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LINC01128 suppresses the progression of recurrent spontaneous abortion via modulation of the miR-515-5p/DNMT1 axis

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Abstract

Objective. To investigate the mechanism of action involved in LINC01128 in serum and decidua tissue of recurrent spontaneous abortion (RSA).

Materials and Method. 49 RSA patients and 50 pregnant women with normal termination of pregnancy were included in the study. Following curettage, decidual tissues and serum samples were stored at -80 °C. Real-time quantitative PCR (RT-qPCR) was performed to detect the expression of genes as well as vascular endothelial growth factor (VEGF) and matrix metalloprotein 2/9 (MMP2 and MMP9). A dual-luciferase report assay (DLR) was performed to verify the targetting interactions between miR-515-5p and LINC01128 or DNA methyltransferase 1 (DNMT1). Cell Counting Kit-8 (CCK8) detected cell proliferation, Transwell observed migration, and flow cytometry recorded apoptosis.

Results. RSA patients contained low LINC01128 and high miR-515-5p in serum and metaphase tissue, and the expression of both was negatively correlated. DLR results showed that these two genes have an antagonistic targetting relationship. LINC01128 could inhibit the expression of miR-515-5p, promote the proliferation and migration of trophoblast cells, reduce apoptosis, and increase the expression of vascular endothelial growth factor, MMP2 and MMP9. DNMT1 is a target gene of miR-515-5p, which is lowly expressed in serum and metaphase tissue of RSA patients, and negatively correlates with miR-515-5p. The expression level of DNMT1 was increased in trophoblast cells transfected with LINC01128.

Conclusions. The LINC01128/miR-515-5p/DNMT1 regulatory axis in trophoblast cells may be associated with the development of RSA.

Key words

miR-515-5p, DNMT1, recurrent spontaneous abortion, LINC01128

INTRODUCTION

Recurrent spontaneous abortion (RSA) is one of the more common gynaecological problems affecting women's health [1], affecting approximately 5% of women of childbearing age globally [2, 3]. The causes of RSA are complex and varied, making it difficult for women of childbearing age who have experienced RSA to have a successful pregnancy, which brings great mental stress and physical burden to the female patients. Up to now, studies have found that causes of RSA include immune factors [4], reproductive structural abnormalities, genetic factors, endocrine disorders, infections, blood-clotting disorders, male-related factors, and psychological factors [5]. The communication between blastocyst trophoblast cells and maternal uterine mucosa in early pregnancy is the basis for ensuring the normal development of the embryo [6], and if the trophoblast cells in early pregnancy are reduced for unknown reasons or invade abnormally, the timely contact between the mother and the foetus cannot be ensured, and preterm abortion is prone to occur. Although many studies have reported factors that cause RSA, few have examined methods to predict RSA in advance. Therefore, the discovery of markers that can predict the progression of RSA and reveal the molecular mechanism of RSA occurrence in the organism is of great significance for the clinical assessment and treatment of RSA.

In recent years, non-coding RNAs (ncRNAs) have been found to play a role in the progression of various diseases. A large number of studies have shown that long non-coding RNAs (LncRNAs) could bind target genes involved in the regulation of reproductive diseases [7]. Moreover, competitive endogenous RNA (ceRNA) network analyses have evidenced that LncRNAs are also the most frequently involved molecules in RSA [8]. Numerous studies have shown that LncRNAs are involved in cell proliferation, invasion, and angiogenesis [9], and their metabolic abnormalities predispose to RSA. However, as a newly identified lncRNA, the functional role of LINC01128 remains largely uncharted. Notably, it has been implicated in modulating vascular smooth muscle cell apoptosis in atherosclerosis and serves as an adverse prognostic indicator in colorectal cancer [10]. Of particular interest, a retrospective analysis found that LINC01128 was significantly down-regulated in

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patients with pre-eclampsia (PE) and that it may contribute to PE, and that it may contribute to PE pathogenesis by regulating blastocyst trophoblast cell function. However, few studies have reported the role of LINC01128 in RSA, and its mechanism in regulating RSA remain unclear. Ove-expression of miR-515-5p inhibits invasion and migration of trophoblast cells in patients with pre-eclampsia [11, 12]. In addition, early laboratory analyses revealed that miR-515-5p may be a LINC01128 target gene. Therefore, it is hypothesized that LINC01128 might be involved in RSA progression through miR-515-5p. It has been shown that DNMT1 is expressed in normal human trophoblast cells and in the dividing phase, but is severely lost in the cells of patients with RSA, which leads to abnormal embryonic development and triggers pre-eclampsia. A study demonstrated a regulatory role for the miR-515-5p/DNMT1 regulatory axis in CRC [13]. However, how miR-515-5p and DNMT1 regulate RSA has not been reported, and the mechanism of action of LINC01128 targeting miR-515-5p/DNMT1 to regulate RSA is unclear.

OBJECTIVE

The aim of the study is to evaluate the diagnostic value of LINC01128 in patients with RSA by analyzing baseline data from 99 patients with miscarriage collected in the clinic. Based on previous studies, an *in vitro* cell model was constructed using trophoblast cells to investigate the mechanism by which LINC01128 function in RSA. The results obtained will reveal another potential mechanism underlying RSA, and provide a theoretical basis for clinical prevention and treatment of this disorder.

MATERIALS AND METHOD

Sample collection. 99 patients who visited Tonglu County Traditional Chinese Medicine Hospital between October 2021 – August 2022 were included in the study: including 49 patients with RSA and 50 pregnant women with normal abortion The patients obtained decidual ceRNA tissue decidual ceRNA tissue was obtained from the patients by curettage after abortion and together with venous blood serum for backup, stored at -80^{0}C. The mean age of the participants was 29.69 ± 3.24, body mass index (BMI) – 23.59 ± 2.96, and all aborted at about 8 weeks. The inclusion criteria for RSA patients were: 1) history of ≥2 spontaneous abortions; 2) no experience of a successful pregnancy; 3) gestational cycle ≤20 weeks; and 4) age between 20 – 45 years. The control group was comprised of healthy pregnant women with concurrent pregnancies and successful experiences who chose to voluntarily terminate their pregnancies around 8 weeks of gestation because they had no reproductive requirements. None of the control population had a history of preterm labour, eclampsia, or ectopic pregnancy, and all subjects had a normal karyotype. Patients with the presence of reproductive tract malformations, structural abnormalities of the uterus, endocrine diseases, infectious vaginal diseases, autoimmune diseases, and chromosomal abnormalities of the embryo, were ruled out in the study.

The study was approved by the Ethics Committee of Tonglu County Traditional Chinese Medicine Hospital, and the subjects signed a written informed consent in accordance with the Declaration of Helsinki.

