for M.D.-Ph.D. research mentoring were identified. DISCUSSION/SIGNIFICANCE OF IMPACT: The CTSI DDC was well received by investigators. The request process fosters collaboration among researchers with similar interests and identifies core laboratory resources that add innovation to ongoing research, funding applications, education, and interinstitutional planning.

2095

Drug screening and hit identification for night blindness with zebrafish

Logan Ganzen and Yuk Fai Leung

OBJECTIVES/SPECIFIC AIMS: Retinitis pigmentosa (RP), also known as night blindness, is an incurable disease which affects ~1 in 4000 individuals globally. Since there are no effective treatment options for RP, the goal of this project is to identify novel drug treatments that can prevent or slow the disease progression. To this end, we optimized a behavioral assay, visual-motor-response (VMR) assay, to investigate rod function (Ganzen *et al.*, *ARVO*, 2017; Ganzen *et al.*, *IJMS*, 2017). This was done utilizing a transgenic zebrafish RP model expressing human rhodopsin with the Q344X mutation. In this study, we used this model to perform a proof-of-concept screen for drugs which may improve the vision of the larvae. METHODS/STUDY POPULATION: To screen for beneficial drugs, the SCREEN-WELL® REDOX library was chosen for screening. This library was selected to identify a compound that may alleviate any excessive oxidative stress in the diseased retina. The Q344X zebrafish line suffers from significant rod degeneration by 7 days postfertilization (dpf) and displayed deficits in VMR under scotopic conditions (Ganzen *et al.*, *ARVO*, 2017). The Q344X larvae were drug treated beginning at 5 dpf at 10 μM. Compounds that were toxic at this concentration were retested at 1 μM. The 5 dpf stage was chosen as most of the rods are intact, and these concentrations were chosen to optimize the drug effect based on similar studies. Hits were identified by assays that provided a robust and reproducible enhancement in the Q344X VMR. The retinae of any drug hits were dissected from larvae crossed with a rod EGFP reporter line and whole-mounted to analyze rod survival via fluorescence. To determine if drug effects were exerted through the retina, eyeless chokh mutant zebrafish were exposed to the drug and tested with the same assay. RESULTS/ANTICIPATED RESULTS: Of the 84 compounds tested, we identified 1 drug that ameliorated the VMR of the Q344X scotopic VMR. Eyeless chokh mutant zebrafish larvae did not exhibit the same VMR when treated with the same drug. Histological analysis suggested increased rod survival in the drug-treated retina of Q344X mutants. DISCUSSION/SIGNIFICANCE OF IMPACT: These results indicate that the vision of the Q344X zebrafish was improved via this beneficial drug treatment. Since eyeless chokh larvae did not respond to the same treatment, the drug likely mediated its positive effects through the Q344X retina, likely by improving rod survival. Together, our results have identified a beneficial drug that may treat RP.

2038

Effects of bilateral frontal transcranial direct current stimulation (tDCS) on the working memory network: An fMRI-tDCS study in healthy older adults

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OBJECTIVES/SPECIFIC AIMS: The study aimed to determine the effects of bilateral frontal active transcranial direct current stimulation (tDCS) at 2 mA for 12 minute Versus sham stimulation on functional connectivity of the working memory network during an fMRI N-Back task. METHODS/STUDY POPULATION: Stimulation was delivered over bilateral frontal dorsolateral prefrontal cortex via and MRI-compatible tDCS device during an fMRI working memory task in healthy older adults in a within-subject design. RESULTS/ANTICIPATED RESULTS: Active stimulation compared with sham resulted in significant increases in functional connectivity in working memory related brain regions during the N-Back task. DISCUSSION/SIGNIFICANCE OF IMPACT: Older adults typically have reduced functional connectivity compared with young adults. Our findings demonstrate that a single session of tDCS can increase functional connectivity of the working memory network in older adults. Based on this mechanism of effect, tDCS may serve as an adjunctive method for interventions aiming to enhance cognitive processes in older adults.

