Increased LINC00922 in preeclampsia regulates the proliferation, invasion, and migration of placental trophoblast cells

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Background: Recent studies have shown that the abnormal expression of long-chain non-coding RNAs (lncRNAs) can significantly affect the biological function of trophoblast cells and lead to the occurrence of preeclampsia (PE). This study explores the expression of lncRNA LINC00922 in PE and its effect on the function of placental trophoblast cells, along with the corresponding molecular mechanism, providing a theoretical basis and molecular target for understanding the occurrence, early diagnosis, and targeted therapy of PE.

Methods: Fluorescence quantitative PCR was used to detect the expression of LINC00922 in 30 cases of PE tissues and normal tissues. The CCK-8 assay, clone formation experiment, and flow cytometry were used to detect the effects of LINC00922 knockdown or overexpression on the proliferation, colony formation, and cell cycle of HTR-8/SVneo placental trophoblast cells. The Transwell assay was used to detect the effects of LINC00922 knockdown or overexpression on the invasion and migration of HTR 8/SVneo cells, and western blot was used to detect the expression of cell cycle-related proteins and invasion and migration-related proteins.

Results: LINC00922 was highly expressed in PE tissues. Knockdown of LINC00922 significantly inhibited the proliferation, invasion, and migration of HTR-8/SVneo cells, along with colony formation and the ability to induce cell cycle arrest in the G0/G1 phase. However, overexpression of LINC00922 had the opposite effect. Knockdown or overexpression of LINC00922 significantly affected the expression of cell cycle-related proteins cyclin-dependent kinase 2 (CDK2), G1/S-specific cyclin-D1 (Cyclin D1), p21, proliferating cell nuclear antigen (PCNA), matrix metallopeptidase 9 (MMP-9), vimentin, and E-cadherin, but had no significant effect on the expression of matrix metallopeptidase 2 (MMP-2).

Conclusions: LINC00922 was highly expressed in PE, and functional experiments showed that LINC00922 could significantly affect the proliferation and invasion abilities of placental trophoblast cells, suggesting that LINC00922 may play an important role in the occurrence, early diagnosis, and treatment of PE.

Keywords: Preeclampsia; LINC00922; cell proliferation; cycle arrest; invasion

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Introduction

Preeclampsia (PE) is a transient disease that is unique to pregnancy, and develops under the influence of genetic inheritance, individual environmental factors, and other factors. If treatment or prevention is not timely, it can seriously endanger the health of mothers and children (1). Pathological diagnosis has shown that the occurrence of the disease may be caused by abnormal immune balance

and immune tolerance between the mother and the fetus, which leads to a decrease in the infiltration capacity of placental trophoblast cells. This results in shallow placental implantation and insufficient placental blood and oxygen supply, causing a local oxidative stress response and producing a large number of virulence factors and inflammatory mediators, so that the vascular endothelium of multiple organs of the body are damaged, vascular spasm appears, and multiple system organs are involved (2,3). The main causes of PE have not been fully elucidated. Some scholars put forward the "two-stage" theory of PE pathogenesis. The first stage is pre-clinical, that is, uterine spiral artery trophoblast recasting disorder, resulting in placental ischemia, hypoxia, release of a variety of placental factors (4). In the second stage, placental factors enter the maternal blood circulation, promoting the activation of systemic inflammatory response and vascular endothelial damage, resulting in the diversified clinical manifestations of PE-eclampsia. The recasting process of the spiral artery mainly depends on the migration and invasion of trophoblast cells and the apoptosis level of vascular endothelial smooth muscle cells that are mediated by trophoblasts. During normal gestation, cell trophoblast cells differentiate into villous trophoblast cells and extravillous trophoblast cells (EVT). EVT includes interstitial extravillous trophoblast cells (iEVT) and endovascular extravillous trophoblast cells (enEVT). iEVT is responsible for infiltrating the endometrial matrix to the inner 1/3 of the myometrium. enEVT enters the uterine spiral arterioles and gradually replaces the smooth muscle cells and endothelial cells in the vascular wall, transforming the artery from a high-resistance low-volume vessel to a low-resistance high-volume vessel, which has improved the blood flow of the placenta and ensured the normal material exchange between the mother and the fetus and the development of the fetus. Therefore, trophoblast cells play a crucial role in the process of arterial recasting, and their proliferation, migration, invasion, differentiation, and apoptosis are co-regulated by various factors (5-7). Therefore, exploring the function of trophoblast cells will further enrich the understanding of the etiology of PE and make a significant contribution to the elucidation of its pathogenesis. At the same time, elucidating the key pathogenic molecules of PE can provide effective diagnostic markers or therapeutic targets for clinical prevention and treatment.

