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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SAT-165

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.

Results: SOAT1 protein expression was heterogenous in this cohort; 38% of ACCs demonstrated strong SOAT1 protein expression while 62% demonstrated weak or absent SOAT1 protein expression. Strong SOAT1 protein expression correlates with known features of high aggressiveness in ACC, such as excessive tumor cortisol secretion (p= 0.007), advanced disease stage [ENSAT 3 and ENSAT 4 (p= 0.009)] and high Ki67 index (0.008). On multivariate analysis, strong SOAT1 protein expression was an independent predictor of lower overall survival (HR 1.71, CI 95% 1.05-2.92; p= 0.04) when considering all cases (n= 107) and of lower progression free survival (HR 3.05, CI 95% 1.05-8.85; p= 0.04) in patients with metastatic disease at diagnosis (n= 22).

Conclusions: Our findings demonstrated that SOAT1 protein expression has prognostic value in ACC and reinforce the importance of investigating SOAT1 as a possible therapeutic target for patients with ACC. Multicentric prospective studies including a larger number of patients are needed in order to validate and consolidate the results found in this cohort.

References:

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Sbiera S, Leich E *et al*. Mitotane inhibits Sterol-O-Acyl Transferase 1 Triggering Lipid-Mediated Endoplasmic Reticulum Stress and Apoptosis in Adrenocortical Carcinoma Cells. Endocrinology. 2015; 156 (11):3895-908.

Bone and Mineral Metabolism

PARATHYROID HORMONE TRANSLATIONAL AND CLINICAL ASPECTS

Characteristics and Outcomes of Severe Hypercalcemia Related Admissions - a Single Centre 5 Years' Experience

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SAT-397

Introduction: Severe hypercalcemia (corrected calcium ≥3.5mmol/L) is typically associated with multi-organ dysfunction and increased mortality. We audited 47 consecutive patients who were admitted to a single tertiary referral center over 5 years period (2014-2019) with severe hypercalcemia. Results: The median age 69 years (10-97yrs); 55% females, and median length of stay was 9 days (1-120). Most patients (30%) were admitted under general medicine and 53% received endocrinology consultation. Renal dysfunction (91%) dominated the clinical presentation, but gastrointestinal abnormalities (70%), neuropsychiatric manifestations (53%), and musculoskeletal involvement (45%) were also very common. PTH was measured in 43/47 patients with 37 PTH independent (calcium level 3.82 mmol/L) and 6 PTH dependent (calcium level 3.70mmol/L) hypercalcemia. Recurrence of the condition within five years was recorded for 5 patients (11%), ten (21%) patients died during the admission, and 4 patients (9%) required ICU admission in PTH independent severe hypercalcaemia, majority are due to malignancy; while none of these outcomes were observed in PTH dependent severe hypercalcaemia. The length of hospital stay is longer in PTH independent (15.5 days) as compared to PTH dependent severe hypercalcaemia (12 days). The most common cause of severe hypercalcemia was malignancy (47%) with multiple myeloma as the most common in 32% followed by lung cancer at 27%. The other non-malignancy causes are calcium supplementation, vitamin D toxicity, and hyperparathyroidism implicated in 13% each. Twenty eight patients (65%) were managed by fluid and antiresorptive agent with 26 patients able to decrease calcium level to <3mmol/L (93%). Eight patients (19%) were managed by fluid alone (not effective in 37%), 9% by antiresorptive agent alone (not effective in 25%), and 16% did not receive any fluid nor antiresorptive agent (not effective in 43%). Conclusion: Similar to previous studies, severe hypercalcaemia is commonly seen in patients with malignancy and associated with significant symptoms, recurrence in 5 years, ICU admission and mortality. Calcium and vitamin D supplementation and hyperparathyroidism are also found to commonly cause severe hypercalcemia. The most effective management is combination of fluid resuscitation and antiresorptive agent to decrease the calcium level to <3mmol/L.

Neuroendocrinology and Pituitary

NEUROENDOCRINOLOGY AND PITUITARY

Peak Stimulated Growth Hormone Is Lower in Subjects with Nonalcoholic Fatty Liver Disease Than Controls of Similar Sex, Age and BMI

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MON-286

Background: Nonalcoholic fatty liver disease (NAFLD), fatty infiltration of the liver in the absence of alcohol use, is a prevalent and serious complication of obesity. Obesity is a state of relative growth hormone (GH) deficiency, and GH has been identified as a candidate disease-modifying target in NAFLD because of its lipolytic and anti-inflammatory properties. However, it is not known whether individuals with NAFLD phenotyped by proton magnetic resonance spectroscopy (1H-MRS), the gold standard imaging modality for assessment of intrahepatic lipid (IHL) content, have lower peak stimulated GH levels as compared to those of similar age, sex and BMI without NAFLD.

Methods: We studied 99 generally healthy adults without diabetes or significant alcohol use, ages 19-67 y and BMI >25 kg/m^2. All subjects underwent 1H-MRS for assessment of IHL content. Using a cutoff of >5.5%, 65 subjects had NAFLD and 34 did not (controls). GHRH-arginine