



Potential role of microRNA-424 in regulating ERR γ to suppress trophoblast proliferation and invasion in fetal growth restriction



Zhiyong Zou, Zhiming He, Jian Cai, Linhuan Huang, Hui Zhu, Yanmin Luo*

Department of Obstetrics & Gynecology, The First Affiliated Hospital of Sun Yat-Sen University, 510080, Guangzhou, People's Republic of China

ARTICLE INFO

Keywords:

Fetal growth restriction
Placenta
miR-424
ERR γ
HSD17B1

ABSTRACT

Background: Abnormal expression of estrogen-related receptor γ (ERR γ) protein is associated with fetal growth restriction (FGR). The upstream regulators of ERR γ are still unknown.

Objective: To evaluate the placental expression level of microRNA-424 (miR-424) and to demonstrate the relationship between miR-424 and FGR.

Methods: The expression levels of miR-424 were detected in FGR and control placentas. HTR-8/SVneo cells were transfected with mimics or inhibitors to increase or decrease the miR-424 expression level, respectively. The transwell and CCK-8 assays were used to determine trophoblast-derived cell line invasion and proliferation. The expression levels of miR-424, ERR γ , and 17 beta-hydroxysteroid dehydrogenase type 1 (HSD17B1) were detected by qRT-PCR and Western blotting. The relationship between miR-424, ERR γ , and HSD17B1 was determined by luciferase reporter assay.

Results: Compared to the normal pregnancy group, FGR placental tissues showed a significantly higher expression level of miR-424. The up-regulation of miR-424 decreased trophoblast-derived cell line invasion and proliferation. Down-regulation of miR-424 enhanced invasive and proliferative abilities of the cell lines. Over-expression of miR-424 reduced ERR γ protein levels and decreased both mRNA and protein levels of HSD17B1. Thus down-regulation of miR-424 induced protein expression of ERR γ and enhanced the mRNA and protein expressions of HSD17B1. MiR-424 probably mediated the expression of ERR γ via binding to sites other than mRNA 3'UTR.

Conclusion: MiR-424 may be associated with the pathogenesis of FGR by modulating trophoblast-derived cell line proliferation and invasion. MiR-424 may play a role in mediating the protein expressions of ERR γ and HSD17B1.

1. Introduction

Fetal growth restriction (FGR) is defined as the failure of the foetus to achieve its optimal growth potential due to various reasons [1]. An estimated 3%–10% of all pregnancies are affected by FGR. Children affected by FGR are at a relatively high risk of complications during infancy; in addition, these children are vulnerable to developmental disorders owing to neurological, cardiovascular, and metabolic disorders [1–4]. FGR may be caused by fetal, maternal, and/or placental factors [5]. Although several studies have investigated the causes of FGR, the etiopathogenesis of FGR still remains unclear. Till date there are no effective preventive or curative measures against FGR, other than expectant management or delivery. Investigating the pathogenesis of FGR is necessary to improve management of this condition.

Estrogen-related receptor γ (ERR γ), is an orphan nuclear receptor that belongs to the ERR/NR3 subfamily. It plays a key role in mitochondrial function and biogenesis [6]. ERR γ is highly expressed in human placenta and is significantly upregulated during differentiation of human trophoblasts [7]. 17 beta-hydroxysteroid dehydrogenase type 1 (HSD17B1) (a human steroid converting enzyme) is a member of 17 beta-HSD family; in a previous study, HSD17B1 was shown to be a predictor of preeclampsia (PE) [8]. In our previous study, ERR γ was found to regulate the expression of HSD17B1; therefore, it may be associated with the pathogenesis and development of FGR [9]. However, the upstream regulator of ERR γ is yet to be identified. Recent studies have revealed that microRNA-320a may regulate the trophoblast function by targeting ERR γ [10,11]. Thus microRNA is a potential upstream regulator of ERR γ that may play a role in the pathogenesis of

* Corresponding author. Department of Obstetrics & Gynecology, the First Affiliated Hospital of Sun Yat-Sen University, 58 Zhong Shan Er Road, Guangzhou, 510080, People's Republic of China.

E-mail address: luoyanm@mail.sysu.edu.cn (Y. Luo).

<https://doi.org/10.1016/j.placenta.2019.07.001>

Received 22 March 2019; Received in revised form 5 June 2019; Accepted 1 July 2019

0143-4004/© 2019 Elsevier Ltd. All rights reserved.

