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.
secretion in metastatic neuroendocrine (NE) cancers. (2) To evaluate the expression pattern of SV2 receptors in NE cancer patient-derived tissues for potential use as targets for recombinant human chromophore (HCR) technology and to assess the in vivo efficacy and toxicity of HCR in a NE cancer liver metastasis mouse model 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 bovine heavy chain (HCR) was generated using an in vitro transcription/translation expression system in E. coli BL21. The HCR 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 HCR and incubated for 72 hours. Following incubation, cell viability was assessed by ATP quantification using the CellTiter-Glo (Promega) assay. Freshly delivered tumors were then injected into polydimethylsiloxane bioreactors in a matrigel and collagen suspension for 3D culture experiments. The viability of 3D cultures incubated with various doses of HCR 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 x 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 HCR on the expression levels of NET marker proteins. Immunohistochemistry: Degrappafinized tissue culture slides were incubated with SV2a primary antibody in 1% BSA and expression levels of NET marker proteins. Immunohistochemistry assay. To create an animal system using the standard cytotoxicity assays as well as we will validate the NET patient-derived 3D spheroids we will test the anticancer activity of HCR in this system using the standard cytotoxicity assays as well as we will validate the NET hormone expression using immunohistochemistry assay. To create a novel animal model of NE cancer progression, we will perform in atrial injection of NET cell lines. In approximately 4 weeks, the animals should develop NE liver metastases based upon our previous experience. HCR-iFPs accumulation in the tumor mass: HCR-iFPs will be injected to 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 125 mg/kg. RESULTS/ANTICIPATED RESULTS: Based on the preliminary data, we expect to detect HCR-iFPs in NE cancer xenografts and in patient-derived 3D explants. Our preliminary data revealed that treating NETs cells with HCR significantly reduced NET peptide expression in 3 days. The results allow us 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 HCR-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 HCR peptide to conjugate with the nanocarrier for targeted drug delivery. In this case HCR peptide can be used as a ligand that specifically binds to NE cancer cells and delivers anticancer drug. 3D NET patient derived explants we expect significant reduction of NET markers and hormones (serotonin and calcitonin) in NE cancer cells upon long-term HCR-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 therapeutic agent for recombinant human chromophore (HCR) technology. DISCUSSION/SIGNIFICANCE OF IMPACT: NE cancers are highly metastatic. NET 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 NET metastases and tumors, 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 hemostatic impairment by the NET cancer. Moreover, other forms of therapy including chemoembolization, radioembolization, cryoablation, and chemotheraphy have had limited efficacy. We hope that our in vitro data on HCR toxicity and specificity to NET, validated in the preclinical models will allow the first in human application of this technology in clinical studies.

DISCUSSION/SIGNIFICANCE OF IMPACT: 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, these 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.

Targeting immunosuppressive myeloid cells to enhance cancer immunotherapy

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OBJECTIVES/SPECIFIC AIMS: Prostate cancer (PCa) is the most common noncurative malignancy in men in the United States. A significant fraction of advanced PCa treated with conventional cytotoxic therapies experience relentless progression to lethal metastatic castration-resistant prostate cancer (mCRPC). The mCRPC 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 promote immunotherapy resistance and metastatic castration-resistant prostate cancer (mCRPC). The mCRPC 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.

TARGETED ECCENTRIC MUSCLE CONTROL TO IMPROVE LOCOMOTION AFTER INCOMPLETE SPINAL CORD INJURY

Kevin O'Brien, D. Michele Basso and James Schmiedeler

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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, these 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.

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