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 3 T 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 the training set. The co-registration accuracy revealed a 94.5% overlap. The co-registration accuracy validation revealed 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 of transforming mouse tumor sections 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 of combining radiology and pathology with machine learning for generating predictive cytological topography (PCT) 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.

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 DSBR 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 findings, we analyzed mRNA expression of PRMT5, AR, and both PRMT5 and AR target genes involved in DSBR 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 DSBR repair, (5) PRMT5 functions as an epigenetic activator of genes involved in DDR, and (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 DSBR 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 DSBR repair through epigenetic activation of multiple target genes involved both in NHEJ and 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 (RAD6, BRC1, BRC2, RAD51, and RAD51API), NHEJ (NHEJ1, Ku80, XRCC4, and DNAAPCs), 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 DSBR repair via both HR and NHEJ, several pieces of evidence in our study suggest that PRMT5 also regulates DSBR 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 DSBR repair by PRMT5 knockdown. Third, PRMT5 knockdown increases IR-induced DSBR 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-translationaly regulates the splicing out of detained-introns (DI) 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 DSBR repair choice process and promotes HR through modification of H2AX. It is therefore likely that PRMT5 regulates repair of IR-induced DSBR via multiple mechanisms. As PRMT5 is overexpressed in many human cancers and its overexpression correlates with poor prognosis, our findings suggest that increased DSBR repair by PRMT5 overexpression in these human cancers may confer survival advantages particularly following DNA damaging treatment. Because targeting DSBR repair has been proven to be an invalid therapeutic approach for cancer treatment, our findings also suggest that PRMT5 targeting may be explored as a monotherapy or in combination therapy with RT or chemotheraphy for cancer treatment.

2467

2342

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 hypothesize we can use to detect biologically relevant levels 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 with metabolic syndrome in a Hispanic pre-teen cohort.
with the development of MetS and identifiable endothelial dysfunction in a
cohort of Hispanic pre-pubertal children. To do so we propose the following
aims: (1) To measure expression of adiponectin and leptin levels in a Hispanic
pre-pubertal cohort and determine their correlation with features of the
MetS. (2) To perform proteomic analysis in a Hispanic pre-pubertal cohort.
(3) Evaluate early onset of endothelial dysfunction and its correlation with
expression of adiponectin and leptin levels in a Hispanic pre-pubertal cohort.

METHODS/STUDY POPULATION: A cross-sectional pilot study will obtain a
bipedal representative sampling of children aged 6–12 years from all
geographical areas of Puerto Rico. Children will be assessed regarding pre-
pubertal status through Tanner staging and later divided into pre-MetS
versus MetS groups as well as controls. MetS will include children meeting 3
or more of the current International Diabetes Federation (IDF) criteria. Pre-
MetS will include children with at least 1 criterion for MetS. Anthropometric
data, blood pressure readings, ultrasound-based noninvasive testing for
endothelial dysfunction, and laboratory assays will be performed to the study
population and data analyzed for correlation. Total adiponectin and leptin
levels will be measured using a commercially available quantitative sandwich
enzyme-linked immunoassay test. The study will be submitted to the
University of Puerto Rico Medical Sciences Campus’ Institutional Review
Board (IRB) for approval. Written consent and assent will be obtained from
parents and children respectively to ensure patient anonymity. RESULTS/
ANTICIPATED RESULTS: We hypothesize that low levels of adiponectin and high
levels of leptin will correlate both with clinical features of the MetS and with
features of the MetS and with

Quantitative structural knee measurements improve
classification of accelerated knee osteoarthritis: Data
from the osteoarthritis initiative

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OBJECTIVES/SPECIFIC AIMS: The aim of this study is to determine
whether quantitative measures of knee structures including effusion, bone
marrow lesions, cartilage, and meniscal damage can improve upon an
existing model of demographic and clinical characteristics to classify
accelerated knee osteoarthritis (AKOA). METHODS/STUDY POPULA-
TION: We conducted a case-control study using data from baseline and
four annual follow-up visits from the osteoarthritis initiative. Participants
had no radiographic knee osteoarthritis (KOA) at baseline. AKOA is
defined as progressing from no KOA to advance-stage KOA in at least 1
knee within 48 months. AKOA knees were matched 1:1 based on sex to (1
participants who did not develop KOA within 48 months and (2
participants who developed KOA but not AKOA. Analyses were person
based. Classification and regression tree analysis was used to determine the
important variables and percent of variance explained. RESULTS/ANTICI-
PATED RESULTS: A previous classification and regression tree analysis
found that age, BMI, serum glucose, and femoral osteal angle explained 31%
of the variability between those who did and did not develop AKOA. Including
structural measurements as candidate variables yielded a model that
included effusion, BMI, serum glucose, cruciate ligament degeneration
and coronal slope and explained 39% of the variability. DISCUSSION/
SIGNIFICANCE OF IMPACT: Knee structural measurements improve
classification of participants who developed AKOA Versus those who did
not. Further research is needed to better classify patients at risk for
AKOA.