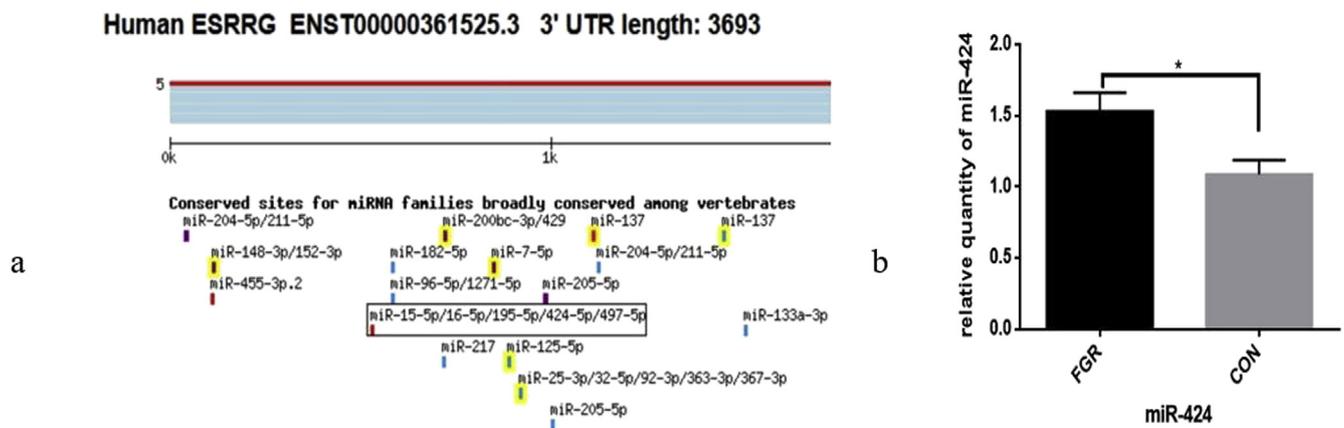


Fig. 1. The predicted miRNAs and the human placental expression of miR-424. Fig. 1a shows miR-424 as one of the upstream regulators of ERR γ . Fig. 1b shows the miR-424 expression levels in placental tissues in the FGR and control groups. Data presented as mean \pm standard error of the mean. * $P < 0.05$. miR-424, microRNA-424; FGR, fetal growth restriction; CON, control group.

FGR.

MicroRNAs (miRNAs/miR), are small single stranded non-coding RNAs (length: 19–25 nucleotides) that can regulate the downstream gene expression via RNA silencing and post-transcriptional regulation [12]. Recent studies have implicated several miRNAs in the causation of placental dysfunction; these include placenta specific miRNAs chromosome 19 microRNA cluster (C19MC), microRNA-210, and microRNA-17-92 [13–17]. Further, abnormal expression levels of these miRNAs may result in several complications during pregnancy such as PE [18], FGR, and gestational diabetes mellitus (GDM) [15,19–21]. Use of several predictive tools, such as TargetScan human7.1 (http://www.targetscan.org/vert_71), miRanda (<http://www.microrna.org/>) and Pictar (<http://pictar.mdc-berlin.de/>) revealed that miR-424 might be one of upstream regulators of ERR γ (Fig. 1a), which suggest miR-424 might regulate the expression of ERR γ and play a role in the development of FGR.

The present study aimed to investigate the potential involvement of miR-424 and ERR γ expressions in trophoblast-derived cell line invasion and proliferation and to assess their precise role in the pathogenesis of FGR. Our findings may provide some evidence to support the important role of miR-424 in the development of FGR and may have implications for the prevention and treatment of FGR.

2. Methods

2.1. Participants and placental tissue collection

Human placental tissues were collected from vaginal or cesarean section deliveries conducted at the First Affiliated Hospital of Sun Yat-Sen University (Guangzhou, Guangdong, China) between January 2016 and December 2017. FGR was defined as estimated fetal weight (EFW) below the 10th percentile for gestational age [22], which was according to the Chinese population references, [23,24] and the formula was developed by Hadlock C [25]. Fetal Doppler abnormalities included reduced, absent or reversed umbilical end-diastolic flow Doppler; or increased middle cerebral artery end-diastolic flow or cerebral distribution; or reduced, absent or reverse flow in the ductus venosus. The method of Doppler examination about umbilical artery (UA) pulsatility index (PI), middle cerebral artery (MCA) and uterine artery (UtA) evaluation was according to the reference. [26,27].

The exclusion criteria for samples were as follows: intrauterine infection, multiple-gestation, pregnancy complicated by pre-existing diseases (including chronic nephritis, cardiovascular, hepatic, or autoimmune diseases), pregnancy complicated by systemic maternal diseases (such as PE and GDM), and fetuses born with congenital abnormalities and aneuploidies.

Based on the presence or absence of FGR, the placentas were categorized into the FGR or control groups, respectively. A total of 39 placentas were collected which included 19 FGR and 20 control cases. The gestational age of foetuses was comparable in the 2 groups. All placental samples were collected within 30 min of delivery and were frozen and stored at -80°C until further processing.

2.2. Cell culture and transfection

The HTR-8/SV neo cell line was purchased (Jennio Biotech Co. Ltd., Guangzhou, China) and the cells were cultured in RPMI 1640 (Gibco, Inc.) medium supplemented with 10% fetal bovine serum (FBS) (Sijiqin, China) without antibiotics for 24 h. MiR-424 mimic (Ribo Inc.), miR-424 inhibitor (*anti*-miR-424; Ribo Inc.), and the negative control miRNA (Ribo Inc., Guangzhou, China) were subsequently transfected into cells, which were seeded onto a 6-well plate (1×10^4 cells/well). The final concentration of cell transfection riboFECTTM CP Reagent (Ribo Inc., Guangzhou, China) was 50 nM and transfection was performed according to the manufacturer's protocol. Cells were further cultured for cell proliferation and transwell assays and were subsequently used for qRT-PCR and Western blot analysis 48 h after transfection. All experiments were conducted in triplicate.

2.3. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from placenta samples and cell lines using kits according to the manufacturer's instructions. The PrimeScript RT Master Mix and SYBR PrimeScript miRNA RT-PCR kits (Takara, Japan) were used for reverse transcription of mRNA and miRNA, respectively. SYBR Premix Ex Taq were used for quantitative real-time PCR using the Applied Biosystems 7500. RPLP0 and U6 were used as internal controls to normalize the relative expression levels of the target genes and miRNAs, respectively (Supplementary Table 1). And it has no expression difference of RPLP0 and U6 in both FGR and control group.

2.4. Western blot

Total protein was extracted from the cells by using radio-immunoprecipitation (RIPA) assay lysis buffer (Beyotime Institute of Biotechnology) and Protease Inhibitor Cocktail (Sigma Aldrich; Merck KgaA). The BCA assay Kit (Beyotime Institute of Biotechnology) was used to detect the concentration of extracted protein. A total of 30 μg was loaded onto each lane and separated using 10% SDS-PAGE and blotted onto polyvinylidene difluoride membranes (Millipore, Biorad). The membrane was subsequently blocked in 5% BSA for 1 h at RT and

incubated overnight at 4 °C with the primary antibodies, ERR γ (0.004 mg/ml; ab82319, Abcam), HSD17B1 (0.000133 mg/ml; ab51045, Abcam), and GAPDH (0.0001 mg/ml; Proteintech Group Inc., China). Following incubation with horseradish peroxidase-conjugated anti-rabbit (0.0006 mg/ml; Proteintech Group Inc., China) secondary antibodies at RT for 1 h, antibody binding signals were detected (Bio-Rad Laboratories Inc., Hercules, CA, USA). The protein bands were subsequently visualized with ImageJ software (version 1.49) (NIH, Bethesda, MD, USA). GAPDH was used to normalize the band intensities.

