RETRACTED ARTICLE: Knockdown of hsa_circ_0058124 inhibits the proliferation of human lung cancer cells by up-regulation of miR-1297

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ABSTRACT
Hsa_circ_0058124 was reported to possess the capacity of enhancing tumorigenesis and invasiveness. This investigation explored the effects of hsa_circ_0058124 on human lung cancer. qRT-PCR was employed for assessing the expression of hsa_circ_0058124 and miR-1297. Cell transfection was conducted for altering the expression of hsa_circ_0058124 and miR-1297. Dual-luciferase reporter gene assay was employed for exploring the relationship between hsa_circ_0058124 and miR-1297. CCK-8 assay, colony formation, flow cytometry, western blot and transwell assay were respectively conducted for exploring the effects of hsa_circ_0058124 silencing and miR-1297 inhibition. The expression of proteins participated in PTEN/AKT and Wnt/β-catenin pathways were determined for exploring the underlying mechanism. Hsa_circ_0058124 was highly expressed in human lung tumour tissues. Besides, hsa_circ_0058124 silencing suppressed cell viability, colony formation, migration and invasion, while enhanced cell apoptosis, which were respectively verified by the regulation of apoptosis-associated and metastasis-related proteins. Additionally, hsa_circ_0058124 silencing inhibited the expression of proteins involved in PTEN/AKT and Wnt/β-catenin pathways including p/t-AKT and β-catenin. miR-1297 was lowly expressed in patients’ tumour tissues and was a target of hsa_circ_0058124. Moreover, the above mentioned effects were prominently abrogated by miR-1297 inhibition. This research verified that hsa_circ_0058124 silencing might achieve its anti-tumour roles via inactivation of PTEN/AKT and Wnt/β-catenin pathways through elevating miR-1297 expression.

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Introduction
Lung cancer is one of the commonest malignancies worldwide with high morbidity and mortality due to tumour proliferation, migration and invasion. Lung cancers include two major categories including small cell carcinoma (SCLC) and non-small cell lung cancer (NSCLC). Among them, NSCLC is the most common type of lung cancer, accounting for over 80% of all lung cancer [1], including large cell lung cancer, lung adenocarcinoma and squamous cell carcinoma. In general, lung cancer is mainly caused by several factors such as genetic factors [2,3], tobacco smoke and air pollution [4–7], and other factors may also be included. Despite of advances in diagnosis and treatment of lung cancer that have been achieved in recent years, the survival rate of patients with lung cancer remains pretty low [8]. Recent years, lung transplantation has been one of the ways to treat lung diseases [9]. Hence, investigating the regulatory mechanism of lung cancer cells is pretty pivotal for the development of new therapeutic targets.

Accumulating literatures demonstrated that circular RNAs (circRNAs) exert extensive functions in tumour genesis, metastasis, invasion, malignant transformation, signal transduction and angiogenesis. For instance, Zou et al. clarified that circ-0067934 overexpression enhanced cell proliferation and NSCLC prognosis [10]. Besides, Chen et al. demonstrated that circRNA hsa_circ_100395 suppressed the progression of lung cancer through regulating microRNA (miR)-1228/TCF21 pathway [11]. Moreover, Ma et al. reported that circMAN2B2 accelerated lung cancer growth by regulating miR-1275/FOXK1 pathway [12]. Furthermore, F-circEA was reported to be a novel liquid biopsy biomarker of NSCLC [13]. Tian et al. specified that hsa_circ_0043256 possessed the capacity of inhibiting cell proliferation and inducing cell apoptosis in NSCLC [14]. Conversely, Li et al. clarified that hsa_circ_0079530 facilitated cell proliferation and invasion of NSCLC cells [15]. In addition, hsa_circ_0058124 was once reported in papillary thyroid cancer elucidating that enhanced tumorigenesis and invasiveness through regulating NOTCH3/GATAD2A axis [16]. Nevertheless, the influences of hsa_circ_0058124 expression in lung cancer still remain unclear.

