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.

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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 $= 40/520\ \mu$s, flip angle $= 12°$, matrix size $= 256^3$, field of view $= 280^3\ mm^3$, 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

secretion in metastatic neuroendocrine (NE) cancers. (2) To evaluate the expression pattern of SV2 receptors in NE cancer patient-derived tissues for prediction of patient response for recombinant HCR (rHCR) treatment. (3) To assess the in vivo efficacy and toxicity of rHCR in a NE cancer liver metastasis mouse models and in the NE patient-derived 3D MicroTumor system. (4) To collect preclinical data to design and conduct a clinical trial with NE cancer patients, a major goal toward translating our discoveries into much needed therapies. METHODS/STUDY POPULATION: Recombinant botulinum heavy chain (rHCR) was produced using an IPTG-inducible expression vector in *E. coli* BL21. The rHCR was His-Tag purified and stored in PBS buffer before usage. Cytotoxicity: H727, TT, and MZ cells were plated at a density of 5000 cells/well in 96-well plates and incubated under standard conditions overnight. The next day, cells were treated with 10, 100, or 500 nmol/L of rHCR and incubated for 72 hours. Following incubation, cell viability was assessed by ATP quantification using the CellTiter-Glo (Promega) assay. Fresh NE tumors were dissociated and injected into polydimethylsiloxane bioreactors in a matrigel and collagen suspension for 3D culture experiments. The viability of 3D cultures incubated with various doses of rHCR was assessed by measuring the uptake of the near-infrared dye IR-783 using an IVIS imaging system. Western blot: H727, TT, and MZ cells were seeded in 6-well plates at a density of 3×10^5 cells/well for 24 hours followed by treatment with 100 nmol/L for 72 hours. Total cellular proteins were isolated and analyzed to assess the level of SV2A expression and the effect of rHCR on the expression levels of NET marker proteins. Immunohistochemistry: Deparaffinized tissue culture slides were incubated with SV2A primary antibody in 1% BSA and incubated overnight at 4°C. Slides were rinsed twice with TBS containing 0.025% Triton, followed by 0.3% H_2O_2 for 15 minutes. Slides were then incubated with HRP-conjugated secondary antibody for 1 hour at room temperature. Detection of protein-protein interaction: Precleared cell lysate was incubated with glutathione-agarose beads in the presence of 10 μg of GST-tagged rHCR for 2 h at 4°C with end-over end mixing. Samples were then centrifuged at 10,000 g for 2 minutes and the supernatant was analyzed by SDS-PAGE. Preclinical models: To allow rHCR testing on NET patient derived cells in a very novel 3D surrogates, sterile collected fresh NET tissues will be obtained from the UAB Tissue Procurement, dissociated into a single cell suspension and injected into a polydimethylsiloxane bioreactors containing extracellular matrix composed of bovine collagen and matrigel. Such 3D cell culture will be maintained in bioreactors with constant supply of media through the microchannels and treated with rHCR at concentrations ranging from 10 nM to 1 mM. Following histologic confirmation of growth and morphology of NET patient-derived 3D surrogates we will test the anticancer activity of rHCR in this system using the standard cytotoxicity assays as well as we will validate the NET hormone expression using immunohistochemistry assay. To create an animal model of NE cancer progression, we will perform intrasplenic injection of NET cell lines. In approximately 4 weeks, the animals should develop NE liver metastases based upon our previous experience. rHCR-iFPs accumulation in the tumor mass: rHCR-iFPs will be injected into the tumor bearing mice after 4 weeks of cells implantation in 1 week interval for total of 4 treatments at the concentrations of 0.125, 1.25, and 12.5 mg/kg. RESULTS/ANTICIPATED RESULTS: Based on the preliminary data, we expect to detect rHCR-iFPs in NE cancer xenografts and in patient derived 3D explants. Our preliminary data revealed that treating NET cells with rHCR significantly reduced NE peptide expression in 3 days. Thus, we expect to see the decrease of NE tumor markers even if the fluorescent detection method is not sensitive enough to monitor the signal. The reduction of the NET markers can be used as an indicator of the rHCR-iFPs uptake by the tumor mass. If HCR exhibit high binding affinity to SV2 receptors in NET models but moderate anticancer efficacy, we plan to use rHCR peptide to conjugate with the nanocarrier for targeted drug delivery. In this case rHCR peptide can be used as a ligand that specifically binds to NE cancer cells and delivers anticancer drug. In 3D NET patient derived explants we expect significant reduction of NET markers and hormones (serotonin and calcitonin) in NE cancer cells upon long-term rHCR-iFPs treatment. In addition, we will perform multiplex protein quantification assay using Luminex to assess the various hormones, cytokines, and growth factors to be repurposed into a diagnostic and detection reagent, or a drug delivery ligand for targeted therapies. DISCUSSION/SIGNIFICANCE OF IMPACT: NE cancers are highly metastatic: NE cancers such as carcinoid, islet cell tumors, and medullary thyroid cancer frequently metastasize to the liver. They are the second most prevalent GI malignancy. Ninety percent of patients with pancreatic carcinoid tumors and 50% of patients with islet cell tumors develop isolated hepatic metastases. Patients with untreated, isolated NE liver metastases have <30% 5-year survival. Thus, there is a critical need for new therapies for NE cancers. Surgery is the only curative therapy available for patients with NE cancers but most cannot be cured: surgical removal is the most effective treatment for NE cancers; however, a very high percentage of patients present with metastatic disease. While surgical resection can be potentially curative, many patients are not candidates for operative intervention due to widespread metastases or the degree of hepatic involvement by the NE cancers. Moreover, other forms of therapy including chemoembolization, radioembolization, cryoablation, and chemotherapy have had limited efficacy. We hope that our in vitro data on rHCR toxicity and specificity to NET, validated in the pre-clinical models will allow the first in-human application of this technology in clinical studies.

