European Journal of Obstetrics & Gynecology and Reproductive Biology 197 (2016) 27–30

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

miRNA and target gene expression in menstrual endometria and early pregnancy decidua

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ARTICLE INFO

Article history:
Received 13 July 2015
Received in revised form 27 October 2015
Accepted 12 November 2015

Keywords:
MicroRNA
Decidua
Menstrual endometrium
Target gene

ABSTRACT

Objective: The role of miRNAs in modulating gene expression in decidualization remains to be determined. We performed a comparative study to identify miRNAs and their potential mRNA targets with different expression levels between endometrium and decidua.
Methods: Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was used to measure the expression of the miR-146b-5p, miR-181b-5p, miR-424, miR-532, miR-199a-3p, miR-423, miR-22-3p, let-7i-5p, and miR-1 and the predicted target genes *IGF2R*, *LEPR*, *SGK1*, *MMP2*, *MMP10*, *LIF*, *IL6*, and *STAT3* in menstrual endometria and early pregnancy decidua.
Results: miR-146b-5p, miR-181b-5p, miR-424, miR-532, and miR-199a-3p were significantly down-regulated in early pregnancy decidua, while miR-423, miR-22-3p, let-7i-5p, and miR-1 were significantly upregulated. In addition, the decidua had significantly lower levels of expression of *LIF*, *IL6*, *MMP2*, *MMP10*, and *IGF2R* and higher levels of expression of *SGK1*, *LEPR*, *PROK1*, and *STAT3* than the menstrual endometria group.
Conclusion: Our results provide new insights into the expression of miRNAs that regulate genes involved in decidualization and the maintenance of early pregnancy.

Introduction

MicroRNAs (miRNAs), an abundant set of evolutionarily conserved RNAs roughly 22 nucleotides long [1], control gene expression by targeting messenger RNAs (mRNAs) for degradation, translational repression, or both [2]. Functional analysis of miRNAs has revealed their influence on the expression of target genes involved in both physiologic and pathologic conditions [3]. Cell cycle progression, proliferation, and differentiation during cyclic changes in the endometrium are among the biological processes regulated by miRNAs [4]. Decidualization is characterized by the transformation of stromal fibroblasts in response to elevated circulating progesterone levels [5].

Acquisition of endometrial receptivity and subsequent decidualization are complex processes involving the expression of numerous molecular mediators. In contrast to most mammals, decidualization of the human endometrium does not require embryo implantation [6]. In a previous study we showed that some miRNAs are abundantly expressed in decidua and that their expression levels differ significantly in normal pregnancy compared to aborted decidua [7]. We demonstrated that early pregnancy decidua from women who have experienced successful implantation exhibit a different miRNA expression profile from that of menstrual endometria (data not shown). In this study, we compared the expression of miRNAs in decidua and menstrual endometria in a larger sample size. We detected increased levels of four miRNAs and decreased levels of five miRNAs in decidua. An analysis of potential target genes of these miRNAs demonstrated that the decidua had significantly lower expression levels of leukemia inhibitory factor (*LIF*), interleukin 6 (*IL6*), matrix metalloproteinase 2 (*MMP2*), matrix metalloproteinase 10 (*MMP10*), and insulin-like growth factor 2 receptor (*IGF2R*) and higher expression levels of serum/glucocorticoid regulated kinase 1 (*SGK1*), leptin receptor (*LEPR*), prokineticin 1 (*PROK1*), and signal transducer and activator of transcription 3 (*STAT3*).

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http://dx.doi.org/10.1016/j.ejogrb.2015.11.003
0301-2115/

Materials and methods

Tissue collection

Endometrial biopsies were collected on the second day of the menstrual cycle from 25 women who suffered from secondary infertility, either for tubal or male factors, and received treatment from October 2011 to April 2012 in the Reproductive unit of Inner Mongolia University Affiliated Hospital (Inner Mongolia, China). Participants ranged in age from 22 to 39 years (mean 31 years). In addition, decidua was collected from 35 women diagnosed with normal pregnancies based on fetal heart activity detected by ultrasound scan who underwent artificial pregnancy termination at 6–8 weeks of gestation. These participants ranged in age from 19 to 39 years (mean 28.6 years). Samples were collected for miRNA and predicted target gene analysis. The decidua was separated from the trophoblast, and each tissue sample was immediately frozen in liquid nitrogen (Supplementary Table S1). Written informed consent had been obtained and this study was approved by the Institute Research Ethics Committee of Inner Mongolia Medical University Affiliated Hospital.

Supplementary Table S1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejogrb.2015.11.003.

