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ORIGINAL ARTICLE

Alpha-enolase 1 knockdown facilitates the proliferation and invasion of villous trophoblasts by upregulating COX-2

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Abstract

Background: Enolase 1 (ENO1) is a metabolic enzyme which participates in pyruvate synthesis and ATP production in cells. Previously, differential expression of ENO1 was discovered in villous tissues between recurrent miscarriage and induced abortion. This study was designed to explore whether ENO1 influences the proliferation and invasion of villous trophoblasts and the related molecular mechanisms.

Methods: First, ENO1 expression in placental villus tissues collected from recurrent miscarriage (RM) patients and women for induced abortion as well as in trophoblast-derived cell lines was detected by RT-qPCR and western blotting. ENO1 localization and expression in villus tissues were further confirmed through immunohistochemistry staining. Then, the effects of ENO1 downregulation on trophoblast Bewo cell proliferation, migration, invasion, and epithelial-mesenchymal transition (EMT) process were evaluated by CCK-8 assay, transwell assay, and western blotting. As for the regulatory mechanism of ENO1, the expression of COX-2, c-Myc and cyclin D1 in Bewo cells after ENO1 knockdown was finally evaluated by RT-qPCR and western blotting.

Results: ENO1 was mainly localized in the cytoplasm, with very small amounts in the nucleus of trophoblast cells. ENO1 expression in the villi tissues of RM patients was significantly increased, when compared with the villous tissues of healthy controls. Furthermore, Bewo cells, a trophoblast cell line with relatively higher expression of ENO1, was used to downregulate the ENO1 expression by ENO1-siRNA transfection. ENO1 knockdown significantly facilitated Bewo cell growth, EMT process, migration, and invasion. ENO1 silencing markedly elevated COX-2, c-Myc, and cyclin D1 expression.

Conclusion: ENO1 may participate in the development of RM via suppressing the growth and invasion of villous trophoblasts via reducing the expression of COX-2, c-Myc, and cyclin D1.

Huaiyun Tang and Linqing Pan contributed equally to this study.

KEYWORDS
COX-2, ENO1, recurrent miscarriage, villous trophoblasts

1 | INTRODUCTION

Recurrent miscarriage (RM) is generally defined as three or more consecutive clinical pregnancy failures with the same partner before 20 weeks of gestation, affecting 2%–5% of pregnant women and with an increasing trend (Koert et al., 2019). However, RM was also identified as two or more consecutive pregnancy losses detected on ultrasound or histopathology, by some reproductive medicine societies (du Fossé et al., 2021; Practice Committee of the American Society for Reproductive Medicine, 2020). Known pathogenic causes of RM include immune factors, infection, endocrine dysfunction, uterine malformation, prothrombotic state, and embryonic or couples' chromosomal abnormalities (Egerup et al., 2016). However, the etiology of about half of RM cases remains unknown. Numerous studies have discovered spiral artery remodeling dysfunction and reduced trophoblast invasion in decidual tissues with RM (Ali et al., 2021; Ding et al., 2019).

Placental trophoblast cell plays a crucial role in pregnancy maintenance and embryonic development. Invasion and endocrine are the two major functions of trophoblast cells. Trophoblasts share similar characteristics with tumor cells, such as increased cell growth, migration, and invasion, as well as gene expression similarities (Mullen, 1998). Normal trophoblast proliferation and invasion is a critical step in normal placentation. Trophoblast cell invasiveness requires tight spatial and temporal regulation. This regulation is pronounced in the first trimester, but attenuates or even disappears in the second and later trimesters. The invasion of extravillous trophoblast (EVT) cells in the uterine epithelium is a prerequisite for placenta formation.

Enolase (ENO) as a metabolic enzyme implicated in pyruvate synthesis, maintaining the level of intracellular ATP and meeting the energy required for life activities. The c-Myc promoter binding protein 1 (MBP-1), as the splice variant of ENO1 (OMIM: 172430), enables DNA-binding transcription activator activity and positively modulates transcription involved in the G1/S transition of mitotic cell cycle (Chen et al., 2021). ENO is widely present on the cytoplasm, nucleus, and the cell membrane (Caturegli et al., 2014). ENO1 is a member of the ENO family. ENO1 plays a key regulatory role in multiple biological processes, including apoptosis, myogenesis, plasmin activity, plasminogen activation, and tumorigenesis (Pancholi & Fischetti, 1998). Due to high similarity in the biological characteristics between tumor cells and trophoblasts, we hypothesize that the mechanism of ENO1 in tumors may also be involved in the occurrence of RM. Furthermore, ENO1 was reported to downregulate COX-2, whose deficiency influences all stages of early pregnancy and results in various impairments (Zhang et al., 2016), as well as repress the transcriptional expression of c-Myc and cyclin D1, which can promote the proliferation and invasion of trophoblast cells (Dai et al., 2012; Sun et al., 2015). Based on the above literature, we hypothesized that ENO1 might restrain trophoblast proliferation, migration and invasion via downregulating the expression of COX-2, c-Myc, and cyclin D1.

This study was designed to observe the effect of ENO1 on villous trophoblast proliferation and invasion and the related molecular mechanisms so as to explore the potential effect of ENO1 on the pathophysiological mechanism of RM.

