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OBJECTIVES/SPECIFIC AIMS: Treatment of acute myeloid leukemia (AML) is challenging, as apoptosis-resistant AML cells often persist within the bone marrow microenvironment despite chemotherapy. The overall goal of our laboratory is to identify and ultimately target the bone marrow factors that protect AML cells. METHODS/STUDY POPULATION: Using cell cultures, we previously reported that SDF-1 (CXCL12), an abundant bone marrow chemokine, induces apoptosis of isolated CXCR4 + AML cells, including freshly isolated bone marrow-derived AML cells from approximately one-third of AML patients. However, co-culture of AML cells with differentiating osteoblasts protected AML cells from apoptosis. RESULTS/ANTICIPATED RESULTS: Histone deacetylase inhibitors (HDACi) abrogated the ability of osteoblasts to protect AML cells and altered expression of matrix mineralization genes including tissue nonspecific alkaline phosphatase (TNAP). A different drug, cyclosporine A (CSA), similarly inhibited osteoblast-mediated protection of AML cells and reduced TNAP expression. Specifically targeting osteoblast TNAP via siRNA was sufficient to prevent osteoblasts from protecting AML cells in co-cultures. In addition, we are targeting TNAP enzymatically. DISCUSSION/SIGNIFICANCE OF IMPACT: Our results indicate that targeting TNAP may be useful in AML treatment to render the bone marrow microenvironment more hostile to leukemic cell survival.

2114

Severity of childhood-onset systemic lupus erythematosus: Impact of preceding and co-existing autoimmune cytopenias (protocol)

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OBJECTIVES/SPECIFIC AIMS: The goals of our study are: (1) To test the hypothesis that the presence of any autoimmune cytopenia (ITP, AIHA, or ES) at time of cSLE diagnosis is associated with decreased risk of developing LN. (1b) To test the hypothesis that there is a lower risk of LN in patients with cSLE and any co-existing autoimmune cytopenia (ITP, AIHA, or ES) who had treatment with immunomodulatory or immunosuppressive therapy (intravenous immunoglobulin, corticosteroids, rituximab, or cyclophosphamide) before diagnosis of cSLE. (2) To test the hypothesis that in patients with cSLE who develop LN, the presence of any co-existing autoimmune cytopenia (ITP, AIHA, or ES) at time of cSLE diagnosis is associated with less severe LN. (3) To test the hypothesis that at the time of cSLE diagnosis, there is a lower incidence of double-stranded DNA (dsDNA) and a higher incidence of ribonucleoprotein autoantibodies in those with co-existing autoimmune cytopenias (ITP, AIHA, or ES). METHODS/STUDY POPULATION: This is a retrospective study of a large cohort of patients from the Emory Children's Center, Children's Healthcare of Atlanta (CHOA) satellite clinics and pediatric rheumatology inpatient services at any of the 3 CHOA hospitals (Egleston, Scottish Rite, and Hughes Spalding) with ICD 9 or ICD 10 codes corresponding to a diagnosis of SLE between January 1, 2000 and January 31, 2015. We will include patients diagnosed at age 2–16 years who meet at least 4 of the 11 American College of Rheumatology (ACR) classification criteria for SLE. We will consider these patients as having cSLE. We will exclude patients with less than 2 years of follow-up data and patients with a pre-existing diagnosis of cSLE who transferred care to our Emory/CHOA center. We will define time of diagnosis as time from initial evaluation for cSLE by a pediatric rheumatologist up to 28 days post cSLE diagnosis. We will define co-existing autoimmune cytopenia as preceding diagnosis of a primary autoimmune cytopenia or the presence of an autoimmune cytopenia at the time of initial evaluation for cSLE and up to 28 days post cSLE diagnosis. We will define AIHA as hemoglobin $\leq$10 g/dL with positive direct Coombs and/or reticulocytosis. We will define ITP as thrombocytopenia $<$100,000/mm^3 and Evans syndrome as concurrent or sequential AIHA and ITP. We will define lupus nephritis (LN) as the presence of urine protein to creatinine ratio $>$ 0.5 in a patient with cSLE and/or biopsy demonstrating LN. IRB approval of the study protocol with waiver of informed consent has been obtained from the CHOA IRB. RESULTS/ANTICIPATED RESULTS: We have approximately 40 newly diagnosed cSLE patients annually; therefore, a study population of 400 patients with cSLE is possible. Therefore, assuming 50% of cSLE patients without autoimmune cytopenias have LN and 22% of cSLE patients with autoimmune cytopenias have LN, at an alpha of 0.05, we will have $>$ 80% power to detect significant differences. We expect to show phenotypic differences in patients with co-existing autoimmune cytopenia and cSLE from other newly diagnosed cSLE patients. We expect that the presence of a co-existing autoimmune cytopenia and cSLE is associated with decreased risk of developing LN. We expect that there will be a decreased prevalence of LN in cSLE patients pretreated with immunosuppression further highlighting that earlier indicators of LN risk and early interventions are necessary. We expect to find decreased severity of LN in patients with a co-existing autoimmune cytopenia at time of cSLE diagnosis. DISCUSSION/SIGNIFICANCE OF IMPACT: Our study will be conducted on one of the largest single-center cohorts of cSLE patients. We will determine whether pediatric patients with SLE and autoimmune cytopenias have a distinct clinical or serological phenotype and less severe disease. Our results will be significant in developing hypothesis for further retrospective or prospective multi-center or large database and immunological studies to understand the relationship of each individual autoimmune cytopenia to cSLE. It will provide the necessary background for further clinical and immunological studies to identify predictive biomarkers of cSLE severity.

