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Relationship between recurrent pregnancy loss and microRNA expression

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Abstract

The miRNAs produced predominantly in the placenta are probably involved in placental differentiation and maintenance of pregnancy. One of the important functions of miR-126 is its involvement in angiogenesis by enhancing the expression of VEGF. Abnormalities of placental vasculature may result in several gestational complications, including pregnancy loss. The current study compared the expression of miR-126 in the plasma of women with recurrent miscarriages and women with healthy pregnancies by Realtime qRT-PCR. The results indicated that the expression difference of miR-21 between the pregnant patients and the controls was statistically significant (p=0.002). This microRNA can be used as a biomarker in diagnosis and progression in recurrent pregnancy.

INTRODUCTION

Recurrent pregnancy loss (RPL) is defined by most clinicians as three or more consecutive miscarriages (Stirrat, 1990). It can be emotionally and physically traumatizing for any couple who experience the repeated loss of their offspring and also live with the anxiety of another miscarriage when they conceive (1). RPL can be divided into the two main categories of maternal and embryological causes. Uterine anomalies, endocrine disorders, thrombophilic disorders, placental anomalies, infection, genetics, immune dysfunction, and exposure to environmental factors are among the maternal causes, whereas chromosomal abnormalities are the most common embryological causes. However, the etiology of RPL remains mostly unknown. To date, the medical evaluation of RPL has focused mainly on the maternal factors; thus, little is known about the role of paternal factors. Several studies have shown that paternally expressed genes may have an impact on embryo implantation and placental proliferation (2). MicroRNAs (miRNAs) are endogenous, short, noncoding molecules which play a role in the mechanism of posttranscriptional gene expression by suppressing the translation of protein-coding genes or cleaving target mRNAs. A peculiar characteristic of miRNAs is represented by the fact that one miRNA can regulate the expression of several genes, while one gene can be targeted by different miRNAs, which means that miRNAs can regulate up to 30% of the human genome. In fact, miRNAs represent important epigenetic mechanisms of regulation that can control complex processes such as cell growth, differentiation, stress response, and tissue remodeling that, under particular conditions, can play a key role in many disease states, including gestational disorders (3-5). In particular, miRNAs may reflect pathological gestational conditions, such as preeclampsia, spontaneous abortion, preterm birth, macrosomia, or low birth weight. Thus, their detection in maternal circulation makes miRNAs good candidate biomarkers to monitor the progression of normal pregnancy and the presence of gestational diseases, for the prevention and treatment of adverse pregnancy outcomes (6).

In recent years, single nucleotide polymorphisms (SNP) in genes coding microRNA (miRNA) have been demonstrated to be associated with RPL. Studies on miRNA expression across several organs have revealed that miRNA expression is tissue-specific, and some miRNAs are also expressed abundantly in placenta (7). Therefore, miRNAs produced predominantly in the placenta are probably involved in placental differentiation and maintenance of pregnancy. Cell-free placental DNA and/or RNA in maternal plasma are possible molecular markers for noninvasive prenatal

monitoring or the early detection of adverse pregnancy-associated outcomes. In this study, the expression of miR-126 in the plasma of women with recurrent miscarriages and in women with healthy pregnancies was compared.

METHODS AND MATERIALS

The study population included 20 women with at least two unexplained consecutive pregnancy losses and 10 healthy controls with at least two live births and no history of pregnancy loss. All subjects were in the age range of 18-35 years and not in a consanguineous marriage. To harvest cell-free plasma, whole blood samples were centrifuged twice at 1200×g for 10 min at room temperature. RNA was extracted immediately after sample collection using a NucleoSpin® miRNA Plasma kit (Norgenbiotek, Canada) according to the manufacturer's protocol. Next, miR-126 was analyzed using real-time qRT–PCR. For this purpose, RealQ Plus Master Mix Green – Ampliqon was used. The PCR primers were as follows: 5-TATGGTTGTTCTCGACTCCTTCAC-3 and 5-TCGTCTGTCGTACCGTGAGTAAT-3 for miR126 and 5-CTCGCT TCGGCAGCACA-3 and 5-AACGCT TCACGAATTTGCGT-3 for U6. Quantitative real-time PCR was conducted on a Rotorgene 6000 System (Corbett Research, Australia). U6 was used to normalize the data of miR-126 expression. The relative expression of miR-126 was calculated using the 2−ΔΔCT method. The data was analyzed by SPSS software (version 16). The independent sample t-test was used for the comparison of means.

