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Gonadotropin Receptor Cross-Talk and Altered Functions in Gonadal and Non-Gonadal Tissues

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Abstract

Reproduction depends on the responses of gonadotropins through their specific receptors. The gonadotropin family has three members; Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), and Human Chorionic Gonadotropin (hCG). These glycoprotein hormones comprise two subunits, an identical α-subunit and a hormone-specific-β subunit. Their cognate receptors (FSHR and LHCGR) are two adrenergic receptor-like family A/rhodopsin-like G-Protein Coupled Receptors (GPCRs) with structurally distinct ligand binding domains. The hCG binds to LHCGR but has a longer half-life and higher affinity to LHCGR. The expression of FSHR and LHCGR is observed in both gonadal and nongonadal cells. In this review, we will be emphasizing the differential expression of gonadotropin receptors in different cells of the human body, their specific responses through cross-talk, and how a defect in the expression and activity of FSHR and LHCGR may alter the responses of FSH and LH/hCG leading to diseases like PCOS, cancer and metabolic disorders.

Keywords: FSHR, Granulosa Cells, LHR, LHCGR, Theca Cells

FSH: Follicle Stimulating Hormone, LH: Luteinizing Hormone, hCG: Human Chorionic Gonadotropin, FSHR: Follicle Stimulating Hormone Receptor, LHR: Luteinizing Hormone Receptor, LHCGR: Luteinizing Hormone/ Chorionic Gonadotropin Receptor, cAMP: cyclic Adenosine Monophosphate, GPCR: G-Protein Coupled Receptor, GDF-9: Growth Differentiation Factor-9, PKA: Protein kinase A, IGF-1: Insulin-like Growth Factor-1, IL-6: Interleukin-6, FORKO: Follicle Stimulating Hormone Receptor Knockout, LuRKO: Luteinizing Hormone Receptor Knockout.

1. Introduction

Gonadotropins are members of a family of glycoprotein hormones, including Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH)/ Human Chorionic Gonadotropin (hCG), and Thyroid Stimulating Hormone (TSH). They are critical regulators of the gonadal axis for the production of sex hormones, namely estrogen, progesterone, and testosterone from gonadal cells (theca cells and granulosa cells) (Figure 1). Under the influence of hypothalamic secretion of Gonadotropin Releasing Hormones (GnRH), the anterior pituitary secretes FSH and LH, and the placenta secretes hCG. These hormones get secreted in several micro-heterogeneous forms based on their molecular weight, half-life, and charge associated with different biological properties[1]. Gonadotropins are dimers with a common alpha subunit, and a hormone-specific beta subunit responsible for specificity of FSH or LH for binding to their cognate receptors[2]. Trophoblast cells of the placenta that express hCG take over the luteotrophic functions of LH during the first trimester of pregnancy to rescue the corpus luteum and maintain the progesterone levels[3].

FSH and LH bind to their specific receptors, the FSH Receptor (FSHR) and LH Receptor (LHCGR), respectively. Both FSHR and LHCGR are members

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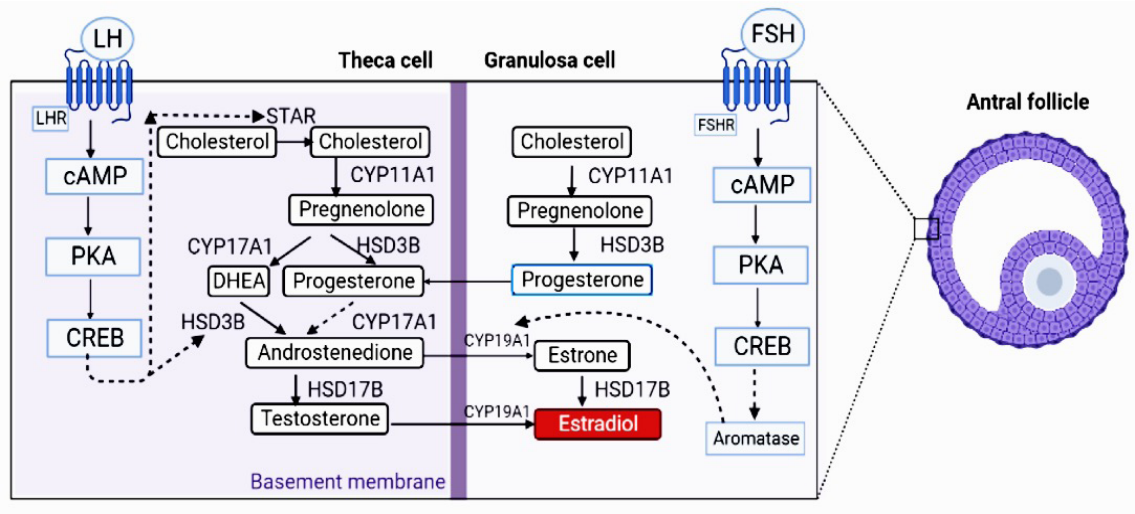

