


Functional Roles of lncRNAs in Recurrent Pregnancy Loss: A Review Study

Shirin Azizidoost, Ph.D.¹, Mahrokh Abouali Gale Dari, M.D.², Farhoodeh Ghaedrahmati, M.Sc.³, Zahra Razani, M.Sc.⁴, Mona Keivan, M.D.⁵, Razieh Mohammad Jafari, M.D.⁶, Mahin Najafian, M.D.⁶, Maryam Farzaneh, Ph.D.^{7*} 

1. Atherosclerosis Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
2. Department of Obstetrics and Gynecology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
3. Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
4. Department of Animal Physiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
5. Fertility and Infertility Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
6. Department of Obstetrics and Gynecology, Fertility, Infertility and Perinatology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
7. Fertility, Infertility and Perinatology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Abstract

Recurrent pregnancy loss (RPL) or recurrent miscarriage is the failure of pregnancy before 20-24 weeks that influences around 2-5% of couples. Several genetic, immunological, environmental and physical factors may influence RPL. Although various traditional methods have been used to treat post-implantation failures, identifying the mechanisms underlying RPL may improve an effective treatment. Recent evidence suggested that gene expression alterations presented essential roles in the occurrence of RPL. It has been found that long non-coding RNAs (lncRNAs) play functional roles in pregnancy pathologies, such as recurrent miscarriage. lncRNAs can function as dynamic scaffolds, modulate chromatin function, guide and bind to microRNAs (miRNAs) or transcription factors. lncRNAs, by targeting various miRNAs and mRNAs, play essential roles in the progression or suppression of RPL. Therefore, targeting lncRNAs and their downstream targets might be a suitable strategy for diagnosis and treatment of RPL. In this review, we summarized emerging roles of several lncRNAs in stimulation or suppression of RPL.

Keywords: Diagnosis, Implantation, lncRNAs, miRNAs, Recurrent Miscarriage

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Introduction

Recurrent pregnancy loss (RPL) is an important issue in reproductive health (1, 2). RPL is generally termed as three or more consecutive and spontaneous death of the fetal before the 20th week of pregnancy or before the fetal weighs 500 g (3). This disorder affects about 5% of couples who are interested in having a child (4). About 50% of women who suffer from this disorder have no significant symptoms on routine checkups (5). Etiology of this disease in 50% of cases remains unclear (6). Several factors such as chromosomes and genetic abnormalities (7, 8), infections (9), endocrine disorders (10), progesterone deficiency (11), uterine defects (12), anatomical disorders, placental abnormalities (13), alcohol and smoking (14), exposure to factors such as

lead, mercury, ethylene oxide and ionizing radiation, and stressful conditions (15) are common established causes of RPL. Recent evidence showed that gene alternations play an important role in the occurrence of RPL (16). Long non-coding RNAs (lncRNAs), by targeting DNA, RNA, and proteins, can affect their transcription and translation (17). Recently, role of lncRNAs in regulating pregnancy and RPL has been considered (18, 19). lncRNAs have different expressions in the chorionic villi of patients with RPL (20). lncRNAs can impact embryonic implantation by targeting trophoblast cell proliferation, migration, invasion and apoptosis (21, 22). Therefore, lncRNAs could be useful biomarkers for the diagnosis and treatment of patients with RPL (23). In this review, we summarized potential roles of lncRNAs in RPL.



