Original Article

Hsa-miR-217 Inhibits the Proliferation, Migration and Invasion in Non-small-cell Lung Cancer Cells Via Targeting SIRT1 and p53/KAI1 Signaling

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Received: 21 September 2019
Accepted: 09 April 2020

DOI: 10.4274/balkanmedj.galenos.2020.2019.9.91

Cite this article is: Jiang W, Hou L, Wei J, Du Y, Zhao Y, Deng X, Lin X. Hsa-miR-217 Inhibits the Proliferation, Migration and Invasion in Non-small-cell Lung Cancer Cells Via Targeting SIRT1 and p53/KAI1 Signaling. Balkan Med J

Background: Brain metastasis is a major cause for cancer death in patients with lung cancer. Sir2uin 1 (SIRT1) and hsa-miR-217 has been identified to mediate the development of non-small cell lung cancer (NSCLC).

Aims: We performed this study to investigate the roles of hsa-miR-217, its target SIRT1, as well as P53/KAI1 axis in the brain metastasis from NSCLC.

Study design: This is a cell culture study.

Methods: Human pulmonary adenocarcinoma brain metastasis cell line PC-14/B were incubated and treated with constructed lentiviral plasmids expressing miR-217 and/or SIRT1. BEAS-2B cell line was used as a control. The targeted regulation of miR-217 to SIRT1 was examined using dual luciferase reporter assay. Cell proliferation, migration, invasion and the expression of relate proteins were detected to examine the effect of miR-217/SIRT1 expression on metastasis.

Results: PC-14/B cells expressed higher SIRT1 and lower p53 and KAI1 compared with BEAS-2B control cells (P<0.05). SIRT1 was a direct target of miR-217. MiR-217 expression suppressed PC-14/B cell invasion (P=0.004), migration (P=0.001) and proliferation (P<0.05), whereas SIRT1 overexpression reversed all processes. SIRT1 expression inhibited P53, KAI1/CD82, matrix metalloproteinase (MMP)-9 and β-catenin but upregulated E-cadherin protein. MiR-217 overexpression induced reverse changes.

Conclusions: Hsa-miR-217 and its target SIRT1 acted as metastasis suppressor and promoter gene in NSCLC, respectively. The hsa-miR-217/SIRT1/P53/KAI1 metastasis regulatory pathway showed novel
and crucial roles in brain metastasis from NSCLC. This axis might be a potential target for the treatment of brain metastasis of lung cancer.

**Keywords:** Brain metastasis, hsa-miRNA-217, lung cancer, PC-14/B cells, sirtuin 1

Brain metastasis, a complication found in about 20-40% of the patients suffering from non-small cell lung cancer (NSCLC) 1. Although surgical therapy, radiotherapy and novel systemic therapy have made strides in the treatment of brain metastasis from lung cancer in the last two decades, the survival rate remains low, with typical lifetime of only months 2. Studies have demonstrated that prophylactic cranial irradiation could decrease the recurrent risk of brain metastasis and intracranial tumor in patients suffering from NSCLC 3, but its efficacy on improving survival outcome in NSCLC patients with brain metastasis remains unknown. Whole-brain radiotherapy (WBRT) is the standard treatment of brain metastasis from cancers including NSCLC. However, WBRT alone has limited survival benefits in patients. The combination of surgery and WBRT extends the survival period of independent treatment, and reduces the mortality associated with the nervous system and local recurrence 4. However, this combination reduces the health-related living quality 4.

Comparing with stereotactic radiosurgery (SRS) or epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) alone, WBRT supplementation benefits the NSCLC patients with 2-4 brain metastases, including controlling cognitive progression and intracranial tumor 5-7. The combination of chemotherapy and WBRT indeed increases the response rate and controls brain metastasis, but also increases toxic and side effect and did not significantly benefit survival 5-8. Targeted therapy is a research hotspot in gene therapy of tumor. The identification of new key genes with potential of inhibiting brain metastasis from lung cancer is indispensable to the development of targeted drugs and precise treatment.

MicroRNAs (miRNAs) and their targets play important roles in the metastasis of cancers. Hsa-miR-217 showed various roles in tumorigenesis, development and drug resistance 9-11. Hsa-miR-217 inhibits laryngeal cancer metastasis via suppressing the expression of its targets including astrocyte elevated gene-1 (AEG-1) and programmed death ligand 1 (PD-L1) 11. The theoretical target gene of hsa-miR-217, Sirtuin 1 (SIRT1) was highly expressed in brain metastasis tissues of NSCLC compared with NSCLC tissues 12. Our primary experiments found that SIRT1 had high expression level in the NSCLC brain metastatic cells compared with normal cells. We thus assumed that hsa-miR-217 might play an important role in brain metastasis from NSCLC via targeting SIRT1.