Long-chain non-coding RNAs (lncRNAs) were once considered as the “noise” in the cell transcriptome, but recent studies have shown that lncRNAs can affect the occurrence and development of PE through changes in the biological function, immune regulation, epigenetic regulation, decidualization, and energy metabolism of trophoblast cells (8,9). For example, lncRNA TDRG1 is abnormally downregulated in the placenta in PE, and overexpression of TDRG1 can promote the proliferation, migration, and invasion of trophoblast cells, while knockdown of TDRG1 can inhibit these processes. Studies on molecular mechanisms have shown that TDRG1 can bind to miR-214-5p, which can regulate the Jagged1 and Notch1 signaling pathways (10). In addition, lncRNAs may be used as targets for predicting and diagnosing PE in the future. For example, the serum levels of lncRNAs (NR_027457, AF085938, G36948, and AK002210) in PE patients were significantly different before and after surgery, so lncRNAs can be used as potential diagnostic biomarkers for PE. Furthermore, lncRNAs such as NR_027457 and G36948 may be involved in the pathogenesis of PE (11). Therefore, identifying and clarifying the lncRNAs that have important regulatory roles in the development of PE is of great significance for the early diagnosis and targeted therapy of PE.

LINC00922 (ID: ENSG00000261742.5, long intergenic non-protein coding RNA 922) is a newly identified lncRNA. Studies have shown that LINC00922 has an important regulatory role in tumor invasion and migration (12,13). Previous studies mainly focused on detecting the expression of LINC00922 in ovarian cancer tissues and cells and studying the effect mechanism of LINC00922 on ovarian cancer (14). Our study focused on the expression of LINC00922 in PE and its effect on placental trophoblast function and its molecular mechanism. The characteristics of invasion and migration of placental trophoblast cells to the maternal decidua are similar to those of malignant cells, but they are strictly limited in time and space. Therefore, this study first detected the expression of LINC00922 in PE tissues, and then explored the effect of LINC00922 on placental trophoblast cells and its molecular mechanism through cell function experiments.

We present the following article in accordance with the MDAR reporting checklist (available at https://dx.doi.org/10.21037/atm-21-4923).

Methods

PE tissue was obtained from PE patients (30 cases) who visited our obstetric department from February 2020 to December 2020. Patients were aged 29–36 years, and

were diagnosed with PE after 34 weeks of gestation. The termination of pregnancy by cesarean section was between 36 and 39 weeks of gestation, and the diagnostic criteria referred to the literature (1). The normal control group comprised 30 pregnant women (aged 22–36 years) with single births who visited our obstetrics department from February 2020 to December 2020, and the termination of pregnancy by cesarean section was between 36 and 39 weeks of gestation. The pregnancy was normal, the embryo or fetus developed normally, and there were no complications. There was no history of adverse pregnancy, primary hypertension, diabetes, chronic nephritis, and thyroid disease. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). All enrolled patients signed informed consent, and the study was approved by the ethics committee of the First Affiliated Hospital of Soochow University (No. 20195845). When pregnancy was terminated, samples (about 200 mg) were collected within the range of 2–3 cm from the maternal surface of the placenta to the umbilical cord attachment. The samples were quickly washed with PBS buffer 3 times and RNA buffer was added, then samples were transferred to the refrigerator at –80 ℃ for storage.

Cell culture

Human placental trophoblast cells, HTR-8/SVneo cells, were obtained from the ATCC in the United States. Cells were cultured in RPMI 1640 medium containing 10% fetal bovine serum (FBS), 100 μg/mL streptomycin, and 100 U/mL penicillin, and were maintained in an incubator at 37 ℃ with 5% CO_2. Subculture was carried out every 2–3 days, and the subculture was carried out at a ratio of 1:2.

Vector construction and transfection

The interference sequence targeting LINC00922 (shRNA-1: 5'-AGUAGACAGAAAC CAUATTT-3', shRNA-2: 5'-CAGAGAAAGGUGGGCAUUATT-3') was synthesized by Shanghai Jikai Gene Chemical Technology Co., Ltd., and the sequences of interference primers were determined with reference to the literature (15). The empty vector of the LINC00922 overexpression plasmid (pcDNA-22) was constructed using a pcDNA3.1 vector, which was synthesized by Shanghai Jikai Gene Chemical Technology Co., Ltd., and packaged with lentivirus.

Cells in good growth state were inoculated in 6-well plates and cultured overnight until they adhered to the wall and the fusion reached 80%. SuperFectin shRNA transfection reagent was used to transfect the designed shRNA-1, shRNA-2, and pcDNA into the cells, and quantitative RT-PCR was performed after 48 hours.

The expression of LINC00922 was detected by RT-PCR

Total RNA was extracted from clinical tissue samples and cultured cells using TRIzol (Invitrogen), and cDNA synthesis was performed using a reverse transcription kit. The ABI7900 fluorescence quantitative PCR instrument and SYBR green (Takara) were used to test the amplification of the target fragment. The relative expression level of LINC00922 was calculated by the ΔΔCt method. GAPDH was the internal reference gene, and the primer sequences of LINC00922 and GAPDH were determined with reference to the literature (15,16).

