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FELLOWSHIP FINAL REPORT

Cross-talk between Follicle stimulating hormone receptor (FSHR), and Luteinizing hormone receptor (LHR) involving Insulin receptor substrates (IRS-1 and IRS-2)

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REPORT INFO

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ABSTRACT

Follicle stimulating hormone (FSH) and luteinizing hormones (LH), the gonadotropins are the regulators of follicle growth, ovulation, and oocyte maturation. An imbalance in their levels or activity is known to cause subfertility or infertility, especially in women with polycystic ovary syndrome (PCOS). In many of these women, LH levels are high along with untimely LH receptor (LHR) expression in granulosa cells in the follicular phase of menstrual cycles. Here, we demonstrate that high LHR activity due to high LH stimulate abnormal cAMP levels. The interaction between FSHR and IRS proteins (IRS-1 or IRS-2) is altered due to high LH/LHR expression/activity. This study demonstrates novel therapeutic targets in women with PCOS. The inhibition of high LHR activity with antagonistic peptides or LHR specific nanobodies would pave a way towards management of hormonal imbalance in women with PCOS.

1-Introduction

Systemic high levels of luteinizing hormone (LH) and/or untimely higher expression of LH receptor (LHR) in granulosa cells in the ovarian follicles of women with polycystic ovary syndrome (PCOS) results in abnormal responses to follicle stimulating hormone (FSH) during the follicular phase [1-2]. Balance in the levels of FSH and LH is critical for normal follicular growth and oocyte maturation. However, FSH-mediated metabolic responses are impaired in granulosa cells of women with PCOS [1]. Most women with PCOS need assisted reproductive technology (ART) for successful oocyte development and conception. However, major challenges still exist in achieving successful pregnancy in these women [3-4]. One of the major issues is the optimization of the relative dose of gonadotropins (FSH and LH/hCG) required for good IVF outcomes.

Our previous work established that FSH stimulates, in an insulin-independent way, the expression and activity of insulin

receptor substrates (IRS-1 and IRS-2), which are important for reproductive and metabolic homeostasis in the ovaries [5]. Further, we showed that higher LH or LHR activity increases the heteromerization of LHR and FSHR in granulosa cells, which impedes the FSH-mediated increase in glucose uptake and glycogen synthesis in an FSH-dependent manner [6-7]. Intriguingly, the direct molecular consequences of LHR and FSHR crosstalk in granulosa cells are unclear.

This study was planned to elucidate the mechanism by which high LH levels may induce abnormal structure-function changes in FSHR, coinciding with a decrease in the sensitivity of FSH affecting IRS-1 and IRS-2 mediated pathways. Here, we also explored the mechanisms that may be involved in the crosstalk between FSHR and LHR under

different hormonal milieu and at higher expression level of LHR, an abnormality present in the ovarian granulosa cells of women with PCOS.

2- Experimental details

In order to investigate the crosstalk between FSHR and LHR, and insulin receptor substrates, HEK293 cells overexpressing either FSHR-RLuc8 or LHR-RLuc8, were co-transfected with different concentrations of the LHR plasmid or FSHR, respectively. Further, differentially transfected HEK293 cells were stimulated with FSH, LH/human chorionic gonadotropin (hCG), or both hormones in different combinations. To mimic the effect of high LH levels as in PCOS patients, high LHR expressing cells were treated with FSH and LH, downstream signaling was monitored using the Live-cell Bioluminescence Resonance Energy Transfer (BRET) technology. HEK293 cells expressing unlabeled LHR and FSHR-RLuc8 were co-transfected with IRS-1-YFP or IRS-2-YFP. The effect of high LH levels or high LHR expression level on IRS-1 and IRS-2 interactions were studied. The results were compared to the levels of cAMP in the presence of different concentrations of FSH and LH.

