hyperinsulinemic clamp studies, both before (1st visit) and after administration of vitamin D or placebo (2nd visit and 3rd visit). Adipose tissue fibrosis and inflammation were quantified by 'real-time' rt-PCR and immunofluorescence. To determine whether vitamin D's effects are mediated through adipocytes, we performed hyperinsulinemic clamp studies and adipose tissue analysis in an adipocyte-specific vitamin D receptor knockout (VDR KO) mouse model. RESULTS/ANTICIPATED RESULTS: 25(OH)D repletion (to >30 ng/ml) was associated with reductions in adipose tissue expression of inflammatory (0.6-0.7-fold decreased expression of TNF-α, IL-6, iNOS and PAI-1) and pro-fibrotic (0.4-0.8-fold decreased expression of TGF-β1, HiF1α, Collagen I, V, VI and MMP7) factors, decreased collagen VI immunofluorescence ($p = 0.02$) and improved hepatic insulin sensitivity in humans, with suppression of endogenous glucose production (EGP) (1.28 ± 0.20 vs 0.88 ± 0.18 mg/kg/min, $p = 0.03$). Compared to wild type (WT), VDR KO mice exhibited increased adipose tissue expression of several pro-inflammatory (Tnf-α, iNos, Pai-1, Mcp-1 and F4/80; 4-10 fold) and pro-fibrotic genes (Tgf-β1, Collagen VI, and Tsp1; 2-4 fold), in concert with hepatic insulin resistance (EGP 10 ± 3 vs 3 ± 2 mg/kg/min in WT, $p = 0.021$). DISCUSSION/SIGNIFICANCE OF IMPACT: Collectively, these complementary human and rodent studies establish a beneficial role of vitamin D to improve hepatic insulin resistance, likely by restraining adipose tissue inflammation and fibrosis. Thus, normalizing 25(OH)D levels could have metabolic benefits in targeted individuals. CONFLICT OF INTEREST DESCRIPTION: **N/A**

4329

Investigating the role of Klotho in neurocognitive outcomes, brain volumes, and white matter changes in pediatric brain tumor survivors

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OBJECTIVES/GOALS: Klotho is a protein linked to improved cognition in aging adults. A specific *KLOTHO* gene variant, KL-VS, increases circulating levels of Klotho. The current study aims to identify if the KL-VS haplotype and Klotho levels are associated with improved neurocognition in pediatric brain tumor survivors. METHODS/STUDY POPULATION: We are actively accruing pediatric brain tumor patients at UCSF alongside an existing multi-institutional cohort study investigating radiation-induced vasculopathies and cognitive outcomes in this population. Normal controls are being enrolled in parallel. Each patient undergoes: 1) single nucleotide polymorphism genotyping to identify KL-VS haplotype status, 2) enzyme-linked immunosorbent assays to measure circulating Klotho, 3) neurocognitive assessments with a computer-based, validated Cogstate battery, and 4) brain volume and white matter lesion segmentation analyses using MRI sequences obtained as part of routine care. RESULTS/ANTICIPATED RESULTS: Genotyping has been performed on 99 enrolled patients. KL-VS heterozygosity was seen in 22.7% of patients. To date, KL-VS status is not associated with neurocognitive outcomes at baseline or Year 1 testing. Association between KL-VS status, circulating Klotho levels, neurocognitive outcomes, brain volume and white matter lesion segmentation analyses is ongoing. We hypothesize that elevated Klotho levels will be associated with improved neurocognition, increased brain volumes in regions of interest and decreased white matter lesion volumes. DISCUSSION/SIGNIFICANCE OF IMPACT: If circulating Klotho leads to improved neurocognition in pediatric brain tumor survivors, Klotho levels might serve as a prognostic biomarker. Furthermore, as Klotho is being investigated for therapeutic indications, it may represent an intervention to prevent cognitive deficits in these patients.