2060

Exploring gene expression signature shared between obese Zucker rat and human cardiac hypertrophy

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OBJECTIVES/SPECIFIC AIMS: Objectives: To determine genes that are shared between human and obese Zucker rat hypertrophic hearts, in order to identify potential early biomarkers and drug target for heart failure. METHODS/STUDY POPULATION: Four age-paired lean and obese Zucker rats were used. The human data are derived from doi:10.1152/physiolgenomics.00122.2016. RESULTS/ANTICIPATED RESULTS: We expect to find genes that are upregulated and downregulated in Zucker rats and humans that present cardiac hypertrophy. DISCUSSION/SIGNIFICANCE OF IMPACT: The genes and proteins determined from this study will provide future directions in order to determine whether obese Zucker rats are a valid model organism for the development of cardiac hypertrophy.

2116

Exploring Müller cell-cone interactions in human fovea using 3-dimensional volume electron microscopy (EM)

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OBJECTIVES/SPECIFIC AIMS: Müller cells, radial glial cells of the retina, are the principal repository of xanthophyll pigment (lutein, zeaxanthin, meso-zeaxanthin), which are modifiable by diet and visible clinically by autofluorescence imaging. To understand the structural basis of xanthophyll visualization in vivo, we used 3-dimensional electron microscopic reconstruction of Müller cells surrounding one cone in a healthy human fovea. METHODS/STUDY POPULATION: From a 21-year-old male organ donor, dissected retinas were rejuvenated by oxygenated Ames medium then fixed in 4% glutaraldehyde. A tissue block 3.5 mm^2 centered on the fovea was prepared for Automated Tape Ultramicrotomy (Kasthuri *et al.*, *Cell* 162: 648–661, 2015). From 1462 serial 65 nm horizontal sections, an area ~250 × 250 μm was imaged at 6 nm *xy* resolution. Images were stitched and aligned. TrackEM software on a pen display was used to trace, reconstruct, and display cone #5 (of 186) and its contacting Müller cells. RESULTS/ANTICIPATED RESULTS: Cone 5 is ensheathed by 2 types of Müller cells, outer and inner (Dacey, *ARVO*, 2016). The outer cell is first seen at the external limiting membrane (ELM) between cones 5 and 17. Moving inward from the ELM, it tightly wraps around cone 5's fiber in a C-shape profile for 78 μm. This Müller cell also intermittently projects to neighboring cones, 2 of which were close to cone 5 at the ELM. As cone 5's axon approaches the pedicle, it contorts into a corkscrew. The outer cell fluidly molds to this changing shape. At this level, this Müller cell doubles in volume to encompass not only cone 5, but also cone 17 and another Müller cell. In the final 17 μm of the block the Müller cell's volume quickly dissipates as it sends a small projection towards the internal limiting membrane, eventually encasing an OFF midget bipolar cell also associated with cone 5. In contrast to this outer cell, an inner Müller cell adjoining cone 5 spans only 19 μm, interacting directly with cone 5 and the outer cell for 3.9 μm. DISCUSSION/SIGNIFICANCE OF IMPACT: Neural-glial relationships in a human fovea are visible through 3-dimensional volume EM. The volume of Müller cells in the fovea was impressive, consistent with a pivotal role in the health of cone photoreceptors and xanthophyll homeostasis. It is possible that individual glia also ensheath the post-receptoral neurons in a cone-driven circuit, supporting the concept that xanthophylls contribute to neural efficiency in vision.

2036

Extracellular matrix as a novel approach to glioma therapy

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OBJECTIVES/SPECIFIC AIMS: Gliomas are the most lethal and common primary tumor type in the central nervous system across all age groups; affected adults have a life expectancy of just 14 months. As glioma cells invade the surrounding

https://doi.org/10.1017/cts.2018.73 Published online by Cambridge University Press