2.5. Proliferation assay

For the cell proliferation assay, the transfected HTR-8/SV neo cells were seeded into 96-well plates (3×10^4 cells/well). Cells were cultivated in RPMI 1640 medium for 3 days. 10 μ L CCK-8 reagent (Dojindo; Japan) was subsequently added to each well and HTR-8/SVneo cells were incubated for 2 h.

2.6. Transwell invasion assay

Cell invasion was assessed using HTR-8/SVneo cells cultured in transwell chambers that were pre-coated with Matrigel. Forty-eight hours after transfection, the cells were seeded into the upper chambers containing medium without serum. The lower chambers were filled with RPMI-1640 medium with 10% FBS. The plates were incubated for 48 h, following which the cells from the serum-free chambers were removed gently and fixed in methanol. Using light microscope, four fields were randomly selected for counting the cell number and imaging.

2.7. Dual-luciferase reporter assay

We used several computational algorithms, including TargetScan human7.1 (http://www.targetscan.org/vert_71), miRanda (<http://www.microrna.org/>) and Pictar (<http://pictar.mdc-berlin.de/>), to predict the upstream regulator microRNA of ERR γ . MiR-424 was selected as a candidate for further evaluation (Fig. 1a). To examine whether ERR γ is directly inhibited by miR-424, its full-length 3'-UTR was introduced into the pGL3-Control luciferase vector (Supplementary Fig. 1).

The 3'-untranslated region (UTR) sequence of ERR γ was amplified and inserted into the luciferase reporter vector T7-enhancer (Promega Corporation, Madison, WI, USA) Supplementary Fig.1.

The primers for wild type ERR γ 3'-UTR were:

5'-GGCGCTCGAGTTAGCAGCAAGACTATGAAG-3'(forward);
5'-AATGCGGCCGCTAACAAATACCTGGAAGAC-3'(reverse);

The primers for mutated ERR γ 3'-UTR were:

5'-CTTCCTTCACGACGAGAAGCTTTTAAAGAGGTC-3'(forward);
5'-AAAAGTTCTCGTCTGAAGGAAGAAAAGAAAG-3'(reverse).

HTR-8/SVneo cells were seeded on to 24-well plates (5×10^5 cells/well) and incubated for 24 h. Wild-type or mutant ERR γ 3'-UTR vectors were subsequently co-transfected with miR-424 mimic into HTR-8/SVneo cells using Lipofectamine 3000 (Invitrogen; Thermo Fisher Scientific Inc.). After incubation for 48 h, cells were lysed and assayed for luciferase activity with the luciferase assay system (Promega Corporation) according to the manufacturer's instructions. Renilla-luciferase was used for normalization.

2.8. Statistical analysis

SPSS software v13.0 (SPSS LP, College Station, TX, USA) was used

for data analysis. Between-group differences were assessed using the Student's *t*-test. The results are shown as mean \pm standard error. $P < 0.05$ was considered indicative of a statistically significant difference.

3. Results

3.1. Clinical characteristics and miR-424 expression level

The clinical characteristics of the two groups are listed in Supplementary Table 2. No significant between-group difference was observed with respect to the gestational age (weeks), maternal age, or cesarean rate. However, the birth weight and placental weight in the FGR group was significantly lower than that in the control group (2260 ± 60.48 vs. 3240 ± 50.55 g, respectively; $P < 0.001$). In addition, compared with control group, the rate of abnormal Doppler result is higher in the FGR group ($P < 0.001$). MiR-424 expression was measured in all 39 placental tissue samples using qRT-PCR; the expression level in FGR placental tissues was significantly greater than that in the control group (Fig. 1b; $P < 0.05$).

3.2. MiR-424 affects the proliferation and invasion of HTR-8/SVneo cells

The transfection efficiency of miR-424 in HTR-8/SVneo cells is shown in Fig. 2a and b. After transfection with miR-424 mimics, the expression of miR-424 in trophoblasts was significantly increased as compared to that in negative control and blank control groups (Fig. 2a, $P < 0.001$). However, the expression of miR-424 was decreased after transfection with miR-424 inhibitors (Fig. 2b, $P < 0.001$). The effect of miR-424 expression on the proliferation and invasive function of HTR-8/SVneo cells is shown in Fig. 2c–j. Overexpression of miR-424 in trophoblast-derived cell line was associated with a decrease in both the proliferative and invasive ability of HTR-8/SVneo cells (Fig. 2c–f). However, these properties of HTR-8/SVneo cells were significantly enhanced after inhibition of the expression of miR-424 (Fig. 2g–j). These results indicated that miR-424 may participate in regulating the function of trophoblast-derived cell line *in vitro*.

3.3. ERR γ may be the downstream effector of miR-424 in HTR-8/SVneo cells

We found that transfection of HTR-8/SVneo cells with miR-424 mimic led to a significant reduction in the protein expressions of both ERR γ and HSD17B1. The protein expressions of both ERR γ and HSD17B1 in the miR-424 inhibitor transfected group were significantly upregulated in comparison to those in the control group (Fig. 3a–f). However, overexpression of miR-424 did not significantly suppress the luciferase activity (Fig. 3g). In addition, inhibiting the endogenous miR-424 by co-transfection with miR-424 inhibitor did not significantly increase the luciferase reporter activity (Fig. 3h). The results indicated that miR-424 may mediate the expression of ERR γ via domains other than 3'-UTR.

