Annals of Clinical Case Reports

Research Article
Published: 17 Feb, 2024

Bioinformatics-Based Analysis of Genes Related to Ferroptosis in Recurrent Spontaneous Abortion

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Abstract

Abnormal decidualization of the endometrium plays a pivotal role in Recurrent Spontaneous Abortion (RSA). Ferroptosis, a form of cell death dependent on iron. However, its effect on RSA remains largely unexplored. Consequently, we examined and validated by utilizing the GSE26787 and GSE165004 the correlation between genes associated with ferroptosis and RSA. Hub genes were identified and a protein-protein interaction network was constructed, together with assessments of functional enrichment of the genes and their association with immune cell recruitment. Fifteen ferroptosis-associated genes associated with RSA were identified. Among these, nine were increased, and six were decreased. GEO and KEGG showed that these genes were involved in cellular responses to stimuli, and the regulation of autophagy, etc. Two hub genes, Furin and NEDD4, were identified. Gene Set Enrichment Analysis indicated that cytokine–cytokine ligand interaction, etc. are common pathways for hub genes. Results from immune infiltration analysis indicated a high infiltration of Type 1 T-helper cells in RSA. External validation demonstrated significant upregulation of Furin and downregulation of NEDD4 in endometrial samples ($p \leq 0.05$). Furin and NEDD4, have the potential as novel and effective markers for the prevention and management of RSA.

Keywords: Recurrent spontaneous abortion; Endometrium; Immune system; Endometrium; Bioinformatics

Introduction

Recurrent Spontaneous Abortion (RSA) occurs relatively frequently in the domains of obstetrics and gynecology and represents the simultaneous occurrence of more than two unexpected abortions with the same companion (Practice Committee of the American Society for Reproductive Medicine 2020) [1]. The leading identified causes encompass maternal immunological effects i.e., autoimmunity and allogeneic response, thrombophilic factors i.e., genetic and acquired predisposition to thrombosis), uterine anatomical anomalies, and endocrine abnormalities [2]. The fundamental causes of around 50% of RSA cases remain unknown, and 80% of these unexpected abortions have strong associations with immune factors [3]. However, RSA is frequently asymptomatic, and therefore, its identification is profoundly challenging. Patients suffer physical and psychological trauma as a result of this condition, which also poses a substantial economic strain on healthcare systems and society [4]. Therefore, it is critical to identify the factors contributing to RSA to obtain a comprehensive understanding of its etiology.

Endometrial decidualization serves as a critical phase in early pregnancy, affecting the initiation of the pregnancy and its sustainability. Developing decidua sustain substantial alterations subsequent to conception as a result of invasive trophoblasts. These changes can either support the development of the embryo or actively reject it [5]. Ferroptosis, a form of cell death differing from necrosis, apoptosis, pyroptosis, and autophagy, was recognized in 2012 as a nonapoptotic death mechanism dependent on iron [6]. Subsequently, ferroptosis has garnered mounting attention due to its unique mechanisms and functions. It is initiated by the interaction of lipid peroxidation, iron toxicity, and plasma membrane dysfunction [7]. Recent findings have suggested the close connection between ferroptosis and endometrial decidualization miscarriage. High levels of iron in the follicular fluid increase the risk of endometriosis-associated infertility, as reported by Ni et al. [8]. Furthermore, increased uterine and placental ferroptosis have been linked to oxidative stress-induced fetal loss [9]. Ferroptosis has been linked to unexpected premature delivery, as demonstrated by Beharier O, and GPX4 inhibition elicits ferroptotic disruption in main human trophoblasts in addition to mouse pregnancies [10]. These findings indicate that ferroptosis correlates with irregularities in endometrial function and embryos, culminating in miscarriage. However, limited knowledge

OPEN ACCESS

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Received Date: *29 Jan 2024*
Accepted Date: *13 Feb 2024*
Published Date: *17 Feb 2024*

Citation:
Han X, Feng Y, Wang L. Bioinformatics-Based Analysis of Genes Related to Ferroptosis in Recurrent Spontaneous Abortion. Ann Clin Case Rep. 2024; 9: 2577.

ISSN: 2474-1655.

exists regarding the iron status of endometrial decidualization in the context of RSA. Consequently, the association between ferroptosis and endometrial activity of the impact on RSA was the primary focus of this investigation.

To identify critical genes and possible pathways related to ferroptosis in endometrial tissue within RSA, we employed widely accessible gene expression datasets and completed bioinformatics analysis in this study. Our approach involved investigating Gene Expression Omnibus (GEO) microarray expression profiles from fertile females and those with RSA, followed by cross-referencing this data with the FerrDB database. Subsequently, we identified differentially expressed genes related to ferroptosis (Ferr-DEGs) and investigated their functions by GO and KEGG analyses. The study included the identification of hub genes, immune cell penetration, single-gene Gene Set Enrichment Analysis (GSEA) analyses. After validating diagnostic efficacy across various datasets, we culminated our investigation by constructing a competing endogenous RNA (ceRNA) interaction network based on hub genes and predicting hub gene-targeted drugs. This research aims to facilitate advances in the identification and management of RSA by providing additional information on the function of ferroptosis in the disease.

Materials and Methods

Data resource

Data were retrieved from RSA-related microarrays (coded as GSE26787 and GSE165004) using the GEO database (https://www.ncbi.nlm.nih.gov/geo/).

The GSE26787 dataset was acquired via the Affymetrix Human Genome U133 Plus 2.0 Array platform GPL570 [HG-U133_Plus_2]. It includes gene profiles from five fertile female individuals (control group) and five individuals with recurrent spontaneous abortion (treatment group) who provided endometrial biopsy samples. The GSE165004 dataset is based on the GPL16699 Agilent-039494 SurePrint G3 Human GE v2 8×60K Microarray 039381 (Feature Number version) platform. This dataset comprises gene expression profiles from 24 fertile female individuals (control group) and 24 individuals experiencing recurrent pregnancy losses (treatment group) who provided endometrial tissue samples.

Additionally, 484 Ferroptosis-Related Genes (FRGs), such as suppressors, drivers, and markers, were identified from the FerrDb database (http://www.zhounan.org/ferrdb/index.html).

Identification of expressed Ferr-DEGs

The GSE26787 dataset was normalized by applying the normalize between Arrays function presented by R software (https://cran.r-project.org/). The limma program was employed to analyze DEGs between the control and RSA groups. These were selected based on the criteria of a cut-off p-value of ≤ 0.05 and |log2FC|>1. DE-FRGs were subsequently designated as the crossover genes between DEGs and FRGs.

GO and KEGG analyses of Ferr-DEG functions

We established relationships between gene IDs and org.Hs.eg SYMB2EG package values *via* the mget function. A cutoff of $p \leq 0.05$ was used in GO and KEGG evaluations of the Ferr-DEGs by applying the R package clusterProfiler.

