Genetics and Development (including Gene Regulation)

G PROTEIN-COUPLED RECEPTOR SIGNALING IN ENDOCRINE SYSTEMS: NOVEL MECHANISMS IN HEALTH AND DISEASE

Identifying Regulatory Elements Within a Novel Enhancer of FSHB Containing Two PCOS-Associated Single Nucleotide Polymorphisms

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Polycystic ovary syndrome (PCOS) is the most common cause of female infertility, affecting approximately 10 percent of women by Rotterdam criteria, and is comorbid with obesity, type II diabetes, hypertension, and non-alcoholic fatty liver disease. As twin studies reveal that genetics account for approximately 70% of PCOS risk, genome-wide association studies (GWAS) can provide powerful insight into PCOS etiology. PCOS GWAS studies from several populations identified a risk locus containing the *FSHB* gene, which encodes the beta subunit of follicle-stimulating hormone (FSH). As FSH supplementation can restore ovulation in some PCOS patients, deficient FSH signaling could be a causative factor of anovulation and potentially other facets of PCOS. Two of the lead single nucleotide polymorphisms (SNPs) in association with PCOS, rs11031005 and rs11031006, fall within a highly conserved genomic region in mammals. We hypothesized that the conserved region (~450 base pairs) enhances *FSHB* transcription, and that one or both PCOS-related SNPs alter its function. We have shown that the conserved region from both human and mouse can act as an enhancer of *FSHB* in LβT2 cells, an immortalized, mouse-derived, mature pituitary gonadotrope cell line, and that its function is altered by the rs11031006 minor allele through modification of an SF1 consensus site. As elimination of the SF1 site reduced but did not completely abolish the function of the enhancer, we continued our investigation to identify additional regulatory sites. Transient transfection of LβT2 cells revealed a possible role for the rs11031005 SNP in *FSHB* regulation, with the minor allele decreasing enhancer-mediated *FSHB* transcription. This effect may be due to decreased binding of an unidentified transcription factor, as gel shift revealed that the rs11031005 minor allele reduced the intensity of a binding complex. Using truncations and sliding deletions, we identified three additional putative transcription factor binding sites with consensus sequences for ZEB1, PTX1, and SMAD. To support a role for the conserved region as an enhancer in native chromatin, we assessed the histone status in LβT2 chromatin. Compared to the proximal *Fshb* promoter, the enhancer-specific marker, H3K4me1, was enriched near the conserved region. Neither promoter/enhancer markers of active (H3K27Ac) or repressed (H3K27me3) chromatin were enriched near the conserved region, although levels of both modifications were consistent with the *Fshb* proximal promoter. Overall, our data support the role of this conserved region as a novel regulator of *FSHB/Fshb* transcription and reveal a possible mechanism to explain the contribution of PCOS-associated SNPs through *FSHB* regulation.

Adrenal

ADRENAL - TUMORS

Sterol O-Acyl Transferase 1 as a Prognostic Marker of Adrenocortical Carcinoma

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Background: Adrenocortical carcinoma (ACC) is a rare endocrine malignancy with an unfavorable prognosis. Despite the poor prognosis in the majority of patients, no improvements in treatment strategies have been achieved, largely due to the rarity of these tumors. Therefore, the discovery of new prognostic biomarkers that could guide and improve the management of patients with ACC is of enormous interest. Sterol-O-Acyl Transferase 1 (SOAT1) is involved in cholesterol esterification in adrenocortical cells. Recently, it was demonstrated that SOAT1 inhibition leads to impaired steroidogenesis and cell viability in ACC [1]. There are no studies so far addressing the impact of SOAT1 protein expression in ACC prognosis and clinical outcomes. ***Methods:*** We evaluated SOAT1 protein expression by immunohistochemistry (ab39327; 1:4000; *Abcam, EUA*) in a tissue microarray of 107 adrenocortical carcinomas (Weiss score ≥ 3) from adult patients treated in a single tertiary center in Brazil. Immunohistochemistry results were evaluated through a semiquantitative approach by two independent pathologists. We aimed to evaluate the correlation of SOAT1 protein expression with clinical and biochemical parameters, surgical specimen histological characteristics, recurrence free-survival, progression free-survival and overall survival.