ORIGINAL ARTICLE: REPRODUCTIVE BIOLOGY

MicroRNA-451 plays a role in murine embryo implantation through targeting Ankrd46, as implicated by a microarray-based analysis

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Objective: To determine the potential microRNA (miRNA) regulators of embryo implantation, as a continuation of genomic and proteomic research.
Design: Laboratory animal research.
Setting: University hospital laboratory.
Animal(s): Adult healthy female C57BL6/J mice (age 6–8 weeks, nonfertile, weighing 18–20 g each).
Intervention(s): Female mice were mated naturally with fertile males to produce pregnancy. Luminal epithelium was collected by laser-capture microdissection during the implantation period. Mouse models of pseudopregnancy, delayed implantation, and artificial decidualization were established.
Main outcome measure(s): The miRNA profile in luminal epithelium was clarified by microarray analysis and validated by real-time reverse transcription polymerase chain reaction (qRT-PCR) in a series of models. Target genes were predicted and confirmed by luciferase activity assay. The role of miRNA in implantation was examined by loss-of-function and gain-of-function of miRNA in vitro and in vivo.
Result(s): A total of 29 and 15 miRNAs were up- and down-regulated, respectively, during the implantation period; 11 of these miRNAs were validated by qRT-PCR. The profile of miR-451 was clarified in a series of models. A dual-luciferase activity assay showed that Ankrd46 was a target gene of miR-451. Loss-of-function by LV-miR-451 sponge or miR-451 inhibitor led to a reduced number of embryo implantations, but had little effect on fertilization.
Conclusion(s): miR-451 was specifically up-regulated during the implantation period, and it may play a major role in embryo implantation by targeting Ankrd46. (Fertil Steril® 2014;■: ■–■. ©2014 by American Society for Reproductive Medicine.)
Key Words: Embryo implantation, miRNA, luminal epithelium, miR-451, Ankrd46

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During the process of embryo implantation, the surface of the endometrium undergoes clear morphological and molecular changes. The uterine environment with respect to implantation is divided into prereceptive, receptive, and nonreceptive phases, and the "window" of murine uterine receptivity is limited to day 4–5 of pregnancy. Even a short delay of on-time implantation may lead to poor pregnancy outcome and spontaneous pregnancy loss (1). Thus, a concurrent transition in endometrial status is critical for successful implantation, and understanding the potential molecular events is helpful in efforts to improve infertility. Accumulated evidence suggests that the "2-way" interaction between blastocyst and endometrium is a complex process involving the regulation of numerous molecular mediators, such as growth factors, cytokines, chemokines, lipids, and adhesion molecules (2). The endometrial expression of these molecules must be synchronized to generate a timely progression of their signaling pathways.

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Based on the fact that the blastocyst-endometrium crosstalk is comprised of complex molecular events, and a microRNA (miRNA) is directly responsible for the repression of hundreds of proteins, we believe that some miRNAs likely play important roles in this 2-way process, via regulation of their target genes. Of particular relevance are several recent microarray analyses focusing on embryo implantation (3–6), and some miRNAs and their targets involved in embryo implantation and associated pathologic processes, such as miR-101/199a-COX2 (7), miR-199a-Grb10 (8), miR-200a-PTEN (9), miR-141-PTEN (10), miR-224-Ptx3 (11), and miR-98-Bcl-xl (12). However, the overlap in results among studies remains less than satisfactory in defining the role of miRNAs.

In our opinion, the unsatisfactory overlap may be due, at least partially, to the histologic heterogeneity of endometrium, which is used as a sample in these studies. Endometrial tissue comprises luminal epithelium, glandular epithelium, and stroma, and the luminal epithelium cells represent only 5%–10% of the total of endometrial cells (13). The difference in genomic profile between luminal epithelium and other components in endometrium has been reported by a series of studies (13–17). Another possibility is that miRNA is up-regulated in luminal epithelium but down-regulated in other cells, and this change may be obscured by the use of whole endometrial tissue. In addition, the difference between implantation and interimplantation sites is an issue that should be addressed. Several microarray studies have shown a different expression profile of messenger RNAs (mRNAs) (17–19) and miRNAs (3, 4) in implantation vs. interimplantation endometrial sites.