2 | MATERIALS AND METHODS

In this study, three parts were included. First, ENO1 (GenBank reference sequence and version number: NC_000001.11) expression in the villous tissues of RM patients was compared with the healthy controls, and the localization of ENO1 in villous tissues was detected. Second, we investigated the biological function of ENO1 via RNA interference-mediated knockdown (loss-of-function approach) using Bewo cells, a trophoblast cell line. Third, the potential regulatory mechanism of ENO1 in RM was explored.

2.1 | Ethical compliance

This study was approved by the Ethics Committee of Lianyungang Maternal and Child Health Hospital (No. LYG-ME2017003). All individual participants signed informed consent prior to the study.

2.2 | Participating cohorts

Eight women who experienced spontaneous abortion three or more times before 12 weeks of pregnancy were included in this study. These pregnancies were confirmed under ultrasound examination that no primitive cardiac tube pulsation was found, and the pregnancy was

terminated by induced abortion. Chromosomal abnormal villi were ruled out by array-CGH diagnosis. Eight women who underwent abortion at the same hospital before 12 weeks of pregnancy without adverse pregnancy were recruited as the controls. Villi tissues were obtained under sterile conditions, washed thoroughly with sterile saline to remove decidual tissue and blood, cut into 0.5 cm in diameter, and stored under liquid nitrogen labeling.

2.3 | Cell line

The trophoblast cell lines including JAR and Bewo cells were obtained from Chinese Academy of Sciences (Beijing, China), and the JEG3 and HTR-8/SVneo cells were obtained from the China Typical Culture Preservation Center (Wuhan University, China). All cells were incubated in the RPMI 1640 medium with 10% fetal bovine serum (FBS), 1% penicillin–streptomycin (Gibco, USA) at 37°C in a 5% CO_2 incubator. Bewo cells were transfected with ENO1 siRNA and ENO1 siRNA-NC, respectively, for 48 h.

2.4 | In vitro experiments

At 48 h after si-ENO1 transfection, Bewo cells were collected. For cell proliferation detection, transfected cells were seeded into 96-well plates (5000 cells/well). Then, 10 μL CCK8 solution (Dojindo, Japan) was added into each well and cultured for 1 h, and the absorbance at 490 nm was measured.

For migration and invasion evaluation, the upper chambers of transwell inserts (8 μM; BD Biosciences) were coated without or with Matrigel (BD Biosciences). Briefly, 1×10^5 cells were seeded into the upper chamber supplement with 200 μL of serum-free medium, and the lower chamber was filled with 600 μL of DMEM containing 10% FBS. After 48 h incubation, migrated or invaded cells on the lower surface of the membranes were fixed with 95% methanol, stained with 0.5% crystal violet, and counted with a microscope (Olympus).

2.5 | Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from villi tissues and trophoblast cells using TRIzol reagent (Invitrogen). cDNA was synthesized using a PrimeScript First Strand cDNA Synthesis Kit (TaKaRa), and qPCR was performed using SYBR Green I mix (Takara). GAPDH served as an internal standard. The results were calculated applying the $2^{-\Delta\Delta Ct}$ method.

2.6 | Western blot analysis

Total protein was extracted from villous tissues and Bewo cells using RIPA buffer. Then, proteins were separated by SDS-PAGE and transferred to PVDF membranes. The PVDF membrane was blocked with 5% skimmed dried milk for 1 h. Primary antibodies (Abcam) against ENO1 (ab227978, 1:1000), E-cadherin (ab76055, 1:1000), N-cadherin (ab76011, 1:5000), Vimentin (ab137321, 1:500), COX-2 (ab179800, 1:1000), c-Myc (ab32072, 1:1000), cyclin D1 (ab16663, 1:200), and GAPDH (ab9485, 1:2500) were added followed by incubation at 4°C overnight, and then the membranes were washed thrice with TBST and incubated with HRP-conjugated secondary antibodies (1:500, Proteintech) for 1 h at 37°C. Detection was performed by enhanced chemiluminescence kit (Beyotime).

2.7 | Immunohistochemistry

After dewaxing, hydrating and 3% hydrogen peroxide treatment, villi tissues were fixed by 10% formaldehyde solution and placed in sodium citrate buffer for antigen retrieval. Then, the tissues were incubated with rabbit-anti-ENO1 antibodies (ab227978, 1:2000, Abcam) at 37°C for 2 h and then with secondary antibody at 37°C for 30 min. After diaminobenzidine (DAB) color development, hematoxylin redyeing, and mounting, tissues were visualized by the ScanScope CS2 system and ATTurbo scanner.

2.8 | Statistical analysis

All experiments were repeated in triplicate. The data were analyzed using SPSS 18.0 software and are expressed as the mean ± SD. Student's *t*-test and one-way ANOVAs were conducted for statistical analysis. A p-value <0.05 was statistically significant.