Cell culture and transfection. The human chorionic trophoblast cells (HTR-8-Svneo) used in the experiments were purchased from Wuhan Procell Biotechnology Co. They were grown in RPMI-1640 (Gibco, USA) medium at 37 °C with a CO2 concentration of 5%. In addition, 1% antibiotics and 10% foetal bovine serum (Gibco, USA) were added to the basal medium. DNA vectors (pcDNA3.1, pcDNA3.1-LINC01128, oe NC, oe DNMT1, DNMT1 Small interfering RNA [si-DNMT1], and small interfering RNA NC [si-NC]), oligonucleotides (miR NC, miR-515-5p mimic, miR-515-5p inhibitor) were introduced into logarithmically growing trophoblast cells by transfection reagents. The cells were incubated in a constant temperature cell culture incubator for subsequent relevant experiments.

Serum and decidua tissue RNA extraction and quantification. Total RNA was extracted from serum and decidua tissues by TRIzol™ (Invitrogen, USA) reagent, which required crushing the decidua tissues using the Tissue Master™ Handheld Tissue Mill (Beyotime, Shanghai, China) in an ice bath. NanoDrop Nucleic Acid Protein Concentration Tester (Thermo, USA) was used to check the quality of RNA. miRNA 1st Strand cDNA Synthesis Kit (Vazyme, Nanjing, China) synthesized cDNA of LINC01128 with miR-515-5p; HiScript II 1st Strand cDNA Synthesis Kit (Vazyme, Nanjing, China) synthesized cDNA of DNMT1 and matrix metalloproteinase 2/9 (MMP2/9), and the reverse transcription reaction conditions were referred to the corresponding instructions. Real-time fluorescence quantitative chain polymerase reaction was carried out using Taq Pro Universal SYBR qPCR Master Mix under the following conditions: 95° C, 30s pre-denaturation reaction, 95 °C, 5s, 60 °C, 20s, and cycling for 40 times, and the reaction was finished at 55 °C for 1min. All experiments were performed with 3 validated replicates, and the experimental data were normalized by using GAPDH as an internal reference gene for LINC01128, vascular trophoblast growth factor (VEGF), MMP2/9, DNMT1, and U6 as an internal reference gene for miR-515-5p. The relative expression of genes was calculated by $2^{-\Delta\Delta Ct}$. Primer sequence information for the relevant genes is given in Supplementary Table 1.

Supplementary Table 1. Primer sequence information

Name		Primer sequence
LINC01128	Forward	5′-CAGAGGAGCTACGAAGGGAG-3′
	Reverse	5′-CTGTGGAATCGCTTGGTACG-3′
miR-515-5p	Forward	5′-TTCTCCAAAAGAAAGCACTTTCTG-3′
	Reverse	5′-CTCGCTTCGGCAGCACA-3′
VEGF	Forward	5′-ATGAACTTTCTGCTGTCTTGG-3′
	Reverse	5′-TCACCGCCTCGGCTTGTCACA-3′
MMP2	Forward	5′-TACAGGATCATTGGCTACACACC-3′
	Reverse	5′-GGTCACATCGCTCCAGACT-3′
MMP9	Forward	5′-GGGACGCAGACATCGTCATC-3′
	Reverse	5′-TCGTCATCGTCGAAATGGGC-3′
DNMT1	Forward	5′-TACCTGGACGCCCTGACCTC-3′
	Reverse	5′-CGTTGGCATCAAAGATGGACA-3′
GAPDH	Forward	5′-AGTAGAGGCAGGGATGATG-3′
	Reverse	5′-TGGTATCGTGGAAGGACTC-3′
U6	Forward	5′-CTCGCTTCGGCAGCACATATACT-3′
	Reverse	5′-ACGCTTCACGAATTT-GCGTGTC-3′

Dual luciferase reporter assay. The prediction results from the ENCORI database (https://starbase.sysu.edu.cn/) showed that miR-515-5p might be the target gene of LINC01128; DNMT1 may have a targeting relationship with miR-515-5p. Wild-type (WT-LINC01128, WT-DNMT1) and mutant (MUT-LINC01128, MUT-DNMT1) plasmids were constructed by the pGL3 vector (Promega, USA), respectively. Transfection reactions were performed by Lipo 3000 in an enzyme-labelled plate, and assayed by Dual-Luciferase® Reporter Assay System (Promega, USA) components after 48h of transfection. Cells were broken up using cell lysate, and 100 µl of lysate was mixed with luciferase in equal volume and added into a 96-well light-shielding plate; the fluorescence intensity was detected by a multifunctional chemiluminescence instrument (REMEX, Xi'an, China) after a few moments of the reaction, and the results of the experiments were normalized by the luminescence value of sea kidney luciferase.

RNA immunoprecipitation (RIP) assay. RIP assays were performed with minor modifications to the standard protocol. Cells were lysed in RIPA buffer containing proteinase inhibitor on ice for 30 min, then centrifuged at 13,000 rpm at 4°C for 15 min. The supernatant was collected and transferred to an RNase-free tube. Magnetic beads were washed with RIPA buffer and incubated with the Ago2 antibody at room temperature for 30 min; equal amounts of IgG were used as the control. After antibody-bead coupling, 500 µl RIPA buffer was added to remove unbound antibodies. The antibody-conjugated beads were re-suspended in 900 µl RIPA buffer, mixed with 100 µl cell lysate, and rotated at 4°C overnight. After washing with RIPA buffer, proteinase K was added to degrade proteins. The RNA in the immunoprecipitation complexes was purified and detected by RT-qPCR.

Cell proliferation assay. Cell proliferation was examined using a Cell Counting kit 8 (Abcam, Cambridge, UK). Cells subjected to different treatments were seeded into 96-well enzyme-labelled plates at a density of 1×10^3 cells/well before the experiment, and 10 µl of CCK8 reagent was added to the cells at 0 h, 24 h, 48 h, and 72 h of transfection, respectively, and the cells incubated at 37°C for 2 h. The absorbance of the solution was detected by a UV spectrophotometer at a wavelength of 450 nm to assess the effect of different transfection conditions on the proliferation of cells.

Transwell cell migration assay. Before the experiment, the cell growth environment was replaced with basic medium starvation treatment for 24 h. 1×10^4 cells were inoculated in the upper chamber of Transwell, and the DNA vector and oligonucleotides were co-transfected into the cells by transfection reagent Lipo 3000. Cell growth medium containing 10% foetal bovine serum was added to the lower chamber and the cells were incubated in a constant temperature incubator for 3 h. The cells were then fixed with 4% paraformaldehyde, stained with 0.1% crystal violet dye, and washed several times with PBS. Cell migration was evaluated by counting the number of migrated cells in 5 random fields of view under an inverted microscope.

Flow cytometry. HTR8/SVneo from different treatment groups were first digested by 0.25% trypsin (Gibco, USA), 500 × g, centrifuged at 4°C to discard the supernatant, cells were washed using pre-cooled PBS, and staining buffer was re-suspended and adjusted to a cell concentration of 1×10^6 cells/mL. 100 µl of single-cell suspension was mixed with an appropriate amount of Annexin V-FITC/PI staining solution (Roche, Switzerland) and added to a flow-through tube, incubated in an ice bath protected from light for 30 min. Unreacted fluorescein background was removed using staining buffer first, and the cells were then washed and resuspended in PBS. Finally, the proportion of apoptotic cells at 488 nm was recorded by a FACSCanto II flow cytometer (BD Bioscience, USA).

