Reproductive BioMedicine Online (2016) 32, 635–641

www.sciencedirect.com
www.rbmonline.com

ARTICLE

Luteinizing hormone/chorionic gonadotrophin receptor overexpressed in granulosa cells from polycystic ovary syndrome ovaries is functionally active

Venkateswarlu Kanamarlapudi [a,*], Uma D Gordon [b], Andrés López Bernal [c]

[a] *Institute of Life Science 1, College of Medicine, Swansea University, Swansea, UK;* [b] *Bristol Centre for Reproductive Medicine, North Bristol NHS Trust, Southmead Hospital, Westbury-on-Trym, Bristol, UK;* [c] *Division of Obstetrics and Gynaecology, School of Clinical Sciences, University of Bristol, Henry Wellcome Laboratories for Integrative Neuroscience and Endocrinology, Bristol, UK*

* Corresponding author. *E-mail address:* k.venkateswarlu@swansea.ac.uk (V Kanamarlapudi).

Professor Venkateswarlu Kanamarlapudi is currently working as Professor of Molecular Cell Biology and Pharmacology at Swansea University, UK. His current research interest is in ADP-ribosylation factor (ARF)6 small GTPase- and PI 3-kinase-mediated signalling pathways in health and disease.

Abstract Polycystic ovarian syndrome (PCOS) is associated with anovulatory infertility. Luteinizing hormone/chorionic gonadotrophin receptor (LHCGR), which is critical for ovulation, has been suggested to be expressed prematurely in the ovarian follicles of women with PCOS. This study aimed to analyse the expression and activity of LHCGR in ovarian granulosa cells from PCOS patients and the involvement of ARF6 small GTPase in LHCGR internalization. Granulosa cells (GC) isolated from follicular fluid collected during oocyte retrieval from normal women ($n = 19$) and women with PCOS ($n = 17$) were used to study differences in LHCGR protein expression and activity between normal and PCOS patients. LHCGR expression is up-regulated in GC from PCOS women. LHCGR in PCOS GC is functionally active, as shown by increased cAMP production upon human gonadotrophin (HCG)-stimulation. Moreover, ARF6 is highly expressed in GC from PCOS patients and HCG-stimulation increases the concentrations of active ARF6. The inhibition of ARF6 activation attenuates HCG-induced LHCGR internalization in both normal and PCOS GC, indicating that there are no alterations in LHCGR internalisation in GC from PCOS. In conclusion, the expression and activation of LHCGR and ARF6 are up-regulated in GC from PCOS women but the mechanism of agonist-induced LHCGR internalization is unaltered.

KEYWORDS: ARF6, cAMP, human granulosa cells, LHCGR, PCOS

http://dx.doi.org/10.1016/j.rbmo.2016.03.003

Introduction

Polycystic ovarian syndrome (PCOS) is one of the commonest disorders of ovulation, affecting 5–10% women of reproductive age (The Rotterdam ESHRE/ASRM-Sponsored Pcos Consensus Workshop Group, 2004). It is characterized by ovulation failure, polycystic ovaries and excessive androgen production, which lead to infertility (The Rotterdam ESHRE/ASRM-Sponsored Pcos Consensus Workshop Group, 2004). PCOS is a complex syndrome involving endocrine and metabolic abnormalities. One of the characteristic endocrine defects is the inappropriate exposure of granulosa cells (GC) of immature follicles to raised concentrations of LH. The elevated LH may prevent follicular maturation and contribute to failure to ovulate (Yong et al., 1992). LH and chorionic gonadotrophin (CG) share the same receptor (luteinizing hormone/chorionic gonadotrophin receptor [LHCGR]) to activate the $G\alpha_s$ subunit of the G-protein/adenylyl cyclase (AC)/cyclic adenosine monophosphate (cAMP) pathway (Choi and Smitz, 2014). Additional coupling of LHCGR through Gq proteins activates phospholipase C, protein kinase B and ERK1/2 pathways (Choi and Smitz, 2014). LHCGR is overexpressed in PCOS GC, which tend to be hypersensitive to LH stimuli with excessive androgen production (Ehrmann et al., 1995). Moreover, previous studies have suggested that LHCGR, which is critical for ovulation, may be induced prematurely in women with PCOS (Jakimiuk et al., 2001; Willis et al., 1998). Despite a plethora of studies on clinical, metabolic, genetic and evolutionary factors in PCOS (Ben-Shlomo and Younis, 2014; Corbett and Morin-Papunen, 2013; Gorry et al., 2006; Williams et al., 2013), only a few studies have advanced our understanding of the physiopathology of this disorder.

