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**

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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.

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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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