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Targeting Homologous Repair to Overcome Genotoxic Therapy Resistance in Pancreatic Cancer

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OBJECTIVES/GOALS: Pancreatic ductal adenocarcinoma (PDAC) is a relatively radioresistant disease, and inhibition of DNA homologous recombination (HR) repair in combination with radiation therapy (RT) is a potentially attractive strategy to overcome radioresistance. We have found that the expression of the HR protein RAD18 is upregulated in PDAC cells. METHODS/STUDY POPULATION: Standard clonogenic assays, Î³H2aX foci staining, HR-GFP reporter assay, and western blot analysis of DNA damage response proteins were performed in MIA-PaCa2 (MP2) and PANC-1 cells following knockdown of RAD18 in cells via short hairpin RNA (shRNA). Drug targeting of RAD18 was achieved through the use of a USP-7 inhibitor, P5091. Cells with or without stable knockdown of RAD18 were implanted orthotopically in the pancreas of athymic nude mice and treated with sham radiation or radiation to a dose of 20 Gy in 5 daily fractions once tumors reached 100-150 mm3. RESULTS/ANTICIPATED RESULTS: Stable knockdown of RAD18 in MP2 and PANC-1 resulted in decreased radiation clonogenic survival in vitro (dose enhancement factor (DEF)=1.52 and 1.51, respectively), decreased DNA repair after radiation as measured by the increased number of Î³H2aX nuclear foci assay at 6, 12, and 24 hours (all p<0.05), decreased HR activation following DNA damage via an HR-GFP reporter assay (p=0.039), and increased tumor growth delay following radiation in vivo (p<0.001). P5091 treatment of both MP2 and PANC-1 resulted in efficient knockdown of RAD18, which was confirmed through western blotting, qRT-PCR, and luciferase reporter assays. P5091 increased radiosensitization, yH2aX nuclear foci remained elevated at 12 and 24 hours (p<0.05), and HR repair was also reduced (p=0.014). DISCUSSION/SIGNIFICANCE: Herein, we show the HR repair protein RAD18, and that modulation of RAD18 expression correlates with in vitro and in vivo radiosensitization through altered HR-mediated DNA repair. USP7 inhibition successfully reduced RAD18 expression and resulted in enhanced radiosensitization.

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The Role of Mechanosensitive Ion Channels in Primary Open Angle Glaucoma

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OBJECTIVES/GOALS: Intraocular pressure is the most significant risk factor for glaucoma. Mechanosensitive proteins may have a critical role in transducing mechanical stimuli that ultimately lead to death of retinal ganglion cells. Our goal is to use genetic and functional approaches to discover mechanosensitive ion channels that mediate the progression of glaucoma. METHODS/STUDY POPULATION: Association data, obtained using a logistic regression model that included age, gender and population substructure as co-variates, for 2,576 SNPs located in the PIEZO1 and PIEZO2 genomic regions were extracted from the NEIGHBORHOOD genome-wide association study results for primary open angle glaucoma (POAG) (3,853 cases and 33,480 controls) and the subset of cases with intraocular pressure (IOP) measurements > 21mmHg (high-tension, HTG) (1868 cases and 33,480 controls). Rare coding PIEZO1 and PIEZO2 variants were evaluated using logistic regression and SNP data from the Human Exome array in 2606 POAG cases and 2606 controls and the subset of 1868 HTG cases and 2606 controls. Immunohistochemistry was used to characterize the expression of Piezo1 and Piezo2 in mouse eye sections. RESULTS/ANTICIPATED RESULTS: Exome data analysis identified two protein-altering variants associated with lower glaucoma risk (P<0.05): a PIEZO1 missense allele (Arg1527His; OR= 0.18, P= 0.001) and a variant disrupting a splice donor site (c.1107 +1G>C; OR=0.38, P= 0.02), that prematurely truncates the protein. Investigation of the NEIGHBORHOOD GWAS dataset identified nominal association with common PIEZO2 variants (minor allele frequency > 0.3) in POAG overall (top SNP rs264179, P= 0.008) and in the HTG subgroup (top SNP rs264160, P= 0.001). The associated PIEZO2 SNPs are significantly associated with gene expression in lymphocytes (P< 1x10-8) with the risk allele correlated with decreased gene expression. Piezo1 and Piezo2 are expressed in many ocular tissues in the mouse, including cornea, ciliary body and retina. DISCUSSION/SIGNIFICANCE: We identify rare, protein-altering PIEZO1 variants associated with lower glaucoma risk and show that Piezo1 and Piezo2 are broadly expressed in the eye. Common variants influencing PIEZO2 expression also show nominal association with POAG risk. Inhibition of Piezo1 or augmentation of Piezo2 could be novel therapeutic strategies for glaucoma.

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Omega-3 and omega-6 fatty acids attenuate platelet reactivity in postmenopausal women

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OBJECTIVES/GOALS: This study aimed to investigate the mechanistic effects of fish oil (âµ-3 fatty acids) or evening primrose oil (âµ-6 fatty acids) supplementation on platelet reactivity in postmenopausal women. METHODS/STUDY POPULATION: Postmenopausal women were recruited from the Ann Arbor community and the University of Michigan Medicine Center. All subjects were recruited under study protocols approved by the University of Michigan IRB between November 2015 and March 2017. We conducted a randomized, double-blind, two-period crossover trial, consisting of a 60-day supplementation period followed by a 14-day washout period in between and at the end of the study. Subjects were treated daily in random order with 2g of fish oil supplement and 2g of evening primrose oil. Blood was drawn at baseline, post-supplementation, and after washout. The effects of fatty acid supplementation on platelet aggregation, dense granule secretion and activation of basal integrin â¹IIbÎ²3 were assessed following supplementation and washout period. RESULTS/ANTICIPATED RESULTS: The study started with 90

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