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Circular RNA hsa_circ_0007059 restrains proliferation and epithelial-mesenchymal transition in lung cancer cells via inhibiting microRNA-378

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ABSTRACT

As newly discovered non-coding RNA (ncRNA), circular RNA (circRNA) has become a research hotspot in manifold cancers. But, the influences of hsa_circ_0007059 in lung cancer remain obscure. Expression of hsa_circ_0007059 in lung cancer tissues was firstly determined through RT-qPCR. After overexpressing hsa_circ_0007059, cell viability, apoptosis, p53/CyclinD1, Bax and Pro/Cleaved-Caspase-3 and EMT-correlative factors (E-cadherin, Vimetin, Twist1 and Zeb1) were tested in A549 and H1975 cells. MiR-378 expression in lung cancer tissues and cells was evaluated after miR-378 mimic transfection. Wnt/β-catenin and ERK1/2 pathways were finally evaluated in A549 and H1975 cells. Inhibition of hsa_circ_0007059 was discovered in lung cancer tissues. Overexpressed hsa_circ_0007059 evidently restrained cell proliferation, elevated p53 and repressed CyclinD1 expression, meanwhile triggered apoptosis and enhanced Bax and Cleaved-Caspase-3 expression. Increased hsa_circ_0007059 abated EMT via enhancement of E-cadherin and inhibition of Vimentin, Twist and Zeb1 in A549 and H1975 cells. MiR-378 was up-regulated in lung cancer tissues, declined by hsa_circ_0007059 overexpression in A549 and H1975 cells. Overexpressed hsa_circ_0007059 hindered Wnt/β-catenin and ERK1/2 pathways via suppressing miR-378 in A549 and H1975 cells. The investigations manifested that hsa_circ_0007059 abated cell proliferation and EMT process in lung cancer cells via inactivation of Wnt/β-catenin and ERK1/2 pathways via suppressing miR-378.

1. Introduction

Lung cancer is a grievous malignant cancer, which is characterized by uncommitted growth of cells in lung tissues [1]. Currently, the management of lung cancer is still faced enormous challenges due to its metastasis and poor prognosis [2,3]. Occupational ailments are a class of specific diseases due to exposure to dust, radioactive substances and other toxic and harmful substances during work activities [4]. Occupational diseases comprise multiple types, such as occupational cancer, occupational poisoning, occupational radiation sickness and pneumoconiosis [5–8]. In term of occupational cancer, the occupational lung cancer is the most commonly occurring cancer, which is caused by diversified hazardous substances, embracing asbestos, chloromethyl ether, dichloromethyl ether, arsenic and its compounds, coke oven emission, hexavalent chromium compounds and erionite, respectively [9–11]. Occupational lung cancer has been aroused the public concern due to the harmfulness and high incidence. Therefore, to further probe the regulatory mechanism of lung cancer cells is of great importance for the clinical remedy of the occupational lung cancer.

Epithelial-to-mesenchymal transition (EMT) is the first step in tumor invasion and metastasis [12], which plays a vital regulatory function in diverse cancers, comprising lung cancer [13]. Evidence has proven that EMT process is closely linked to the poor prognosis of lung cancer [14]. In the process of EMT, there are many important biomarkers (E-cadherin, Vimentin, Twist and Zeb1), which also play an important regulatory role in lung cancer. E-cadherin is a specific marker of epithelial cells, and its expression level gradually decreases during the development of EMT. Research from Yao et al. revealed that TLE1 could accelerate EMT in A549 lung cancer cells via repression of E-cadherin [15]. Additionally, Vimentin is also a crucial EMT biomarker, which joins in mediating the interaction between cytoskeletal protein with cell adhesion molecules, thereby affecting cell adhesion, migration and invasion in tumor cells [16]. Besides, Twist and Zeb1 are both participate in regulating EMT process, thereby influencing lung cancer cells growth [17]. Therefore, researches focused on the role of EMT in lung cancer cell metastasis and its regulatory mechanism will provide valuable information for the early diagnosis and prognosis evaluation of lung cancer.

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Circular RNA (circRNA) is a recently discovered peculiar non-coding RNA (ncRNA), which is ubiquitous in human cells [18]. As a newfangled endogenous ncRNA, circRNA has been deemed to be a pivotal adjustor in the multifarious biological processes [19]. Additionally, circRNAs have testified to be connected with the generation and blossom of cancers. An emerging research uncovered that hsa_circ_001569 acted as a positive controller to expedite colorectal cancer cell proliferation and invasion [20]. A near-term microarray profile research of circRNAs testified that hsa_circ_0014130 was an innovative circRNA biomarker in non-small cell lung cancer (NSCLC) [21]. Moreover, Ma et al. attested that circ_MAN2B2 could potentiate cell proliferation and invasion in lung cancer cells through modulation of miR-1275/Forkhead box k1 (Foxk1) axis [22]. A momentous research revealed that repression of hsa_circ_0007059 was observed in oral squamous cell carcinoma (OSCC) cells, which mediated the malignant behavior through adjusting AKT and mTOR pathways [23]. But, whether hsa_circ_0007059 affects the progression of lung cancer remains unreported.

Herein, the research surveyed the expression of hsa_circ_0007059 in lung cancer tissues concurrently probed the functions in lung cancer A549 and H1975 cell proliferation, apoptosis and EMT process. The latent mediatory mechanism was uncovered through exploration of Wnt/β-catenin and ERK1/2 pathways. Understanding the functions of hsa_circ_0007059 in lung cancer might afford neoteric alternative curative options for this cancer.

2. Materials and methods

2.1. Clinical specimens

The lung cancer tissues (n = 20) and the adjacent non-tumor tissues (n = 20) were gathered from the patients with lung cancer from Weifang People's Hospital (Weifang, China) aging from 32 to 57 from August 2017 to December 2018. The 7 cases of lung cancer caused by asbestos, the 2 cases caused by chlorine methyl ether, 1 case caused by double chlorine methyl ether, 4 cases caused by arsenic and its compounds, 4 cases caused by coke oven dissipation, 1 case caused by hexavalent chromium compounds and 1 case caused by erionite. All these participators were un-received any therapies before surgery, and personally signed the informed consents. The study was supported by the Medical Ethics Committee of the Weifang People's Hospital (Weifang, China).