3 | RESULTS

3.1 | ENO1 expression in villi tissues and trophoblast cells

ENO1 expression in the villous tissue of RM patients and the control group was first detected. The mRNA and protein levels of ENO1 were significantly higher in RM patients than in controls (Figure 1a–c). Furthermore, ENO1 expression in four human trophoblast-derived cell lines was also evaluated by PCR and western blotting analyses,

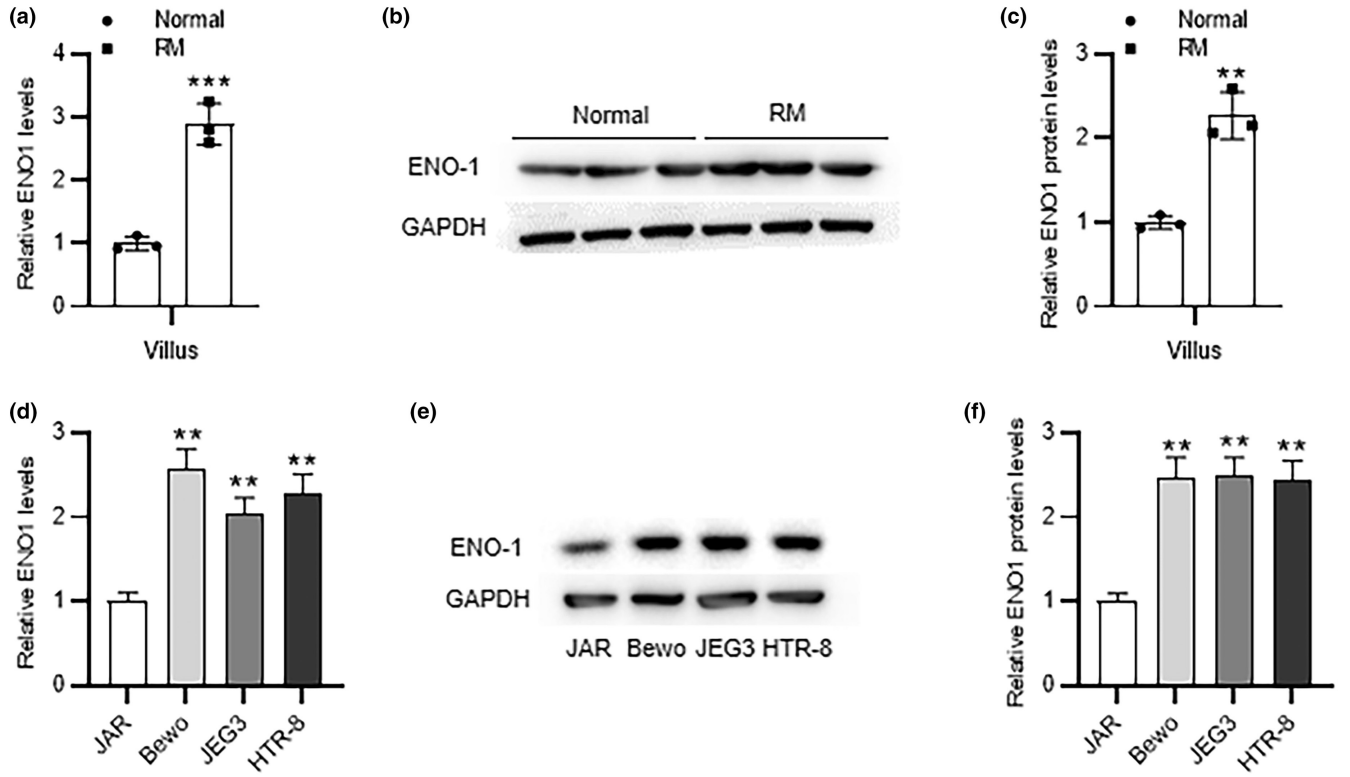

FIGURE 1 ENO1 expression in villous tissues and trophoblast cells. (a–c) ENO1 mRNA and protein levels in villous tissue of patients with RM compared with that of women for induced abortion were detected by qRT-PCR and western blot analyses; (d–f) ENO1 mRNA and protein levels in trophoblast-derived cell lines were tested by qRT-PCR and western blot analyses. $^{**}p < 0.01$; $^{***}p < 0.001$. RM, recurrent miscarriage.

which revealed that Bewo cells had the highest mRNA and protein levels (Figure 1d–f). Therefore, Bewo cells were selected for the subsequent experiments that investigated the effects of ENO1 on trophoblast proliferation and invasion.

3.2 | Cellular localization of ENO1 in villi tissues

Then, ENO1 localization and expression in villus tissues were measured by immunohistochemistry. The RM group displayed weaker expression of ENO1, and positive signals were observed mainly in the cytoplasm, cell membrane and nucleus of trophoblast cells. The cell membranes of villous trophoblast cells in RM patients were dark brown, while those of villous trophoblast cells in the control group appeared light yellow (Figure 2).

3.3 | ENO1 knockdown facilitated Bewo cell proliferation and invasion

Si-ENO1 was transfected into Bewo cells to knock down ENO1. As shown in Figure 3a, ENO1 mRNA and protein levels were reduced in Bewo cells transfected with si-ENO1, but not changed after si-NC transfection (Figure 3a–c). CCK-8 assay revealed that Bewo cell viability was notably enhanced after downregulating ENO1 (Figure 3d). The Transwell chambers were employed to evaluate the effects of ENO1 silencing on the migratory and invasive capabilities of Bewo cells. After transfection with si-ENO1, the number of villous trophoblast cells that passed into the lower chamber was significantly elevated (Figure 3e,f), suggesting that ENO1 markedly promoted the migration and invasion of villous trophoblast cells. Additionally, the protein levels of EMT markers influenced by ENO1 knockdown were evaluated. As shown by western blot analysis, ENO1 knockdown led to a marked reduction in E-cadherin level and a remarkable elevation in Vimentin and N-cadherin levels (Figure 3g,h). Thus, the EMT process was facilitated following ENO1 downregulation.

