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The Observation of FSH's Cellular Internalization

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Abstract

Follicle stimulating hormone (FSH) is a kind of glycoprotein gonadotropin, and plays an important role in the diagnosis and treatment of infertility. Follicle stimulating hormone receptor (FSHR) is a kind of G protein coupled receptor (GPCR), found in the ovary and testes, and its activation is required for the hormonal operation during the breeding period. In this study, an experimental model of FSHR mediated FSH into cell membrane, which exhibited a phenomenon of fluorescent localized on cell surfaces internalized into cell interior, was established to verify biological activity of FSH.

Keywords

FSH Receptor, Target Binding, Celluar Internalization

1. Introduction

Human follicle stimulating hormone (hFSH), synthesized and secreted by the pituitary gland, is a kind of glycoprotein gonadotropin [1]. It is heterologous dimers glycoprotein that composed of FSHα subunit and FSHβ subunit [2]. FSHα and FSHβ subunits are both involved in receptor binding and signal mediating. The binding of FSHα and FSHβ subunits has a direct effect on the activity of hormones [3]. Separating FSHα subunit and FSHβ subunit will cause the FSH to lose biological activity [4].

The role of FSH in male and female is not the same. In female body, the main function of FSH is to stimulate the development of the follicle, ovulation and endometrial growth. Because FSH is a macromolecular protein, it requires receptor follicle stimulating hormone receptor (FSHR) to enter into the cell membrane. FSH's receptor is presented in the granulosa cells of the follicle [5]. In male body, FSH can stimulate sperm production and promote the mature of sperm by the synergy of Luteinizing Hormone (LH) and androgen, to promote the mature of sperm [6] [7].

According to the role of FSH, that played in the human body, it is mainly used in clinical treatment of infertility. At present, there are three kinds of follicle stimulating

hormone: Urinary and Pituitary source follicle stimulating hormone and recombinant human Follicle stimulating hormone (rhFSH). Because the purification of Urinary and Pituitary source follicle stimulating hormone is difficult, it Contains other Miscellaneous protein, has certainly side effects. With the development of gene engineering, domestic and foreign scholars have conducted a lot of researches on rhFSH. RhFSH's high purity expression and high safety performance, makes its application scope and value increase. Currently, the mainly products on the market are rhFSH produced by genetic engineering. The aim of genetic engineering expressed recombinant human follicle stimulating hormone protein is to explore its medicinal value and to use in clinical research in the future.

In this study, the positive biological activity of FSH standard sample was verified by cell experiment *in vitro*. Only if there be a suitable protein structure, the protein would have a corresponding biological function. FSH is a macromolecular protein, its signal transmission is dependent on specific receptor. FSH needs to be bound to the receptor at least in a region of the receptor binding domain and the resulting effect. The target protein that mediates FSH into the cell is FSHR. The cellular internalization of FSH standard sample can provide reference for the expression of rhFSH protein's activity detection.

2. Targeted Binding Receptor

In this experiment, the cells were transfected the expression vector of pSNAPf-ADRβ2-FSHR. Labeled FSHR protein was expressed on the cell membrane and then added FSH protein standard sample. If the FSHR protein is able to identify FSH, it can be mediated into the cell membrane. In this way, fluorescent tagged FSHR proteins enter the cell from the cell membrane, and form the phenomenon of intrinsic fluorescence.

2.1. Cell Staining

Digest the cells that express fusion protein stablely and inoculate in 96-well plate. Start SNAP-Surface 549 staining until the density of HEK-293 cells grew to 80%, put 96-well plate in carbon dioxide to incubator cells for a moment. Remove stain and wash the cells with PBS three times to remove the remaining stain (Notice: Wash the cells slowly, its better not to lose cells). Observed the stained cells under the microscope. Photo recorded.

2.2. rhFSH Targeted Combination FSHR

Add the FSH standard sample, and put 96-well plate in carbon dioxide to incubator cells for a moment. Observation under fluorescence microscope. Photo recorded.

3. Results

3.1. Comparison of Transfection and Non Transfection of HEK-293T Cells

FSHR protein was expressed on the surface of cell membrane of HEK-293 cells trans-

fected with pSNAPf-ADRβ2-FSHR vector. After staining, the cell membrane formed a layer of red fluorescence (**Figure 1**). No red fluorescence was obserbered in the cell membrane of the non transfected HEK-293 cells. (**Figure 2**) It indicated that the fusion protein was on the cell membrane.

3.2. Results of FSH and FSHR Targeted Binging

The FSH protein standard was added to the HEK-293 cells after staining. Incubated for a period of time in incubator, the fluorescence of the HEK-293 cells membrane became dark and irregularly, and red fluorescence appeared in cell interior. The red fluorescence on the cells membrane is internalized into the cells (**Figure 3**). And there is no internalization in the cells that without adding FSH (**Figure 4**).

According to the addition of different FSH concentrations, as well as the level of protein activity and incubation time, the degree of internalization was incongruent (**Figure 5** and **Figure 6**). Higher protein activity and concentration of added, relatively higher degree of intrinsic fluorescence and more cell fluorescence moved from the membrane to the cell interior.

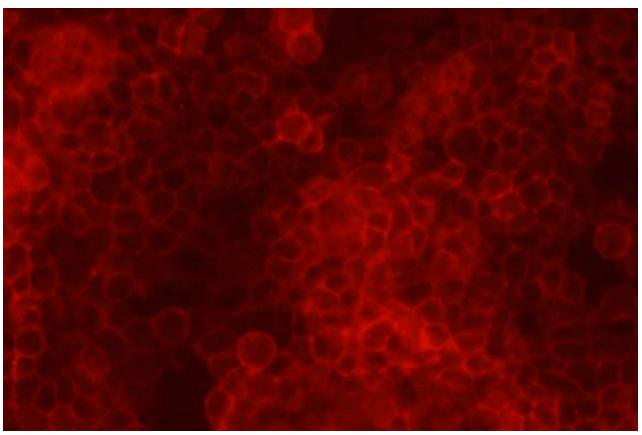

Figure 1. HEK-293 cells transfected with pSNAPf-ADRβ2-FSHR vector.

