which enables high-quality images with lower tracer activity in this translational animal model. Future research will apply the same methodology to other anatomical targets as well as to the use of different tracers. Preclinical nuclear medicine imaging using ultra-low tracer doses, demonstrated the potential to obtain reasonable quality images and diminishing radiation surveillance in accordance with as low as reasonably achievable tracer levels.

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## Urinary tract infections in children with kidney allografts: Risk factors and clinical consequences

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OBJECTIVES/SPECIFIC AIMS: Background: Renal transplantation (tx) is the optimal treatment for end-stage renal disease (ESRD) in children, but post-tx urinary tract infections (UTIs) may cause morbidity and reduce allograft survival. Objectives: To quantify the number and risk factors for UTIs in pediatric kidney tx recipients in preparation for an analysis of the morbidity and impact of UTIs on allograft survival. METHODS/STUDY POPULATION: Methods: We identified all patients who underwent kidney tx between 2001 and 2016 (n = 390) at Children's Healthcare of Atlanta (CHOA). Patients were included if they had >1 year of follow-up at CHOA. We conducted an IRB-approved, retrospective review of patient demographics, medical history, and tx outcomes in the 5 years following tx. RESULTS/ANTICIPATED RESULTS: Results: Of the 205 records reviewed to date, we identified 176 eligible patients (61.9% male). Mean age at tx was 11.7 ± 5.5 years. In total, 58.5% had a deceased and 41.5% had a living kidney donor. Obstructive uropathy was the etiology of ESRD in 21.0%. Mean UTIs in all patients was 1.1/patient ± 2.7. On preliminary analysis, patients with a history of obstructive uropathy were more likely to develop a UTI than patients without (45.9% vs. 25.2%, $p = 0.014$). There is a trend to more UTIs in patients with a history of obstructive uropathy compared with patients without (2.1 ± 3.5 vs. 0.9 ± 2.4, $p = 0.055$). In males, there were more UTIs in patients with a history of obstructive uropathy compared to patients without (1.7 ± 2.9 vs. 0.5 ± 1.5, $p = 0.024$). In all, 23.2% of all patients were on UTI prophylaxis post-tx; trimethoprim-sulfamethoxazole was the prophylactic antibiotic in 54.5%. DISCUSSION/SIGNIFICANCE OF IMPACT: Conclusions: UTIs are common post kidney tx in children, especially in those with a history of obstructive uropathy. The associated morbidity and impact on graft survival are unknown.

2010

## Use it but still lose it: Exploring age-related changes in skeletal stem cell location and activation in response to physical stimulation