2.2. Cell culture and stimulation

A549 (ATCC® CCL-185™) and H1975 (ATCC® CRL-5908™) lung cancer cell lines were gained from American Type Culture Collection (ATCC, Rockville, MD, USA). The A549 and H1975 cells were fostered in ATCC-formulated Kaighn's Modification of Ham's F-12 medium (F-12 K, ATCC® 30–2004™) and RPMI-1640 medium (ATCC® 30–2001™), which both comprised 10% fetal bovine serum (FBS, ATCC® 30–2020™). In a constant temperature incubator (37 °C) with 95% air and 5% CO_2, A549 and H1975 cells were incubated for 24 h before exploiting in the next experiments. For induction of EMT process, A549 and H1975 cells disposed with 10 ng/mL TGFβ1 for 48 h.

2.3. Cell transfection

The overexpressed vector of hsa_circ_0007059 (pLCDH-hsa_circ_0007059) was synthesized by Geneseed Co., Ltd. (Guangzhou, China), meanwhile the miR-378 mimic (mimic sense 5′-AGC UGG UGU UGU GAA UCA GGC CG-3′; mimic antisense 5′-GCC UGA UUC ACA ACA CCA GCU UU-3′) and its related control (NC mimic) were synthesized by GenePharma (Shanghai, China). The above synthesized vectors were utilized for A549 and H1975 cells transfection through utilizing Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) on the basis of the instruction book. Forty eight post-transfection later, the transfected A549 and H1975 cells were harvested and prepared for the application in the follow-up experiments.

2.4. Cell viability assay

After transfection with overexpressed vector of hsa_circ_0007059 and miR-378 mimic, the viabilities of A549 and H1975 cells were evaluated through exploiting Cell Counting Kit-8 (CCK-8, Sigma, St Louis, MO, USA) assay. The transfected A549 and H1975 cells were seeded in 96-well plate and cultured with 10 μL CCK-8 solution for 1 h at 37 °C. The absorbance was assessed through adopting a Microplate Reader (Dynatech, Chantilly, Virginia, USA) at 450 nm.

2.5. Cell apoptosis assay

After transfection with overexpressed vector of hsa_circ_0007059 and miR-378 mimic, A549 and H1975 cells were gathered and rinsed with 4 °C PBS. Afterward, 400 μL 1 × binding buffer was supplemented to re-suspend A549 and H1975 cells, and the concentration of cells was adjusted to 1×10^6/mL. Subsequently, 5 μL Annexin V-FITC and 5 μL PI (Biosea, Beijing, China) were supplemented and fostered with A549 and H1975 cells for 15 min under the absence of light condition. Ultimately, the reaction tube was added 300 μL 1 × binding buffer and then loaded onto a flow cytometry instrument (FACSAria II, BD, USA) for the assessment of cell apoptosis.

2.6. Real-time quantitative PCR (RT-qPCR)

The TRIzol reagent (TaKaRa, Dalian, China) was utilized to extra total RNA from lung cancer tissues and transfected A549 and H1975 cells. The 5 μL extracted RNA served as a template was utilized to reverse transcribed into cDNA via adopting PimeScript RT master mix (TaKaRa). The expression levels of hsa_circ_0007059 and miR-378 were evaluated via RT-qPCR assay with Applied Biosystems™ TaqMan and Taqman microRNA RTKit (ABI, Foster City, CA, USA). The housekeeping genes of GAPDH and U6 were selected for the standardization of hsa_circ_0007059 and miR-378 expression. The data in these experiments were calculated by adopting the $2^{-\Delta\Delta Ct}$ method [24]. The correlative prime sequences utilized in the experiment were as followed: hsa_circ_0007059, Forward: 5′-GAG ACA GTA GCC ATC CAG C-3′; Reverse: 5′-TGA TCT GAG TCC AGG TGT T-3′; miR-138, Forward: 5′-ACA CTC CAG CTG GGA GCT GGT GTT GTG AAT C-3′; Reverse: 5′-TGG TGT CGT GGA GTC G-3′; GAPDH, Forward: 5′-TCA AGG CTG AGA ACG GGA AG-3′; Reverse: 5′-TCG CCC CAC TTG ATT TTG GA-3′; U6, Forward: 5′-CTC GCT TCG GCA GCA CA-3′; Reverse: 5′-AAC GCT TCA CGA ATT TGC GT-3′.

2.7. Western blot assay

For extraction of the total protein, A549 and H1975 cells were gathered after 48 h transfection and mixed with RIPA lysis buffer (Beyotime, Shanghai, China) encompassing protease inhibitor (Roche, San Francisco, USA). The concentration of extracted protein was detected via BCA™ Protein Assay Kit (Beyotime). The proteins were subjected to SDS-PAGE for electrophoretic separation. Subsequently, the semi-dry blotter method was carried out for transferring the protein to nitrocellulose membranes (Millipore, Billerica, MA, USA). After sealing with 5% BSA comprising 0.1% Tween 20 for 1 h, the membranes were incubated with the primary antibodies all night along at 37 °C. The primary antibodies of p53 (ab31333), CyclinD1 (ab226977), Bax (ab32503), Pro-Caspase-3 (ab184787), Cleaved-Caspase-3 (ab49822), E-cadherin (ab15148), Vimentin (ab137321), Twist1 (ab49254), Zeb1 (ab245283), Wnt3a (ab219412), β-catenin (ab32572), t-ERK1/2 (ab184699), p-ERK1/2 (ab214362) and β-actin (ab16039, Abcam, Cambridge, UK) were exploited in above-mentioned experiment.

Afterward, the appropriate secondary goat anti-rabbit antibody (ab205718, Abcam) was utilized to co-cultivate the nitrocellulose membranes for extra 1 h at circumambient temperature. The enhanced chemiluminescence (ECL) reagent (Pierce, IL, USA) was adopt for visualization of the bands. The gel imaging system (Bio-Rad, Hercules, CA, USA) was implemented for scanning the chemiluminescent signals.

2.8. Statistical analysis

The outcomes from the current research were expressed as the mean ± standard deviation (SD). The data for statistics were gained through implementation of SPSS 19.0 statistical software (IBM, Armonk, NY). Student *t*-test and ANOVA with Tukey's multiple comparisons test were exploited for the calculation of the data in two groups or multiple groups. *P* value < 0.05 hinted a significant difference consequence.

