



FSH stimulates IRS-2 expression in human granulosa cells through cAMP/SP1, an inoperative FSH action in PCOS patients



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ABSTRACT

Follicle stimulating hormone (FSH) plays a central role in growth and differentiation of ovarian follicles. A plethora of information exists on molecular aspects of FSH responses but little is known about the mechanisms involved in its cross-talk with insulin/IGF-1 pathways implicated in the coordination of energy homeostasis in preovulatory granulosa cells (GCs). In this study, we hypothesized that FSH may regulate IRS-2 expression and thereby maintain the energy balance in GCs. We demonstrate here that FSH specifically increases IRS-2 expression in human and rat GCs. FSH-stimulated IRS-2 expression was inhibited by actinomycin D or cycloheximide. Furthermore, FSH decreases IRS-2 mRNA degradation indicating post-transcriptional stabilization. Herein, we demonstrate a role of cAMP pathway in the activation of IRS-2 expression by FSH. Scan and activity analysis of IRS-2 promoter demonstrated that FSH regulates IRS-2 expression through SP1 binding sites. FSH stimulates SP1 translocation into nucleus and its binding to IRS-2 promoter. These results are corroborated by the fact that siRNA mediated knockdown of IRS-2 decreased the FSH-stimulated PI3K activity, p-Akt levels, GLUT4 translocation and glucose uptake. However, FSH was not able to increase IRS-2 expression in GCs from PCOS women undergoing IVF. Interestingly, IRS-2 mRNA expression was downregulated in GCs from the PCOS rat model. Taken together, our findings establish that FSH induces IRS-2 expression and thereby activates PI3K, Akt and glucose uptake. Crucially, our data confirms a molecular defect in FSH action in PCOS GCs which may cause deceleration of metabolism and follicular growth leading to infertility. These results lend support for a therapeutic potential of IRS-2 in the management of PCOS.

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1. Introduction

Follicle stimulating hormone (FSH) plays a central role in the growth and differentiation of ovarian follicles to preovulatory phenotype. A plethora of information exists on physiological responses of FSH in preantral and antral follicles, however much remains to be understood regarding its role in the regulation of glucose metabolism in preovulatory follicles. The cross-talk between FSH and insulin/IGF-1 signaling pathways is known to be implicated in the coordination of energy homeostasis and reproduction [1–3]. Various studies have shown that FSH stimulates many different signaling cascades, including the canonical cAMP/PKA pathway and those mediated by different kinases such as

PI3K, Akt/PKB, and ERK1/ERK2 in a G protein dependent or independent manner [3–9]. Implication of the FSH-stimulated cAMP pathway in the activation of various genes and transcription factors has profound significance in follicle maturation [3,9]. The involvement of the cAMP pathway in the activation of insulin receptor substrate-2 (IRS-2) expression has also been shown in pancreatic β cells [10]. However, there have been no reports on the ability of the FSH-stimulated cAMP pathway to regulate the expression of IRS-2 in granulosa cells (GCs).

The IRS proteins are the classical substrates and the signaling intermediates of the insulin and insulin-like growth factor 1 (IGF-1) receptor [11,12]. It has been well characterized that IRS proteins are expressed in the human GCs and regulate many important ovarian functions of insulin/IGF-1 [13,14]. IRS-2 has been known to mediate metabolic and reproductive functions. In addition, studies have also emphasized its role in growth and proliferation [15–18]. The emerging correlation between cancer and IRS-2 over expression substantiates the requirement of understanding the regulation of IRS-2 in human diseases. The specific role of IRS-2 protein in the regulation of reproductive functions has been foreshadowed by knockout studies [15].

Abbreviations: GCs, granulosa cells; FSH, follicle stimulating hormone; IRS, insulin receptor substrate; SP1, specificity protein 1; PCOS, polycystic ovary syndrome; PKA, protein kinase A; PKB/Akt, protein kinase B; cAMP, cyclic adenosine mono phosphate; Act D, actinomycin D; CHX, cycloheximide.

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