comfortable for use in human subjects. DISCUSSION/SIGNIFICANCE OF IMPACT: This novel technique allows for an inexpensive, noninvasive, and reproducible ocular blood flow imaging platform. By optimizing this technique, we can proceed with our future plans for a pilot study to compare our imaging technique with the current standard, paving the way for future clinical studies.

2429

Optogenetic stimulation of corticotropin-releasing hormone expressing neurons in Barrington's nucleus recapitulates the social stress voiding phenotype in mice

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OBJECTIVES/SPECIFIC AIMS: Voiding postponement is common cause of LUT dysfunction in which children void infrequently with large volumes. This condition is modeled in mice that are subjected to social stress who show decreased voiding frequency and increased voided volumes along with increases in corticotropin-releasing hormone (CRH) expression in Barrington's nucleus (BN) (i.e., the pontine micturition center). Optogenetics is a technique to selectively stimulate cells or neurons of interest via light activated channel receptors [i.e., channel-2 rhodopsin (ChR2)]. Here we examined the effects of optogenetic manipulation of CRH BN neurons on the in vivo voiding phenotype and urodynamics in awake mice. We hypothesized that stimulating these neurons at higher frequencies (10–50 Hz) would lead to CRH-dependent alterations in voiding phenotype (i.e., larger voided volumes and longer intermicturition intervals. METHODS/STUDY POPULATION: Double transgenic mice expressing ChR2 in CRH cells were generated using the Cre-lox recombinase system and had fiberoptic probes implanted into BN at 8 weeks of age. The mice also underwent simultaneous catheter placement into the bladder for in vivo cystometry. In vivo cystometry before and during optogenetic stimulation at various frequencies was performed 5–7 days postoperatively. Saline was perfused at 10 μL/minute and baseline stable voiding cycles were established. Bladder capacity, threshold pressure, voiding pressure, and voided volume were recorded at baseline and at each optogenetic setting. In some mice, the protocol was repeated in the presence of CRH antagonist (NBI 30775). RESULTS/ANTICIPATED RESULTS: Fiberoptic stimulation (470 nm at 25 and 50 Hz) produced a significant rise in the intermicturition interval, bladder capacities and increased void volumes. This effect was especially pronounced in females in whom bladder capacity and intermicturition interval more than doubled at 50 Hz stimulation. Fluoroscopic images confirmed complete bladder emptying with each void. The increased bladder capacity at higher frequencies (25 and 50 Hz) was CRH-dependent as injection of a CRH antagonist (NBI 30775) blocked the optogenetic effect. Control non-double mice showed no effects from optogenetic stimulation. DISCUSSION/SIGNIFICANCE OF IMPACT: Our results suggest that optogenetic stimulation of CRH-expressing neurons in BN at high frequency (25 and 50 Hz) inhibits micturition and recapitulates the voiding phenotype seen in socially stressed mice (large, infrequent voids). Lower frequencies of optogenetic stimulation (2 and 10 Hz) had no effects on cystometry parameters or voiding phenotype. In addition, females had a greater response to optogenetic stimulation compared with males with larger bladder capacities and longer intermicturition intervals. The changes in voiding phenotype seen were CRH dependent as blockage of the CRH receptor prevented changes in cystometry parameters with optogenetic stimulation. Further elucidation of these and other neural subpopulations in BN are warranted to understand micturition and how it may be manipulated in disease states such as voiding postponement and acute urinary retention.

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Personalized models of distal airway epithelial-stromal unit in COPD