Figure 1. Signaling cascade of FSHR and LHCGR associated with the production of estradiol from androgens.

of the rhodopsin/2-adrenergic receptor-like family A. These GPCRs have a short carboxy-terminal intracellular domain, a transmembrane domain consisting of seven membrane-spanning alpha-helices, and a large extracellular amino-terminal domain[4]. FSHR is an essential component in controlling ovarian function with its role in follicle formation and stimulating estrogen production. FSH stimulates aromatase enzyme (CYP19A1) in granulosa cells, which converts androgens to estrogens. Granulosa cells express ERs, and estrogen plays an autocrine/paracrine role in follicular growth. The LHCGR regulates steroidogenesis in theca cells, oocyte maturation, and ovulation[5].

The receptor is activated when gonadotropin hormones bind to the orthosteric site of its GPCR receptor, which is positioned in a large horseshoe-shaped extracellular domain with Leucine-Rich Repeats (LRRs). The interaction of the ligand triggers a two-step activation mechanism that includes the remodelling of the receptor conformation. The alpha and beta subunits engage with a sulfated tyrosine residue in the hinge region of FSHR, thereby stabilising the ligand-receptor complexes to initiate the coupling of the Gαs protein to the gonadotropin receptor and activation of the downstream canonical cAMP/PKA cascade[2,6]. All the gonadotropins activate the cAMP/PKA signaling pathway in target cells, associated with trophic effects, mitotic functions, and occasionally apoptosis[3]. Extracellular-regulated kinase 1 and 2 (ERK1/2) are activations that occur downstream of PKA. Moreover, the transcription of target genes, including the Steroidogenic Acute Regulatory Protein (StAR), is induced by the activation of phosphorylation of the cAMP-Responsive Element-Binding protein (CREB) to promote steroidogenesis in target cells[2,7] (Figure 1).

The classical pathway of FSH through cAMP/PKA is critical for the growth of follicles and the production of estradiol. The activation of FSHR or LHCGR by binding with FSH and LH, respectively, activates several other

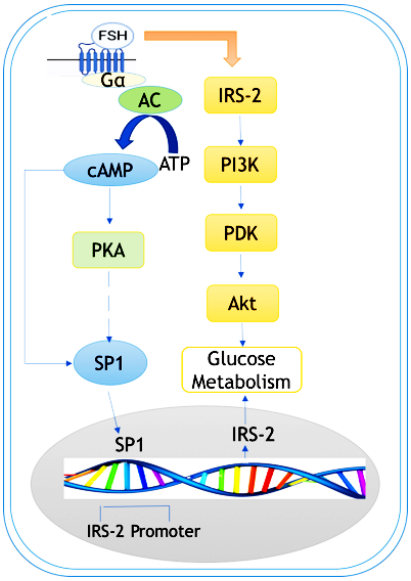

Figure 2. IRS-2 mediated pathway of FSH in granulosa cells involved in follicular growth and metabolism.

downstream signaling pathways that ultimately lead to successful folliculogenesis and ovulation. The Gαq/11 and inositol triphosphate pathways may also be activated by FSHR and LHCGR[8]. Other pathways, such as the ERK pathway and IRS-2/PI3K/AKT pathway in human granulosa cells, play crucial role in metabolism and follicular growth[9] (Figure 2). FSH is more effective than LH or hCG in increasing the glucose uptake and storage in granulosa cells; however, only FSH increases the glycogen synthesis independent of insulin by activating glycogen synthase via the IRS-2/PI3K/Akt2 pathway[9,10] (Figure 2).

In PCOS women, impairment of the FSHR-IRS-2 pathway due to high LH or genetic reduction in FSHR activation leads to reduced FSH-stimulated glucose uptake and glycogen production in granulosa cells[9]. The Genome-Wide Association Studies (GWAS) performed on the Han Chinese population revealed that FSHR and LHCGR, along with nine other genes, confer significant susceptibility to PCOS. These genes were also confirmed to pose a risk of PCOS in European women[11-13]. A study by Hwang *et al.* on Korean women has also confirmed that PCOS is associated with the gene variants of FSHR, DENN Domain Containing 1A (DENND1A) and glycogen synthase 2 (GYS2)[14,15].