Isolation of total RNA and analysis of miRNA and target gene expression by RT-PCR

The expression of the miRNAs miR-146b-5p, miR-181b-5p, miR-424, miR-532, miR-199a-3p, miR-423, miR-22-3p, let-7i-5p, and miR-1 and their predicted target genes *IGF2R*, *LEPR*, *SGK1*, *MMP2*, *MMP10*, *LIF*, *IL6*, and *STAT3* was analyzed in human early pregnancy decidua and menstrual endometria. Candidate genes involved in pregnancy maintenance were identified from a previous study [7] (Table 1).

Total RNA, including miRNA, was extracted using RNAiso Plus (Takara, Japan). Complementary DNA (cDNA) was generated using 1 μg total RNA from each sample, the MicroRNA RT Kit, RT Reagent Kit with gDNA Eraser (Takara, Japan), and RT primers. The SYBR Green PCR Kit (Takara, Japan) was used to perform quantitative RT-PCR (qRT-PCR) reactions in triplicate, according to the manufacturer's instructions, on an ABI Prism 7300HT (Amersham-Pharmacia, Piscataway, NJ). PCR primers are provided in Supplementary Table S2. MicroRNA and mRNA levels were normalized to the expression levels of U6 and β-actin, respectively. Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method [8].

Table 1
Candidate miRNAs and the predicted target genes.

miRNA	Target gene symbol
miR-146b-5p	*LEPR*
miR-181b-5p	*SGK1*
	STAT3
	LEPR
miR-424	*SGK1*
	STAT3
	LEPR
miR-532	*STAT3*
	LEPR
	SGK1
miR-30a	*MMP2*
miR-423	*LIF*
	MMP2
miR-22-3p	*LIF*
miR-1	*LIF*
	IL6
	IGF2R
	MMP10

Supplementary Table S2 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejogrb.2015.11.003.

MiRNA target prediction

MIREAP was used to predict mRNA targets (http://sourceforge.net/projects/mireap/) [9]. Bioinformatics predictions were ranked using the scores on the site based on the predicted efficacy of targeting.

Statistical analysis

Statistical analyses were performed with SPSS software (version 16.0; SPSS Inc., Chicago, IL, USA). Two-tailed *t*-tests and nonparametric tests were used to compare miRNA and mRNA expression levels determined by RT-PCR in human tissue samples, and P-value < 0.05 was considered indicative of statistical significance.

Results

miR-146b-5p, miR-181b-5p, miR-424, miR-532, and miR-199a-3p were significantly downregulated in decidua, while miR-423, miR-22-3p, let-7i-5p, and miR-1 were significantly upregulated (Fig. 1). Decidua had significantly lower levels of *LIF*, *IL6*, *MMP2*, *MMP10*, and *IGF2R* expression and significantly higher levels of *SGK1*, *LEPR*, *PROK1*, and *STAT3* than menstrual endometria (Figs. 2 and 3). *SGK1*, *LEPR*, and *STAT3* are predicted targets of miR-181b-5p, miR-424, and miR-532, respectively. *LIF* is a predicted target of miR-423, miR-22-3p, and miR-1, and *MMP2* is also a predicted target of miR-423 and miR-22-3p. Finally, *IL6*, *IGF2R*, and *MMP10* are predicted targets of miR-1.

Discussion

There is growing evidence to indicate that specific miRNAs are involved in the development and progression of early pregnancy, regulating a broad number of signaling pathways in endometrial tissues that impact inflammation, local estrogen biosynthesis [10], progesterone resistance [11], cell invasion, extracellular matrix remodeling, angiogenesis [12], and epigenetic regulation. In this study, miR-146b-5p, miR-181b-5p, miR-424, miR-532, and miR-199a-3p were downregulated and miR-423, miR-22-3p, let-7i-5p, and miR-1 were upregulated in early pregnancy decidua relative to menstrual endometria. The roles of these miRNAs in decidualization are for the most part unknown and their potential interaction with target genes involved in events leading up to decidualization requires further study.

An miRNA's function is defined by its effect on the genes it targets. Global gene expression data comparing endometria from in vitro fertilization patients with repeated implantation failure and from fertile women during the implantation window has revealed a number of differentially expressed genes [13]. Decidualization has been attributed to a variety of genes, including *MMP*s [14], *IL*s [15], *LIF*, and *SGK1* [16]. In this study, we report higher expression of *SGK1*, *LEPR*, *PROK1*, and *STAT3* and lower expression of *LIF*, *IL6*, *MMP2*, *MMP10*, and *IGF2R* in decidua relative to menstrual endemotria. We noted the prevalence of factors involved in intercellular signaling in our data set, including *SGK1*, *STAT3*, *LIF*, *IL6*, and *IGF2R*, as well as a hormone receptor, *LEPR*. Our data support previous reports of the importance of several of these genes in decidualization, discussed in more detail below. Furthermore, our results provide guidance for future investigations into a role for miRNAs in regulating genes involved with pregnancy maintenance.