4. Discussion

Our study indicates that increased expression of miR-424 may be associated with FGR. Further miR-424 may play a role in regulating the proliferation and invasion of the trophoblast-derived cell line, which might be mediated via the ERR γ pathway.

4.1. Dysfunction of placental miR-424 is associated with FGR

Placental hypoxia is related to the dysfunction of trophoblasts and can result in inadequate placentation during the first trimester; in addition, placental hypoxia is implicated in many complications, such as PE and FGR [28,29]. Placental hypoxia results in altered expression of

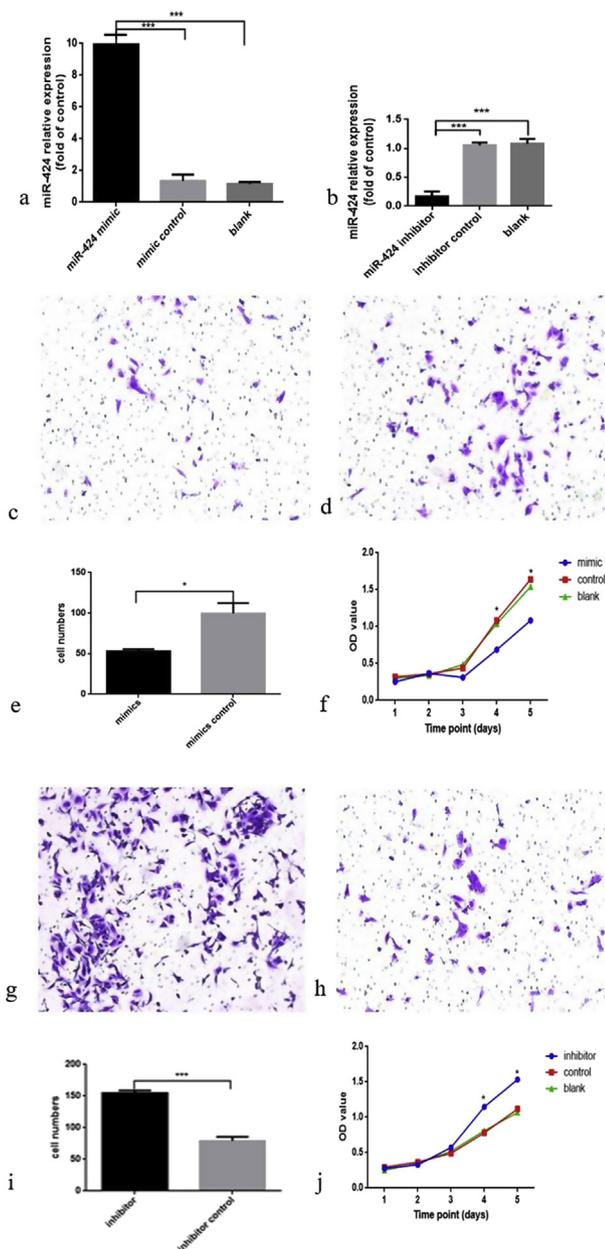


Fig. 2. The relationship between miR-424 and trophoblast proliferation and invasion. Fig. 2a and b shows the transfection efficiency of miR-424 in HTR-8/SVneo cells. Fig. 2c and d shows that overexpression of miR-424 inhibited the trophoblast invasion, and Fig. 2e is the quantification of Fig. 2c and d. Fig. 2g and h shows the enhanced invasion ability of cells after down-regulation of miR-424, and Fig. 2i is the quantification of Fig. 2g and h. Fig. 2f and j shows the proliferation ability of cells after overexpression or down-regulation of miR-424. Mean (\pm standard error) values from three independent experiments are presented. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control. miR-424, microRNA-424.

several hypoxia induced miRNAs including miR-424 [30]. A recent study indicated that miR-424 may play a role in placental hypoperfusion; the expression of miR-424 in choriocarcinoma and endothelial cell lines was enhanced in hypoxic environment [30]. In a study by Whitehead et al. [31], fetal hypoxia was associated with a 2.7-fold increased expression of miR-424 in the maternal blood. In addition, FGR was associated with a 3.6-fold increase in the expression of miR-424 in the maternal blood. Huang et al. [21] also found increased expression levels of miR-424 in FGR placenta, which could bind to mitogen-activated protein kinase 1 (MEK1).

Our results also revealed increased miR-424 expression in FGR placenta as compared to that in the gestational age-matched control group. This further underlines the relationship between high expression of miR-424 and the development of FGR. Moreover, previous studies have shown an association between miR-424 expression and carcinogenesis, which is mediated via the proliferation and invasion of cancer cells [32–34]. This suggests a potential effect of miR-424 on the function of trophoblasts. In our study, overexpression of miR-424 reduced trophoblast-derived cell line invasion and proliferation; however, inhibition of miR-424 in trophoblast-derived cell line attenuated this effect. These findings indicate that high expression level of miR-424 is related to the development of FGR by regulating the trophoblast-derived cell line proliferation and invasion.

4.2. The potential relationship between miR-424 and $ERR\gamma$

In this study, we found that miR-424 may influence the protein expression of $ERR\gamma$ and indirectly mediate the expression of HSD17B1. This indicates a potential regulatory relationship between miR-424 and $ERR\gamma$. However, the luciferase reporter assay indicated a weak possibility of combination between the $ERR\gamma$ 3'UTR and miR-424. This result does not exclude the possibility of binding sites in another area of $ERR\gamma$ (other than 3'UTR). We found no significant alteration in the mRNA expression of $ERR\gamma$; the changes in protein expression suggest miRNA post-translational regulation.

