Decoding the functional significance of follicle stimulating hormone glycosylation variants

Follicle stimulating hormone (FSH) and its G protein-coupled receptor (FSHR) are essential for the coordination of reproductive functions. As such, they are a primary target of most assisted reproductive technologies, thus understanding the physiology regulating their function is paramount. Two naturally occurring glycoforms of FSH have been identified-hyperglycosylated FSH (FSH24) and hypoglycosylated FSH (FSH21), based on their ASN glycosylation pattern. The secretion of FSH21 and FSH24 has been shown to be differentially regulated during the menstrual cycle, suggesting functional dichotomy of FSH21 and FSH24 within the ovary. Additionally, FSH24 and FSH21 display differential binding kinetics to FSHR and potency at activating cAMP, the principle G protein-dependent pathway of FSH/FSHR, suggesting an important role for the differential actions and activities of FSH glycoforms in regulating FSHR function. This study aimed to (1) determine how the differential effects of FSH21 and FSH24 are mediated by the FSHR, and (2) determine additional differential signalling pathways/novel targets of FSH21 and FSH24. As we have previously shown di/oligomerisation of the luteinising hormone receptor to be an important modality for regulating signal strength, we determined if FSH21 and FSH24 differentially modulated FSHR di/oligomerisation via the super resolution imaging technique, photoactivated dye-localisation microscopy. In HEK293 cells transiently expressing FSHR, acute 2-minute treatment with 30ng/ml FSH21 resulted in a significant decrease in the number of FSHR homomers observed in comparison to basal, however, no signficant difference from basal was observed with FSH24 treatment. Analysis of the individual FSHR homomeric forms revealed an enrichment in dimeric population and decrease in higher order FSHR oligomers following FSH21-treatment, suggesting the enhanced signalling properties of FSH21 may be mediated by alterations in FSHR associations. Phosphokinase screening identified novel differential targets of FSH21 and FSH24. These data support the distinct roles of FSH glycoforms and suggest this may be mediated via modulating FSHR di/oligomerisation.