Cell viability was detected by CCK-8

Transfected cells in logarithmic growth phase were collected and diluted into 4×10^4/mL cell suspensions using RPMI 1640 medium with 10% FBS, then inoculated in a 96-well plate (100 μL/well). Five replicates were set in each group and the culture conditions were 37 ℃ with 5% CO_2. The cells were cultured for 24, 48, and 72 hours, 10 μL CCK-8 solution was added to each well, and the cells were cultured for another 1 hour. Then, the cell viability was detected.

Clone formation experiment

Transfected cells in logarithmic growth phase were collected and diluted into 10^3/mL cell suspensions using RPMI 1640 medium containing 10% FBS, then inoculated in 6-well plates (600 μL/well). The medium was changed every 3 days. After 21 days, the medium was discarded and cells were washed once with PBS, then 1% formaldehyde was added to fix the cells for 10 minutes. Cells were washed once with PBS, 0.1% crystal violet solution was added for staining for 15 minutes, and the clone formation rate was analyzed.

Cell cycle was detected by flow cytometry

According to the instructions of the kit, RNase A/propidium iodide (PI) was used to evaluate the cell cycle (KeyGEN,

China). First, trypsin was used to digest the cells which were transfected for 24 hours, then cells were prepared into single-cell suspensions. The cell precipitates were collected by centrifugation, suspended in 500 μL 70% cold ethanol, and fixed at 4 ℃ overnight. Then, the cells were washed with pre-cooled PBS 3 times, and 500 μL of pre-cooled PI/RNase A staining solution was added. The cells were gently mixed and incubated at room temperature away from light for 60 minutes. The percentages of cells at the G0/G1, S, and G2/M stages were analyzed by BD flow cytometry.

Invasion and migration experiment

Transfected cells in logarithmic growth phase were collected and digested by trypsin. Cells were diluted into 10^5/mL cell suspensions using RPMI 1640 medium containing 1% FBS. Then, 200 μL of cells were inoculated into the upper chamber of a Transwell (8 μm, Corning, USA), and RPMI 1640 medium containing 15% FBS was added to the lower chamber. The Transwell was incubated at 37 ℃ for 36 hours. Then, the cells in the upper chamber were gently wiped with cotton swabs, fixed with methanol for 30 minutes, and stained with 0.1% crystal violet solution for 20 minutes. The chamber was washed with PBS 3 times, and the number of cells penetrating the filter membrane was observed under a light microscope. For the invasion experiment, the upper chamber of the Transwell was coated with Matrigel (BD Biosciences, USA), and subsequent procedures were performed following those for migration.

Western blot detection

Transfected HTR-8/SVneo cells were collected by centrifugation at 4 ℃. RIPA lysis buffer was added to the cell precipitates, whisk gently and mix well, and lysed on ice. Protein concentration was determined by the BCA protein quantification method. Protein electrophoresis was performed with 12% polyacrylamide gel electrophoresis (SDS-PAGE), then protein was transferred to a PVDF membrane. The PVDF membrane was incubated with 5% skimmed milk blocking solution at room temperature for 2 hours, then incubated overnight with the primary antibody at 4 ℃. anti-CDK2 (1:800; Abcam, Shanghai, China), anti-Cyclin D1 (1:800; Abcam), anti-p21 (1:1,000; Abcam), anti-PNCA (1:800; CST, Shanghai, China), anti-matrix metallopeptidase 9 (MMP-9) (1:1,000, CST), anti-matrix metallopeptidase 2 (MMP-2) (1:1,000, CST), anti-E-cadherin (1:1,000, CST), anti-vimentin (1:1,000, CST), anti-GAPDH (1:1,000, CST). Subsequently, the membrane was incubated with HRP-conjugated secondary antibody at room temperature for 2 hours, then was imaged and analyzed in a gel imager.

Statistical analysis

SPSS 20.0 statistical software was used for analysis. Data were expressed as mean ± standard deviation (SD). Comparison between groups was performed by the *t*-test or analysis of variance, and $P<0.05$ was considered statistically significant.

Results

LINC00922 was highly expressed in PE tissues

Firstly, the expression level of LINC00922 in 30 cases of PE tissues and 30 cases of normal tissues was detected by fluorescence quantitative PCR. The results showed that compared with normal tissues, LINC00922 was highly expressed in PE tissues (*Figure 1A*), which suggested that LINC00922 may play an important regulatory role in the occurrence and development of PE. The effects of overexpression or knockdown of LINC00922 on the related biological functions of cells were further investigated using placental trophoblast cells (HTR-8/SVneo). The results of fluorescence quantitative PCR showed that compared with the blank control (shRNA-NC), the expression level of LINC00922 was significantly decreased after transfection of shRNA-1 and shRNA-2 interference sequences that targeted LINC00922 knockdown, and shRNA-2 showed higher knockdown efficiency ($P<0.01$). Therefore, shRNA-2 was selected for the knockdown experiment in the follow-up study (*Figure 1B*).