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Targeted eccentric motor control to improve locomotion after incomplete spinal cord injury

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OBJECTIVES/SPECIFIC AIMS: Incomplete spinal cord injury typically results in life-long disability, often in the form of profound loss of locomotion capability. Individuals who have experienced incomplete spinal cord injury exhibit persistent eccentric motor deficits, which are particularly prevalent in the weight acceptance phase of gait and emphasized in sagittal plane knee motion and frontal plane hip motion. METHODS/STUDY POPULATION: Motion analysis can capture the kinematic and joint-level deficits of these individuals, but it is impossible to directly calculate the contributions of individual muscles to weight acceptance due to the complexity of the musculoskeletal system. Instead, those muscle contributions must be simulated in order to approximate muscle power during locomotion. RESULTS/ANTICIPATED RESULTS: The traditional method for driving these simulations with electromyography readings is unavailable for individuals who have neuromuscular deficits (e.g., spasticity or paralysis), due to the need to generate reliable maximum voluntary isometric contractions for baseline purposes. Instead, this research develops a novel method for using resting electromyography data to drive musculoskeletal simulations using a muscle activation threshold paradigm. DISCUSSION/SIGNIFICANCE OF IMPACT: The simulation results of this method more closely resemble experimental results, but further simulation refinement is needed to fully capture the true muscle activity.

2025

Targeting immunosuppressive myeloid cells to enhance cancer immunotherapy

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OBJECTIVES/SPECIFIC AIMS: Prostate cancer (PCa) is the most common noncutaneous malignancy in men in the United States. A significant fraction of advanced PCa treated with androgen deprivation therapy experience relentless progression to lethal metastatic castration-resistant prostate cancer (mCRPC). The PCa tumor microenvironment is comprised of a complex mixture of epithelial and stroma cell types engaged in multifaceted heterotypic interactions functioning to maintain tumor growth and immune evasion. We recently uncovered the important role played by myeloid-derived suppressor cells (MDSCs) to mediate tumor immune evasion in aggressive PCa (Wang, Lu *et al.*, Cancer Discovery, 2016). Immune checkpoint blockade (ICB) has elicited durable therapeutic responses across a number of cancer types. However, the impact of ICB on mCRPC has been disappointing, which may signal the need to combine mechanistically-distinct ICB agents and/or override immunosuppression in the tumor microenvironment. Our objective is to determine if robust immunotherapy responses in mCRPC may be elicited by the combined actions of ICB agents together with targeted agents that neutralize MDSCs yet preserve T cell function. METHODS/STUDY POPULATION: We created a novel embryonic stem cell-based chimeric mouse model of mCRPC engineered with signature mutations to study the response to single and combination immunotherapy. The efficacy studies were followed with detailed mechanistic investigation and clinical validation. RESULTS/ANTICIPATED RESULTS: Consonant with early stage clinical trials experience, anti-CTLA4 or anti-PD1 monotherapy failed to impact disease progression. Similarly, modest anti-tumor activity was observed with combination ICB as well as monotherapy with targeted agents including Cabozantinib (tyrosine kinase inhibitor), Dactolisib (PI3K/mTOR inhibitor), and Dasatinib (tyrosine kinase inhibitor). In contrast, mCRPC primary and metastatic disease showed robust responses to dual ICB treatment together with either Cabozantinib or Dactolisib, but not with Dasatinib which impaired T cell infiltration in the tumor. Detailed intratumoral immune profiling with mass cytometry (CyTOF) showed that combined ICB and Cabozantinib or Dactolisib was associated with significant depletion of MDSCs. Cabozantinib and Dactolisib blocked the PI3K signaling activity in MDSCs and reduced their immunosupppresive activity. Mechanistically, the combination efficacy was due to the upregulation of IL-1RA and suppression of MDSC-promoting cytokines secreted by mCRPC cells. DISCUSSION/SIGNIFI-CANCE OF IMPACT: We demonstrated that an antibody cocktail targeting CTLA4 and PD1 was insufficient to generate effective anti-tumor response, but combination of ICB with targeted therapy that inactivates PI3K signaling displayed superior synergistic efficacy through impairing MDSCs in the tumor microenvironment. These observations illuminate a clinical path hypothesis for combining ICB with MDSC-targeted therapies in the treatment of mCRPC. Importantly, conclusions from the study on PCa may have implications in combination immunotherapy for aggressive breast cancer which is also largely resistant to immune checkpoint blockade and replete with immunosuppressive myeloid cells.