Analysis of Differential Glycosylation Patterns of Human FSH

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Abstract. Follicle stimulating hormone (FSH) is a glycoprotein hormone with two subunits, α and β, and is required for gamete development. Our data suggest that estrogen is responsible for inhibiting the glycosylation of FSHβ in reproductive-age women, thus producing a di-glycosylated FSH with higher biological activity than the tetra-glycosylated form. The difference in glycosylation of two subunits is suspected to be due to activity of different oligosaccharyltransferase (OST) isoforms. OSTs are responsible for the first step in N-glycosylation. Factors including signal peptide hydrophobicity of α and β maybe contribute to selective usage of OST, and hence modulate N-glycosylation. Therefore our hypothesis is that N-glycosylation of FSH subunits is regulated by the differential interactions between OST isoforms and the signal peptides of each subunit, and the differential interaction is modulated by hormones such as estrogen. To test our hypothesis, we will genetically engineer chimeric hFSH subunits by swapping the signal peptide sequences of α and β. Constructs with the chimeric sequences will be introduced into immortalized gonadotrope cell lines. FSH glycoforms expressed in the cell lines will be examined using Western Blot. If our hypothesis is correct, then we would expect to detect unglycosylated α subunit in the transfected cell lines. Different hormones such as estrogen will be used to treat the cell lines and the difference in FSH subunit glycosylation 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 gametes at ovulation in women. FSH is composed of two subunits, alpha (α) and beta (β). Both subunits can be decorated with oligosaccharide branches at two residues. What is known as the tetraglycosylated glycoform is therefore the result. Another potential glycosylation pattern is that of a diglycosylated glycoform, in which only the α-subunit carries the two oligosaccharide branches. Studies indicate that the tetraglycosylated form is biologically more potent than that of the diglycosylated glycoform. These two isoforms circulate in women at varying ratios through time. 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. Data suggests that activity of estradiol selectively inhibits FSHβ glycosylation, while having no apparent effect on glycosylation patterns of FSHα. The result of decreased estradiol concentrations at menopause is an increased abundance of tetraglycosylated FSH, and therefore decreased biological action of the same amounts of the secreted hormone. The action of estradiol is the suspected mechanism by which inhibition or modulation of the activity of oligosaccharyltransferase (OST), occurs. OST is the enzyme responsible for the addition of 15-residue oligosaccharide branches onto the emerging polypeptide chain as it enters the mammalian ER and thereby responsible for glycosylation ratios. Studies have indicated that two isoforms of OST exist, and selective usage of each isoform is believed to be correlated to the hydrophobicity of the signal peptides of each of the FSH subunits. Therefore our hypothesis is that selective usage of OST isoforms is related to the signal peptide of each subunit and is modulated by activity of estradiol. To test our hypothesis, we will construct chimeras of each FSH subunit by swapping their signal peptides. These chimeras will be introduced into immortalized human gonadotrope cell lines and the cell lines will be treated with estradiol. If our hypothesis is correct, then we should detect a non-glycosylated α subunit, and a large amount of glycosylated β subunit. 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

Expression vectors containing hFSHα (hα/pSVL), hFSHβ (pKR8) and the mammalian expression vector pCI-neo have been purified using Qiagen midi plasmid kit. After treatment with chimeras, cells will be grown in a medium containing G418 to select for the cells that are transfected with the selection vector. Radioimmunoassay (RIA) will be used to identify antibiotic-resistant cells that actively express the hormone. Once a stable cell line has been identified, FSH-expressing LβT2 cells will be incubated in the presence and absence of 17-estradiol. After three days, the conditioned medium will be collected. FSH concentration will be measured by RIA using National Hormone and Pituitary Program kits. FSH and 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 hFSH glycoform abundance will be determined by Western blot analysis. The signal peptide sequence was amplified with primers FSH SP-F and FSH SP-R using pKR8 as a DNA template, and the mature peptide sequence will be amplified with primers FSH-F and FSH-R using hα/pSVL as a DNA template. PCR products will be cloned into pGEM-T EASY vector followed by sequence confirmation. Restriction sites have been introduced into the primers for easy cloning. Following sequence confirmation, each fragment will be digested from isolated pGEM-T EASY with complementary ends for the subcloning procedure. Signal peptide sequences will be ligated to opposite mature peptide sequences producing chimeric sequences. The chimeric sequence will be cloned into the pCI neo mammalian expression vector at EcoRI NheI, and Sall sites.

The chimeric constructs will be used to transfect CHO cells following standard procedures. Stable cell lines will be isolated based on G418 resistance. Proteins will be purified from the conditioned medium of the transfected cell lines and assayed for FSH expression by RIA. Once protein expression is confirmed, Core B will extract FSH subunits and analyze N-glycosylation patterns. Changes of N-glycosylation in response to hormone treatment will be examined. The project is now at the stage in which the chimeras are being constructed, where currently each fragment, being the signal peptides and mature peptides, need to be ligated together and then cloned into the pCI neo expression vector.

3. Conclusion

Currently, the project is in its juvenile stages, as construction of the chimeras is still underway. This knowledge will ideally provide insight into the avenue by which menopause occurs, and to a more broad extent, insight into reproductive ageing, as similar mechanisms may exist in other tissue types or other species.

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