Development of the Protein–Protein Interaction (PPI) network in RSA and determination of hub genes

The database known as STRING (http://string-db.org/) was applied to examine the interactions between multiple Ferr-DEGs. Cytoscape (version 3.10.0, http://cytoscape.org/) was used for developing and displaying the networks. In accordance with Degree algorithms, the cytoHubba plug-in was used to determine the top 5 genes. By applying the Glmnet package and Least Absolute Shrinkage and Selection Operator (LASSO) regression, Hub Ferr-DEGs were generated from the overlap between the top 5 genes in the PPI network and disease-related genes. ROC curves for the detection of RSA in relation to the genes of hub Ferr-DEGs have been produced using the pROC package.

Shared gene identification of hub genes in RSA

Importing hub Ferr-DEGs into the GeneMANIA database (http://genemania.org/) allowed us to identify genes that may share functions with hub genes based on their interactions.

Single-gene GSEA

GSEA algorithm is a computational method employed to evaluate consistent and substantial disparities between two biological datasets comprising a predetermined set of genes. The clusterProfiler program was applied to execute GSEA on hub Ferr-DEGs.

Evaluation of the invasion of immune cells in RSA

We used the single-sample GSEA (ssGSEA) algorithm to evaluate immune cell infiltration in patients with RSA within the GSE26787 dataset. GSVA package was used for the above analyses.

External validation

To validate our findings, we utilized a second dataset, GSE165004, obtained from the GEO database. We verified expression differences between the two groups and established ROC curves for the hub genes to determine cutoff values and calculate the Area Under the Curve (AUC) to assess the clinical diagnostic significance of key genes.

Detection of hub gene-targeted drugs

We predicted hub gene-targeted drugs using the DGIdb database to construct regulatory networks.

Construction of ceRNA network

We designed a ceRNA network and screened the ceRNA regulatory network based on the competitive scoring of gene mRNA in the miRanda, miRDB, and TargetScan databases. This process allowed us to create two ceRNA modulatory networks for DEGs *via* Cytoscape.

Results

Identification of Ferr-DEGs in RSA

By applying predefined criteria, we detected 1357 DEGs between samples from fertile females and those with RSA in the GSE26787 dataset. Volcano graphs illustrating these DEGs with remarkable accuracy of distinguished fertile females and RSA samples, and heatmaps of the top 50 highly expressed genes and the top 50 genes are shown (Figure 1A, 1B). To investigate FRGs differentially expressed in RSA, we retrieved 484 FRGs from the FerrDb database. Approximately 15 FRGs were recognized as Ferr-DEGs subsequent overlapping of DEGs and FRGs into consideration. These Ferr-DEGs consisted of nine upregulated and six downregulated genes (Figure 1C). The expression patterns of these 15 Ferr-DEG s in the GSE26787 dataset are presented in Figure 1D.

GO and KEGG analyses of Ferr-DEG functions

We employed the R package clusterProfiler for GO and KEGG

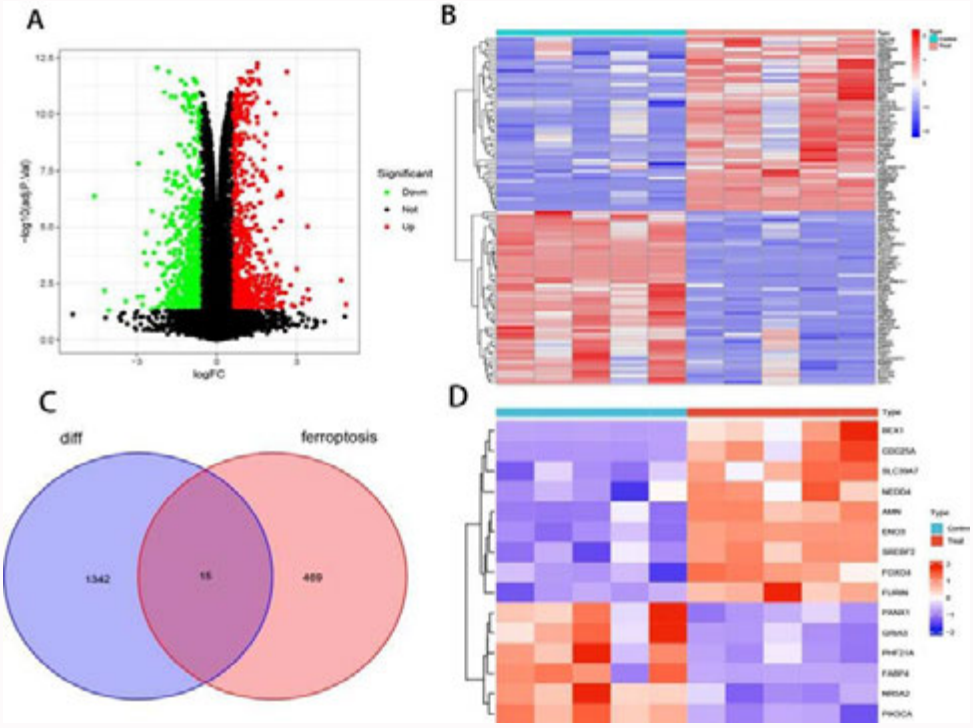

Figure 1: Detection of Differentially Expressed Ferr-DEGs. (A) Volcano diagrams illustrate genes whose expression is considerably different. Genes that are substantially decreased are denoted by green dots, genes that are significantly elevated are indicated by red dots, and genes that do not differ by any means are denoted by black dots. (B) Heatmap representing the DEG expressions as displayed in GSE26787. (C) Venn diagram illustrating the Ferr-DEGs. (D) Heatmap showing the expression of the 15 Ferr-DEGs expressions in GSE26787 RSA dataset.