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OBJECTIVES/SPECIFIC AIMS: The objective of this study is to develop patient-derived "personalized" organotypic models of human distal airways, in which basal stem cells (BCs) isolated from the pre-/terminal conducting airway region are co-cultured with autologous stromal cells from the same region to reproduce patient-specific distal airway epithelial-stromal units and their remodeling in COPD. METHODS/STUDY POPULATION: We established a protocol to isolate and propagate epithelial BCs, fibroblasts, and endothelial cells from the distal airways of normal and COPD lung donors. Heterogeneous cellular and molecular phenotypes in the human distal airways were characterized using immunofluorescence and single-cell RNA sequencing. Patient-specific distal airway epithelial-stromal units were reconstructed by co-culturing BCs and autologous stromal cells using an air-liquid interface-based airway wall model and a bronchosphere-based 3D distal airway organoid assay. RESULTS/ANTICIPATED RESULTS: Histologic analysis of derived epithelial-stromal units revealed heterogeneous patient-specific phenotypes characterized by hypo-/hyper-/metaplastic lesions (hypo-regenerative phenotype, mucous cell hyperplasia, squamous metaplasia, distal-to-proximal repatterning) in the epithelial compartment, accompanied, in some samples, by stromal remodeling. Candidate epithelial-stromal cross-talk mechanisms were identified using quantitative real-time RT-PCR analysis of autologous epithelial and stromal compartments of established patient-specific distal airway unit models. DISCUSSION/SIGNIFICANCE OF IMPACT: Epithelial and stromal cells isolated from distal airways of subjects with and without COPD can be assembled into functional, organ-level tissue which mimics the architecture of human distal airways and, in patients with COPD, reproduces several distal airway remodeling phenotypes. Patient-specific models of distal airway epithelial-stromal cross-talk established in this study can be used to identify candidate pathways that mediate disease-relevant airway remodeling and potentially utilized as pre-clinical platforms for developing personalized therapeutic approaches to suppress the progression of distal airway remodeling in chronic lung diseases, including COPD.

2375

Pharmacokinetics of phosphatidylethanol 16:0/20:4 homolog in human blood after consumption of 0.4 and 0.8 g/kg alcohol in a laboratory clinical study

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OBJECTIVES/SPECIFIC AIMS: The purpose of this study was to characterize the pharmacokinetics of phosphatidylethanol (PEth) 16:0/20:4 homolog in uncoagulated, human blood samples taken from 18 participants in a clinical laboratory setting after consumption of 2 doses of ethanol. METHODS/STUDY POPULATION: Male and female participants received either 0.4 or 0.8 g/kg oral doses of ethanol during a 15-minute period. Blood samples were collected before and throughout 6 hours immediately after alcohol administration, then after 2, 4, 7, 11, and 14 days of administration day. PEth 16:0/20:4 levels were quantified by liquid mass spectrometry. Breath ethanol concentrations were measure concurrently with each blood collection during the administration day, as well as transdermal ethanol concentrations monitored constantly before, during and after ethanol administration day. RESULTS/ANTICIPATED RESULTS: (1) Single doses of 0.4 and 0.8 g ethanol/kg produced proportional increases in BrAC and PEth 16:0/20:4 levels; (2) the increase of Peth 16:0/20:4 from base line to Cmax was less than either PEth 16:0/18:1 or PEth 16:0/18:2 during the 6-hour period after ethanol administration; (3) the mean rate of formation of PEth 16:0/20:4 was lower than those of the other 2 homologs; (4) the mean half-life of PEth 16:0/20:4 was 2.18 days, which was shorter than that of either PEth 16:0/18:1 and PEth 16:0/18:2, which were 6.80 and 6.62, respectively. DISCUSSION/SIGNIFICANCE OF IMPACT: The results of this study further confirm that PEth homologs are a sensitive biomarker for ethanol consumption. The measurement of three PEth homologs appears to provide additional information about the level and time frame of drinking.

2555

Predictive cytological topography (PiCT): A radiopathomics approach to mapping prostate cancer

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OBJECTIVES/SPECIFIC AIMS: The objective of this study is to use machine Learning techniques to generate maps of epithelium and lumen density in MRI