Recently, mounting investigations have suggested that miRNAs exert essential roles in the pathogenesis of NSCLC. For instance, Pan et al. revealed that downregulation of miR-24-3p resulted in etoposide-cisplatin resistance through regulating autophagy-associated gene 4A [17]. Besides, Huang et al. specified that miR-5100 enhanced tumour growth by...
regulating Rab6 in lung cancer [18]. In addition, Li et al. figured out that miR-185 overexpression notably suppressed cell proliferation, migration and invasion in vitro, and inhibited tumour growth in vivo [19]. Bu and Luo revealed that miR-1297 mimic enhanced cell proliferation in NSCLC [20], while Zhang et al. specified that co-transfection with miR-1297 and pcDNA-GFP-TRIB2-3’UTR vector inhibited cell proliferation in human lung adenocarcinoma [21]. Nevertheless, the exact role of miR-1297 in the progression of lung cancer is still not well understood.

In the current research, we tried to investigate the molecular regulatory mechanism of lung cancer. The outcomes illustrated that hsa_circ_0058124 silencing prominently repressed cell viability, colony formation, cell migration, invasion and activation of phosphatase and tensin homologue deleted on chromosome 10/protein kinase B (PTEN/ AKT) and Wnt/β-catenin pathways, but enhanced cell apoptosis. However, these effects were all partially reversed by miR-1297 inhibition. This research may offer new clues for seeking for the future therapeutic target of lung cancer.

Materials and methods

Clinical specimens

Henan Provincial People’s Hospital (Zhengzhou, China) provided the clinical human lung cancer tissues and the corresponding adjacent non-tumor tissues from 20 patients aged 59.7–72.4. General information of these patients was listed in Table 1. The informed consents were attained from each patient. This investigation was approved by the Ethics Committee of Henan Provincial People’s Hospital.

Cell culture

A549 and H1975 human lung cancer cell lines (American Type Culture Collection, Rockville, MD) were maintained in Dulbecco’s modified Eagle medium (DMEM, BBI Solution, Crumlin, UK) with the existence of 10% FBS (BBI Solution, Crumlin, UK) in a circumstance of 5% CO2 at 37 °C. The human bronchial epithelial cell lines (16HBE) were acquired from Bio-Rad Biological Technology Co. Ltd. (Shanghai, China) and maintained in RPMI 1640 (Lonza, Basel, Switzerland) with the existence of 10% FBS (BBI Solution, Crumlin, UK) under 5% CO2 and 37 °C condition. The culture medium was replaced every other day.

Quantitative reverse-transcription polymerase chain reaction (qRT-PCR)

Total cellular RNA was isolated from cells and patients’ non-tumour and tumour tissues by the use of RNAiso Plus (TAKARA, Beijing, China) with the existence of DNase I (Thermo Fisher Scientific, Waltham, MA). Afterwards, reverse transcription and qRT-PCR were respectively fulfilled by utilizing PrimeScript™ RT Reagent Kit (Perfect Real Time) and SYBR Premix Ex Taq™ II (Tli RNaseH Plus) (TAKARA, Beijing, China). The relative expression of hsa_circ_0058124 and miR-1297 were normalized to GAPDH and U6, respectively. The primer sequences used in this investigation were displayed in Table 2.

Cell transfection

Small-interfering (si)-hsa_circ_0058124, si-negative control (NC), miR-1297 inhibitor (in) and NC in were all synthesized by Sangon Biotech (Shanghai, China). Then, these sequences were respectively transfected into A549 and H1975 cells by utilizing Lipofectamine 3000 (Thermo Fisher Scientific, Waltham, MA). The harvest time of these transfected cells was set to 48 h post transfection.