normal parenchyma they remodel the composition and ultrastructure of the surrounding extracellular matrix (ECM), suggesting that the native (i.e., "normal") microenvironment is not ideal for their survival and proliferation. Recent reports describe suppressive and/or lethal effects of mammalian ECM hydrogels derived from normal (nonneoplastic) sources upon various cancer types. ECM-based bioscaffolds placed at sites of neoplastic tissue resection in humans have never been reported to facilitate cancer recurrence. The objective of the present research is to evaluate mammalian ECM as a novel approach to glioma therapy. METHODS/STUDY POPULATION: ECM hydrogels from porcine dermis, small intestine, and urinary bladder were produced as described previously. Primary glioma cells were graciously supplied by Drs. Nduka Amankulor and Johnathan Engh, and U-87 MG were ordered through ATCC. Cells were plated onto tissue culture plastic at ~60% confluence and allowed to attach for 24 hours before treatment. The saline-soluble fraction (SSF) of ECM was obtained by mixing lyophilized, comminuted ECM with 0.9% saline for 24 hours then filtering the resulting mixture through a 10 kDa molecular weight cutoff column. All assays and kits were followed according to the manufacturer's instructions. Cell viability was measured via MTT assay (Vybrant® MTT Cell Proliferation Assay, Invitrogen) and by live/dead staining (LIVE/DEAD® Cell Imaging Kit, Invitrogen). Time lapse videos were created by taking images every 20 minutes for 18 hours (phase-contrast) or every 10 minutes for 12 hours (darkfield). NucView reagent was ordered from Biotium. Temozolomide was ordered through Abmole. All in vivo work was conducted according to protocols approved by the University of Pittsburgh's IACUC office. RESULTS/ANTICIPATED RESULTS: ECM hydrogels derived from porcine dermis, small intestine, or urinary bladder all decreased the viability of primary glioma cells in vitro, with urinary bladder extracellular matrix (UBM) having the most dramatic effects. The SSF of UBM (UBM-SSF), devoid of the fibrillar, macromolecular components of ECM, was sufficient to recapitulate this detrimental effect upon neoplastic cells in vitro and was used for the remainder of the experiments described herein. In a cell viability assay normalized to the media treatment, non-neoplastic CHME5 and N1E-115 cells scored 103% and 114% after 48 hours when treated with UBM-SSF and 2 primary high-grade glioma cell types scored 17% and 30.5% with UBM-SSF (n = 2). Phase-contrast time-lapse video showed CHME5 and HFF thriving in the presence of UBM-SSF for 18 hours while most primary glioma cells shriveled and died within this time. Darkfield time-lapse video of wells containing Nucview dye, fluorescent upon cleavage by active caspase-3, confirmed that within 12 hours most primary glioma cells underwent apoptosis while CHME5 and HFF did not. In culture with primary astrocytes, high grade primary glioma cells, and U-87 MG glioma cells for 24 hours, UBM-SSF was found to significantly increase the population of primary astrocytes compared with media ($p < 0.05$) while decreasing the 2 glioma cell types to approximately one-third as many cells as the media control ($p < 0.0001$). A dose-response of temozolomide from 0 to 10,000 μM showed that when treating 2 non-neoplastic cell types (CHME5 and HFF) and 2 types of primary glioma cell there was no difference in survivability at any concentration. Contrasted to this, a dose-response of UBM-SSF from 350 to 7000 μg/mL showed that the non-neoplastic cells survived significantly better than the glioma cells at concentrations of 875 μg/mL and upward ($p < 0.05$). In preliminary animal experiments, large primary glioma tumors in the flanks of athymic nude mice were resected and replaced with either UBM SSF or Matrigel (an ECM product of neoplastic cell origin). After 7 days the resection sites with UBM-SSF had little tumor regrowth if any compared with the dramatic recurrence seen in the Matrigel injection sites (n = 2). In a separate survival study comparing PBS to UBM-SSF injections in the flank-resection model, all animals given PBS had to be sacrificed at 9, 11, and 11 days (n = 3) whereas animals given UBM-SSF were sacrificed at 15, 24, and 39 days (n = 3), indicating a moderate increase in survival due to the UBM-SSF. DISCUSSION/SIGNIFICANCE OF IMPACT: Since the introduction of the pan-cytotoxic chemotherapeutic agent TMZ in 2005, the standard of care for patients with glioblastoma multiforme has not improved. These findings indicate that non-neoplastic ECM contains potent bioactive regulators capable of abrogating malignancy. Our in vitro data suggest these molecules appear to have no deleterious effect on non-neoplastic cells while specifically inducing apoptosis in glioma cells. Our in vivo data suggest that these molecules may be useful in delaying glioma recurrence, thus resulting in extended lifespan. Delivering soluble fractions of ECM to a tumor site may represent a novel approach to glioma therapy, sidestepping traditional cytotoxic therapies in favor of utilizing putative endogenous anti-tumor pathways.

