all-cause mortality. Selection of Subjects: We will include all patients admitted to the JPNATC Trauma and Neurosurgical ICUs intubated and mechanically ventilated and meeting the definition of Berlin definition of ARDS8. We will collect data for a total of 12 months. RESULTS/ANTICIPATED RESULTS: Due to gaps in reporting, the incidence, mortality, and practice-based management algorithms applied in trauma patients suffering from ARDS in India is unknown. We hypothesize that the overall incidence of trauma-related ARDS is higher, and the fraction of patients managed with evidence-based therapies is lower than global reported averages. DISCUSSION/SIGNIFICANCE OF IMPACT: Although the true incidence of ARDS in trauma subjects in India is currently unknown, we suspect that it is much higher than reported. Such data are important in identification of resource allocation including ICU bed and mechanical ventilator availability, particularly in a resource-limited environment. This proposal will aid in the development of research infrastructure at JPNATC, contribute to capacity building, and the establishment of a Clinical Research unit at the Apex Institute. Finally, a provision to develop a consortium and trauma quality improvement program among the existing trauma centers in New Delhi to disseminate important research findings and guidance to the rest of India is a future benefit of the study.

2261

Tumor suppressor RARRES1 regulates cell survival by modulating mitochondrial energetics

Sara Maimouni[1], Mi-Hye Lee[2], You-Me Sung[2], Chokri Ouaari[3] and Stephen Byers[2]

[1] Georgetown University – Howard Universities; [2] Lombardi Comprehensive Cancer Center; [3] University of the District of Columbia

OBJECTIVES/SPECIFIC AIMS: One of the driving mechanisms of cancer progression is the reprogramming of metabolic pathways in intermediary metabolism. Cancers increase their energy expenditure by increasing ATP production for utilization in anabolic pathways to increase production of proteins, nucleic acids and lipids. The Warburg effect, where cancer cells predominantly use aerobic glycolysis rather than oxidative phosphorylation to produce ATP, was long thought to be the main initiating pathway in increasing tumor burden. However, compelling new evidence shows that there exists metabolic heterogeneity among and within tumors. Mitochondrial respiration often plays a major role in tumor progression, as many different cancers contain a subpopulation of slow-cycling tumor-initiating cells that are multidrug-resistant and dependent on oxidative phosphorylation. These cells represent a target for cancer therapy. In this study, we identification a novel endogenous regulator of mitochondrial respiration, retinoic acid receptor responder 1 (RARRES1). METHODS/STUDY POPULATION: We assessed the metabolic phenotype of RARRES1-depleted normal epithelial cells through metabolomics, a flux analyzer and blotting for phosphorylation of AMP kinase, a major regulator of energy homeostasis. We further examined mitochondrial energetics by staining the mitochondria with TMRM and Mito-Tracker. We then analyzed the apoptotic phenotype of epithelial cells with depletion of RARRES1 with fluorescence-activated cell sorting analysis of annexin V-staining. RESULTS/ANTICIPATED RESULTS: Remarkably, fluorescence-activated cell sorting analysis of annexin V-stained epithelial cells with depletion of RARRES1 were resistant to all studied modes of cell death, implying an effect on a fundamental cell process. By using proteomics, metabolomics, cellular and molecular analyses, our data show that RARRES1 regulates mitochondrial membrane potential and subsequently alters 1-carbon metabolism by modulating the function of the mitochondrial voltage-dependent anion channel. We believe this is the first example of a tumor suppressor protein that functions to directly regulate mitochondrial energetics. Using an extracellular flux analyzer, our data also show that depletion of RARRES1 causes an increase in mitochondrial respiration and ATP production, thus enhancing biosynthetic pathways that drive the pathogenicity and survival of cancer. The metabolic and anti-apoptotic phenotype of RARRES1-depleted cells was reversed by treatment of metformin, a mitochondrial inhibitor. DISCUSSION/SIGNIFICANCE OF IMPACT: These data lay the foundation for metabo-therapy of the many tumor types that exhibit RARRES1 depletion and may have the added benefit of targeting drug-resistant tumor-initiating cells.

2213

Tumor suppressors p53 and ARF control oncogenic potential of triple-negative breast cancer cells by regulating RNA editing enzyme ADAR1

Che-Pei Kung[1], Emily A. Bross[2], Catherine E. Kuzmicki[2], Michael Benjamin[2], Leonard B. Maggi Jr[2] and Jason D. Weber[2]

[1] Washington University in St. Louis; [2] Institute of Clinical and Translational Sciences, Washington University in St. Louis