Characteristics of long non-coding RNAs

So far, 61533 total human genes are characterized, 19982 of which are related to the protein-coding genes, 18811 are specific to the lncRNA genes, and 7567 was found to be related to the small ncRNA genes (genecodegenes.org/human/stats.html). ncRNAs are categorized into structural (rRNAs and tRNAs) and regulatory (small, medium, and lncRNAs) ncRNAs (24). lncRNAs (>200 nt) with a similar structure to mRNAs are transcribed by RNA polymerase II. lncRNAs have transcriptional termination codons or stop codons with the potential to signal ending translation, therefore lncRNAs cannot construct a full-length protein (17, 25, 26). They can be categorized into long intergenic ncRNAs, sense lncRNAs, antisense lncRNAs, intronic lncRNAs and bidirectional lncRNAs (27-29). lncRNAs, as decoys, can bind to microRNAs (miRNAs) or transcription factors to block their functions (30, 31). lncRNAs, as the guide molecules, bind to the regulatory proteins and guide them to the specific target area (32). Besides, lncRNAs as the scaffold molecules provide a central platform for different types of macromolecular complexes (33) (Fig.1). Studies showed that lncRNAs may play role in pregnancy pathologies, such as miscarriage (34, 35).

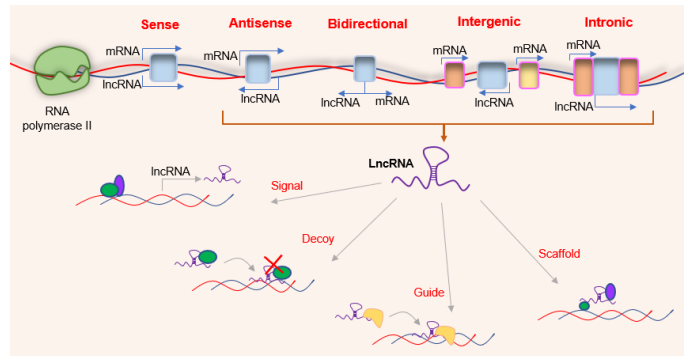


Fig.1: Biogenesis and functions of long non-coding RNAs (lncRNAs). lncRNAs could be classified into intergenic, sense, antisense, intronic, and bidirectional lncRNAs. They can act as molecular signals to regulate transcription, bind to microRNAs, chromatin modifiers or transcription factors to affect transcription and translation, and as the scaffold molecules providing a central platform.

Functional roles of lncRNAs in recurrent pregnancy loss

It has been reported that lncRNAs present essential roles in the stimulation or suppression of placental trophoblast invasion into endometrial stromal cells, by targeting various miRNAs and transcription factors (36) (Table 1). Here, we summarized potential roles of several lncRNAs in RPL (Fig.2).

Table 1: Functional roles of lncRNAs in recurrent pregnancy loss

lncRNA	Expression in RPL	Target		Result	Ref
		Suppression	Stimulation		
TCL6	EGFR	Suppress the proliferative ability of trophoblasts	(37)
SNHG7-1	Down	miR-34a	Wnt/ β -catenin	Suppress the proliferative and invasive ability of trophoblasts to induce RPL progression	(38)
Lnc-SLC4A1-1	Up	TNF- α /IL-1 β	NF- κ B/CXCL8	Stimulate apoptosis and immune responses in trophoblast cells	(18)
H19	Down	miR-106a-5p	VEGFA	High invasion of extravillous trophoblast cells, regulate angiogenesis, induce apoptosis	(39)
		miRNA let-7	ITGB3	Suppress trophoblast cell adhesion and invasion	(40)
		miR-675	NOMO1	Suppress trophoblast cell proliferation	(41)
EPB41L4A-AS1	...	HIF-1 α	VDAC1	Inhibition of the aerobic glycolysis and cell growth, and induction of apoptosis	(42)
TINCR	Progression of miscarriage	(43)
MALAT-1	Down	Reduce cell migration and proliferation, regulate angiogenesis, and induce apoptosis	(44)
ANRIL	Progression of miscarriage	(45)
HULC	Progression of miscarriage	(46)
Lnc-49a	Down	CD49a	IFN- γ	Reduce the adhesion, migration, and cytotoxic activity of dNK cells, stimulate the progression of miscarriage	(47)
MEG3	Down	RASA1	Ras-MAPK	Reduce implantation, proliferation, invasion, and induce apoptosis of primary trophoblasts	(48)
ZEB2-AS1	...	CST3	...	Accelerate proliferation, migration, and invasion of chorionic trophoblasts	(49)
SOX2OT	Progression of miscarriage	(50)
NEAT1	Up	miR-373	FLT1	Reduce proliferation, migration, invasion, and colony formation of trophoblast cells, and accelerate apoptosis	(51)
CCAT2	Reduce risk of miscarriage	(52)
HOTAIR	Down	PIK3-AKT	TTP	Suppress trophoblast cell proliferation, migration, and invasion	(53)
Lnc-HZ08	Up	PI3K/p-AKT/P21/CDK2	...	Suppress trophoblast cell proliferation, migration, and invasion	(54)
Lnc-HZ01	Up	MXD1	METTL14	Suppress trophoblast cell proliferation and induce risk of miscarriage	(55)