Data analysis. The results of the data information were analyzed by SPSS 23 and GraphPad Prism 9 software. Three valid replications were performed, and statistical results were expressed as mean ± standard deviation ($\bar{x}\pm S$). Distribution of the data was evaluated using the Shapiro-Wilk test to assess whether the data followed a normal distribution. All quantitative data included in the present study were confirmed as conforming to a normal distribution. Comparisons between groups were made using an independent samples t-test and analysis of variance (ANOVA), and *post hoc* tests were performed on the results of the analyzed data by Tukey's method. The diagnostic value of LINC01128 and miR-515-5p was assessed by constructing receiver operating characteristic (ROC) curves. Pearson correlation analysis was performed to assess the correlation between the expression of LINC01128, miR-515-5p, and DNMT1 in serum and decidual tissues of patients with RSA. Multiple comparisons were adjusted by the Bonferroni correction where appropriate. $P < 0.05$ represents a statistically significant difference in data results.

RESULTS

Expression of LINC01128 and miR-515-5p in different patients. Baseline characteristics were initially compared between RSA patients and the controls. No significant disparities were revealed in age, body mass index, or gestational age between the 2 cohorts ($P>0.05$) (Suppl. Tab. 2). Quantitative results demonstrated a notable decrease in serum LINC01128 (Fig. 1A) and a marked up-regulation of miR-515-5p (Fig. 1B) in the serum of RSA patients compared with healthy controls. Pearson correlation analysis revealed an apparent negative correlation between the LINC01128 and miR-515-5p expression in the serum of RSA patients (r=-0.6971; *P<0.0001)* (Fig. 1C). The results of decidua tissue indicated that LINC01128 was expressed lowly in RSA patients (Fig. 1D), whereas miR-515-5p was highly expressed (Fig. 1E). Secondly, there was also a markedly negative correlation between LINC01128 and miR-515-5p expression in decidua tissues (r=-0.7811; *P<0.0001)* (Fig. 1F).

Supplementary Table 2. Basic clinical information of the patient

Characteristic	Control (n=50)	RSA (n=49)	P value
Age (years)	29.20±3.19	30.18±3.30	0.135
BMI (kg/m²)	23.30±3.12	23.88±2.80	0.330
Gestation age (weeks)	7.98±0.52	8.08±0.61	0.381

BMI – body mass index; $P < 0.05$ represents a statistically significant difference

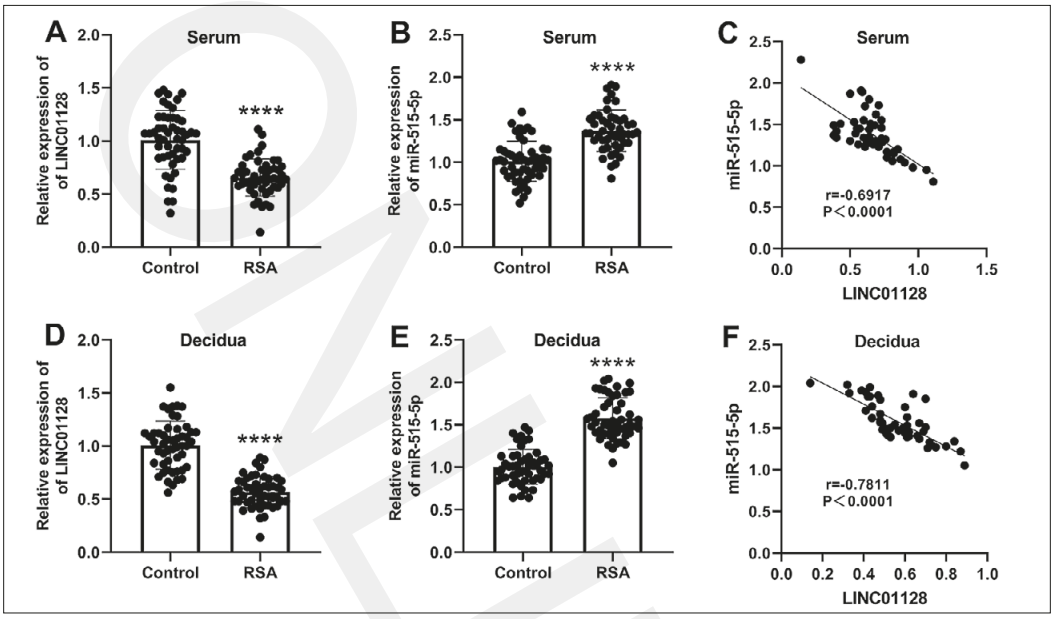

Figure 1. LINC01128 and miR-515-5p expression in RSA and normal abortion patients. (A) Serum LINC01128 level in RSA and normal abortion (control) patients. (B) Serum miR-515-5p level in RSA and control. (C) Correlation analysis between serum LINC01128 and miR-515-5p expression in RSA patients. (D) LINC01128 levels in decidua tissue of RSA and control group. (E) miR-515-5p levels in decidua tissue of RSA and control group. (F) Correlation between LINC01128 and miR-515-5p expression in decidua tissue of RSA patients.
* *P<0.05*, ** *P<0.01*, *** *P<0.001*, **** *P<0.0001* vs. control

Diagnostic value of LINC01128 and miR-515-5p expression in RSA patients. Based on the above results, the clinical value of LINC01128 and miR-515-5p was assessed by analyzing the ROC curve constructed from their expression levels. The results showed that LINC01128 yielded an AUC of 0.8580 (CI: 0.7780–0.9379; *P<0.0001*), with a sensitivity of 87.76% and a specificity of 82.00% (Fig. 2A). This result demonstrated that LINC01128 has diagnostic value for RSA. For miR-515-5p, the AUC was 0.8590 (CI: 0.7851–0.9329; *P<0.0001*), with a sensitivity of 83.67%, and a specificity of 82.00% (Fig. 2B). This demonstrated that miR-515-5p also had diagnostic value for RSA.

miR-515-5p is a possible target gene of LINC01128. The ENCORI database predicted that miR-515-5p might be a target gene of LINC01128 (Fig. 3A). The results of the dual luciferase reporter assay explained that cellular fluorescence activity decreased when miR-515-5p mimic and WT-LINC01128 were transfected, whereas cellular fluorescence activity was enhanced in the presence of miR-515-5p inhibitor. However, none of the changes in fluorescence activity were significantly different in endothelial cells containing MUT-LINC01128 (Fig. 3B). When compared to the negative control IgG, both LINC01128 and miR-515-5p showed significant enrichment on the Ago2 antibody (*P*<0.001) (Fig. 3C). In addition, post-treatment cell analysis illustrated that LINC01128 expression was obviously increased in HTR8/SVneo after transfection with LINC01128 (*P<0.001)* (Fig. 3D), and miR-515-5p was clearly decreased (*P<0.01*) (Fig. 3E). However, after transfection of pcDNA-LINC01128+ miR mimic, only the expression of miR-515-5p was upregulated, and the expression of LINC01128 was not significantly changed. This result again demonstrates that miR-515-5p in endothelial cells is present downstream of and regulated by LINC01128.