In the current study, we adopted a microarray-based strategy to identify the differentially expressed miRNAs in luminal epithelium at implantation sites. Laser-capture microdissection (LCM) was used to decrease the histologic heterogeneity of endometrial tissue (20), which can lead to more-extensive and accurate representation of the miRNA profile. We clarify the role of miR-451 in mouse embryo implantation, and demonstrate that Ankrd46 is a direct target of miR-451. The loss-of-function of miR-451 in vivo leads to a reduced number of embryo implantations, but it has little effect on fertilization.

MATERIALS AND METHODS

Animal and Specimen Preparation

The Institutional Animal Care and Treatment Committee of Sichuan University approved all studies herein. Mice were housed in a semibarrier animal care facility according to institutional guidelines for laboratory animals. Adult, healthy, female C57BL6/J mice (6–8 weeks old, nonfertile, weighing 18–20 g each) were mated naturally with fertile males to produce pregnancy (1 male for every 2 females). The morning of finding a vaginal plug was designated as day 1 of pregnancy. Implantation was assumed to be taking place on day 5.

The implantation sites were made visible as blue bands by intravenous injection of 1% Chicago Blue B dye solution (0.1 ml per mouse), and the regions between blue bands were defined as interimplantation sites. Anesthetized pregnant mice were killed via euthanasia, and the implantation and interimplantation segments in uterine specimens were separated by careful dissection with clean cuts. The specimens were cut open longitudinally to expose the uterine lumen. The endometrial tissue was easily stripped from the uterine myometrium with ophthalmic forceps. Each sample was immediately frozen in liquid nitrogen for subsequent experiments.

Establishment of Animal Models

Pseudopregnancy was induced by mating adult nonfertile females with vasectomized males, with female pseudopregnancy confirmed by presence of a vaginal plug. To induce artificial decidualization, on day 5 of pseudopregnancy, when the uterus was optimally sensitized for artificial deciduogenic stimuli, 25 μl of olive oil was infused into the lumen of 1 uterine horn. The contralateral uterine horn, which was not infused, served as a control. On day 8 of pseudopregnancy, the anesthetized mice were killed via euthanasia, and the uterine tissues were isolated and frozen in liquid nitrogen.

To induce delayed implantation, on day 4, the pregnant mice were ovariectomized and intraperitoneally injected with progesterone (1 mg per mouse) to maintain delayed implantation on days 5 and 6. On day 7, the mice were injected with progesterone (1 mg per mouse), combined with 17β-estradiol (0.1 μg per mouse), to terminate delayed implantation. The anesthetized mice were killed via euthanasia 24 hours later, and the uterine tissues were isolated and frozen in liquid nitrogen. To confirm that the mice receiving only progesterone were in a state of delayed implantation, uterine flushing was collected from day 5 to day 7 and examined for the presence of hatched blastocysts. Three parallel mice were prepared for each model.

Laser-Capture Microdissection

Freshly frozen specimens were cut into 8-μm sections, fixed in 70% ethanol, washed with RNase-free water, and stained with Arcturus HistoGene staining solution (Sigma-Aldrich). The sections were then dehydrated in 75%, 95%, and 100% ethanol, followed by incubation in xylene. The sections were air dried, and LCM was performed with a Pix Cell II LCM System equipped with Laser Capture Microscope (Arcturus Engineering, Inc.) to capture luminal epithelial cells. A single LCM cap (Capture Transfer Film, Arcturus) was used for each section. Each LCM cap containing captured luminal epithelial cells was tightly fitted to a centrifuge tube containing lysis buffer, and cells dissected from various sections from a single biopsy were pooled into a single tube and stored at –80°C.

RNA Extraction, Labeling, and miRNA Microarray Hybridization

Total RNA from luminal epithelial cells captured by the LCM was extracted and purified using mirVana miRNA Isolation Kit (Ambion) following manufacturer instructions and checked for an RNA integrity number to inspect RNA integration by an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc.). MicroRNA molecules in total RNA were labeled with an miRNA Complete Labeling and Hyb Kit (Agilent Technologies, Inc.), according to manufacturer instructions. MicroRNA expression profiling was analyzed with the Agilent 8×60K