The regulating mechanism of the miRNAs is complex. MiRNAs typically bind to the RNA 3'UTR site to regulate the protein expression of genes [35]. However, several studies have shown that miRNAs can combine with other domains including the RNA 5'UTR or open reading frames (ORFs), to alter translation of genes [36–42]. In cancer cells, some miRNAs have been shown to bind to both 3'UTR and 5'UTR for post-translational inhibition of the expression of major histocompatibility complex class I (MHC-1) chain related protein B (MICB) [38]. In a study by Lee et al. [36], primate-specific miRNA, miR-605, was shown to bind to 5'UTR of the target mRNA to mediate the change in the protein expression. Moretti et al. [37] demonstrated that miR-2 can bind to 3'UTR, 5'UTR, and ORFS; binding to these three sites was shown to have a similar inhibitory effect on translation. These studies indicate that miRNAs can also bind to mRNA 5'UTR or other domains, in addition to 3'UTR.

Previous studies have also found this miRNA regulatory mechanism without concomitant changes at the mRNA level; according to the authors, the changes in protein level probably affect ribosomal binding [43,44]. Nonetheless, miR-424 may have a complicated regulatory mechanism and more studies are required to further investigate the mechanisms that mediate the changes in $ERR\gamma$.

4.3. Limitations

The present study has several limitations. First, we conducted studies only on the trophoblast cell lines and third-trimester placenta. In the future, we need to perform experiments on primary trophoblast cells and detect the expression level of miR-424, $ERR\gamma$, and HSD17B1 in the first trimester placenta. Although the specific expression level of miR-424 in the early trimester placenta is unknown, future studies could investigate if the over-expression of miR-424 in first-trimester may lead to the extra-villous dysfunction to cause FGR. Second, we only detected one $ERR\gamma$ 3'UTR loci by luciferase report assay; further studies on other 3'UTR or other domains including 5'UTR area are necessary. In addition, larger and high-throughput analyses of plasma miRNAs are required prior to clinical application of these and similar discoveries. Future studies are required to investigate the mechanisms by which upregulation of miRNA-424 affects the expression levels of $ERR\gamma$ and HSD17B1 in FGR placenta.

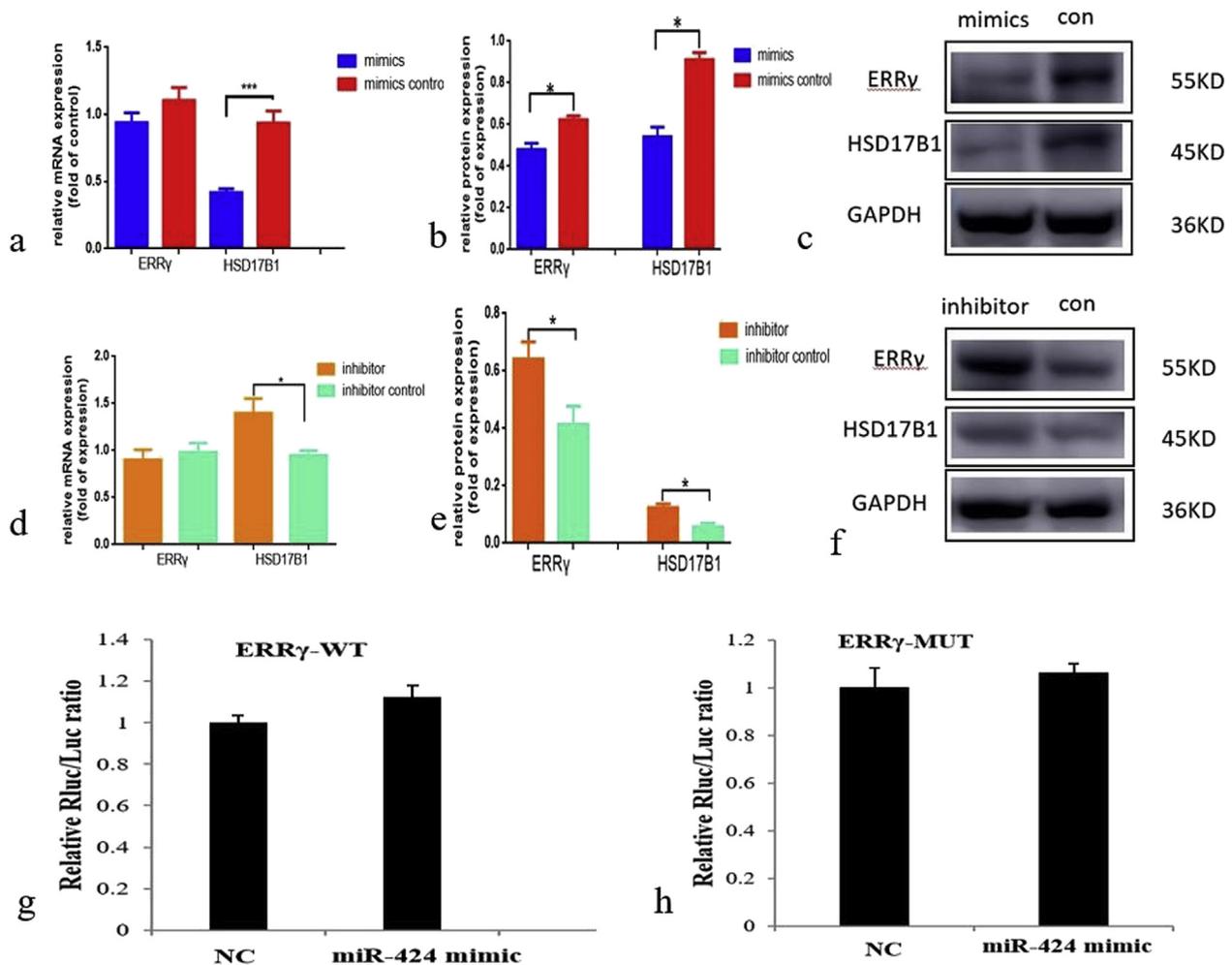


Fig. 3. The probable regulation among miR-424 and ERR γ . Fig. 3a–c shows that induction of miR-424 expression in trophoblasts decreased the protein level of ERR γ and HSD17B1 without any significant change in ERR γ mRNA expression. Fig. 3d–f shows that the protein levels of ERR γ and HSD17B1 were increased after reducing miR-424 expression. Fig. 3g and h indicate the weak combination between ERR γ mRNA 3'UTR and miR-424. Mean (\pm standard error) values from three independent experiments are presented. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. ERR γ , Estrogen-related receptor γ ; HSD17B1, 17 beta-hydroxysteroid dehydrogenase type 1; miR-424, microRNA-424.

