RETRACTED ARTICLE: Repression of lncRNA-SVUGP2 mediated by EZH2 contributes to the development of non-small cell lung cancer via brisking Wnt/β-catenin signal

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ABSTRACT
To grab the possible impact of lncRNA-SVUGP2 in the biology and process of non-small cell lung cancer (NSCLC). Sixty paired NSCLC tumour and the adjacent non-tumour lung tissues were collected for detection of lncRNA-SVUGP2. lncRNA-SVUGP2 expression in NSCLC cells (SK-MES-1, A549, SPC-A1, and NCI-H1975) was also detected. lncRNA-SVUGP2 was overexpressed and depressed in A549 and H1975 cells, and the effects of lncRNA-SVUGP2 dysregulation on cell biological performances including viability, colony formation, apoptosis, migration and invasion were grabbed. Furthermore, the regulatory association of lncRNA-SVUGP2 vs. EZH2 in H1975 cells, as well as the association between lncRNA-SVUGP2 and Wnt/β-catenin pathway, was explored. lncRNA-SVUGP2 was depressed in NSCLC tissues and cells. Overexpression of lncRNA-SVUGP2 depressed proliferation, induced apoptosis, and suppressed migration and invasion of A549 and H1975 cells. In addition, lncRNA-SVUGP2 was repressed by EZH2 and was inversely correlated with EZH2 levels in H1975 cells. Repression of lncRNA-SVUGP2 potentially participated in the oncogenic function of EZH2. Besides, overexpression of lncRNA-SVUGP2 depressed the briskness of Wnt/β-catenin signal in H1975 cells. Our data reveal that lncRNA-SVUGP2 is under-expressed in NSCLC cells and the reduced expression of lncRNA-SVUGP2 may enhance the development and process of NSCLC by interacting with EZH2 and activating Wnt/β-catenin pathway.

Introduction
Lung cancer remains to be one of the most familiar respiratory system cancers among both men and women worldwide [1,2]. Therefore, statistic referred that the accounted percentage of non-small-cell lung cancer (NSCLC) is approximately 80–85% and, unfortunately, more than half of these patients are diagnosed with metastatic disease [3,4]. NSCLC has an extremely poor prognosis with a dismal 11% of 5-year overall survival rate [5,6]. There are three histologic subtypes of NSCLC, including squamous-cell carcinoma, adenocarcinoma, and large-cell lung carcinoma [7]. Although considerable progress has been archived in the diagnosis and treatment of NSCLC, the incidence and mortality in patients with NSCLC are still high [8]. Therefore, elucidation of the key molecular mechanism underlying NSCLC is of great importance to greatly improve the clinical outcomes.

It is reported that only a small scale (1–2%) of the genome encodes proteins although over 70% of the human genome is actively transcribed [9,10]. Long non-coding RNAs (lncRNAs), RNA transcripts longer than 200 bp in length, are the major part of transcribed noncoding RNA [11,12]. LncRNAs are found to be involved in a diverse aspect of the biology, such as gene expression regulation [13], chromatin structure[14], epigenetic control [15], and splicing [16]. In addition, aberrant levels of lncRNAs can also play a key role in the pathological progression of many diseases, including a variety of human cancers [17–19]. The following lncRNAs are reported to have participated in the development and process of NSCLC, such as UCA1 [20], SBF2-AS1 [21], MALAT1 [22], CCAT2 [23] and GASS [24]. Furthermore, several lncRNAs are suggested as novel predictors, promising biomarkers or targets for the diagnosis or treatment of NSCLC [25,26]. Given the crucial role of lncRNAs in biology and etiology, further identification of novel lncRNAs underlying NSCLC development is imperative. LncRNA-SVUGP2 is recently found to have a decreased expression in hepatocellular carcinoma samples and can exhibit tumour-suppressive effects in this malignancy [27]. However, the possible impact of lncRNA-SVUGP2 in other cancers including NSCLC is largely unknown.