RPL; Recurrent pregnancy loss, TNF- α ; Tumour necrosis factor α , IL; Interleukin, HIF-1 α ; Hypoxia-inducible factor 1 α , VDAC1; Voltage-dependent anion channel 1, VEGFA; Vascular endothelial growth factor A, RASA1; Ras p21 protein activator 1, CST3; Cystatin C, TTP; Tristetraprolin, EGFR; Epidermal growth factor receptor, IFN- γ ; Interferon- γ granzyme B, ITGB3; Integrin β 3, NOMO1; Nodal modulator 1, FLT-1; Fms-like tyrosine kinase-1, and METTL14; Methyltransferase 14, N6-Adenosine-Methyltransferase Subunit.

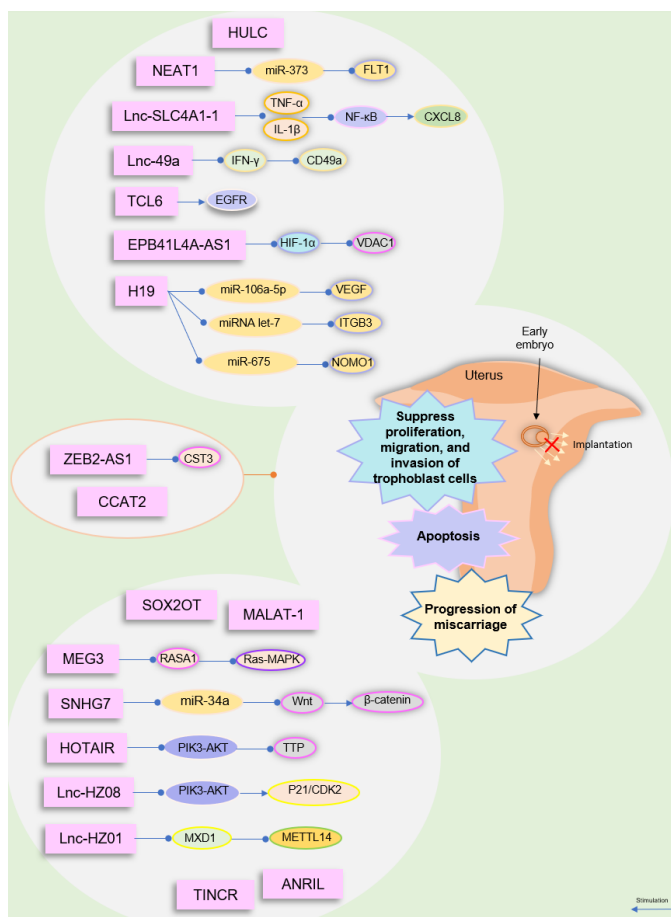


Fig.2: lncRNAs by targeting several miRNAs/mRNAs play important roles in the induction or suppression of recurrent pregnancy loss (RPL). Some lncRNAs, including TCL6, SNHG7, H19, lnc-49a, lnc-SLC4A1-1, EPB41L4A-AS1, TINCR, MALAT-1, ANRIL, HULC, MEG3, HOTAIR, and SOX2OT present critical roles in RPL development. These lncRNAs can inhibit trophoblastic cell proliferation, migration, invasion, and induce apoptosis. In contrast, two lncRNAs such as CCAT2 and ZEB2-AS1 target trophoblastic cell proliferation, migration, and invasion for reduction of the risk of miscarriage.

