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Assessment of the expression of mir−29c, mir−200a and mir−145 in endometrial tissue and the downstream molecules in infertile patients with endometriosis

Xiao−Mei Shu✉

Gynecology Department, Leshan Maternal and Child Health Hospital in Sichuan Province, Leshan City, Sichuan Province, 614000

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ABSTRACT

Objective: To study the expression of mir-29c, mir-200a and mir-145 in endometrial tissue and analyze the downstream molecules in infertile patients with endometriosis. **Methods:** Female patients with infertility caused by endometriosis who were treated in Leshan Maternal and Child Health Hospital between May 2014 and February 2017 were selected as the infertility group of the research, and female patients with infertility caused by male factors over the same period were selected as the control group of the research. Endometrial tissue was collected to detect the expression of mir-29c, mir-200a, mir-145, HOXA-10 and HOXA-11 as well as downstream molecules and adhesion molecules. **Results:** mir-29c, mir-200a and mir-145 expression in endometrial tissue of infertility group were significantly higher than those of control group; HOXA-10, HOXA-11, integrin v β 3, IGFBP-1, CD44V6, N-cadherin and FAK mRNA expression in endometrial tissue of infertility group were significantly lower than those of control group and negatively correlated with mir-29c, mir-200a and mir-145 expression while E-cadherin and FUT4 mRNA expression were significantly higher than those of control group and positively correlated with mir-29c, mir-200a and mir-145 expression. **Conclusion:** The highly expressed mir-29c, mir-200a and mir-145 in endometrial tissue can regulate the expression of HOXA-10 and HOXA-11 as well as downstream molecules and adhesion molecules, and influence the endometrial receptivity in infertile patients with endometriosis.

1. Introduction

Endometriosis is that the active endometrium appears in other parts than the intima covered by theuterine cavity and abnormally grows[1]. It is a common cause of female infertility in clinic, and the decline of endometrial receptivity is closely related to the occurrence of infertility or decreased pregnancy rate in patients with endometriosis[2,3]. The regulation of endometrial receptivity involves multiple molecules and links, HOXA-10, HOXA-11, adhesion molecules and immune molecules can all affect the endometrial receptivity to the embryo, and the abnormal expression a variety of molecules will affect the endometrial receptivity[4,5]. Although the relationship between endometrial receptivity and infertility is recognized, it is still unclear about the specific mechanism that regulates the receptivity regulators in the endometrium. MicroRNA (miRNA) is a small molecular RNA that is involved in the regulation of post-transcriptional control of genes, and the mir-29c, mir-200a and mir-145 in local tissues are confirmed to be closely related to the regulation of endometrial receptivity. The expression of mir-29c, mir-200a and mir-145 in the endometrial tissue of infertile patients with endometriosis were specifically analyzed, and the downstream molecules of miRNA were explored in the following studies.

✉Corresponding author: Xiao-Mei Shu, Gynecology Department, Leshan Maternal and Child Health Hospital in Sichuan Province, Leshan City, Sichuan Province, 614000.

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2. Research subjects and methods

2.1 General information of research subjects

A total of 56 female patients with infertility caused by endometriosis who were treated in Leshan Maternal and Child Health Hospital between May 2014 and February 2017 were selected as the infertility group of the research, they conformed to

the diagnosis for endometriosis and received in vitro fertilization - embryo transfer therapy, and those with infertility caused by tubal and ovarian factors were eliminated. A total of 38 female patients with infertility caused by male factors over the same period were selected as the control group of the research, were not with the infertility caused by female reproductive system factors and received in vitro fertilization - embryo transfer therapy. Infertility group were 25-34 years old, the course of disease was 6-15 months; control group were 23-33 years old and the course of disease was 7-16 months. There was no statistically significant difference in general information between the two groups (P>0.05).

2.2 Research methods

2.2.1 Clinical sample collecting

Endometrial tissue was collected from two groups of patients in the process of in vitro fertilization - embryo transfer therapy, washed with saline for 3-5 times, then frozen in the liquid nitrogen for 30 min, then taken out and placed in a -70 ℃ cryogenic refrigerator.

2.2.2 miRNA expression detecting

Endometrial tissue of infertility group and control group was taken, miRNA extraction kit and miRNA special reverse transcription kit were used to separate total miRNA and reversely transcribe it into cDNA, fluorescence quantitative PCR kit was used to amplify target miRNA mir-29c, mir-200a and mir-145 and reference gene U6, and the mir-29c, mir-200a and mir-145 expression were calculated according to the amplification curve.