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OBJECTIVES/SPECIFIC AIMS: Our goal is to assess age-related changes in osteogenic stem cell populations of bone tissue. We hypothesize that aging mice have reduced osteogenic capacity in response to physical stimulation due to aging-associated decline in osteoprogenitor cell number and their proliferative capacity. METHODS/STUDY POPULATION: Mechanical loading: The NYU School of Medicine Institutional Animal Care and Use Committee approved all procedures. The response of tibial periosteal cells to physical stimulation or mechanical loading was assessed in 16-week-old adult (n = 6) and aged 78-week-old female (n = 4) mice subjected to 4 consecutive days of strain-matched axial compressive loading (1400 μm, 120 cycles, 2 Hz). Whole Mount Staining: Baseline periosteal cell numbers and nuclear morphology were assessed by whole bone DAPI staining of the antero-medial region of the tibiae in adult and aged mice (n = 6). Immunohistochemistry: Tibiae were fixed in 4% PFA, decalcified in 19% EDTA, OCT-embedded, and thickly sectioned (150 μm) at midshaft. Sca1 + , Prrx1 + , and Ki67 + cell numbers were quantified by simultaneous fluorescent immunohistochemical staining from loaded and nonloaded contralateral tibiae. Nonimmune species specific serum served as negative controls. Imaging: 3D image datasets of the periosteum at the antero-medial region of the tibial midshaft were acquired by multi-photon and confocal microscopy. Quantification of Sca1 + , Prrx1 + , and Ki67 + cells was carried out using Particle Analysis software (ImageJ) and Imaris 7.4.2 Surface Rendering Statistics functions. Cell number was normalized to periosteal area (~0.04 $mm^2$). A Student *t*-test determined significance at $p < 0.05$. RESULTS/ANTICIPATED RESULTS: At baseline, aged periosteal cell nuclei (DAPI + ) area (14% decrease, $p < 0.0001$), nuclei number, and Prrx1 + cell number (22% decrease) was significantly lower compared with adult mice. In loaded adult mice, Prrx1 + but not Sca1 + cell number increased significantly (35%, $p = 0.0115$). Proliferating Sca1 + (top panel) and Prrx1 + (top panel) cells also increased with loading, 62%, $p = 0.0253$ and 115%, $p = 0.0004$, respectively, in adult but not aged mice. The percentage of Prrx1 + cells undergoing proliferation (co-expressing Ki67 + ) in the total Prrx1 + cell population increased significantly with loading (bottom panel). Aged mice did not exhibit significant differences in loaded versus nonloaded controls for all other outcomes. Our data suggest fundamental changes in periosteal cell morphology, number and response to mechanical loading with aging. The significant increase in total Prrx1 + cell number and the number of Prrx1 + cells undergoing proliferation with loading in adult mice, suggest that the Prrx1 + cell population expands through proliferation. In fact, loading resulted in a 2-fold increase in the percentage of Prrx1 + preosteogenic cells undergoing proliferation. Accordingly, the significant age-related decrease in Prrx1 + cells may explain, in part, the attenuation of load-induced bone formation in aged mice. Loading resulted in greater numbers of proliferating Sca1 + cells (the more primitive cell) in adult mice, though this represented only a small percentage (<10%) of the total Sca1 + population. Mechanical loading expands the Prrx1 + pre-osteogenic cell population, but not the more primitive Sca1 + population. However, this load-induced osteogenic effect in the periosteum is not observed in aged mice, which may explain age-related diminishment of load-induced bone formation. DISCUSSION/SIGNIFICANCE OF IMPACT: Mechanical loading presents an inexpensive treatment for increasing bone mass and bone strength, but may be insufficient to prevent or reverse age-related bone loss due to reduced numbers of osteogenic progenitors in the periosteum. Therapeutic approaches targeting the osteogenic capacity of periosteal cells will be required to address declining mechanoresponsiveness with age.

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## Using real-time functional magnetic resonance imaging (fMRI) neurofeedback as a tool for demonstrating therapeutic efficacy in cognitive behavioral therapy

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OBJECTIVES/SPECIFIC AIMS: The purpose of this study was to provide individuals who have experience with cognitive behavioral therapy (CBT) with a demonstration of how using their therapeutic strategies affects their brain activity. Two challenges that face CBT and other cognitive therapies are (1) sustaining the gradual, incremental behavioral changes characteristic of the treatment and (2) measuring associated biological changes. These challenges may impede treatment efficacy and may negatively affect treatment outcomes, including patient discontinuation of CBT. Ideas for addressing these issues include providing patients with (1) a more immediate indicator of therapy effectiveness as well as (2) a biological index of behavioral change. In this study, we aimed to provide participants with an index of biological change based on therapeutic experiences via use of real-time functional magnetic resonance imaging (rtfMRI) neurofeedback. METHODS/STUDY POPULATION: We recruited participants who had already completed cognitive therapy as part of a clinical trial for depression at the University of North Carolina at Greensboro (n = 13). In the present experiment, participants were asked to provide a list of negative autobiographical memories or worries as well as cognitive strategies they use to cope with negative moods. The task consisted of COUNT, MEMORY, and STRATEGY trials (30 s each). During baseline COUNT trials, participants counted backwards (e.g., 300–4). During MEMORY trials, they viewed phrases previously developed describing their negative autobiographical memories/worries. During STRATEGY trials participants viewed a strategy they use to help them process the memory/worry. First, a localizer run was completed to determine a unique region of interest for each participant. We identified peak activation within the cingulate cortex to the contrast of MEMORY (STRATEGY + COUNT). Although the task was the same, no neurofeedback was displayed during the localizer run. During the feedback runs, participants were shown neurofeedback from the cingulate cortex following both the MEMORY and STRATEGY trials. This activation was