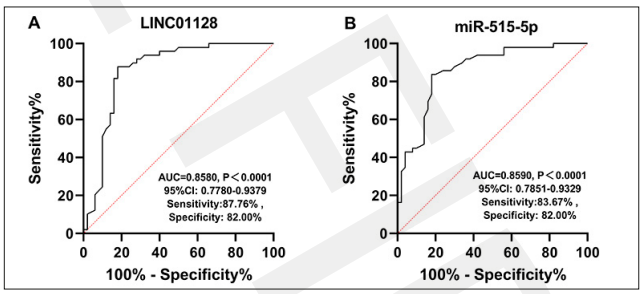

Figure 2. ROC curves of serum LINC01128 and miR-515-5p for prediction of RSA. (A) ROC curves assessing the diagnostic value of LINC01128 for prediction of RSA. (B) ROC curves assessing the diagnostic value of miR-515-5p for prediction of RSA. AUC denotes the area under the curve of the enclosure, CI stands for the confidence interval, i.e., the interval within which a sample's true value has a 95% probability of occurring of the interval.
* *P<0.05*, ** *P<0.01*, *** *P<0.001*, **** *P< 0.0001*

LINC01128 affects cellular function and inflammation levels by regulating miR-515-5p. Based on these results, the effects of LINC01128 and miR-515-5p on HTR8/SVneo cell function were further investigated. The results showed that the proliferation was significantly increased following transfection with LINC01128 alone, whereas proliferation was markedly inhibited by co-transfection with the miR-515-5p mimic (*P<0.05*) (Fig. 4A). Transwell results demonstrated that LINC01128 overexpression promoted cell migration, while miR mimic suppressed migration (*P<0.0001*) (Fig. 4B). Flow cytometry analysis showed that transfection with pcDNA-LINC01128 reduced apoptosis, but the co-administration of miR-515-5p mimic induced apoptosis (Fig. 4C). In addition, it was found that the use of miR mimic resisted the LINC01128 overexpression-induced increase in VEGF and up-regulation of MMP2 and MMP9 (*P<0.01*) (Fig. 4D-F).

LINC01128 is involved in RSA progression through the miR-515-5p/DNMT1 axis. The prediction results showed that DNMT1 might be the target gene of miR-515-5p (Fig. 5A). The fluorescence activity decreased when the cells contained wild-type vector (WT-DNMT1) and miR-515-5p mimic and increased in the presence of miR-515-5p

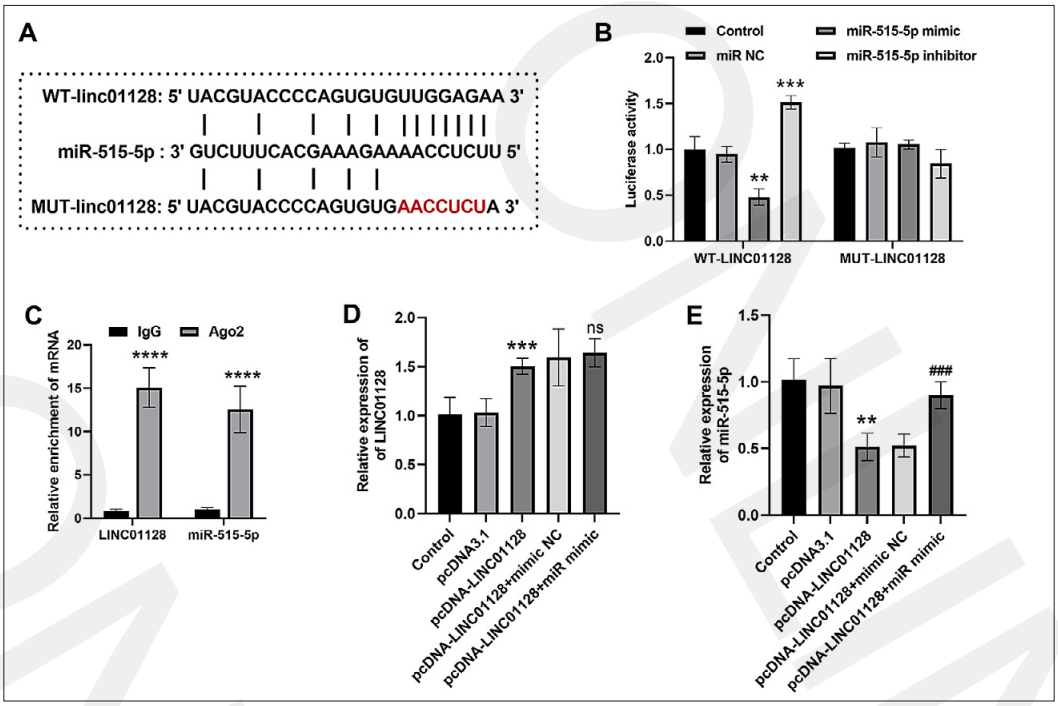

Figure 3. Validation of the targeting relationship between LINC01128 and miR-515-5p. (A) Interactive target site map of LINC01128 and miR-515-5p; (B) Dual luciferase report assay to validate the targetting relationship between LINC01128 and miR-515-5p. (C) RIP assay analyzed the targetting relationship between miR-515-5p and LINC01128. (D) Expression level of LINC01128 in the cells after transfection of LINC01128 and miR mimic. (E) miR-515-5p expression level in cells after transfection of LINC01128 and miR mimic.
* *P<0.05*, ** *P<0.01*, *** *P<0.001*, **** *P<0.0001* vs. miR NC& pcDNA3.1. ### *P<0.001* vs. pcDNA-LINC01128+ mimic NC

