Analysis of Differential Glycosylation Patterns of Human FSH

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Abstract. Follicle stimulating hormone (FSH) is a glycoprotein hormone with subunitsαandβ, and is required for gamete development. Differential glycosylation may produce diglycosylated FSH with higher biological activity, and is suspected to be generated via the action of oligosaccharyltransferase(OST) isoforms. Signal peptide hydrophobicity of α and β maycontribute to selective usage of OST, and hence modulate N-glycosylation. We hypothesize that N-glycosylation of FSH subunits is regulated via differential interactions between OST isoforms and subunit signal peptides and modulated by estrogen. To test our hypothesis, we will engineer chimeric hFSH subunits by swapping the signal peptide sequences of α and β. Constructs with the chimeric sequences will be transfected into cell lines, and expressed FSH will be examined.

1. Introduction

The hormone FSH is partially responsible for the development of follicles, which in turn are responsible for the production of viable ova at ovulation in women. FSH is composed of two subunits, an alpha (α) and beta (β) and both subunits may be decorated with oligosaccharide branches at two residues on each subunit [1]. In humans, the FSHα is continually glycosylated, whereas there seem to be detectable variations in FSHβ glycosylation. Preliminary data suggests that activity of estradiol selectively inhibits FSHβ glycosylation, while having no apparent effect on glycosylation patterns of FSHα. This action produces a di-glycosylated glycoform of FSH, being more biologically potent than the tetra-glycosylated form in which both subunits carry two oligosaccharides. Previous studies have indicated that a shift in the ratio of glycoforms of FSH occurs as women age, and may suggest a potential avenue by which menopause and the cessation of reproductive viability occurs [2]. In other words, as estradiol concentrations decrease in premenopausal, or menopausal women, the abundance of tetra-glycosylated FSH increases, and thereby decreasing the relative amount of highly potent FSH. Oligosaccharyltransferase (OST)is the enzyme responsible for the addition of oligosaccharide branches onto the emerging polypeptide chain as it enters the mammalian endoplasmic reticulum (ER). Studies have indicated that two isoforms of OST exist, and selective usage of each isoform is believed to be coorelated to the hydrophobicity of the signal peptides of each FSH subunit [3]. Therefore, our hypothesis is that selective usage of OST isoforms is based on the signal peptide sequence of each subunit, and is modulated by activity of estradiol. To test our hypothesis, we have constructed chimeras of each FSH subunit by swapping their signal peptide sequences. These chimeras are used to transfect an immortalized rat gonadotrope cell line (GH3 cells). Once transfected cells expressing the hormone have been isolated, we will examine the hormone expression profile under different conditions, specifically various concentrations of estradiol. If our hypothesis is correct, then we should detect a non-glycosylated α subunit, and a large amount of glycosylated β subunit in the transfected cells. In addition, we should also be able to modify the amount of glycosylated α subunit by administering varying concentrations of estradiol.

2. Experiment, Results, Discussion, and Significance

Initially, expression vectors containing hFSHα (hα/pSVL), hFSHβ (pKR8) and the mammalian expression vector pCI-neo purified using Qiagen midi plasmid kit were used to amplify FSH subunits for ligation into a cloning vector. The cloned subunits, after bacterial amplification, would be isolated, digested out of the cloning vector, and ligated together to form the chimeric sequences. Following ligation, these sequences would be cloned into the mammalian expression vector pCI-neo, containing a viral promoter for constitutive, or continual, expression. This expression vector would then be transfected into GH3 cells, and the expressed hormone would be released from the cell into the conditioned media and harvested. Cells were grown in medium containing G418 to select for the cells that are transfected with the selection vector. Once a stable cells have been identified, FSH-expressing GH3 cells will be incubated in the presence and absence of 17-estradiol. After two days, the conditioned medium will be collected, and replaced with fresh media. FSH concentration will be measured by RIA using National Hormone and Pituitary Program kits. FSH will be purified by immunoaffinity chromatography and the

heterodimer fraction will be separated from unassociated subunit by Superdex 75 gel filtration. Di-glycosylated and tetra-glycosylated hFSHglycoform abundance will be determined by Western blot analysis.