TCL6

lncRNA TCL6 stands for lncRNA T-cell leukemia/lymphoma 6 which is located in the breakpoint cluster region on chromosome 14q32. It has been reported to be involved in the preeclampsia pathogenesis by suppressing proliferative ability of trophoblasts (56, 57). Recently, the real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis reported that overexpressed lncRNA-TCL6 of placental villus was correlated with threatened abortion compared to normal pregnancy. Regarding its aberrant expression, biological investigation of lncRNA TCL6 using si-lncRNA TCL6-1 revealed that knock-down of lncRNA TCL6 could significantly induce proliferation and invasion of trophoblasts. Epidermal growth factor receptor (EGFR) signaling plays an essential role in early human embryo implantation and pregnancy disorders. There is a negative correlation between lncRNA TCL6 and the EGFR pathway. In this regard, inhibitory role of the lncRNA TCL6 on proliferation activity of trophoblast cells could be inverted by EGFR knock-down. Taken together, lncRNA

TCL6 could induce early abortion and inhibit placental implantation by targeting the EGFR signaling (37).

SNHG7

Despite the regulatory role of lncRNAs in trophoblast proliferation and apoptosis, function of SNHG7 in RPL remains challenging (58). Small nucleolar host gene 7 (SNHG7) is a 2176 base pairs (bp) lncRNA on chromosome 9q34.3 (59). Gene expression analysis of SNHG7, using qRT-PCR, reported its down-regulated expression in recurrent spontaneous abortion (RSA) villi compared to normal pregnancy villi. SNHG7 silencing was also shown to prohibit trophoblast proliferation by MTT assay, along with increase apoptosis using flow cytometry, thereby confirming SNHG7 function in RPL development (38). Since the discovery of lncRNAs as indirect regulators of mRNA expression levels using sequestering miRNA, several studies identified SNHG7 as a molecular target of miR-34a (60, 61). In this regard, inhibition of miR-34a reversed suppressed proliferation of trophoblasts which was induced by SNHG7 silencing (38). It has been shown that WNT1 through the Wnt/ β -catenin pathway, acted as a miR-34a down-stream target. Dysregulated expression of the Wnt/ β -catenin pathway might be correlated to RPL pathogenesis (62). SNHG7 silencing was also reported to modulate proliferation and invasion of trophoblasts with the Wnt/ β -catenin pathway inactivation. Due to the involvement of SNHG7/miR-34a/Wnt/ β -catenin axis in RPL progression, the latter could be underlying potential molecular targets for RPL treatment (38).

lnc-SLC4A1-1

lnc-SLC4A1-1 was found to target NF- κ B and CXCL8, inhibiting the inflammatory response by TNF- α and IL-1 β . Therefore, high expression of this lncRNA can trigger apoptosis and stimulate immune responses in trophoblast cells (18).

H19

H19 is a lncRNA with 2.3 kbp length in the cytoplasm. High expression of H19 was reported during embryonic development and its low expression after birth (63). H19 can modulate adhesion and invasion of trophoblasts within early pregnancy (40, 64). Reduced expression of H19 resulted in low invasion of extravillous trophoblast cells, associated with the pathogenesis of both intrauterine growth restriction and preeclampsia (65). Significant expression of H19 in trophoblast of early pregnant women modulated the angiogenic ability of extravillous trophoblast cells by targeting the miR-106a-5p/VEGFA axis (66). Decreased expression of H19 in placental villi tissue of RPL patients showed the same expression trend in anti-apoptotic Bcl-2 level along with a negative impact on pro-apoptotic Bax expression. Therefore, H19 regulates RPL pathogenesis through inducing apoptosis and can be presented as a novel therapeutic target for RPL patients (39). In

another study, H19 was reported to block miRNA let-7 and stimulated expression of integrin β 3 (ITGB3), as the target gene of let-7. It was also shown that the H19/let-7/ITGB3 axis could accelerate adhesion and invasion ability of trophoblast cells. Therefore, low expression of H19 triggered RPL progression (40). Besides, H19 has been found to target miR-675 and upregulate nodal modulator 1 (NOMO1) protein expression to induce cell proliferation and phosphorylation of Smad 2. Therefore, low expression of this lncRNA was participated in the proliferation of trophoblast cells (41).