SIRT1-mediated p53 signaling has been validated in various cells 13-14. SIRT1 is a NAD-dependent deacetylase which deacetylates and inhibits its physiological substrate p53 13. The SIRT1-p53 signaling pathway plays important roles in the metastatic progression of cancers like prostate cancer 15 and esophageal squamous cancer 16. In addition, a target of p53, the metastasis suppressor gene KAI1/CD82, showed therapeutic potential in NSCLC 17. However, there was no direct report showing the association between hsa-miR-217/SIRT1/p53/KAI1 pathway and brain metastasis from NSCLC.

We performed this study to investigate the roles of hsa-miR-217 and it target gene SIRT1 in the brain metastasis from NSCLC. The cell proliferation, migration, and invasion of PC-14/B cells transfected with hsa-miR-217 and SIRT1 expressing plasmids were detected to evaluate the effect of hsa-miR-217/SIRT1/p53/KAI1 pathway on cell metastasis. This study would provide a novel insight into the mechanism of hsa-miR-217/SIRT1/p53/KAI1 pathway-mediated brain metastasis from
NSCLC.

MATERIALS AND METHODS

Cell Culture
The human pulmonary adenocarcinoma brain metastasis cell line PC-14/B was obtained from Shanghai Maisha Biotechnology Co., Ltd (Shanghai, China). The human bronchial epithelial cell line BEAS-2B was a gift from the Department of Physiology, the Second Military Medical University Shanghai, China. The 293 T cell line was purchased from the cell bank of the Chinese Academy of Sciences (Beijing, China). PC-14/B and BEAS-2B cells were cultured in RPMI1640 medium (Invitrogen, Shanghai, China) supplemented with 10% fetal bovine serum (FBS) and 293T cells were cultured in DMEM (Invitrogen) plus 10% FBS. All cells were maintained at 37°C in 5% CO₂.

Plasmids and vectors
Human genomic DNA was extracted from PC-14/B cells and used for amplification of the precursor sequence of has-miR-217. The PCR product was inserted into linear pCDH-EF1-GFP vector (System Biosciences, Mountain View, CA, USA; pcDH-miR-217) through double enzyme digestion (EcoRI and BamHI) and was then transformed into Top10 competent cells (Takara, Tokyo, Japan). The CDS sequence of SIRT1 was amplified and cloned into the pcDH-CMV lentiviral expressing vector (Promega; pcDH-SIRT1) through double enzyme digestion (HindIII and BamHI) methods. The constructed vectors were validated by DNA sequencing. The mimics, inhibitor and negative control of hsa-miR-217 were chemically synthesized by Shanghai Sangon (Shanghai, China).

Preparation of recombinant lentivirus
Twenty-four hours before transfection, 293T cells were co-transfected with pcDH-miR-217 (2 μg) or pcDH-SIRT1 vector (2 μg) and 10μg pPACK Packaging Plasmid Mix (System Biosciences) using Lipofectamine 2000 (Invitrogen). Cells were then incubated in the DMEM with 1% FBS for 48 h, followed by harvest, centrifugation (5000 × g at 4°C for 5 min), and filter. The titer of packaged lentiviruses was determined using gradient dilution.

Overexpressing hsa-miRNA-217 and SIRT1 in PC-14/B cells though a lentiviral approach
The suspension of PC-14/B cells (logarithmic phase) was prepared using trypsin digestion (Promega), and the density of viable cells was determined. Cells were collected, resuspended, and then seeded into 6-well plates with a density of 2×10⁶ cells/well. Cells were cultured in RPMI1640 medium overnight at 37°C in 5% CO₂. Then, the medium was replaced with fresh medium supplemented with Lv-miRNA-217 and/or Lv-SIRT1 vectors with a multiplicity of infection of 10. The infection efficiency as well as the hsa-miR-217 and SIRT1 content were determined at 72 h post infection.