2464

Sexual dimorphism in a mouse model of syndromic thoracic aortic aneurysm

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OBJECTIVES/SPECIFIC AIMS: Pre-clinical and clinical observations have noted that increased aortic dilation is associated with male sex. Using an experimental model of severe, syndromic thoracic aortic aneurysms, we quantify aortic dilation and elastin stability in male Versus female mice. METHODS/STUDY POPULATION: Ascending aortas from male and female FBN1mgR/mgR mice and their wild type littermates were assessed every 4 weeks from 6 to 18 weeks of age by ultrasound. Measurements were taken luminal edge to luminal edge in diastole. At termination, aortas were harvested for RT-PCR analysis of extracellular matrix genes. Aortas were serially sectioned and elastin fragmentation was imaged by auto-fluorescence. RESULTS/ANTICIPATED RESULTS: At 12 weeks of age, differences of aortic diameters between male and female FBN1mgR/mgR mice were significantly different (2.24 ± 0.43 vs. 1.57 ± 0.22 mm; $p = 0.002$), while there were no significant differences between sexes of wild type littermates (1.29 ± 0.13 vs. 1.23 ± 0.08 mm; $p = 0.71$). Male sex was associated with increased elastin but not fibrillin-1 mRNA expression. Ascending aortas from male and female FBN1mgR/mgR mice significantly differed in the degree of elastin fragmentation (2.76 vs. 1.85 breaks/ 100 μm aorta; $p = 0.03$). DISCUSSION/SIGNIFICANCE OF IMPACT: Sexual dimorphism of thoracic aortic dilation observed in human TAA patients was recapitulated in the fibirllin-1 hypomorphic mouse model of syndromic thoracic aortic aneurysms. Differences in this mouse model could be explained by the differential expression of extracellular matrix genes.

2394

Sodium-glucose transporter 2 is a novel diagnostic and therapeutic target for early-stage lung adenocarcinoma

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OBJECTIVES/SPECIFIC AIMS: Lung cancer claims 160,000 lives in the United States every year, and lung adenocarcinoma (LADC) is the most frequent type. Early diagnosis is crucial. Computed tomography (CT) is very sensitive in identifying early-stage lung nodules, but has low specificity. Increased glucose uptake is a hallmark of cancer measurable in vivo by fluorodeoxyglucose (FDG) positron-emission tomography (PET). FDG PET is widely used for cancer staging but has low sensitivity in the diagnosis of solitary lung nodules. We have previously identified an alternative glucose transporter, SGLT2, expressed in different types of cancer but not detected by FDG PET. SGLT2 activity can be measured in vivo with the PET tracer methyl-4-fluorodeoxyglucose (Me4FDG). The objective of this study was to test the hypothesis that SGLT2 is a novel diagnostic and therapeutic target in FDG-negative, early stage LADC. METHODS/STUDY POPULATION: To study glucose transporter expression in LADC, we performed immunohistochemistry with SGLT2- and GLUT1-specific antibodies in human lung pre-malignant lesions and LADC samples. To verify the possibility of detecting SGLT2 activity in vivo, we performed microPET imaging with the SGLT-specific tracer Me4FDG in a Kras-driven, p53-null genetically engineered mouse model and in patient-derived xenografts