5. Conclusion

In conclusion, miR-424 may be associated with the pathogenesis of FGR by modulating trophoblast proliferation and invasion. MiR-424 may play a role in mediating the protein expressions of ERR γ and HSD17B1.

Conflicts of interest

None declared.

Funding source

This work was supported by the National Natural Science Foundation of China (No. 81741015).

Acknowledgements

We express our gratitude for the kind help of Prof. Qun Fang from The First Affiliated Hospital of Sun Yat-Sen University for providing the HTR-8/SVneo cell line.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.placenta.2019.07.001>.

References

- [1] L.M. Nardoza, A.C. Caetano, A.C. Zamarian, J.B. Mazzola, C.P. Silva, V.M. Marcal, T.F. Lobo, A.B. Peixoto, E. Araujo Junior, Fetal growth restriction: current knowledge, Arch. Gynecol. Obstet. 295 (2017) 1061–1077 <http://doi.org/10.1007/s00404-017-4341-9>.
- [2] D.J. Barker, Fetal growth and adult disease, Br. J. Obstet. Gynaecol. 99 (1992) 275–276 <http://doi.org/10.1111/j.1471-0528.1992.tb13719.x>.
- [3] D.J. Barker, A.R. Bull, C. Osmond, S.J. Simmonds, Fetal and placental size and risk of hypertension in adult life, Bmj 301 (1990) 259–262 <http://doi.org/10.1136/bmj.301.6746.259>.
- [4] D.J. Barker, P.D. Gluckman, K.M. Godfrey, J.E. Harding, J.A. Owens, J.S. Robinson, Fetal nutrition and cardiovascular disease in adult life, Lancet 341 (1993) 938–941 [http://doi.org/10.1016/0140-6736\(93\)91224-A](http://doi.org/10.1016/0140-6736(93)91224-A).
- [5] M.G. Neerhof, Causes of intrauterine growth restriction, Clin. Perinatol. 22 (1995) 375–385 [http://doi.org/10.1016/S0095-5108\(18\)30289-6](http://doi.org/10.1016/S0095-5108(18)30289-6).
- [6] D.J. Heard, P.L. Norby, J. Holloway, H. Vissing, Human ERRgamma, a third member of the estrogen receptor-related receptor (ERR) subfamily of orphan nuclear receptors: tissue-specific isoforms are expressed during development and in the adult, Mol. Endocrinol. 14 (2000) 382–392 <http://doi.org/10.1210/mend.14.3.0431>.
- [7] Y. Takeda, X. Liu, M. Sumiyoshi, A. Matsushima, M. Shimohigashi, Y. Shimohigashi, Placenta expressing the greatest quantity of bisphenol A receptor ERR{gamma} among the human reproductive tissues: predominant expression of type-1