3.4 | Knockdown of ENO1 elevated the expression of COX-2, c-Myc, and cyclin D1

Finally, to assess whether ENO1 mediates the pathophysiology of RM via COX-2, RT-qPCR, and western blot analysis were conducted. The results illustrated that ENO1

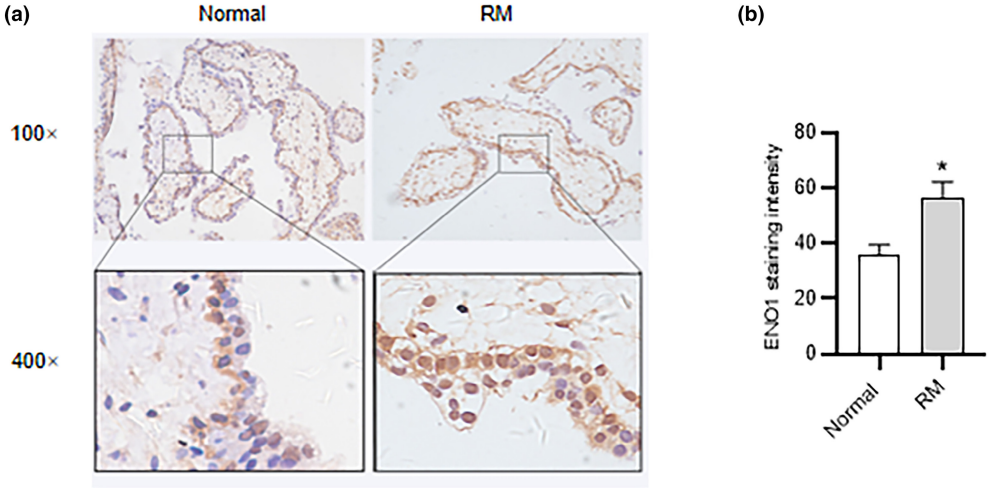

FIGURE 2 ENO1 localization and expression in villous tissues of RM patients. (a and b) Immunohistochemistry analysis of ENO1 stain from placental villus of RM patients and women for induced abortion. The cell membranes of villous trophoblast cells in patients with RM were dark brown, while those in the control group appeared light yellow. RM, recurrent miscarriage.

silencing elevated the mRNA and protein levels of COX-2, c-Myc, and cyclin D1 (Figure 4a–c). Therefore, ENO1 is implicated in RM development through modulating the COX-2, cyclin D1, and c-Myc expression.

4 | DISCUSSION

ENO1, also known as α-enolase, is one of the most important enzymes in enolase family (Principe et al., 2017). ENO1 has been demonstrated to play an essential regulatory role in biological processes including apoptosis, myogenesis, plasmin activity, plasminogen activation, and tumor development (Li et al., 2021). The currently known biological functions of ENO1, such as autoimmune response endocrine activity, plasminogen activity, and oncogene enhancing cell migration and invasion, are highly similar to the molecular mechanisms of RM (Gou et al., 2021). Previously, ENO1 was reported to be highly expressed in the decidua of RM patients in the first trimester (Ye et al., 2019). In the EVT, the overexpression of ENO-1 followed by its translocation to the cell surface could cause the appearance of direct-acting anti-ENO-1 autoimmune antibodies in patients with recurrent miscarriage, together with the decrease in hCG expression (Ye et al., 2019). In this study, ENO1 expression was significantly increased in the villous tissues of the RM group, compared with the control group. Next step, we observed that trophoblast cell proliferation, migration, and invasion ability were enhanced after knockdown of ENO1. Immunohistochemical investigations displayed that ENO1 is expressed in the cytoplasm, membrane, and nucleus of Bewo cells, and ENO1 expression was elevated in the RM group versus in the control group. However, the molecular mechanisms behind these phenomena remain to be fully understood.

In this study, we found that the villous trophoblast cell membrane coloration in the RM group was obviously darker than that in the control group, indicating that ENO1 is highly expressed on the cell membrane of trophoblast cells of RM patients. Previously, ENO1 located on the surface of cell membranes was discovered to play a significant role in the plasmin system and identified as a plasminogen-binding molecule (Wang et al., 2021). ENO1 acts as a plasminogen receptor pancreatic ductal adenocarcinoma cells and promotes cell survival, migration, and transfer through synergy with integrin and urokinase plasminogen activator receptor (uPAR) (Principe et al., 2017). ENO1 on the surface of lung cancer cells facilitates extracellular matrix degradation and cell invasion. Targeting surface ENO1 is a promising approach to inhibit tumor metastasis (Hsiao et al., 2013). The interaction of plasmin with ENO1 contributes to the pathophysiological process, such as cell invasion, inflammation, and tumor metastasis.

Embryo implantation depends on the dynamic balance between numerous fibrinolytic and anti-fibrinolytic-associated protein factors synthesized and secreted by early placental trophoblasts (Sela et al., 2013). The balance can effectively dissolve the endometrial tissue, which is conducive to trophoblast cell invasion and placenta formation (Sela et al., 2013). In this study, ENO1 expression is upregulated on the cell membrane of trophoblast cells in RM. Building on results of previous studies of ENO1, we suppose that ENO1 may repress trophoblast cell migration and invasion via attenuating the action of the fibrinolytic system, thereby resulting in the occurrence of RM.