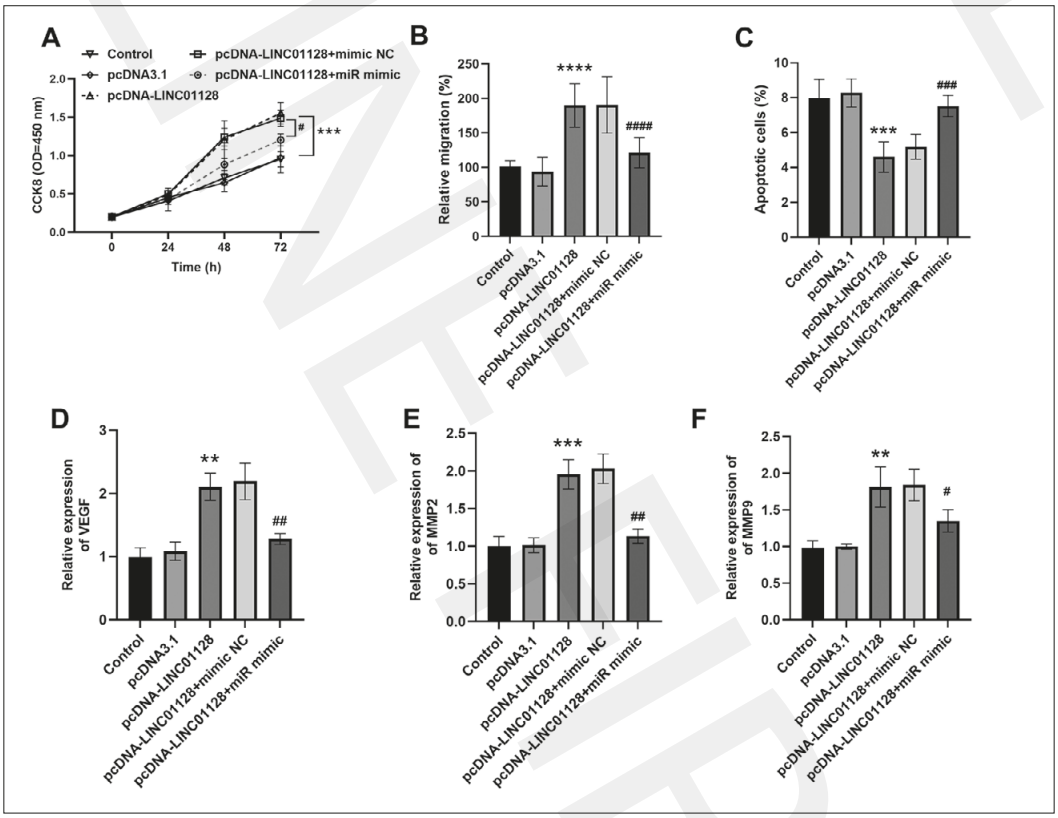

Figure 4. Effect of transfection of LINC01128 and miR mimic on cell function and inflammatory factors. Effects of co-transfection of LINC01128 and miR mimic on cell proliferation (A) migration (B), and apoptosis (C) conditions; expression levels of VEGF (D), MMP2 (E), and MMP9 (F) in cells after transfection of LINC01128 and miR mimic.
* *P<0.05*, ** *P<0.01*, *** *P<0.001*, **** *P<0.0001* vs. pcDNA3.1. # *P<0.05*, ## *P<0.01*, ### *P<0.001*, #### *P<0.0001* vs. pcDNA-LINC01128+ miR mimic

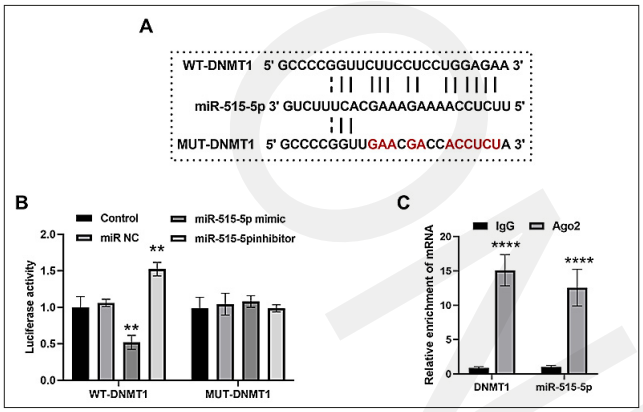

Figure 5. Validation of miR-515-5p targetting relationship with DNMT1. (A) Interactive target site map of miR-515-5p and target gene DNMT1. (B) Changes in intracellular fluorescence activity after co-transfection of miR-515-5p with wild-type (WT-DNMT1) and mutant-type (MUT-DNMT1) containing vectors. (C) RIP assay analyzed the targeting relationship between DNMT1 and miR-515-5p. ** *P<0.01,* **** *P <0.0001* vs. miR NC or IgG

inhibitor (*P<0.01*) (Fig. 5B). However, there was no significant difference in the change of cellular fluorescence activity in the presence of the mutant vector (MUT-DNMT1). Compared to the negative control IgG, both DNMT1 and miR-515-5p showed significant enrichment on the Ago2 antibody (*P*<0.001) (Fig. 5C). Quantitative results indicated that DNMT1 was significantly downregulated in both serum (*P<0.0001*) (Fig. 6A) and decidua tissue (*P<0.0001*) (Fig. 6B) of RSA patients. Moreover, Pearson correlation analysis revealed that miR-515-5p was negatively correlated with DNMT expression in serum (r=-0.8141; *P<0.0001*) (Fig. 6C) and decidua tissue (r=-0.7699; *P<0.0001*) (Fig. 6D). By transfection of different substances, it was observed that overexpression of LINC01128 significantly up-regulated DNMT1 expression; transfection of pcDNA-LINC01128+ miR mimic resulted in the down-regulation of DNMT1 expression, and the level of DNMT1 was significantly up-regulated again when transfected with pcDNA+ miR mimic+ oe DNMT1 (*P*<0.01) (Fig. 6E). This result demonstrates that LINC01128 may target the regulation of the miR-515-5p/DNMT1 axis in trophoblast cells.

The influence of DNMT1 on cellular function was investigated further. Different, small interfering RNAs substantially downregulated DNMT1 expression (Fig. 7A). Among them, si-DNMT1#1 had the most significant downregulation effect and was chosen for subsequent research. Compared with si-NC, DNMT1 interference notably inhibited cell proliferation (*P*<0.001) (Fig. 7B). DNMT1 silencing not only suppressed cell migration but also promoted apoptosis (*P*<0.05) (Fig. 7C-D). Additionally, DNMT1 reduction significantly prevented VEGF elevation and lowered MMP2 and MMP9 mRNA levels (*P*<0.005) (Fig. 7E-F).

DISCUSSION

RSA is an obstetric problem that seriously threatens the health of women of childbearing age, affecting the harmony of 2% of couples, an average, per year [14]. Due to the heterogeneity and specificity of RSA, the etiology is diverse and complex, and the pathogenic mechanism is not clear. Some studies have shown that LncRNAs encapsulated in vesicles are involved in the regulation of female reproduction [15], and