Luciferase reporter assay
Target Scan was used to predict the miRNA-mRNA target pair. The total RNA was extracted from PC-14/B cells and reversely transcribed into cDNA samples. The luciferase reporter plasmids were constructed by cloning the wild and mutant 3’-UTR of homo sapiens SIRT1 (NM_012238.4) gene into the pGL3-promotor vector (Promega, Madison, WI, USA; pGL-WT-SIRT1 or pGL-MT-SIRT1) using XbaI digestion. Hsa-miR-217 mimics, inhibitor, and NC were separately transfected into 293T cells together with pGL-WT-SIRT1 or pGL-MT-SIRT1 vectors using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. Forty-eight hours later, 293T cells were harvested and luciferase assays were conducted using a dual luciferase reporter assay system (Promega).

Cell proliferation examination
Cell viability was detected using the Cell Counting Kit-8 (CCK-8) assay (Dojindo, Japan). After infection for 72 hour, a total of 1×10⁵ PC-14/B cells/well (uninfected or infected) were seeded into
96-well plates and incubated for 12, 24, 48 and 72 h. Cell viability assay was performed using 10 μl/well CCK-8 solution following the manufacturer's protocol. A microplate reader (MultiSkan Spectrum Thermo Electron Corporation, Waltham, MA, USA) was used for the reading the absorbance at 450 nm. All experiments were performed in triplicates. Blank PC-14/B cells and cells infected with Lv-control vectors were used as negative controls.

**Cell invasion assay**

Cell invasion ability was determined using a QCMTM 24-well Fluorimetric Cell Invasion Assay kit (Chemicon, Temecula, CA, USA). The insert polycarbonate membrane was coated with a thin layer of ECFMatrixTM that occluded the pores (8-μm). RPMI1640 medium (500 μl) supplemented with 10% FBS was filled into the lower chamber. After 72 h incubation, invaded cells were fixed using paraformaldehyde (4%) and stained by diaminophenylindane (DAPI). Each experiment was conducted with three duplicates.

**Wound-healing assay**

Cell migration ability was determined using the wound-healing assay. In brief, 1×10^5 cells/well were seeded in 6-well plates and maintained till the formation of confluent monolayer cells. Then, a 200 μl pipette tip (Axygen, Corning, USA) were using for the scratching. Cells were incubated under normal conditions (37°C, 5% CO2) with fresh serum-free medium for 48 h. Photographs were taken using a computer-assisted microscope (Nikon, Japan) at 24 h and 48 h post scratching. Each experiment was conducted with three duplicates.

**Western blot assay**

Total cellular protein was extracted from PC-14/B cells at 72 h post infections using lysis buffer (Beyotime Institute of Biotechnology, Shanghai, China), followed by western blotting analysis of SIRT1, P53, and KAI1(CD82), as well as matrix metalloproteinase (MMP)-9, E-cadherin and β-catenin using the standard methods. In brief, protein samples were separated by 10% SDS-PAGE, electrotransferred onto PVDF membranes (Millipore). Primary incubation was performed using specific antibodies (1: 200 ~600) at 4°C for 12 h. All antibodies were purchased from Cell Signaling Technology (CST, Danvers, MA, USA). GAPDH (1: 1200, Boster) was used as the reference protein. Horseradish peroxidase-conjugated goat anti-rabbit/rat IgG secondary antibodies (1: 20000, Boster Biotechnology, Wuhan, China) were used for secondary incubation. Image-Pro Plus 6.0 software (Media Cybernetics Inc., Bethesda, MD, USA) was used for the digital analysis.

**Real-time PCR measurement**

Total RNA was extracted using the trizol reagent (Invitrogen), followed by the synthesis of cDNA using the M-MMLV reverse transcription kit (TaKaRa, China) with specific primers:

5’-GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACTCCAA -3’ (hsa-miR-217) and 5’-TACCTTGCGAAGTGCTTAAAC-3’ (U6 snRNA (NM_001101.3)), respectively.

The amplification of the miR-217 and U6 (control gene) was performed on a PCR Thermal Cycler Dice Real Time System using the SYBR® PrimeScript PCR Kit (TaKaRa) and the amplification primers listed in Table 1. For the examination of the relative expression of SIRT1, P53 and KAI1 mRNAs, total RNA was reversely transcribed into cDNA with OligoDT (TaKaRa). PCR amplification was performed using the PCR primers in Table 1. The expression of miRNA and mRNAs was analyzed with the 2-ΔΔCT method, and all values were normalized to an endogenous U6 or GAPDH control.

**Statistical analysis**

SPSS statistical software (16.0 for Windows) and GraphPad Prism 6.0 software were employed for statistical analyses. All data were expressed as means ± standard deviation (SD; Supplementary file 1).
The difference was analyzed using two tailed t-test (between two groups) and one-way analysis of variance (ANOVA, among more than three groups) followed with Tukey test. Differences with \( P < 0.05 \) were considered as statistically significant.

