carried by the larger apo(a) size in all subjects (r=0.139, p=0.0361). In race/ethnicity analyses, a significant association was seen for African-Americans (r=0.268, p=0.0199), but not for Caucasians. In contrast, there were no significant associations of PCSK9 with isoform-specific Lp(a) levels for the smaller apo(a) sizes in all participants nor in ethnic-specific analyses. Of note, PCSK9 levels were significantly negatively associated with the larger apo(a) isoform sizes in all participants (r=-0.139, p=0.0366). Although significant in both groups, heritability of PCSK9 level was higher in Caucasians than in African-Americans (47% vs. 22%, respectively). **Conclusions:** Among African-Americans, but not Caucasians, PCSK9 levels were associated with isoform-specific Lp(a) levels carried on larger, but not smaller, apo(a) sizes. The findings illustrate a diverging relationship of PCSK9 with isoform-specific Lp(a) levels.

Tumor Biology

TUMOR BIOLOGY: DIAGNOSTICS, THERAPIES, ENDOCRINE NEOPLASIAS, AND HORMONE DEPENDENT TUMORS

Clonal Status of Multigland Disease Primary Hyperparathyroidism

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SUN-137

Abstract: Primary hyperparathyroidism (PHPT) is a common endocrine disorder that arises due to single or multiple parathyroid gland disease (MGD). The molecular mechanism(s) of parathyroid neoplasia are incompletely understood and both monoclonal (mono-X) and polyclonal (poly-X) parathyroid tumors have been described using methylation-sensitive PCR of X-linked Human Androgen Receptor (HUMARA) alleles. Our previous investigations of parathyroid tumor clonal status has shown that poly-X tumors are common and are associated with MGD in patients with non-familial PHPT (Shi *et al.* 2014 & 2018). This work examined the clonal status of the dominant gland and the clonal relationship of multiple tumors from the same patient has not been examined. The goal of the current study was to determine the clonal relationship of parathyroid tumors from PHPT patients with MGD. Banked parathyroid tissues from twenty-nine PHPT patients with MGD were examined in this study. Clonal status (mono-X vs poly-X) of multiple abnormal parathyroid glands from each patient was determined using a modification of the HUMARA assay used in our prior work. Briefly, methylation-sensitive PCR of HUMARA alleles was performed followed by fragment analysis using Capillary-Electrophoresis performed. Raw fragment sizing data analyzed using Peak Scanner software. Classification of samples as either mon-X or poly-X was made as described in (Shattuck *et al.*) Of 29 PHPT patients with MGD, 13 (45%) had pure mono-X, 5 (17%) had pure poly-X, and 11 (38%) had a mixture of mono-X and poly-X tumors. Five of 29 patients had three or more abnormal glands evaluated: 3 had mixture of poly-X and mono-X, 2 had pure mono-X tumors, and none were pure polyclonal-X. Eighteen (62%) out of 29 patients had paired upper or lower double adenomas. Of these, 9 (50%) were pure mono-X, 4 were pure poly-X, and 5 were mixed mono-X/poly-X. In 2 patients with multiple mono-X tumors, allele distribution was not the same in different abnormal glands. Our previous work has demonstrated that among patients with non-familial PHPT, poly-X parathyroid tumors are common and are associated with MGD. Our new data extend these findings to show that the clonal relationship between multiple parathyroid tumors from the same patient is complex and may reflect the emergence of single or multiple tumors from a background of parathyroid hyperplasia, or other mechanism(s). Future studies to explore the mechanisms behind these apparent clonal relationships are warranted and ongoing. **Reference:** (1) Shi et al., PNAS 2014, 201319742. (2) Shi et al., Surgery 2018, 9-14. (3) Shattuck. N Engl J Med 2005, 2406-12.

Thyroid

THYROID NEOPLASIA AND CANCER

Calcitonin-Based Thyroidectomy Is a Safe Approach in Patients with Germline RET Mutation and Permits to Delay Surgery in Children

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MON-490

Introduction: Medullary thyroid cancer (MTC) arises from C cells secreting calcitonin. In familial MTC cases, a germline RET mutation is discovered in 98% of cases. Nowadays, an early diagnosis and radical surgery are the only curative approach. However, thyroidectomy in children is associated with a higher rate of surgical adverse events, compared to surgery in adults. The best clinical approach in patient harboring germline RET mutation (gene carriers, GC) is still undefined. Methods and materials: since 1994 to 2018 we identified 174 GC by RET screening. 56 GC underwent total thyroidectomy and lymph node dissection for the evidence of high calcitonin levels at the first clinical evaluation, whereas 27 GC underwent surgery for high stimulated calcitonin levels during the active surveillance (median 16 months, range 13-118). 90 GC are still in follow up. Results: In the group of 27 GC patients who underwent surgery during the active surveillance, 15 GC had only C cells hyperplasia (CCH) foci and 12 were affected by MTC. These carcinomas were all confined to the thyroid, without any lymph node and distant metastasis. All these patients are still in clinical remission, after a median follow-up of 4 years (range 1-11). At time of the surgery, the patients affected by MTC were significantly older than patients harboring only CCH (median 49 vs 30 years old, respectively). Among these 27 GC, 7 were diagnosed as GC when they were younger than 18 years (median 7 years old, range 2-18) and they underwent surgery after a median

period of 3 years (range 1-10 years), when they were all older than 7 years. In this group, 6 of 7 were affected by CCH and only one case by a microMTC. There were not any persistent surgical adverse events and all of them are still in clinical remission. 41 of 90 GC, who are still in active surveillance, were younger than 18 years at time of RET screening: nowadays, 10/41 are older than 18 years and 15/41 are older than 14 years, all with calcitonin still in the normal range. Conclusions: we demonstrated that the calcitonin-based thyroidectomy is a safe approach in GC. Intriguingly, this approach seems to be interesting especially in children in order to perform still an early and safe surgery but when they are older, possibly adults.