3. Results

3.1. Down-regulation of hsa_circ_0007059 presented in lung cancer tissues

Initially, the research gathered the tumor tissues and adjacent tissues from a total of twenty patients with lung cancer, RT-qPCR assay was carried out for the determination of hsa_circ_0007059 expression. Results in Fig. 1A revealed that hsa_circ_0007059 expression was clearly repressed in lung cancer tissue as contrasted to that in the adjacent tissues ($P < 0.001$). The lung cancer tissues from patients with different stages (Stage I, Stage II and Stage III) were utilized to further examine the relationship between hsa_circ_0007059 and staging of tumors. There are five patients with Stage I, nine patients with Stage II and six patients with Stage III. The results showed that, the higher the stage, the lower the expression level of hsa_circ_0007059 ($P < 0.01$ or $P < 0.001$, Fig. 1B). The findings manifested that abnormal expressed hsa_circ_0007059 in lung cancer tissues might be implicated in the pathogenesis of lung cancer.

3.2. Overexpression of hsa_circ_0007059 impeded cell proliferation and induced apoptosis in lung cancer cells

A549 and H1975 lung cancer cells were transfected with circRNA expression vector to overexpress hsa_circ_0007059 expression, the efficiency of cell transfection was emerged in Fig. 2A ($P < 0.001$). After overexpression of hsa_circ_0007059, cell proliferation and apoptosis were evaluated in A549 and H1975 cells. Restrained cell viability ($P < 0.001$) and elevated apoptosis ($P < 0.001$) triggered by overexpressed hsa_circ_0007059 were observed in A549 and H1975 cells (Fig. 2B and C). In the meantime, elevation of p53 ($P < 0.001$) and down-regulation of CyclinD1 ($P < 0.01$ or $P < 0.001$) caused by overexpressed hsa_circ_0007059 were observed in A549 and H1975 cells (Fig. 2D-2F). Outside of this, enhancements of Bax and Cleaved-Caspase-3 ($P < 0.001$) triggered by overexpressed hsa_circ_0007059 were also displayed in A549 and H1975 cells (Fig. 2G-2I). All these discoveries manifested that hsa_circ_0007059 hindered lung cancer cell growth via impeding cell proliferation and augmenting apoptosis.

3.3. Overexpression of hsa_circ_0007059 abated EMT process in lung cancer cells

After transfection with overexpressed vector of hsa_circ_0007059, A549 and H1975 cells were disposed with TGFβ1 for the induction of EMT process. The correlated factors of E-cadherin, Vimentin, Twist1 and Zeb1 were evaluated via implementing western blot assay. In A549 cells, we discovered that overexpressed hsa_circ_0007059 notably cut down Vimentin, Twist1 and Zeb1 expression ($P < 0.001$), meanwhile elevated E-cadherin expression (Fig. 3A and B). Similarly, the repression of Vimentin, Twist1 and Zeb1 ($P < 0.001$) and elevation of E-cadherin ($P < 0.05$) triggered by overexpressed hsa_circ_0007059 were also observed in H1975 cells (Fig. 3C-3D). These data implied that hsa_circ_0007059 suppressed EMT process in lung cancer cells.

3.4. Overexpression of hsa_circ_0007059 repressed miR-378 expression in lung cancer cells

We next also probed the expression of miR-378 in the tumor tissue and adjacent tissues from a total of twenty patients with lung cancer. Enhancement of miR-378 was obviously presented in lung cancer tissues as relative to that in adjacent tissues ($P < 0.001$, Fig. 4A). Moreover, the higher the stage, the higher the expression level of miR-378 ($P < 0.05$, $P < 0.01$ or $P < 0.001$, Fig. 4B). After transfection with the overexpressed vector of hsa_circ_0007059, we discovered that miR-378 expression was dramatically declined in A549 and H1975 cells ($P < 0.001$, Fig. 4C). The investigations uncovered that overexpression of hsa_circ_0007059 restrained miR-378 expression in lung cancer cells. In accordance of these results, we supposed that miR-378 might join in modulating the functions of hsa_circ_0007059 in lung cancer cell growth and EMT process.

3.5. Overexpression of miR-378 overturned the functions of hsa_circ_0007059 in lung cancer cell growth

For further exploration of above conjecture, the expression vectors of miR-378 mimic and NC mimic were transfected into A549 and H1975 cells. Fig. 5A revealed that the elevation of miR-378 was exhibited in A549 and H1975 cells after miR-378 mimic transfection ($P < 0.001$), manifesting that miR-378 mimic was successfully transfected into A549 and H1975 cells. Subsequently, we discovered that overexpression of miR-378 distinctly overturned the inhibitory function of hsa_circ_0007059 in cell viability in A549 ($P < 0.01$) and H1975 cells ($P < 0.001$, Fig. 5B). Likewise, the promoting function of hsa_circ_0007059 in cell apoptosis was also reversed by miR-378 overexpression ($P < 0.001$, Fig. 5C). Additionally, the enhancement of p53, the inhibition of CyclinD1, simultaneously the elevations of Bax and Clevaed-Caspase-3 triggered by hsa_circ_0007059 overexpression were all eliminated by overexpression of miR-378 ($P < 0.01$ or $P < 0.001$,

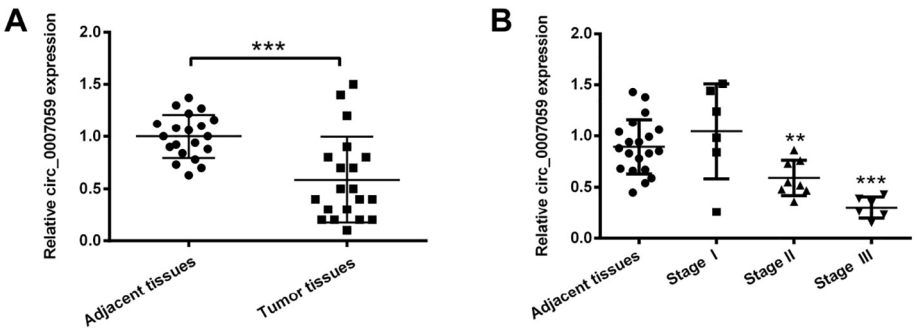

Fig. 1. Repression of hsa_circ_0007059 was exhibited in lung cancer tissues.
(A) The tumor tissues and adjacent tissues from a total of 20 patients with lung cancer were gathered, RT-qPCR assay was implemented for the evaluation of hsa_circ_0007059 expression. (B) The tumor tissues from the five lung cancer patients with Stage I, nine patients with Stage II and six patients with Stage III were collected, and RT-qPCR assay was re-performed for the determination of hsa_circ_0007059 expression.
$^{**}P < 0.01$, $^{***}P < 0.001$.