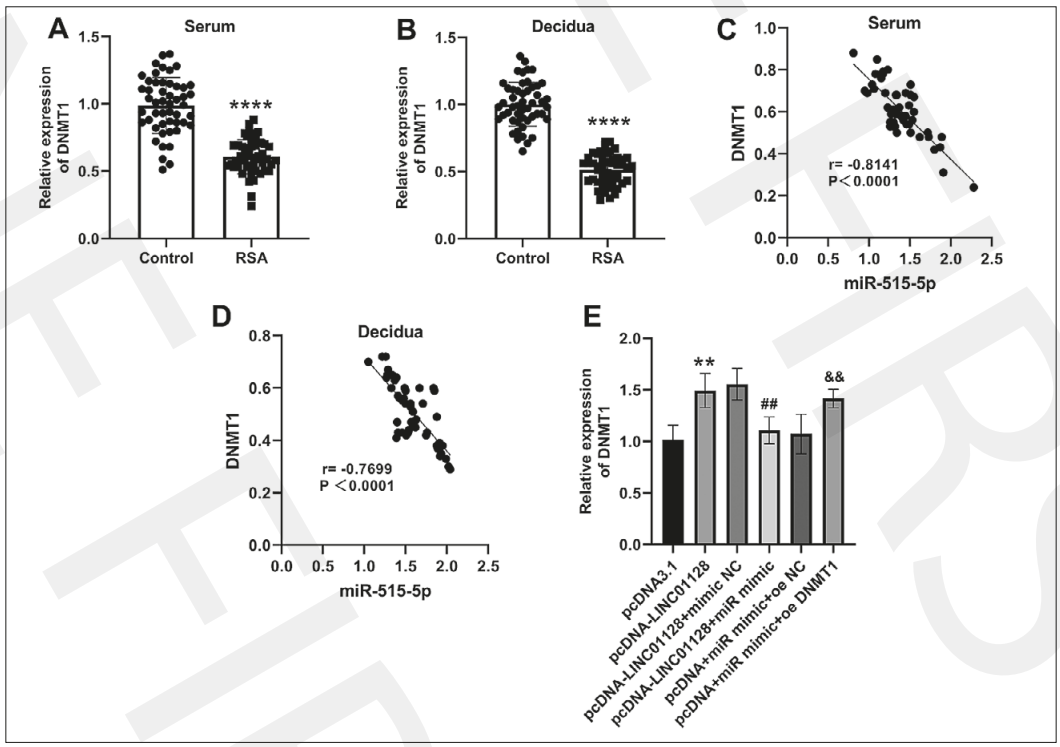

Figure 6. DNMT1 expression changes in RSA and normal abortion patients. (A) DNMT1 expression level in serum of RSA and control group. (B) DNMT1 expression level in decidua tissue of RSA and control group. (C) Correlation analysis of miR-515-5p in serum of RSA patients and DNMT1 expression. (D) Correlation analysis of miR-515-5p and DNMT1 expression in decidua tissue of RSA patients. (E) DNMT1 expression levels in cells after transfection with LINC01128, LINC01128 + miR mimic, and miR mimic + oe DNMT1, respectively. * *P<0.05*, ** *P<0.01*, *** *P<0.001*, **** *P<0.0001* vs. control & pcDNA3.1. ## *P<0.01* vs. pcDNA-LINC01128+ mimic NC. && *P<0.01* vs. pcDNA+ miR mimic+ oe NC

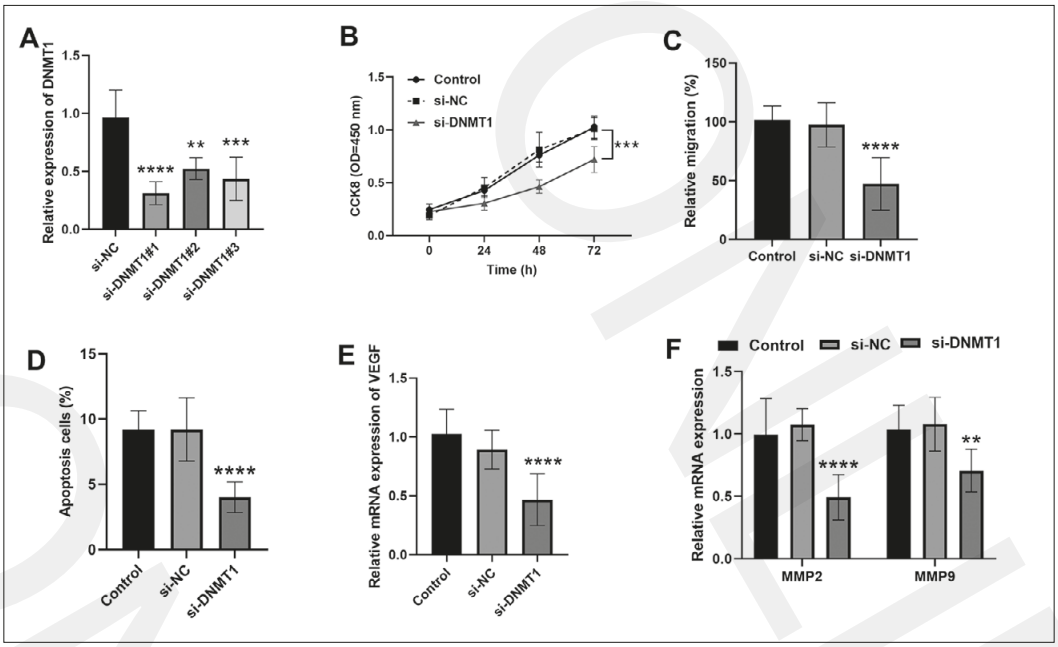

Figure 7. DNMT1 silencing impaired HTR8/SVneo cell function. (A) DNMT1 expression levels following transfection with different DNMT1 targeting siRNAs. (B-D) Changes in cell proliferation, migration, and apoptosis after DNMT1 interference, assessed via CCK-8, Transwell, and flow cytometry assays. (E-F) RT-qPCR analysis of the effects of DNMT1 silencing on VEGF, MMP2, and MMP9 mRNA expressions in cells. ** *P<0.01*, *** *P<0.001*, **** *P<0.0001* vs. control

LncRNAs can also regulate embryonic development and pregnancy complications [16, 17] via specific target genes. For example, dysregulation of LncSNHG12 contributes to RSA by inhibiting trophoblast migration via the Dio2/Snail [18]. In addition, transcriptomic sequencing revealed that many LncRNAs are dysregulated in RSA patients [19]. LINC01128 was found to be dysregulated in patients with eclampsia [20], but its mechanism of action in RSA is unclear. The results of the current study prompted the finding that low levels of LINC01128 and high levels of miR-515-5p were present in RSA patients, and the expression of both suggested an apparent negative correlation in serum and decidua tissues of RSA patients. This regulatory relationship is the 'driver' of RSA progress. ROC results demonstrated that both LINC01128 and miR-515-5p may have clinical diagnostic value in predicting the progression of RSA.

LINC01128 acts as a miRNA sponge to regulate mRNA expression. Recent research reveals it can also bind RNA-binding proteins, potentially broadening its regulatory role in RSA. In pre-eclampsia (PE), LINC01128 regulates migration and invasion in human chorionic trophoblast cells via miR-16 [20]. Similar to how H19 overexpression influences RSA apoptosis by targeting miR-29a-3p/SOCS3 [21], the current study suggests that LINC01128 may also play a crucial role in RSA pathogenesis through its interaction with miRNAs. It has been reported that miR-515-5p assists in predicting early-onset RSA and guide clinical decision-making [22]. Moreover, emerging evidence shows that miR-515-5p can be regulated by long non-coding RNAs other than LINC01128, suggesting a complex regulatory network in which it is involved. This complexity may contribute to its diverse functions in different physiological and pathological conditions. The target site map of biosignature analysis indicated that miR-515-5p and LINC01128 had multiple binding sites, and the dual luciferase reporter assay indicated the existence of a targeting interplay between the two. All these findings confirm the conjecture that LINC01128 has the potential to target miR-515-5p to participate in the RSA process.