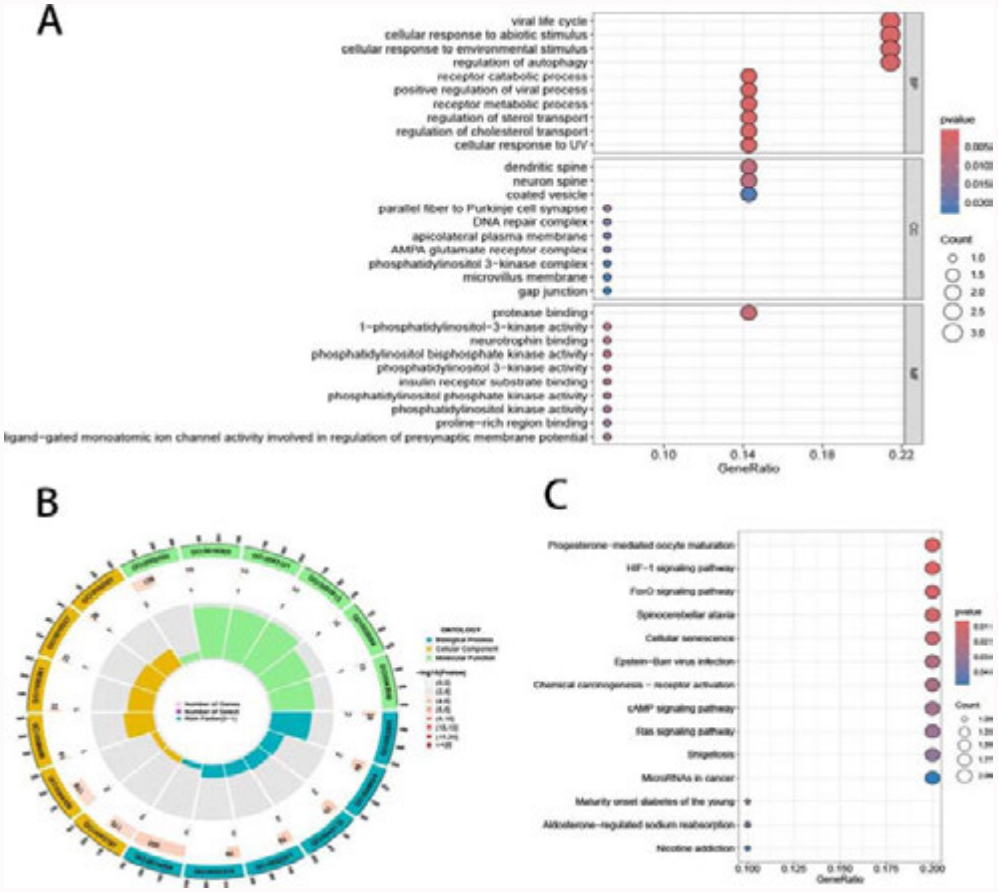

Figure 2: GO and KEGG analyses of Ferr-DEG functions. (A) Bubble diagram showing GO annotations; (B) Circle diagram showing the top six BP, CC, MF. (C) Bubble diagram showing KEGG pathway enrichment.

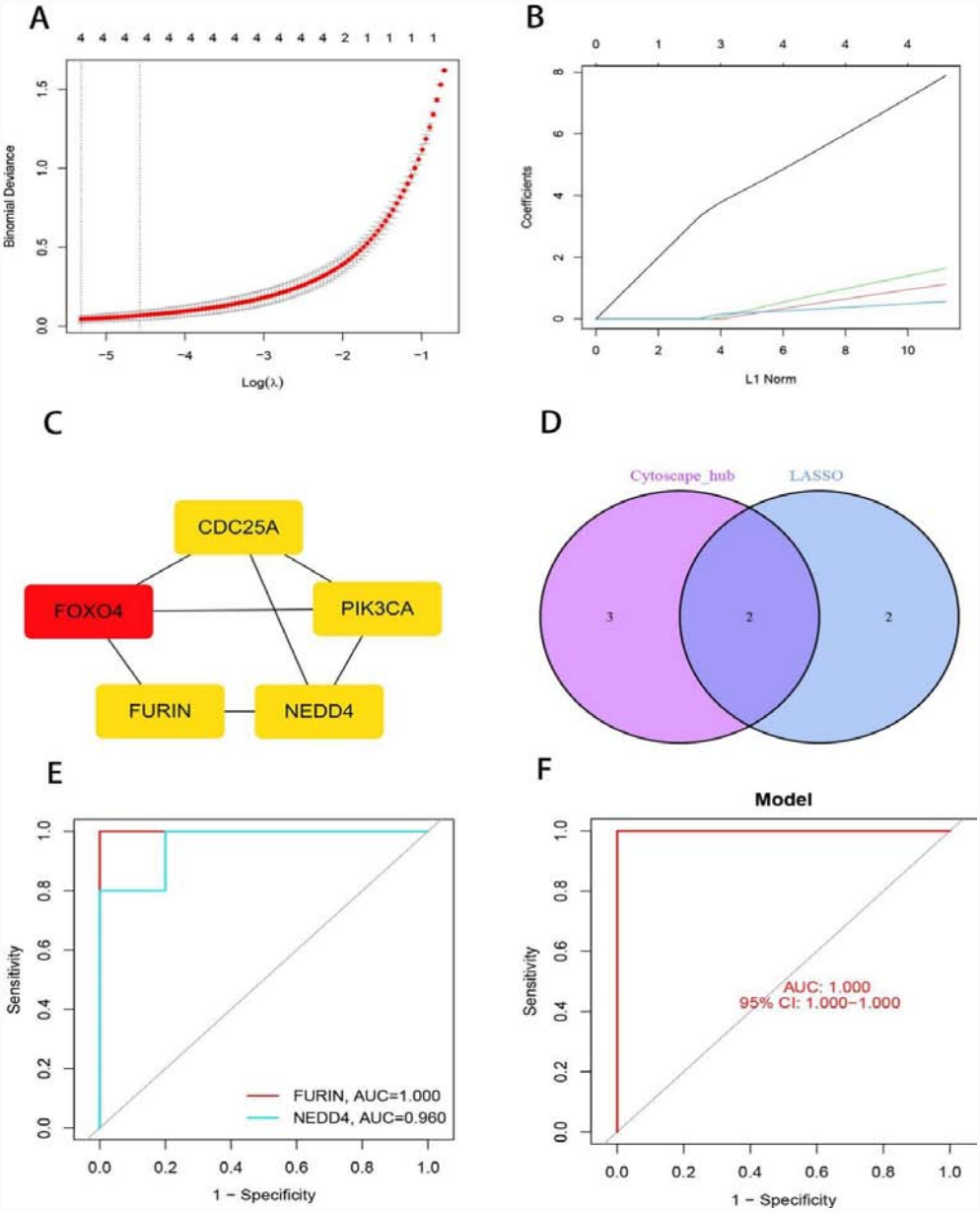

Figure 3: LASSO algorithm for disease profile gene analysis. (A) Profiles of LASSO coefficients for four Ferr-DEGs; (B) 10-time cross-validation of the LASSO model to select tuning factors. (C) The top five genes PPI network of Ferr-DEGs; (D) Venn diagram depicting intersecting genes of PPI networks and LASSO algorithm genes. (E) ROC curve of two individual hub Ferr-DEGs; (F) ROC curve for the multigene model.

enrichment analyses on the 15 Ferr-DEGs. In the GO analysis, the DEGs were significantly enriched in various categories. For Biological Processes (BP), the enrichment included the viral life cycle, cellular response to abiotic stimulus, and regulation of autophagy (Figure 2A). In the Cellular Component (CC) and Molecular Function (MF) categories, the DEGs exhibited enrichment in distinct processes (Figure 2B). For KEGG pathway analysis, enrichment was observed in pathways associated with progesterone-mediated oocyte maturation, hypoxia-inducible factor -1 signaling, and Forkhead box O (FoxO) signaling (Figure 2C).

