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Screening of miRNAs in human follicular fluid reveals an inverse relationship between microRNA-663b expression and blastocyst formation

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KEY MESSAGE

MIR-663B expression levels in follicular fluid were significantly inversely related to blastocyst formation and grading. MIR-663B might constitute a promising biomarker of the follicular microenvironment that could be used to predict IVF prognosis and enhance female infertility management.

A B S T R A C T

Research Question: Are miRNAs found in follicular fluid related to blastocyst formation from the corresponding oocytes?

Design: In this study, 91 individual follicular fluid samples from single follicles containing mature oocytes from 91 women were collected and classified into group 1 (n = 38) with viable blastocysts, and group 2 (n = 53) with no blastocyst. TaqMan human miRNA cards and quantitative reverse transcription polymerase chain reaction were used to identify differently expressed follicular fluid miRNAs between the two groups.

Results: We found MIR-663B to be significantly differentially expressed in follicular fluid of oocytes that yielded viable blastocysts versus those that did not develop into blastocysts (14.16 ± 7.00 versus 23.68 ± 17.02; P = 0.019), as well as for those which develop into blastocysts with good morphology versus those with poor morphology (11.69 ± 3.49 versus 20.16 ± 9.33; P = 0.003).

Conclusions: MIR-663B expression levels in human follicular fluid samples were significantly negatively related to viable blastocyst formation and may become an objective evaluation criterion for embryo development potential after IVF.

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Introduction

One of the key steps in assisted reproduction technology is the selection of the embryos with the highest development potential. Currently, the selection of day-3 embryos for fresh transfer is based on the morphological index, mainly including the numbers of blastomeres and the fragmentation rate of the embryos. This method, however, relies heavily on the experience of embryologists, and little consensus has been achieved between different IVF centres. Blastocyst culture can be used as an approach to choose good embryos, but extending embryo culture has raised some concerns about safety and cost (Martins et al., 2017).

Follicular fluid provides an important microenvironment for the development, maturation and ovulation of oocytes. Follicular fluid contains various types of hormones, proteins, metabolites and molecules that affect the developmental potential of oocytes (Chang et al., 2002; Enien et al., 1995; Mantzoros et al., 2000; Oyawoye et al., 2003; Pasqualotto et al., 2004; Wang et al., 2006; Wu et al., 2007). Micro RNAs (miRNAs) are one type of RNA molecule in follicular fluid (Sang et al., 2013). Recently, we, and others, have found that miRNAs play vital roles in reproductive diseases and in predicting embryo developmental potential (Feng et al., 2015; Rayner et al., 2010; Schetter et al., 2008; Zampetaki et al., 2010). In addition, we previously found that some miRNAs in the follicular fluid were associated with early cleavage embryo development in mice (Feng et al., 2015). Thus far, however, the role of follicular fluid miRNAs related to blastocyst formation has not been reported.

In the present study, we collected follicular fluid from the very first single aspirated follicle containing mature oocytes from patients scheduled to undergo intracytoplasmic sperm injection (ICSI). Fertilization outcome, embryo development and blastocyst morphology scoring on day 5 after insemination were recorded and evaluated. We produced differentiated miRNA expression profiles and used quantitative reverse transcription polymerase chain reaction (qRT-PCR) to identify miRNAs associated with blastocyst development.

Materials and methods

Patient characteristics

This study included 91 women who were undergoing ICSI procedures and blastocyst culture at the Shanghai Ji Ai Genetics and IVF Institute affiliated with Fudan University between July 2015 and March 2016. Written informed consent was obtained for the use of each of the follicular fluid samples at the time of oocyte retrieval, and the study was approved by the Institute Review Committee of Obstetrics and Gynecology Hospital of Fudan University (number: Kyy2015-13; date: 24 February 2015).

Exclusion criteria included male factor with azoospermia; severe oligozoospermia; and teratozoospermia; patients scheduled to undergo preimplantation genetic testing; polycystic ovary syndrome; and endometriosis. All patients were younger than 40 years, and their body mass index was less than 30 kg/m^2. The baseline hormone level was evaluated in each patient at day 3 of the menstrual cycle.

Ovarian stimulation and cycle monitoring

Ovarian stimulation was carried out using the antagonist protocol. Gonadotrophin stimulation by recombinant FSH) (Gonal-F, Serono, Switzerland) was started from the second day of the menstrual cycle. The starting dose of gonadotrophin was 150–300 IU/day, according to patient age and body weight. Monitoring was started on day 5 of stimulation, and the gonadotrophin dose was adjusted based on serum oestradiol concentrations and ovarian response observed by ultrasound. When the leading follicles reached 14 mm in diameter, 0.25 mg of cetrorelix (Merck-Serono, Germany) was added and continued daily until the day of HCG administration. Ten thousand international units of HCG (Livzon Pharmaceutical Group, Zhuhai, China) were administered 34–36 h before follicular puncture.

The sampling of follicular fluid and oocyte collection

The follicular fluid sample was collected by transvaginal ultrasound-guided puncture of an 18- to 20-mm follicle. Each sample was obtained from the patient's first aspirated follicle. The supernatant was collected after centrifugation at 300 g for 10 min. Typically, 4–6 ml of follicular fluid could be collected from the very first aspirated follicle of each patient. The samples were stored at –80°C until the RNA extraction. The cumulus oocyte complex associated with the sampled follicular fluid was isolated and the surrounding cumulus cells removed by hyaluronidase digestion. Fertilization was not attempted if the oocyte was immature (germinal vesicle or metaphase I) or in atresia and only mature metaphase II oocytes underwent ICSI. We only included one oocyte from each patient in this study.

Day-3 embryo and blastocyst evaluation

After ICSI, oocytes were incubated at a constant temperature incubator at 37°C, 5% O2, and 5% CO2. Fertilization was complete within 14–16 h after ICSI treatment. If two pronuclei were observed, the oocyte was regarded as normally fertilized. If any other number of pronuclei was observed, the oocyte was considered to be abnormally fertilized. Culture continued only if the oocyte was normally fertilized. Embryo quality was evaluated 3 and 5 days after insemination. Routine examination of day-3 embryo quality included the number of blastomeres, the degree of fragmentation and the uniformity of blastomeres (Liu et al., 2017). Embryo morphology was scored as follows: grade 1, no fragments and equal blastomeres; grade 2, less than 20% fragmentation, equal or unequal blastomeres; grade 3, equal or unequal blastomeres, 20–50% fragments; grade 4, equal or unequal blastomeres, over 50% fragments. Embryos with more than five cells and fragments less than 50% were assessed as available day-3 embryos. Embryos with at least seven blastomeres, grade 1 or grade 2 were defined as excellent day-3 embryos (Hardarson et al., 2001). The percentage of blastocyst formation was determined, and each blastocyst was assigned a score using the system established by Gardner et al. (2000) Briefly, blastocysts were given a numerical score from 1 to 6 on the basis of their degree of expansion and hatching status, as follows: 1, an early blastocyst with a blastocoel that is less than half the volume of the embryo; 2, a blastocyst with a blastocoel that is half of or greater than half the volume of the embryo; 3, a full blastocyst with a blastocoel completely filling the embryo; 4, an expanded blastocyst with a blastocoel volume larger than that of the early embryo, with a thinning zona; 5, a hatching blastocyst with the trophectoderm starting to herniate through the zona; and 6, a hatched blastocyst, in which the blastocyst has completely escaped from the zona. For blastocysts graded as 3 to 6, i.e., full blastocysts onward, the development of the inner cell mass was assessed as follows: A, tightly packed, many cells; B, loosely grouped, several cells; or C, very few cells. The

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