Results

The results indicated that the difference in miR-21 expression between pregnant patients and the controls was statistically significant with a decrease of 0.54-fold. Figure 1 illustrates the fold-change comparison of miR-126 in patients and controls (p = 0.002).

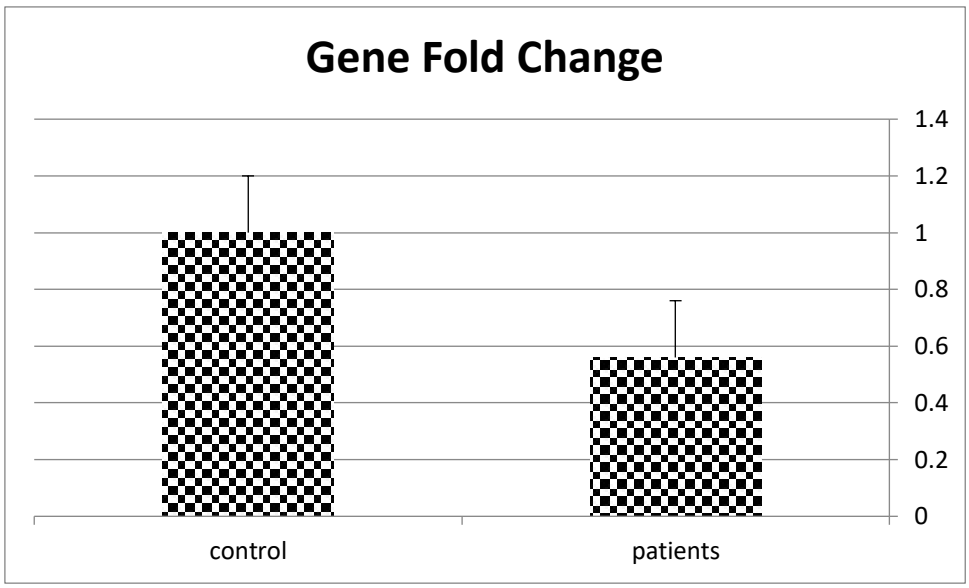

Fig. 1. Fold change of miR-126 between RPL and healthy groups

DISCUSSION

Recurrent pregnancy loss (RPL), which is defined as two pregnancy losses that occur before 20 weeks gestation, is relatively common, occurring in approximately 1–5% of women (8). The underlying cause is often unclear, although numerous factors may contribute to RPL, including environmental and immunological factors, blood coagulation disorders, and genetics. MicroRNAs (miRNAs) are 19–25-nucleotide single-strand non-coding RNA species that induce post-transcriptional gene silencing and mediate translational repression through binding to target mRNA, leading to subsequent mRNA degradation (9). The recent elucidation of the miRNA function has provided new insight into the regulation of gene expression. Key molecules involved in miRNA biogenesis, such as DROSHA, XPO5, and DICER, have been identified in trophoblast cells, confirming that the miRNA biogenesis pathway is active in human placenta. It has been demonstrated that the human placenta produces a large number of miRNAs which are involved in placental development. In addition, miRNAs regulate uterine gene expression which is associated with inflammatory responses during the peri-implantation period and participates in maternal-fetal immune tolerance (10). Numerous reports have demonstrated the association of aberrant miRNA expression with various human diseases related to

reproductive conditions. Moreover, accumulating evidence from recent reports shows that various miRNAs are associated with one or more aspects of pregnancy and pregnancy outcomes. For instance, Renthal et al. (2010) showed that the miR200 family regulates uterine quiescence and contractility during pregnancy and labor (Renthal et al., 2010) (11). Mice knockout studies of miRNA biogenesis proteins (e.g., Dicer1 and Ago2) have confirmed that miRNAs are crucial for reproduction (Hong et al., 2008; Morita et al., 2007) (12, 13). Chakrabarty et al. (2007) showed that a number of miRNAs are specifically expressed during the peri-implantation and pre-implantation periods in mice (6). The current results showed decreased levels of miR-126 in RPL cases compared to the corresponding healthy controls with a 0.54-fold decrease (p=0.002). One of the important functions of miR-126 is its involvement in angiogenesis by enhancing the expression of VEGF. Abnormalities in placental vasculature may result in several gestational complications, including pregnancy loss, intrauterine fetal death, intrauterine growth restriction, and preeclampsia. VEGF plays an essential role in fetal and placental angiogenic development, and diminished placental trophoblastic VEGF has been described in the decidual endothelium of spontaneous miscarriages (Su et al., 2011) (14). The current results are consistent with those of Dai et al. (2011), who showed that collapsed blood vessels and cranial hemorrhages occurred in zebrafish with reduced miR-126 abundance, and mice deficient in miR-126 exhibited delayed angiogenic sprouting, widespread hemorrhaging, and partial embryonic lethality (Dai et al., 2011) (5). This microRNA can be used as a biomarker for the diagnosis and progression recurrent pregnancy.