Screening of hub Ferr-DEGs in RSA

LASSO regression was used to identify four genes (Figure 3A, 3B). Further analysis of the top five proteins in the PPI network resulted in the selection of two hub Ferr-DEGs (Figure 3C, 3D). At the level of single-gene expression, the AUCs of the ROC curves for RSA diagnosis were 1.000 for Furin and 0.960 for Neuronal precursor cell-Expressed Developmentally Downregulated 4 (NEDD4) (Figure 3E). The AUC for the multigene expression model was 1.000 (Figure 3F).

Shared gene identification results

Using GeneMANIA, we identified genes that may share functions with the hub genes. Shared gene identification results indicated the involvement of NOTCH3, ADAMTS4, GRB10, PROZ, and ISG15. Furin was mainly predicted to function in peptidase inhibition, whereas NEDD4 was associated with the modulation of the signaling pathway of the receptor of vascular endothelial growth factor and the modulation of cellular response against stimulus of growth factor (Figure 4)

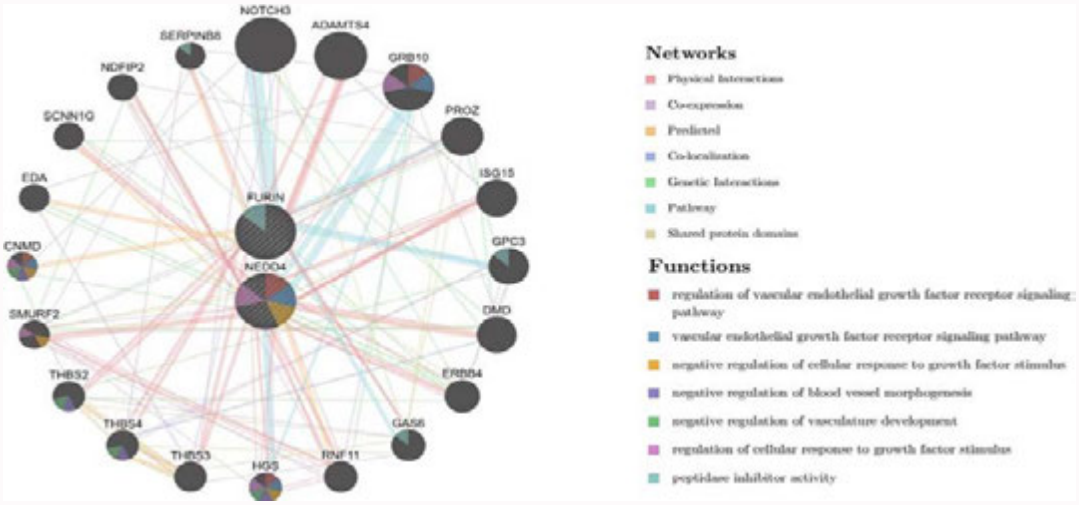

Figure 4: Predicting function of hub Ferr-DEGs using GeneMANIA.

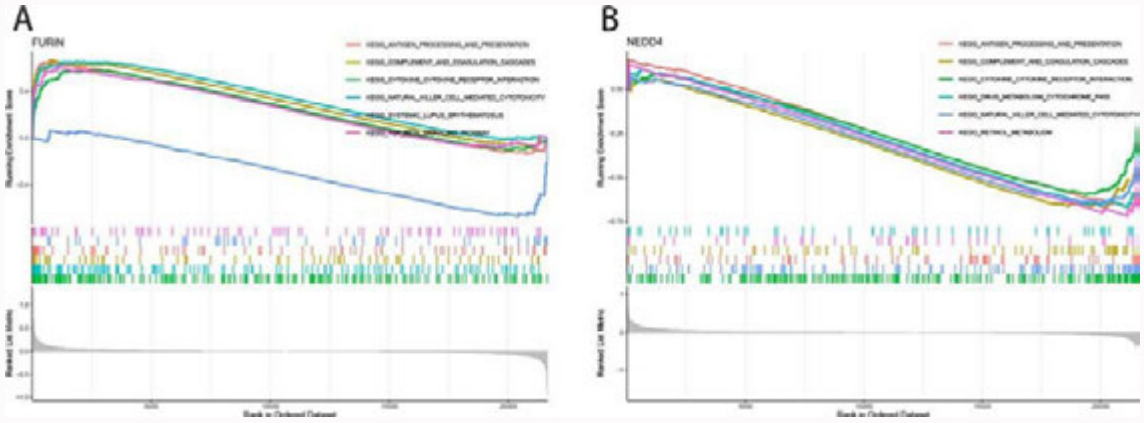

Figure 5: GSEA of hub Ferr-DEGs in RSA. (A) Furin; (B) NEDD4.

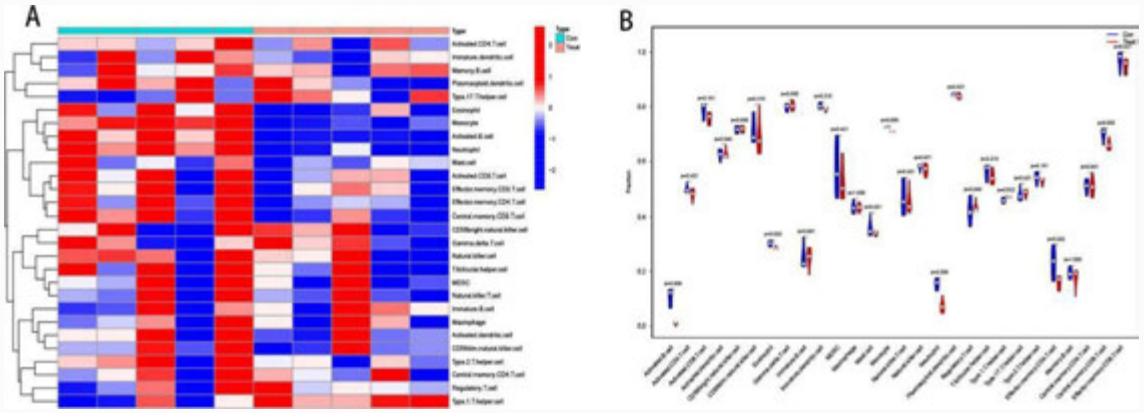

Figure 6: Invasion of immune cells in RSA. (A) Heatmap demonstrating a connection between the treatment and the control group with respect to the expression of invasion levels of 28 distinct types of immune cells. (B) Violin plot illustrating the variation in the level of invasion of 28 distinct immune cell types between the treatment and control groups.