4198

Investigating the Role of Rab27B in Non-Small Cell Lung Cancer Tumor Initiating Cells

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OBJECTIVES/GOALS: Rab27B, a small GTPase, functions in exosome formation and secretion. Rab27B is overexpressed in non-small cell lung cancer (NSCLC) and predicts patient survival; however, little is known about its importance in NSCLC cells. Here, we investigated the role of Rab27B in NSCLC tumor initiating cells. METHODS/STUDY POPULATION: Tumor initiating cells (TICs) were enriched in a panel of NSCLC cell lines using low adherence spheroid cultures. QPCR and immunoblot analysis were used to compare Rab27B mRNA and protein expression, respectively, in adherent bulk cancer cells and TIC cultures. Lentiviral-packaged short hairpin RNAs (shRNAs) were used to knockdown Rab27B in PC9 and H1299 NSCLC TICs. The effects of Rab27B knockdown on PC9 and H1299 TIC expansion, transformed growth, and invasion were analyzed by MTT cell proliferation, soft agar colony formation, and Boyden chamber assays respectively. RESULTS/ANTICIPATED RESULTS: Quantitative PCR and immunoblot analysis showed that Rab27B expression is elevated in NSCLC TICs when compared to adherent bulk cancer cells. Efficient knockdown of Rab27B was achieved in PC9 and H1299 NSCLC TICs using two independent shRNA constructs. Rab27B knockdown cells exhibited decreased expansion as spheroid cultures, transformed growth, and invasion when compared to non-target shRNA control cells. Future experiments will focus on determining the importance of Rab27B in TIC exosome production and *in vivo* tumor growth and metastasis. DISCUSSION/SIGNIFICANCE OF IMPACT: Our results show that Rab27B is important in NSCLC TIC growth and invasion. Further studies are needed to determine the mechanism of Rab27B action. TICs have been linked to enhanced tumorigenic properties, suggesting that Rab27B could be a good candidate for therapeutic targeting of NSCLC TICs.

4249

Markers of mitochondrial biogenesis, fusion and architecture are disturbed in PBMC from war veterans with posttraumatic stress disorder (PTSD)

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OBJECTIVES/GOALS: The aim of this study was to define the transcription profiles of the molecular markers of mitochondrial biogenesis and fusion/architecture, and the markers of mtDNA copy numbers in the peripheral blood mononuclear cells (PBMCs) from

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war veterans with/without post-traumatic stress disorder (PTSD). METHODS/STUDY POPULATION: The peripheral blood mononuclear cells (PBMCs) from war veterans with/without post-traumatic stress disorder (PTSD) were used to monitor transcription profile of the molecular markers of mitochondrial biogenesis and fusion/architecture, as well as the markers of mtDNA copy numbers. The human male immortalized monocytes were exposed *in vitro* to hormonal markers of PTSD in order to monitor the effects of each particular hormonal marker on the molecular markers of mitochondrial biogenesis and fusion/architecture, as well as the markers of mtDNA copy numbers. RQ-PCR analyses were used to define transcriptional profile of above mentioned markers. RESULTS/ANTICIPATED RESULTS: The transcription profiles of above mentioned markers were disturbed, with high individual variability within the groups. A significant increase in the expression of the *PPARGC1A* transcript was observed in a group of subjects with current PTSD, as well as in the subjects with "life-time" PTSD, compared to healthy controls. *PPARGC1B, NRF2 and MFN2* transcripts increased only in PBMCs of "life-time"-PTSD, while the level of transcripts for other investigated genes and the ratio of markers of mtDNA copy numbers showed no significant difference between groups. The *in vitro* results showed parallelism with the results obtained using the PBMCs from the subjects of the PTSD study. DISCUSSION/SIGNIFICANCE OF IMPACT: Although preliminary (the analysis require a larger number of subjects), the results are first findings and a solid base for further extensive multidisciplinary research in order to clarify the molecular mechanisms for the prevention and treatment of trauma-induced pathological conditions.