In addition, we constructed the overexpression vector according to the sequence of LINC00922 and transfected it into HTR-8/SVneo cells. The effect of LINC00922 overexpression in transfected cells was detected by fluorescence quantitative PCR. The results showed that the expression level of LINC00922 was significantly increased after transfection with the overexpression vector of LINC00922 compared with the empty vector group (pcDNA-NC) (*Figure 1C*). The above experiments showed that the overexpression and knockdown vectors of LINC00922 were successfully constructed and transfected into HTR-8/SVneo cells, providing strong support for subsequent functional studies.

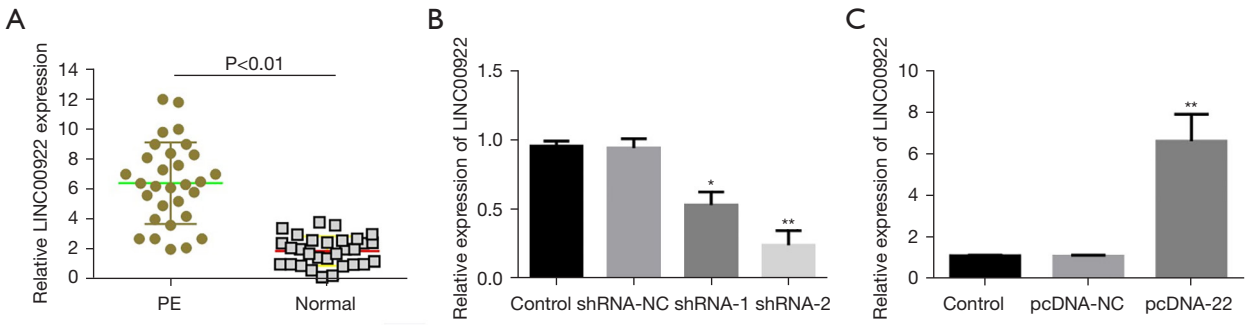

Figure 1 LINC00922 was highly expressed in preeclampsia (PE) tissues. (A) The expression of LINC00922 in PE tissues and normal tissues was detected by fluorescence quantitative PCR. (B) The knockdown efficiency of LINC00922 in placental trophoblast cells was detected by fluorescence quantitative PCR. (C) The overexpression level of LINC00922 in placental trophoblast cells was detected by fluorescence quantitative PCR. *, P<0.05; **, P<0.01. PE, preeclampsia.

The effect of LINC00922 on the proliferation of placental trophoblast HTR-8/SVneo cells

Firstly, HTR-8/SVneo cells transfected for 48 hours were counted and inoculated into 96-well plates. Then, cell activity was assessed by the CCK-8 assay after 0, 24, 48, and 72 hours of culture. The results showed that compared with the control group, the proliferation of cells in the shRNA-NC group was not significantly changed. However, the proliferation of cells was significantly inhibited after 48 hours of LINC00922 (shRNA-2) knockdown (P<0.01), and the inhibition was especially significant after 72 hours (P<0.01). Compared with the blank control group, the proliferation ability of cells in the empty vector group (pcDNA-NC) was not significantly changed, and the overexpression of LINC00922 (pcDNA-22) significantly promoted the proliferation ability of cells after culture for 72 hours (P<0.05) (*Figure 2A*).

The clone formation experiment results showed that compared with the blank control group, there was no significant change in the number of cell colonies in the shRNA-NC group, while knocking down LINC00922 (shRNA-2) could significantly inhibit the clone formation ability of cells (P<0.05). Compared with the blank control group, the number of cell colonies in the empty vector group (pcDNA-NC) did not change significantly, while overexpression of LINC00922 (pcDNA-22) could significantly promote the clone formation of cells (P<0.05) (*Figure 2B*). Furthermore, flow cytometry was used to test whether knockdown or overexpression of LINC00922 would affect the cell cycle. The results showed that compared with the control group, the cell cycle distribution of the shRNA-group (shRNA-NC) was not significantly changed, while knockdown of LINC00922 (shRNA-2) significantly blocked the cell cycle at the G1/G0 phase, and the corresponding S phase and G2/M were significantly decreased (P<0.05) (*Figure 2C*). Compared with the blank control group, the cell cycle distribution of the empty vector group (pcDNA-NC) did not change significantly, while overexpression of LINC00922 (pcDNA-22) could significantly promote cells in the S and G2/M phases (P<0.05) (*Figure 2C*), and the corresponding cell number in the G1/G0 phase was significantly reduced.

