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Original Article

Relevance of MicroRNA-122 to pathogenesis of preeclampsia in rats

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Abstract: Aims: This study aims to investigate the role of MicroRNA-122 (miR122) in preeclampsia development. Methods: miR122 and its target gene potassium channel modulatory factor 1 (KCMF1) expressions were compared in placentas respectively from healthy pregnant and preeclampsia rats. The effects of miR122 on expression of KCMF1 was investigated in HTR8/SVneo cells. The role of miR122 and KCMF1 in cell migration and invasion was investigated. Results: The levels of KCMF1 were significantly lower in preeclamptic placenta tissues, which was inversely correlated with the level of miR122. KCMF1 was validated as the target of miR122 using realtime PCR and Western blotting in HTR8/SVneo cells. miR122 inhibited the migration, invasion and proliferation of trophoblast cells, and this inhibition was abrogated by the overexpression of KCMF1. The tumor necrosis factor-α could upregulate miR122 while suppressing KCMF1 expression in HTR8/SVneo cells. Conclusion: Aberrant miR122 expression may contribute to preeclampsia by interfering with KCMF1-mediated signaling.

Keywords: Preeclampsia, MicroRNA-122, potassium channel modulatory factor 1, TNF-α

Introduction

Preeclampsia, a major contributor to maternal morbidity worldwide [1], is a pregnancy-specific syndrome manifested by the onset of hypertension and proteinuria after the 20th week of gestation. Abnormal placenta development has been generally accepted as the initial cause of the disorder [2, 3]. Defects of trophoblast cell function, such as reduced proliferation [4], excessive apoptosis [5], aberrant differentiation [6], limited migration and invasion of the uterus, and poor remodeling of spiral arteries [7], have been considered to be associated with preeclampsia. However, the pathogenesis and molecular mechanisms of this disorder is not clearly understood.

MicroRNAs (miRNAs) are a subclass of short (20-23 nucleotides in length), endogenous, non-coding, single-stranded RNAs that regulate gene expression post-transcriptionally [8-10]. miRNAs-mediated gene silencing is via RNA-induced silencing complex (RISC), inducing translational repression or degradation of targeted mRNAs. Recently, numerous investigations have confirmed the important roles of miRNAs in the regulation of human cancer [11, 12], as well as in physiological function including immune responses [13], cellular proliferation, differentiation, and apoptosis [14, 15]. Recent studies, mostly microarray based miRNA profiles have suggested that dysregulation of miRNAs in placental tissues is involved in the pathogenesis of preeclampsia [16-19]. In addition, miRNAs may partly regulate implantation and placentation as well as different processes such as angiogenesis, apoptosis or cell cycle of placental cells [20]. Recently, microRNA-122 (miR122) has been found to be upregulated in preeclamptic placentas compared with normal placentas, indicating a possible association of this small molecule with the placental pathology of preeclampsia [21]. However, the function of miR122 in the development of the placenta remains elusive.

Potassium channel modulatory factor 1 (KCMF1) was first mentioned in 2010, which could be a potential factor in the regulation of potassium channels [22]. It was also named FIGC (basic fibroblast growth factor-induced gene in

gastric cancer) for its upregulation by fibroblast growth factor receptor 2 signaling pathways in gastric cancer cells [23-25]. Previous literature proved that KCMF1 was involved in the proliferation, migration, and invasion in epithelial cancers [24]. According to previous bioinformatic analyses, potassium channel modulatory factor 1 (KCMF1) is one of the commonly predicted targets of miRNAs. KCMF1 may play an important role in the development of preeclampsia as a target gene of miR-210 [26].

Based on the evidence noted above, we propose that miR122 might regulate trophoblast cell migration and invasion by targeting KCMF1 and that the pathway may play a role in the development of preeclampsia. To test this hypothesis, we used preeclampsia rats and a human trophoblast cell line, HTR8/SVneo, to investigate the mechanisms by which miR122 exerts its function via KCMF1.

Materials and methods

Animals

All procedures involving animals in this study were performed in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee of the University of Xi'an Jiaotong University. Animal experiments were conducted on timed-pregnant Sprague Dawley rats that were housed under a 12:12-h light-dark cycle and fed a standard laboratory chow diet.

Reduced uterine perfusion in the pregnant rat

A modification of the method by Eder and McDonald [27] as previously described was used to reduce uterine perfusion by 35% to 45%. Briefly, rats were anesthetized with 2% isoflurane delivered by an anesthesia apparatus. At day 14 of gestation, a silver clip (0.203-mm ID) was placed around the lower abdominal aorta above the iliac bifurcation. Because compensation of blood flow to the placenta occurs in the pregnant rat through an adaptive increase in uterine blood flow, both right and left uterine arteries were clipped (0.100-mm ID). When the clipping procedure resulted in total reabsorption of the fetuses, rats were excluded from data analyses. Control pregnant rats were sham operated.

Cell line and culture condition

Immortalized human trophoblast cell line, HTR8/SVneo, was obtained from ATCC and cultured according to the supplier's protocols using RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin. The cells were incubated at 37°C in a 5% CO_2 atmosphere until they reached confluence. Then the cells were randomly assigned into 4 groups: Untreated Cell (NC), miR122 mimics transfection (miR122 mimics), miR122 mimics transfection (miR122 inhibitor) and miR122 mimics transfection plus KCFM1 overexpression (miR122 mimics + pKCFM1).