2. Differences in LH and HCG Responses through LHCGR

LH and hCG bind to the same receptor that is designated as LHCGR. However, hCG has a higher affinity than LH, and there are differences in the structure and function of these hormones. The β-subunit of LH has one Asn-linked glycosylation. In contrast, hCGβ contains two N-linked sites (Asn-13 and Asn-30), which promote the dimerization of LHCGR, activating it weakly[16]. Further, the glycosylation of Asn-78 of hCG-β is involved in its binding with LHCGR, while the glycosylation of Asn-52 is important for normal receptor activation. hCGβ has a unique carboxyl-terminal peptide with four O-linked glycosylations, which increases the half-life of hCG (24h), which is ten-fold more than LH (2.4h). hCG is more effective than LH in the production of cAMP and the involvement of β-arrestin in the downstream signalling of LHCGR[17]. Differences in amino acid sequence between the rodent and human LHCGR receptors have little to no impact on the rates at which hLH or hCG bind[18]. An exon 10 deletion in LHCGR, which causes structural and spatial rearrangements at the hinge region of the receptor, is known to alter LH- and hCG-induced signalling in distinct ways[19]. This loss affects LH signaling while leaving hCG signaling unaffected, indicating differences in the interactions and functions of the hCG and LH receptors. Recent research has revealed that the hinge region of the receptor interacts differently with hCG and LH and that only hCG can cause both cis- and trans-activation of human LHCGR[20,21]. Both hCG and LH were shown to have a similar rate of association ($3\times10^{8\,-1}min^{-1}$) with LHCGR but distinct dissociation rates (25 hours for hCG and approx. 9 hours for hLH), which suggests that hCG resides for a longer time on its receptor[18]. Their real-time cAMP measurements demonstrated that the two hormones had different kinetics, with Recombinant hCG (rhCG) generating faster cAMP responses than Recombinant Human LH (rhLH). The findings implied that the discrepancies between rhCG- and rhLH responses may be explained by the slower dissociation rate of rhCG compared to that of rhLH and/or the variations in hormone-receptor complex conformations. A restricting element for maximum progesterone synthesis is β-arrestin. rhLH, like rhCG, is a full agonist on LHCGR for the generation of cAMP and testosterone but only a partial agonist for the recruitment of β-arrestin and the creation of progesterone[17].

3. Expression of Gonadotropin Receptors in Gonadal Cells

3.1 FSHR Expression in Granulosa Cells

The expression of FSHR can be observed in the ovarian granulosa cells of growing follicles in females and is not detected in primordial follicles in ovaries. FSHR expression in granulosa cells is observed in the secondary follicle stage[22]. As the follicle develops from the preantral to the antral stage, FSHR expression increases, and peaks in the mid-follicular phase, and the expression may be twice that of the luteal phase[23]. Antral follicular development gets impaired in a hypophysectomised rat lacking FSH secretion while having FSHR expression[24]. In FSHR knockout mice, antral follicular development was suppressed[25,26]. The expression of FSHR is also regulated by androgens and an oocyte-derived Growth Differentiation Factor 9 (GDF9). A study showed that in Rhesus monkey, the androgen receptors get co-expressed with FSHR in

the same follicles[27]. Administration of testosterone to the rhesus monkey induces the mRNA expression of FSHR in granulosa cells, while FSH was shown to stimulate the expression of androgen receptors[28]. GDF9 is a crucial factor that contributes to the growth of the follicles from the preantral stage to the antral stage by causing androgen production in the theca cells[29]. In an *in vitro* follicular system, knockdown GDF9 suppressed the expression of FSHR and induced the apoptosis of granulosa cells, which led to arrested follicular growth. However, this arrest was rescued by adding GDF9 or androgen, suggesting that GDF9 critically regulates FSHR expression in granulosa cells and is achieved via androgen signalling[30]. In the rat granulosa cell culture system, estrogen alone did not increase LHCGR or alter FSHR expression, but it did promote LHCGR expression in the presence of FSH by stabilizing the LHR mRNA[26].

3.2 LHCGR Expression in Theca and Granulosa Cells

The normal ovarian cycle involves dynamic changes in LHCGR expression. LHCGR is expressed in both the ovarian granulosa and thecal cells in females; however, expression in granulosa cells is very low during the follicular phase, and its expression peaks during preovulatory stage in response to FSH[4,31]. When follicles grow in response to FSH, estrogen, and other paracrine stimuli, LHCGR expression is further increased to facilitate successful ovulation[5]. LHCGR expression is momentarily downregulated as estrogen-producing granulosa cells differentiate into luteal cells during the preovulatory LH surge. The mid-luteal phase is when LHCGR expression is at its highest, and progesterone production is also increased. The LHCGR levels decrease as the corpus luteum regresses[4].

