

a picture of severe acute pancreatitis. Laboratory tests showed hypercalcemia (13.9 mg/dL, normal 8.5-10.6 mg/dL) and elevated lipase (1134 U/L, normal 13-60 U/L); however, a magnetic resonance cholangiopancreatography showed no biliary obstruction. Further testing revealed markedly elevated PTH (>5000 pg/ml, normal 15-65 pg/ml), and subsequent neck ultrasound showed a solitary mass on the left side of the neck. Despite maximum medical treatment, the patient continued to rapidly decompensate and passed away rapidly. Autopsy examination revealed a picture of severe acute pancreatitis including a markedly enlarged necrotic pancreas (360 grams, normal: 60-100 grams), extensive omental fat necrosis, ascites, and dusky discoloration of the abdominal organs. A well-circumscribed mass (6.6 x 3.5 x 1.5 cm) was found on the superior aspect of the left thyroid lobe. The tumor showed parathyroid cell proliferation admixed with banding fibrosis, no unequivocal invasion into the surrounding capsule, blood vessels or perineural spaces, and no evidence of lymph node involvement or distant metastasis, consistent with a diagnosis of APA. Her cause of death was the left neck APA and its associated sequelae of significant hypercalcemia and acute pancreatitis. **Conclusion:** This patient had asymptomatic hypercalcemia for months prior to presentation with PTH/hypercalcemic crisis, highlighting the importance of ruling out primary hyperparathyroidism in the assessment of patients with asymptomatic hypercalcemia. While it's generally appreciated that parathyroid carcinomas usually cause more profound hypercalcemia and are more likely to cause fatality from metabolic complications, and that APAs follow a generally more benign course, this case shows that APAs can grow into significantly large lesions and could follow a severe and abrupt clinical course if not surgically removed.

Neuroendocrinology and Pituitary HYPOTHALAMIC-PITUITARY DEVELOPMENT AND FUNCTION

Single-Cell Sequencing Identifies Novel Regulators of Thyrotrope Populations and POU1F1-Independent Thyroid-Stimulating Hormone Expression

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We implemented single-cell RNA sequencing (scRNAseq) technology as a discovery tool to identify factors enriched in differentiated thyrotropes. Thyroid-stimulating hormone (TSH) is produced in the pars distalis of the anterior pituitary (AP) and primarily acts on the thyroid gland to regulate metabolism through T3/T4. However, TSH is also produced by cells in the pars tuberalis (PT), which is comprised of a thin layer of cells that extends rostrally from the pars distalis along the pituitary stalk to the median eminence in the hypothalamus. TSH produced by PT thyrotropes acts on hypothalamic tanycytes to regulate seasonal reproduction. PT thyrotropes likely descend from rostral tip thyrotropes that arise at e12.5 of mouse development, which transcribe the TSH beta subunit (*Tshb*) without detectable expression

of the transcription factor POU1F1. POU1F1 is required for *Tshb* transcription in thyrotropes of the adenohypophysis, and it acts synergistically with GATA2 to drive cell fate. The molecular mechanisms driving *Tshb* expression independently of *Pou1f1* in PT thyrotropes are unclear.

Thyrotropes are the least abundant endocrine cell-type in the pituitary gland. We used genetic labeling and fluorescence-activated cell sorting (FACS) to enrich for thyrotropes for single-cell sequencing. We performed scRNAseq on 7-day-old GFP-positive pituitary cells from *Tshb-Cre; R26-LSL-eYFP* and intact whole pituitaries, recovering more than 15,000 cells altogether. We observe two distinct populations of cells expressing *Tshb*. The larger thyrotrope population has approximately twenty fold higher levels of *Tshb* and five fold higher *Cga* transcripts than the smaller population, and they are also distinguished by expression of *Pou1f1*, TSH-releasing hormone receptor (*Trhr*), and deiodinase 2 (*Dio2*), consistent with expectations for AP thyrotropes. The smaller thyrotrope population does not express *Pou1f1*, but those cells are characterized by expression of TSH receptor (*Tshr*) and melatonin receptor 1A (*Mtnr1a*), consistent with expectations for PT thyrotropes. They express mildly increased levels of *Eya3* and *Six1*, although these genes are expressed in other cell-types including AP thyrotropes, stem cells, and gonadotropes. They have two-fold higher levels of *Gata2* transcripts and uniquely express the transcription factor *Sox14*. SOX14 is a SoxB2 family transcription factor that counteracts the transcriptional activity of SoxB1 family members, such as *Sox2*. In conclusion, our scRNAseq has identified novel markers of PT thyrotropes and unveils novel insights into the similarities and differences in the development and function of pituitary thyrotrope subpopulations.

Genetics and Development (including Gene Regulation)

GENETICS AND DEVELOPMENT AND NON-STEROID HORMONE SIGNALING I

Familial 46, XY Complete Female External Sex Development with a Non-Mosaic Inherited SRY Gene Variation

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SUN-712

Abstract

Context: SRY, an architectural transcription factor containing a SOX-related high-mobility group (HMG) box, initiates testis formation in the mammalian bipotential gonadal ridge. Inherited human SRY mutations can be associated with differences in sexual differentiation (DSD) with variable phenotypes in a family.

