few reports have been shown in Cushing's disease (CD). It suggests a presence of glucocorticoid (GC)-driven positive-feedback loop. However, the underlying mechanism remains elusive. Here we present a case of CD showing clear clinical and pathophysiological evidences of GC positive-feedback using ex vivo 3-dimensional (D) culture method.

Case: A 62-year-old woman manifested typical Cushing's symptoms, including moon face, central obesity, hypertension, hypokalemia, and vertebral fractures. Endocrinological data were consistent with a diagnosis of CD; morning plasma ACTH 299 pg/mL, serum F 28 μg/dL, midnight serum F 43 μg/dL, and 24hr urinary free cortisol 988 μg/day. CRH test showed a slight increase in plasma ACTH levels (1.4 fold), and pituitary MRI revealed a 14 mm macroadenoma invading into the left cavernous sinus. Interestingly, both 1 mg and 8 mg DST showed a paradoxical increase in serum cortisol levels (27.7→64.7μg/dL and 17.17→35.68μg/dL, respectively). These data indicated a presence of positive-feedback response to GC in the tumor. Indeed, after the initiation of metyrapone (1,000 mg/day) administration for the treatment of hypercortisolemia, plasma ACTH levels were decreased to 147.5 pg/mL accompanied with the decrease in serum F levels to 4.12 μg/dL. Moreover, pituitary tumor obviously shrank during the metyrapone treatment. Thereafter, we undertook transsphenoidal surgery and plasma ACTH and serum F levels decreased to 35.8 pg/mL and 7.6μg/dL, respectively.

ex vivo studies: To prove the presence of the positive feedback response and explore the underlying mechanisms, we performed a primary culture experiment using the tumor and applied 3D culture method. The resected tumor tissue was enzymatically digested, dispersed and embedded into the Matrigel. Then cells were treated with Dex (0.1-10 nM), and ACTH concentrations were measured after 72 hrs. Interestingly, ACTH levels significantly increased by 10 nM Dex treatment at 72 h (129 %, $p < 0.01$), indicating a paradoxical response to Dex in the tumor ex vivo.

Conclusions: To our knowledge, this is the first case of CD who showed clinically obvious GC positive-feedback that was proved by ex vivo 3D primary culture cell models. Notably, tumor shrinkage was observed during the metyrapone treatment, suggesting that GC positive-feedback mechanisms also involve tumor proliferation. Further investigation is required for elucidating the underlying mechanisms.

Genetics and Development (including Gene Regulation)

GENETICS AND DEVELOPMENT AND NON-STEROID HORMONE SIGNALING II

The Cellular and Molecular Landscape of Hypothalamic Patterning and Differentiation

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The hypothalamus is a central regulator of physiological homeostasis. During development, multiple transcription factors coordinate the patterning and specification of hypothalamic nuclei. However, the molecular mechanisms controlling hypothalamic patterning and cell fate specification are poorly understood. To identify genes that control these processes, we have used single-cell RNA sequencing (scRNA-Seq) to profile mouse hypothalamic gene expression across multiple developmental time points. We have further utilised scRNA-Seq to phenotype mutations in genes that play major roles in early hypothalamic patterning. To first understand hypothalamic development, hypothalami were collected at both embryonic (E10-E16, E18) and postnatal (PN4, PN8, PN14, PN45) time points. At early stages, when the bulk of hypothalamic patterning occurs (E11-E13), we observe a clear separation between mitotic progenitors and postmitotic neural precursor cells. We likewise observed clean segregation among cells expressing regional hypothalamic markers identified in previous large-scale analysis of hypothalamic development. This analysis reveals new region-specific markers and identifies candidate genes for selectively regulating patterning and cell fate specification in individual hypothalamic regions. With our rich dataset of developing mouse hypothalamus, we integrated our dataset with the Allen Brain Atlas *in situ* data, publicly available adult hypothalamic scRNA-Seq dataset to understand hierarchy of hypothalamic cell differentiation, as well as re-defining cell types of the hypothalamus.

We next used scRNA-Seq to phenotype multiple mutant lines, including a line that has been extensively characterised as a proof of concept (*Ctnnb1* overexpression), and lines that have not been characterised (*Nkx2.1*, *Nkx2.2*, *Dlx1/2* deletion). We show that this approach can rapidly and comprehensively characterize mutants that have altered hypothalamic patterning, and in doing so, have identified multiple genes that simultaneously repress posterior hypothalamic identity while promoting prethalamic identity. This result supports a modified columnar model of organization for the diencephalon, where prethalamus and hypothalamus are situated in adjacent dorsal and ventral domains of the anterior diencephalon. These data serve as a resource for further studies of hypothalamic development and dysfunction, and able to delineate transcriptional regulatory networks of hypothalamic formation.

Lastly, using our mouse hypothalamus as a guideline, we are comparing dataset of developing chicken, zebrafish and human hypothalamus, to identify evolutionarily conserved and divergent region-specific gene regulatory networks. We aim to use this knowledge and information of key molecular pathways of human hypothalamic development and produce human hypothalamus organoids.