Transfection

For transient transfection experiments, the cells were seeded in 6-well plates at 1.5×10^5 cells per well in complete medium. Twenty-four hours following seeding, miR122 mimics, miR122 inhibitor and the KCMF1 expressing plasmid were transfected into the cells using Lipofectamine 2000 reagent according to the manufacturer's instruction (Invitrogen, Carlsbad, CA). Cells were subjected to transwell invasion assay or RNA/protein extraction. The mature microRNA mimics for miR122, miR122 inhibitor, the scramble control (NC) and the KCMF1 plasmid were designed and purchased from GenePharma (Shanghai, China).

RNA extraction and quantitative real-time PCR

For determination of placental miR122 and KCMF1 levels in pregnant rats, animals were euthanized with carbon dioxide inhalation at day 21 of gestation, and placentas were isolated, weighed and quickly frozen in liquid nitrogen and stored at 80°C. Total RNA from cells and placental tissues were extracted using the RNeasy Protect mini-kit supplied by Qiagen, as outlined in the instructions provided by the manufacturer. Real-time PCR was used, as previously described, to determine tissue miR122 and KCMF1 levels. Briefly, cDNA was synthesized from 1 μg of RNA with Bio-Rad Iscript cDNA reverse transcriptase, and real-time PCR was performed using the Bio-Rad SYBR Green Supermix and iCycler. The mRNA and miRNA expression were calculated using the $2^{-\Delta\Delta CT}$ method, where ΔCT indicated the subtraction of the CT of GAPDH from the mRNA or miRNA of interest, and ΔΔCT was calculated by subtracting the ΔCT.

Western blot analysis

Protein extracts were prepared from cells and placental tissues using RIPA lysis buffer. Cell or tissue lysates were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Western blotting was carried out as described previously. The primary antibodies used were mouse anti-human KCMF1 monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA), mouse anti-human GAPDH antibody (Ambion, Austin, Texas, USA) and horseradish peroxidase-conjugated secondary antibody (Promega, Madison, WI). Signals were detected using an Enhanced Chemiluminescence Plus kit (Thermo Scientific, Rockford, USA) and visualized after exposure to a Kodak film. The membranes were then scanned and signal intensities were analyzed by the Gel-Pro Analyzer (software version 4.0; United Bio). The relative densities of the KCMF1 were determined by normalization with the density value of GAPDH in the same blot.

Dual luciferase assay

HTR8/SVneo cells were plated into 24-well plates with a density of 4.5×10^4 cells/well. Transfection was performed 24 hours after seeding using Lipofectamine 2000 (Invitrogen) with 100 ng of pMIR-REPORT plasmid construct, 10 ng of pRL-TK control vector (encoding Renilla luciferase) and 40 nM of miR-122. Five hours after transfection, the cells were recovered in complete medium. Luciferase activities were measured by the Dual-Glo luciferase assay system according to the manufacturer's instructions (Promega, Madison, Wisconsin, USA) 48 hours after transfection.

Transwell assay

Transwell cell migration and invasion assay is a method that measures the capacity of cell motility and invasiveness toward a chemoattractant gradient. Quantitative cell migration assays were performed using a modified Boyden chamber (Costar-Corning, New York, USA) with 8.0-μm pore polycarbonate filter inserts in 24-well plates as described previously. 48 hours after transfection, cells were treated with 10 μg/ml Mitomycin C for another 2 h to avoid any influence of cell growth on the results of the transwell assay. Then the cells were trypsinized and seeded into transwell insert at 5×10^4 cells per insert. The top chambers contained RPMI 1640 medium supplemented with 1% FBS, and the lower chambers were loaded with RPMI-1640 medium plus 10% FBS. The cells were fixed and stained with crystal violet for 10 min at 28 hours later. Cells on the upper surface of the membrane were completely removed by washing the chambers 5 times with dH_2O, and all remaining cells represent cells that have migrated. Migration was assessed by counting the number of stained cells from 10 random fields at × 200 magnification. Cell invasion assay was performed similarly, except that transwell inserts were matrigel-coated.

Scratch test

Scratch test was carried out to determine the cell protrusion and migration ability of cells. HTR8/SVneo cells were planted in a 6-well culture plates and incubated overnight to a density of 60%-70%. Cells were transfected by miR122 mimics, miR122 inhibitor and miR122 mimics + KCFM1 plasmid respectively according to the protocol mentioned above. Cell monolayers were then scratched with a 100 μL yellow pipette tip and washed with PBS three times to remove detached cells. The wounded areas were imaged using an Olympus microscope and marked at different times. The size of the wounded areas was then quantitated using ImageJ Version 1.41 software (National Institutes of Health). Experiments were carried out in triplicate at least three times.

Tumor necrosis factor-α treatment on HTR8/SVneo cell

To determine the effects of tumor necrosis factor-α (TNF-α) on expression of miR122 and KCMF1, the HTR8/SVneo cells were treated with recombinant human TNF-α (100 ng/ml, Sigma St. Louis. MO) for 24 hours in serum-free RPMI-1640 medium. To determine the mRNA and protein level of miR122 and KCMF1, cell RNA and protein was extracted and quantitative real-time PCR and western blot were performed according to the methods mentioned above.