LINC00922 can significantly regulate the expression of cell cycle-related proteins in HTR-8/SVneo placental trophoblasts

We further explored the effects of knockdown or overexpression of LINC00922 on the expression of cell cycle-related proteins in HTR-8/SVneo placental trophoblasts. Since knocking down LINC00922 can significantly cause cell cycle arrest in the G0/G1 phase and can inhibit cell proliferation, we detected the expression of cyclin-dependent kinase 2 (CDK2), G1/S-specific cyclin-D1 (Cyclin D1), cyclin-dependent kinase inhibitor 1A (p21), and proliferating cell nuclear antigen (PCNA). The results of western blot showed that the expression levels of p21 and PCNA in the shRNA group (shRNA-NC) were significantly different from those of the blank control group. Compared with the blank control group or shRNA-group (shRNA-NC), knockdown of LINC00922 (shRNA-2) significantly promoted the expression of p21 and inhibited the expression of CDK2, Cyclin D1, and PCNA, thus

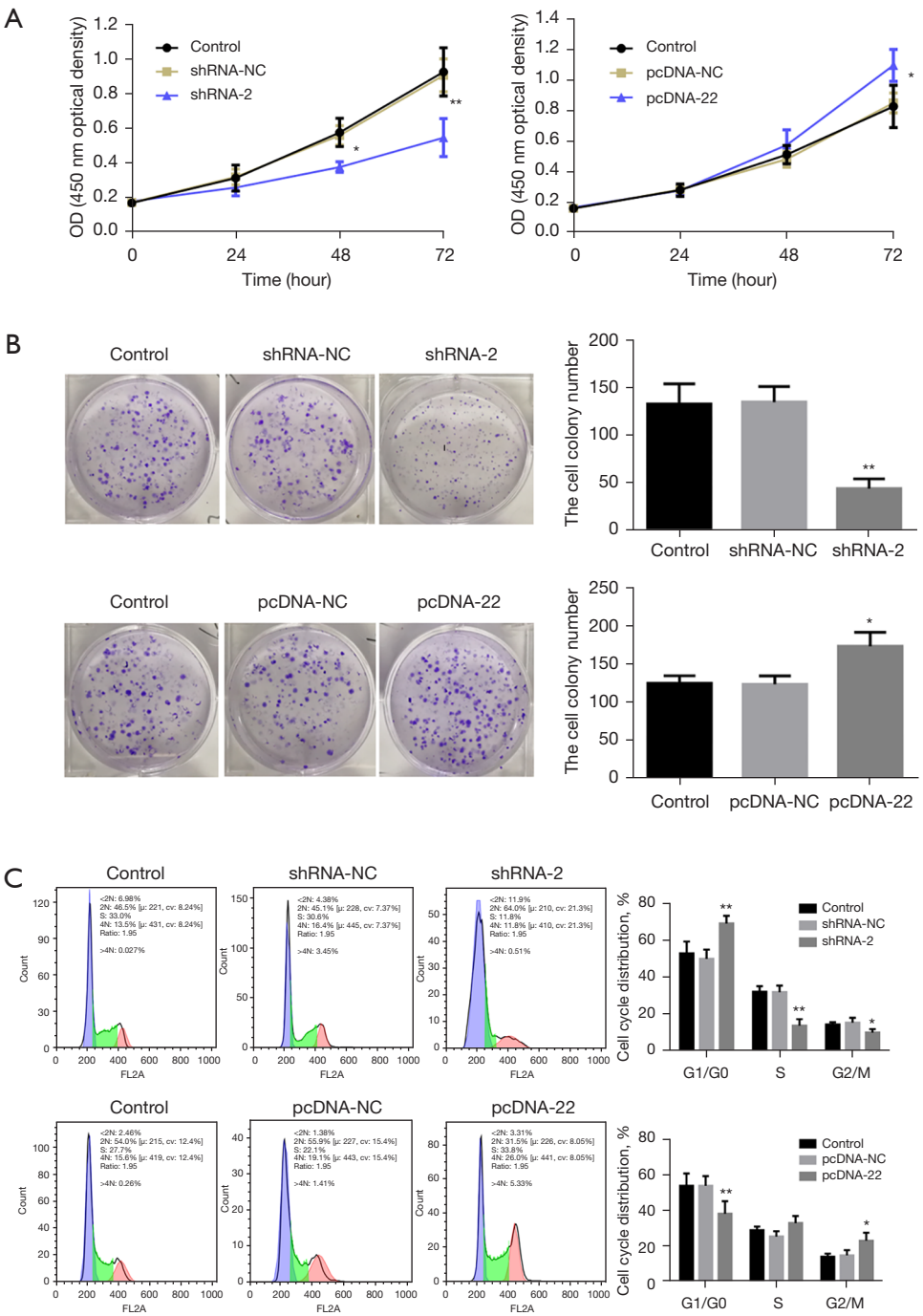

Figure 2 Effect of LINC00922 on the proliferation of HTR-8/SVneo cells. (A) The effect of knockdown or overexpression of LINC00922 on cell proliferation was detected by the CCK-8 assay. (B) The effect of knockdown or overexpression of LINC00922 on the clone formation ability of cells was detected by the clone formation assay. (C) The effects of knockdown or overexpression of LINC00922 on cell cycle distribution were detected by flow cytometry. *, P<0.05; **, P<0.01.

