and in mice treated with bleomycin in combination with the peptide. Further, to differentiate the crosslinking activity of LOX from other potential effects, primary human fibroblasts were cultured with rLOX in the presence of the inhibitor, beta-aminopropionitrile. The expression levels of ECM (collagen and fibronectin), pro-fibrotic factors (IL-6 and TGF-beta), and transcription factor (c-Fos) were examined by real-time PCR, ELISA, immunoblotting, or hydroxyproline assay. RESULTS/ANTICIPATED RESULTS: LOX mRNA was increased in lung tissues and matching fibroblasts of SSc patients. rLOX-induced ECM production in vitro and ex vivo in lung fibroblasts and in human lung tissues maintained in organ culture, respectively. Additionally, TGF-beta and bleomycin induced ECM production, LOX mRNA expression and activity. Endostatin peptide abrogated these effects. In vivo, rLOX synergistically exacerbated pulmonary fibrosis in bleomycin-treated mice. The inhibition of LOX catalytic activity by beta-aminopropionitrile failed to abrogate LOX-induced ECM production. LOX increased the production of IL-6. IL-6 neutralization blocked the effects of LOX. Further, LOX induced c-Fos expression and its nuclear localization. DISCUSSION/SIGNIFICANCE OF IMPACT: LOX expression and activity were increased with fibrosis in vitro, ex vivo, and in vivo. LOX induced fibrosis via increasing ECM, IL-6 and c-Fos translocation to the nucleus. These effects were independent of the crosslinking activity of LOX and mediated by IL-6. Our findings suggest that inhibition of LOX may be a viable option for the treatment of lung fibrosis. Further, the use of human lung in organ culture establishes the relevance of our findings to human disease.

2045

The role of TGFβ in driving early cystic fibrosis lung disease

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OBJECTIVES/SPECIFIC AIMS: Transforming growth factor-beta (TGFβ) is a genetic modifier of cystic fibrosis (CF) lung disease. TGFβ's pulmonary levels in young CF patients and its mechanism of action in CF are unknown. We examined TGFβ levels in children with CF and investigated responses of human airway epithelial cells (AECs) and mice to TGFβ. METHODS/STUDY POPULATION: TGFβ levels in bronchoalveolar lavage fluid from CF patients ($n = 15$) and non-CF control patients ($n = 21$) < 6 years old were determined by ELISA. CF mice and non-CF mice were intratracheally treated with an adenoviral TGFβ1 vector or PBS; lungs were collected for analysis at day 7. Human CF and non-CF AECs were treated with TGFβ or PBS for 24 hours then collected for analysis. RESULTS/ANTICIPATED RESULTS: Young CF patients had higher bronchoalveolar lavage fluid TGFβ than non-CF controls ($p = 0.03$). Mouse lungs exposed to TGFβ demonstrated inflammation, goblet cell hyperplasia, and decreased CFTR expression. CF mice had greater TGFβ-induced lung mechanics abnormalities than controls; both CF human AECs and CF mice showed higher TGFβ induced MAPK and PI3K signaling compared with controls. DISCUSSION/SIGNIFICANCE OF IMPACT: For the first time, we show increased TGFβ levels very early in CF. TGFβ drives CF lung abnormalities in mouse and human models; CF models are more sensitive to TGFβ's effects. Understanding the role of TGFβ in promoting CF lung disease is critical to developing patient specific treatments.