In the current research, we studied whether the levels of lncRNA-SVUGP2 were dysregulated in NSCLC tissues and cells. In addition, lncRNA-SVUGP2 was overexpressed and knocked down in A549 and H1975 cells, and the effects of lncRNA-SVUGP2 dysregulation on cell viability, colony-formation, apoptosis, migration and invasion were explored to reveal the role of lncRNA-SVUGP2 in NSCLC. Moreover, it is reported that the functions of lncRNAs are achieved by interaction with some protein-coding genes [28]. EZH2, a histone methyltransferase subunit of a polycomb repressor complex,
is highly expressed in many tumours and enhances the proliferation and metastasis of NSCLC cells [29]. The regulatory relationships between IncRNA-SVUGP2 and EZH2 in NSCLC cells were thus evaluated. Furthermore, Wnt/β-catenin pathway is a key regulatory principle involved in the development and process of NSCLC [30,31], the association between IncRNA-SVUGP2 and Wnt/β-catenin signal in NSCLC cells was also elucidated to reveal the regulatory principle of IncRNA-SVUGP2 in NSCLC. Our efforts will offer a novel view of the diagnosis and therapy of NSCLC.

Materials and methods

Tissue collection

Between December 2010 and October 2017, 60 NSCLC patients who underwent surgery at our hospital were recruited in this study. The diagnosis of NSCLC was pathological confirmed and classified according to World Health Organization criteria. None of the patients received local or systemic treatments before surgery. The paired tumour and adjacent non-tumour tissues (ANTT) were collected from these patients, followed by being snap-frozen in liquid nitrogen and storing at −80°C until use. The informed consent was gained from each patient, and this research obtained the approval of the local research ethics committee.

Cell lines and culture

A normal human bronchial epithelial cell line 16HBE, an NSCLC squamous carcinomas cell line SK-MES-1, and three NSCLC adenocarcinoma cell lines A549, SPC-A1 and NCI-H1975 were gained from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (IBCBCA, Shanghai, China). Cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco, CA, USA) mixed with 10% fetal bovine serum (FBS, Gibco), 100 U/mL penicillin and 100 mg/mL streptomycin (Invitrogen, Carlsbad, CA, USA), and then cultured at 37°C in humidified air with 5% CO₂.

Lentivirus transfection

Using the forward primer (Xhol) of CCGCTCGAGCA TTGAAGAAATTTAAGTGAGTTTTACC and reverse primer (BamHI) of CCGGGGATCCTCCAGCCAGCTGATCGTTTG, the sequences of SVUGP2 was amplified from the cDNA of A549/H1975 cells using PrimerSTAR Max premix (TaKaRa, Japan) and then inserted into the PGMLV-PA6 vector (Sangon, Shanghai). 293FT cells were co-transfected with VSVG, PAX2 and PGMLV by means of the FuGene transfection reagent (Roche Diagnostics, Mannheim, Germany). At 48 and 72 h after transfection, 293FT cells were harvested, filtered to remove cell debris and used for subsequent infection. A549/H1975 cells (2 × 10⁵) were seeded into 6-well plates and cultured overnight. For infection, the medium was then replaced with the supernatant containing virus and 8 μg/mL polybrene (Sangon) and then used for incubating cells for 8 h. Afterwards, fresh medium was added to replace the medium and the infected cells were selected using 1 μg/mL puromycin.

Real-time quantitative PCR (qPCR)

We isolated the total RNA from cells by Trizol reagent (Sangon), and then we quantified the purity of the isolated samples using SMA 400 UV-VIS (Merinton, Shanghai, China). Reverse-transcription reactions using total RNA as the template were performed using an M-MLV Reverse Transcriptase kit (Invitrogen). On Rotor-Gene RG-3000A (Corbett Life Science, Sydney, Australia), real-time qPCR was carried out using SYBR Green PCR kit (Toyobo, Osaka, Japan) protocol. After determining CT values of all samples, 2⁻ΔΔCT method was chosen for assessing the relative gene expression levels [32] on the referenced β-actin for RNAs.