After agonist stimulation, LHCGR internalises through ARF6 small GTPase-mediated β-arrestin-clathrin-dynamin pathway (Hunzicker-Dunn et al., 2002; Kanamarlapudi et al., 2012b). LHCGR internalization and recycling regulate the density of cell-surface receptors and thereby the sensitivity of the cells to LH (Bhaskaran and Ascoli, 2005). ARF6 is a member of the ARF family of small GTPases that regulates membrane trafficking by cycling between the active GTP- and inactive GDP-bound states (Donaldson and Jackson, 2011). ARF are activated by guanine nucleotide exchange factors (GEF) and inactivated by GTPase-activating proteins (GAP). Among the six known mammalian ARF isoforms (ARF1–6), ARF1 and ARF6 are the best characterized. ARF1 localizes to and acts at the Golgi, whereas ARF6 localizes to and acts at the cell periphery. ARF6 mediates cell-surface-receptor internalization and the actin cytoskeleton reorganization beneath the plasma membrane (Donaldson and Jackson, 2011). The activation of ARF1–5, but not ARF6, is inhibited by the fungal toxin brefeldinA (BFA). However, ARF6 activation by the cytohesin family of ARF GEF is inhibited by a chemical inhibitor, secinH3 (Hafner et al., 2006).

This article focuses on functional mechanisms of gonadotrophin response in PCOS GC to complement previous studies on clinical and genetic factors. The aim of this study was to analyse the expression and activity of LHCGR and ARF6, and measure ARF6-mediated LHCGR internalization in GC from normal and PCOS women.

Materials and methods

GC isolation and culture

GC were obtained from follicular fluid collected during oocyte retrieval from women undergoing IVF/intracytoplasmic sperm injection (ICSI) at the Bristol centre for Reproductive Medicine. Aspirated follicular fluid is an excellent source of granulosa-lutein cells that retain functional Gs-adenylyl cyclase and Gq-phospholipase C coupling (Asboth et al., 2001; Carrasco et al., 1997). To obtain a sufficient number of GC for some experiments, follicular fluid from several individual follicles were combined. The normal group included 19 women (age range 25–43, mean 34.2 years) undergoing IVF/ICSI with regular menses, unexplained infertility, tubal factor or male factor infertility and normal ovarian morphology on ultrasound. The PCOS group comprised 17 women (age range 24–41, mean 35.6 years) with irregular periods, infertility, hyperandrogenism and morphological appearance of polycystic ovaries; the diagnosis was made according to the established guidelines (The Rotterdam ESHRE/ASRM-Sponsored Pcos Consensus Workshop Group, 2004). The body mass index (BMI; kg/m^2) in the normal (22.5 ± 7) and PCOS (23.3 ± 3.5) groups was similar. The duration of infertility at 48 months (range 36–72) was significantly longer in the PCOS group than in the normal group (36, range 24–48 months; $P = 0.006$). Serum FSH (IU/l) concentrations were similar in the two groups: 6.5 ± 1.6 versus 6 ± 1.3 for normal and PCOS, respectively. The study was conducted with local research ethics committee approval (the project [ID 1974] approved on 10/03/2008) and written informed consents were obtained from all participants.

Both groups of women received gonadotrophin-releasing hormone analogue nasal spray (Suprefact, Aventis Pharma, Kent, UK) for pituitary desensitization. Serum oestradiol was measured to confirm pituitary suppression. Following this, FSH (human menopausal gonadotrophin; Menopur, Ferring, UK) was used to induce follicular maturation, which was monitored with vaginal ultrasound scans. The total dose of FSH (IU) used was 2428 ± 978 for the normal group and 2536 ± 1189 for the PCOS group; the difference was not statistically significant. When at least three follicles reached the size of ≥17 mm, 6500 IU recombinant human chorionic gonadotrophin (HCG) (Ovitrelle, Merck Serono, Feltham, UK) was administered to complete follicular/oocyte maturation. Transvaginal ultrasound oocyte retrieval was undertaken 36 h after HCG administration. The clinical pregnancy rates were similar in both normal and PCOS groups (39% in women under 35 years old and 24% in women over 39 years old).

GC were isolated from the pooled follicular fluid and washed twice with phosphate-buffered saline containing 0.5% bovine serum albumin (PBS-BSA) by centrifugation at 400*g* for 10 min. The GC were resuspended in 10 ml Dulbecco's modified Eagle's medium (DMEM) and over layered onto 12.5 ml sterile 35% Percoll in PBS-BSA and centrifuged for 30 min at 400*g* to obtain GC as the layer at the interface of medium and Percoll (Asboth et al., 2001). GC were then washed three times with PBS-BSA and resuspended in DMEM containing 2 mmol/l glutamine, 100 U/ml penicillin, 0.1 mg/ml streptomycin and 10% fetal calf serum (complete medium), and cell number and viability were determined by Trypan Blue (0.4%) staining. This method routinely obtains $1.5–3 \times 10^6$ cells with 75–85%