EPB41L4A-AS1

lncRNA EPB41L4A-AS1 is located on the 5q22.2 chromosome, while it is down-regulated in tumors. Its down-regulation in malignancies started aerobic glycolysis, called the Warburg effect, inducing fast tumor growth (42). Trophoblasts as rapid-growing cells require aerobic glycolysis to support their fast growth. Although the biological function of EPB41L4A-AS1 in cancers is evident, its functional effect on early pregnancy placental tissue and RPL needs to be elucidated. EPB41L4A-AS1 expression was found to be upregulated in placental tissue in early RPL compared to the normal pregnancy. Recent mRNA microarray analysis reported a significant increase in glycolysis-related genes inconsistent with the expression of fatty acid oxidative phosphorylation genes (67). Both glycolysis along with fatty acid oxidative phosphorylation process were involved in extravillous trophoblasts metabolism that was impaired in abortion (68). Increased expression of EPB41L4A-AS1 prohibited HIF-1 α as a cell cycle transcription factor and induced VDAC1 expression as a key apoptosis regulatory protein (42, 69, 70). Altogether, overexpression of EPB41L4A-AS1 was responsible for the disrupted metabolic program in human villous trophoblast through inhibition of the aerobic glycolysis and cell growth, and promotion of apoptosis that might be key reasons of RPL (42).

TINCR

Terminal differentiation-induced non-coding RNA (TINCR) is a 3.7 kb lncRNA located on human chromosome 19. TINCR is involved in epidermal and somatic tissue differentiation and progression of miscarriage. Although the association of genetic susceptibility of some lncRNAs in RPL has been found (71), there was no significant correlation between the TINCR gene rs2288947 A>G polymorphism and RPL (43).

MALAT-1

Metastasis-associated lung adenocarcinoma transcript-1 (MALAT-1) is the lncRNA with more than 8000 bp length. It is up-regulated in invasive placentation (72). Expression of MALAT-1 was shown to be decreased in women with RPL as well as reducing cell migration and proliferation, while it induced apoptosis (44). The rs619586 G variant

of MALAT-1 decreased susceptibility to RPL (73).

ANRIL

Recently, ANRIL has been introduced as a new large antisense lncRNA with 403 kb length locating on the chromosome 9p21 locus. ANRIL polymorphism is correlated with susceptibility to miscarriage (74, 75). The Rs2151280 GG allele of ANRIL was correlated with an increased risk of RPL and it might be associated with initiation of abortion (45).

HULC

Highly upregulated in liver cancer (HULC) is a lncRNA with 500 nucleotides located on chromosome 6p24.3. It is highly expressed in human hepatocellular carcinoma (76). This lncRNA regulates cell invasion, proliferation and migration (77). SNP genotyping of lncRNA HULC showed that three variant genotypes of the HULC, including rs1041279 C>G, rs7770772 G>C and rs17144343 G>A, reduced RPL. Such protective impact was very noticeable in women aged under 35 as well as those with at least four abortions. Therefore, lncRNA HULC might be a potent biomarker for diagnosis of RPL (46).

lnc-49a

lnc-49a is a novel lncRNA, positively regulated CD49a expression of human decidual natural killer (dNK) cells. This lncRNA was reported to reduce adhesion, migration, and cytotoxic activity of dNK cells, while accelerated interferon- γ granzyme B (IFN- γ) expression (47). Therefore, lnc-49a could stimulate progression of RPL, by suppressing cellular migration and invasion.