Cell viability assay

We assessed the cell viability via the MTT assay [33]. Approximately 2 × 10³ A549/H1975 cells were cultured in each well of a 96-well plate and maintained overnight. At various times following the treatment, 20 μL of 5 mg/mL of MTT solution (Sangon) was added to each well at 37°C. After 4 h of incubation, the supernatant was swept after centrifugation, and 150 μL of dimethyl sulfoxide was added to adequately dissolve the formazan precipitates. The OD470 value of each well was then measured with an MRX II absorbance reader (DYNEX Technologies, Chantilly, Virginia, USA).

Colony-forming assay

The colony-forming abilities of cells were evaluated by colony-forming assay [33]. Following different transfections, A549/H1975 cells (400 cells/well) were harvested, resuspended in RPMI-1640 medium mixed with 10% FBS, then plated into each well of a 6-well plate, and then maintained under standard culture conditions for 2 weeks. Followed by fixation with absolute methanol and staining with crystal violet, the forming colonies with a diameter over 2 mm were statistically calculated.

Flow cytometry for detecting apoptotic cells

We carried out tests to assess the cell apoptosis abilities in this study using Annexin V-FITC Apoptosis Detection Kit (TaKaRa). Following different transfections, A549/H1975 cells (1 × 10⁵ cells/mL) were obtained, then the cells were washed with PBS buffer and resuspended in 100 μL binding buffer. Then, cells were treated with Annexin V and propidium iodide (PI). The apoptosis analysis was carried out by the BD LSRII Flow Cytometer System (BD Biosciences, Mississauga, ON) within 1 h and the apoptotic cells were analyzed by FACSDiva Software (BD Biosciences).
Cell migration and invasion tests

We carried out tests to find the cell migration and invasion abilities using Transwell chambers (8 μm pore size; Costar, Switzerland) [34]. The only difference was that Transwell chambers used for detecting cell invasion were precoated with 1 mg/mL of Matrigel (BD Biosciences). After 48 h of different transfections, A549 or H1975 cells (8 × 10^4 cells/mL) were suspended in 200 μL of fresh RPMI-1640 medium without FBS and then added to the upper compartment of Transwell chambers. Next, 600 μL of medium containing 10% FBS was filled into the lower compartment as a chemo-attractant. The Transwell chambers continued to be incubated for 24 h. Afterwards, the translocated cells into the lower compartment of Transwell chambers that were defined as migrated or invaded cells were treated in cold ethanol, stained with 0.1% crystal violet, and then counted under a light microscope.

Chromatin immunoprecipitation (ChIP) assay

We analyzed the interaction between EZH2 and SVUGP2 by carrying out a ChIP assay using ChIP Chromatin Immunoprecipitation Kit (Millipore, Bedford, MA, USA) [35]. In brief, cross-linked chromatin was sonicated into 200- to 1000-bp fragments. Next, the chromatin was immunoprecipitated by incubation with normal mouse immunoglobulin G (IgG) and anti-EZH2 antibody (BD Biosciences). IgG was chosen as the negative control. qPCR was carried out using the SYBR Green PCR kit (Toyobo, Osaka, Japan).

Western blot

Following different transfections, A549/H1975 cells were collected and total proteins were then extracted by lysing in cell lysis buffer (Beyotime, Haimen, China). After quantification with a BCA protein assay kit (Sangon), an equal amount of isolated proteins (30 μg/lane) were subjected on 12% SDS-polyacrylamide gels for separation, and then the separated proteins were blotted onto polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). By incubation with the primary antibodies to Bax, Bcl-2, pro-caspase-3, pro-caspase-9, cleaved-caspase-3, cleaved-caspase-9, snail, Vimentin, E-cadherin, N-cadherin, EZH2, Axin, TCF4, β-catenin and β-actin (1:1000 dilution, Abcam, Cambridge, UK) overnight at 4°C and horseradish peroxidase-labeled secondary antibody (1:1000 dilution) for 1 h at 37°C, immunoblotting of the membranes was performed. The protein signals were revealed by enhanced chemiluminescence (ECL) method using an Odyssey Infrared Imaging System (LI-COR, Lincoln, NE, USA). β-actin was chosen as the control.