To further explore the mechanism of action of LINC01128/miR-515-5p in RSA, HTR8/SVneo cells were selected, which can respond to changes in the embryonic trophoblast, for *in vitro* functional verification. The experimental results showed that miR-515-5p expression was reduced in cells after transfection with pcDNA-LINC01128. In trophoblast cells co-transfected with pcDNA-LINC01128+miR mimic, LINC01128 expression remained unchanged, whereas miR-515-5p expression was significantly upregulated. This result further confirmed that miR-515-5p is a downstream target of LINC01128. Cell function assay showed that transfection of LINC01128 inhibited miR-515-5p expression in cells, promoted cell proliferation and migration, and reduced apoptosis. The addition of LINC01128 promoted the expression of VEGF and up-regulated the level of MMP2/9. However, when LINC01128 was co-transfected with miR-515-5p mimic, apoptosis was increased, and proliferation and migration were attenuated, a result that is consistent with the discussion of in the literature. For example, Zhao XY et al [20] reported that LINC01128 was able to inhibit pre-eclampsia by promoting trophoblast proliferation through miR-16 [20]. Inflammation functions as a fundamental pathological regulator in diverse disease processes by modulating immune activation, tissue remodelling, and cellular function [23, 24]. Dysregulated inflammation critically impairs trophoblast activity, disrupts uterine microenvironmental homeostasis, and compromises embryo implantation, thereby contributing to the pathogenesis of RSA.

The results obtained in the current study suggest that LINC01128/miR-515-5p can participate in RSA progression by regulating cellular functions and inflammation levels. VEGF is crucial for promoting angiogenesis and facilitating vascular remodelling in early pregnancy. It also contributes to oocyte maturation, trophoblast proliferation, embryo

implantation, and placental vascular development. A deficiency or downregulation of VEGF can disrupt the formation of the villous vascular network or impair cellular infiltration and differentiation, potentially leading to miscarriage [25]. Additionally, research has linked VEGF factors to the clinical-pathological features of RSA [26]. MMPs, which are Zn^{2+}-dependent endopeptidases, participate in ECM degradation. Elevated MMP levels during mid-RSA imply their involvement in RSA pathogenesis through ECM breakdown. Studies have also shown that MMP2/9 expression has an effect on uterine artery blood flow in patients with RSA, and it has the potential to be a predictive marker for RSA [27].

These studies demonstrate that the expression of VEGF and MMPs [28] may influence RSA progression. Notably, for the first time, the LINC01128/miR-515-5p has been identified as a possible upstream regulator of this pathway, which may play a pivotal role in the diagnosis and treatment of RSA. This axis may operate by inducing excessive uterine ECM degradation, inhibiting trophoblastic tissue migration, and ultimately contributing to RSA progression.

LncRNAs can participate in the disease progression of chorionic cells, in which the abnormal expression of miRNAs is closely related to cell proliferation, apoptosis, and other processes. Numerous studies have shown that miR-515-5p can target HDAC2 to affect trophoblast cell function in pre-eclamptic patients [12], and it can also regulate trophoblast cell invasion and proliferation through XIAP [11]. The above findings suggest that miR-515-5p is involved in the regulation of HTR8/SVneo cell function as a target gene of LINC01128 in the progression of RSA. The analysis results showed that DNMT1 is a possible target gene of miR-515-5p, and the fluorescence analysis results proved that there is a target-interaction relationship between the two. Furthermore, recent studies have confirmed that DNMT1 promotes trophoblast proliferation, migration, and invasion by inhibiting MEG3, while suppressing apoptosis; its downregulation exacerbates miscarriage-associated phenotypes [29]. Consistent with these findings, the current study revealed that DNMT1 is lowly expressed in both serum and decidual tissue samples from patients with RSA, and its expression shows a significant positive correlation with that of LINC01128. Functional experiments further demonstrated that DNMT1 knockdown significantly impairs trophoblast cell proliferation, migration, and invasion, promotes apoptosis, and downregulates the expression of MMP2, MMP9, and VEGF. These results suggest that low DNMT1 expression directly contributes to trophoblast cell dysfunction, thereby facilitating the occurrence and progression of RSA. Notably, study is the first to show that DNMT1 expression was persistently elevated in cells transfected with pcDNA-LINC01128, whereas it was markedly suppressed in cells co-transfected with pcDNA-LINC01128 and miR-515-5p mimic. The results of this study demonstrated that a regulatory relationship of LINC01128 targetting miR-515-5p/DNMT1 does exist in HTR8/SVneo cells. So far, this study has also found that LINC01128 and miR-515-5p may be important for RSA progression and have diagnostic value in predicting RSA progression. The signalling pathway LINC01128 targets miR-515-5p/DNMT1 to inhibit cell proliferation and VEGF expression and promote apoptosis existing in trophoblast cells. This finding has some theoretical significance and clinical value for the progress of diagnosis and treatment of RSA.

The current study confirmed the aberrant expression of LINC01128, miR-515-5p, and DNMT1 in RSA patients. It also identified the regulatory axis of LINC01128/miR-515-5p/DNMT1 in HTR8/SVneo cells, which provides new insights into the molecular mechanism underlying RSA. From a clinical perspective, the dysregulated expression of LINC01128 and miR-515-5p in early pregnancy suggests their potential as novel non-invasive diagnostic biomarkers for RSA, especially in peripheral blood-based panels. Moreover, targetting this axis may represent a promising therapeutic strategy for RSA by restoring trophoblast function.

Limitations of the study. Several limitations of this study should be acknowledged. First, the relatively small clinical sample size (49 RSA patients and 50 healthy controls) may have limited the statistical power and generalizability of the findings. In addition, due to experimental design constraints, the in-depth functional validation of the target genes was not fully performed. In the future, further investigations will be conducted using the small interfering RNAs (siRNAs), specific inhibitors [12, 30] and clinical cohorts, to verify and extend the conclusions reached by the authors.

CONCLUSIONS

In conclusion, this study demonstrates that LINC01128 and miR-515-5p exhibit promising diagnostic value for predicting RSA progression. Furthermore, the study identified a regulatory relationship between LINC01128, miR-515-5p, and DNMT1 in trophoblast cells, which may be associated with RSA pathogenesis by modulating downstream factors, including VEGF and MMPs.