**RESULTS**

*Expression SIRT1, P53 and KAI1 in PC-14/B cells*

Compared with BEA2-2B cells, PC-14/B cells had lower mRNA levels of P53 (\( P=0.0039 \)) and KAI1 (\( P=0.0034 \)), and insignificant higher SIRT1 mRNA level (\( P=0.196 \); Fig. 1A). Western blotting revealed that PC-14/B cells had lower levels of P53 (\( P=0.0007 \)) and KAI1 protein (\( P=0.0007 \)) and higher level of SIRT1 protein (\( P=0.0003 \)) than BEA2-2B (Fig. 1B). All these data suggested that the abnormal expression of SIRT1, P53 and KAI1 in PC-14/B cells.

*Prediction and validation of the hsa-miRNA-217-SIRT1 pair*

TargetScan 6.1 prediction showed there was a hsa-miR-217 target region (seeding area, 5'-AUGCAGUA-3'; Fig. 2A) in the SIRT1 3′-UTR region. Luciferase reporter assays showed that the addition of hsa-miR-217 mimics into 293T cells carrying pGL-WT-SIRT1 vectors significantly reduced the luciferase light intensity (fire/Reni; \( P=0.0007 \); Fig. 2B) but not the cells carrying the mutant 3′-UTR of SIRT1 (pGL-MT-SIRT1 vectors). The addition of hsa-miR-217 inhibitor enhanced the luciferase light intensity by contrast (\( P=0.0114 \); Fig. 2B). These results suggested the direct target relationship between hsa-miR-217 and SIRT1.

*Lentiviral infection of PC-14/B cells*

The expression of GFP in PC-14/B cells at 72 h post infection (Lv-miRNA-217 and/or Lv-SIRT1, MOI=10) was observed under inverted fluorescent microscope (Fig. 3A). As shown in Fig. 3B, Lv-SIRT1 infection increased SIRT1 mRNA by 5.28±0.33 folds (\( P<0.0001 \)) and Lv-miR-217 increased miR-217 by 8.43±1.12 folds (\( P=0.001 \)) at 72 h after infections. The levels of miR-217 and SIRT1 mRNA in the cells co-infected with Lv-SIRT1 and Lv-miR-217 lentiviruses were as high as those in the cells infected with Lv-miRNA-217 or Lv-SIRT1 alone (\( P > 0.05 \)).

*Effect of SIRT1 and hsa-miR-217 expression on invasion, proliferation and migration in PC-14/B cells*

The overexpression of hsa-miR-217 significantly reduced the invaded PC-14/B cell number (\( P=0.004 \)) and cell migration rate (\( P=0.001 \)) compared with controls (Fig. 4A and B). The overexpression of exogenous SIRT1 obviously increased the invaded cell number (\( P=0.027 \)) in PC-14/B cells compared with controls. In addition, the addition of Lv-SIRT1 and Lv-miR-217 reversed hsa-miR-217-induced suppression on cell invasion and migration (\( P < 0.05 \); Fig. 4A and B). Cell proliferation assay showed that hsa-miR-217 overexpression significantly inhibited the proliferation of PC-14/B cells at 48 and 72 h (\( P < 0.05 \) vs. Control and Lv-control). By contrast, the overexpression of exogenous SIRT1 increased PC-14/B cell proliferation (\( P < 0.05 \) vs. Control) and rescued PC-14/B cells from hsa-miR-217-mediated inhibition in cell proliferation (\( P=0.005 \) at 48 h, and \( P=0.006 \) at 72 h; Fig. 4C). All these findings suggested that hsa-miR-217 inhibited the proliferation, invasion and migration in PC-14/B cells via inhibiting SIRT1.

*Effect of hsa-miR-217 on the p53/KAI1 pathway and the expression of relevant proteins*

Western blot analysis showed the expression of hsa-miR-217 inhibited SIRT1 expression in PC-14/B cells (\( P=0.50 \) vs. Control) and Lv-SIRT1 infection increased SIRT1 protein level (\( P=0.0001 \) vs. Control). There was no difference in SIRT1 level between cells transfected with Lv-SIRT1 and Lv-miRNA-217+Lv-SIRT1 (\( P > 0.05 \)) due to the lack of the wild 3′UTR in exogenous SIRT1, and the loss target of hsa-miR-217. The overexpression of hsa-miR-217 increased P53 (\( P<0.0001 \)) and KAI1
protein level (P<0.0001) in PC-14/B cells compared with controls, and the infection of Lv-SIRT1 decreased the expression of P53 (P=0.006) and KAI1 proteins significantly (P=0.050 vs. control; Fig. 5B and C). No difference was observed in the expression level of P53 and KAI1 between Lv-SIRT1 and Lv-miR-217+Lv-SIRT1 cells (P > 0.05; Fig. 5B and C).