Cell counting kit-8 (CCK-8) assay

Both of the transfected and un-transfected cells were respectively inoculated in 96-well plates at the density of 5000 cells each well. Then, cell viability was evaluated by utilizing CCK-8 kit (Dojindo Laboratories, Kumamoto, Japan). Briefly, 10 μl of CCK-8 solution was supplied to each well and the mixture was maintained in a humidified circumstance of 5% CO2 at 37 °C for 1 h. Finally, a Microplate Reader (Bio-Rad, Hercules, CA) was employed for detecting the absorbance of each well at 450 nm.

Colony formation assay

Cells were inoculated in six-well plates at the density of 3000 cells each well. Subsequently, the cells were maintained in a humidified incubator containing 5% CO2 at 37 °C. The culture medium was replaced every other day. After 14 days, the culture was terminated and the results were observed. After rising, cells were first fixed in 10% methanol (Aladdin, Beijing, China) and then stored in 100% methanol. After that, the cells were stained with 0.1% crystal violet (Aladdin, Beijing, China) for 10 min. Finally, a Microplate Reader (Bio-Rad, Hercules, CA) was employed for detecting the absorbance of each well at 570 nm.
China) for 30 min, and then stained in 5% crystal violet (Solarbio, Beijing, China) for another 30 min. Finally, the washing operation was repeated again and the number of clones was counted under a microscope (Olympus, Tokyo, Japan).

Apoptosis assay

Cell apoptosis analysis was fulfilled by employing Annexin V-fluorescein isothiocyanate (FITC) double staining method (Sigma-Aldrich, St. Louis, MO) with a flow cytometer (Beckman Coulter, Brea, CA). Both of the transfected and untransfected cells were inoculated in six-well plates at the density of 100,000 cells each well. Briefly, the cells were first suspended in binding buffer, and then Annexin V-FITC (10 μl) and PI (5 μl) were respectively supplied to each well. Finally, flow cytometry analysis was performed after the mixtures reacted in dark condition for 15 min.

Western blot

Total cellular proteins were isolated by the use of RIPA lysis buffer (Beyotime, Shanghai, China) with the presence of protease inhibitors (Thermo Fisher Scientific, Waltham, MA). Then, the concentrations of extracted proteins were quantified by utilizing a Nanodrop 2000 system (Thermo Scientific, Waltham, MA). Afterwards, the sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was utilized for separating the extracted proteins. After rinsing with phosphate-buffered saline (PBS), the target proteins were transferred onto the nitrocellulose (NC) membrane and subjected to blocking for 2 h at 25 °C. Then, primary antibodies (1:1000) directly against Bcl-2 (ab196495, Abcam, Cambridge, UK), Bax (ab263897), cleaved-PARP (ab4830), matrix metalloproteinase (MMP)-9 (ab73734), matrix metallo-proteinase inhibitor (TIMP)-1 (ab61224), Vimentin (ab137321), PTEN (ab31392), p-AKT (ab8933), t-AKT (ab64148), β-catenin (ab2365) and GAPDH (ab22555) incubation were performed at 4 °C overnight. Thereafter, HRP-labelled secondary antibody (ab205718, 1:2000; Abcam) incubation was performed at 25 °C for 1 h. Subsequently, ECL reagent was provided and covered the membrane surface to develop colour. Finally, the signals were detected with the help of LAS-3000 (FUJIFILM, Tokyo, Japan) and the greyscale analysis was fulfilled by utilizing quantity-one software (Bio-Rad, Hercules, CA).

Migration and invasion assay

Cell migration was determined by utilizing a two-chamber migration assay with a pore size of 8 μm. First, 200 μl of cells suspended in serum-free medium (1.5 × 10^5/ml) were provided to the upper chamber, and 600 μl of DMEM medium replenished with 10% FBS was provided to the lower chamber. Subsequently, the transwell chamber (Corning, Corning, NY) was incubated in a circumstance of 5% CO2 at 37°C for 24 h. After that, the non-traversed cells on the upper surface of membrane filter were erased. Then, the cells in the lower chamber were fixed in 4% paraformaldehyde (Aladdin, Shanghai, China) for 20 min after rinsing with PBS. Thereafter, 0.2% crystal violet (Aladdin, Shanghai, China) staining was conducted for 15 min, and then the traversed cells in the lower chamber were counted with the help of an inverted phase contrast microscope (Olympus, Tokyo, Japan).