of LADC. Finally, we performed therapeutic trials in genetically engineered and patient-derived mouse models of LADC with the FDA-approved SGLT2 inhibitor canagliflozin. RESULTS/ANTICIPATED RESULTS: We observed a switch in the modality of glucose transport during lung carcinogenesis: SGLT2 was highly expressed in pre-malignant lesions and well-differentiated LADC, whereas GLUT1 was upregulated in advanced, poorly differentiated lesions. This pattern was observed both in human samples and in murine models. This observation led us to hypothesize that early-stage LADCs are often negative on FDG PET because this imaging modality does not detect the activity of SGLT2, which is expressed in early lesions. Therefore, we performed PET imaging with the tracer Me4FDG, that measures SGLT2 activity, in our mouse model, and observed that Me4FDG accumulated in small nodules that were negative with FDG. We confirmed the functionality of SGLT2 in human LADC by Me4FDG PET in patient-derived xenografts. To investigate the role of SGLT2-mediated glucose uptake in the early stages of LADC development, we treated both genetically engineered mice and patient-derived xenografts with FDA-approved SGLT2 inhibitors, showing that SGLT2 inhibition effectively reduced LADC growth and prolonged survival in mouse models. In addition, Me4FDG uptake predicted response to SGLT2 inhibition. DISCUSSION/SIGNIFICANCE OF IMPACT: Our results show that sodium-dependent glucose transport is a critical metabolic supply strategy in the early stages of lung adenocarcinoma development, and that Me4FDG is a novel biomarker of early LADC and of SGLT-dependent tumor growth. The discovery of SGLT2 in LADC highlighted the need for a re-interpretation of FDG-negative lung nodules, which might rely on SGLT2 for glucose uptake, and therefore may be detected by the new tracer Me4FDG. We anticipate our findings will lead to clinical studies evaluating Me4FDG as a diagnostic tracer for solitary lung nodules and early LADC, and as a biomarker for the selection of patients eligible for treatment with SGLT2 inhibitors.

2118

Solid-state MRI as a nonradiative alternative to computed tomography for craniofacial imaging