Statistical analysis

Data in this study are expressed as the mean ± SEM. All experiments were repeated at least 3

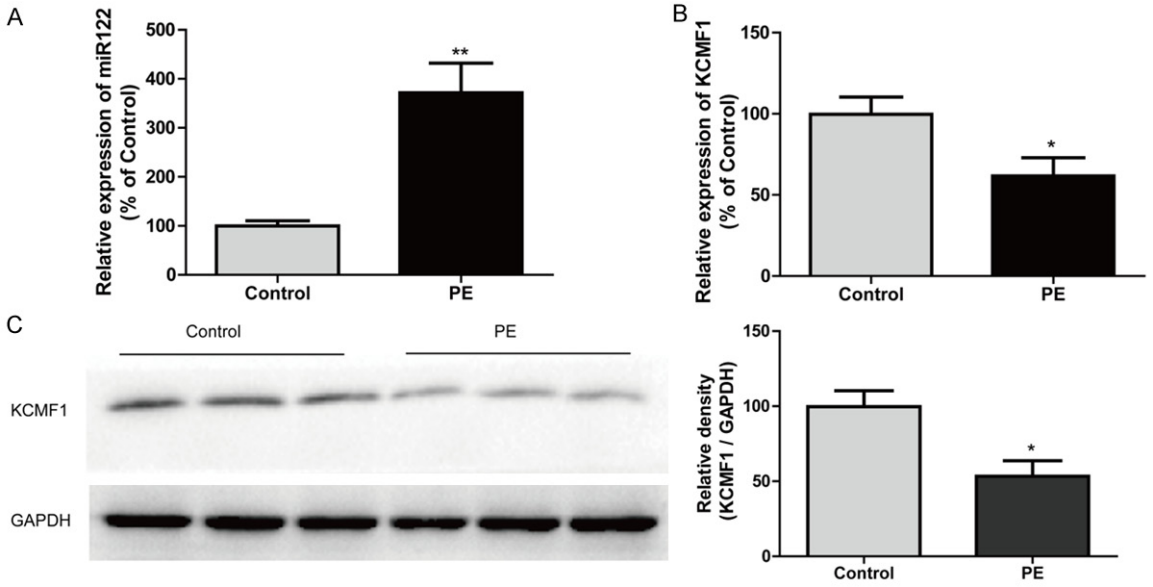

Figure 1. Differential expression patterns of miR122 and KCMF1 in placentas derived from severe preeclamptic (PE) rats and normal pregnant rats (control). Quantitative real-time polymerase chain reaction (A, B) and Western blotting experiments (C) were performed to measure the expression levels of miR122 and KCMF1 of the placentas derived from PE rats (n=6) and from normal pregnant rats (n=6). The data are presented as the means ± SEM; **P*<0.05; ***P*<0.01.

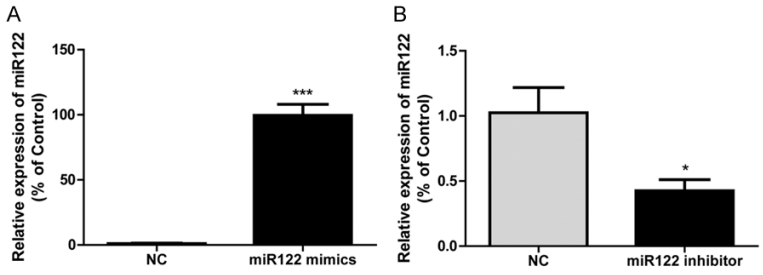

Figure 2. mRNA expression levels of miR122 of HTR8/SVneo cells transfected by miR122 mimics (A) and miR122 inhibitor (B). The data are presented as the means ± SEM; **P*<0.05; ****P*<0.001.

times. Statistical analysis was performed using one-way ANOVA for multi-group comparison or two-sided Student t test for two-group comparison, and differences with P<0.05 were considered statistically significant. All statistical analyses were performed using SPSS 22.0 (IBM, Armonk, NY).

Results

Expressions of KCMF1 and miR122 exhibited an inverse correlation in placentas from rats with preeclampsia

We examined KCMF1 and miR122 expression levels in control and PE placentas using quantitative real-time polymerase chain reaction. The results showed thatthe level of miR-122 was significantly higher in the PE placentas than in that of the normal pregnant controls (**Figure 1A**; *P*<0.01). The mRNA level of KCMF1 was significantly lower in PE placentas than in the corresponding control groups (**Figure 1B**; *P*<0.05). The KCMF1 protein level, as examined using Western blotting, was also decreased in PE placentas (**Figure 1C**). These data suggested an inverse correlation in miR122 and KCMF1 expressions in the placentas of the studied individuals.

Validation of KCMF1 as the target of miR122 in human trophoblast cells

Data of quantitative real-time polymerase chain reaction showed that the level of miR122 increased 100 fold after miR122 mimics transfection (**Figure 2A**) and decreased by 60% after miR122 inhibitor transfection compared with the negative control cells (**Figure 2B**). As shown in **Figure 3A** and **3B**, the levels of both KCMF1