2090

TL1 team approach to osteosarcoma cell detection

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OBJECTIVES/SPECIFIC AIMS: The objective of our collaboration is to develop a strong transdisciplinary team consisting of microfluidics engineers, cancer biologists, and clinicians, to identify cell surface markers capable of detecting circulating osteosarcoma cells (COC) using microfluidic devices. Our goals are 3-fold: (1) Identify cell surface markers unique to osteosarcoma (OS) for COC isolation, (2) develop a Geometrically Enhanced Mixing (GEM) device to isolate COCs, and (3) Evaluate the efficacy of GEM device to detect COCs in OS patients under treatment. The long-term goal is to utilize this cell detection approach to correlate the presence of COC with metastatic incidence. METHODS/STUDY POPULATION: To identify a marker to capture COCs we are utilizing flow cytometry and microfluidic capture devices. Flow cytometry will be used to evaluate the relative expression of epithelial cell adhesion molecule (EpCAM), CD45, cell surface vimentin (CSV), insulin-like growth factor 2 (IGF2R), interleukin 11 receptor subunit alpha (IL-11Rα), ganglioside 2 (GD2), and receptor activator of nuclear factor κ-B (RANK) on a panel of OS cell lines. These cell surface markers were selected based on an extensive review of OS cell surface markers. OS cell capture efficacy will be assessed by passaging a known concentration of OS cells through a GEM microfluidic device coated with antibodies targeting the selected marker, as indicated by flow cytometry. Once captured, COCs on the device will be analyzed and the capture efficiency for the indicated marker will be measured. ANOVA will be used to determine any significant difference in capture efficiency between marker types. Once an optimal marker or panel of markers has been selected we will conduct capture studies using OS cell spiked blood samples followed by clinical samples obtained from OS patients. In clinical samples, COC detection will be validated using the FDA approved triple immunocytochemistry technical definition of a circulating tumor cell (CTC). This will enable COCs to be differentiated from the normal whole blood cell population by selecting for CD45 −, EpCAM +, and cytokeratin + cells. RESULTS/ANTICIPATED RESULTS: Our preliminary studies have shown that on our microfluidic device, EpCAM, a marker commonly used to identify circulating tumor cells in other cancer settings, has a poor capture efficiency (15.9% + 7.7%) for HU09 OS cells while the same setup with EpCAM has a capture efficiency of 56.9% + 2.7% for BXPc-3 pancreatic cells. We therefore anticipate our flow cytometry studies to show a low expression of EpCAM and CD45 for OS cell lines, while showing a moderate to high expression of CSV, IGF2R, IL-11Rα, GD2, and RANK. We expect to show a 60%–80% capture efficiency for markers selected for COC capture. Currently, CSV and GD2 are particularly promising as markers based on previously published studies. DISCUSSION/SIGNIFICANCE OF IMPACT: OS is the most common primary bone tumor and the third leading cause of pediatric cancer deaths. At diagnosis 80% of patients will present with metastasis, however only 20% of these cases are clinically detectable. Innovative strategies to identify patients at risk of metastasis would allow for stratification of intervention therapies. Currently, tumor recurrence and metastasis are primarily dependent on diagnostic-imaging modalities such as computerized tomography or positron emission tomography scans. Unfortunately, these imaging modalities can only detect tumor masses of significant size (106 tumor cells). Liquid biopsies are a novel alternative to current diagnostic imaging systems to monitor metastatic incidence and treatment efficacy. The detection of CTCs through routine blood sampling has the potential to be used clinically for earlier detection, monitoring the treatment of metastatic cancers and surveying the effect of therapeutic interventions on metastasis. To date, the majority of the studies on CTCs have evaluated their presence in carcinomas. Although sarcomas are rare, they generally have a poor prognosis. This study will address one of the unmet medical needs in the field of CTC detection; the identification of cell surface OS makers to improve binding specificity, increase purity, and maintain a high capture efficiency. This phase of our proposal will evaluate the most abundant and conserved markers across a panel of OS cell lines. Once a marker or panel of markers is selected, we will begin to develop a microfluidic device that can be used clinically to detect CTCs in this disease setting.

2303

Trauma-related acute respiratory distress syndrome (ARDS) in India: Current incidence and management strategies

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OBJECTIVES/SPECIFIC AIMS: Aim 1: To determine the true incidence of trauma-related acute respiratory distress syndrome (ARDS) in India. We propose to perform a prospective observational study to determine the incidence of ARDS in India. Aim 2: To perform a preliminary assessment of risk factors for ARDS in the Indian trauma population. We will leverage these findings against the global ARDS data to provide a foundation for further interventional studies. Aim 3: To evaluate the current management strategies and patient outcomes from ARDS in trauma subjects admitted to the Jai Prakash Narayan Apex Trauma Center (JPNATC). These findings will identify areas in need of practice-based performance improvement in ARDS therapies in India. METHODS/STUDY POPULATION: This application proposes an observational study of trauma patients with ARDS, a population that continues to have substantial in-hospital mortality. The approximate number of ICU-admitted trauma cases for the study period is 1700. Specific data elements to be collected include patient demographics, comorbidities, mechanism of injury, Injury Severity Score, risk factors for ARDS, sequential organ failure and assessment scores, vital signs, laboratory values, and evidence-based treatments received, including mechanical ventilation and adjunctive therapies. Outcome data will include discharge location, ICU and hospital length of stay and

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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

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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

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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

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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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