GSEA results

GSEA results indicated that hub Ferr-DEGs were associated with pathways such as interaction between cytokines, cytotoxicity induced by NK, complement and coagulation cascades, and antigen processing and representation (Figure 5A, 5B).

Immune cell invasion in control and RSA

Analysis of infiltration of immune cells exhibited that in samples from patients with RSA, Type 1 T helper cells exhibited higher infiltration than in control samples. Furthermore, activated B cells, eosinophils, monocytes, neutrophils exhibited low infiltration in

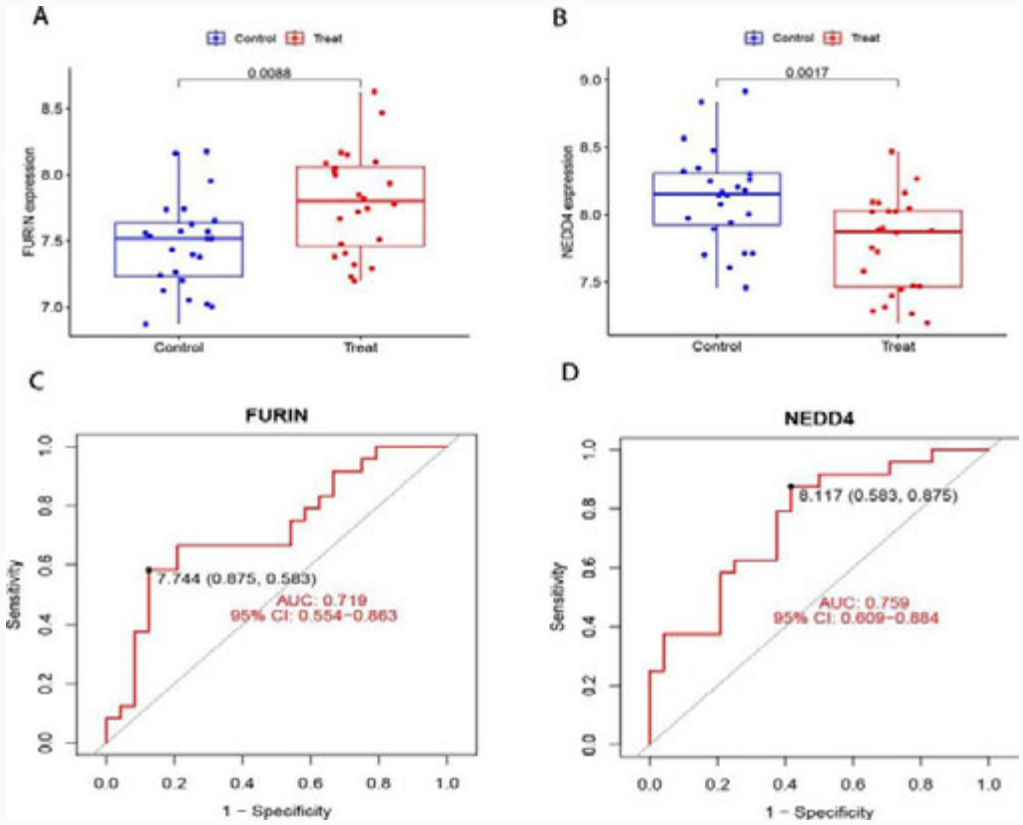

Figure 7: External validation of hub genes. (A–B) Comparison of hub gene expression between the control and treatment groups, p<0.05. (C–D) ROC curves for the hub genes, with AUC values exceeding 0.7.

patients with RSA (Figure 6A, 6B).

External validation of hub genes

The results of the previous biological analyses were validated using another RSA dataset, GSE165004, obtained from the GEO database. This validation confirmed that the expression of the Furin gene was substantially elevated in RSA relative to the control samples (p<0.05). Conversely, NEDD4 levels were markedly decreased in RSA relative to the controls (p<0.05). The results are presented in Figure 7.

Hub gene-targeted drugs

We explored potential drugs targeting hub genes through the DGIdb database. Interestingly, we found that the gene NEDD4 interacts with WARFARIN (Figure 8), a coumarin-based oral anticoagulant primarily used for anticoagulation in the body.

Networks of ceRNA containing hub genes

Subsequently, we examined the miRanda, miRDB, and TargetScan databases to detect possible modulatory micro RNAs (miRNAs) for the Furin and NEDD4 transcripts. The Furin network included 15 miRNAs and 83 long noncoding RNAs (lncRNAs), whereas the NEDD4 network included 10 miRNAs and 54 lncRNAs (Figure 9).

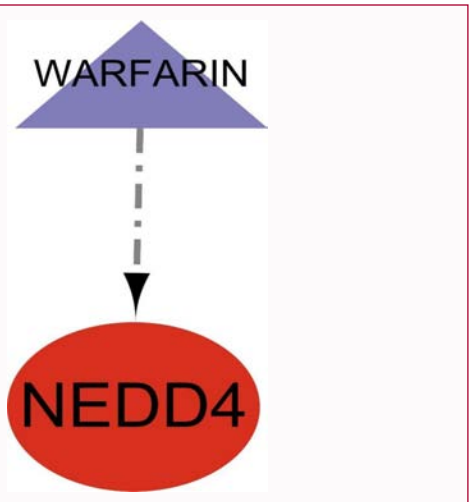

Figure 8: Hub gene-targeted drugs prediction.

Discussion

RSA, with an incidence rate of 1% to 5%, is prevalent but often underestimated [11]. Recent research has shed light on the pathologic factors associated with RSA, revealing the potential significance of impaired endometrial decidualization in its pathogenesis [12]. Decidualization comprises the intricate conversion of endometrial stromal cells into decidual cells. This conversion is controlled by a variety of physiological variables, including growth factors, intracellular signaling, and ovarian steroid hormones (estrogen, progesterone, prolactin).