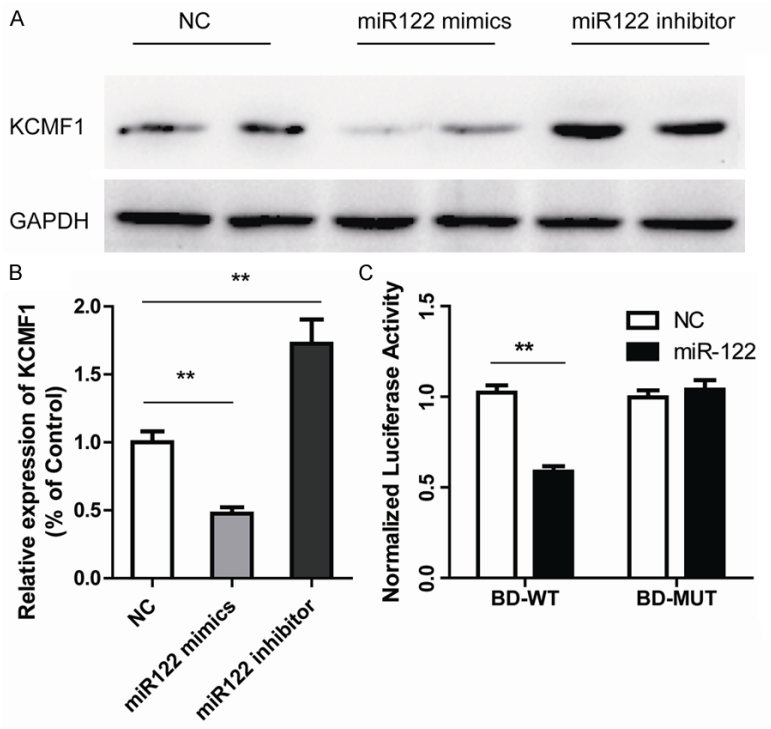

Figure 3. Effects of miR122 on the expression of KCMF1 in HTR8/SVneo cells and validation of KCMF1 as a target gene of miR-122. Western blotting experiments (A) and quantitative real-time polymerase chain reaction (B) were performed to measure the expression levels of KCMF1 of the HTR8/SVneo cells transfected by miR122 mimics and miR122 inhibitor. (C) Luciferase assay result of HTR8/SVneo cells transfected with BD-WT and BD-MUT reporter constructs with miR-122 or negtive control were shown. The data are presented as the means ± SEM; $^{**}P<0.01$.

protein and mRNA were 70% and 50% lower in HTR8/SVneo cells transfected with miR122 mimics than the negative control cells. On the other hand, the expression of KCMF1 were higher after miR122 inhibitor transfection compared with the negative control cells. These data strongly suggest that KCMF1 may be the target gene of miR122 in human trophoblast cells. We constructed a luciferase reporter vector by cloning a 240-bp DNA fragment, including binding site (BD) of the 3'-UTR in human KCMF1 mRNA downstream of the firefly luciferase reporter gene (the reporter vector was named BD-WT). A point mutation was incorporated into the binding sites of the 3'-UTR in the KCMF1 gene to generate BD-MUT reporter vector. As shown in **Figure 3C**, miR-122 mimics significantly reduced the relative luciferase activity of the BD-WT construct by ≈40% compared with the scramble control but did not influence the luciferase activity of the BD-MUT construct. These data strongly suggest that KCMF1 can be the target gene of miR-122 in human trophoblast cells and that the 3'-UTR region in the KCMF1 gene is the real binding site for miR-122.

Effects of miR122 and KCMF1 on cell migration and invasion in human trophoblast cells

The effect of miR122 on trophoblast cell migration and invasion were further examined. As shown in **Figure 4A**, transfection with miR122 mimics or inhibitors could clearly repress or induce the cell migration (**Figure 4Aa-d**) and invasion (**Figure 4Ae-h**) in HTR8/SVneo cells. The resultant data revealed that miR-122 had less trophoblast cell migration and invasion. We performed a rescue experiment by transfecting HTR8/SVneo cells with miR122 mimics together with a KCMF1-expressing plasmid (pKCMF1). Interestingly, the repressing effect of miR122 was abrogated by the over-expressed KCMF1. The quantitative result was show in **Figure 4B**. The data indicated that miR122 could mediate the migration and invasion alterations in trophoblasts through an miR122-KCMF1 pathway.

Effects of miR122 and KCMF1 on cell proliferation and migration in human trophoblast cells

We also evaluated the effects of miR122 on the proliferation and migration ability of human trophoblast cells with *in vitro* scratch assay (**Figure 5**). As shown in **Figure 5**, miR122 significantly decreased the proliferation of HTR-8/SVneo by 36% compared to the corresponding negative control and inhibition of miR122 significantly improved the proliferation of HTR-8/SVneo by 40% compared to the corresponding negative control. Similar to the transwell results, over-expression of KCMF1 could rescue the proliferation-repressing effect of miR122 in HTR8/SVneo Cells. According to the combined