Cell invasion was determined by utilizing the same transwell chamber as the cell migration assay. But the upper chamber was covered with 50 μl of matrigel matrix (Becton, Dickinson and Company, Franklin Lakes, NJ) 4–6 h before conducting invasion experiments. The remaining procedures were consistent with cell migration.

Dual-luciferase reporter gene assay

The mutated (mut) and wild-type (wt) 3’-untranslated region (UTR) of hsa_circ_0058124 were respectively produced by PCR. Then, these sequences were respectively cloned into the luciferase reporter plasmid psiCHECK2 (Promega, Madison, WI). Afterwards, co-transfections of the recombinant plasmids containing hsa_circ_0058124-mut/hsa_circ_0058124-wt and the sequences of miR-1297 mimic/NC mimic were respectively completed with the help of Lipofectamine 3000 (Thermo Fisher Scientific, Waltham, MA). Finally, cell lysis was performed and luciferase intensity assessment was conducted by utilizing fluorescence spectrophotometer (Hitachi, Tokyo, Japan).

Statistical analysis

Each experiment was repeated for three times. Data were expressed as mean ± standard deviation (SD). GraphPad 6.0 (GraphPad Software, La Jolla, CA) was utilized for statistical analysis. One-way analysis of variance (ANOVA) and Student’s t test were respectively utilized for comparisons among multi-groups and between two groups. p < .05 was considered as statistically significant.

Results

Hsa_circ_0058124 was highly expressed in patients’ lung cancer tissues

The expression of hsa_circ_0058124 was examined in patients’ lung cancer tissues to determine whether it exerts a role in the development of human lung cancer. Observations showed that the expression level of hsa_circ_0058124 was dramatically higher in the tumor tissues than its expression in the adjacent non-tumor tissues (p < .001, Figure 1), which suggested that it exactly played a role in the development of human lung cancer.

Hsa_circ_0058124 expression was silenced in lung cancer cell lines A549 and H1975

To evaluate the expression of hsa_circ_0058124 in lung cancer cells comparing to 16HBE cells, we respectively tested its levels in these cells. Results showed that the levels of hsa_circ_0058124 in lung cancer cells were both remarkably
enhanced comparing to 16HBE cells (both \( p < .01 \), Figure 2(A)). For further determining the influences of hsa_circ_0058124 expression on the development of human lung cancer, we silenced its expression in lung cancer cell lines A549 and H1975. Observations illustrated that hsa_circ_0058124 expression was pronouncedly suppressed both in A549 (\( p < .01 \)) and H1975 cells (\( p < .01 \), Figure 2(B)) compared with control, which indicated that they were successfully silenced in A549 and H1975 cells.

**Hsa_circ_0058124 silencing suppressed cell viability and colony formation while enhanced apoptosis**

After silencing hsa_circ_0058124 expression, several cell characteristics were examined including cell viability, colony formation and cell apoptosis in A549 and H1975 cells. Observations showed that cell viabilities were prominently suppressed by hsa_circ_0058124 silencing in both A549 (\( p < .01 \)) and H1975 (\( p < .01 \), Figure 3(A)) cells relative to control. Besides, the colony formation was also decreased (both \( p < .01 \), Figure 3(B)) by hsa_circ_0058124 silencing. However, cell apoptosis was pronouncedly enhanced by hsa_circ_0058124 silencing in A549 (\( p < .001 \)) and H1975 (\( p < .001 \), Figure 3(C)) cells relative to control, which were evidenced by the elevated expression of apoptosis-related Bax (both \( p < .001 \)) and cleaved-PARP (both \( p < .001 \)), and the reduced expression of Bcl-2 (both \( p < .01 \), Figure 3(D–F)) compared to control. The above observations hinted that hsa_circ_0058124 silencing notably inhibited cell viability and colony formation, but enhanced cell apoptosis.