- ERRgamma isoform, *J. Biochem.* 146 (2009) 113–122 <http://doi.org/10.1093/jb/mvp049>.
- [8] A. Ohkuchi, O. Ishibashi, C. Hirashima, K. Takahashi, S. Matsubara, T. Takizawa, M. Suzuki, Plasma level of hydroxysteroid (17-beta) dehydrogenase 1 in the second trimester is an independent risk factor for predicting preeclampsia after adjusting for the effects of mean blood pressure, bilateral notching and plasma level of soluble fms-like tyrosine kinase 1/placental growth factor ratio, *Hypertension research*, *Off. J. Jpn. Soc. Hypertens.* 35 (2012) 1152–1158 <http://doi.org/10.1038/hr.2012.109>.
- [9] H. Zhu, L. Huang, Z. He, Z. Zou, Y. Luo, Estrogen-related receptor gamma regulates expression of 17beta-hydroxysteroid dehydrogenase type 1 in fetal growth restriction, *Placenta* 67 (2018) 38–44 <http://doi.org/10.1016/j.placenta.2018.05.012>.
- [10] T. Gao, M. Deng, Q. Wang, MiRNA-320a inhibits trophoblast cell invasion by targeting estrogen-related receptor-gamma, *J. Obstet. Gynaecol. Res.* 44 (2018) 756–763 <http://doi.org/10.1111/jog.13560>.
- [11] R.H. Liu, Q. Meng, Y.P. Shi, H.S. Xu, Regulatory role of microRNA-320a in the proliferation, migration, invasion, and apoptosis of trophoblasts and endothelial cells by targeting estrogen-related receptor gamma, *J. Cell. Physiol.* 234 (2018) 682–691 <http://doi.org/10.1002/jcp.26842>.
- [12] M. Selbach, B. Schwanhauser, N. Thierfelder, Z. Fang, R. Khanin, N. Rajewsky, Widespread changes in protein synthesis induced by microRNAs, *Nature* 455 (2008) 58–63 <http://doi.org/10.1038/nature07228>.
- [13] L. Xie, J.F. Mouillet, T. Chu, W.T. Parks, E. Sadovsky, M. Knofler, Y. Sadovsky, C19MC microRNAs regulate the migration of human trophoblasts, *Endocrinology* 155 (2014) 4975–4985 <http://doi.org/10.1210/en.2014-1501>.
- [14] R.B. Donker, J.F. Mouillet, T. Chu, C.A. Hubel, D.B. Stolz, A.E. Morelli, Y. Sadovsky, The expression profile of C19MC microRNAs in primary human trophoblast cells and exosomes, *Mol. Hum. Reprod.* 18 (2012) 417–424 <http://doi.org/10.1093/molehr/gas013>.
- [15] R. Luo, X. Shao, P. Xu, Y. Liu, Y. Wang, Y. Zhao, M. Liu, L. Ji, Y.X. Li, C. Chang, J. Qiao, C. Peng, Y.L. Wang, MicroRNA-210 contributes to preeclampsia by downregulating potassium channel modulatory factor 1, *Hypertension* 64 (2014) 839–845 <http://doi.org/10.1161/HYPERTENSIONAHA.114.03530>.
- [16] P. Kumar, Y. Luo, C. Tudela, J.M. Alexander, C.R. Mendelson, The c-Myc-regulated microRNA-17–92 (miR-17–92) and miR-106a363 clusters target hCYP19A1 and hGCM1 to inhibit human trophoblast differentiation, *Mol. Cell. Biol.* 33 (2013) 1782–1796 <http://doi.org/10.1128/MCB.01228-12>.
- [17] G. Fu, G. Ye, L. Nadeem, L. Ji, T. Manchanda, Y. Wang, Y. Zhao, J. Qiao, Y.L. Wang, S. Lye, B.B. Yang, C. Peng, MicroRNA-376c impairs transforming growth factor-beta and nodal signaling to promote trophoblast cell proliferation and invasion, *Hypertension* 61 (2013) 864–872 <http://doi.org/10.1161/HYPERTENSIONAHA.111.203489>.
- [18] L. Li, S. Wang, H. Li, J. Wan, Q. Zhou, Y. Zhou, C. Zhang, microRNA-96 protects pancreatic beta-cell function by targeting PAK1 in gestational diabetes mellitus, *Biofactors* 44 (2018) 539–547 <http://doi.org/10.1002/biof.1461>.
- [19] L. Anton, A.O. Olarerin-George, N. Schwartz, S. Srinivas, J. Basteck, J.B. Hogensch, M.A. Elovitz, miR-210 inhibits trophoblast invasion and is a serum biomarker for preeclampsia, *Am. J. Pathol.* 183 (2013) 1437–1445 <http://doi.org/10.1016/j.ajpath.2013.07.021>.
- [20] Y. Gao, R. She, Q. Wang, Y. Li, H. Zhang, Up-regulation of miR-299 suppressed the invasion and migration of HTR-8/SVneo trophoblast cells partly via targeting HDAC2 in pre-eclampsia, *Biomed. Pharmacother.* 97 (2018) 1222–1228 <http://doi.org/10.1016/j.biopha.2017.11.053>.
- [21] L. Huang, Z. Shen, Q. Xu, X. Huang, Q. Chen, D. Li, Increased levels of microRNA-424 are associated with the pathogenesis of fetal growth restriction, *Placenta* 34 (2013) 624–627 <http://doi.org/10.1016/j.placenta.2013.04.009>.
- [22] ACOG practice bulletin No. 204: fetal growth restriction, *Obstet. Gynecol.* 133 (2019) e97–e109 <http://doi.org/10.1097/AOG.0000000000003070>.
- [23] T.N. Leung, M.W. Pang, S.S. Daljit, T.Y. Leung, C.F. Poon, S.M. Wong, T.K. Lau, Fetal biometry in ethnic Chinese: biparietal diameter, head circumference, abdominal circumference and femur length, *Ultrasound in obstetrics & gynecology*, *Off. J. Int. Soc. Ultrasound Obstet. Gynecol.* 31 (2008) 321–327 <http://doi.org/10.1002/uog.5192>.
- [24] Y.K.Y. Cheng, J. Lu, T.Y. Leung, Y.M. Chan, D.S. Sahota, Prospective assessment of INTERGROWTH-21(st) and World Health Organization estimated fetal weight reference curves, *Ultrasound in obstetrics & gynecology*, *Off. J. Int. Soc. Ultrasound Obstet. Gynecol.* 51 (2018) 792–798 <http://doi.org/10.1002/uog.17514>.
- [25] F.P. Hadlock, R.B. Harrist, R.S. Sharman, R.L. Deter, S.K. Park, Estimation of fetal weight with the use of head, body, and femur measurements—a prospective study, *Am. J. Obstet. Gynecol.* 151 (1985) 333–337 [http://doi.org/10.1016/0002-9378\(85\)90298-4](http://doi.org/10.1016/0002-9378(85)90298-4).