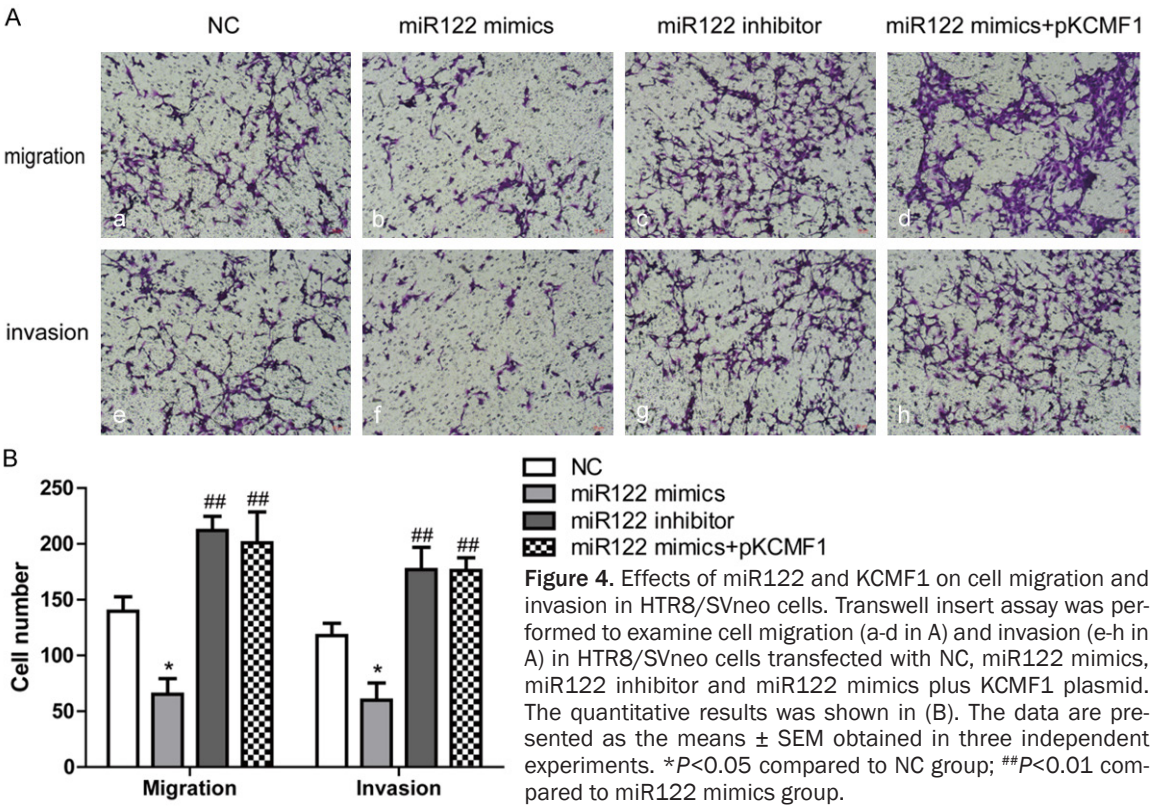

Figure 4. Effects of miR122 and KCMF1 on cell migration and invasion in HTR8/SVneo cells. Transwell insert assay was performed to examine cell migration (a-d in A) and invasion (e-h in A) in HTR8/SVneo cells transfected with NC, miR122 mimics, miR122 inhibitor and miR122 mimics plus KCMF1 plasmid. The quantitative results was shown in (B). The data are presented as the means ± SEM obtained in three independent experiments. **P*<0.05 compared to NC group; ##*P*<0.01 compared to miR122 mimics group.

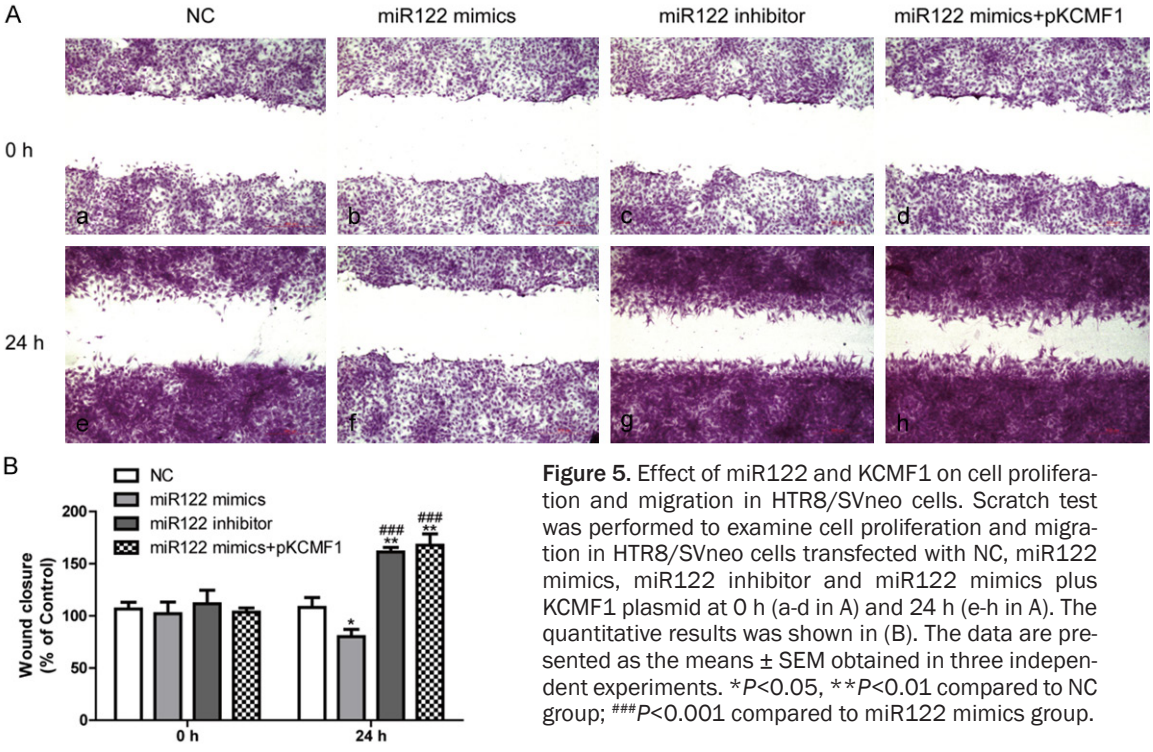

Figure 5. Effect of miR122 and KCMF1 on cell proliferation and migration in HTR8/SVneo cells. Scratch test was performed to examine cell proliferation and migration in HTR8/SVneo cells transfected with NC, miR122 mimics, miR122 inhibitor and miR122 mimics plus KCMF1 plasmid at 0 h (a-d in A) and 24 h (e-h in A). The quantitative results was shown in (B). The data are presented as the means ± SEM obtained in three independent experiments. **P*<0.05, ***P*<0.01 compared to NC group; ###*P*<0.001 compared to miR122 mimics group.

results of transwell assay and scratch assay, miR122 could inhibited the migration and invasion abilities of trophoblasts through an miR122-KCMF1 pathway.