2446

Feasibility, acceptability, and appropriateness of the menstrual cup for short-term non-surgical management of vesicovaginal fistula (VVF) among potential users and stakeholders

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OBJECTIVES/SPECIFIC AIMS: To examine how women with OF in Ghana develop strategies for coping in the absence of access to successful surgical repair. To assess the feasibility, acceptability, and appropriateness of an innovation to support coping among women with OF seeking care in a health facility in Ghana. To examine the perceived facilitators and barriers to implementation among additional OF stakeholders regarding the innovation. METHODS/STUDY POPULATION: This study uses a sequential exploratory mixed methods design. The population of study is women in Ghana living with obstetric fistula, as well as additional fistula stakeholders (programmers, policy makers, community leaders). To get an understanding of usual leakage, women carried out at baseline a pad test, where they wore a sanitary pad for 2 hours and leaked freely. We subtracted the dry pad weight from the wet pad weight to estimate urine leakage in mL. Then women inserted the cup for 2 hours and again wore a pad and urine leakage was estimated. Acceptability among women with vesicovaginal fistula was measured by questionnaire. Acceptability among additional stakeholders was examined by semistructured interview. Appropriateness was assessed among the user, additional stakeholders, and organizational setting. RESULTS/ANTICIPATED RESULTS: We observed a 61% mean reduction in leakage with the cup which was also perceived by cup users as a reduction in wetness. Notably, one participant who had 4 previous surgical attempts, experienced a 78% reduction in leakage. No adverse events attributable to use of the cup were observed, unlike some of the strategies women currently use to manage leakage. Acceptability was high as most women could easily insert, remove, and wear the cup over the 2-hour period and fistula stakeholders indicated the innovation content and complexity were acceptable. In community interviews, women shared various coping and self-care strategies to manage their leaking, other related impairments, and stigma. Women using the cup in the health facility expressed that it was useful. Additional stakeholders found the cup a low-cost, low-tech solution to supplement existing programs. Within the stakeholder interviews we heard that the cultural norms and existing activities of the potential implementation partners align with the innovation approach. Stakeholders revealed various implementation facilitators and barriers. The facilitators to implementation reported in the interviews were related to the intervention and organization characteristics in particular. Stakeholders perceived a relative advantage to self-management. Stakeholders had concerns regarding whether women would find the insertable device acceptable and appropriate—questioning whether potential users would have access to water, soap, and safe space to empty cup. DISCUSSION/SIGNIFICANCE OF IMPACT: The innovation is efficacious, acceptable, adds to current coping strategies, and fits within existing fistula programs. Stakeholders pre-implementation perceptions highlight the importance of partnerships and the need for an evidence base related to effectiveness, acceptability, and cost. Challenges to address include access to resources within these contexts (water, soap, and safe space) and development appropriate counseling message.

2103

Fecal bile acids, fecal short-chain fatty acids, and the intestinal microbiota in patients with irritable bowel syndrome (IBS) and control volunteers

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OBJECTIVES/SPECIFIC AIMS: Objectives and goals of this study will be to: (1) compare fecal microbiota and fecal organic acids in irritable bowel syndrome (IBS) patients and controls and (2) investigate the association between colonic transit and fecal microbiota in IBS patients and controls. METHODS/STUDY POPULATION: We propose an investigation of fecal organic acids, colonic transit and fecal microbiota in 36 IBS patients and 18 healthy controls. The target population will be adults ages 18–65 years meeting Rome IV criteria for IBS (both diarrhea- and constipation-predominant, IBS-D and IBS-C) and asymptomatic controls. Exclusion criteria are: (a) history of microscopic colitis, inflammatory bowel disease, celiac disease, visceral cancer, chronic infectious disease, immunodeficiency, uncontrolled thyroid disease, liver disease, or elevated AST/ALT > 2.0 × the upper limit of normal, (b) prior radiation therapy of the abdomen or abdominal surgeries with the exception of appendectomy or cholecystectomy >6 months before study initiation, (c) ingestion of prescription, over the counter, or herbal medications affecting gastrointestinal transit or study interpretation within 6 months of study initiation for controls or within 2 days before study initiation for IBS patients, (d) pregnant females, (e) antibiotic usage within 3 months before study participation, (f) prebiotic or probiotic usage within the 2 weeks before study initiation, (g) tobacco users. Primary outcomes will be fecal bile acid excretion and profile, short-chain fatty acid excretion and profile, colonic transit, and fecal microbiota. Secondary outcomes will be stool characteristics based on responses to validated bowel diaries. Stool samples will be collected from participants during the last 2 days of a 4-day 100 g fat diet and split into 3 samples for fecal microbiota, SCFA, and bile acid analysis and

https://doi.org/10.1017/cts.2018.73 Published online by Cambridge University Press