Several metabolic pathways linked to iron, lipid, and amino acid metabolism, as well as degradation, including ubiquitin-mediated proteasomal degradation and macroautophagy, are dependent on ferroptosis, an iron-dependent form of cell death triggered by lipid peroxidation [13]. It is noteworthy that RSA patients exhibit both low serum iron levels and local iron deposition within the decidua. Research has indicated that inhibiting ferroptosis in decidual

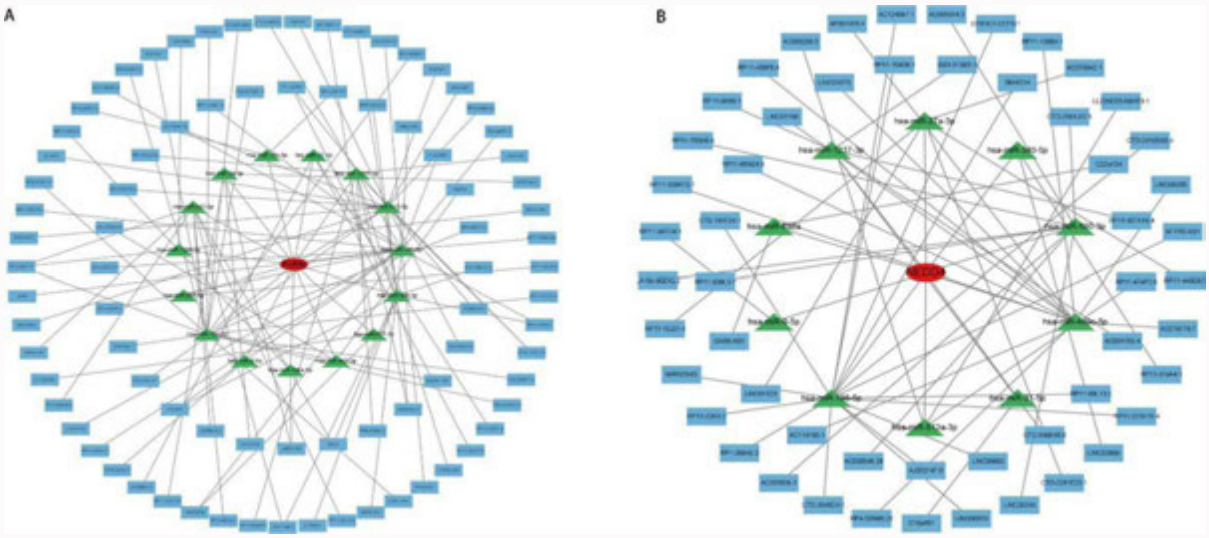

Figure 9: Networks of ceRNAs containing hub genes. (A) Networks of Furin comprising 15 miRNAs and 83 lncRNAs. (B) The NEDD4 network comprising 10 miRNAs and 54 lncRNAs.

ferroptosis and reversing embryo loss in unplanned abortions are effective methods [14], indicating the possibility of a relationship between RSA and ferroptosis. Despite this, little research has been conducted on the molecular processes and signaling pathways that initiate ferroptosis in RSA. Therefore, further analysis of FRGs associated with RSA is essential to establish potential diagnostic criteria and therapeutic targets.

Here, 15 Ferr-DEGs related to RSA were identified in the GSE26787 dataset, of which nine were upregulated and six downregulated. Subsequent GO and KEGG examinations indicated that these 15 Ferr-DEGs are crucially involved in cellular response to stimulus, regulation of autophagy, signaling pathways of FoxO, the cyclic Adenosine Monophosphate (cAMP), and progesterone-mediated oocyte maturation. These pathways may have significant relevance to the progression of RSA. Growing evidence indicates that the autophagy machinery is essential for the functioning of ferroptosis. A critical element of the ferroptotic reaction is ferritinophagy, which involves the removal of ferritin, the main iron-storing protein, via autophagy [15]. Therefore, the FOXO family, known for its diverse cellular functions, regulates genes essential for decidualization [16,17]. Furthermore, cAMP, a vital secondary messenger in intracellular signal transduction, is critical for decidualization resulting from hormonal changes, with cAMP, in combination with progesterone, being the most effective inducer of endometrial decidualization [18,19]. Dysregulation in these pathways may lead to impaired endometrial decidualization.

Pregnancy presents a substantial challenge to the functionality of the maternal immune system. Both the mother and the fetus must be protected from such immune attacks. Numerous immune cells assemble at the interface between the mother and fetus during early pregnancy. These cells consist of dendritic cells, macrophages, NK cells, and various subsets of T-cells [20,21]. These cells are pivotal for vascular remodeling of the decidua, maintaining maternal immune balance, and regulating mesenchymal cell proliferation and differentiation, thereby facilitating endometrial transformation into decidua. The precise mechanisms that immune cell regulated endometrial decidualization remain to be fully elucidated, along with the potential for therapeutic intervention in these processes. It is plausible that RSA is linked to disturbances in the regulation of immune cell infiltration. Our analysis demonstrated higher infiltration of Type 1 T helper cells and lower infiltration of activated B cells, eosinophils, monocytes, and neutrophils in patients with RSA. Gu H reported significantly higher Th1 (IFN-g) and Th1/Th2 ratios in RSA patients [22], consistent with our results. Th1 cells are integral components of the immune response, performing pivotal functions in immune rejection and tolerance, and imbalances in this ratio affect the function of the decidua, leading to miscarriage [23].

Aberrant gene expression has been widely recognized as a substantial determinant in RSA and a critical indicator of pregnancy complications [24,25]. Therefore, we conducted a meticulous screening of ferroptosis-related characteristic genes within endometrial samples. Through the integration of the LASSO algorithm and the PPI network, we identified two hub Ferr-DEGs, namely Furin and NEDD4. We evaluated the co-expression patterns and interacting proteins of these hub genes in order to further elucidate their functions in RSA. Among the top five co-expression patterns, NOTCH3, ADAMTS4, GRB10, PROZ, and ISG15 emerged as notable. The functions of 20 interacting proteins were substantially enhanced in the two hub genes, based on our analysis. These proteins were primarily involved in regulating cellular responses to growth factor stimuli and the vascular endothelial growth factor receptor signaling pathway. Furthermore, GSEA revealed that interaction between cytokines, cytotoxicity induced by NK cells, complement, and coagulation networks were identified as common pathways related to hub Ferr-DEGs. Notably, abnormal gene expression in cytokine–cytokine receptor-associated pathways suggest immune cell dysfunction in cases of repeated pregnancy loss. NK cells, in particular, are crucially involved in the initiation of inflammation and restoration [26,27]. They contribute to recurrent miscarriage through pathways such as SP1-CASP3-PARP1 [28]. The primary function of dNK cells at the maternal-fetal interface is to preserve an immune-tolerant microenvironment. In contrast, the polarization of dNK cells, which normally attains immune tolerance polarization *via* interaction with extravillous trophoblasts, was significantly disrupted in the context of RSA, as demonstrated by Pan et al. *via* single-cell sequencing evaluation [29]. Many investigations have demonstrated

associations between abnormal numbers and subsets of NK cells to RSA. It appears that NK cells are substantially disrupted in RSA, suggesting NK cell activity as a possible prognostic marker for RSA [30].