Statistical analysis

Statistical analyses were performed with SPSS version 18.0 (SPSS, Chicago, IL, USA). The measurement data collected from three repeated tests were presented as mean ± standard deviation (SD). Two-tailed t-test or post-hoc Tukey test was applied for comparisons between different groups. The correlation analysis between lncRNA-SVUGP2 level and EZH2 level in H1975 cells was analyzed by the Pearson correlation coefficient. We chose P-values < .05 to be of significance.

Results

LncRNA-SVUGP2 was under-expressed in NSCLC tissues and cells

The level of SVUGP2 in tumour tissues and in the ANTT was analyzed. The levels of lncRNA-SVUGP2 in NSCLC tissues were markedly lower relative to that in ANTT (P < .01, Figure 1(A)). Moreover, consistent findings were obtained that lncRNA-SVUGP2 expression was also significantly lowly expressed in an NSCLC squamous carcinomas cell line SK-MES-1, and three NSCLC adenocarcinoma cell lines A549, SPC-A1, and NCI-H1975 relative to that in 16HBE (P < .05, Figure 1(B)).

Overexpression of lncRNA-SVUGP2 inhibited proliferation, induced apoptosis and suppressed migration and invasion of A549 and H1975 cells, whereas suppression of lncRNA-SVUGP2 exhibited opposite effects

To further study the possible impact of lncRNA-SVUGP2 in NSCLC, lncRNA-SVUGP2 was overexpressed in A549 and H1975 cells, followed by an investigation of cell biological processes. The levels of lncRNA-SVUGP2 in A549 and H1975 cells transfected with pcDNA-SVUGP2 were significantly higher than those in A549 and H1975 cells transfected with blank vector pcDNA3.1 (P < .001, Figure 1(C)), implying that lncRNA-SVUGP2 was successfully overexpressed in two kinds of cells. To assess cell proliferation, MTT and colony-forming assays were carried out. The viabilities and colony-forming abilities of A549 and H1975 cells transfected with pcDNA-SVUGP2 were remarkably inhibited in comparison with those of A549 and H1975 cells transfected with control (P < .05, Figure 1(D,E)), indicating that overexpression of lncRNA-SVUGP2 significantly inhibited NSCLC cell proliferation. Moreover, the percentage of apoptotic A549 and H1975 cells in pcDNA-SVUGP2 group was obviously enhanced related to that in pcDNA3.1 group (P < .001, Figure 1(F)); the levels of Bax/Bcl-2, cleaved/pro-caspase-3, and cleaved/pro-caspase-9 in pcDNA-SVUGP2 group were higher than those in the pcDNA3.1 group (Figure 1(G)). These data implied that high level of lncRNA-SVUGP2 promoted NSCLC cell apoptosis. Furthermore, the total number of migrated and invaded A549 and H1975 cells in the pcDNA-SVUGP2 group were significantly lower than that in the pcDNA3.1 group (P < .05, Figure 1(H)); and overexpression of lncRNA-SVUGP2 dramatically inhibited EMT by enhancing E-cadherin level and depressing the levels of N-cadherin, vimentin, and snail (Figure 1(I)). These findings indicated that the up-expressed lncRNA-SVUGP2 suppressed NSCLC cell migration and invasion.

