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Increased expression of PGRN protein in follicular fluid and mRNA in granulosa cells in overweight patients with polycystic ovary syndrome

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ABSTRACT

Background: Obesity and low-grade chronic inflammation play critical roles in pathological process of PCOS. PGRN is an adipokine and was recently reported that it could induce a low-grade chronic inflammatory state and plays a functional role in insulin resistance associated with obesity. The overall goal of the present study was to evaluate the levels of PGRN in follicular fluid (FF) and the expression of PGRN in granulosa cells (GCs) with respect to the quality of the oocytes both in patients with PCOS and in the normal ovary during COH cycles.
Methods: Ninety-two patients underwent IVF-ET were divided into four groups based on body mass index: normal-weight PCOS group; overweight PCOS group; non-overweight control group and overweight control group. FF samples and GCs were collected at the time of oocyte retrieval. The PGRN, TNF-α, IL-6 and MCP-1 levels were measured by ELISA, and the mRNA expression of PGRN in GCs was detected by real-time polymerase chain reaction.
Results: Analysis of PGRN expression revealed that PGRN levels in FF and the mRNA expression of PGRN in GCs were higher in patients with PCOS than in control patients, and higher in overweight patients than in the normal-weight patients; PGRN in FF of PCOS was positively correlate with basal testosterone and FF TNF-α, but negative correlation with retrieved oocytes number
Conclusion: This study suggests that PGRN may be a crucial determinant of fertilization success for PCOS patients.

Background

Polycystic ovary syndrome (PCOS), one of the most common endocrine and metabolic disorders of women in reproductive age, is characterized by ovulatory infertility, polycystic ovaries, hyperandrogenism, low-grade chronic inflammation, insulin resistance (IR) and obesity [1]. Much of the published literature states that PCOS patients exhibit some degree (50–70%) of insulin resistance [2]. And varying degrees (60–70%) of obesity, especially abdominal obesity [3,4]. It has been verified that low-grade chronic inflammation is a key link between obesity and insulin resistance in PCOS patients [5], the mechanisms underlying this association are of critical importance in the pathological process of PCOS but it's still not clear.

Follicular fluid (FF) provides an important and suitable microenvironment for the development of oocytes. FF is involved in follicular development, early embryo development and implantation [6]. Numerous studies had demonstrated that a variety of

Abbreviations: BMI, Body mass index; CRP, C-reactive protein; E_2, Estradiol; ELISA, The enzyme-linked immunosorbent assay; FBG, Fasting plasma glucose; FF, Follicular fluid; FIN, Fasting blood insulin; FSH, Follicle-stimulating hormone; GCs, Granulosa cells; GnRHant, Gonadotropin-releasing hormone antagonist; hCG, Human chorionic gonadotrophin; HFD, High fat diet; HOMA-IR, Homeostasis Model Assessment-linsulin resistance; ICSI, Intracytoplasmic sperm injection; IL-6, Interleukin-6; IVF-ET, In vitro fertilization-embryo transfer; IR, Insulin resistance; LH, Luteinizing hormone; MCP-1, Monocyte chemotactic protein-1; OHSS, Ovarian hyperstimulation syndrome; P, Progesterone; PCOS, Polycystic ovary syndrome; PGRN, Progranulin; T, Testosterone; TNF)-α, Tumor necrosis factor-α; TNFR, Tumor necrosis factor receptor.

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proinflammatory cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-8, IL-11, monocyte chemotactic protein 1 (MCP-1), leukemia inhibiting factor and the IL-1 system, are crucially involved in folliculogenesis [7], implantation [8] and follicular maturation [9–11]. In addition, in-vitro studies have shown that physiologically levels of recombinant adiponectin induce ovarian gene expression and steriodogenesis in mammalian ovaries [9]. There is accumulative evidence showing direct effects of adipokine on the late stages of folliculogenesis [9].

Recently it was reported that progranulin (PGRN, also called PGRN-epithelin precursor, proepithelin), an adipokine, could mediate high fat diet (HFD) induced insulin resistance and obesity [12]. PGRN has proinflammatory properties and has been identified as a novel marker of chronic inflammation in obesity and type 2 diabetes where circulating PGRN levels were significantly elevated [13]. Furthermore, it was reported that PGRN could induce a low-grade chronic systemic inflammatory state which plays a functional role in insulin resistance associated with obesity.

It was reported that PGRN was specifically localized in granulosa cells (GCs) in pig developing follicles [14]. As an adipokine and proinflammatory cytokine, we hypothesize that PGRN protein may be correlated to obesity, insulin resistance and follicular development in PCOS patients. Nevertheless, there is no published study on the role of PGRN expression during the development of oocytes in overweight patients with PCOS. The purpose of our study was to evaluate the levels of PGRN protein in FF and granulosa cells in overweight patients with PCOS and to analyze the relationship between PGRN with IR, obesity and follicular development in PCOS patients.