2.2.3 Gene expression detecting

Endometrial tissue of infertility group and control group was taken and added in Trizol lysate to extract total RNA, cDNA first-strand synthesis kit was used for reverse transcription from total RNA to cDNA, fluorescence quantitative PCR kit was used to amplify target genes HOXA-10, HOXA-11, integrin v β 3, IGFBP-1, CD44V6, E-cadherin, FUT4, N-cadherin, FAK and housekeeping gene β -actin, and the target gene mRNA expression was calculated according to amplification curve.

2.3 Statistical methods

SPSS 19.0 software was used to input data, measurement data between two groups was by t test, the correlation between two data was by Pearson test and P<0.05 indicated statistical significance in differences in test results.

3. Results

3.1 mir-29c, mir-200a and mir-145 expression in endometrial tissue

Analysis of mir-29c, mir-200a and mir-145 expression in endometrial tissue of infertility group and control group was as follows: mir-29c, mir-200a and mir-145 expression in endometrial tissue of infertility group were significantly higher than those of control group. Differences in mir-29c, mir-200a and mir-145 expression in endometrial tissue were statistically significant between infertility group and control group (P<0.05).

3.2 HOXA-10, HOXA-11 and downstream molecule expression in endometrial tissue

Analysis of HOXA-10 and HOXA-11 as well as downstream molecules v β 3 and IGFBP-1 expression in endometrial tissue of infertility group and control group was as follows: HOXA-10, HOXA-11, integrin v β 3 and IGFBP-1 mRNA expression in endometrial tissue of infertility group were significantly lower than those of control group. Pearson correlation analysis showed that HOXA-10, HOXA-11, integrin v β 3 and IGFBP-1 mRNA expression in endometrial tissue of infertility group were negatively correlated with mir-29c, mir-200a and mir-145 expression.

3.3 Adhesion molecule expression in endometrial tissue

Analysis of adhesion molecules CD44V6, E-cadherin, FUT4, N-cadherin and FAK expression in endometrial tissue of infertility group and control group was as follows: CD44V6, N-cadherin

Table 1.
Comparison of mir-29c, mir-200a and mir-145 expression in endometrial tissue.

Groups	n	mir-29c	mir-200a	mir-145
Infertility group	56	2.37±0.35	2.21±0.29	2.61±0.38
Control group	38	1.02±0.14	1.01±0.15	0.98±0.13
T		12.485	11.038	15.684
P		<0.05	<0.05	<0.05

Table 2.
Comparison of HOXA-10, HOXA-11 and downstream molecule expression in endometrial tissue.

Groups	n	HOXA-10	HOXA-11	Integrin v β 3	IGFBP-1
Infertility group	56	0.36±0.07	0.42±0.06	0.24±0.04	0.37±0.07
Control group	38	1.04±0.16	0.97±0.12	1.02±0.13	1.05±0.11
T		19.393	12.328	24.595	10.208
P		<0.05	<0.05	<0.05	<0.05

Table 3.
Comparison of adhesion molecule expression in endometrial tissue.

Groups	*n*	CD44V6	E-cadherin	FUT4	N-cadherin	FAK
Infertility group	56	0.35±0.07	2.41±0.34	2.19±0.35	0.28±0.04	0.42±0.07
Control group	38	1.01±0.14	0.96±0.13	1.05±0.15	1.03±0.14	0.98±0.13
T		19.284	15.676	12.038	25.685	13.416
P		＜0.05	＜0.05	＜0.05	＜0.05	＜0.05

and FAK mRNA expression in endometrial tissue of infertility group were significantly lower than those of control group while E-cadherin and FUT4 mRNA expression were significantly higher than those of control group. Pearson correlation analysis showed that CD44V6, N-cadherin and FAK mRNA expression in endometrial tissue of infertility group were negatively correlated with mir-29c, mir-200a and mir-145 expression while E-cadherin and FUT4 mRNA expression were positively correlated with mir-29c, mir-200a and mir-145 expression.

4. Discussion

Endometrial receptivity change is an important factor of endometriosis to cause female sterility[6,7], but it is still unclear about the specific mechanism regulating the endometrial receptivity in patients with endometriosis. MiRNA is a kind of non-coding small molecular RNA that is involved in post-transcriptional regulation of many genes and can produce corresponding biological effects through changes in gene expression levels. In endometrial tissue, mir-29c, mir-200a and mir-145 are three important miRNAs that adjust the endometrial tissue receptivity to the embryo, which can cause endometrial decidualization and synchronization with embryo implantation, and help embryo implantation[8]. In the study, analysis of the changes in the above three miRNA expression in endometrial tissue of patients with infertility caused by endometriosis showed that mir-29c, mir-200a and mir-145 expression in endometrial tissue of infertility group were significantly higher than those of control group. This indicates that the high expression of mir-29c, mir-200a and mir-145 in endometrial tissue of patients with endometriosis is related to the occurrence of infertility; the high expression of mir-29c, mir-200a and mir-145 in the endometrium can regulate the expression of the downstream target genes to influence the endometrial receptivity, and thus cause the occurrence of infertility.