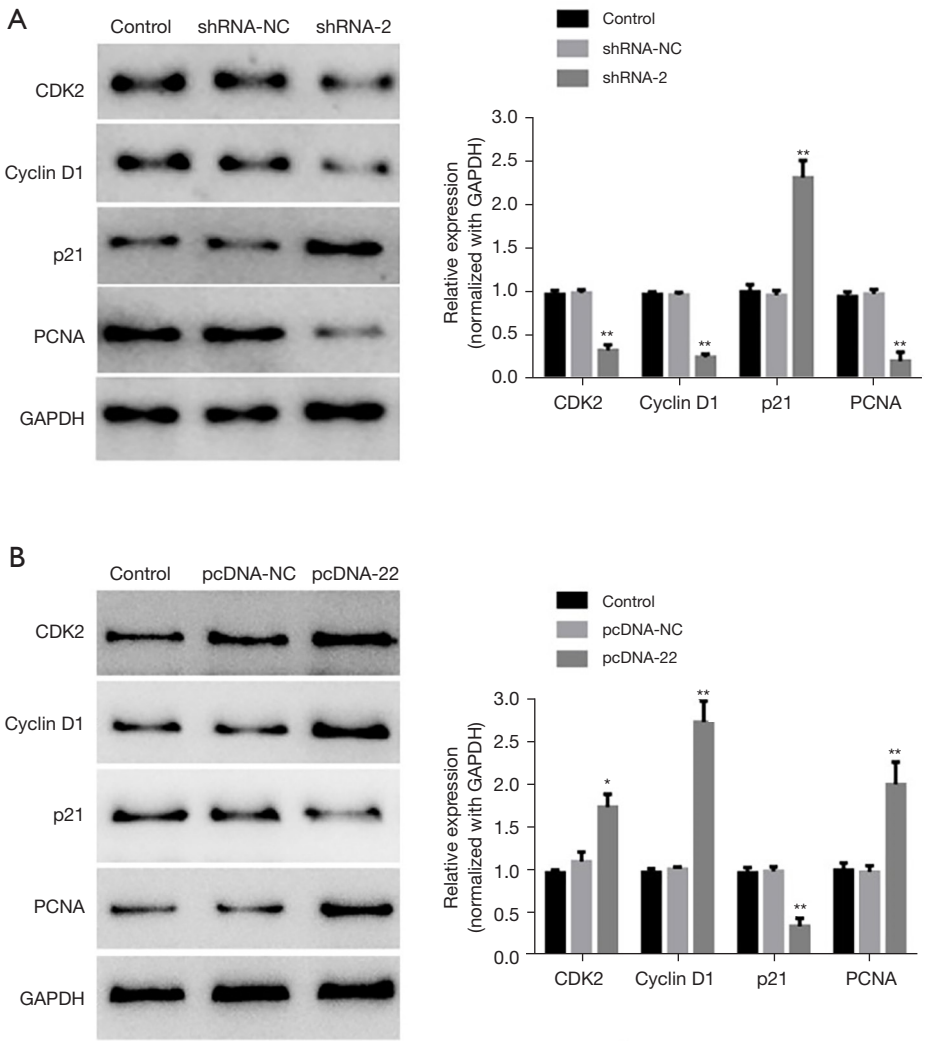

Figure 3 The effect of LINC00922 on the expression of cell cycle-related proteins in HTR-8/SVneo cells was detected by Western blot. (A) The effects of knockdown of LINC00922 on cell proliferation-related proteins CDK2, Cyclin D1, p21, and PCNA. (B) The effects of overexpression of LINC00922 on cell proliferation-related proteins CDK2, Cyclin D1, p21, and PCNA. *, P<0.05; **, P<0.01. CDK2, cyclin-dependent kinase 2; Cyclin D1, G1/S-specific cyclin-D1; PCNA, proliferating cell nuclear antigen.

inhibiting the cell cycle (*Figure 3A*).

To explore the effect of overexpression of LINC00922 in HTR-8/SVneo cells, CDK2 and Cyclin D1 were detected, which promoted cells from the G0/G1 phase to the S and G2/M phases. Western blot results showed that compared with the blank control group, there was no significant change in the expression of related proteins in the empty vector group (pcDNA-NC), while the overexpression of LINC00922 (pcDNA-22) significantly promoted the expression of CDK2, Cyclin D1, and PCNA, and inhibited the expression of p21 (*Figure 3B*).