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space. METHODS/STUDY POPULATION: Methods: We prospectively recruited 39 patients undergoing prostatectomy for this institutional review board (IRB) approved study. Patients underwent MP-MRI before prostatectomy on a 3T field strength MRI scanner (General Electric, Waukesha, WI, USA) using an endorectal coil. MP-MRI included field-of-view optimized and constrained undistorted single shot (FOCUS) diffusion weighted imaging with 10 *b*-values ($b = 0$, 10, 25, 50, 80, 100, 200, 500, 1000, and 2000), dynamic contrast enhanced imaging, and T2-weighted imaging. T2 weighted images were intensity normalized and apparent diffusion coefficient maps were calculated. The dynamic contrast enhanced data was used to calculate the percent change in signal intensity before and after contrast injection. All images were aligned to the T2 weighted image. Robotic prostatectomy was performed 2 weeks after image acquisition. Prostate samples were sliced using a 3D printed slicing jig matching the slice profile of the T2 weighted image. Whole mount samples at 10 µm thickness were taken, hematoxylin and eosin stained, digitized, and annotated by a board certified pathologist. A total of 210 slides were included in this study. Lumen and epithelium were automatically segmented using a custom algorithm written in MATLAB. The algorithm was validated by comparing manual to automatic segmentation on 18 samples. Slides were aligned with the T2 weighted image using a nonlinear control point warping technique. Lumen and epithelium density and the expert annotation were subsequently transformed into MRI space. Co-registration was validated by applying a known warp to tumor masks noted by the pathologist and control point warping the whole mount slide to match the transform. Overlap was measured using a DICE coefficient. A learning curve was generated to determine the optimal number of patients to train the algorithm on. A PLS algorithm was trained on 150 random permutations of patients incrementing from 1 to 29 patients. Slides were stratified such that all slides from a single patient were in the same cohort. Three cohorts were generated, with tumor burden balanced across all cohort. A PLS algorithm was trained on 2 independent training sets (cohorts 1 and 2) and applied to cohort 3. The input vector consisted of MRI values and the target variable was lumen and epithelium density. The algorithm was trained lesion-wise. Trained PiCT models were applied to the test cohort voxel-wise to generate 2 new image contrasts. Mean lesion values were compared between high grade, low grade, and healthy tissue using an ANOVA. An ROC analysis was performed lesion-wise on the test set. RESULTS/ANTICIPATED RESULTS: Results: The segmentation accuracy validation revealed $R = 0.99$ and $R = 0.72$ ($p < 0.001$) for lumen and epithelium, respectively. The co-registration accuracy revealed a 94.5% overlap. The learning curve stabilized at 10 patients with a root mean square error of 0.14, thus the size of the 2 independent training cohorts was set to 10, leaving 19 for the test cohort. DISCUSSION/SIGNIFICANCE OF IMPACT: We present a technique for combining radiology and pathology with machine learning for generating predictive cytological topography (PiCT) maps of cellularity and lumen density prostate. The voxel-wise approach to mapping cellular features generates 2 new interpretable image contrasts, which can potentially increase confidence in diagnosis or guide biopsy and radiation treatment.

2467

PRMT5 is a master epigenetic regulator to promote repair of radiation-induced DNA damage