4564

Nilotinib alters microRNAs that regulate specific autophagy and ubiquitination genes in the cerebrospinal fluid of Parkinson's patients†

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OBJECTIVES/GOALS: Our preclinical data demonstrate that the principal effects of nilotinib, a multi-tyrosine kinase inhibitor, in models of neurodegeneration is clearance of misfolded proteins via autophagy. Here we aimed to evaluate the effects of nilotinib on microRNAs in the cerebrospinal fluid of Parkinson's disease patients. METHODS/STUDY POPULATION: Cerebrospinal fluid (CSF) was collected as part of an open label phase I (NCT02281474) (n = 12, 300 mg nilotinib taken orally once daily for 6 months), and a phase II randomized, double-blind, placebo-controlled study (NCT02954978) (n = 75, randomized 1:1:1 into placebo, 150 mg or 300 mg nilotinib taken orally once daily for 12 months). RNA was isolated from CSF and Indexed sequencing libraries were prepared from total RNA plus miRNA. Next generation whole-genome sequencing (single-end 1x75 bp, 25 million raw reads per sample) was performed to identify miRNAs significantly differentially expressed (fold-change $\geq$ 2, Benjamini-Hochberg FDR p-value $\leq$ 0.05 or Empirical Bayes FDR $\leq$ 0.05) with treatment compared to baseline. RESULTS/ANTICIPATED RESULTS: Next generation whole-genome sequencing of microRNAs in the CSF demonstrated that nilotinib significantly increases microRNAs that specifically regulate expression of autophagy and ubiquitination genes in individuals with Parkinson's disease. In the open label phase I, samples, 28 microRNAs found to regulate autophagy and ubiquitination genes, were significantly altered with treatment (Benjamini-Hochberg FDR p-value $\leq$ 0.05). In the phase II randomized, double-blind, placebo-controlled study samples, we verified several of those 28 candidate microRNAs had been significantly deferentially expressed with treatment (Empirical Bayes FDR p-value $\leq$ 0.05). DISCUSSION/SIGNIFICANCE OF IMPACT: Our data provide robust evidence that nilotinib's effects on misfolded protein clearance is via autophagy and CSF miRNA sequencing is a valid biomarker of nilotinib's effects in a definitive phase III study to investigate nilotinib in Parkinson's and other neurodegenerative diseases. CONFLICT OF INTEREST DESCRIPTION: Charbel Moussa is listed as an inventor on several Georgetown University patents for the use of tyrosine kinase inhibitors as a treatment for neurodegenerative diseases

4306

Periodontal disease and the oral microbiome in antiretroviral-treated patients with HIV

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OBJECTIVES/GOALS: People living with HIV, despite antiretroviral therapy (ART), have increased burden of inflammatory and aging-related comorbidities such as periodontitis. Oral microbiota have been linked to periodontitis, but not in the context of HIV. We aim to compare relationships between the oral microbiome and periodontal disease in HIV+ vs healthy controls. METHODS/STUDY POPULATION: In an ongoing cohort study we have been recruiting pre- and post-menopausal women with HIV+ on ART for $\geq$6 months and HIV- controls matched by menopausal status (target n = 30 per arm; currently HIV+: n = 30 post- and 9 pre-M; HIV-: n = 15 post- and 6 pre-M). Patients age <18 or on antibiotics within 3 mos., except prophylaxis, are excluded. Patients provide saliva, then subgingival plaque collection during a dental examination through scaling from six index teeth. Standard CDC/AAP classifications of periodontitis are used. We will perform 16S rRNA and ITS sequencing to profile bacterial and fungal communities in saliva and plaque. Linear mixed effect regression and differential abundance analyses will be used to identify microbial and mycobial oral signatures of periodontal disease severity in HIV+ and HIV- populations. RESULTS/ANTICIPATED RESULTS: We found a markedly high prevalence of severe periodontal disease in HIV+ women despite ART (59%, compared to 11% in HIV- controls). In post-menopausal women with HIV, saliva bacterial α- and β-diversity in the saliva differed significantly with periodontal disease severity. Fungal α-diversity was also significantly lower in plaque from teeth with severe loss of tissue attachment (CAL $\geq$4 mm). We identified bacterial and fungal taxa significantly enriched in post-menopausal HIV+ women with severe compared to no or mild periodontitis. We hypothesize, similarly, associations between the oral microbiome and periodontitis in HIV- controls. However, we expect overall diversity metrics to be significantly altered in HIV+ compared to HIV- patients, indicating long-term dysbiosis despite treatment with ART. DISCUSSION/SIGNIFICANCE OF IMPACT: Contrasting associations between the oral microbiome and periodontal disease with respect to HIV will provide evidence for the role of microbiota in accelerated aging phenotype caused by HIV. Our results would also provide rationale for interventions

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