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 at Children’s Hospital of Atlanta. 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% of patients with a history of obstructive uropathy compared with patients without (45.9% vs. 25.2%, p = 0.014). In males, there were 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 females, there were more UTIs in patients with a history of obstructive uropathy compared with patients without (1.7 ± 2.9 vs. 0.5 ± 1.5, p = 0.024). In all, 23.3% 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 post-tx; trimethoprim-sulfamethoxazole was the prophylactic antibiotic in 54.5%. A Student t-test determined significance at p < 0.05. RESULTS/ANTICIPATED RESULTS: At baseline, aged periosteal cell nuclei (DAPI +) were absent in 19% of patients. Mechanical loading expanded 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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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 tibia 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 tibia 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. Scil +, 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 tibia midshaft were acquired by multi-photon and confocal microscopy. Quantification of Scil +, Prrx1 +, and Ki67 + cells was carried out using Particle Analysis Software (ImageJ) and Imaris 7.4.2. Surface Rendering Statistics function. Cell number was normalized to periosteal area (~0.04 mm²). 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 Scil + cell number increased significantly (35%, p = 0.0012). Proliferating Scil + (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 significantly increased 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 + pre-osteogenic 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 Scil + cells (the more primitive cell) in adult mice, though this represented only a small percentage (<10%) of the total Scil + population. Mechanical loading expands the Prrx1 + pre-osteogenic cell population, but not the more primitive Scil + 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 therapists 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 were presented with stimuli and asked to maintain their focus on the stimulus. They were also instructed to use strategies they use to help them process the memory/worry. First, a localizer run was performed to determine the 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 applied 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
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 vesicles (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, migration, and Fas ligand (FasL)-induced apoptosis, and the induction of mitotic arrest and differentiation with 3-Methyl-D-Nitro-N-nitrosoguanidine (MNNG) or cigarette smoke extract (CSE) were assessed by flow cytometry, annexin V staining, and soft agar assays, respectively. For SOCS3 rescue, epithelial cells were treated with neutralizing alveolar macrophages-derived MVs (isolated via ultracentrifugation) or synthetic unilamellar liposomes containing human recombinant SOCS3 for at least 1 hour before assaying.

RESULTS/ANTICIPATED RESULTS: SOCS3 expression was significantly reduced in BALF sampled to have NSCLC as compared with healthy volunteers (186.6±26.74 vs. 395.6±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 neutral 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.

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 based on immunohistochemical staining for the checkpoint target protein PD-L1 in the original surgery or biopsy sample. However, such negative results may be caused by tumor sampling error or the clinical evolution of most patients’ cancer. Alternatively, circulating tumor cells (CTCs) allow serial, non-invasive sampling of the current tumor status throughout a patient’s clinical course. 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. PD-L1 expression was additionally verified by interferon-mediated up-regulation. CTCs isolated from bladder cancer patient samples via density centrifugation were assessed via interferon-mediated up-regulation. A case study will be presented that illustrates 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 increased symptomatic response.