Materials and methods

Ethical approval and patient consent

We strictly obeyed the Declaration of Helsinki for Medical Research involving human subjects during the project and informed consent was obtained from each patient before their inclusion in the study. The study was approved by the Ethical Research Committee of Renmin Hospital of Wuhan University (reference number. WHR13102.) and was conducted in accordance with the institutional guidelines.

Subjects

Patients who undergwent in vitro fertilization(IVF) or intracytoplasmic sperm injection (ICSI) procedures were recruited from the outpatient clinics of the Reproductive Medicine Center in Renmin Hospital of Wuhan University (Wuhan, China) between May 2014 and January 2015.

In this study we analyzed 39 patients with PCOS and 53 age-matched ovulatory women as controls. PCOS was diagnosed by the Rotterdam 2003 criteria. When two out of the following three features were present: oligoovulation and/or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovaries on ultrasound examination (the presence of 12 or more follicles measuring 2–9 mm in diameter and/or ovarian volume >10 cm^3). Oligoanovulation was defined as the presence of oligomenorrhea (menstrual cycles of >35 days) or amenorrhea (lack of menstrual period for 6 months or more) [15]. The following etiologies of patients in control group were included: male factors (sperm abnormalities) and female factors (tubal factor), and all patients with normal ovarian reserve. Patients who had taken oral contraceptive agents for at least 3 months before the study, or with a previous diagnosis of endometriosis, immune infertility, history of pelvic tuberculosis, ovarian surgery, macroprolactin or thyroid dysfunction were excluded.

Design

The subjects were divided into four groups based on body mass index (BMI): normal weight PCOS group (group A, BMI < 25 kg/m^2, n = 24); overweight PCOS group (group B, BMI ≥ 25 kg/m^2, n = 15); normal weight control group (group C, BMI < 25 kg/m^2, n = 35) and overweight control group (group D, BMI ≥ 25 kg/m^2, n = 18).

Determination of estradiol (E_2), follicle stimulating hormone(FSH), luteinizing hormone (LH), Testosterone(T), fasting plasma glucose (FBG) and fasting blood insulin(FIN) concentrations

Serum levels of E2, LH, FSH, T, FIN were measured using an Immulite® 2500 immunoassay analyzer (Siemens, Munich, Germany). Prior to each test, the Immulite® 2500 was calibrated with three control samples containing low, medium and high concentrations of the appropriate hormones. FBG was analyzed by enzymatic assays using an autoanalyzer (Siemens, Munich, Germany). HOMA used the following formula: HOMA-IR = fasting insulin (uU/ml)/fasting Glu (mmol/L)/22.5 [16]. A value $\geq$2.14 was considered to be indicative of IR [17].

Ovarian stimulation, FF sampling, granulosa cells and oocyte collection

PCOS patients took a consecutive cycle of oral contraceptive agents (Diane-35, Schering, Berlin, Germany) for pre-treatment at D5, then patients in all four groups underwent a long treatment protocol with gonadotropin-releasing hormone agonist administration in the midluteal phase at D21, followed by ovarian stimulation with recombinant FSH(Gonal-f®, Merck Serono, Geneva, Switzerland; or Puregon®; Schering-Plough Corporation, Kenilworth, Nj, USA) at D3. Following confirmation of ovarian suppression and when at least three follicles had reached a mean diameter of 18 mm under transvaginal ultrasound examination (GE Logiq 400 Pro, GE Healthcare Life Sciences, Shanghai, China), 10,000 IU human chorionic gonadotropin (hCG; Livzon Pharmaceutical Group Inc.) was administered subcutaneously. After 34–36 h, oocytes were retrieved by Aloka ProSound SSD-3500 ultrasound-guided transvaginal puncture (Hitachi-Aloka Medical, Ltd., Guangzhou, China). An individual aspiration was conducted to collect the oocytes,FF was collected from ovarian follicles that were $\geq$14 mm, and was pooled for each patient. FF samples were centrifuged at 2,000 x *g* for 10 min, and the supernatants were stored at -80° C for further analysis.

For extraction of mural granaulosa cells, the pellets were digested by 0.2% hyaluronidase (Sigma, St. Louis, MO, USA) for 10 min at 37 °C and then resuspended in PBS. The suspension was pipetted and layered onto Ficoll-Paque (Roche, Basel, Swit) and centrifuged for 20 min at 1,000 x *g*. Cells at the interface were removed and washed twice with PBS; the resulting pellets were stored at -80° C until assays were performed.

Assessment of oocyte morphology and maturation

Oocytes isolated from FF samples were evaluated. The cumulus oophorus and corona radiata were removed from the oocytes by mechanical pipetting in SynVitro Flush containing 300 IU/ml hyaluronidase (Sigma-Aldrich) for up to 1–2 min depending on the extent of cumulus investment. Nuclear maturation of the oocytes was determined by the identification of the first polar body. On day 2 or 3, the oocytes were sorted into four categories, based on their morphologic appearance, zonal thickness, cytoplasmic