**Hsa_circ_0058124 silencing suppressed cell migration and invasion**

In addition to the above-mentioned characteristics, cell migration and invasion were also examined. Observations suggested that both cell migration and invasion were prominently repressed by hsa_circ_0058124 silencing in A549 (both \( p < .01 \)) and H1975 (both \( p < .01 \), Figure 4(A,B)) cells relative to control. These outcomes were subsequently testified by the declined expression of MMP-9 (both \( p < .01 \)) and Vimentin (both \( p < .01 \)), and the increased expression of TIMP-1 (both \( p < .01 \), Figure 4(C–E)) in A549 and H1975 cells compared to control. Collectively, the above-mentioned outcomes indicated that hsa_circ_0058124 silencing pronouncedly suppressed cell migration and invasion.

**miR-1297 was a target of hsa_circ_0058124**

Substantial previous literatures have pointed out that miR-1297 impacts the proliferation of NSCLC and lung adenocarcinoma cells [20,21]; therefore, we deduced that its expression might be associated with the development of lung cancer. Herein, to verify this deduction, we respectively tested its expression in patients’ tumour and non-tumour tissues. Outcomes showed that the level of miR-1297 in tumour tissues was distinctly lower than non-tumour tissues (\( p < .001 \), Figure 5(A)), which implied that miR-1297 may play a part in the development of lung cancer. Considering that the expression of hsa_circ_0058124 was also validated to be differentially expressed in tumour and non-tumour tissues, we suspected that there might exerted a correlation between...
hsa_circ_0058124 and miR-1297 expression. Therefore, we respectively determined the expression of miR-1297 in non-treated and hsa_circ_0058124 silenced lung cancer cells. Data showed that hsa_circ_0058124 silencing remarkably increased miR-1297 expression both in A549 (\( p < .01 \)) and H1975 cells (\( p < .01 \), Figure 5(B)) relative to cells transfected with si-NC group, which hinted that hsa_circ_0058124 silencing may achieve its roles through mediating the expression of miR-1297. For further verifying this speculation, dual-luciferase reporter gene assay was performed. miR-1297 was first

Figure 3. Hsa_circ_0058124 silencing suppressed cell viability and colony formation while enhanced apoptosis. A549 and H1975 cells were respectively transfected with si-NC and si-circ. (A) Cell viabilities were both pronouncedly suppressed by hsa_circ_0058124 silencing in si-circ transfected A549 and H1975 cells. (B) Colony formation capacities were both pronouncedly suppressed by hsa_circ_0058124 silencing in si-circ transfected A549 and H1975 cells. (C) Cell apoptosis were both pronouncedly facilitated by hsa_circ_0058124 silencing in si-circ transfected A549 and H1975 cells. (D–F) The expression of apoptosis-associated Bax and cleaved-PARP was both pronouncedly facilitated, while the expression of Bcl-2 was prominently reduced by hsa_circ_0058124 silencing in si-circ transfected A549 and H1975 cells. CTRL: control; si: small-interfering; NC: negative control; circ: hsa_circ_0058124; PARP: poly-ADP-ribose polymerase; **\( p < .01 \), ***\( p < .001 \).