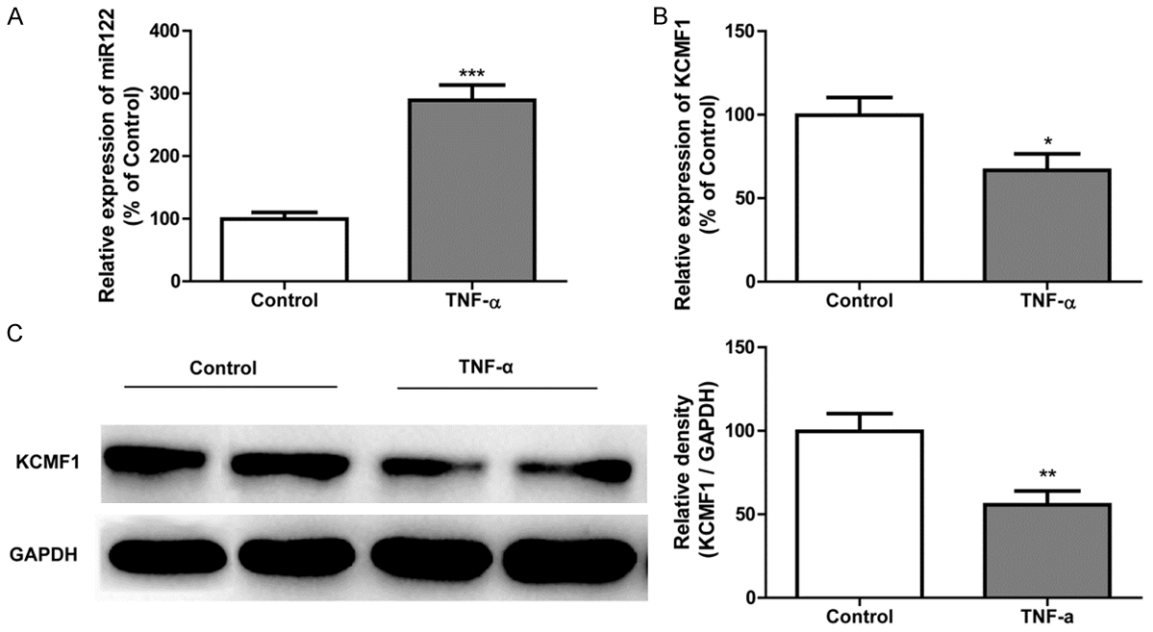

Figure 6. Regulation of the expressions of miR122 and KCMF1 by TNF-α in HTR8/SVneo cells. Quantitative real-time polymerase chain reaction to reveal the expressions of miR122 (A) and KCMF1 (B) in HTR8/SVneo cells treated with TNF-α. Western blot analysis showing alterations in KCMF1 protein levels in HTR8/SVneo cells treated with TNF-α (C). The values are presented as the means ± SEM. The data are presented as the means ± SEM; **P*<0.05, ***P*<0.01, ****P*<0.001.

TNF-α increases miR122 expression but down-regulates KCMF1

The effects of TNF-α on expression of miR122 and KCMF1 in human trophoblast cells were also investigated in the present study. As shown in **Figure 6**, TNF-α significantly increased the expression of miR122 by 3-fold over the control (**Figure 6A**; *P*<0.001), whereas it decreased KCMF1 mRNA and protein levels to 70% to 55% of the control (**Figure 6B**, *P*<0.05 and **Figure 6C**, *P*<0.01). The inverse change pattern of miR122 and KCMF1 on TNF-α treatment in human trophoblast cells further indicated the functional correlation of these two molecules.

Discussion

microRNAs have been proved irregularly expressed in preeclamptic placentas compared with normal placentas [28, 29]. However, the mechanisms involving the dysregulated microRNAs participation in the pathogenesis of preeclampsia remaines unclear. According to previous literature, miR122 is significantly overexpressed in preeclamptic placentas compared with normal placentas, indicating a possible association of this small molecule with the placental pathology of preeclampsia [21]. However, the function of miR122 in the development of the preeclamptic placenta remains unclear. Bioinformatics research showed that KCMF1 is one of the targets of miR-122, which is also involved in the development of placenta. Therefore, we proposed that miR122-KCMF1 pathway may be involved in regulating preeclampsia development.

In the present study, several lines of evidence support that KCMF1 is a target gene of miR122 in human trophoblasts. Firstly, the KCMF1 expression is inversely correlated with miR122 expressions in the placentas of the studied preeclampsia rats. Secondly, the KCMF1 expression could be suppressed by miR122 mimics in HTR8/SVneo cells, while the inhibitory effect of miR122 could be reversed by miR122 inhibitor transfection. Thirdly, functional study in HTR8/SVneo cells demonstrated that miR122 could inhibit the proliferation, migration and invasion of cells. Importantly, the overexpression of KCMF1 could well abrogate the repressing effects of miR122 on HTR8/SVneo cells. These findings strongly suggest that KCMF1 is a critical miR122 target gene, at least participating in mediating the regulation of cell proliferation,

migration and invasion in human trophoblast cells. One of the most important causal factors of preeclampsia is abnormal placental development, especially the shallow invasion of trophoblasts into the decidual stroma and spiral arteries during early gestation [30]. In all, the results in the present study indicated that the involvement of miR122-KCMF1 pathway in the preeclamptic process.