In addition, hsa-miR-217 overexpression suppressed the expression of MMP-9 (P=0.091) and E-cadherin protein (P=0.074 vs. control; Fig. 5D and E), but upregulated β-catenin in PC-14/B cells (P=0.001 vs. controls; Fig. 5F). The addition of the Lv-SIRT1, however, increased MMP-9 (P<0.0001) and E-cadherin (P<0.0001) expression and decreased β-catenin protein expression (P=0.016) significantly (Fig. 5D-F). These results suggested that hsa-miR-217 expression promoted PC-14/B cells epithelial-mesenchymal transition (EMT) via the miR-217/SIRT1-mediated P53/KAI1 and MMP-9 signaling pathways.

**DISCUSSION**

Great expectations have been shown in oncology for the utility of miRNA as cancer biomarkers and therapeutic targets. To date, few studies are reported to describe expression and function of miRNAs targeting SIRT1 in brain metastases from NSCLC. In the present study, we primarily focused on the interaction of hsa-miRNA-217-SIRT1 pair and its function in the metastasis of NSCLC cells. Hsa-miR-217 functioned as a tumor suppressor gene in several human cancers via regulating its targets like EZH2 and E2F3. Our present study suggested that the expression of hsa-miR-217 showed inhibitory effect on the migration, invasion and proliferation in PC-14/B cells via targeting SIRT1, suggesting the potential roles of hsa-miR-217/SIRT1 signaling in brain metastasis from NSCLC.

SIRT1 is NAD+ dependent histone deacetylase which belongs to the Sirtuin family. Sirtuin family members (SIRT1 to SIRT7) are wildly expressed, located at different parts in the cell, and are involved in cell proliferation, inflammation and metabolism. Among the sirtuin family members, SIRT1 has the longest amino acid sequence and is the one best studied. Recent studies suggest that SIRT1 could both promote and inhibit tumorigenesis and is closely related to NSCLC. Lin et al. have found that SIRT1 is expressed during the progress of NSCLC, especially in patients with squamous cell carcinomas, and might serve as a prognostic indicator for NSCLC. Shin et al. demonstrated that hypoxic inactivation of the SIRT1-AMPK pathway led to cisplatin and doxorubicin resistance, and a SIRT1 activator srt1720 could augment the antitumor effects of cisplatin, which was blocked by administration of an AMPK inhibitor compound C, suggesting the regulatory effect of SIRT1/AMPK on drug resistance in lung cancer under hypoxia. In addition, Gong et al. indicated that the combination of SIRT1/2 could predict the survival of NSCLC patients. They also detected that high SIRT1 correlated with shorter recurrence-free survival (RFS) time, while high SIRT2-3 and SIRT5-7 expressions were associated with longer RFS time. We confirmed the overexpression of SIRT1 served as a tumor promoter gene by promoting the invasion, migration and proliferation of PC-14/B cells, but inhibited EMT. In consistent with our study, Han et al found that the SIRT1 expression was in positive regulation of NSCLC cell migration; Li et al indicated that SIRT1 protected NSCLC against osteopontin-induced EMT by inhibiting NF-κB pathway activation. Han et al detected that the knockout of SIRT1 effectively inhibited the migration of A549 cells and SIRT1 was highly expressed in brain metastasis tissues of NSCLC. These results revealed the crucial roles of SIRT1 in brain metastasis from NSCLC by acting as an oncogene.