Figure 4. Hsa_circ_0058124 silencing suppressed cell migration and invasion. A549 and H1975 cells were respectively transfected with si-NC and si-circ. (A) Cell migrations were both pronouncedly suppressed by hsa_circ_0058124 silencing in si-circ transfected A549 and H1975 cells. (B) Cell invasions were both pronouncedly suppressed by hsa_circ_0058124 silencing in si-circ transfected A549 and H1975 cells. (C–E) The expression of metastasis-related TIMP-1 was pronouncedly facilitated, while the expression of MMP-9 and Vimentin was pronouncedly suppressed by hsa_circ_0058124 silencing in si-circ transfected A549 and H1975 cells. CTRL: control; si: small-interfering; NC: negative control; circ: hsa_circ_0058124; MMP-9: matrix metalloproteinase 9; TIMP-1: tissue inhibitor of metalloproteinase-1; **\( p < .01 \), ***\( p < .001 \).
overexpressed in lung cancer cells \((p < .001, \text{Figure 5(C)})\), and further results showed that the luciferase activity was markedly decreased after co-transfection of miR-1297 mimic and \text{hsa}_circ_0058124-wt \text{3'UTR} \((p < .05, \text{Figure 5(D)})\), while mutation of the \text{3'UTR} of \text{hsa}_circ_0058124 abrogated this suppressive effect on luciferase activity. These data manifested that miR-1297 was a target of \text{hsa}_circ_0058124. 

\text{Hsa}_circ_0058124 silencing suppressed cell viability and colony formation while enhanced cell apoptosis by elevating miR-1297 expression 

For further validating the roles of miR-1297 in the regulating process of \text{hsa}_circ_0058124 silencing, we silenced miR-1297 in both A549 and H1975 cells. Data displayed in Figure 6(A) suggested that miR-1297 was successfully inhibited in both A549 and H1975 cells \((p < .01)\) compared to cells transfected with NC in group. Besides, observations showed that miR-1297 inhibition observably reversed the suppressive effects on cell viabilities and colony formation triggered by \text{hsa}_circ_0058124 silencing in A549 and H1975 cells \((p < .05, \text{Figure 6(B,C)})\) relative to si-circ + NC in transfected group. Moreover, miR-1297 inhibition observably ameliorated the elevating effects on cell apoptosis triggered by \text{hsa}_circ_0058124 silencing in A549 and H1975 cells \((p < .05, \text{Figure 6(D)})\) relative to si-circ + NC in transfected group. These results were verified by the expression of apoptosis-associated proteins in A549 and H1975 cells. The outcomes displayed that miR-1297 inhibition observably revered the promoting effects on Bax \((p < .05)\) and cleaved-PARP \((p < .05)\) expression, and suppressive effect on Bcl-2 expression \((p < .05, \text{Figure 6(E–G)})\) relative to si-circ + NC in transfected group. Overall, these outcomes hinted that \text{hsa}_circ_0058124 silencing might achieve its inhibitory effects on cell viability and colony formation and promoting effects on cell apoptosis by elevating miR-1297 expression.

\text{Hsa}_circ_0058124 silencing suppressed cell migration and invasion through increasing miR-1297 expression 

The influences of miR-1297 silencing on cell migration and invasion were also investigated in A549 and H1975 cells. Observations showed that miR-1297 inhibition partially reversed \text{hsa}_circ_0058124 silencing-triggered inhibitory effects on cell migration \((p < .05)\) and invasion \((p < .05, \text{Figure 7(A,B)})\) relative to si-circ + NC in transfected group in both A549 and H1975 cells. These outcomes
were subsequently validated by the detections of migration and invasion-related proteins. The results suggested that miR-1297 inhibition partially reversed the suppressive effects on MMP-9 (all $p<.05$) and Vimentin (all $p<.05$) expression, and the promoting effect on TIMP-1 (all $p<.05$, Figure 7(C–E)) expression relative to si-circ + NC in transfected group. Collectively, the above-mentioned observations indicated that hsa_circ_0058124 silencing might realize its suppressive effects on cell migration and invasion through increasing miR-1297 expression.