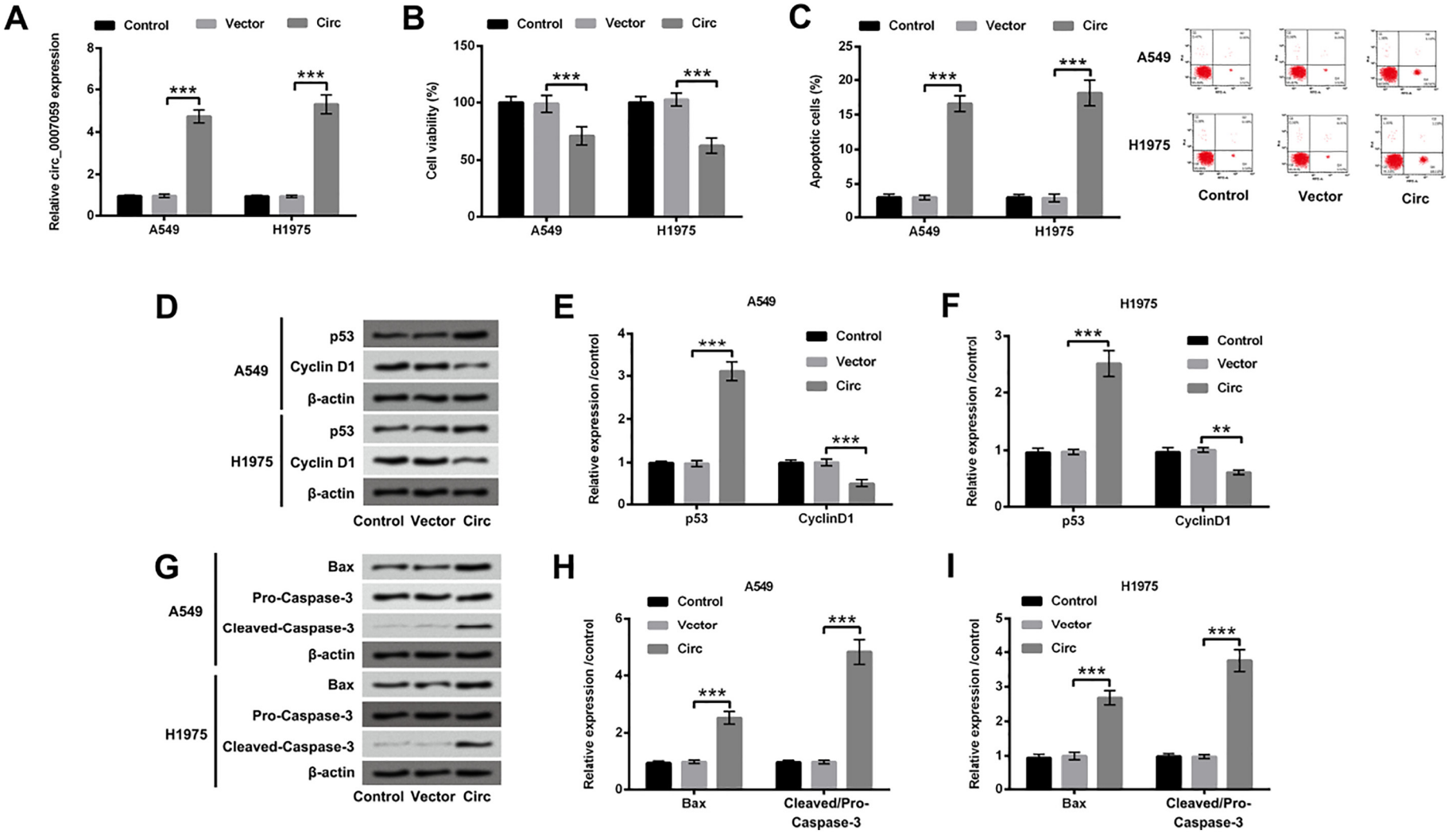

Fig. 2. Overexpression of hsa_circ_0007059 hindered cell proliferation and accelerated apoptosis in lung cancer cells.
A549 and H1975 cells were transfected with the overexpressed vector of hsa_circ_0007059 and the connected control vector, then (A) cell transfection efficiency, (B) cell viability and (C) apoptosis were tested via carrying out RT-qPCR, CCK-8 and flow cytometry assays. Protein levels of (D–F) p53, CyclinD1 and (G–I) Bax, Pro-Caspase-3 and Cleaved-Caspase-3 were evaluated via implementation of western blot assay in both A549 and H1975 cells.
$^{***}P < 0.001$.