Functional studies of miR122 have been largely performed in cancer cells, and the data demonstrated its participation in regulation of cell apoptosis, having targets which are as well proapoptotic as antiapoptotic [31]. Human placental trophoblast cells possess similar properties to tumor cells in terms of proliferation and invasion, although their behaviors are temporally and spatially restricted during pregnancy [32]. Our finding of the inhibition on human trophoblast cell proliferation and invasion by miR122 was consistent with its effects on human esophageal cancer cell growth [33] and hepatocellular carcinoma [34].

Local oxygen tension and inflammation have been proved to be the primary determinants of the preeclapsia development [35]. Several cytokines, including interleukin-2, interferon-γ, and TNF-α, are established mediators of immune maladaptation in preeclampsia [36]. Further more, TNF-α has been reported as one of the regulators of miR122 in other cell type [37]. TNF-α has been shown to decrease the motility of HTR-8/SVneo cells in vitro [38] and to inhibit the cell migration and invasion. In the present study, we found that TNF-α significantly increased the expression of miR122 in HTR-8/SVneo cells, while decreased the KCMF1 expression. We assume that the increased miR122-KCMF1 pathway may also be involved in the immune maladaptation at the fetal maternal interface during the occurrence of preeclampsia.

In conclusion, the present study provides new evidence about the functions of miR122 and KCMF1 in the human preeclampsia development. Our findings strongly suggest that aberrantly expressed miR122, via downregulating KCMF1, may play an important role in the development of preeclampsia.

Disclosure of conflict of interest

None.

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References

[1] Grotegut CA. Prevention of preeclampsia. J Clin Invest 2016; 126: 4396-4398.
[2] Broughton Pipkin F and Roberts JM. Hypertension in pregnancy. J Hum Hypertens 2000; 14: 705-724.
[3] Thompson LP, Pence L, Pinkas G, Song H and Telugu BP. Placental hypoxia during early pregnancy causes maternal hypertension and placental insufficiency in the hypoxic guinea pig model. Biol Reprod 2016; 95: 128.
[4] Redline RW and Patterson P. Pre-eclampsia is associated with an excess of proliferative immature intermediate trophoblast. Hum Pathol 1995; 26: 594-600.
[5] Liu X, Hu Y, Zheng Y, Liu X, Luo M, Liu W, Zhao Y and Zou L. EPHB4 regulates human trophoblast cell line HTR-8/SVneo function: implications for the role of EPHB4 in preeclampsia. Biol Reprod 2016; 95: 65.
[6] Fisher SJ. The placental problem: linking abnormal cytotrophoblast differentiation to the maternal symptoms of preeclampsia. Reprod Biol Endocrinol 2004; 2: 53.
[7] Cui Y, Wang W, Dong N, Lou J, Srinivasan DK, Cheng W, Huang X, Liu M, Fang C, Peng J, Chen S, Wu S, Liu Z, Dong L, Zhou Y and Wu Q. Role of corin in trophoblast invasion and uterine spiral artery remodelling in pregnancy. Nature 2012; 484: 246-250.
[8] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.
[9] Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009; 136: 215-233.
[10] Xie T, Huang M, Wang Y, Wang L, Chen C and Chu X. MicroRNAs as regulators, biomarkers and therapeutic targets in the drug resistance of colorectal cancer. Cell Physiol Biochem 2016; 40: 62-76.
[11] Liu N, Li J, Zhao Z, Han J, Jiang T, Chen Y, Hou N and Huang C. MicroRNA-302a enhances 5-fluorouracil-induced cell death in human colon cancer cells. Oncol Rep 2017; 37: 631-639.
[12] Takikawa T, Masamune A, Yoshida N, Hamada S, Kogure T and Shimosegawa T. Exosomes derived from pancreatic stellate cells: MicroRNA signature and effects on pancreatic cancer cells. Pancreas 2017; 46: 19-27.
[13] Vencken SF and Greene CM. Toll-like receptors in cystic fibrosis: impact of dysfunctional mi-

