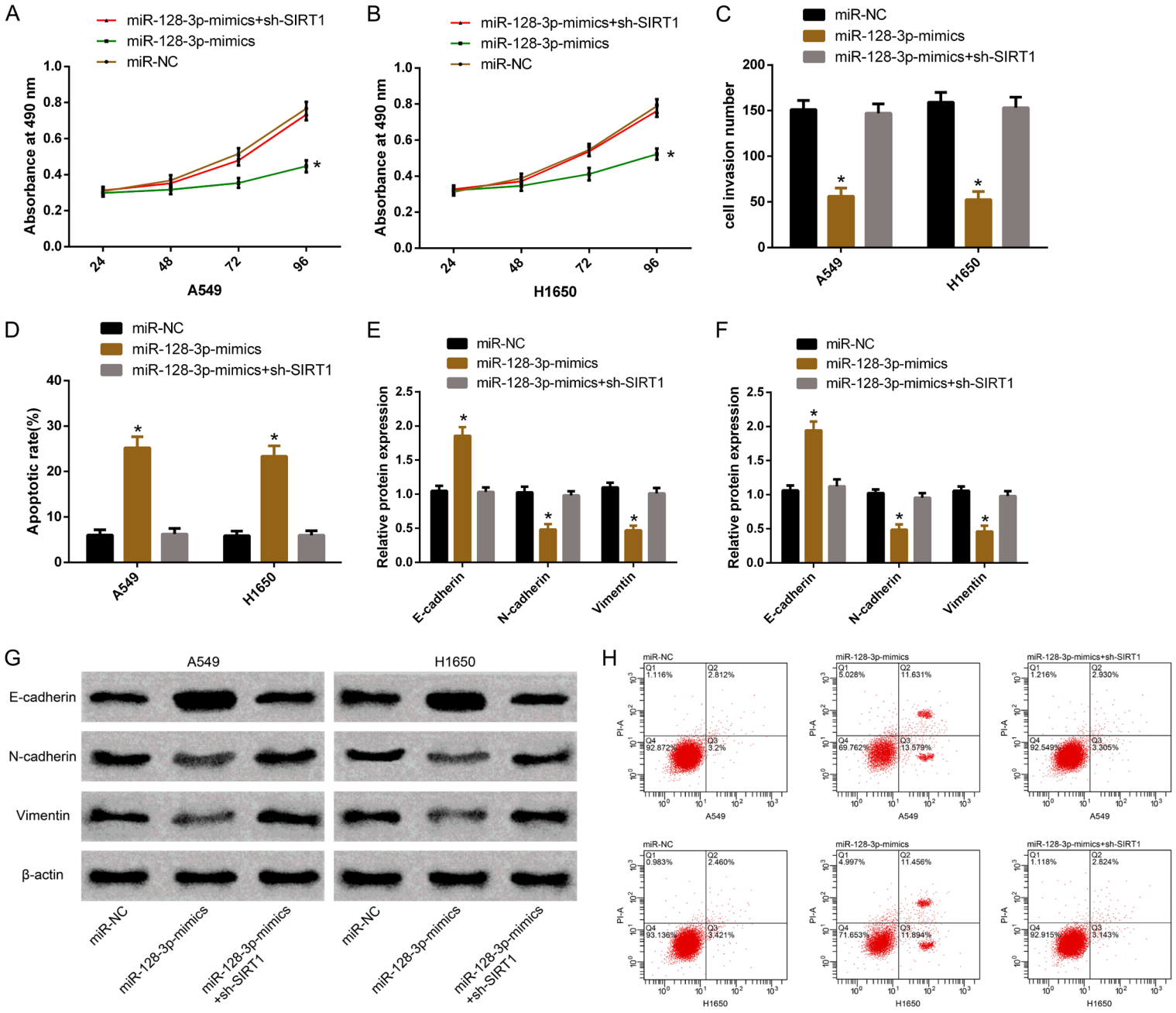
A
miR-128-3p-mimics+sh-SIRT1
miR-128-3p-mimics
miR-NC
Absorbance at 490 nm
A549
B
H1650
C
cell invasion number
D
Apoptotic rate(%)
E
Relative protein expression
E-cadherin
N-cadherin
Vimentin
F
G
β-actin
H
Q1
Q2
Q3
Q4
PI-A

