



MicroRNA in follicular fluid: new molecular markers of oocyte quality in cancer patients

Stefania Gieri¹, Cinzia Di Pietro², Marilena Vento³, Giuseppa Scandurra⁴, Giusi I. Forte⁵, Giorgio Russo⁴, Paolo Scollo⁴

¹ SIGO/MSD researcher, Italy

² Department Gian Filippo Ingrassia, Biologia Cellulare e Molecolare, Genetica, Genomica Giovanni Sichel, Università di Catania, Italy

³ Servizio di PMA/Azienda Ospedaliera Cannizzaro, Catania, Italy

⁴ Division of Gynecological and Medical Oncology, Department of Maternal and Child, Cannizzaro Hospital, Catania, Italy

⁵ Institute of Molecular Bioimaging and Physiology, National Research Council, Cefalù, Palermo, Italy

ABSTRACT

Objective: to investigate the expression profile of microRNA in cancer patients, before radio/chemotherapy, compared with age-matched healthy patients.

Patient(s): patients with malignant disease, younger than 35 years, with a good ovarian reserve and who didn't receive any prior treatment within comparative studies with healthy, age-matched patients whose primary infertility is due to a male factor.

Main Outcome Measure(s): fast real time PCR system to investigate the expression miRNA profiles. 2-CT method to calculate relative gene expression levels between the two groups of patients. Bioinformatic tools for miRNA target prediction and pathway analysis.

Results: our ongoing research project aims to identify highly represented miRNA in follicular fluid of cancer patients compared with healthy group. Our results will improve knowledge of the different signature which have a strong effect on reproductive health.

Keywords: microRNA, molecular markers, oncofertility.

SOMMARIO

Obiettivo: analizzare la significatività dei microRNA in pazienti affetti da cancro, prima della radio/chemioterapia, rispetto a pazienti sani di pari età.

Pazienti: soggetti con malattia maligna, di età inferiore a 35 anni, con una buona riserva ovarica e che non abbiano ricevuto alcun trattamento precedentemente all'interno di studi comparativi con pazienti sani, di pari età la cui sterilità primaria è dovuta ad un fattore maschile.

Misure principali di risultato: sistema PCR veloce in tempo reale per studiare l'espressione dei profili miRNA; metodo 2-CT per calcolare i relativi livelli di espressione genica tra i due gruppi di pazienti; strumenti bioinformatici per la previsione dei miRNA e analisi del percorso.

Risultati: il nostro progetto di ricerca si propone di individuare i miRNA altamente rappresentati nel liquido follicolare di pazienti affetti da cancro rispetto al gruppo sano. I nostri risultati miglioreranno la conoscenza di diversi parametri che hanno un forte effetto sulla salute riproduttiva.

INTRODUCTION

Oncofertility is a term coined for fertility preservation in cancer patients. Improvement in cancer management and increasing survival rates has created a need for oncofertility. Current data suggest that for most tumors posttreatment pregnancy does not increase the risk of cancer

progression or obstetric or neonatal outcome⁽¹⁾. The emphasis therefore has moved from providing life to providing quality of life⁽²⁾.

During physiological reproductive aging the primordial follicle count declines rapidly. This is associated with decreased oocyte quality, increased aneuploidy, and reduced fertility and fecundity⁽³⁻⁴⁾. Reduced ovarian reserve and reproductive potential can be quantified by changes in markers including reduced antral follicle count (AFC), increased serum FSH

Correspondence to: stefaniagieri@msn.com

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(follicular stimulating hormone), reduced AMH (antimüllerian hormone) and reduced inhibin-B⁽⁹⁾.

At the time the effect of malignancy on ovarian function remains unclear. Several retrospective studies have addressed the potential impaired ovarian reserve due to malignancy. A review explained different effect on ovarian reserve in cancer patients compare with healthy controls. For example, Pal et al compared the in vitro fertilization (IVF) outcomes of 5 women with malignancy with 12 women with tubal factor infertility and found that cancer was associated with a reduction in oocyte maturity and quality and decreased fertilization rate. The authors postulated a possible detrimental biological effect of the malignancy on the oocytes. In another study, Lawrenz et al compared a group of women with Hodgkin's and non-Hodgkin's lymphoma (pretreatment) with healthy controls. They found lower serum AMH levels as well as decreased response to IVF treatment in those with disease, suggesting that malignancy itself may be deleterious to the ovarian reserve. Agarwal et al. discussed a possible adverse association between the neoplasia with an increased metabolic state and hypothalamic dysfunction, causing infertility.