Radiofrequency renal denervation attenuates kidney
fibrosis in spontaneously hypertensive rats

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OBJECTIVES/SPECIFIC AIMS: The goal of this study was to investigate
whether RF-RDN attenuates renal fibrosis and inflammation in SHR with
established hypertension. METHODS/STUDY POPULATION: Twenty-two-
week-old SHR received bilateral RF-RDN or Sham-RDN (Biosense Webster
Stockert 70 generator and RF-probe). Four weeks later, SHR were sacrificed and
paraffin sections of kidneys were stained for fibrosis by Masson’s
trichrome staining. Kidney tissue were homogenized for measurement of
cytokines levels by ELISA. RESULTS/ANTICIPATED RESULTS: The results
showed that Sham-RDN treated SHR had extensive fibrosis as demonstrated
by moderate thickening of Bowman’s capsule, collagen deposition in
glomerulus, extensive tubulointerstitial fibrosis, and segmental
glomerulosclerosis. In contrast, RF-RDN significantly reduced each of these pathological
components in kidney cortex and medulla as compared with Sham-
RDN treated kidneys. In addition, C57BL6/J DCD+ T cells and CDD+ T cells in the kidney of SHR as measured by flow
cytometry. Meanwhile, kidney tissue levels of IL-17, INFγ, MIP-3a, TNF-α,
and TGF-β were decreased as compared with respective levels in Sham-RDN.
DISCUSSION/SIGNIFICANCE OF IMPACT: Together, these findings demonstr-
ate that removal of the influence of heightened renal sympathetic activity by
RF-RDN reduces kidney inflammatory markers and attenuates renal fibrosis in
hypertensive SHR.

Regulation of retinal protein O-GluN Acylation by
angiotensin-(1-7) and cAMP
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OBJECTIVES/SPECIFIC AIMS: Increased retinal protein O-GlcNAcylation
occurs in response to hyperglycemia and contributes to diabetic retinopathy.
Renin-angiotensin system (RAS) blockers reduce the incidence of diabetic
retinopathy. Beneficial effects of RAS blockers are often attributed to production of angiotensin-(1-7) (Ang1-7). The objective here is to determine
the impact of Ang1-7 on retinal protein O-GlcNAcylation. METHODS/STUDY
POPULATION: C57/BL6 mice were fed a high-fat diet for 8 weeks and then
treated for 3 weeks with either a vehiclecontrol, the RAS blocker captopril, or
captopril and the Ang1-7 receptor antagonist A779. R28 cells were used to
assess levels of O-GlcNAcylated proteins in response to Ang1-7, and the role of
cAMP was investigated with addition of forskolin, 6-Bnz-cAMP-AM, and 8-
pCPT-2-O-Me-cAMP-AM to cell culture medium. RESULTS/ANTICIPATED
RESULTS: Captopril attenuated retinal protein O-GlcNAcylation in mice fed a
high-fat diet. This effect was reversed by A779. Ang1-7 attenuated protein
O-GlcNAcylation and increased cAMP levels. Forskolin and the EPAC selective
cAMP analog 8-pCPT-2-O-Me-cAMP-AM, but not the PKA selective cAMP
analog 6-Bnz-cAMP-AM, attenuated O-GlcNAcylation. Inhibition of EPAC
blocked the effect of forskolin, whereas inhibiting PKA did not. DISCUSSION/
SIGNIFICANCE OF IMPACT: This study demonstrates a novel role for Ang1-7
in the retina and identifies a potential EPAC-dependent mechanism that
regulates protein O-GlcNAcylation. Thus, future therapeutics targeted at an
Ang1-7/EPAC axis in retina may be used to address DRC.

Relationship power imbalance and history of male
partner HIV testing among pregnant women in
central Uganda

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