Figure 5. Effects of co-transfection of miR-128-3p-mimics+sh-SIRT1 on the biological function of lung carcinoma. A, B. Proliferation of A549 and H1650 transfected with miR-128-3p-mimics+sh-SIRT1 showed no statistical difference compared with those transfected with miR-NC cells, but was increased compared with those cells transfected with miR-128-3p-mimics. C. Invasion of A549 and H1650 cells transfected with miR-128-3p-mimics+sh-SIRT1 showed no difference compared with those transfected with miR-NC cells, but was increased compared with those cells transfected with miR-128-3p-mimics. D. Apoptosis of A549 and H1650 transfected with miR-128-3p-mimics+sh-SIRT1 showed no difference compared with those transfected with miR-NC cells, but was increased compared with those cells transfected with miR-128-3p-mimics. E, F. Expression of E-cadherin, N-cadherin, and Vimentin transfected with miR-128-3p-mimics+sh-SIRT1 showed no difference compared with those transfected with miR-NC cells, but E-cadherin was increased and N-cadherin and Vimentin were increased compared with those cells transfected with miR-128-3p-mimics. G. WB image. H. Flow cytometry. *denotes $P<0.05$.

ies support that SIRT1 promotes the process of EMT in a variety of tumor cells [23-25]. This paper has demonstrated that SIRT1 can be targeted by miR-128-3p to prevent the progression of lung carcinoma. Therefore, we hypothesized that miR-128-3p can also influence the EMT process of lung carcinoma cells through SIRT1. We inquired into the effects of miR-128-3p and SIRT1 on EMT in lung carcinoma cells by detecting the changes of EMT markers in this study. The results showed that increasing miR-128-3p or decreasing SIRT1 can up-regulate E-cadherin or down-regulate N-cadherin and Vimentin in A549 and H1650 cells. What's more, miR-128-3p-mimics+sh-SIRT1 was found to prevent changes of E-cadherin, N-cadherin, and Vimentin caused by transfection of miR-128-3p-mimics. This indicates that miR-128-3p can also inhibit lung carcinoma EMT through SIRT1.

This study proved miR-128-3p prevents metastasis of lung carcinoma through targeting SIRT1, reflecting the promising value of the miR-128-3p/SIRT1 axis in treating lung carcinoma. Another study has found that SIRT1 can regulate EMT process in oral cancer through Smad4/MMP7 pathway [26]. Therefore, we hypothesized that miR-128-3p inhibits EMT and invasion of lung carcinoma through the SIRT1/Smad4/MMP7 pathway, which will be explored in future experimental designs. In addition, this paper only discussed the functions of miR-128-3p/SIRT1 axis in lung carcinoma *in vitro,* and *in vivo* experiments will be performed in subsequent studies to improve the regulatory network of this axis.

In conclusion, miR-128-3p can inhibit EMT and invasion of lung carcinoma cells through SIRT1, providing experimental support for miR-128-3p as a therapeutic target for lung carcinoma.

Disclosure of conflict of interest

None.

Address correspondence to: Jichen Zhang, Thoracic and Cardiovascular Surgery of Taizhou First People's Hospital, No. 218 Hengjie Road, Huangyan District, Taizhou 318020, Zhejiang Province, China. E-mail: zhanjieji74714@163.com

References

[1] Sperduto PW, Yang TJ, Beal K, Pan H, Brown PD, Bangdiwala A, Shanley R, Yeh N, Gaspar LE, Braunstein S, Sneed P, Boyle J, Kirkpatrick JP, Mak KS, Shih HA, Engelman A, Roberge D, Arvold ND, Alexander B, Awad MM, Contessa J, Chiang V, Hardie J, Ma D, Lou E, Sperduto W and Mehta MP. Estimating survival in patients with lung cancer and brain metastases: an update of the graded prognostic assessment for lung cancer using molecular markers (lung-molGPA). JAMA Oncol 2017; 3: 827-831.

[2] Zappa C and Mousa SA. Non-small cell lung cancer: current treatment and future advances. Transl Lung Cancer Res 2016; 5: 288-300.

[3] Didkowska J, Wojciechowska U, Manczuk M and Lobaszewski J. Lung cancer epidemiology: contemporary and future challenges worldwide. Ann Transl Med 2016; 4: 150.

[4] Tian L, Shen D, Li X, Shan X, Wang X, Yan Q and Liu J. Ginsenoside Rg3 inhibits epithelial-mesenchymal transition (EMT) and invasion of lung cancer by down-regulating FUT4. Oncotarget 2016; 7: 1619-1632.

[5] Chen L, Nan A, Zhang N, Jia Y, Li X, Ling Y, Dai J, Zhang S, Yang Q, Yi Y and Jiang Y. Circular RNA 100146 functions as an oncogene through direct binding to miR-361-3p and miR-615-5p in non-small cell lung cancer. Mol Cancer 2019; 18: 13.

[6] Thomson DW and Dinger ME. Endogenous microRNA sponges: evidence and controversy. Nat Rev Genet 2016; 17: 272-283.