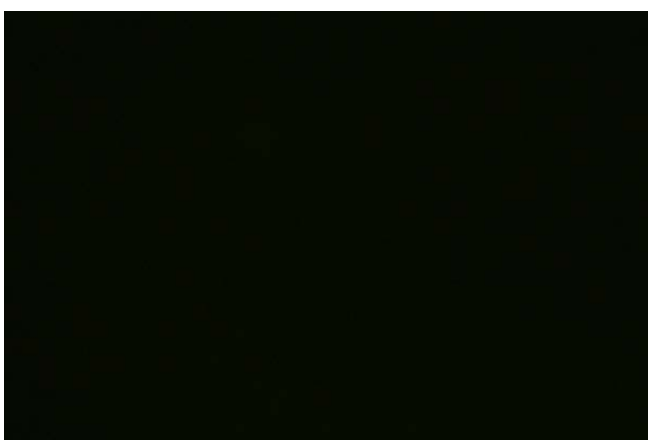

Figure 2. HEK-293 cells without vector.

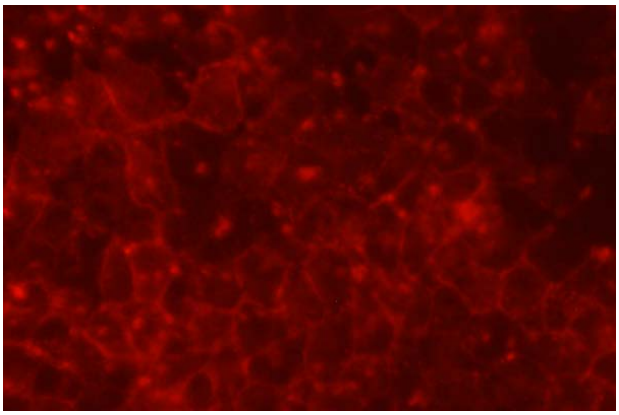

Figure 3. HEK-293 cells adding FSH.

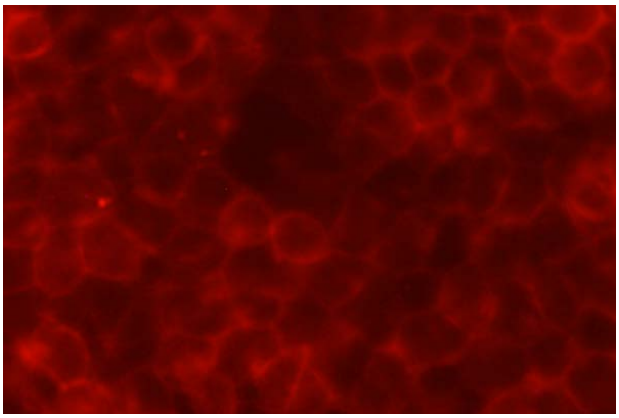

Figure 4. HEK-293 cells without adding FSH.

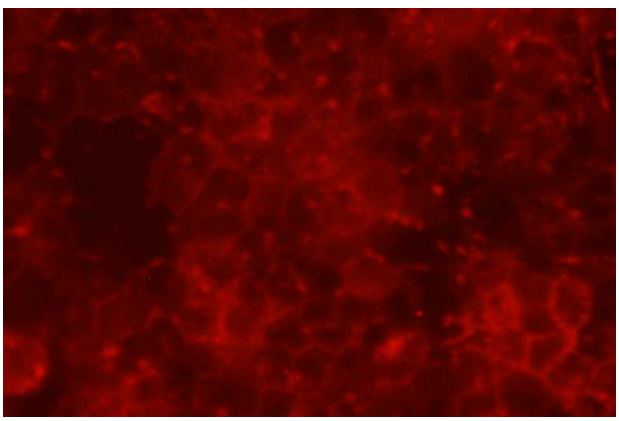

Figure 5. HEK-293 cells adding low concentration of FSH.

4. Discussion

In this study, the establishment of cell fluorescence system was used to make the target protein targeted binding receptor and to verify the biological activity of FSH. External target protein targeted binding receptor FSHR proved that FSH has the functional domain that can be combined with the target protein, and FSHR can mediate rhFSH

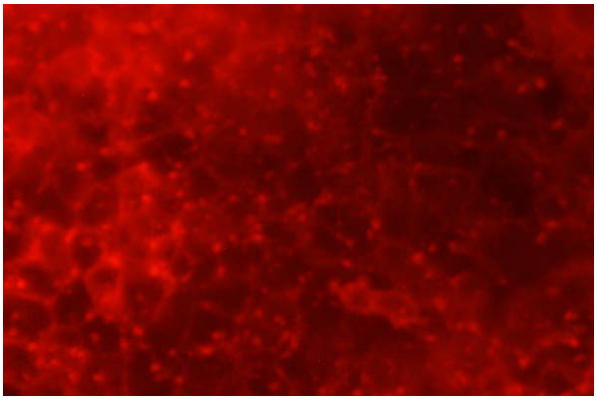

Figure 6. HEK-293 cells adding high concentration of FSH.

entry into cells to play the biological function of FSH.

Target protein targeted binding receptor is to directly observe whether the FSH can be combined with its receptor FSHR. And the method is fast and convenient.

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