Effect of LINC00922 on the invasion and migration of HTR-8/SVneo placental trophoblasts

Existing studies have shown that the onset of PE mainly involves "shallow implantation" caused by insufficient infiltration and migration of placental trophoblast cells (17). Therefore, this study further explored whether LINC00922 affected the invasion and migration abilities of HTR-8/SVneo placental trophoblasts. The Transwell assay results showed that compared with the blank control group, the migration and invasion abilities of shRNA-NC cells were not significantly changed. Knockdown of LINC00922

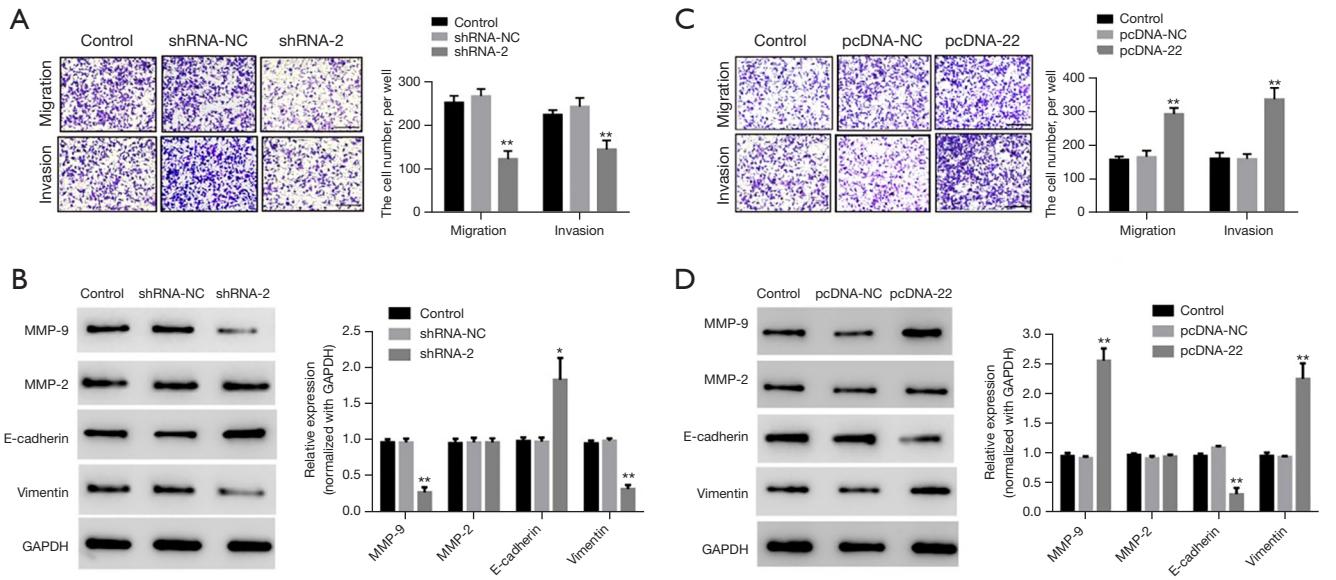

Figure 4 The effect of LINC00922 on the invasion and migration of HTR-8/SVneo cells. (A) The effect of knockdown of LINC00922 on cell invasion and migration was detected by the Transwell assay (crystal violet staining, scale bar =100 μm). (B) The effect of knockdown of LINC00922 on cell invasion-related proteins was detected by Western blot. (C) The effect of overexpression of LINC00922 on cell invasion and migration was detected by the Transwell assay (crystal violet staining, scale bar =100 μm). (D) The effect of overexpression of LINC00922 on cell invasion-related proteins was detected by western blot. *, P<0.05; **, P<0.01. MMP-9, matrix metallopeptidase 9; MMP-2, matrix metallopeptidase 2.

(shRNA-2) significantly inhibited the invasion and migration abilities of HTR-8/SVneo cells (*Figure 4A*). The effects of knockdown or overexpression of LINC00922 on the expression of matrix remodeling- and invasion-related proteins in HTR-8/SVneo cells were further explored. Western blot results showed that compared with the blank control group, the expression of related proteins in the shRNA-group (shRNA-NC) was significantly changed. Knockdown of LINC00922 (shRNA-2) significantly inhibited the expression of MMP-9 and vimentin and promoted the expression of E-cadherin in cells, but had no significant effect on the expression of MMP-2 (*Figure 4B*).

LINC00922 was overexpressed in HTR-8/SVneo cells, and the Transwell assay results showed that the migration and invasion abilities of cells in the empty vector group (pcDNA-NC) were not significantly changed compared with the blank control group. The overexpression of LINC00922 (pcDNA-22) could significantly promote the invasion and migration of cells (*Figure 4C*). LINC00922 was overexpressed in HTR-8/SVneo cells and western blot results showed that compared with the blank control group, the expression of related proteins in the empty vector group (pcDNA-NC) was not significantly changed, while the overexpression of LINC00922 (PCDNA-22) significantly promoted the expression of MMP-9 and vimentin, and inhibited the expression of E-cadherin, but had no significant effect on the expression of MMP-2 (*Figure 4D*).