LHCGR expression in the granulosa cells is induced after FSH stimulation, even though it was previously visible in the theca cells from the time that it first appeared in the secondary follicle[32-34]. After FSH induces LHCGR expression, many intra-ovarian factors work in coordination to boost it and promote follicular development to prepared for the LH surge and subsequent ovulation (Figure 3). These local factors involve activin, Interleukin-6 (IL-6), Insulin-like Growth Factor-1 (IGF-1), and estrogen[26]. The ovarian granulosa cells express the growth factor activin, a member of the TGF-β superfamily, along with the activin receptor. Activin has been seen to strongly increase FSHR and CYP19A1 expression in rat granulosa cells, where it increases the FSH-induced LHCGR expression. As the follicle grows, the granulosa cells' production of activin is decreased while their production of inhibin and follistatin (an activin-binding protein) is increased. In the rat ovarian granulosa cell culture system, adding activin to the culture media caused an abrupt increase in FSHR expression. However, activin production was sharply reduced after FSH stimulation of the cells. These results suggest that activin is secreted chiefly by granulosa cells before FSH stimulation and may play a role in maintaining the gonadotropin receptors[25,26]. On the other hand, in the theca cells, activin significantly reduced androgen synthesis that LH and IGF-1 stimulated.

IGF-1 expression in the ovary is in growing and fully developed follicles and is localized in the same region as FSHR and aromatase. Only the antral follicles with a diameter of 3-5 mm were found to express IGF-1 in human samples. On the other hand, from the primary

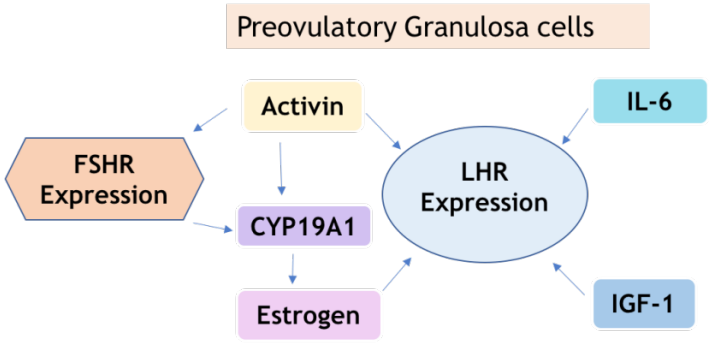

Figure 3. Intra-ovarian factors that boost LHR/LHCGR expression to promote follicular development and ovulation.

to the preovulatory stage, its receptor was present in most follicles. IGF-1 significantly increased LHCGR expression in the rat granulosa cell culture system after FSH stimulation. In this phenomenon, IGF-1 prolonged the half-life of the mRNA rather than improving the transcription of LHCGR mRNA. The addition of IGF-1 also increased the expression of FSHR[35]. FSH increases the secretion of Interleukin-6 (IL-6) from rat granulosa cells[36,37]. In rat granulosa cell culture, when IL-6 was additionally provided, FSH-induced expression of LHCGR was dramatically increased. IL-6 is a cytokine that regulates humoral immunity and was initially discovered as a B-cell differentiation factor.

4. Expression of Gonadotropin Receptors in Non-Gonadal Cells

Recent research documenting the development of functional ectopic gonadotropin receptors, FSHR and LHCGR, which are thought to be important in non-reproductive processes, has cast doubt on this long-held belief on their role in reproduction[2]. The expression of functional FSHR has been found on various non-gonadal tissues such as fat, bone, brain, adrenal glands, endothelial cells, uterus, placenta, umbilical cord, and several types of cancer tissues[38]. It was found in endometriotic lesions, where FSH induced the expression of CYP19A1 and the production of estrogen[39]. The extragonadal effects of FSH/FSHR activation get aggravated in menopausal women with high FSH and LH levels. The risks associated with the abnormal non-gonadal effects of FSHR include bone loss, obesity, cardiovascular risk, and cancer risk[40]. The modulation of bone mass and adipose tissue by FSH-induced signals was observed in genetically altered or antibody-treated mouse models[41,42]. Studies have shown a connection between FSH levels, osteoporosis, and postmenopausal increase in fat mass. In a study based on the Chinese population, low levels of FSH were linked to higher cardio-metabolic risk and cardiovascular disorders[43].