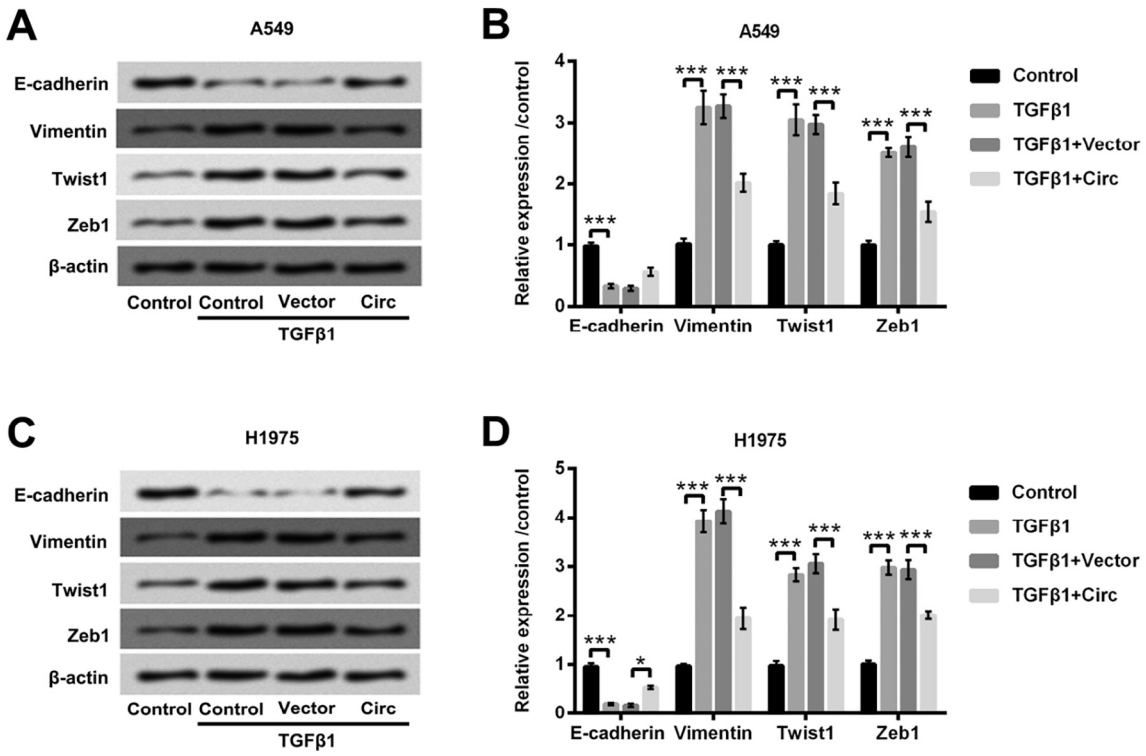

Fig. 3. Overexpression of hsa_circ_0007059 restrained EMT process in lung cancer cells.
A549 and H1975 cells were transfected with the overexpressed vector of hsa_circ_0007059 and the connected control vector, and then treated with 10 ng/mL TGFβ1 for 48 h. (A and B) Protein levels of E-cadherin, Vimentin, Twist1 and Zeb1 in the processed A549 cells were estimated through execution of western blot assay. (C and D) Protein levels of E-cadherin, Vimentin, Twist1 and Zeb1 in the processed H1975 cells were evaluated through utilization of western blot assay.
$^{*}P < 0.05$, $^{***}P < 0.001$.

Fig. 5D-5I). The data hinted that overexpression of miR-378 reversed the functions of hsa_circ_0007059 in lung cancer cell growth.

3.6. Overexpression of miR-378 reversed the functions of hsa_circ_0007059 in EMT process in lung cancer cells

For further exploration of whether miR-378 joins in modulation of EMT process, A549 and H1975 cells were transfected with miR-378 mimic and received TGFβ1 exposure. In Fig. 6A and B, we discovered that the up-regulation of E-cadherin and the down-regulation of Vimentin, Twist1 and Zeb1 ($P < 0.001$) triggered by overexpressed hsa_circ_0007059 were both eliminated by miR-378 overexpression in A549 cells. Moreover, Fig. 6C and D disclosed the same results in H1975 cells as that overexpression of miR-378 reversed the functions of hsa_circ_0007059 overexpression in E-cadherin, Vimentin, Twist1 and Zeb1 expression ($P < 0.001$). These explorations uncovered that miR-378 overexpression abolished the functions of hsa_circ_0007059 in EMT process in lung cancer cells.

3.7. Overexpression of hsa_circ_0007059 blocked Wnt/β-catenin and ERK1/2 pathways through suppression of miR-378

Functions of hsa_circ_0007059 in Wnt/β-catenin and ERK1/2 pathways were finally analyzed for further disclosing the possible

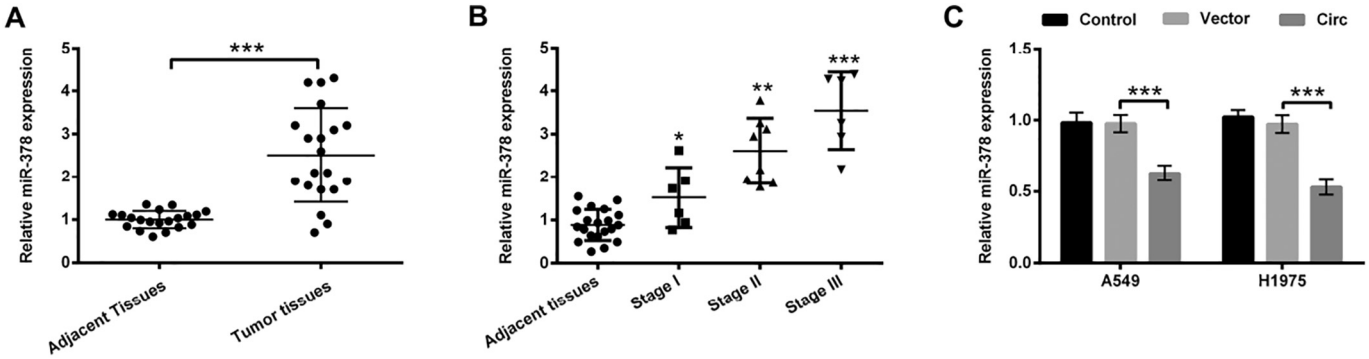

Fig. 4. Overexpression of hsa_circ_0007059 restrained miR-378 expression in lung cancer cells.
(A) The tumor tissues and adjacent tissues from 20 patients with lung cancer were gathered, RT-qPCR assay was carried out for the assessment of miR-378 expression. (B) The tumor tissues from the five lung cancer patients with Stage I, nine patients with Stage II and six patients with Stage III were collected, and RT-qPCR assay was executed again for the evaluation of miR-378 expression. (C) A549 and H1975 cells were transfected with the overexpressed vector of hsa_circ_0007059 and the connected control vector, RT-qPCR assay was adopted again for the detection of miR-378 expression.
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