Discussion

PE is the leading cause of morbidity and perinatal mortality worldwide, and results in 60,000 maternal deaths every year (18). Up to now, the etiology of PE has remained unclear. PE is a common cause of fetal growth restriction, fetal malformation, and maternal death, so it is of great significance to explore the pathogenesis of PE. LncRNAs are an important type of regulatory RNA which are involved in the regulation of a variety of biological functions, and are abnormally expressed in many diseases (19,20). Recent studies have shown that lncRNAs are closely related to the function of trophoblast cells in PE (21). This study first found that LINC00922 was highly expressed in PE tissues. Knockdown of LINC00922 could significantly inhibit the proliferation and clone formation ability of

HTR-8/SVneo cells, induce cell cycle arrest in the G0/G1 phase, and inhibit the invasion and migration of cells. However, overexpression of LINC00922 had the opposite effect. Knockdown or overexpression of LINC00922 could significantly affect the expression of cell cycle and invasion-related proteins, suggesting that LINC00922 may play an important role in the development, early diagnosis, and treatment of PE.

Recent studies have shown that the abnormal expression of lncRNAs can significantly affect the occurrence and development of PE, and these lncRNAs are expected to be biomarkers for the early diagnosis or treatment of PE (22,23). A previous study showed that lncRNA SPRY4-IT1 was highly expressed in placental tissue and could regulate the proliferation, migration, apoptosis, and tube formation of HTR-8/SVneo cells, which provided evidence for the role of lncRNAs in PE at the cellular level for the first time, and has initiated further study (24). LncRNA MEG3 was downregulated in PE placental tissues, which inhibited the migration of trophoblast cells and promoted their apoptosis. It also affected the expression of NF-κB, caspase 3, and Bax proteins, which may be related to the failure of uterine spiral artery remodeling, leading to the occurrence of PE (25). LncRNA MALAT1 was highly expressed in placental implantation and/or penetration, and its downregulation inhibited the invasion of choriocarcinoma trophoblast cell lines BeWo, JAR, and JEg-3, suggesting that MALAT1 may be involved in the regulation of trophoblast cell invasion during the development of late invasive placenta (26). Therefore, lncRNAs can play important regulatory roles in the occurrence and development of PE through a variety of pathways, and may gradually become biomarkers for PE. This study found that LINC00922 was highly expressed in PE tissues. Further studies showed that knocking down LINC00922 could significantly inhibit the proliferation and clone formation ability of HTR-8/SVneo cells and cause cell cycle arrest, while overexpression of LINC00922 had the opposite effect. This suggests that LINC00922 has an important effect on the proliferation of placental trophoblast cells.

The cell cycle is the main process of normal cell division and proliferation in the body, and is regulated by a variety of cell cycle regulatory proteins. The abnormal expression of these proteins may lead to cell cycle disorder, affecting normal body functions (27,28). For example, lncRNA CCAT1 was highly expressed in PE, which could promote the progression of PE by inhibiting the expression of CDK4 (16). LncRNA SNHG12 promoted the transition of trophoblast cells from the G0/G1 phase to S phase, and then promoted the occurrence of PE (29). This study further showed that knockdown of LINC00922 significantly inhibited the expression of cell cycle-related proteins CDK2, Cyclin D1, and PCNA, and promoted the expression of p21, while overexpression of LINC00922 had the opposite effect. Previous studies have shown that p21 binds to CDK2 and inhibits its protein activity, leading to cell cycle arrest in the G1/S phase (30).

PE is characterized by impaired trophoblast cell invasion and spiral arterial transformation, leading to progressive uterine placental hypoxia. The abnormal migration and invasion abilities of EVT is an important cause of the development of PE (31). For example, PE-related lncRNA INHBA-AS1 promotes the occurrence of PE by affecting the proliferation, invasion, and migration of placental trophoblast cells through the CENPB/TRAF1 signaling pathway (22). This study showed that knockdown of LINC00922 could significantly inhibit the invasion and migration abilities of HTR-8/SVneo cells. Molecular mechanism studies showed that knockdown of LINC00922 could significantly inhibit the expression of MMP-9 and vimentin, and promote the expression of E-cadherin in cells. However, it had no significant effect on the expression of MMP-2. The results of this study are consistent with previous reports, that is, inhibition of MMP-9/2 expression can significantly inhibit the invasion and migration abilities of placental trophoblast cells (32). However, how LINC00922 affects the expression of these proteins remains unclear. Therefore, we infer that LINC00922 may regulate the expression of downstream target genes through intermediate miRNAs or regulating gene transcription in PE tissues. In subsequent experiments, we will further reveal the specific molecular regulatory mechanism of LINC00922 in PE and clarify the regulation of target genes.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/atm-21-4923). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). All enrolled patients signed informed consent, and the study was approved by the ethics committee of the First Affiliated Hospital of Soochow University (No. 20195845).

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