croRNA on innate immune responses in the cystic fibrosis lung. J Innate Immun 2016; 8: 541-549.
[14] Li M, Chen W, Zhang H, Zhang Y, Ke F, Wu X, Zhang Y, Weng M, Liu Y and Gong W. MiR-31 regulates the cisplatin resistance by targeting Src in gallbladder cancer. Oncotarget 2016; 7: 83060-83070.
[15] Vallabhaneni KC, Hassler MY, Abraham A, Whitt J, Mo YY, Atfi A and Pochampally R. Mesenchymal stem/stromal cells under stress increase osteosarcoma migration and apoptosis resistance via extracellular vesicle mediated communication. PLoS One 2016; 11: e0166027.
[16] Mouillet JF, Chu T and Sadovsky Y. Expression patterns of placental microRNAs. Birth Defects Res A Clin Mol Teratol 2011; 91: 737-743.
[17] Betoni JS, Derr K, Pahl MC, Rogers L, Muller CL, Packard RE, Carey DJ, Kuivaniemi H and Tromp G. MicroRNA analysis in placentas from patients with preeclampsia: comparison of new and published results. Hypertens Pregnancy 2013; 32: 321-339.
[18] Murphy MS, Tayade C and Smith GN. Maternal circulating microRNAs and pre-eclampsia: challenges for diagnostic potential. Mol Diagn Ther 2017; 21: 23-30.
[19] Biro O, Nagy B and Rigo J Jr. Identifying miRNA regulatory mechanisms in preeclampsia by systems biology approaches. Hypertens Pregnancy 2017; 36: 90-99.
[20] Fu G, Brkic J, Hayder H and Peng C. MicroRNAs in human placental development and pregnancy complications. Int J Mol Sci 2013; 14: 5519-5544.
[21] Lasabova Z, Vazan M, Zibolenova J and Svecova I. Overexpression of miR-21 and miR-122 in preeclamptic placentas. Neuro Endocrinol Lett 2015; 36: 695-699.
[22] Beilke S, Oswald F, Genze F, Wirth T, Adler G and Wagner M. The zinc-finger protein KCMF1 is overexpressed during pancreatic cancer development and downregulation of KCMF1 inhibits pancreatic cancer development in mice. Oncogene 2010; 29: 4058-4067.
[23] Jang JH. FIGC, a novel FGF-induced ubiquitin-protein ligase in gastric cancers. FEBS Lett 2004; 578: 21-25.
[24] Tan Z, Zhang S, Li M, Wu X, Weng H, Ding Q, Cao Y, Bao R, Shu Y, Mu J, Ding Q, Wu W, Yang J, Zhang L and Liu Y. Regulation of cell proliferation and migration in gallbladder cancer by zinc finger X-chromosomal protein. Gene 2013; 528: 261-266.
[25] Zou J, Mi L, Yu XF and Dong J. Interaction of 14-3-3sigma with KCMF1 suppresses the proliferation and colony formation of human colon cancer stem cells. World J Gastroenterol 2013; 19: 3770-3780.
[26] Luo R, Shao X, Xu P, Liu Y, Wang Y, Zhao Y, Liu M, Ji L, Li YX, Chang C, Qiao J, Peng C and Wang YL. MicroRNA-210 contributes to preeclampsia by downregulating potassium channel modulatory factor 1. Hypertension 2014; 64: 839-845.
[27] Eder DJ and McDonald MT. A role for brain angiotensin ii in experimental pregnancy-induced hypertension in laboratory rats. Clinical and experimental hypertension. Part B: Hypertension in Pregnancy 1987; 6: 431-451.
[28] Zhu XM, Han T, Sargent IL, Yin GW and Yao YQ. Differential expression profile of microRNAs in human placentas from preeclamptic pregnancies vs normal pregnancies. Am J Obstet Gynecol 2009; 200: 661, e661-667.
[29] Enquobahrie DA, Abetew DF, Sorensen TK, Willoughby D, Chidambaram K and Williams MA. Placental microRNA expression in pregnancies complicated by preeclampsia. Am J Obstet Gynecol 2011; 204: 178, e112-121.
[30] Chelbi ST and Vaiman D. Genetic and epigenetic factors contribute to the onset of preeclampsia. Mol Cell Endocrinol 2008; 282: 120-129.
[31] Ma L, Liu J, Shen J, Liu L, Wu J, Li W, Luo J, Chen Q and Qian C. Expression of miR-122 mediated by adenoviral vector induces apoptosis and cell cycle arrest of cancer cells. Cancer Biol Ther 2010; 9: 554-561.
[32] Bai Y, Yang W, Yang HX, Liao Q, Ye G, Fu G, Ji L, Xu P, Wang H, Li YX, Peng C and Wang YL. Downregulated miR-195 detected in preeclamptic placenta affects trophoblast cell invasion via modulating ActRIIA expression. PLoS One 2012; 7: e38875.
[33] Zhang HS, Zhang FJ, Li H, Liu Y, Du GY and Huang YH. Tanshinone A inhibits human esophageal cancer cell growth through miR-122-mediated PKM2 down-regulation. Arch Biochem Biophys 2016; 598: 50-56.
[34] Yang YM, Lee CG, Koo JH, Kim TH, Lee JM, An J, Kim KM and Kim SG. Galpha12 overexpressed in hepatocellular carcinoma reduces microRNA-122 expression via HNF4alpha inactivation, which causes c-Met induction. Oncotarget 2015; 6: 19055-19069.
[35] Hung TH, Skepper JN, Charnock-Jones DS and Burton GJ. Hypoxia-reoxygenation: a potent inducer of apoptotic changes in the human placenta and possible etiological factor in preeclampsia. Circ Res 2002; 90: 1274-1281.
[36] Bernardi F, Guolo F, Bortolin T, Petronilho F and Dal-Pizzol F. Oxidative stress and inflammatory

markers in normal pregnancy and preeclampsia. J Obstet Gynaecol Res 2008; 34: 948-951.
[37] Kagiya T and Nakamura S. Expression profiling of microRNAs in RAW264.7 cells treated with a combination of tumor necrosis factor alpha and RANKL during osteoclast differentiation. J Periodontal Res 2013; 48: 373-385.
[38] Todt JC, Yang Y, Lei J, Lauria MR, Sorokin Y, Cotton DB and Yelian FD. Effects of tumor necrosis factor-alpha on human trophoblast cell adhesion and motility. Am J Reprod Immunol 1996; 36: 65-71.