At menopause, women's serum FSH levels dramatically increase. FSH receptors are expressed on osteoclasts, osteoclast precursors, and mesenchymal stem cells but not osteoblasts[42,44,45]. Sex hormone replacement therapy may be able to reverse obesity after menopause. As adipose tissues produce functional FSH receptors[46], the benefits of sex hormone replacement might be mediated by a comparable decrease in serum FSH. Several studies have indicated FSHR expression in various tumor cells such as from the prostate, ovary, breast, thyroid, colon, neuroendocrine pancreas, urinary bladder, kidney, lung, liver, stomach, testis, and pituitary cancer, as well as soft tissue sarcomas[47,48]. FSHR expression is increased in tumour vessel cells by FSH[47,48]. FSHR expression was reported in endothelial cells in thyroid and neuroendocrine tumors[49-51]. Further, FSH promoted proliferation, migration, invasion, and angiogenesis in epithelial cancer cells[47,48].

4.1 LHCGR Expression in Non-Gonadal Cells

The extragonadal expression of LHCGR has been reported in the uterus, skin, kidney, placenta, glial cells, adipose tissue, pancreas, breast, adrenals, thyroid, neural retina, cone photoreceptors, and neuroendocrine

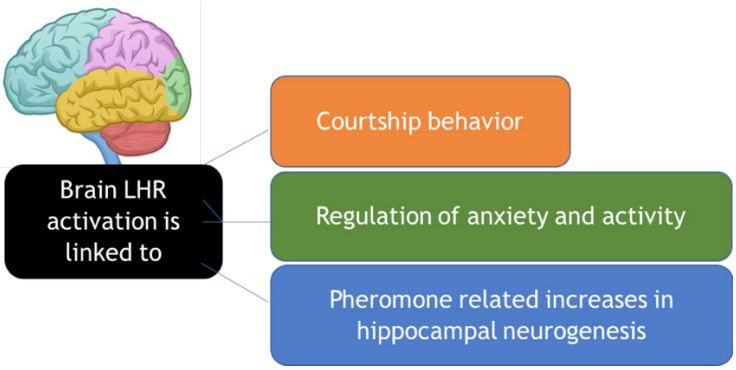

Figure 4. Processes induced by LHCGR activation in the brain.

cells[8]. LHCGR expression was discovered in the mouse adrenal cortex[52], supporting earlier research showing that postmenopausal women's adrenal glands have LHCGR expression[53]. Cortisol synthesis and Cushing's syndrome are related to abnormal LHCGR expression in the adrenal gland[54,55]. Human uterine tissues were also shown to express functional LH receptors[2,56]. LHCGR is expressed in the brain, in areas within the cortex related to cognitive functions, and in the hippocampus (Figure 4). Even in human brain microglial cells, immunoreactivity to LHCGR was discovered, and it was positively associated with a higher risk of developing Alzheimer's disease[57]. LHCGR activation in brain is speculated to be linked to specific processes affecting behaviour. More recently, it was proposed that LHCGR expression is present in the retina, where LH and hCG would have similar effects on vascular endothelial growth factor[58].

4.2 LHCGR Expression in Cancer

Transgenic mice overexpressing human LHCGR (hLHR) had morphological and molecular changes (increased proliferation and trans-differentiation in the endometrial layer) in uterus. In these mice, there was upregulation of genes linked to cell cycle control and downregulation of genes connected to the immune system and xenobiotic metabolism. Older LHCGR transgenic mice developed uterine tumour masses that mimicked endometrial cancer[59]. LH promotes cell migration and invasion in breast cancer cells with functional LHCGR through modulation of multiple kinases, activating actin cytoskeletal proteins. LH causes paxillin phosphorylation and transport to the plasma membrane, where focal adhesion complexes are formed. This process is initiated through rapid extragonadal LHCGR signalling to Src/FAK/paxillin resulting in the phosphorylation/activation of the nucleation promoter factors cortactin and N-WASP. As a result, there is an increase in cell motility and the invasive properties which promote the tumour cell metastatic dissemination[60].

5. Conclusion

The discovery of FSHR and LHCGR has led to significant advancements in understanding gonadotropin action in gonadal and non-gonadal tissues; however, the significance of nongonadal receptors requires more investigation. The expression of FSHR and LHCGR is correlated with PCOS, cancer, and metabolic disorders. Much needs to be discovered about the cross-talk between FSHR and LHCGR due to changes in the FSH: LH ratio or their receptor levels. The therapeutic utilization of rhFSH and rhLH has much potential to improve the consequences of hormonal anomaly and infertility.

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