OBJECTIVES/SPECIFIC AIMS: Triple-negative breast cancer (TNBC) accounts for one-fifth of the breast cancer patient population. The heterogeneous nature of TNBC and lack of options for targeted therapy make its treatment a constant adventure. The deficiency of tumor suppressors p53 and ARF is one of the known genetic signatures enriched in TNBC. Crucial questions remain about how TNBC is regulated by these genetic alterations. METHODS/STUDY POPULATION: In order to address this issue, we established p53/ARF-defective murine embryonic fibroblast and mammary epithelial cell to study the molecular and phenotypic consequences. Moreover, transgenic mice were generated to investigate the effect of p53/ARF deficiency on mammary tumor development in vivo. RESULTS/ANTICIPATED RESULTS: Increased proliferation and transformation capability were observed in p53/ARF-defective cells, and an aggressive form of mammary tumor was also seen in $p53^{-/-}ARF^{-/-}$ mice. Gene expression profiling and knock-down experiments using shRNAs were conducted to identify inflammatory marker ISG15 and RNA-editing enzyme ADAR1 as potential culprits for the elevated oncogenic potential. Interestingly, we found that the overexpression of ISG15 and ADAR1 is also prevalent in human TNBC cell lines. Reducing ADAR1 expression abrogated the oncogenic potential of human TNBC cell lines, while non-TNBC cells are less susceptible. DISCUSSION/SIGNIFICANCE OF IMPACT: These results indicate critical roles played by the tumor suppressors p53 and ARF in the pathogenesis of TNBC, likely through regulating ADAR1-mediated RNA modifications. Further understanding of this pathway promises to shed light on genetics-driven vulnerabilities of TNBC and inform development of more effective therapeutic strategies.

2516

Ultra-low Na18F tracer dosing for preclinical skeletal imaging enables new concepts in digital PET/CT

Maria I. Menendez, Richard Moore, Katherine Binzel, Michael Friel, Jun Zhang, Rebecca Jackson and Michael Knopp

The Ohio State University

OBJECTIVES/SPECIFIC AIMS: The aim of this study was to assess the ultra-dose Na18F dPET protocol feasibility for skeleton imaging in a canine model with reduced radiation dose and preserved quantitative characteristics. We hypothesized that administering an ultra-low Na18F dose would provide suitable image quality while reducing subject's exposure to radiation. METHODS/STUDY POPULATION: In total, 13 adult male beagles [weight (kg) mean ± SD; 14.3 ± 2.2] were scanned. The dogs were administered 3 different Na18F doses: 3 (standard dose/SD), 1 (low dose/LD), and 0.05 (ultra-low dose/ULD) mCi. Imaging started ≃45 minutes post injection for ≃ 33 minute total acquisition time. Covering the whole body, 11 bed positions, acquiring 120 (3 mCi) and 180 (1, 0.05 mCi) seconds per bed position. All imaging was performed on a digital photon counting system (Philips Vereos, pre-commercial release). PET list mode data were reconstructed using Time-of-flight with 4, 2, and 1 mm^3 voxel volumes. Point spread function, and Gaussian filtering were applied. Two experienced blinded readers evaluated image sets overall quality, tissue characterization, and quality of background in the whole body skeleton. Three-dimensional (3D) regions of interest (ROI) were traced over the distal femur, first lumbar vertebra, and a portion of the liver, recording standard uptake values (SUVmax and SUVmean). RESULTS/ANTICIPATED RESULTS: All the scans and reconstructions were successfully completed in all subjects. Decreasing Na18F dose from the standard dose (3 mCi) to the ultra-low dose/ULDO (0.05 mCi), demonstrated acceptable image quality and quantification. Ultra-low dose Na18F SUVmean values for the 3D ROIs reported (mean ± SD) 2.6 ± 0.7, 2.5 ± 1.1, 9 ± 1.6, and 0.6 ± 0.3 from the right and left distal femur, first lumbar vertebra, and a portion of the liver, respectively. When compared the SD with the LD and ULD, dPET demonstrated acceptable image quality and definition for qualitative overall assessment. This was also found for the overall quantitative ROI assessment of the healthy canine skeletons. DISCUSSION/SIGNIFICANCE OF IMPACT: Ultra-low dose Na18F at a level of 50 μCi for a 14 kg canine appears to be diagnostically feasible and a robust option to reduce (60-fold) radiotracer doses in a translational animal model using a dPET system. Furthermore, it allows us to move preclinical nuclear medicine imaging forward with substantial reduced exposure levels while preserving image quality. Both visual and quantitative results indicate that the standard-dose bone Na18F dPET can be decreased with a satisfactory diagnostic image quality. Ultra-low Na18F dose is indeed important for younger populations, control patients, and nononcological diseases/conditions. Favorable pharmacokinetics of Na18F (such as high bone uptake, minimal binding to serum proteins, rapid single-pass extraction, and fast clearance from the soft tissues) in addition to the technological capabilities of dPET/CT demonstrated feasibility enabling dose reduction strategies. Ultra-low dose has diagnostic reproducibility and lower radiation burden compared with higher fixed dose techniques in current available guidelines [Society of Nuclear Medicine and Molecular Imaging; SNMMI (5–10 mCi)]. Na18F dPET/CT provides higher sensitivity and diagnostic accuracy,

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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.

2366

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

Annie Farrell[1], Larry Greenbaum[2] and Traci Leong[3]
[1] Emory University; [2] School of Medicine and Children's Hospital of Atlanta, Emory University; [3] Rollins School of Public Health, Emory University

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

Pamela C. Zuckerman[1], Chao Liu[2,3] and Alesha B. Castillo[2,3]
[1] H + H Clinical and Translational Science Institute, New York University, New York, NY, USA; [2] New York University, New York, NY, USA; [3] Veterans Affairs New York Harbor Healthcare System, New York, NY, USA

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

Kathryn Dickerson, Katherine E. MacDuffie, Jeff MacInnes, Kari M. Eddington, Timothy J. Strauman and R. Alison Adcock
Duke University

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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