Furin, a serine protease, is ubiquitously expressed across all cell lines and tissues. It has been observed to cleave γ- and γ-ENaC at their external domains during the maturation of newly formed channels *via* the biosynthetic/Golgi pathway [31]. Consequently, it participates in the synthesis of various proteins and receptors, including bacterial endotoxins, viral envelope glycoproteins, growth factors, matrix metalloproteinases, and proteins associated with complement and coagulation [32]. Freyer C. determined that the human endometrium exhibited the highest Furin levels during menstruation, with the lowest levels during proliferation [33]. The failed abortion group exhibited increased immunoreactivities to Furin in comparison to the control group [34], which aligns with our findings of increased expression in patients with RSA. Dong S demonstrated that Furin overexpression attenuated induced ferroptosis-like injury by activating Nrf2 and upregulating Gpx4 in ulcerative colitis [35]. As such, while the role of Furin in RSA is promising, it requires further verification in future research endeavors.

NEDD4 is a strongly conserved HECT E3 ubiquitin ligase. It is involved in many biological events by facilitating proteasomal degradation through the recognition of PPxY motifs in substrates. These substrates can be found in the plasma membrane or within the nucleus [36]. NEDD4 is involved in several physiological processes, and studies have suggested that it may serve as an oncogene by promoting cancer cell formation. The critical role of NEDD4 in tumorigenesis is demonstrated through its regulation of various substrates, such as Epithelial Sodium Channel (ENaC) [37], Notch [38,39], large tumor suppressor kinase 1 [40], among others. Targeting NEDD4 may, therefore, offer possible and therapeutic approach to treating malignancies in humans [41]. Also, Di X demonstrated that levels of NEDD4 and ENaC, both of which are associated with SGK1 signaling, were decreased in the decidual tissue of the RSA group [42].

In order to provide additional evidence regarding the clinical diagnostic efficacy of these two genes, endometrial tissues were obtained from 24 fertile females (control group) and 24 individuals with recurrent pregnancy losses (treatment group) for validation from the GSE165004 dataset. The pattern of gene expression exhibited by these two RSA-associated genes was similar to that observed in GSE26787. Specifically, the expression of Furin was upregulated, whereas NEDD4 was downregulated in RSA. ROC curve analysis further supports the diagnostic potential of these two hub genes for recurrent miscarriage. Lastly, we conducted an analysis of marker genes for gene-targeted drugs and constructed a ceRNA network. It is noteworthy that we found only one gene that interacts with NEDD4 in the context of targeted drugs, which is WARFARIN. The ceRNA interaction network, which was assembled *via* Furin and NEDD4, comprised a variety of miRNAs and lncRNAs. Noncoding RNAs are known to contribute in RSA, and therefore, the identified drugs and noncoding RNAs are prospective subjects for further study.

In summary, this study identified two hub Ferr-DEGs as possible therapeutic targets and diagnostic markers for RSA. Specifically, we found that Furin was significantly upregulated, whereas NEDD4 was significantly downregulated in endometrial samples. Furthermore, our study has predicted a set of signaling pathways, noncoding RNAs, and drugs that may modulate these hub genes. However, these findings are currently based on bioinformatics data analysis and external validation, lacking empirical data and support from clinical evidence. Therefore, further investigation is necessary to gain insight into the post-translational alterations, metabolic, and epigenetic processes that control the process of endometrial decidualization. This will aid in further verifying the roles of FRGs and regulatory pathways underlying RSA resulting from impaired decidualization. Prospectively, novel approaches to the prevention and management of deleterious consequences of pregnancy might be developed by inhibiting ferroptosis in patients diagnosed with RSA.

Funding

This work was supported by grants from Sponsored by the Natural Science Foundation of Xinjiang Uygur Autonomous Region (2021D01F25).

References

1. Practice Committee of the American Society for Reproductive Medicine. Definitions of infertility and recurrent pregnancy loss: A committee opinion. Fertil Steril. 2020;113(3):533-5.
2. Deng T, Liao X, Zhu S. Recent advances in treatment of recurrent spontaneous abortion. Obstet Gynecol Surv. 2022;77(6):355-66.
3. Abdollahi E, Tavasolian F, Ghasemi N, Mirghanizadeh SA, Azizi M, Ghoryani M, et al. Association between lower frequency of R381Q variant (rs11209026) in IL-23 receptor gene and increased risk of Recurrent Spontaneous Abortion (RSA). J Immunotoxicol. 2015;12(4):317-21.
4. Quenby S, Gallos ID, Dhillon-Smith RK, Podesek M, Stephenson MD, Fisher J, et al. Miscarriage matters: the epidemiological, physical, psychological, and economic costs of early pregnancy loss. Lancet. 2021;397(10285):1658-67.
5. Gellersen B, Brosens JJ. Cyclic decidualization of the human endometrium in reproductive health and failure. Endocr Rev. 2014;35(6):851-905.
6. Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, et al. Ferroptosis: An iron-dependent form of nonapoptotic cell death. Cell. 2012;149(5):1060-72.
7. Chen X, Kang R, Kroemer G, Tang D. Ferroptosis in infection, inflammation, and immunity. J Exp Med. 2021;218(6):e20210518.
8. Ni Z, Li Y, Song D, Ding J, Mei S, Sun S, et al. Iron-overloaded follicular fluid increases the risk of endometriosis-related infertility by triggering granulosa cell ferroptosis and oocyte dysmaturity. Cell Death Dis. 2022;13(7):579.
9. Hu M, Zhang Y, Ma S, Li J, Wang X, Liang M, et al. Suppression of uterine and placental ferroptosis by N-acetylcysteine in a rat model of polycystic ovary syndrome. Mol Hum Reprod. 2021;27(12):gaab067.
10. Beharier O, Tyurin VA, Goff JP, Guerrero-Santoro J, Kajiwara K, Chu T, et al. PLA2G6 guards placental trophoblasts against ferroptotic injury. Proc Natl Acad Sci U S A. 2020;117(44):27319-28.
11. Zhou LY, Zhang HX, Lan YL, Li Y, Liang Y, Yu L, et al. Epidemiological investigation of risk factors of the pregnant women with early spontaneous abortion in Beijing. Chin J Integr Med. 2017;23(5):345-9.
12. Sang Y, Li Y, Xu L, Li D, Du M. Regulatory mechanisms of endometrial decidualization and pregnancy-related diseases. Acta Biochim Biophys Sin (Shanghai). 2020;52(2):105-15.
13. Chen X, Li J, Kang R, Klionsky DJ, Tang D. Ferroptosis: machinery and regulation. Autophagy. 2021;17(9):2054-81.
14. Sun F, Cui L, Qian J, Li M, Chen L, Chen C, et al. Decidual stromal cell ferroptosis associated with abnormal iron metabolism is implicated in the pathogenesis of recurrent pregnancy loss. Int J Mol Sci. 2023;24(9):7836.
15. Rochette L, Dogon G, Rigal E, Zeller M, Cottin Y, Vergely C. Lipid

peroxidation and iron metabolism: Two corner stones in the homeostasis control of ferroptosis. Int J Mol Sci. 2022;24(1):449.