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OBJECTIVES/SPECIFIC AIMS: We recently reported that PRMT5 epigenetically activates androgen receptor (AR) in prostate cancer cells. Because targeting AR signaling through androgen deprivation therapy is clinically used as a radiosensitization approach to treat high-risk prostate cancer, our finding raised an exciting possibility that targeting PRMT5 may improve RT for prostate cancer patients. Contrary to our expectation, targeting PRMT5 sensitized both AR expressing and AR negative (AR−) prostate cancer cell lines to radiation. The goal of our study was therefore to determine the role of PRMT5 in repair of IR-induced DSBs and to translate these findings to improving radiation therapy for cancer patients in general (not just prostate cancer patients). METHODS/STUDY POPULATION: The majority of experiments were basic science experiments analyzing PRMT5's role in the DNA damage response in normal and cancer cell lines. For example, to extend our findings and determine if PRMT5's role in DSB repair is conserved across multiple cell types, we performed similar experiments in AR− prostate cancer cells, luminal breast cancer cells, glioblastoma cells, and human embryonic kidney cells. To determine the clinical significance of our finding, we also analyzed mRNA expression of PRMT5, AR, and both PRMT5 and AR target genes involved in DSB repair across 43 clinical cancer data sets. RESULTS/ANTICIPATED RESULTS: (1) Targeting PRMT5 sensitizes prostate cancer cells to IR in an AR-independent manner, (2) PRMT5 regulates the repair of IR-induced DSBs in an AR-independent manner, (3) RNA-seq analysis reveals that PRMT5 likely regulates genes involved in the DNA damage response, (4) PRMT5 activates expression of several genes in the DDR including those involved in DSB repair, (5) PRMT5 functions as an epigenetic activator of genes involved in DDR, (6) PRMT5 is required for NHEJ, HR, and G2-Arrest upon IR treatment, (7) Upregulation of PRMT5 correlates with formation and repair of IR-induced DSBs, (8) PRMT5's role in repair of IR-induced DSBs is conserved in several normal and cancer cell types, and (9) PRMT5 expression correlates with expression of DSB repair proteins in clinical cancer samples. DISCUSSION/SIGNIFICANCE OF IMPACT: In summary, we provide evidence that PRMT5 is a master epigenetic regulator of IR-induced DSB repair through epigenetic activation of multiple target genes involved both HR and NHEJ as well as G2 arrest. Interestingly, the majority of genes regulated by PRMT5 are well-characterized, "core repair proteins" involved in HR (RAD51, BRCA1, BRCA2, RAD51D, and RAD51AP1), NHEJ (NHEJ1, Ku80, XRCC4, and DNAPKcs), and G2 arrest (Cdk1, CDC25C, CCNB2, and WEE1), which may explain why PRMT5 is essential to repair IR-induced DSBs in several cell lines. Although AR may also regulate DSB repair via both HR and NHEJ, several pieces of evidence in our study suggest that PRMT5 also regulates DSB repair independent of AR. First, PRMT5 targeting sensitizes both AR + and AR − prostate cancer cells to IR. Second, exogenous expression of AR only partially rescues the impairment of IR-induced DSB repair by PRMT5 knockdown. Third, PRMT5 knockdown increases IR-induced DSB in AR − DU145 cells and several other cancer cell lines and normal cells. Fourth, PRMT5 expression correlates positively with the expression of its target genes in multiple human cancer tissues. During preparation of this project, Braun *et al.* reported that PRMT5 post-translationally regulates the splicing out of detained-introns (DI)s of genes to modulate gene expression. However, analysis of their data showed that the majority of DEGs we identified either do not contain DIs or DI splicing was not affected by targeting PRMT5. In addition, Clarke *et al.* reported that PRMT5 participates in the DSB repair choice process and promotes HR through methylation of RUVBL1. It is therefore likely that PRMT5 regulates repair of IR-induced DSB via multiple mechanisms. As PRMT5 is overexpressed in many human cancers and its overexpression correlates with poor prognosis, our findings suggest that increased DSB repair by PRMT5 overexpression in these human cancers may confer survival advantages particularly following DNA damaging treatment. Because targeting DSB repair has been proven to be a valid therapeutic approach for cancer treatment, our findings here also suggest that PRMT5 targeting may be explored as a monotherapy or in combination therapy with RT or chemotherapy for cancer treatment.

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Protein production as an early pharmacodynamics biomarker for RNA-targeting therapies

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OBJECTIVES/SPECIFIC AIMS: We aimed to develop an assay to measure new protein synthesis after Antisense Oligonucleotide treatment, which we hypothesized to be the earliest biochemical identification of RNA-targeting therapy efficacy. METHODS/STUDY POPULATION: We treated 2 transgenic animal models expressing proteins implicated in neurodegenerative disease: human tau protein (hTau) and human superoxide dismutase 1 (hSOD1), with ASO against these mRNA transcripts. Animals received isotope-labeled 13C6-Leucine via drinking water to label newly synthesized proteins. We assayed target protein synthesis and concentration after ASO treatment to determine the earliest identification of ASO target engagement. RESULTS/ANTICIPATED RESULTS: hTau ASO treatment in transgenic mice lowered hTau protein concentration 23 days post-treatment in cortex (95% CI: 0.05%–64.0% reduction). In the same tissue, we observed lowering of hTau protein synthesis as early as 13 days (95% CI: 29.4%–123%). In hSOD1 transgenic rats, we observed lowering of 13C6-leucine-labeled hSOD1 in the cerebrospinal fluid 30 days after ASO treatment compared with inactive ASO control (95% CI: 12.0%–48.4%). DISCUSSION/SIGNIFICANCE OF IMPACT: In progressive neurodegenerative diseases, it is crucial to develop measurements that identify treatment efficacy early to improve patient outcomes. These data support the use of stable isotope labeling of amino acids to measure new protein synthesis as an early pharmacodynamics measurement for therapies that target RNA and inhibit the translation of proteins.

2520

Proteomics in the early diagnosis of metabolic syndrome in a Hispanic pre-teen cohort

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OBJECTIVES/SPECIFIC AIMS: The objective of the present study is to determine if decreased adiponectin and increased leptin levels are associated

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