or guide biopsy and radiation treatment. Interpretable image contrasts, which can potentially increase cancer detection, are generated through a voxel-wise approach to mapping cellular features. This results in two new predictive cytological topography (PiCT) maps of cellularity and lumen density that are used to guide biopsy and radiation treatment.

The size of the two independent training cohorts was set to 10, leaving 19 for the test set. The learning curve stabilized at 10 patients with a root mean square error of 0.14. Accuracy validation revealed 87% sensitivity and 95% specificity. A learning curve was generated to determine the optimal number of patients to train the algorithm on. A PLS algorithm was trained on 150 undistorted single-shot (FOCUS) diffusion-weighted imaging with 10(DSb)s and T2-weighted imaging. T2-weighted images were intensity normalized and apparent diffusion coefficient maps were calculated. The dynamic contrast-enhanced image 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 3D 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 data set. The 10 independent test cohorts 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 cohorts. 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 maps were applied to test cohorts and used to generate 2 new interpretive 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 interpretive 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, we found 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 findings, we 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, BRC1, BRC2, RAD51D, and RAD51AP1), NHEJ (NHEJ1, Ku80, XRC4C, 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 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– DUX45 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-translational regulation is the splicing out of retained-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 DI 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 H3K4me1. 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, these findings also suggest that PRMT5 targeting may be explored as a monotherapy or in combination therapy with RT or chemotherapy for cancer treatment.