Objective: To describe the clinical, histopathological and molecular features of a novel inherited *SRY* allele (pMet64Val; consensus box position 9) observed within an extensive pedigree: two 46, XY sisters with primary amenorrhea (16 and 14 years of age; probands P1 and P2), their normal father and brother, and an affected paternal XY grandaunt.

Design, Setting, Participants and Outcome Measurements: Following DNA sequencing to identify the *SRY* mutation, hormonal studies of the probands and histopathological examination of their gonads were performed. Functional consequences of p.Met64Val (and other mutations at this site) were also investigated.

Results: Breast development in P1 and P2 was Tanner II and IV, respectively. Müllerian structures and gonads resembling ovaries were found in each sister. Histopathology revealed gonadal dysgenesis, gonadoblastoma and dysgerminoma. AMH/MIS, P450 SCC, and P450 aromatase were expressed in gonadoblastoma tissues. Variant p.Met64Val impaired *Sox9* transcriptional activation associated with attenuated occupancy of the testis-specific enhancers *Enh13* and *TESCO*.

Conclusion: The partial biological activity of p.Met64Val *SRY*, maintained at the threshold of *SRY* function, rationalizes opposing paternal and proband phenotypes (the “father-daughter paradox”). Sex steroids biosynthesis by gonadoblastoma may delay genetic diagnosis and recognition of gonadal tumors. Quantitative assessment of inherited *SRY* alleles highlights the tenuous transcriptional threshold of developmental decision-making in the bipotential gonadal ridge.

Reproductive Endocrinology

CLINICAL STUDIES IN FEMALE REPRODUCTION I

Prolactin Is Expressed in Uterine Leiomyomas and May Promote Their Growth

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SAT-003

Prolactin is Expressed in Uterine Leiomyomas and May Promote Their Growth

(DiMauro A., Dahal A., Okeke I., Lerman I., Amitrano A., Seger C., Kumar D., Bhagavath B., Taya M., Hammes S. R.) Uterine leiomyomas, commonly referred to as fibroids, are benign, estrogen sensitive smooth muscle tumors that occur in the myometrium of the uterine wall. Leiomyomas are common, as it is estimated that 60% of reproductive-aged women are affected, and 80% of women develop leiomyomas during their lifetime. In fact, uterine leiomyomas are the leading cause of abnormal menstrual bleeding or menstrual pain, as well as the number one reason for hysterectomy. Novel treatment options are necessary as current treatments are limited to anti-estrogen therapy or hysterectomy. Estrogen is known to have an effect on

the etiology of leiomyomas, but little is known about the proliferative roles of other hormones in leiomyomas. One hormone of interest is prolactin (PRL) which is primarily secreted from the pituitary to regulate lactation, but has been linked to proliferation in breast cancer, perhaps via local prolactin production in breast tissue. With this background, we examined local PRL production and its effects on leiomyomas. RNA isolation and quantitative PCR of human leiomyoma samples (n=20) relative to adjacent myometrium in the same patients confirmed significant expression of both PRL (p= 0.0028) and dopamine receptor D2, a known regulator of PRL production in the pituitary (p<0.0001), with no difference in prolactin receptor expression. Using both immunohistochemistry and immunofluorescence of human leiomyomas samples, we find increased prolactin expression in leiomyomas when compared to adjacent myometrium or control uteri. These results suggest that leiomyomas contain cells producing PRL, which in turn may promote signaling in smooth muscle leiomyoma cells to regulate proliferation. Accordingly, we find that PRL robustly activates STAT5 and MAPK signaling in the rat leiomyoma cell line ELT3. Functional assays were also conducted to evaluate the ability of PRL to induce migration, invasion and proliferation of ELT3 cells. Together, our findings suggest that local prolactin production in leiomyomas may promote their growth, migration, invasion and proliferation. It is possible that this local production is mediated by the dopamine receptor D2. Thus, anti-PRL therapy or dopamine receptor D2 modulation may prove useful in treating this prevalent and often debilitating disease.

Bone and Mineral Metabolism

BONE DISEASE FROM BENCH TO BEDSIDE

Sex Is a Strong Variable in the Mineral Metabolism Defects and Endocrine Dysfunction Associated with the Murine Adenine Diet Model of Chronic Kidney Disease (CKD).

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The adenine diet is widely used in animal models to produce a tubulointerstitial fibrosis and inflammation that mimics human CKD in many aspects. These include the biochemical manifestations hyperphosphatemia and anemia, as well as endocrine dysfunction with elevated FGF23 and hyperparathyroidism. Male rodents are known to be less tolerant of adenine diet regimen than females, however the underlying mechanisms driving the sex differences remain unclear. Additionally, much of the data for adenine studies arises from rats, whereas mice are more commonly used in laboratory settings and are far easier to manipulate genetically. To this end, as part of a larger study to test the effects of iron-handling in CKD, we assessed the biochemical,