Furthermore, we also suppressed the level of lncRNA-SVUGP2 in A549/H1975 cells (P < .01, Figure 2(A)). Moreover,
LncRNA-SVUGP2 was down-regulated in NSCLC tissues and cells and overexpression of lncRNA-SVUGP2 inhibited proliferation, induced apoptosis, and suppressed migration and invasion of A549 and H1975 cells. (A) qPCR analysis showed the expression levels of lncRNA-SVUGP2 in NSCLC tissues and adjacent non-tumour lung tissues. (B) qPCR analysis showed the expression levels of lncRNA-SVUGP2 in a normal human bronchial epithelial cell line 16HBE, a NSCLC squamous carcinomas cell line SK-MES-1, and three NSCLC adenocarcinoma cell lines A549, SPC-A1, and NCI-H1975. Statistical analyses were performed with SPSS version 18.0 (SPSS, Chicago, IL, USA). * P < .05 and ** P < .01 compared with corresponding controls. (C) qPCR analysis showed the expression levels of lncRNA-SVUGP2 in A549 and H1975 cells after transfection. (D) MTT assay showed cell viability of A549 and H1975 cells after transfection. (E) Colony-forming assay showed the colony-forming abilities of A549 and H1975 cells after transfection. (F) Flow cytometry showed that the percentage of apoptotic A549 and H1975 cells after transfection. (G) Western blot displayed that the expression levels of Bax/Bcl-2, cleaved/pro-caspase-3, and cleaved/pro-caspase-9 in A549 and H1975 cells after transfection. (H) Transwell assay showed the number of migrated and invaded A549 and H1975 cells after transfection. (I) Western blot displayed that the expression levels of EMT markers (E-cadherin, N-cadherin, vimentin and snail) in A549 and H1975 cells after transfection. A549 and H1975 cells were transfected with pcDNA-SVUGP2 and pcDNA3.1. Data collected from three repeated experiments were presented as mean ± SD. *P < .05; **P < .01; ***P < .001 compared with pcDNA3.1 group.
suppression of lncRNA-SVUGP2 significantly promoted the viabilities and colony-formation of A549/H1975 cells (P < .05, Figure 2(B–C)). However, there were no significant changes in the percentage of apoptotic A549/H1975 cells between si-SVUGP2 and si-NC groups (Figure 2(D)), as well as the levels of Bax/Bcl-2, cleaved/pro-caspase-3, and cleaved/pro-caspase-9 (Figure 2(E)). Furthermore, suppression of lncRNA-SVUGP2 markedly increased the number of migrated/invaded A549/H1975 cells (P < .05, Figure 2(F)) and enhanced EMT through decreasing E-cadherin level and increasing the level of N-cadherin, vimentin, and snail (Figure 2(G)). These data indicated that depression of lncRNA-SVUGP2 promoted NSCLC cell proliferation, migration and invasion, which were opposite with the effects of lncRNA-SVUGP2 overexpression.

**LncRNA-SVUGP2 was repressed by EZH2 and was inversely correlated with EZH2 levels in H1975 cells**

Accumulating pieces of evidence have confirmed that EZH2 is highly expressed in many tumours and promotes the progression of a variety of cancers, such as prostate cancer [36,37]. We thus grabbed the regulatory correlation of EZH2 vs. lncRNA-SVUGP2. The results of ChIP assay displayed that EZH2 was highly enriched in the promoter region of lncRNA-SVUGP2 (P < .001, Figure 3(A)). Subsequently, EZH2 expression was overexpressed and depressed in H1975 cells by transfection with pc-EZH2 and si-EZH2, and the high transfection efficiency was carried out furtherly (P < .01, Figure 3(B)). Moreover, the results of ChIP test displayed that knockdown of EZH2 by transfecting si-EZH2 could significantly decrease the levels of H3K27me3 at the promoter region of lncRNA-SVUGP2 (P < .001, Figure 3(C)). Furthermore, lncRNA-SVUGP2 was up-expressed in the si-EZH2 group and down-regulated in the pc-EZH2 group significantly (P < .01, Figure 3(D)). Besides, the correlation analysis displayed that the level of lncRNA-SVUGP2 was inversely associated with EZH2 in H1975 cells (P < .001, Figure 3(E)).

**Repression of lncRNA-SVUGP2 was involved in the oncogenic function of EZH2**

EZH2 was pointed out to be crucial in promoting the proliferation and metastasis of NSCLC cells [29]. We thus tested whether lncRNA-SVUGP2 was involved in NSCLC via interacting with EZH2. Results displayed that co-transfection of pc-lncRNA-SVUGP2 and pc-EZH2 simultaneously markedly changeover the impacts of pc-lncRNA-SVUGP2 alone on H1975 cell viability (P < .05, Figure 4(A)), colony-formation (P < .05, Figure 4(B)), apoptosis (P < .001, Figure 4(C)), the expression changes of Bax/Bcl-2, cleaved/pro-caspase-3, and cleaved/pro-caspase-9 (Figure 4(D)), migration and invasion (P < .01, Figure 4(E)), and the levels of EMT markers (Figure 4(F)). These data indicated that repression of lncRNA-SVUGP2 was potentially associated with the oncogenic effect of EZH2.