Neuroendocrinology and Pituitary

ADVANCES IN NEUROENDOCRINOLOGY

Angiotensin II Stimulates Microglia Cell Inflammatory Responses

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SUN-254

Angiotensin II Stimulates Microglia Cell Inflammatory Responses

Angiotensin II (**AngII**) is the principal effector molecule of the renin-angiotensin system (**RAS**). It's effects on the cardiovascular and renal system are well-documented. AngII acts mainly via interaction with the AngII type-1 receptor (**AT1R**). Disordered levels of AngII lead to hypertension and cardiovascular disease. Increasing evidence suggests that AngII may also play a role in the pathophysiology of neurodegenerative diseases through unclear mechanisms. We investigated AngII, AT1R and AT2R levels in a mouse model of neurodegenerative disease, the experimentally induced autoimmune encephalomyelitis (**EAE**) mouse. In EAE mice, AngII and AT1R gene expression in brain tissue were significantly increased when compared to control mice (3.2 folds ±1.9, $p<0.05$, n=5; and 2.6 folds ±1.1, $p<0.01$, n=5 respectively). In addition, iNOS mRNA expression by qRT-PCR was likewise upregulated in EAE mice compared to control (3.4 ± 1.4 folds, $p<0.01$, n=5). We then studied the effects of AngII in human microglial cells (HMC3) -resident innate immune cells of the central nervous system (**CNS**). In HMC3 cells, treatment with AngII up-regulated the expression IL-6 (3.9 folds ± 1.2, $p<0.01$, n=4) and increased IL-6 concentration by 83% ($p<0.05$, n=4) by ELISA; effects that were blocked by the AT1R antagonist, Losartan. Also, AngII induced TNF-α production, increasing its concentration by 90% ($p<0.05$, n=4), an increase that was blocked by Losartan. We also quantified Nitric Oxide (**NO**) production by using Griess Reagent and reactive oxygen species (**ROS**) production by the MUSE Oxidative Stress assay. In these cells, NO and ROS production were significantly increased by AngII ($p<0.05$, n=4) and treatment with Losartan reduced their production ($p<0.05$, n=4). In addition, AngII treatment induced iNOS overexpression (2.5 folds ±0.8, $p<0.05$, n=4); results that are consistent with increases in the EAE mice. These data suggest that AngII can activate microglia cell inflammatory responses and as such may contribute to the pathophysiology of CNS inflammation and neurodegenerative diseases.

Genetics and Development (including Gene Regulation)

GENETICS AND DEVELOPMENT AND NON-STEROID HORMONE SIGNALING II

Single-Cell RNA-Sequencing Deciphers POMC Neuron Destiny

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MON-709

The hypothalamus is one of the critical brain nodes regulating body weight and energy homeostasis. Within this node, *Pomc* neurons sense nutrient and hormonal signals to release melanocortin peptides that induce satiety, whereas AgRP/NPY neurons exert opposite effects by releasing AGRP that promotes feeding. Immature neurons in the hypothalamic ventricular zone start to express *Pomc* at E10.5, reach a maximum number at E14.5 and then decrease to stabilize at E18.5. However, it remains elusive how *Pomc* expressing precursors adopt their final cell fates. Therefore, the goal of this study was to decipher the temporal sequence of transcription factor (TF) expression leading to the terminal differentiation of POMC neurons. Red fluorescent cells collected from dissociated hypothalami of *Pomc-tDimer-dsRed* mice at six critical developmental time points - E11.5, E13.5, E15.5, E17.5, P5 and P12- were FACS sorted for the 10X genomics scRNA-seq pipeline. Unsupervised cell clustering identified 11 distinct clusters based on their transcriptional profiles. Eight of the clusters were highly-enriched for neuronal signature genes and were further characterized based on their transcript levels for *Pomc* (high, medium or low) and other distinct feature genes. Cells in the $Pomc^{high}$ cluster expressed genes identified previously to modulate *Pomc* expression, including *Isl1, Nkx2-1,* and *Tbx3,* together with several novel candidate TFs. Unexpectedly, *Nr5a1*, the ventromedial hypothalamic nucleus marker gene encoding SF1, was highly expressed in the $Pomc^{high}$ cluster at early stages. One of the $Pomc^{low}$ clusters highly expressed *Otp*, *Agrp, Npy*, *Sst* and *Calcr* while a second was highly enriched with *Tac2, Kiss1, Pdyn*, *Prlr, Ar* and *Esr1* transcripts. All the clusters showed direct correlations of embryonic stage with the expression of progressively more mature markers of differentiation, thereby extending previous reports of these clusters based on single time points. Moreover, our results uncovered five novel *Pomc* neuron clusters with unique patterns of TF gene expression. For comparison of these data to the adult hypothalamus, we performed a TRAP-Seq study using $Pomc^{CreERT}$,$Rosa26^{eGFP\text{-}L10a}$ mice. *Prdm12 and Tbx3* were among the most highly differentially expressed TFs in the POMC neuron affinity purified translatome. Similarly, *Cited1, Npy2r,* and *Asb4* were highly expressed in both the $Pomc^{high}$ cluster and the TRAP-Seq derived POMC translatome. This comprehensive molecular