HOXA10 and HOXA11 in the HOX family are important members that regulatef endometrial receptivity, and their downstream molecules integrin v β 3 and IGFBP-1 are involved in the regulation of embryo implantation process[9,10]. Integrin v β 3 is a molecule that regulates endometrium function and makes it in receptive state, which is beneficial for embryo implantation[11]; IGFBP-1 can be combined with IGF-1 to transport IGF-1, which helps the IGF-1 to exert the biological effect of promoting embryonic development in the local endometrium[12]. In the study, analysis of the HOXA-10, HOXA-11 and downstream molecule expression in endometrial tissue of patients with infertility caused by endometriosis showed that HOXA-10, HOXA-11, integrin v β 3 and IGFBP-1 mRNA expression in endometrial tissue of infertility group were significantly lower than those of control group. This indicates that the expression deletion of signaling molecules HOXA-10 and HOXA-11 as well as the decreased expression of downstream molecules integrin v β 3 and IGFBP-1 in endometrial tissues of patients with endometriosis are related to the occurrence of infertility. Further analysis of the correlation of miRNA with HOXA-10, HOXA-11 and downstream molecule expression in endometrial tissue of patients with infertility caused by endometriosis showed that HOXA-10, HOXA-11, integrin v β 3 and IGFBP-1 mRNA expression were negatively correlated with mir-29c, mir-200a and mir-145 expression. It means that the high expression of mir-29c, mir-200a and mir-145 in endometrial tissue of patients with infertility caused by endometriosis can inhibit the expression of signaling molecules HOXA-10 and HOXA-11 aswell as the downstream molecules integrin v β 3 and IGFBP-1, and then influence the endometrial receptivity and cause infertility through the changes of HOXA-10, HOXA-11 and downstream molecule expression.

The adhesion between the embryo and the endometrium is an important biological process for the completion of embryo implantation, which is regulated by a variety of pro-adhesion molecules and anti-adhesion molecules. CD44V6 is a variant of CD44, which mainly degrades hyaluronic acid to promote cell adhesion and infiltration[13,14]; the E-cadherin and N-cadherin in cadherin famlity have different biological effects, the former can sustain the intercellular polarity and inhibit cell movement and adhesion, and the latter can promote cell movement and help cell adhesion and invasion[15,16]; FAK is a type of non-receptor tyrosine kinase that mediates the adhesion between cells and extracellular matrix through Ras/MAPK pathway[17,18]; FuT4 can catalyze the synthesis of fucosylation oligosaccharide Lewis oligosaccharides-Y and mediate apoptosis, which is not conducive to embryo implantation. In the study, analysis of adhesion molecule expression in endometrial tissue of patients with infertility caused by endometriosis showed that CD44V6, N-cadherin and FAK mRNA expression in endometrial tissue of infertility group were significantly lower than those of control group while E-cadherin and FUT4 mRNA expression were significantly higher than those of control group. This indicates that the low expression of pro-adhesion molecules and the high expression of anti-adhesion molecules in endometrial tissues of patients with endometriosis are related to the occurrence of infertility. Further analysis of the correlation of miRNA with adhesion molecule expression in endometrial tissue of patients with infertility caused by endometriosis showed that

mir-29c, mir-200a and mir-145 expression in endometrial tissue of infertility group were negatively correlated with CD44V6, N-cadherin and FAK mRNA expression, and positively correlated with E-cadherin and FUT4 mRNA expression. It means that the high expression of mir-29c, mir-200a and mir-145 in endometrial tissue of patients with infertility caused by endometriosis can inhibit the expression of pro-adhesion molecules and increase the expression of anti-adhesion molecules so as to influence the adhesion between the embryo and the endometrium and cause infertility.

The mir-29c, mir-200a and mir-145 are highly expressed in endometrial tissue of infertile patients with endometriosis; highly expressed mir-29c, mir-200a and mir-145 can inhibit the expression of HOXA-10, HOXA-11 and downstream molecules, and also inhibit the expression of pro-adhesion molecules and increase the expression of anti-adhesion molecules so as to influence the endometrial receptivity and cause infertility.

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