[7] Zhao L, Yu H, Yi S, Peng X, Su P, Xiao Z, Liu R, Tang A, Li X, Liu F and Shen S. The tumor sup-

pressor miR-138-5p targets PD-L1 in colorectal cancer. Oncotarget 2016; 7: 45370-45384.
[8] Li G, Luo J, Xiao Q, Liang C and Ding P. Predicting microRNA-disease associations using label propagation based on linear neighborhood similarity. J Biomed Inform 2018; 82: 169-177.
[9] Zhao L, Li R, Xu S, Li Y, Zhao P, Dong W, Liu Z, Zhao Q and Tan B. Tumor suppressor miR-128-3p inhibits metastasis and epithelial-mesenchymal transition by targeting ZEB1 in esophageal squamous-cell cancer. Acta Biochim Biophys Sin (Shanghai) 2018; 50: 171-180.
[10] Yu D, Green B, Marrone A, Guo Y, Kadlubar S, Lin D, Fuscoe J, Pogribny I and Ning B. Suppression of CYP2C9 by microRNA hsa-miR-128-3p in human liver cells and association with hepatocellular carcinoma. Sci Rep 2015; 5: 8534.
[11] Zhao J, Li D and Fang L. MiR-128-3p suppresses breast cancer cellular progression via targeting LIMK1. Biomed Pharmacother 2019; 115: 108947.
[12] Pan J, Zhou C, Zhao X, He J, Tian H, Shen W, Han Y, Chen J, Fang S, Meng X, Jin X and Gong Z. A two-miRNA signature (miR-33a-5p and miR-128-3p) in whole blood as potential biomarker for early diagnosis of lung cancer. Sci Rep 2018; 8: 16699.
[13] Maiese K. SIRT1 and stem cells: in the forefront with cardiovascular disease, neurodegeneration and cancer. World J Stem Cells 2015; 7: 235-242.
[14] Guan Y, Rao Z and Chen C. miR-30a suppresses lung cancer progression by targeting SIRT1. Oncotarget 2018; 9: 4924-4934.
[15] Duan K, Ge YC, Zhang XP, Wu SY, Feng JS, Chen SL, Zhang LI, Yuan ZH and Fu CH. miR-34a inhibits cell proliferation in prostate cancer by downregulation of SIRT1 expression. Oncol Lett 2015; 10: 3223-3227.
[16] Wang D, Shi W, Tang Y, Liu Y, He K, Hu Y, Li J, Yang Y and Song J. Prefoldin 1 promotes EMT and lung cancer progression by suppressing cyclin A expression. Oncogene 2017; 36: 885-898.
[17] Livak KJ and Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2 (-Delta Delta C (T)) method. Methods 2001; 25: 402-408.
[18] Rupaimoole R and Slack FJ. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. Nat Rev Drug Discov 2017; 16: 203-222.
[19] Gareev IF, Novicova LB and Beylerli OA. Circulating microrPas as new potential biomarkers for the diagnosis of high-grade gliomas. Zh Nevrol Psikhiatr Im S S Korsakova 2019; 119: 86-90.
[20] Motta MC, Divecha N, Lemieux M, Kamel C, Chen D, Gu W, Bultsma Y, McBurney M and Guarente L. Mammalian SIRT1 represses forkhead transcription factors. Cell 2004; 116: 551-563.
[21] Ye Z, Fang B, Pan J, Zhang N, Huang J, Xie C, Lou T and Cao Z. miR-138 suppresses the proliferation, metastasis and autophagy of non-small cell lung cancer by targeting Sirt1. Oncol Rep 2017; 37: 3244-3252.
[22] Ding XM. MicroRNAs: regulators of cancer metastasis and epithelial-mesenchymal transition (EMT). Chin J Cancer 2014; 33: 140-147.
[23] Choupani J, Mansoori Derakhshan S, Bayat S, Alivand MR and Shekari Khaniani M. Narrower insight to SIRT1 role in cancer: a potential therapeutic target to control epithelial-mesenchymal transition in cancer cells. J Cell Physiol 2018; 233: 4443-4457.
[24] Qin T, Liu W, Huo J, Li L, Zhang X, Shi X, Zhou J and Wang C. SIRT1 expression regulates the transformation of resistant esophageal cancer cells via the epithelial-mesenchymal transition. Biomed Pharmacother 2018; 103: 308-316.
[25] Yu S, Zhou R, Yang T, Liu S, Cui Z, Qiao Q and Zhang J. Hypoxia promotes colorectal cancer cell migration and invasion in a SIRT1-dependent manner. Cancer Cell Int 2019; 19: 116.
[26] Sun X, Li Y, Yu J, Pei H, Luo P and Zhang J. miR-128 modulates chemosensitivity and invasion of prostate cancer cells through targeting ZEB1. Jpn J Clin Oncol 2015; 45: 474-482.