**LncRNA-SVUGP2 modulated Wnt/β-catenin signaling in H1975 cells**

Previous studies pointed out that Wnt/β-catenin signal is crucial in the development and process of NSCLC [30,31], we thus explored whether the abnormal level of lncRNA-SVUGP2 in NSCLC will be archived by modulating Wnt/β-catenin signal in H1975 cells. The data uncovered that overexpression of lncRNA-SVUGP2 accelerated Axin1 expression and depressed the levels of TCF4 and β-catenin (Figure 4(G)), indicating that overexpression of lncRNA-SVUGP2 suppressed the briskness of Wnt/β-catenin signal in H1975 cells. However, knockdown of lncRNA-SVUGP2 had opposite effects on this pathway (Figure 4(G,H)).

**Discussion**

Increasing pieces of evidence have confirmed that lncRNAs are key regulators in cancer biology, diagnosis and prognosis [38,39], thereby, exploring the important lncRNAs involving in NSCLC will facilitate to designing effective strategies for the diagnosis and treatment of this disease. Several lncRNAs are involved in NSCLC development. For instance, lncRNA ANRIL is shown to promote proliferation and inhibit apoptosis of NSCLC cells through silencing KLF2 and P21 expression [40]; and low expression of TUBA4B increases cell proliferation in NSCLC and predicts poor prognosis [41]. In this research, we discovered that lncRNA-SVUGP2 was under-expressed in NSCLC tissues and cells. Also, overexpression of lncRNA-SVUGP2 depressed cell proliferation but induced apoptosis of NSCLC cells. These data indicate the suppressed role of lncRNA-SVUGP2 in NSCLC.

Also, our data displayed that the up-expressed lncRNA-SVUGP2 suppressed migration and invasion abilities of NSCLC cells, whereas suppression of lncRNA-SVUGP2 exhibited opposite effects. Metastasis is a multilevel process that is mediated by altered expression of many genes. EMT is pointed out to be crucial in tumour invasion and metastasis [42]. E-cadherin, an EMT marker, is found to be significantly associated with lymphnode metastasis and vascular invasion in patients with NSCLC [43]. In this study, overexpression of lncRNA-SVUGP2 dramatically suppressed EMT by enhancing the level of E-cadherin but depressing the levels of N-cadherin, vimentin, and snail. Hence, we deduced that lncRNA-SVUGP2 may regulate the migration and invasion of NSCLC cells via modulating EMT markers.

Furthermore, as another aspect of the present analysis, our results showed the lncRNA-SVUGP2 was repressed by EZH2 and was inversely correlated with EZH2 levels in H1975 cells. As a histone methyltransferase subunit of a polycomb repressor complex, EZH2 is pointed out that it can promote malignant behaviours of NSCLC cells and thereby may serve as a prognostic marker for patients with NSCLC [44]. A meta-analysis also revealed that EZH2 high level was correlated to poor prognosis of overall survival in patients with NSCLC [45]. Moreover, EZH2-mediated epigenetic depression of lncRNA SPRY4-IT1 contributes to NSCLC development [29]. Our results showed that repression of lncRNA-SVUGP2 was possibly correlated to the oncogenic impact of EZH2. It can,
Figure 2. Suppression of IncRNA-SVUGP2 promoted proliferation, inhibited apoptosis and promoted migration and invasion of A549 and H1975 cells. A549 and H1975 cells were transfected with si-SVUGP2 and si-NC. (A) qPCR analysis showed the expression levels of IncRNA-SVUGP2 in A549 and H1975 cells after transfection. (B) MTT assay showed cell viability of A549 and H1975 cells after transfection. (C) Colony-forming assay showed the colony-forming abilities of A549 and H1975 cells after transfection. (D) Flow cytometry showed that the percentage of apoptotic A549 and H1975 cells after transfection. (E) Western blot displayed that the expression levels of Bax/Bcl-2, cleaved/pro-caspase-3, and cleaved/pro-caspase-9 in A549 and H1975 cells after transfection. (F) Transwell assay showed that the number of migrated and invaded A549 and H1975 cells after transfection. (G) Western blot displayed that the expression levels of EMT markers (E-cadherin, N-cadherin, vimentin and snail) in A549 and H1975 cells after transfection. Data collected from three repeated experiments were presented as mean ± SD. *P < .05; **P < .01; ***P < .001 compared with si-NC group.
therefore, be speculated that EZH2-mediated epigenetic repression of lncRNA-SVUGP2 may contribute to NSCLC development.

