Neuroendocrinology and Pituitary

NEUROENDOCRINOLOGY AND PITUITARY BASIC RESEARCH ADVANCES

Potential Role for the RASD1 Glucocorticoid-Responsive Gene in Corticotroph Tumorigenesis

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Introduction: Originally identified due to its dexamethasone inducibility in mouse corticotropinoma AtT20 cells, RASD1 is a receptor-independent activator of G-proteins, via guanine nucleotide exchange factor (GEF) activity. It remains unclear, however, whether, and if so, how RASD1 mediates the effects of glucocorticoids on corticotroph cells. We identified a rare germline *RASD1* variant and investigated its functional effects *in vitro*. **Methods:** We screened 209 CD patients (94.3% pediatric) studied at the at the National Institutes of Health Clinical Research Center between 1997 and 2018 by germline whole-exome sequencing (WES) only (n=157), germline and tumor WES (n=27), and/or *RASD1* droplet digital PCR germline copy number variant (CNV) analysis (n=201). Corticotropinoma DNA was available in 72 patients to screen for *USP8* hotspot variants by Sanger sequencing. A *RASD1* variant was identified and functionally characterized. **Results:** We studied 119 female (56.9%) and 90 (43.1%) male CD cases, including 197 pediatric (≤18 years at disease onset) and 12 adult patients. *USP8* defects were present in 19.4% (14/72) of cases. No *RASD1* CNVs were found. A rare (with a minor allele frequency of 0.0022% in gnomAD v3) heterozygous germline missense *RASD1* variant, c.580A>C, p.M194L was detected in one male sporadic case. Neither *USP8* variants nor loss of heterozygosity at the *RASD1* variant position were observed in the patient's microadenoma. The wild type and p.M194L RASD1 transiently overexpressed proteins displayed similar short half-lives (<1 h) by cycloheximide chase in HEK293 cells, as well as cytoplasmic localization by immunocytofluorescence in AtT20 cells. A CRISPR/Cas9 *Rasd1* knockout AtT20 cell line displayed reduced *Pomc* expression compared with the parental cell line at the mRNA level (*Actb*-normalized absolute quantification 5.80±0.92 vs 9.62±0.7, P=0.005). Viability of the cell lines did not differ significantly by MTT assay. Overexpression of p.M194L resulted in increased accumulation of phospho-CREB S133 (1.83±0.8 vs 1±0.2 in empty vector control, P=0.0390) as well as a non-significant increase in *Pomc* expression in wild type, but not in *Rasd1* knockout AtT20 cells by immunoblot band densitometry. **Conclusions:** We found an infrequent *RASD1* variant in one CD patient. *Rasd1* seems to have a role within the intracellular signaling pathways controlling *Pomc* expression. Overexpression of the p.M194L variant caused phospho-CREB S133 activation, suggesting increased GEF activity for this variant. Interestingly, another variant at the same position, p.M194I, was found in the COSMIC database (COSS2121715) as a somatic change in cutaneous malignant melanoma. Further studies are required to better define the role of *RASD1* in corticotroph physiology and its possible involvement in tumorigenesis.

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Prenatal Androgen Excess Impairs Sexual Behavior in Adult Female Mice: Perspective on Sexual Dysfunction in PCOS

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Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders worldwide, affecting 5-20% of reproductive aged women [1]. PCOS is characterised by androgen excess, oligo- or anovulation, and polycystic ovarian morphology [1]. PCOS patients also experience sexual dysfunction, including decreased sexual desire, increased sexual dissatisfaction and gender dysphoria [2-4]. The origins of PCOS-related sexual difficulties remain unidentified, but may be related to impaired central mechanisms regulating sexual behaviours. Prenatally androgenized (PNA) mice recapitulate the PCOS phenotype and exhibit alterations in the neuronal network regulating reproductive function [5], providing a powerful, pathology-based model to unravel the biological origins of sexual dysfunction in PCOS. Here, we aimed to determine whether female sexual behaviours are impaired in the PNA mouse model of PCOS. To model PCOS, female dams received injections of dihydrotestosterone (PNA) or oil vehicle (VEH) daily from gestational day 16-18. Adult female offspring were ovariectomized and implanted with a silastic capsule of estradiol to examine the female-typical sexual behaviour: lordosis as well as partner preference. We also examined a potential masculinisation of the brain by replacing the estradiol implant by a testosterone implant then testing the female for male-like sexual behaviours. PNA females exhibited significantly reduced lordosis behaviour compared to VEH females ($p<0.01$). In contrast, partner preference and male-like sexual behaviour were not different between PNA and VEH females. In addition, using Open-field test and elevated-plus maze, we observed no effect of prenatal androgen exposure on locomotion and anxiety. These results highlight, for the first time, that prenatal exposure to the non-aromatisable androgen, DHT, impairs female receptivity only without masculinisation. These findings support the use of the PNA mouse model of PCOS to identify the neuronal targets of prenatal androgen action and to determine the mechanisms by which prenatal androgen excess