REFERENCE

1. Wilcox AJ, Weinberg CR, O'Connor JF, Baird DD, Schlatterer JP, et al. (1988) Incidence of early loss of pregnancy. N Engl J Med 319: 189–194.

2. Stirrat GM (1990) Recurrent miscarriage. II: Clinical associations, causes, and management. Lancet 336: 728–733.

3. Practice Committee of the American Society for Reproductive Medicine (2012) Evaluation and treatment of recurrent pregnancy loss: a committee opinion. Fertil Steril

4. an Q, Luo X, Toloubeydokhti T, Chegini N (2007) The expression profile of micro-RNA in endometrium and endometriosis and the influence of ovarian steroids on their expression. Mol Hum Reprod 13: 797–806.

5. Bin, Y., Tianmei, Q., Yongjun, W., Songlin, Z., Guohui D., Fei, D., Xiaosong G. (2012). miR-182 inhibits Schwann cell proliferation and migration by targeting FGF9 and NTM, respectively at an early stage following sciatic nerve injury. Nucleic Acids Research, 40(20): 10356-10365.

6. Chakrabarty, A., Tranguch, S., Daikoku, T., Jensen, K., Furneaux, H., Dey, S.K. (2007). MicroRNA regulation of cyclooxygenase-2 during embryo implantation. Proceedings of NationalAcademy of Science(USA), 104 (38): 15144-15149.

7. Dai, Y., Diao, Z., Sun, H., Li, R., Qiu, Z., Hu, Y. (2011). MicroRNA-155 is involved in the remodelling of human-trophoblast-derived HTR8/SVneo cells induced by lipopolysaccharides. Human Reproduction, 26(7):1882-1891.

8. Practice Committee of the American Society for Reproductive Medicine. Definitions of infertility and recurrent pregnancy loss. Fertil Steril. 2008; 90(5 Suppl):S60.

9. Amin-Beidokhti M, Mirfakhraie R, Zare-Karizi S, Karamoddin F. The role of parental microRNA alleles in recurrent pregnancy loss: an association study. Reprod Biomed Online. 2016: 34(3):325–30

10. Fu G., Brkic J., Hayder H., Peng C. (2013). MicroRNAs in Human Placental Development and Pregnancy Complications. International Journal of Molecular Sciences 14, 5519-5544.

11. Renthal, N.E., Chen, C.C., Williams, K.C., Gerard, R.D., Prange-Kiel J., Mendelson C.R. (2010). miR200 Family and targets, ZEB1 and ZEB2, modulate uterine quiescence and contractility during pregnancy and labor. Proceedings of National Academy of Science (USA),107(48):20828-20833.

12. Hong, X., Luense, L.J., McGinnis, L.K., Nothnick, W.B., Christenson, L.K.(2008). Dicer1 is essential for female fertility and normal development of the female reproductive system. Endocrinology 149 (12), 6207-6212.

13. Morita, S., Horii, T., Kimura, M., Goto, Y., Ochiya, T., Hatada, I. (2007). One Argonaute family member. Eif2c2 (Ago2), is essential for development and appears not to be involved in DNA methylation. Genomics, 89 (6):687-696.

14. Sun, H.X., Zeng D.Y., Li, R.T., Pang, R.P., Yang, H., Hu, Y.L., Zhang, Q., Jiang, Y., Huang, L.Y., Tang, Y.B., Yan, G.J., Zhou, J.G. (2012). Essential role of microRNA-155 in regulating endothelium-dependent vasorelaxation by targeting endothelial nitric oxide synthase. Hypertension, 60(6):1407-1414.