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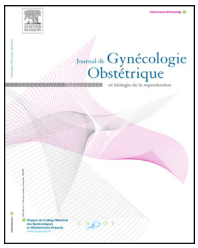

ORIGINAL ARTICLE

The WNT/β-catenin signaling pathway may be involved in granulosa cell apoptosis from patients with PCOS in North China

La voie de signalisation/β-caténine peut être impliquée dans l'apoptose des cellules granulosa de patients souffrant du SOPK en Chine du Nord

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KEYWORDS
WNT/β-catenin signaling pathway;
Granulosa cells;
Polycystic ovary syndrome

Summary
Introduction. — Investigate the expressions of WNTs in granulosa cells of PCOS in North China, and explore the possible mechanism.
Material and methods. — Patients with PCOS ($n = 40$) and controls without PCOS ($n = 20$) were enrolled into the study. The levels of serum sex hormone were detected by chemiluminescent enzyme immunoassay. RT-qPCR was used to measure mRNA levels of WNT family members (WNT1, WNT3, WNT4, WNT5, sFRP4 and sFRP5). The levels of Bax and Bcl-2 were measured by enzyme-linked immunosorbent assay.
Results. — The levels of PROG, TES and LH/FSH had difference ($p < 0.05$) on third day of menstrual cycle between PCOS and control groups. After injection of HCG, the levels of LH, E_2 and PROG had significant difference ($p < 0.05$). The PCOS group had higher transcript levels of WNT1, WNT3 and WNT4 ($p < 0.05$), compared to controls. The level of secreted frizzled-related protein (sFRP4) was lower level in PCOS patients ($p < 0.05$). In addition, we found a significant reduction of the β-catenin as well as the downstream targets (survivin and BMP4) ($p < 0.05$). The level of Bax was significantly higher in PCOS group than in the control group ($p < 0.05$). Above all, the WNT/β-catenin signaling pathway might be involved into granulosa cell apoptosis, which may provide a strategy for clinical treatment.

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MOTS CLÉS
Voie de signalisation WNT/β-caténine ;
Cellules granulosa ;
Syndrome des ovaires polykystiques

Résumé
Introduction. — Étudier l'expression des WNT dans les cellules granulosa chez les patients souffrant du SOPK en Chine du Nord et explorer le mécanisme possible.
Matériel et méthodes. — Des patients souffrant de SOPK ($n = 40$) et des témoins sans SOPK ($n = 20$) participaient à l'étude. Un dosage enzymo-immunologique par chimioluminescence était utilisé pour déterminer le taux plasmatique d'hormones sexuelles. RT-qPCR était utilisé pour mesurer les taux des ARN-messagers des membres de la famille WNT (WNT1, WNT3, WNT4, WNT5, sFRP4 et sFRP5). Les taux de Bax et de Bcl-2 étaient déterminés par Elisa.
Résultats. — Au troisième jour du cycle, les taux de PROG, de TES et de LH/FSH montraient des différences entre le groupe SOPK et le groupe témoin ($p < 0,05$). Après injection d'HCG, les taux de LH, d'E_2 et de PROG montraient une différence significative. Les taux des transcripts de WNT1, de WNT3 et de WNT4 étaient plus élevés dans le groupe SOPK ($p < 0,05$) comparé au groupe témoin. Le taux de sFRP4 était plus bas dans le groupe SOPK ($p < 0,05$). Nous avons trouvé, en plus, une réduction significative de la β-caténine et d'autres cibles en aval (survivine et BMP4) ($p < 0,05$). Le taux de Bax était plus élevé dans le groupe SOPK que dans le groupe témoin ($p < 0,05$). Surtout, la voie de signalisation WNT/β-caténine serait impliquée dans l'apoptose des cellules granulosa, suggérant une stratégie thérapeutique éventuelle.

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder with reproductive dysfunction and abnormal metabolic in women, which affects 5—10% of women of reproductive age [1]. It is thought to be one of the leading causes of female subfertility [2—4]. PCOS is characterized with symptoms such as infertility, hirsutism, a high level of androgens, irregular menstrual cyclicity, obesity, insulin tolerance. Although there are a large number of antral follicles in ovarian of PCOS patients, mature follicles can't be produced periodically and its pathology mechanisms are unclear.

During the development of mammalian ovarian follicles, a limited number of follicles are selected for ovulation, while the rest undergo a degenerative process called physiological atresia. Primordial follicles and primary follicles atresia are mainly caused by the apoptosis of oocyte [5]. At the late stage of follicular development, atresia of a locking cavity follicles and mature follicles may be induced by apoptosis of oocyte itself or ovarian granulosa cells [6]. There are two kinds of mechanism of apoptosis [7], one is induced by the combination of cell surface receptor with death factor, the other is mediated by mitochondrion. However, the underlying mechanism of follicular atresia in PCOS remains unclear. The identification of the signaling pathways involved in granulosa cell apoptosis not only better understand how to control follicular development in mammalian, but also further clarify the association between PCOS and ovarian infertility or assisted reproduction outcomes [8].

β-catenin, a multifunctional protein, is crucial in process of proliferation, growth, differentiation and survival [9]. It is the core component of WNT pathway [10]. In vitro luteinized granulosa cells toxicity model, Sanchez A.M. et al. found WNT4 upregulation and WNT3 down-regulation of WNT pathway may be targeted to regulate follicle development, ovulation, and luteinization in order to limit the follicular damage caused by chemotherapy [11]. Recently, Gupta P.S. et al. established an intriguing regulatory of WNT signaling pathway, which is associated with follicular growth and steroidogenesis, in FSH action on bovine granulosa cells. In non lactating Holstein dairy cows, granulosa cells were isolated from F1 (largest follicle; future dominant) and F2 (second largest follicle; future subordinate) follicles [12]. It provides novel insight on the hormonal regulation of expression of WNT system in granulosa cells.

In recent years, the incidence of PCOS was higher, due to environmental and social factors. In the study, we examined the relation of WNT/β-catenin signaling pathway in granulosa cell to apoptosis based on survival-related genes, survivin and bone morphogenic protein 4 (BMP4). The purpose of the study is to investigate the how WNT/β-catenin signaling pathway is altered in granulosa cell of PCOS in North China, and explored the mechanism. It will provide therapeutic target in assisted reproduction technology.

Material and methods

Ethics statement

All subjects were Han ethnic from Shanxi Province in North China. Written informed consent was obtained from all participants, and the studies were approved by the Institutional ethics committee of the Shanxi Women and Children Health Hospital.

Patient selection

A prospective case-control study was designed including 40 PCOS patients and 20 controls, undergoing in vitro fertilization (IVF) in the center of Reproductive Medicine of Shanxi Women and Children Health Hospital from September 2013 to October 2014.

Inclusion criteria for selection of cases [13]. The Rotterdam Criteria was used to diagnose PCOS and excluded other androgen excess disorders: Cushing's syndrome, androgen secreting tumors, thyroid diseases, hyperprolactinemia, drug-induced androgen excess, and other causes for anovulation. Controls with male infertility were selected including

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