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represented on a signal bar display and represented the average cingulate activation during the trial. Unlike many rtfMRI studies, the purpose here was not for participants to interact with the neurofeedback directly. Rather, a feedback summary was shown to participants after each MEMORY and STRATEGY trial as an index of how brain activity changed in response to negative memories/worries and therapeutic strategies. Our goal was not for participants to learn to self-regulate the cingulate cortex, but rather to provide participants with a metacognitive demonstration of strategy efficacy. Participants were given detailed instructions regarding the task design, the role of the cingulate cortex in depression, as well as the hypothesized direction of activation during the MEMORY and STRATEGY phases to help them interpret the neurofeedback. RESULTS/ANTICIPATED RESULTS: Results revealed that "stronger neurofeedback" (defined as the difference between STRATEGY vs. MEMORY trials) correlated with self-reported strategy efficacy ratings immediately following the scan session ($p < 0.05$). More importantly, stronger neurofeedback predicted both self-reported strategy efficacy and frequency of use 1 month following the MRI session ($p < 0.05$). Importantly, this relationship was specific to only those strategies used inside the scanner; and no such relationship was observed at baseline. Neuroimaging results revealed that during the MEMORY phase, activation within inferior frontal gyrus and supramarginal gyrus correlated with baseline BDI score (whole brain, cluster corrected with FSL Flame 1 to $p < 0.05$). During the STRATEGY phase, the periaqueductal gray nucleus, insula, and temporal pole predicted self-reported frequency of strategy use 1 month post-scan session (whole brain, cluster corrected with FSL Flame 1 to $p < 0.05$). DISCUSSION/SIGNIFICANCE OF IMPACT: We believe this study holds promise to provide a powerful demonstration for individuals that strategies used to cope with negative moods can produce significant changes in their brain.

2165

## Vesicular secretion of suppressor of cytokine signaling 3 by alveolar macrophages is dysregulated in NSCLC patients and its provision inhibits epithelial cell transformation and tumor cell function

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OBJECTIVES/SPECIFIC AIMS: Insufficient endogenous expression of suppressor of cytokine signaling 3 (SOCS3) with subsequent over-activation of its target, the transcription factor STAT3, has been associated with tumorigenesis and cancer development in the lung and other organs. We have observed that a "backup" source of SOCS3 in the lung, namely that secreted in microvesicles (MVs) by alveolar macrophages, is reduced in the bronchoalveolar lavage fluid (BALF) of KRAS mutant mice harboring lung tumors. Here we sought to evaluate levels of SOCS3 in BALF of a cohort of non-small cell lung cancer (NSCLC) patients and to test the effects of vesicular SOCS3 administration on tumor cell transformation and function as potential therapeutic strategy. METHODS/STUDY POPULATION: In total, 22 BALF samples were obtained from healthy volunteers (n = 11) as well as patients undergoing diagnostic bronchoscopies for suspected lung cancer (n = 11). SOCS3 levels in the BALF were determined by ELISA after brief sonication to disrupt vesicles. In vitro experiments utilized the human adenocarcinoma cell line (A549) or human G12V mutant KRAS-expressing rat lung epithelial cells (RLE-G12V). Proliferation, Fas ligand (FasL)-induced apoptosis, and chemical transformation with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) or cigarette smoke extract (CSE) were assessed by CyQuant assay, annexin V staining, and soft agar assay, respectively. For SOCS3 rescue, epithelial cells were treated with natural alveolar macrophages-derived MVs (isolated via ultracentrifugation) or synthetic unilamellar liposomes containing human recombinant SOCS3 for at least 1 hour before assay. RESULTS/ANTICIPATED RESULTS: SOCS3 levels were significantly reduced in BALF samples of patients determined to have NSCLC as compared with healthy volunteers ($186.6 \pm 26.74$ vs. $395.6 \pm 74.31$ pg/mL, $p = 0.015$, n = 11). Addition of exogenous SOCS3-containing liposomes had the capacity to significantly inhibit MNNG and cigarette smoke extract-induced transformation and colony formation in soft agar. Exogenous SOCS3 provided in liposomes or in natural MVs significantly induced apoptosis (both in the presence and absence of FasL) and inhibited basal proliferation of A549 cells. DISCUSSION/SIGNIFICANCE OF IMPACT: These data identified a novel dysregulation of immune surveillance in the form of decreased SOCS3 secretion in the tumor-bearing lung that may contribute to tumorigenesis via sustained STAT3 activation. Future studies will focus on the mechanism underlying this defect and whether rescuing SOCS3 secretion can inhibit cancer progression in vivo.