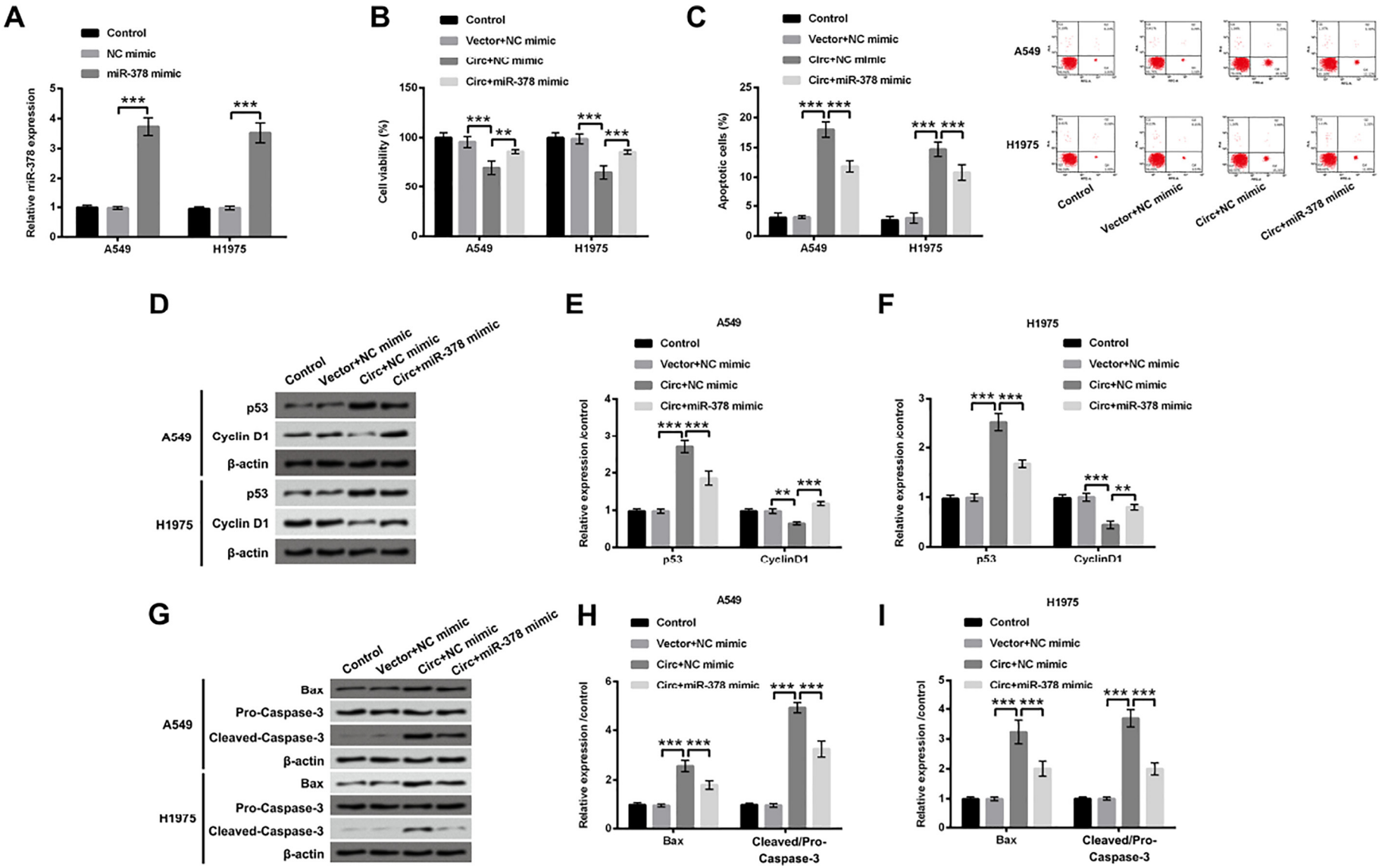

Fig. 5. Overexpression of hsa_circ_0007059 impeded lung cancer cell growth via suppression of miR-378.
(A) A549 and H1975 cells were transfected miR-378 mimic and NC mimic, cell transfection efficiency was evaluated via execution of RT-qPCR. After co-transfection with miR-378 mimic and overexpressed vector of hsa_circ_0007059, (B) cell viability and (C) apoptosis were measured via execution of CCK-8 and flow cytometry assays. Protein levels of (D-F) p53, CyclinD1 and (G-I) Bax, Pro-Caspase-3 and Cleaved-Caspase-3 were evaluated via implementation of western blot assay in both A549 and H1975 cells.
$^{**}P < 0.01$, $^{***}P < 0.001$.