Quintero et al compared 50 women with malignancy with predicted good response to ovarian stimulation with 50 age-matched controls that underwent IVF for male factor infertility. The number of oocytes and mature oocytes and the number of fertilized oocytes were comparable between the two groups. However, significant differences were demonstrated in the dose and length of gonadotropin stimulation required, suggesting possible damage of the functional ovarian reserve in women with malignancy. Moreover, recently Oktay et al. stated that women with breast and ovarian cancer, carriers of BRCA1 mutation, may respond poorly to ovarian stimulation. On the other hand, other studies have failed to demonstrate these differences between women with malignancy and the control group⁽⁶⁻⁸⁾.

For female patients, methods for fertility preservation have been developed and are currently classified into clinically established methods such as cryopreservation of oocytes, whereas ovarian tissue cryopreservation (OTC) is still considered experimental by international collaborative work groups⁽⁸⁾. Pregnancy rate obtained with cryopreserved oocytes after vitrification is 20%⁽⁹⁾. The number of oocytes retrieved and their quality are imperative factors predicting the potential efficacy of the fertility preservation procedure⁽¹⁰⁾. Comparing the

expression profile of messenger RNAs in single vitrified-thawed oocytes with that of fresh collected oocytes was assessed the biologic quality of oocytes. Di Pietro et al. proved for the first time the absence of statistically significant variation⁽¹¹⁾.

Unfortunately these data refer only to healthy infertile women whereas data on cancer patients are still lacking. Therefore, the effect of cancer on the ovarian response before IVF for fertility preservation has lacked consensus⁽¹⁰⁾. It is reasonable to think that some biochemical characteristics of the follicular fluid (FF) surrounding the oocyte may play a critical role in determining oocyte quality and its analysis may provide useful information on pathways involved on follicle differentiation and development⁽¹²⁾. The discovery of FF miRNA could open up the possibility to use these molecules as biomarkers of oocyte quality. Santonocito et al. identified 37 microRNAs upregulated in FF. These miRNA are involved in critically important pathways for follicle growth and oocyte maturation. Specifically, nine of them target and negatively regulate mRNAs expressed in the follicular microenvironment encoding inhibitors of follicle maturation and meiosis resumption⁽¹³⁾.

MATERIALS AND METHODS

Patients: We are collecting FF samples from healthy women and from cancer patients selected by an IVF center (Servizio di PMA, Azienda Ospedaliera Cannizzaro, Catania). Patients must fulfill all the following criteria: healthy women younger than 35 years whose primary infertility is due to a male factor; this excluded pathologies that could influence oocyte quality (e.g., endometriosis, polycystic ovaries, and ovarian insufficiency) and cancer patients younger than 35 years with a cancer diagnosis, with a good ovarian reserve (FSH, AMH) and who didn't receive any prior treatment. The patients must sign an informed consent including the use of collected samples. The study is exempted from Institutional Review Board approval because patients are included in IVF program.

OVARIAN STIMULATION AND SAMPLE COLLECTION

For healthy patients hormone stimulation is performed by treatment with GnRH agonists (triptorelin or buserelin), followed by ovarian stimulation with recombinant FSH and hMG.

Stimulation is monitored using both serum and E2 concentrations as well as ultrasound measurements of follicle numbers and diameters. When follicles reach a diameter >18 mm and serum E2 concentration per follicle reach 150–200 ng/L, ovulation is induced with 10,000 IU of hCG. Transvaginal ultrasound-guided aspiration of ovarian follicles is performed 34–36 hours after hCG injection. FF samples are centrifuged for 200 at 2,800 rpm at 4°C to remove follicular cell residues and any blood traces; the supernatant is immediately transferred into a clean polypropylene tube and stored at -20°C for further analysis. Samples with massive blood contamination are excluded from further analysis. FF of individual follicles is kept separated until decumulation of the oocytes to collect only the FF in which nuclear mature oocytes (metaphase II) are identified. Most cancer patients are treated with a GnRH antagonist-based protocol since this protocol provides the shortest delay of the cancer treatment and the lowest risk of impending ovarian hyper stimulation syndrome (OHSS) by induction of ovulation with GnRH agonist. Specifically for this patients group ovarian stimulation protocol changes depending on malignancy and on patient's menstrual cycle day.

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METHODS

At the time we are waiting to collect all the necessary samples. miRNA isolation, reverse transcription and preamplification will be done to profile the expression of miRNA in cancer patients compared with healthy group. Expression profiles are investigated by using TaqMan Low-density array technology in a 7900HT fast real time PCR system. After that it will be possible expression data analysis. To calculate relative gene expression levels between the two patients groups is applied the 2-CT method. Bioinformatic analysis (miRNA targets prediction and pathways analysis) will be performed to identify miRNA involved in signaling pathways critically important for follicle growth, oocyte maturation, and early embryo development.

RESULTS

Our research project is ongoing. Expression data analysis will lead to identification of miRNA highly represented in follicular fluid and involved in the regulation of ovarian follicular pathways of cancer patients. Therefore these identified miRNA could represent non invasive molecular markers of oocyte quality.

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