MEG3

lncRNA maternally expressed gene 3 (MEG3) is located on chromosome 14q32.3 and modulated proliferation and invasion of trophoblasts (78). A recent analysis in aborted villous tissue showed a dramatic decrease level of MEG3 expression, compared to the normal pregnant women. This was negatively associated with the unexplained RPL. Analysis of possible down-stream targets of MEG3 identified the Ras p21 protein activator 1 (RASA1) gene that was overexpressed in villous tissue of aborted women in comparison to normal villous tissue (48). RASA1 obstructed the Ras signaling by binding to the activated form of Ras (Ras-GTP), thereby repressed this pathway (79, 80). Indeed, the Ras-MAPK signaling modulated cell proliferation and apoptosis. MEG3 was shown to stimulate the Ras-MAPK signaling via suppressing RASA1 in embryonic villi of women with unexplained RSA. It can be suggested that an under-expressed level of MEG3 can increase implantation, proliferation, invasion and suppress apoptosis in aborted primary trophoblasts (48).

ZEB2-AS1

lncRNA zinc finger E-box binding homeobox 2 antisense RNA1 (ZEB2-AS1) is involved in the prediction

and progression of cancers (81). It has been reported that ZEB2-AS1 targeted cystatin C (CST3) which was associated with trophoblast inactivity along with the susceptibility to developing preeclampsia (82, 83). Both FISH assay and qRT-PCR analysis revealed low expression of ZEB2-AS1 level in recurrent aborted mice. In addition, CST3 expression was dramatically upregulated in the placental tissue of aborted mice. Furthermore, CST3 overexpression inhibited proliferative activity, migration capacity, and invasion of mouse chorionic trophoblasts. In contrast, high expression of ZEB2-AS1 prohibited CST3 expression, thereby promoting the biological function of mouse chorionic trophoblast cells. Therefore, ZEB2-AS1 can be introduced as a protective lncRNA against RPL via promoting trophoblast activity through suppression of CST3 expression (49).

SOX2OT

lncRNA SOX2 overlapping transcript (SOX2OT) mapping on the *SOX2* gene was found to represent an oncogenic activity in the pathogenesis of breast cancer (84, 85). Since the association of abortion as one type of reproductive risk factor for breast cancer (86), it is hypothesized that lncRNA SOX2OT has a possible correlation with RPL (87). It has been suggested that the SOX2OT polymorphism functions as an increased risk factor for RPL with no dramatic relationship to the number of abortion in different age (50).

NEAT1

Nuclear-enriched abundant transcript-1 (NEAT1) lncRNA is located on chromosome 11q13 and binds with various paraspeckle bodies (88). Expression of NEAT1 was demonstrated to be increased in placental samples of preeclampsia than the control group. During preeclampsia, NEAT1, as a competing endogenous RNAs (ceRNAs), can bind with miR-373 and enhance expression of Fms-like tyrosine kinase-1 (FLT-1). Therefore, the NEAT1/miR-373/FLT-1 may provide a new approach for understanding the pathogenesis of RPL by targeting trophoblast cell proliferation and apoptosis (51).

CCAT2

Colon cancer-associated transcript 2 (CCAT2) is a lncRNA with 1752 bp located on chromosome 8q24.21 which represents an oncogenic role in colorectal cancer. Effect of CCAT2 polymorphism on RPL indicated a reduced risk of RPL of the CCAT2 rs6983267 allele, and provided new lncRNA to protect RPL especially in women younger than 35 years old (52).