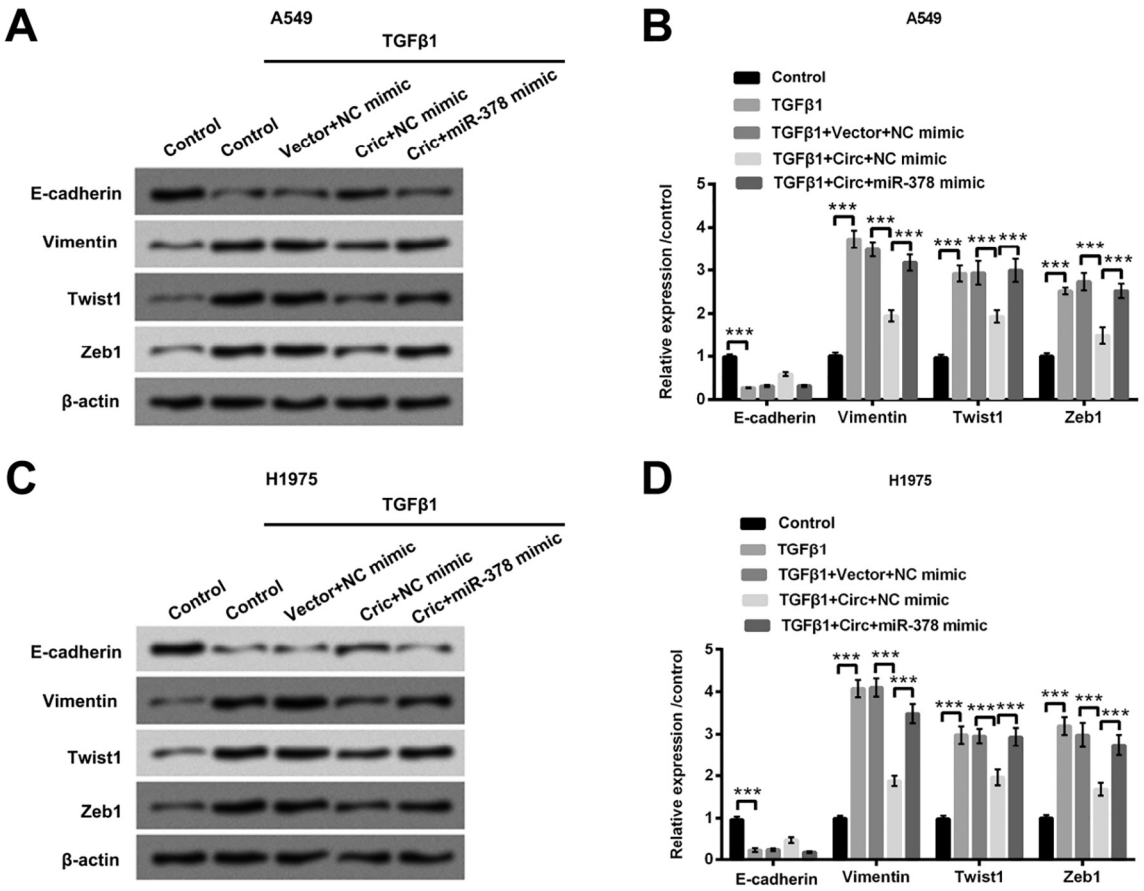

Fig. 6. Overexpression of hsa_circ_0007059 repressed EMT process via repression of miR-378 in lung cancer cells.
A549 and H1975 cells were co-transfected with miR-378 mimic and overexpressed vector of hsa_circ_0007059, and subsequently managed with 10 ng/mL TGFβ1 for 48 h. (A and B) Protein levels of E-cadherin, Vimentin, Twist1 and Zeb1 in the disposed A549 cells were evaluated via implementation of western blot assay. (C and D) Protein levels of E-cadherin, Vimentin, Twist1 and Zeb1 in the disposed H1975 cells were detected via utilization of western blot assay.
***$P < 0.001$.

molecular mechanisms. Western blot assay revealed that overexpressed hsa_circ_0007059 apparently refrained Wnt3a and β-catenin expression in A549 and H1975 cells ($P < 0.001$, Fig. 7A-7C). In like manner, we discovered that hsa_circ_0007059 overexpression also declined p-ERK1/2 expression in A549 ($P < 0.01$) and H1975 cells ($P < 0.05$, Fig. 7D-7F). Whereas, after miR-378 mimic transfection, we found that the repressive function of hsa_circ_0007059 overexpression in Wnt3a, β-catenin and p-ERK1/2 were both overturned in A549 and H1975 cells ($P < 0.05$ or $P < 0.001$, Fig. 7A-7F). These findings indicated that overexpression of hsa_circ_0007059 hindered Wnt/β-catenin and ERK1/2 pathways via repression of miR-378 in lung cancer cells.

4. Discussion

CircRNAs are a class of RNAs with covalent ring structure, which have been proven to be connected with the stages and metastasis of cancers, and also possess diversified especial biological functions in physiological processes [25,26]. In consideration of the high stability and tissue specificity, circRNA is hopeful to become an underlying biomarker for prediction and therapy of cancers [27]. Nonetheless, the influences and modulatory mechanism of circRNA in lung cancer only have a handful of studies reported. In the current research, we probed the functions of hsa_circ_0007059 in lung cancer cell growth and EMT process. Repressed hsa_circ_0007059 was firstly discovered in lung cancer tissues. Function analysis disclosed that overexpression of hsa_circ_0007059 abated cell proliferation, triggered apoptosis and restrained EMT process in A549 and H1975 cells. Additionally, miR-378 was found to ascend in lung cancer tissues but hsa_circ_0007059 overexpression repressed miR-378 expression in A549 and H1975 cells. MiR-378 overexpression evidently reversed the functions of hsa_circ_0007059 in lung cancer cell growth and EMT process. Finally, we discovered that overexpressed hsa_circ_0007059 hindered Wnt/β-catenin and ERK1/2 pathways through repression of miR-378 in A549 and H1975 cells.

Generally, the existing studies concentrated on circRNAs in lung cancer can be roughly divided into biomarker and functional mechanism studies. In biomarker studies, Yao et al. disclosed that the up-regulation of hsa_circ_100876 was intently linked to lymph node metastasis and tumor stages of lung cancer [28]. Luo et al. noticed that the elevation of hsa_circ_0000064 was observed in lung cancer tissues and A549 and H1229 lung cancer cell lines and the exceptional expression was also connected to the clinical characteristics of lymph node metastasis and TNM stage [29]. In functional mechanism study, a crucial research corroborated that hsa_circRNA_103809 aggrandized lung cancer cell proliferation and invasion in vitro and accelerated tumor formation in vivo through sponging miR-4302 [30]. In regard to hsa_circ_0007059, it is only reported to restrain cell growth, migration, and invasion in OSCC cell lines of SCC15 and CAL27 [23]. Similar with the aforementioned research, we discovered that hsa_circ_0007059 was declined in lung cancer tissues and hsa_circ_0007059 overexpression refrained cell proliferation, augmented apoptosis and relieved the process of EMT in A549 and H1975 cells. The findings suggested that hsa_circ_0007059 might be a possible biomarker in lung cancer.