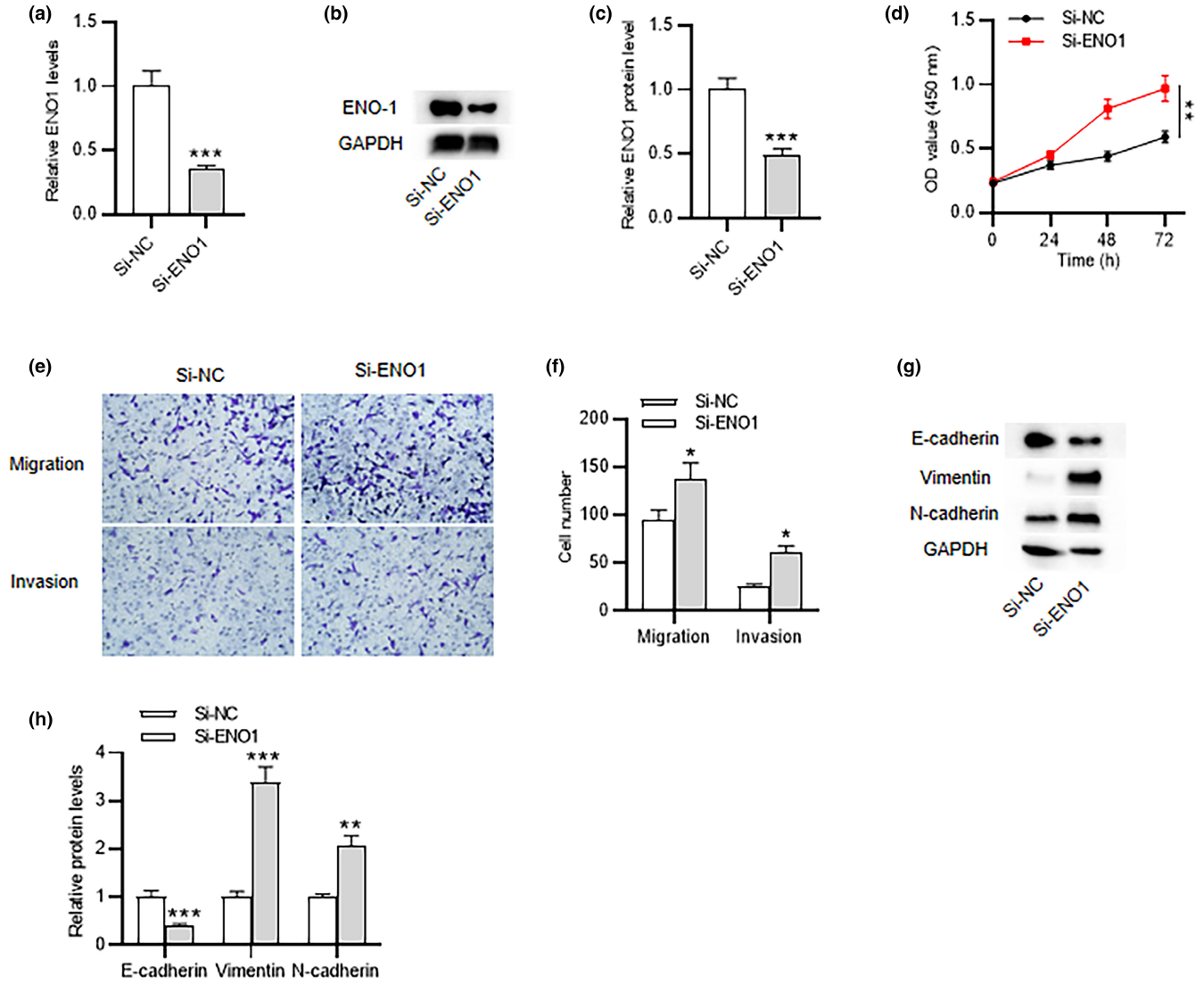

FIGURE 3 Effects of ENO1 on trophoblast proliferation, migration and invasion. (a–c) qRT-PCR and western blot analyses of ENO1 level in ENO1-downregulated Bewo cells. (d) CCK8 assay of Bewo cell proliferation after ENO1 depletion. (e and f) Transwell chamber assays were employed to evaluate Bewo cell migratory and invasive capabilities affected by ENO1 knockdown. (g and h) Western blot analysis of the expression of EMT markers in ENO1-downregulated Bewo cells. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

However, further research should be performed in order to strengthen this speculation.

Trophoblast cell dysfunction can cause many pregnancy-associated diseases including choriocarcinoma, premature delivery, spontaneous abortion, eclampsia, preeclampsia, and intrauterine growth restriction (Zhang et al., 2015). Herein, we detected and discovered that the proliferation, migration and invasion of Bewo cells were notably promoted after ENO1 knockdown, which is consistent with the impacts of ENO1 on the biological behaviors of tumor cells. Previously, MBP-1, the splice variant of ENO1, was reported to bind to the COX-2 promoter of chromosomal DNA to regulate promoter activity in the living cell environment (Hsu et al., 2009). In gastric cancer, MBP1 knockdown facilitated tumor cell growth, migration, and invasion through increasing COX-2 expression (Hsu et al., 2009). COX-2, being a highly inducible enzyme, modulates angiogenesis, mitogenesis, differentiation, and inflammation (Mitchell & Warner, 1999; Tsujii et al., 1998; Wu et al., 2005). The deficiency of COX-2 influences all stages of early pregnancy and results in various impairments (Lim et al., 1997). COX-2 plays a pivotal role in human decidualization and its reduced expression threatens vascularization and decidualization of the endometrial stroma, which contribute to the occurrence of preeclampsia (Zhang et al., 2016). In the present study, we discovered that COX-2 expression was greatly elevated following ENO1 depletion. Therefore, downregulated ENO1 alleviated the development of RM