16. Kajihara T, Brosens JJ, Ishihara O. The role of FOXO1 in the decidual transformation of the endometrium and early pregnancy. Med Mol Morphol. 2013;46(2):61-8.

17. Vasquez YM, Mazur EC, Li X, Kommagani R, Jiang L, Chen R, et al. FOXO1 is required for binding of PR on IRF4, novel transcriptional regulator of endometrial stromal decidualization. Mol Endocrinol. 2015;29(3):421-33.

18. Muñoz-Fernández R, De La Mata C, Requena F, Martín F, Fernandez-Rubio P, Llorca T, et al. Human predecidual stromal cells are mesenchymal stromal/stem cells and have a therapeutic effect in an immune-based mouse model of recurrent spontaneous abortion. Stem Cell Res Ther. 2019;10(1):177.

19. Vagena E, Ryu JK, Baeza-Raja B, Walsh NM, Syme C, Day JP, et al. A high-fat diet promotes depression-like behavior in mice by suppressing hypothalamic PKA signaling. Transl Psychiatry. 2019;9(1):141.

20. Vento-Tormo R, Efremova M, Botting RA, Turco MY, Vento-Tormo M, Meyer KB, et al. Single-cell reconstruction of the early maternal-fetal interface in humans. Nature. 2018;563(7731):347-53.

21. Wang XQ, Zhou WJ, Hou XX, Fu Q, Li DJ. Trophoblast-derived CXCL16 induces M2 macrophage polarization that in turn inactivates NK cells at the maternal-fetal interface. Cell Mol Immunol. 2018;15(12):1038-46.

22. Gu H, Li L, Du M, Xu H, Gao M, Liu X, et al. Key gene and functional pathways identified in unexplained recurrent spontaneous abortion using targeted RNA sequencing and clinical analysis. Front Immunol. 2021;12:717832.

23. Nakagawa K, Kwak-Kim J, Ota K, Kuroda K, Hisano M, Sugiyama R, et al. Immunosuppression with tacrolimus improved reproductive outcome of women with repeated implantation failure and elevated peripheral blood TH1/TH2 cell ratios. Am J Reprod Immunol. 2015;73(4):353-61.

24. Brew O, Sullivan MH, Woodman A. Comparison of normal and pre-eclamptic placental gene expression: A systematic review with meta-analysis. PLoS One. 2016;11(8):e0161504.

25. Houshmand-Oeregaard A, Hjort L, Kelstrup L, Hansen NS, Broholm C, Gillberg L, et al. DNA methylation and gene expression of TXNIP in adult offspring of women with diabetes in pregnancy. PLoS One. 2017;12(10):e0187038.

26. Zitti B, Bryceson YT. Natural killer cells in inflammation and autoimmunity. Cytokine Growth Factor Rev. 2018;42:37-46.

27. Kucuksezer UC, Aktas Cetin E, Esen F, Tahrali I, Akdeniz N, Gelmez MY, et al. The role of natural killer cells in autoimmune diseases. Front Immunol. 2021;12:622306.

28. Liu Y, Chen P, Fei H, Li M, Li X, Li T. Natural killer cells contributed to recurrent miscarriage by SP1-CASP3-PARP1. Int Immunopharmacol. 2021;93:107424.

29. Pan D, Liu Q, Du L, Yang Y, Jiang G. Polarization disorder of decidual NK cells in unexplained recurrent spontaneous abortion revealed by single-cell transcriptome analysis. Reprod Biol Endocrinol. 2022;20(1):108.

30. Zhang X, Wei H. Role of decidual natural killer cells in human pregnancy and related pregnancy complications. Front Immunol. 2021;12:728291.

31. Kleyman TR, Eaton DC. Regulating ENaC's gate. Am J Physiol Cell Physiol. 2020;318(1):C150-C162.

32. Nakayama K. Furin: A mammalian subtilisin/Kex2p-like endoprotease involved in processing of a wide variety of precursor proteins. Biochem J. 1997;327(Pt 3):625-35.

33. Freyer C, Kilpatrick LM, Salamonsen LA, Nie G. Pro-protein Convertases (PCs) other than PC6 are not tightly regulated for implantation in the human endometrium. Reproduction. 2007;133(6):1189-97.

34. Ozbilgin K, Turan A, Kahraman B, Atay C, Vatansever S, Uluer ET, et al. Distribution of furin, TNF-α, and TGF-β2 in the endometrium of missed abortion and voluntary first trimester termination cases. Anal Quant Cytopathol Histpathol. 2015;37(2):123-33.

35. Dong S, Lu Y, Peng G, Li J, Li W, Li M, et al. Furin inhibits epithelial cell injury and alleviates experimental colitis by activating the Nrf2-Gpx4 signaling pathway. Dig Liver Dis. 2021;53(10):1276-85.

36. Ingham RJ, Gish G, Pawson T. The Nedd4 family of E3 ubiquitin ligases: Functional diversity within a common modular architecture. Oncogene. 2004;23(11):1972-84.

37. Staub O, Dho S, Henry P, Correa J, Ishikawa T, McGlade J, et al. WW domains of Nedd4 bind to the proline-rich PY motifs in the epithelial Na+ channel deleted in Liddle's syndrome. Embo J. 1996;15(10):2371-80.

38. Sakata T, Sakaguchi H, Tsuda L, Higashitani A, Aigaki T, Matsuno K, et al. Drosophila Nedd4 regulates endocytosis of notch and suppresses its ligand-independent activation. Curr Biol. 2004;14(24):2228-36.

39. Wilkin MB, Carbery AM, Fostier M, Aslam H, Mazaleyrat SL, Higgs J, et al. Regulation of notch endosomal sorting and signaling by Drosophila Nedd4 family proteins. Curr Biol. 2004;14(24):2237-44.

40. Salah Z, Cohen S, Itzhaki E, Aqeilan RI. NEDD4 E3 ligase inhibits the activity of the Hippo pathway by targeting LATS1 for degradation. Cell Cycle. 2013;12(24):3817-23.

41. Yang Y, Luo M, Zhang K, Zhang J, Gao T, Connell DO, et al. Nedd4 ubiquitylates VDAC2/3 to suppress erastin-induced ferroptosis in melanoma. Nat Commun. 2020;11(1):433.

42. Di X, Hao Y, Duan Z, Ma Y, Cao Y, Tan Z, et al. Activation of SGK1/ENaC signaling pathway improves the level of decidualization in unexplained recurrent spontaneous abortion. Reprod Sci. 2023;30(11):3273-84.