REFERENCES

1. Wen X, Dong P, Liu J, et al. Role of Immune Inflammation in Recurrent Spontaneous Abortions. J Inflamm Res. 2024;17:9407–22. http://doi.org/10.2147/JIR.S488638
2. van Wely M. Series of overviews on miscarriage and recurrent miscarriage. Fertil Steril. 2023;120(5):932–3. http://doi.org/10.1016/j.fertnstert.2023.09.006
3. Ibrahaim Y, Hotaling J. Sperm Epigenetics and Its Impact on Male Fertility, Pregnancy Loss, and Somatic Health of Future Offspring. Semin Reprod Med. 2018;36(3–04):233–5. http://doi.org/10.1055/s-0039-1688453
4. Ye X, Ma C, Guo W, et al. Metabolomic analysis reveals potential role of immunometabolism dysregulation in recurrent pregnancy loss. Front Endocrinol (Lausanne). 2024;15:1476774. http://doi.org/10.3389/fendo.2024.1476774
5. Hendriks E, MacNaughton H, MacKenzie MC. First Trimester Bleeding: Evaluation and Management. Am Fam Physician. 2019;99(3):166–74.
6. Davalieva K, Bozhinovski G, Kiprijanovska S, et al. Proteomics Analysis of Human Chorionic Villi Reveals Dysregulated Pathways That Contribute to Recurrent Pregnancy Loss. Proteomics Clin Appl. 2024;18(6):e202400020. http://doi.org/10.1002/prca.202400020
7. Lihua C, Hua S, Wenzhan W, et al. Expression and clinical significance of lncRNA PART1 in patients with unexplained recurrent pregnancy loss. Gynecol Endocrinol. 2024;40(1):2375582. http://doi.org/10.1080/09513590.2024.2375582
8. Mu F, Liu C, Huo H, et al. The relationship between Sjogren's syndrome and recurrent pregnancy loss: a bioinformatics analysis. Reprod Biomed Online. 2024;49(6):104363. http://doi.org/10.1016/j.rbmo.2024.104363
9. Huang Y, Zhou Z, Zhang J, et al. lncRNA MALAT1 participates in metformin inhibiting the proliferation of breast cancer cell. J Cell Mol Med. 2021;25(15):7135–45. http://doi.org/10.1111/jcmm.16742
10. Zhou X, Li Y, Wu L, et al. Upregulated lncRNA LINC01128 in colorectal cancer accelerates cell growth and predicts malignant prognosis through sponging miR-363-3p. J Cancer Res Clin Oncol. 2024;150(5):276. http://doi.org/10.1007/s00432-024-05804-4

11. Nunode M, Hayashi M, Nagayasu Y, et al. miR-515-5p suppresses trophoblast cell invasion and proliferation through XIAP regulation in preeclampsia. Mol Cell Endocrinol. 2023;559:111779. http://doi.org/10.1016/j.mce.2022.111779
12. Zhang K, Zhang H, Gao S, et al. Effect and mechanism of microRNA-515-5p in proliferation and apoptosis of trophoblast cells in preeclampsia via manipulating histone deacetylase 2. Mol Reprod Dev. 2023;90(1):59–66. http://doi.org/10.1002/mrd.23649
13. Mao G, Zhou B, Xu W, et al. Hsa_circ_0040809 regulates colorectal cancer development by upregulating methyltransferase DNMT1 via targeting miR-515-5p. J Gene Med. 2021;23(12):e3388. http://doi.org/10.1002/jgm.3388
14. Uta C, Tirziu A, Zimbru EL, et al. Alloimmune Causes of Recurrent Pregnancy Loss: Cellular Mechanisms and Overview of Therapeutic Approaches. Medicina (Kaunas). 2024;60(11). http://doi.org/10.3390/medicina60111896
15. Tang C, Hu W. Biomarkers and diagnostic significance of non-coding RNAs in extracellular vesicles of pathologic pregnancy. J Assist Reprod Genet. 2024;41(10):2569–84. http://doi.org/10.1007/s10815-024-03268-6
16. Sheng F, Sun N, Ji Y, et al. Aberrant expression of imprinted lncRNA MEG8 causes trophoblast dysfunction and abortion. J Cell Biochem. 2019;120(10):17378–90. http://doi.org/10.1002/jcb.29002
17. Jia N, Li J. Noncoding RNAs in Unexplained Recurrent Spontaneous Abortions and Their Diagnostic Potential. Dis Markers. 2019;2019:7090767. http://doi.org/10.1155/2019/7090767
18. Wang Z, Yan S, Liao S, et al. Dysregulated lncSNHG12 suppresses the invasion and migration of trophoblasts by regulating Dio2/Snail axis to involve in recurrent spontaneous abortion. Biochem Pharmacol. 2024;229:116459. http://doi.org/10.1016/j.bcp.2024.116459
19. Huang Y, Hao J, Liao Y, et al. Transcriptome sequencing identified the ceRNA network associated with recurrent spontaneous abortion. BMC Med Genomics. 2021;14(1):278. http://doi.org/10.1186/s12920-021-01125-4
20. Zhao X, Liu F, Zhang J, et al. LINC01128 – miR-16 interaction regulates the migration and invasion of human chorionic trophoblast cells. Hypertens Pregnancy. 2021;40(2):152–61. http://doi.org/10.1080/10641955.2021.1917602
21. Qian Z, Luo K, Zhang M, et al. Overexpression of long non-coding RNA H19 enhances cell viability and inhibits apoptosis in recurrent spontaneous abortion by targeting the miR-29a-3p/SOCS3 axis. In Vitro Cell Dev Biol Anim. 2025;61(8):946–55. http://doi.org/10.1007/s11626-024-00922-2
22. Chen X, Wang X, Yuan Y, et al. Construction and validation of nomogram prediction model for recurrent spontaneous abortion based on the expression of MALAT1, miR-515-5p, and MCL1 mRNA. Front Med (Lausanne). 2025;12:1558818. http://doi.org/10.3389/fmed.2025.1558818
23. Fudalej P, Al-Jeabory M, Pruc M, et al. A comprehensive evaluation and meta-analysis of the efficacy of autologous conditioned serum vs platelet-rich plasma in knee osteoarthritis treatment. Ann Agric Environ Med. 2025;32(4):497–503. http://doi.org/10.26444/aaem/202310
24. Miciuda P, Jerzak M, Olbrot N, et al. Review of current knowledge about infections accompanied by paediatric acute lymphoblastic leukemia with emphasis on challenges regarding the treatment of refugees. Ann Agric Environ Med. 2025;32(1):46–51. http://doi.org/10.26444/aaem/195393
25. Ju Y, Hou X, Wang Y, et al. Correlation of VEGF, HIF-1alpha, and MMP2 expression in placental villi among patients with recurrent spontaneous abortion. Obstet Gynecol Sci. 2025;68(5):408–17. http://doi.org/10.5468/ogs.24176
26. Bagheri A, Kumar P, Kamath A, Rao P. Association of angiogenic cytokines (VEGF-A and VEGF-C) and clinical characteristic in women with unexplained recurrent miscarriage. Bratisl Lek Listy. 2017;118(5):258–64. http://doi.org/10.4149/BLL_2017_051
27. Li H, Ding L, Ding J, Huang F. The Influence of CD40/CD40L, MMP2/MMP9 on Uterine Artery Blood Flow and Their Expression in Recurrent Miscarriage. Altern Ther Health Med. 2025;31(1):222–6.
28. Wu XM, Li YX, Zheng HS, et al. The effect and mechanism of low-molecular-weight heparin on the decidualization of stromal cells in early pregnancy. J Matern Fetal Neonatal Med. 2024;37(1):2294701. http://doi.org/10.1080/14767058.2023.2294701
29. Liu S, Wang Y, Guo Y, et al. MiR-204-5p promoted maternally expressed gene 3 (MEG3) through SP1-mediated DNMT1 pathway in trophoblast cells in recurrent miscarriage. Adv Clin Exp Med. 2025;34(11):1841–9. http://doi.org/10.17219/acem/195925
30. Wu W, Li Y, He J, et al. Resveratrol shields against cisplatin-induced ototoxicity through epigenetic lncRNA GAS5 modulation of miR-455-5p/PTEN pathway. Int Immunopharmacol. 2024;138:112464. http://doi.org/10.1016/j.intimp.2024.112464

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