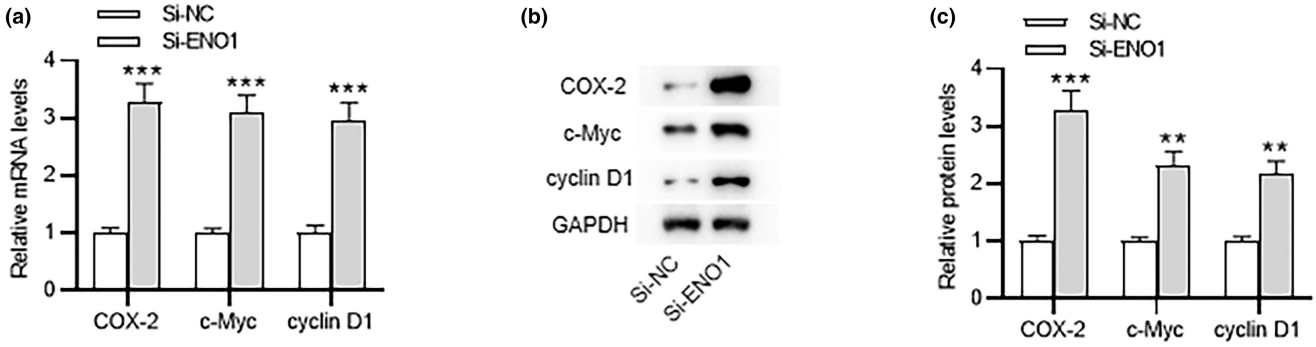

FIGURE 4 Effects of ENO1 knockdown on the expression of COX-2, c-Myc and cyclin D1. (a–c) RT-qPCR and western blot analyses of the expression of COX-2, c-Myc and cyclin D1 in Bewo cells after downregulating ENO1. $^{**}p < 0.01$; $^{***}p < 0.001$.

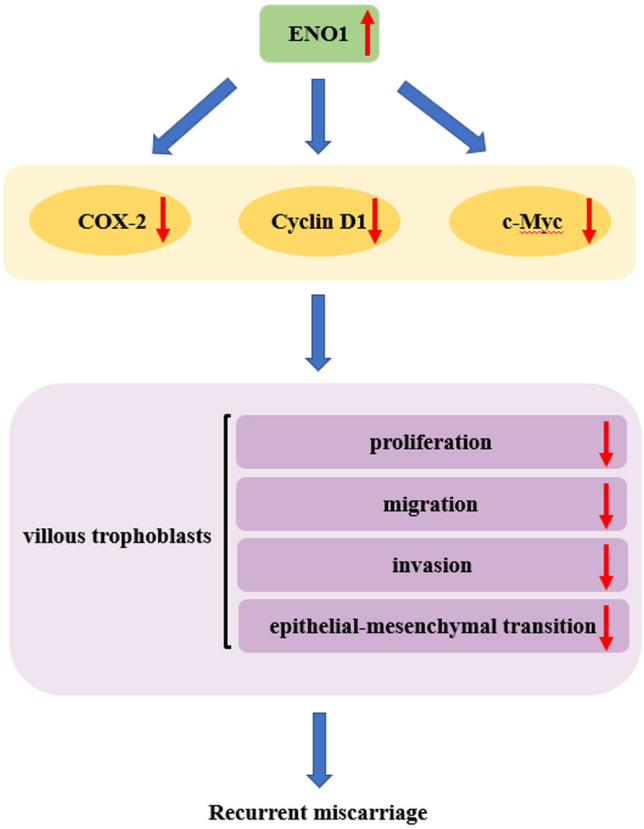

FIGURE 5 The schematic diagram illustrating the molecular mechanism involved in the regulation of ENO1 for RM development.

by promoting COX-2 expression. In addition, MBP-1 overexpression was also found to suppress the expression of c-Myc and cyclin D1, both of which can promote the growth and invasion of trophoblast cells (Hsu et al., 2009). For example, c-Myc abrogated the suppression of CRY2 overexpression on trophoblast cell migration and invasion, thereby alleviating the progression of recurrent spontaneous abortion (Wu et al., 2020). TSLP activates the STAT3 pathway to stimulate the expression of c-Myc, thereby inducing trophoblast proliferation (Pu et al., 2012). Cyclin D1, as a protein implicated in cell cycle control, determines cell proliferation and apoptosis resistance (Musgrove et al., 2011). Cyclin D1 is expressed in cytotrophoblast and EVTs in the placentas of normal pregnancy and will increase over the gestational age implying its pivotal role in regulating human placental development and functions (DeLoia et al., 1997). Reduction of cyclin D1 in preeclampsia placentas leads to the decrease in trophoblast cell proliferation and migration (Yung et al., 2008).