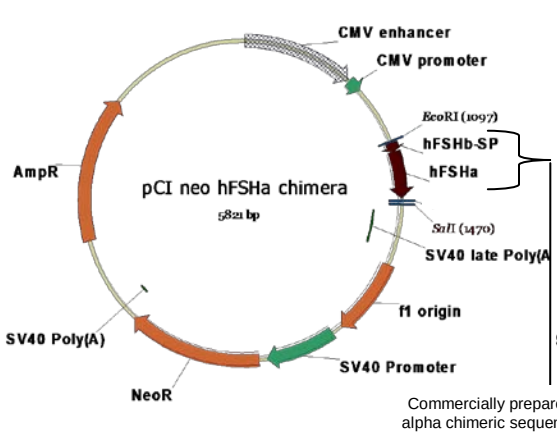

Figure 1.pCI neo containing the □□ chimera.

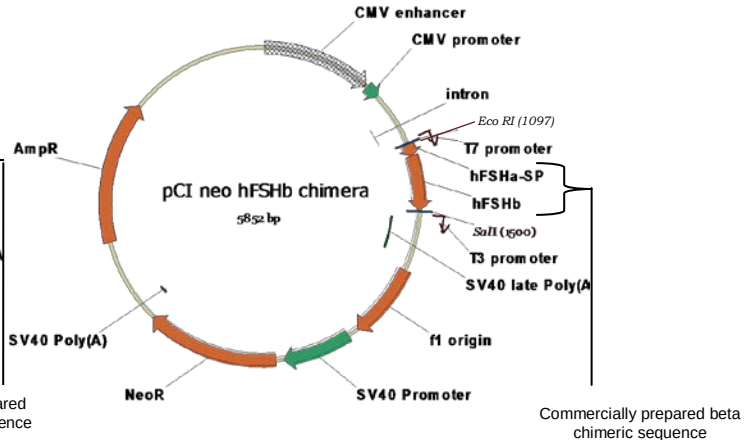

Figure 2.pCI neo containing the β chimera.

Currently, due to difficulty in traditional cloning methods, the constructs have been commercially prepared, cloned into pCI neo, and transfected into rat pituitary tumor cells (GH3) using a liposomal transfection reagent from Biontex. As mentioned previously, stable cell lines would have been isolated based on G418 resistance. However, cell loss due to gradual lack of cell adherence to the culture vessels yielded small amounts of conditioned media for analysis, so a second transfection using BD Biosciences Primaria culture flasks and plates, noted as providing increased cell adherence due to chemically treated cell culture surface, was performed. Proteins wereattempted to be purified from the conditioned medium of the transfected cell lines and assayed for FSH expression viaradioimmunoassay (RIA). Complications believed to be caused by the presence of serum proteins in the conditioned media have produced unreliable RIA results. Avenues to remove serum from media are currently being explored, such as removing proteins using a column, or culturing cells briefly in serum free media. Cells exposed to selection media containing G418 underwent necrosis in extremely high quantities, however some survivors seem to be currently replicating. It may be necessary to do multiple transfections concurrently to isolate enough protein for analysis if cell numbers fall again. Once protein expression is confirmed, extracts of FSH subunits will be prepared and N-glycosylation patterns analyzed. Changes of N-glycosylation in response to hormone treatment will be examined. Following confirmation that hormone is being secreted, the hormone subunits will be analyzed using imunoaffinity chromatography andWestern blot.

3. Conclusion

This knowledge will ideally provide insight into the avenue by which menopause occurs, and to a more broad extent, insight into reproductive ageing. Additionally, as glycosylation exists as a means of post transcriptional modification that applies to many proteins, this experiment may give insight into a significant modification mechanism.

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