Figure 6. Hsa_circ_0058124 silencing suppressed cell viability and colony formation while enhanced cell apoptosis by elevating miR-1297 expression. A549 and H1975 cells were respectively transfected with NC in, miR-1297 in, si-NC + NC in, si-circ + NC in and si-circ + miR-1297 in. (A) miR-1297 was successfully inhibited in A549 and H1975 cells. (B) miR-1297 inhibition observably alleviated hsa_circ_0058124 silencing-triggered suppressive effects on cell viabilities of A549 and H1975 cells. (C) miR-1297 inhibition observably alleviated hsa_circ_0058124 silencing-triggered suppressive effects on colony formation abilities of A549 and H1975 cells. (D) miR-1297 inhibition observably alleviated hsa_circ_0058124 silencing-triggered promoting effects on cell apoptosis of A549 and H1975 cells. (E–G) miR-1297 inhibition observably alleviated hsa_circ_0058124 silencing-triggered promoting effects on apoptosis-associated Bax and cleaved-PARP expression, and the depressive effect on Bcl-2 expression in both A549 and H1975 cells. CTRL: control; si: small-interfering; NC: negative control; circ: hsa_circ_0058124; in: inhibitor; miR-1297: microRNA-1297; PARP: poly-ADP-ribose polymerase. *$p<.05$, **$p<.01$, ***$p<.001$. 

**Figure 7.**...
Hsa_circ_0058124 silencing inactivated PTEN/AKT and Wnt/β-catenin pathways by elevating miR-1297 expression

In order to probe into the underlying mechanisms of the regulating effects of hsa_circ_0058124 silencing, the expression of pivotal proteins participated in PTEN/AKT and Wnt/β-catenin pathways were investigated. Results showed that hsa_circ_0058124 silencing dramatically enhanced the expression of PTEN/AKT pathway-associated PTEN (both $p < .01$), while suppressed the ratio of p/t-AKT (both $p < .01$) in both A549 and H1975 cells compared with si-NC + NC in transfected groups. However, these effects were pronouncedly reversed by miR-1297 inhibition in these two cell lines (all $p < .05$; Figure 8(A–C)). Besides, outcomes suggested that the expression of β-catenin (both $p < .05$) involved in Wnt/β-catenin pathway was both significantly repressed by hsa_circ_0058124 silencing relative to si-NC + NC in transfected groups. Nevertheless, the suppressive effects were dramatically reversed by miR-1297 inhibition in A549 and H1975 cells comparing to si-circ + NC in transfected cells (both $p < .01$, Figure 8(D–F)). These observations illustrated that hsa_circ_0058124 silencing might inactivate PTEN/AKT and Wnt/β-catenin pathways through elevating miR-1297 expression.

Discussion

In this investigation, cell viability, colony formation, cell migration and invasion were observably suppressed, while cell apoptosis was notably promoted after hsa_circ_0058124 silencing in A549 and H1975 cells. In addition, the expression of PTEN involved in PTEN/AKT pathway were prominently enhanced, while p/t-AKT and β-catenin involved in Wnt/β-catenin pathway were all observably suppressed by hsa_circ_0058124 silencing. Further trials validated that miR-1297 was a target of hsa_circ_0058124. Moreover, these above mentioned effects were all partially abolished by miR-1297 inhibition. These observations hinted that hsa_circ_0058124 silencing might exert its anti-cancer roles via inhibiting PTEN/AKT and Wnt/β-catenin pathways through elevating miR-1297 expression.

Recent years, emerging literature has illustrated the regulating roles of circRNAs in lung cancer. For instance, Ma et al. figured out that knockdown of circMAN2B2 observably suppressed cell proliferation and invasion in A549 and H1299 lung cancer cells [12]. Besides, Zou et al. revealed that cell proliferation was significantly repressed by circ-0067934 knockdown in NSCLC cells [10]. Moreover, Jiang et al. specified that knockdown of hsa_circ_0007385 pronouncedly inhibited cell proliferation, migration and invasion of NSCLC cells in vitro [22]. Likewise, Zhu et al. figured out that silencing hsa_circ_0013958 pronouncedly suppressed cell proliferation, migration and invasion, while induced cell apoptosis in lung adenocarcinoma [23]. In addition, Liu et al. proved that hsa_circRNA_103809 knockdown notably inhibited cell proliferation and invasion in vitro and suppressed tumour growth of lung cancer in vivo through sponging miR-4302 [24]. Besides, Tan et al. illustrated that F-circEA-2a facilitated cell migration and invasion in NSCLC [25]. Similar observations were also observed in our investigation demonstrating that silencing hsa_circ_0058124 dramatically suppressed the cell proliferative, colony formation, cell migratory and invasive
capabilities. Nevertheless, it promoted the apoptosis of A549 and H1975 cells. These observations illustrated that hsa_circ_0058124 knockdown exerted inhibiting roles in the progression of lung cancer.