3- Results and discussion

This research is focused on delineating the direct impact of high LH through its receptor/ high LHR expression on the molecular mechanisms of FSH/FSHR adversely affected in women with PCOS. This study demonstrates that IRS-1 or -2 interact with FSHR and LHR; however, the proximity varies in the presence of different levels of LH or FSH. We need to study the impact of high cAMP levels on FSH responses and the interaction between FSHR and IRS-1 and IRS-2. This study is the first of its kind to establish adverse molecular changes in the signaling components of FSH and LH and their cross-talk with IRS-1 and IRS-2 in the presence of high LH. The results of these experiments validated LHR as a therapeutic target and emphasized the development of an LHR antagonist for the treatment of women with PCOS and high LH levels.

Here, we show that a high LHR activity results in the activation of the classical pathway with an increase in cAMP. High LHR expression not only attenuated FSHR activity on heteromerization with FSHR, however still increased the cAMP levels and that may be the cause of desensitization of FSHR. We studied the *in vitro* effects of hCG in place of LH on FSH-mediated interactions of IRS-1 and IRS-2 in HEK293 cells co-expressing both FSHR, LHR, IRS-1 or -2 using different FSHR and LHR stimulation protocols. BRET changes were measured in HEK-293 cells co-expressing RLuc8-labelled FSHR, LHR, and YFP-labelled IRS-1 or -2 in different combinations. Here, we show that a high LHR activity results in the activation of the classical pathway with an increase in cAMP. This approach has opened new avenues for future research on the therapeutic use of gonadotropins in IVF.

Malfunction of FSH signaling pathways can be associated with genetic variants of its β subunit or receptors and/or different LH and FSH micro heterogeneous forms due to differential glycosylation of FSH and/or female age [4]. However, in addition to the above reasons, another abnormality causes infertility and is common in women with PCOS is the higher LH:FSH ratio. This study demonstrated the consequences of the hormonal imbalance as well as abnormal receptor density. FSH-mediated insulin-independent metabolic pathways were adversely impacted and that can be a key to the development of insulin resistance in women with PCOS. With this information, new strategies were used through the designing of peptides for targeting the LH/LHR-mediated dysregulation. One of the peptide was found to inhibit the LHR activity both in terms of inhibiting the interaction of LH with LHR and the production of cAMP by LH-stimulated LHR. However, whether this peptide can inhibit heterodimerization of LHR and FSHR is not yet established. Further, more research is required to establish the activity level of unliganded LHR at high density and its impact on granulosa cells. This study indicates that LHR inhibition in PCOS granulosa cells may have impact on the FSH sensitivity and glucose metabolism to improve fertility and enhance pregnancy outcomes in women with PCOS using ART.

PCOS is a complex endocrinopathy associated with subfertility/infertility and frequent pregnancy losses. High LH and/or untimely higher expression of LHR in granulosa cells of some women with PCOS impairs FSH-mediated follicular growth and oocyte maturation. FSH-stimulated expression and activity of IRS-2, important for reproductive and metabolic homeostasis, is impaired in women with PCOS. International Committee for Monitoring Assisted Reproductive Technologies (ICMART-2017) specifically considered the importance of reduced gonadotropin action in infertile women [9]. Our studies here have highlighted a new mechanism by which gonadotropin responses are attenuated in women with PCOS and are responsible for aggravating infertility in these women.

4- Conclusion

Our findings unveil previously unknown mechanisms underlying gonadotropin action in follicular growth and metabolism. FSHR and LHR upon stimulation with FSH and/or LH, interact with IRS-1 and IRS-2, and these signaling molecules transmit signals to the metabolic pathways. These metabolic responses of FSHR are adversely affected by high LHR/LH activity. These findings confirmed the potential of LHCGR as the important drug targets in women with PCOS.