To be honest, there exist some limitations in our study. First, even though our results showed that ENO1 knockdown substantially increased COX-2, c-Myc and cyclin D1 expression in trophoblast Bewo cells, there lack further assays to validate that upregulation of COX-2, c-Myc, and cyclin D1 expression can improve Bewo cell proliferation and invasion. Second, our study only included in vitro results, and animal models of RM are required to be established to confirm the therapeutic effects and regulatory mechanism of ENO1 knockdown on EVT cell dysfunction in vivo.

In summary, the elevated ENO1 may result in trophoblast dysfunction and participate in the development of RM by downregulating COX-2, c-Myc, and cyclin D1 expression (Figure 5). In future, ENO1 could be a potential target of the diagnosis and treatment for RM after more and well-designed studies.

AUTHOR CONTRIBUTIONS

Huaiyun Tang and Linqing Pan designed experiment and reviewed the manuscript. Huaiyun Tang, Linqing Pan, and Jiayin Liu performed research. Lisha Tang and Jiayin Liu contributed new reagents. Huaiyun Tang and Linqing Pan analyzed data and wrote the paper.

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CONFLICT OF INTEREST STATEMENT

The authors declare they have no competing interests.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article.

ETHICS STATEMENT

This study was approved by the Ethics Committee of Lianyungang Maternal and Child Health Hospital (No. LYG-ME2017003). All individual participants signed informed consent.

CONSENT FOR PUBLICATION

The author declares that all work described here has not been published before (except in the form of an abstract or as part of a published lecture, review, or thesis) and that its publication has been approved by all co-authors.

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REFERENCES

Ali, S., Majid, S., Ali, M. N., Taing, S., Rehman, M. U., & Arafah, A. (2021). Cytokine imbalance at materno-embryonic interface as a potential immune mechanism for recurrent pregnancy loss. *International Immunopharmacology*, *90*, 107118.

Caturegli, P., De Remigis, A., & Rose, N. (2014). Hashimoto thyroiditis: Clinical and diagnostic criteria. *Autoimmunity Reviews*, *13*, 391–397.

Chen, X., Lu, Z., Chen, Y., Wu, R., Luo, Z., Lu, Q., Guan, N., & Chen, D. (2021). Deletion of the MBP1 gene, involved in the cell cycle, affects respiration and pseudohyphal differentiation in *Saccharomyces cerevisiae*. *Microbiology Spectrum*, *9*(1), e0008821.

Dai, Y., Qiu, Z., Diao, Z., Shen, L., Xue, P., Sun, H., & Hu, Y. (2012). MicroRNA-155 inhibits proliferation and migration of human extravillous trophoblast derived HTR-8/SVneo cells via down-regulating cyclin D1. *Placenta*, *33*(10), 824–829.

DeLoia, J., Burlingame, J., & Krasnow, J. (1997). Differential expression of G1 cyclins during human placentogenesis. *Placenta*, *18*(1), 9–16.

Ding, J., Cheng, Y., Zhang, Y., Liao, S., Yin, T., & Yang, J. (2019). The miR-27a-3p/USP25 axis participates in the pathogenesis of recurrent miscarriage by inhibiting trophoblast migration and invasion. *Journal of Cellular Physiology*, *234*(11), 19951–19963.

du Fossé, N. A., van der Hoorn, M. P., de Koning, R., Mulders, A. G. M. G. J., van Lith, J. M. M., le Cessie, S., & Lashley, E. E. L. O. (2021). Toward more accurate prediction of future pregnancy outcome in couples with unexplained recurrent pregnancy loss: Taking both partners into account. *Fertility and Sterility*, *117*, 144–152.

Egerup, P., Kolte, A. M., Larsen, E. C., Krog, M., Nielsen, H. S., & Christiansen, O. B. (2016). Recurrent pregnancy loss: What is the impact of consecutive versus non-consecutive losses? *Human Reproduction (Oxford, England)*, *31*(11), 2428–2434.

Gou, Y., Li, F., Huo, X., Hao, C., Yang, X., Pei, Y., Li, N., Liu, H., & Zhu, B. (2021). ENO1 monoclonal antibody inhibits invasion, proliferation and clone formation of cervical cancer cells. *American Journal of Cancer Research*, *11*(5), 1946–1961.

Hsiao, K. C., Shih, N. Y., Fang, H. L., Huang, T. S., Kuo, C. C., Chu, P. Y., Hung, Y. M., Chou, S. W., Yang, Y. Y., Chang, G. C., & Liu, K. J. (2013). Surface α-enolase promotes extracellular matrix degradation and tumor metastasis and represents a new therapeutic target. *PLoS ONE*, *8*(7), e69354.

Hsu, K. W., Hsieh, R. H., Wu, C. W., Chi, C. W., Lee, Y. H., Kuo, M. L., Wu, K. J., & Yeh, T. S. (2009). MBP-1 suppresses growth and metastasis of gastric cancer cells through COX-2. *Molecular Biology of the Cell*, *20*(24), 5127–5137.

Koert, E., Malling, G. M. H., Sylvest, R., Krog, M. C., Kolte, A. M., Schmidt, L., & Nielsen, H. S. (2019). Recurrent pregnancy loss: Couples' perspectives on their need for treatment, support and follow up. *Human Reproduction (Oxford, England)*, *34*(2), 291–296.

Li, H. J., Ke, F. Y., Lin, C. C., Lu, M. Y., Kuo, Y. H., Wang, Y. P., Liang, K. H., Lin, S. C., Chang, Y. H., Chen, H. Y., Yang, P. C., & Wu, H. C. (2021). ENO1 promotes lung cancer metastasis via HGFR and WNT signaling-driven epithelial-mesenchymal transition. *Cancer Research*, *81*, 4094–4109.