A previous investigation pointed out that miR-1297 possessed crucial roles in tumour suppression in lung cancers [21]. Besides, Chen et al. clarified that miR-1297 overexpression prominently inhibited cell proliferation, migration and invasion, while enhanced cell apoptosis in pancreatic cancer [26]. In addition, Liu et al. elucidated that miR-1297 overexpression prominently restrained the proliferative, migratory and invasive capabilities, while promoted the apoptosis capability of hepatocellular carcinoma cells. However, these effects could be abolished by miR-1297 inhibition [27]. Moreover, Ma et al. revealed that miR-1275 could prominently abolish the inhibitory effects on cell proliferation and invasion triggered by circMAN2B2 knockdown in A549 and H1299 lung cancer cells [12]. Besides, the results showed that cell proliferation was enhanced while cell apoptosis was declined by miR-1297 overexpression in human cervical carcinoma [28]. As for colony formation, Wang et al. revealed that miR-1297 reduced colony formation capacity of glioma cells [29]. Similar observations were also found in the current investigation showing that miR-1297 expression was significantly elevated by hsa_circ_0058124 silencing. Furthermore, the suppressive effects on cell viability and colony formation and the promoting effects on cell apoptosis triggered by hsa_circ_0058124 silencing were pronouncedly abolished by miR-1297 inhibition. Considering our present findings and aforementioned literatures, a conclusion could be drawn demonstrating that hsa_circ_0058124 silencing repressed cell viability and colony formation, while enhanced cell apoptosis through augmenting miR-1297 expression.

Numbers of previous investigations have indicated that miRNAs and circRNAs participate in the progression of lung cancer through regulating PTEN/AKT and Wnt/β-catenin pathways. For instance, Wan et al. revealed that cirITCH inhibited cell proliferation of lung cancer through suppressing Wnt/β-catenin pathway [30]. In addition, Qu et al. clarified that miR-33b suppressed the cell growth, invasion and epithelial–mesenchymal transition of lung adenocarcinoma through inactivating Wnt/β-catenin/ZEB1 pathway [31]. Besides, Cui et al. found that SOX9 exerted oncogenic effects on NSCLC via activation of Wnt/β-catenin pathway by working as a target of miR-101-3p [32]. Furthermore, Bu and Luo figured out that miR-1297 mimic transfection accelerated cell viability and proliferation of A549 cells through blocking the activation of PTEN/AKT/Skp2 pathway [20]. Additionally, Yang et al. specified that miR-1297 inhibited cell growth through suppressing PTEN/P38K/AKT pathway in testicular germ tumour [33]. In our investigation, similar results were also observed demonstrating that hsa_circ_0058124 silencing blocked the activation of PTEN/AKT and Wnt/β-catenin pathways through declining the expression of p/t-AKT and β-catenin. However, subsequent miR-1297 inhibition pronouncedly abolished the inhibitory effects. These observations indicated that hsa_circ_0058124 silencing might suppress PTEN/AKT and Wnt/β-catenin pathways by elevating the expression of miR-1297.
Collectively, this investigation hinted that hsa_circ_0058124 silencing might suppress cell viability, colony formation, cell migration and invasion, while facilitate cell apoptosis via suppressing the activation of PTEN/AKT and Wnt/β-catenin pathways by elevating the expression of miR-1297.

Disclosure statement
The authors declare that they have no competing interests.

Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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