5- Perspectives of future collaborations with the host laboratory

Here, we have understood the consequences of hormonal imbalance and untimely high LHR expression in ovarian granulosa cells simulated *in vitro* studies. High LHR activity can adversely impact FSH-mediated insulin-independent metabolic responses and may contribute to intraovarian insulin resistance in women with PCOS. Further, high LHR activity mediated high cAMP levels adversely impacted the FSHR-mediated signaling pathways and its interaction with IRS-1 and IRS-2. We need to understand the role of downstream signaling components, such as prolonged Ca2+ response and engagements of β-arrestins, in the attenuation of FSH interactions with IRS-1 and -2 by high LH/LHR expression [8]. β-arrestins are involved in the GPCR desensitization processes, and it is pertinent to ask whether β-arrestins could communicate between the FSH and LH receptors in the presence of high LH levels/high LHR density. Two main driving forces control β-arrestin recruitment to GPCRs: agonist-induced modification of the receptor conformation and G-protein-coupled receptor kinase (GRK)-mediated phosphorylation of the ligand-occupied receptor. No antagonist of LHR are available in the market and therefore, novel peptides were designed and synthesized. preliminary experiments study established antagonistic potential of at least one of the peptides however, whether these peptides have the inhibitory effect on the heterodimerization of FSHR and LHR needs to be studied.

Furthermore, is the high LHR expression at the preovulatory stage a signal for desensitization of FSHR? Does untimely high expression of LHR during the follicular phase, as in women with PCOS, cause untimely desensitization of FSHR, a consequence of the change in the proximity of IRS proteins to FSHR or LHR? Whether a LH-LHR binding inhibitory peptide could reverse these abnormalities? Answers to these questions will provide insights into the physiology and therapeutic potential of LHR. Deciphering the involved mechanisms is required to be done in future. We propose the potential of intracellular LHR-VHH nanobodies available at the French group, to mimic the results obtained with synthetic peptides [9]. These VHH nanobodies to LHR can be used to validate the proposed therapeutic intervention of high LHR activity.

6- Articles published in the framework of the fellowship

Singh R et. al. Impact of abnormal LH receptor activity on insulin-independent metabolic responses of FSH receptor: New targets for pharmacological intervention of polycystic ovary syndrome. Manuscript in preparation 2026

Chahal N et al. Direct impact of gonadotropins on glucose uptake and storage in preovulatory

granulosa cells: Implications in the pathogenesis of polycystic ovary syndrome. Metabolism. 2021 Feb; 115:154458.

Bosch E, et al. Reduced FSH and LH action: implications for medically assisted reproduction. Hum Reprod. 2021 May 17;36(6):1469-1480.

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8- References

1. Kaur S, et al. Differential gene expression in granulosa cells from polycystic ovary syndrome patients with and without insulin resistance: identification of susceptibility gene sets through network analysis. J. Clin Endo Metab. (JCEM), 2012;97: E2016-21.
2. Casarini L, Crépieux P. Molecular Mechanisms of Action of FSH. Front Endocrinol (Lausanne). 2019 May 14 ;10 :305.
3. Singh R et al. Gonadotropins as pharmaco logical agents in assisted reproductive technology and polycystic ovary syndrome. Trends Endocrinol Metab. 2023 Apr;34(4):194-215.
4. Bosch E, et al. Reduced FSH and LH action: implications for medically assisted reproduction. Hum Reprod. 2021 May 17;36(6):1469-1480.
5. Anjali G, et al. FSH stimulates IRS-2 expression inhuman granulosa cells through cAMP/SP1, an inoperative FSH action in PCOS patients. Cell Signal. 2015 Dec;27(12):2452-66.
6. Chahal N, Crepieux P et al. Direct impact of gonadotropins on glucose uptake and storage in preovulatory granulosa cells: Implications in the pathogenesis of polycystic ovary syndrome. Metabolism. 2021; 115:154458.
7. Feng X et al. Heterodimerization between the lutropin and follitropin receptors is associated with an attenuation of hormone-dependent signaling. Endocrinology. 2013;154(10):3925-30.
8. Tréfier A, et al. G protein-dependent signaling triggers a β-arrestin-scaffolded p70S6K/ rpS6 module that controls 5'TOP mRNA translation. FASEB J. 2018;32(3): 1154-1169.
9. Raynaud P. Crépieux P et al. (2022). Intracellular VHH to monitor and modulate GPCR signaling. *equal contribution. Front. Endocrinol. 13, 1048601.