Functionally, recent researches have certified that circRNA

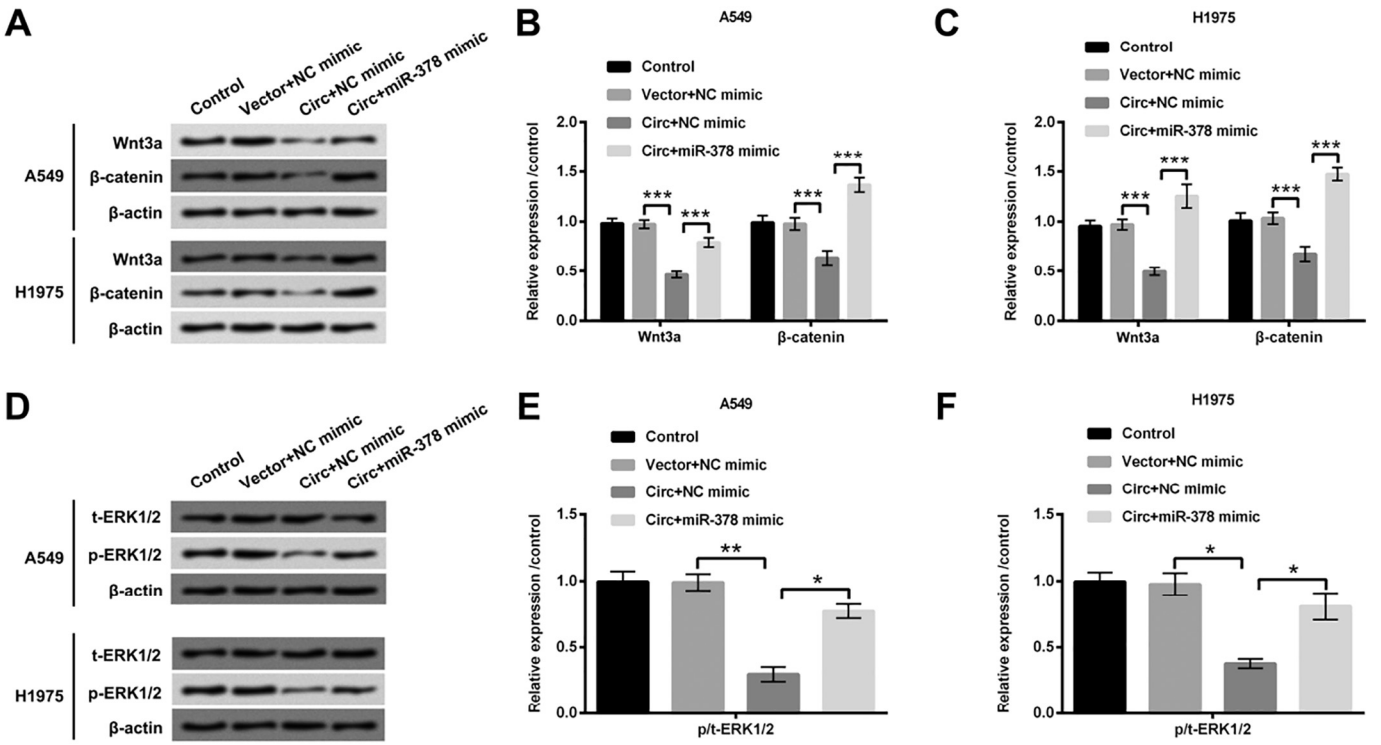

Fig. 7. Overexpression of hsa_circ_0007059 hindered Wnt/β-catenin and ERK1/2 pathways via suppression of miR-378.
A549 and H1975 cells were co-transfected with miR-378 mimic and overexpressed vector of hsa_circ_0007059, (A-C) protein levels of Wnt3a and β-catenin were tested in the processed A549 and H1975 cells via utilization of western blot assay; (D-F) protein levels of t-ERK1/2 and p-ERK1/2 were estimated in the processed A549 and H1975 cells via implementation of western blot assay.
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

molecules are rich in the binding sites of miRNA, which serve as miRNA sponge and further relieve the inhibition of miRNA on its target genes [31,32]. Su et al. utilizing the bioinformatics software (https://circinteractome.nia.nih.gov) forecasted that miR-593, miR-383 and miR-188-3p might be the latent targets of hsa_circ_0007059 [23]. MiR-378 is a versatile regulator in the variety of cancers, which can affect cancer cell proliferation, metastasis and differentiation [33–35]. In lung cancer, miR-378 has been testified to augment cell proliferation in H1299 cells and to modulate vascularization and metastasis in NCI-H292 cells [36,37]. In our research, we observed that miR-378 was elevated in lung cancer tissues, meanwhile hsa_circ_0007059 overexpression dropped off miR-378 expression in A549 and H1975 cells. On the basis of the phenomenon, we speculated that miR-378 might participate in modulation of the influences of hsa_circ_0007059 in lung cancer cells. Just as we thought, miR-378 overexpression obviously inverted the influences of hsa_circ_0007059 in cell proliferation, apoptosis and EMT process in A549 and H1975 cells. In the existing research, miR-378 has been reported to regulate lung cancer cells proliferation via suppression of FOXG1 [36]. Additionally, Ho et al. disclosed that miR-378 could adjust tumor invasion, migration and angiogenesis in lung adenocarcinoma via targeting RBX1 and CRKL [38]. Therefore, we have reason to guess that miR-378 affects the functions of hsa-circ-0007059 might through regulating or targeting several relevant mRNAs. Further researches are still necessary for testifying this conjecture. Furthermore, whether hsa_circ_0007059 exerts the repressive functions via sponge of miR-378 is also still required to further research.

Exceptional activated Wnt/β-catenin pathway shows a conclusive role in the initiation, evolution and metastasis of lung cancer [39]. Current research uncovered that hsa_circ_0002052 overexpression damaged the progression of osteosarcoma progression through hindering Wnt/β-catenin pathway via mediating miR-1205/APC2 axis [40]. Additionally, hsa_circ_ITCH has been testified to restrain cell proliferation through impeding Wnt/β-catenin pathway in lung cancer cells [41]. Activated ERK1/2 pathway in lung cancer has been also certified to be linked to the lymph node metastases and tumor staging of lung cancer [42]. One research from Zhong et al. disclosed that hsa_circ_MYLK accelerated EMT process, facilitated cell growth and angiogenesis in bladder cancer cells through modulating Ras/ERK pathway [43]. Whereas, whether Wnt/β-catenin and ERK1/2 pathways affects the functions of hsa_circ_0007059 in lung cancer cells remains confusing. In the research, we concluded that hsa_circ_0007059 overexpression impeded Wnt/β-catenin and ERK1/2 pathways via hindering miR-378 expression in A549 and H1975 cells. The observations implied that hsa_circ_0007059 possessed an antitumor activity in lung cancer might through inactivating Wnt/β-catenin and ERK1/2 pathways via repression of miR-378.

In short, the research depicted that hsa_circ_0007059 inactivated Wnt/β-catenin and ERK1/2 pathways through suppressing miR-378, and thus prohibited cell proliferation and EMT process in lung cancer cells. The investigations suggested that hsa_circ_0007059 might be a newly discovered biomarker in lung cancer. Further exploration of the functions of hsa_circ_0007059 in the progression of lung cancer might emerge great implication for the development of fresh RNA-based cancer diagnosis and therapy.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Availability of data and materials

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

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