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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