2125

## What genes are involved in the brain food reward circuitry: Findings from a large candidate gene analysis

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OBJECTIVES/SPECIFIC AIMS: The food reward circuitry regulates hedonic eating especially in relation to palatable hypercaloric foods, which can lead to chronic overeating and consequent overweight and obesity. Evidence supports that there is considerable overlap within the brain reward circuitry between palatable hypercaloric food intake and substance addiction. The goal of this study was to identify associations between addiction-related genes and body mass index. We hypothesized that addiction-related genes potentially participate in the food reward circuitry if they are associated with obesity traits. METHODS/STUDY POPULATION: A secondary analysis was conducted with 1093 African American adolescents and young adults from the New Mother's Study. Anthropometric, genetic, demographic and lifestyle measurements were available at the 18-year follow-up assessments. A total of 1350 single nucleotide polymorphisms mapped to 127 addiction-related genes were assessed. A total of 186 ancestry informative markers were used to adjust for population stratification. Generalized estimating equation models were used to identify genetic associations, including additive, dominant, and recessive models, and control for correlations within families. RESULTS/ANTICIPATED RESULTS: The participants ranged from 15 to 23 years of age. Of them, 42.7% were overweight or obese. Significant associations with body mass index were identified for 13 single nucleotide polymorphisms mapped to 11 addiction-related genes, including LEP ($p$ 0.027–<0.001). Most of these genes are involved in dopaminergic, opioidergic, serotonergic pathways, and stress. DISCUSSION/SIGNIFICANCE OF IMPACT: Our results support the role of dopaminergic and opioidergic pathways in the food reward circuitry, and suggest a potential involvement of serotonergic pathways and genes related to stress in the food reward circuitry. Further investigation of the identified genes will facilitate delineation and understanding of the brain food reward system and its relationship with obesity.

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## Validation of a novel PD-L1 assay for bladder cancer circulating tumor cells

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OBJECTIVES/SPECIFIC AIMS: Bladder cancer patients being considered for immune checkpoint blockade are often judged on immunohistochemical staining for the checkpoint target protein PD-L1 in the original surgery or biopsy sample. However, sampling error or the clinical evolution of most patients' cancer can render the original PD-L1 assessment no longer accurate. In contrast, circulating tumor cells (CTCs) allow serial noninvasive sampling of the current tumor status throughout a patient's clinical course including those with the highest metastatic potential. We therefore sought to develop a method for quantifying PD-L1 expression in CTCs towards addressing inherent limitations of current UC management. METHODS/STUDY POPULATION: This work utilizes both cancer cell lines as well as patient samples. Positive and negative control cancer cell lines were assessed via "industry standard" antibodies for PD-L1 expression via Western blots and immunofluorescence, and a threshold-based method was developed for reliable quantification. PDL-1 expression was additionally verified via interferon-mediated up-regulation. CTCs isolated from bladder cancer patient samples via a density centrifugation method were then assessed for PD-L1 via the same antibodies. RESULTS/ANTICIPATED RESULTS: We will show preliminary preclinical and clinical data that validates the sensitivity and specificity of our assay. A case study will be presented that illustrate the potential useful of the novel approach we describe and which should be complementary to current clinical practices. In a patient with metastatic bladder cancer, this method effectively detected the PD-L1 expression in CTCs taken at a time coincident to when the patient derived an

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