Adrenal

PROGRESS IN ADRENAL CORTEX AND MEDULLA RESEARCH

The Study of Cell Senescence in Cortisol-Producing Adrenocortical Adenomas

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IntroductionAging is associated with the pathogenesis of many endocrine disorders such as cardiovascular diseases and diabetes. Cell senescence has been reported as one of their mechanisms. In addition, stress responsiveness has been reported to be associated with cell senescence. In addition, some genetic abnormalities such as mitochondrial DNA (mtDNA) damages or telomere shortening, have been detected in some endocrine disorders. Cortisol is a well-known stress-induced hormone and closely associated with aging. We previously reported that cortisol-producing adenoma (CPA) more abundantly expressed cell senescent markers such as p16 and p21 than other hormone-producing adrenocortical adenomas. However, the detailed pathophysiology of cell senescence and its association with histological features in CPAs have remained virtually unknown. Therefore, we analyzed cell senescent markers (telomere length, mtDNA copy number, mtDNA deletion and p16 and p21 immunoreactivity) and analyzed their correlation with clinicopathological factors in CPA patients. **Methods & Materials**Forty CPA cases was immunohistochemically evaluated. Twenty CPA, ten adjacent ZF and six non-functional adenoma (NFA) were examined for mtDNA abnormalities. mtDNA deletion was evaluated by nested-PCR and mtDNA copy number and telomere length were measured using real-time PCR. **Results**

p21 immunoreactivity was significantly higher in CPA than that of adjacent ZF (P=0.0001) and significantly inversely correlated with tumor size (P=0.0004). Telomere length was much longer in CPA than that in adjacent ZF(P=0.0038), and NFA (P=0.0018). mtDNA copy number of NFA was significantly higher than that of CPA and adjacent ZF (P=0.0038). mtDNA copy number of compact cells was significantly higher than that of clear cells (P=0.0432). mtDNA copy number of compact cells was positively correlated with urinary free cortisol (UFC) (P=0.0428) and plasma cortisol (F) (P=0.0609). mtDNA copy number of clear cells were inversely correlated with F (0.0497). 4977 bp mtDNA deletion was more frequently detected in CPA (54%) and in adjacent ZF (50%) than in NFA (17%).**Discussion**

Results of our present study did reveal that CPA harbored more senescent phenotype as demonstrated by abundant p16 and p21, marked telomere shortening, frequent mtDNA 4977bp deletion and relatively low mtDNA copy number, possibly caused by long-term exposure of excessive cortisol in situ compared to NFA. In addition, clear tumor cells could represent more senescent histological phenotype because of their lower mtDNA copy numbers. This is the first study to demonstrate that compact tumor cells were biologically more active than clear tumor cells and could reflect clinical cortisol biosynthesis, resulting in marked functional intratumoral heterogeneity in CPAs.

Bone and Mineral Metabolism

PARATHYROID HORMONE TRANSLATIONAL AND CLINICAL ASPECTS

Safety and Efficacy of Conventional Therapy with Calcium and Activated Vitamin D in Patients with Chronic Post-Operative Hypoparathyroidism: Results of a Cross-Sectional Case-Control Study

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Introduction: Conventional therapy of chronic post-operative hypoparathyroidism (PO-HypoPT) with calcium and activated vitamin D is suboptimal and associated with several complications, including impairment of the quality of life. Aim of this study was to compare clinical, biochemical and instrumental parameters in 120 patients who underwent total thyroidectomy for differentiated thyroid cancer, 60 with PO-HypoPT (Group A) treated with conventional therapy and 60 without (Group B), matched for age and sex, followed a tertiary referral center. Matherials and methods: An "ad hoc" CRF was used to collect epidemiological, clinical (symptoms, treatment) and biochemical data (total and ionized calcium, albumin, phosphate, magnesium, calcium/phosphate product, creatinine, 25-OH vitamin D, PTH, TSH, eGFR, 24-h urinary calcium and creatinine), and renal ultrasound. Results: The median duration of PO-HypoPT was 7 years (IQR 4-13). All patients of group A were treated with calcitriol (median 0.5 μg/daily; IQR 0.5-1.0), and 33/60 (55%) were also given calcium carbonate supplementation (median 1000 mg/daily; IQR 500-1000). Hypocalcemia related symptoms were more frequent in group A (27/60 - 45%) than in group B (1/60 - 1.7%) ($p<0.01$). Total and ionized serum calcium [median 8.9 (IQR 8.5-9.1) vs 9.3 (IQR 9.0-9.5) mg/dl; median 1.16 (IQR 1.1-1.2) vs 1.23 (IQR 1.21-1.27) mmol/L] ($p<0.01$), magnesium [median 1.9 (IQR 1.8-2.0) vs 2 (IQR 1.9-2.1) mg/dl - $p<0.01$] and PTH [median 10 (IQR 8-13) vs 29 (IQR 22-35) pg/ml - $p<0.01$] were significantly lower in Group A vs Group B. Conversely, serum phosphate [median 3.7 (IQR 3.4-4.1) vs 3.3 (IQR 3.0-3.6) mg/dl - $p<0.01$], calcium-phosphate product [median 33 (IQR 30-36) vs 30 (IQR 27-34) - $p=0.012$] and 25-OH vitamin D [median 34.1 (IQR 29.2-41.3) vs 26.7 (IQR 18.1-33.4) - $p<0.01$) were significantly higher in Group A vs Group B. Twenty-four hour urinary calcium was higher in group A [median 248 mg (IQR 166-363)] than in group B [median 165 mg (IQR 94-229)] ($p<0.01$). The rate of nephrolithiasis was significantly higher in group A (21/60 pts - 35%) than in group B (7/60 pts - 11.7%) ($p<0.01$). Moreover, there was a significant correlation of neprholithiasis with 24h urinary calcium but not with total and ionized serum calcium. Conclusions: This cross-sectional case-control study confirms that treatment of chronic PO-HypoPT with conventional therapy is suboptimal, even in a tertiary referral center, and associated with an increased risk of nephrolithiasis. Following the recent publication of treatment guidelines, the question