Comprehensive Cancer Center; 3 University of the District of Columbia

Benjamin 2, Leonard B. Maggi Jr 2 and Jason D. Weber 2

RESULTS/ANTICIPATED RESULTS: Remarkably, depletion of RARRES1 with phosphorylation. These cells represent a target for cancer therapy. In this study, we tumor-initiating cells that are multidrug-resistant and dependent on oxidative and within tumors. Mitochondrial respiration often plays a major role in tumor production for utilization in anabolic pathways to increase production of proteins, 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 subgroup 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 identified 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 I-carnitine 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 pathogenic and survival of cancer. The metabolic and 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.

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 subgroup 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 identified 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 I-carnitine 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 pathogenic and survival of cancer. The metabolic and 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.

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

Annie Farrell1, Larry Greenbaum2 and Traci Loeng3

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 = 205) 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/APPROACHES: 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, 23.3% 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 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.

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

Pamela C. Zuckerman1, Chao Liu2,3 and Alesha B. Castillo2,3

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 was performed on the tibial midshaft of adult and aged mice (n = 6). Immunohistochemistry: Tibiae were fixed in 4% PFA, decalcified in 1% EDTA, OCT-embedded, and thinly sectioned (150 μm) at midshaft. Scalc1+, Prx1+ 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 Scalc1+, Prx1+ and, Ki67+ cells was carried out using Particle Analysis Software (ImageJ) and Imaris 7.4.2. Surface Rendering Statistics function was used. 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 Prx1+ cell number (22% decrease) was significantly lower compared with adult mice. In loaded adult mice, Prx1+ but not Scalc1+ cell number increased significantly (35%, p = 0.0115). Proliferating Scalc1+ (top panel) and Prx1+ (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 Prx1+ cells undergoing proliferation (co-expressing Ki67+) in the total Prx1+ 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 Prx1+ cell number and the number of Prx1+ cells undergoing proliferation with loading in adult mice, suggest that the Prx1+ cell population expands through proliferation. In fact, loading resulted in a 2-fold increase in the percentage of Prx1+ osteoprogenic cells undergoing proliferation. Accordingly, the significant age-related decrease in Prx1+ cells may explain, in part, the attenuation of load-induced bone formation in aged mice. Loading resulted in greater numbers of proliferating Scalc1+ cells (the more primitive cell) in adult mice, though this represented only a small percentage (<10%) of the total Scalc1+ population. Mechanical loading expands the Prx1+ pre-osteogenic cell population, but not the more primitive Scalc1+ 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.