HOTAIR

lncRNA hox antisense intergenic RNA (HOTAIR) has been demonstrated to participate in normal cell development and its dysregulation involved in cancer progression (89). Low expression of HOTAIR is reported in trophoblasts from RPL women compared to those with

normal pregnancy. HOTAIR modulated invasion and migration capacity of trophoblast, thereby enhanced the RPL pathology (71). Tristetraprolin (TTP) stands as a tandem zinc-finger mRNA binding protein and modulated stability of various targets by binding to specific mRNAs. Upregulated TTP disrupted trophoblast invasion in patients with RPL, through HOTAIR destabilization. Therefore, HOTAIR/TTP can be a possible target for management of trophoblast cell invasion (53).

lnc-HZ01

lnc-HZ01 is a novel lncRNA that stimulates occurrence of miscarriage by enhancing the protein stability of MAX dimerization protein 1 (MXD1). c-JUN is a transcription factor of MXD1 that enhances MXD1 stability in the nucleus of trophoblast cells with the USP36 enzyme. METTL14 is a pivotal methyltransferase that can promote lnc-HZ01 RNA stability in villous tissues through m6A methylation on lnc-HZ01. Besides, lnc-HZ01 has been demonstrated to regulate trophoblast cells proliferation by up-regulating mRNA and protein levels of eukaryotic translation initiation factor (4E EIF4E). In human villous tissues, MXD1 could stimulate 4E EIF4E transcription and lnc-HZ01 in a positive self-feedback loop. Therefore, lnc-HZ01 repressed trophoblast cell proliferation and stimulated RPL through the MXD1/EIF4E pathway (55).

lnc-HZ08

lnc-HZ08 is a highly expressed lncRNA in human trophoblast cells. High expression of lnc-HZ08 could reduce PI3K level in trophoblast cells by targeting the PI3K/p-AKT/p-P21/CDK2 pathway. Therefore, this lncRNA was displayed to induce miscarriage via suppressing trophoblast cells proliferation, migration, and invasion (54).

Conclusion

RPL is one of the most serious clinical problems in reproductive health that affects family well-being dramatically. Consequently, clinical research should focus on how to improve the pregnancy success rate for women with recurrent miscarriage. In recent years, lncRNAs have become a focus of research because they regulate the occurrence and development of a wide variety of diseases. In this study, we literature the most recent advances in lncRNAs research and potential molecular pathways involved in RPL progression. These studies provided only a little information, while many of the functions of lncRNAs remain unknown. Despite the advancements of ncRNAs research in recent years, ncRNAs have the following limitations for clinical use: i. In the circulatory system, ncRNAs are often dynamic and samples from patients over time only provide an indication of the current expression status, ii. function of the most ncRNAs are explored at the cellular level, and animal experiments to investigate the functions and mechanisms of ncRNAs are urgently needed, and iii. It is difficult to know whether ncRNAs will affect expression of the other genes when

used as a targeted therapy, while it requires more clinical experimental evidence.

It can be concluded that silencing lncRNAs, including TCL6, SNHG7, H19, EPB41L4A-AS1, TINCR, MALAT-1, ANRIL, lnc-49a, lnc-SLC4A1-1, HULC, PVT1, HOTAIR, MEG3, and SOX2OT, as well as silencing transcription and inflammatory factors such as NF- κ B, IFN- γ , EGFR, VDAC1, VEGF, and β -catenin may prevent RPL development. Besides, functional roles of some lncRNAs (ZEB2-AS1 and CCAT2), miRNAs (miR-34a, miRNA let-7, miR-675, and miR-106a-5p), and transcription and inflammatory factors (RASA1, HIF-1 α , CST3, TNF- α , IL-1 β , and TTP) in the stimulation of trophoblastic cell proliferation, migration, and invasion should be considered as novel biomarkers or potential inducers for reduction of the risk of miscarriage.

Taken together, we recommend that researchers conduct larger population samples to demonstrate clinical potential of lncRNAs in diagnosing and treating RPL. In addition, to use RNA for disease treatment, more efficient and cost-effective methods for synthesizing and purifying RNA are needed.

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Authors' Contributions

Sh.A., M.A.G.D., F.Gh., Z.R., R.M.J., M.K., M.N., M.F.; Have made contributions to the writing of the manuscript. All authors have approved the submitted version of the article and agreed to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work. All authors read and approved the final manuscript.

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