- [26] D. Arduini, G. Rizzo, Normal values of Pulsatility Index from fetal vessels: a cross-sectional study on 1556 healthy fetuses, *J. Perinat. Med.* 18 (1990) 165–172 <http://10.1515/jpme.1990.18.3.165>.
- [27] A.A. Baschat, U. Gembruch, The cerebroplacental Doppler ratio revisited, *Ultrasound in obstetrics & gynecology*, *Off. J. Int. Soc. Ultrasound Obstet. Gynecol.* 21 (2003) 124–127 <http://doi.org/10.1002/uog.20>.
- [28] J. Stanek, Hypoxic patterns of placental injury: a review, *Arch. Pathol. Lab Med.* 137 (2013) 706–720 <http://doi.org/10.5858/arpa.2011-0645-RA>.
- [29] A. Fujimaki, K. Watanabe, T. Mori, C. Kimura, K. Shinohara, A. Wakatsuki, Placental oxidative DNA damage and its repair in preeclamptic women with fetal growth restriction, *Placenta* 32 (2011) 367–372 <http://doi.org/10.1016/j.placenta.2011.02.004>.
- [30] J.F. Mouillet, R.B. Donker, T. Mishima, T. Cronqvist, T. Chu, Y. Sadovsky, The unique expression and function of miR-424 in human placental trophoblasts, *Biol. Reprod.* 89 (2013) 25 <http://doi.org/10.1095/biolreprod.113.110049>.
- [31] C.L. Whitehead, W.T. Teh, S.P. Walker, C. Leung, L. Larmour, S. Tong, Circulating MicroRNAs in maternal blood as potential biomarkers for fetal hypoxia in-utero, *PLoS One* 8 (2013) e78487 <http://doi.org/10.1371/journal.pone.0078487>.
- [32] Y. Zhou, Q. An, R.X. Guo, Y.H. Qiao, L.X. Li, X.Y. Zhang, X.L. Zhao, miR424-5p functions as an anti-oncogene in cervical cancer cell growth by targeting KDM5B via the Notch signaling pathway, *Life Sci.* 171 (2017) 9–15 <http://doi.org/10.1016/j.lfs.2017.01.006>.
- [33] L. Boldrup, P.J. Coates, G. Laurell, T. Wilms, R. Fahraeus, K. Nylander, Downregulation of miRNA-424: a sign of field cancerisation in clinically normal tongue adjacent to squamous cell carcinoma, *Br. J. Canc.* 112 (2015) 1760–1765 <http://doi.org/10.1038/bjc.2015.150>.
- [34] C. Zhao, X.B. Wang, Y.H. Zhang, Y.M. Zhou, Q. Yin, W.C. Yao, MicroRNA-424 inhibits cell migration, invasion and epithelial-mesenchymal transition in human glioma by targeting KIF23 and functions as a novel prognostic predictor, *Eur. Rev. Med. Pharmacol. Sci.* 22 (2018) 6369–6378 http://doi.org/10.26355/eurrev_201810_16049.
- [35] L. Guo, Y. Liu, Y. Guo, Y. Yang, B. Chen, MicroRNA-423-5p inhibits the progression of trophoblast cells via targeting IGF2BP1, *Placenta* (2018), <http://doi.org/10.1016/j.placenta.2018.12.003>.
- [36] I. Lee, S.S. Ajay, J.I. Yook, H.S. Kim, S.H. Hong, N.H. Kim, S.M. Dhanasekaran, A.M. Chinnaiyan, B.D. Athey, New class of microRNA targets containing simultaneous 5'-UTR and 3'-UTR interaction sites, *Genome Res.* 19 (2009) 1175–1183 <http://doi.org/10.1101/gr.089367.108>.
- [37] F. Moretti, R. Thermann, M.W. Hentze, Mechanism of translational regulation by miR-2 from sites in the 5' untranslated region or the open reading frame, *RNA* 16 (2010) 2493–2502 <http://doi.org/10.1261/rna.2384610>.
- [38] W. Wongfieng, A. Jumnainsong, Y. Chamgramol, B. Sripa, C. Leelayuwat, 5'-UTR and 3'-UTR regulation of MICB expression in human cancer cells by novel microRNAs, *Genes (Basel)* 8 (2017), <http://doi.org/10.3390/genes8090213>.
- [39] M.H. van der Ree, J.M. de Vree, F. Stelma, S. Willemse, M. van der Valk, S. Rietdijk, R. Molenkamp, J. Schinkel, A.C. van Nuenen, U. Beuers, S. Hadi, M. Harbers, E. van der Veer, K. Liu, J. Grundy, A.K. Patick, A. Pavlicek, J. Blem, M. Huang, P. Grint, S. Neben, N.W. Gibson, N.A. Kootstra, H.W. Reesink, Safety, tolerability, and antiviral effect of RG-101 in patients with chronic hepatitis C: a phase 1B, double-blind, randomised controlled trial, *Lancet* 389 (2017) 709–717 [http://doi.org/10.1016/S0140-6736\(16\)31715-9](http://doi.org/10.1016/S0140-6736(16)31715-9).
- [40] S. Atambayeva, R. Niyazova, A. Ivashchenko, A. Pyrkova, I. Pinsky, A. Akimiyazova, S. Labeit, The binding sites of miR-619-5p in the mRNAs of human and orthologous genes, *BMC Genomics* 18 (2017) 428 <http://doi.org/10.1186/s12864-017-3811-6>.
- [41] J.G. Li, Y. Ding, Y.M. Huang, W.L. Chen, L.L. Pan, Y. Li, X.L. Chen, Y. Chen, S.Y. Wang, X.N. Wu, FAMLF is a target of miR-181b in Burkitt lymphoma, *Braz. J. Med. Biol. Res.* 50 (2017) e5661 <http://doi.org/10.1590/1414-431X20175661>.
- [42] V. Huang, R.F. Place, V. Portnoy, J. Wang, Z. Qi, Z. Jia, A. Yu, M. Shuman, J. Yu, L.C. Li, Upregulation of Cyclin B1 by miRNA and its implications in cancer, *Nucleic Acids Res.* 40 (2012) 1695–1707 <http://doi.org/10.1093/nar/gkr934>.
- [43] B. Wightman, I. Ha, G. Ruvkun, Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in *C. elegans*, *Cell* 75 (1993) 855–862 [http://doi.org/10.1016/0092-8674\(93\)90530-4](http://doi.org/10.1016/0092-8674(93)90530-4).
- [44] F.I. Diaz-Perez, U. Hiden, M. Gauster, I. Lang, V. Konya, A. Heinemann, J. Logl, R. Saffery, G. Desoye, S. Cvitic, Post-transcriptional down regulation of ICAM-1 in fetoplacental endothelium in GDM, *Cell Adhes. Migrat.* 10 (2016) 18–27 <http://doi.org/10.1080/19336918.2015.1127467>.