Besides, overexpression of lncRNA-SVUGP2 depressed the briskness of Wnt/β-catenin signal in H1975 cells, whereas knockdown of lncRNA-SVUGP2 had opposite effects on this pathway. Wnt/β-catenin signal has been confirmed to be crucial in modulating the process of NSCLC. β-Catenin was found to be abnormally expressed in 51% of adenocarcinomas and in 94% of squamous CLC samples, and its overexpression is found to be associated with improved prognosis [46]. Sun et al. demonstrated that lncRNA NEAT1 played an oncogenic role in NSCLC by regulating the Wnt signal[47]. Zhang et al. confirmed that miR-770 inhibited tumourigenesis
Figure 4. Repression of lncRNA-SVUGP2 was potentially involved in the oncogenic function of EZH2. H1975 cells were transfected with pcDNA3.1, pcDNA-SVUGP2, and pcDNA-SVUGP2 * pcDNA-EZH2. (A) MTT assay showed cell viability of H1975 cells after transfection. (B) Colony-forming assay showed the colony-forming abilities of H1975 cells after transfection. (C) Flow cytometry showed that the percentage of apoptotic H1975 cells after transfection. (D) Western blot displayed that the expression levels of Bax/Bcl-2, cleaved/pro-caspase-3, and cleaved/pro-caspase-9 in H1975 cells after transfection. (E) Transwell assay showed that the number of migrated and invaded H1975 cells after transfection. (F) Western blot displayed that the expression levels of EMT markers (E-cadherin, N-cadherin, vimentin and snail) in H1975 cells after transfection. (H) LncRNA-SVUGP2 modulated Wnt/β-catenin signaling in H1975 cells. (I) LncRNA-SVUGP2 modulated Wnt/β-catenin signaling in H1975 cells. (J) LncRNA-SVUGP2 modulated Wnt/β-catenin signaling in H1975 cells. (K) Western blot showed the expression of Axin1, TCF4 and β-catenin in H1975 cells transfected with pcDNA-SVUGP2, pcDNA3.1, si-SVUGP2 and si-NC. Data collected from three repeated experiments were presented as mean ± SD. *P < .05; **P < .01; ***P < .001 compared with pcDNA-SVUGP2 group. (G) LncRNA-SVUGP2 modulated Wnt/β-catenin signaling in H1975 cells. (H) LncRNA-SVUGP2 modulated Wnt/β-catenin signaling in H1975 cells. (I) LncRNA-SVUGP2 modulated Wnt/β-catenin signaling in H1975 cells.
and EMT in NSCLC via regulating WNT/β-catenin signal [48]. Wu et al. revealed that knockdown of TC-1 promoted the susceptibility of NSCLC cells to radiosensitivity via the Wnt/β-catenin signal [49]. Furthermore, aberrant expression of Wnt1/β-Catenin is considered as an independent poor prognostic marker of NSCLC after surgery [50]. Notably, the strategy that could target Wnt/β-catenin signal may be a potential anti-cancer therapeutics [51]. Given the key role of Wnt/β-catenin signal in NSCLC, we speculate that overexpression of lncRNA-SVUGP2 may hinder the development of NSCLC by inhibiting the briskness of Wnt/β-catenin signal, which may be an effective strategy for NSCLC therapy.

To sum up, our data display that lncRNA-SVUGP2 is downregulated in NSCLC cells and the reduced expression of lncRNA-SVUGP2 mediated by EZH2 may contribute to the development of NSCLC by activating Wnt/β-catenin signal. lncRNA-SVUGP2 may have the ability to be a latent target for NSCLC therapy. Further studies, aiming at verifying the conclusion of this research, must be carried out.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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