SIRT1 is a NAD-dependent deacetylase and p53 is a physiological substrate of it. The roles of SIRT1-inhibited p53 signaling have been identified in various diseases including osteoarthritis, liver ischemia-reperfusion injury, and cancers. Pan et al revealed that the expression of nicotinamide
phosphoribosyl transferase (NAMPT) was a poor prognostic marker for patients with colon cancer. The increased NAMPT expression was found in subjects with rectal localized colorectal cancer compared with colon localized cancer. Mechanistically, NAMPT suppression induced colon cancer cell proliferation inhibition was mediated by repressing SIRT1 and Cyclin D1/E1/E2/E3, and upregulating p53, p21 and Caspase-3. Similarly, Ye et al. reported that the miR-34a expression inhibited human esophageal squamous cancer cells growth via inhibiting SIRT1 and upregulating p53 and p21. KAI1 is a metastasis suppressor gene and a direct target of tumor suppressor p53. Our study demonstrated that the expression of SIRT1 inhibited P53 and KAI1. These studies suggested that the SIRT1-inhibition activated p53/KAI1 signaling was crucial for the growth and metastasis inhibition of cancer cells.

KAI1 play crucial roles in suppressing metastasis via regulating various mechanisms including cell motility, proliferation, fusion and other signaling pathways like EGFR and Wnt. KA11 expression was correlated with poor survival in NSCLC patients. The activation of KAI1 in colorectal carcinoma inhibited the invasion and migration of cancer cells. Lee et al. suggested that the expression of KAI1 could suppress the fibronectin adhesion-induced EMT in prostate cancer cells. An interesting result in our study was that SIRT1-mediated metastasis was accompanied by two reverse facts: MMP-9 activation together with EMT and β-catenin signaling suppression. EMT, which could be activated by MMP-9 and Wnt, is crucial for cancer cell invasion and metastasis. However, the results in our study suggested the hsa-miRNA-217/SIRT1/P53/KAI1-mediated EMT in PC-14/B cells was modulated by β-catenin signaling pathway, instead of MMP-9 axis. The activated MM-9 in PC-14/B cells might contribute to the increased cell invasion, migration and metastasis. Our study suggested the hsa-miR-217 and SIRT1 played as a metastasis suppressor and promoter in NSCLC, respectively. SIRT1-mediated metastasis was related with the suppression of p53/KAI1 and β-catenin signalings. The hsa-miR-217/SIRT1/p53/KAI1 axis played crucial roles in PC-14/B cells metastasis. Our study provides a novel insight into the role of hsa-miR-217/SIRT1 axis in human brain metastases from NSCLC. This axis might be a promising molecular target for therapy of brain metastases from NSCLC.

Supplementary file: Supplementary file 1. The original value, mean, standard deviation, and p values of experiment data generated during experiment.

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FIG. 1. Expression of SIRT1, P53 and KAI1 in PC-14/B and BEAS-2B cells. A, the mRNA level of SIRT1, P53 and KAI1 in PC-14/B and BEAS-2B cells by qPCR. B, the protein levels of SIRT1, P53 and KAI1 in the two cells by western blotting. Upper: representative blots. Lower: the fold change of the optical density of the target bands. Data are expressed as the mean ± SD of at least three independent experiments. **P < 0.01 vs. controls.

FIG. 2. Verification of interaction between hsa-miR-217 and SIRT1. A, predicted binding site of hsa-miR-217 in the 3'UTR region of the SIRT1 gene. B, 293T cells transfected with pGL3-WT-SIRT1 or pGL3-MT-SIRT1 lentivirus and with or without miR-217 mimic or miR-217-inhibitor. The histogram indicates relative firefly luciferase activities. Error bars represent standard deviation and were obtained from at least three independent experiments. *P < 0.05 and **P < 0.01.
FIG. 3. Overexpression of hsa-miRNA-217 and SIRT1 in PC-14B. A, PC-14/B cells were infected with lentivirus and subjected to fluorescence microscopy to observe GFP. B, qPCR analysis of the relative expression level of hsa-miR-217 and SIRT1 at 72 h post infection. Data are expressed as the mean ± SD of at least three independent experiments. **P < 0.01 vs. controls.

FIG. 4. Effect of hsa-miRNA-217 and SIRT1 expression on PC-14/B cell invasion, cell proliferation and migration. Cells were infected with lentiviruses for another 72 h after identification of infection. A, cell invasion assay. Upper, invaded cells were stained by DAPI. Lower, the statistical analysis of invaded cell numbers. B, wound-healing assay. Upper, scratch wide at 24 and 72 h post infection. Lower, the statistical analysis of migrated cell numbers. *P < 0.05 and **P < 0.01. C, cell viability assay. **P < 0.01 vs. controls.
FIG. 5. Expression of the pathway-related proteins. A-F, the fold change of SIRT1, P53, KAI1, MMP-9, E-cadherin and β-catenin protein in transfected cells. * P<0.05 and ** P<0.01 vs. controls.
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