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OBJECTIVES/SPECIFIC AIMS: Computed tomography (CT) enables 3-dimensional (3D) visualization of cortical bone structures with high spatial resolution, and thus has been the gold-standard method for evaluation and diagnosis of craniofacial skeletal pathologies. However, ionizing radiation and, in particular, repeated scanning for presurgery and postsurgery assessments, is of concern when applied to infants and young children. Recent advances in solid-state MRI allow the capture of the short-T2 signals in cortical bone while suppressing the signal from soft-tissue protons having T2 relaxation time 1–2 orders of magnitude longer (50–100 ms). One approach, a dual-radiofrequency (RF) pulse and ultrashort echo time (UTE) imaging based method, exploits different sensitivities of bone and soft tissue to different RF pulse widths and TEs. This study aims to demonstrate the feasibility of producing 3D renderings of the human skull and visualization of cranial sutures using the bone-selective MRI technique in comparison to CT. METHODS/STUDY POPULATION: Imaging technique: Two RF pulses differing in duration and amplitude are alternately applied in successive repetition time (TR) along the pulse train. Within each TR, 2 echoes are acquired. Acquisition of the first echo starts at the ramp-up of the encoding gradient (TE1), allowing for capture of signals with very short lifetimes (bone), while that of the second starts after a longer delay (TE2). In total, 4 echoes are obtained: ECHO11 (RF1TE1), ECHO12 (RF1TE2), ECHO21 (RF2TE1), and ECHO22 (RF2TE2). During reconstruction, ECHO11 is combined with ECHO21 and ECHO12 is combined with ECHO22, resulting in 2 images. The subtraction of these 2 images yields an enhanced bone contrast. Data acquisition/processing: The pulse sequence described above was applied for MR imaging of a human cadaveric skull and 2 adult human subjects in vivo, at 3T field strength (Siemens Prisma, Erlangen, Germany). Imaging parameters: TR/TE1/TE2 = 7/0.06/2.46 ms, RF1/RF2 durations = 40/520 μs, flip angle = 12°, matrix size = 2563, field of view = 2803 mm^3, voxel size = 1.1 mm isotropic, number of radial spokes = 25,000, and scan time = 6 minutes. Segmentation of bone voxels was performed using ITK-SNAP in a semi-automatic fashion, leading to 3D renderings of the skull. For comparison, a CT scan was also performed in the human cadaveric skull with 1 mm isotropic resolution. Validation: The biometric accuracy was assessed by measuring eight anatomic distances: (1) Maximum craniocaudal aperture of the right orbit. (2) Maximum craniocaudal aperture of the left orbit. (3) Maximum height of the mandible from chin point in the midline. (4) Maximum cranial length (5) Maximum cranial width. (6) Maximum height of piriform aperture. (7) Distance between lateral most aspect of mandibular condyles. (8) Distance between lateral most aspect of posterior hard palate in both CT- and MRI-based 3D renderings of the human cadaveric skull using Mimics software (Materialise®, Ghent, Belgium). These distances were compared with those directly measured on the cadaveric skull. RESULTS/ANTICIPATED RESULTS: Compares CT with the proposed MRI method on cadaveric human skull images, along with corresponding 3D renderings. Compared with CT, the 3D rendered images maintain most features over the entire head (e.g., zygomatic arch), except for appearance of some artifacts in the mandibular region. In vivo head images in 2 adult subjects: axial magnitude images and 3D rendering. In the axial images, bone voxels as well as the inner table of the cranium are clearly visualized, and cranial and spinal bone structures are well depicted in the 3D renderings. Some voxels were erroneously included or excluded in the renderings. The mean difference in measurements of the 8 anatomic distances was 6, 4, and 2 mm when comparing MRI Versus CT, MRI Versus in situ, and CT Versus in situ, respectively. DISCUSSION/SIGNIFICANCE OF IMPACT: Bone proton magnetization exhibits a substantial level of signal decay during the relatively long duration of RF2 due to its very short T2 relaxation time. In contrast, soft-tissue retains nearly the same level of signal intensities over all echoes. Thus, subtraction of ECHO22 from ECHO11, when compared with the difference between ECHO11 and ECHO12, enhances bone contrast from soft tissue. The proposed, dual-RF dual-echo 3D UTE imaging technique produces isotropic high-resolution bone-specified images in the whole head within a clinically feasible imaging time (6 min), leading to clear visualization of craniofacial skeletal structures. These are key components necessary for translation to the clinical setting. Optimization of postprocessing for more realistic 3D renderings and thus accurate anatomic measurements is currently being implemented. The proposed method's potential as a nonradiative alternative to CT will then be thoroughly evaluated in pediatric patients.

2029

Surface display of chimeric proteins for exosome imaging and capturing in mammalians

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OBJECTIVES/SPECIFIC AIMS: Exosomes are living nanoscale vesicles that can shuttle large amounts of bioactive cargo for intercellular communication. The potential of these nanovesicles to serve as both biomarkers for disease diagnosis and vehicles for delivery of therapeutics has only begun to be explored. To realize these potentials, molecular tools for effective exosome tracking and capturing must be invented in order to advance basic research and clinical translation. METHODS/STUDY POPULATION: We utilize a surface display strategy that enables exosome modification in living mammalian systems. By reconfiguring the surface protein CD63 or viral envelope glycoprotein VSV-G, we generate 3 topologically distinctive protein chimeras for exosome imaging and capture in mammalian systems. RESULTS/ANTICIPATED RESULTS: We have shown that these genetically encoded protein chimeras have the ability to correctly target and integrate into exosomes in cultured human cells. Furthermore, we have demonstrated that the secreted exosomes could be successfully captured by an affinity peptide intentionally displayed on the outer surface of exosomes. DISCUSSION/SIGNIFICANCE OF IMPACT: Our study highlights the potential of these fusion proteins for exosome tracking and provides novel genetic tools for exosome research and translation, one of which is loading protein therapeutics for targeted delivery.

2018

Synaptic vesicle 2 receptors as a novel targets for neuroendocrine cancer therapy

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OBJECTIVES/SPECIFIC AIMS: (1) To delineate the function of the heavy-chain receptor binding domain (HCR), a portion of botulinum neurotoxin type A (BoNT/A) and synaptic vesicle 2 (SV2) signaling pathway, which provide a novel multipurpose biologic with potential clinical applications in tumor detection/imaging, inhibition of tumor progression, and reduction of bioactive hormone