Lim, H., Paria, B. C., das, S. K., Dinchuk, J. E., Langenbach, R., Trzaskos, J. M., & Dey, S. K. (1997). Multiple female reproductive failures in cyclooxygenase 2-deficient mice. *Cell*, *91*(2), 197–208.

Mitchell, J., & Warner, T. (1999). Cyclo-oxygenase-2: Pharmacology, physiology, biochemistry and relevance to NSAID therapy. *British Journal of Pharmacology*, *128*(6), 1121–1132.

Mullen, C. (1998). Review: Analogies between trophoblastic and malignant cells. *American Journal of Reproductive Immunology*, *39*(1), 41–49.

Musgrove, E., Caldon, C. E., Barraclough, J., Stone, A., & Sutherland, R. L. (2011). Cyclin D as a therapeutic target in cancer. *Nature Reviews. Cancer*, *11*(8), 558–572.

Pancholi, V., & Fischetti, V. (1998). Alpha-enolase, a novel strong plasmin(ogen) binding protein on the surface of pathogenic streptococci. *The Journal of Biological Chemistry*, *273*(23), 14503–14515.

Practice Committee of the American Society for Reproductive Medicine. (2020). Definitions of infertility and recurrent pregnancy loss: A committee opinion. *Fertility and Sterility*, *113*(3), 533–535.

Principe, M., Borgoni, S., Cascione, M., Chattaragada, M. S., Ferri-Borgogno, S., Capello, M., Bulfamante, S., Chapelle, J., di Modugno, F., Defilippi, P., Nisticò, P., Cappello, P., Riganti,

C., Leporatti, S., & Novelli, F. (2017). Alpha-enolase (ENO1) controls alpha v/beta 3 integrin expression and regulates pancreatic cancer adhesion, invasion, and metastasis. *Journal of Hematology & Oncology*, *10*(1), 16.

Pu, H., Duan, J., Wang, Y., Fan, D. X., Li, D. J., & Jin, L. P. (2012). Thymic stromal lymphopoietin promotes the proliferation of human trophoblasts via phosphorylated STAT3-mediated c-Myc upregulation. *Placenta*, *33*(5), 387–391.

Sela, H. Y., Goldman-Wohl, D. S., Haimov-Kochman, R., Greenfield, C., Natanson-Yaron, S., Hamani, Y., Revel, A., Lavy, Y., Singer, O., Yachimovich-Cohen, N., Turetsky, T., Mandelboim, O., Reubinoff, B., & Yagel, S. (2013). Human trophectoderm apposition is regulated by interferon γ-induced protein 10 (IP-10) during early implantation. *Placenta*, *34*(3), 222–230.

Sun, M., Chen, H., Liu, J., Tong, C., & Meng, T. (2015). MicroRNA-34a inhibits human trophoblast cell invasion by targeting MYC. *BMC Cell Biology*, *16*, 21.

Tsujii, M., Kawano, S., Tsuji, S., Sawaoka, H., Hori, M., & DuBois, R. N. (1998). Cyclooxygenase regulates angiogenesis induced by colon cancer cells. *Cell*, *93*(5), 705–716.

Wang, D., Fu, Y., Fan, J., Wang, Y., Li, C., Xu, Y., Chen, H., Hu, Y., Cao, H., Zhao, R. C., He, W., & Zhang, J. (2021). Identification of alpha-enolase as a potential immunogenic molecule during allogeneic transplantation of human adipose-derived mesenchymal stromal cells. *Cytotherapy*, *24*(4), 393–404.

Wu, K. K., Liou, J., & Cieslik, K. (2005). Transcriptional control of COX-2 via C/EBPbeta. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *25*(4), 679–685.

Wu, L., Cheng, B., Liu, Q., Jiang, P., & Yang, J. (2020). CRY2 suppresses trophoblast migration and invasion in recurrent spontaneous abortion. *Journal of Biochemistry*, *167*(1), 79–87.

Ye, Y., Kuhn, C., Kösters, M., Arnold, G. J., Ishikawa-Ankerhold, H., Schulz, C., Rogenhofer, N., Thaler, C. J., Mahner, S., Fröhlich, T., Jeschke, U., & von Schönfeldt, V. (2019). Anti α-enolase antibody is a novel autoimmune biomarker for unexplained recurrent miscarriages. *eBioMedicine*, *41*, 610–622.

Yung, H., Calabrese, S., Hynx, D., Hemmings, B. A., Cetin, I., Charnock-Jones, D. S., & Burton, G. J. (2008). Evidence of placental translation inhibition and endoplasmic reticulum stress in the etiology of human intrauterine growth restriction. *The American Journal of Pathology*, *173*(2), 451–462.

Zhang, D., Chang, X., Bai, J., Chen, Z. J., Li, W. P., & Zhang, C. (2016). The study of cyclooxygenase 2 in human decidua of preeclampsia. *Biology of Reproduction*, *95*(3), 56.

Zhang, Q., Yu, S., Huang, X., Tan, Y., Zhu, C., Wang, Y. L., Wang, H., Lin, H. Y., Fu, J., & Wang, H. (2015). New insights into the function of Cullin 3 in trophoblast invasion and migration. *Reproduction*, *150*(2), 139–149.

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