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Cadherin complexes recruit PIWIL2 to suppress transposons and pro-tumorigenic transformation

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ABSTRACT IMPACT: This study has uncovered a novel surprising mechanism involving the epithelial adherens junctions and transposon regulation that can deepen our understanding of tumorigenesis. OBJECTIVES/GOALS: Recent studies show that genomic instability in 50% of tumors can be attributed to increased transposon activity, but the the reasons for this activity are unknown. We have evidence of a novel mechanism linking adherens junctions with transposon regulation. We hypothesize that adherens junctions suppress transposons to maintain genomic integrity. METHODS/STUDY POPULATION: We observed co-localization of PIWIL2 with adherens junction components of well differentiated epithelial breast, kidney and colon cell lines MCF10A, MDCK and CACO2, respectively, through immunofluorescence staining, confocal microscopy, and co-immunoprecipitation studies. Breast cancer cell lines MCF7 and MDA-231 were also observed using immunofluorescence to determine the localization of PIWIL2 in cancer cell lines. shRNA knockdown of PIWIL2 in MCF10A cells, followed by western blot, immunofluorescence, and qRT-PCR was performed to confirm the knockdown, observe if transposons were upregulated, and determine the extent of DNA damage to the genome by the marker gamma-H2AX. RNA-seq will be performed to determine piRNA sequences and possible targets of PIWIL2. RESULTS/ANTICIPATED RESULTS: Our data have revealed an interaction of E-cadherin and p120 catenin, core components of adherens junctions, with PIWIL2, a member of the Argonaute family of proteins and a key component of the piRNA processing pathway that is responsible for transposon silencing. piRNAs (PIWI-interacting RNAs) are a distinct class of small RNAs that bind to PIWI proteins, and aid in transposon degradation. We found co-localization of PIWIL2 with E-cadherin and p120 catenin at adherens junctions of well-differentiated epithelial cells, whereas this association was lost in cancer cells. Furthermore, our data show that E-cadherin depletion results in mis-localization of PIWIL2 and TDRD1, another member of the PIWI complex. E-cadherin depletion also results in upregulation of transposons and ?-H2AX, an indicator of DNA damage. DISCUSSION/SIGNIFICANCE OF FINDINGS: Since both loss of junctional integrity and increased transposon activity are universal events in cancer, this study has the potential to further our understanding of the causes of tumorigenesis. Understanding the mechanisms of transposon regulation has the potential to lead to a therapeutic target in the future.

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Triazole-based reversible inhibitors of spermine oxidase and implications for amelioration of neuronal injury

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ABSTRACT IMPACT: This project aims to investigate the impact of spermine oxidase inhibition on amelioration of neuronal injury. OBJECTIVES/GOALS: Our group has recently described a series of triazole-based reversible inhibitors of spermine oxidase (SMOX) (Holshouser et al. 2019). The purpose of the current project is to optimize our most promising inhibitors by structural modification, and to determine whether they can reduce oxidative damage in models of neuronal injury. METHODS/STUDY POPULATION: A small number of SMOX inhibitors have been described in the literature, however, currently available inhibitors lack selectivity for the enzyme and are associated with dose-limiting toxicity. For this project we used multiple medicinal chemistry techniques to synthesize novel triazole-based analogs of our most potent inhibitors as potential SMOX inhibitors. In addition, we plan to utilize virtual and physical screening methods to identify new potential scaffolds. Compounds with demonstrated activity against SMOX via enzymatic assay will then be evaluated in a cell-based model of neuronal injury. In a preliminary study, we investigated the ability of hydrogen peroxide to induce SMOX expression in an SH-SY5Y neuroblastoma cell line using western blot. RESULTS/ANTICIPATED RESULTS: We found that cellular SMOX protein increases in response to hydrogen peroxide exposure in a dose-dependent manner, indicating that this may be a viable cellular model for testing the efficacy of our experimental compounds. To extend these studies, we have developed a SMOX enzymatic assay that will be used for high-throughput screening of commercial libraries, as well as the South Carolina Compound Collection (SC3), which contains 100,000 proprietary, fully annotated analogs. As hits are identified, they will be synthesized and evaluated for potency and selectivity as SMOX inhibitors. The most potent and selective compounds will then be evaluated in our cellular model of neuronal injury. DISCUSSION/SIGNIFICANCE OF FINDINGS: Studies have linked the overexpression of SMOX and the production of associated toxic byproducts with increased susceptibility to excitotoxic stress and neuronal injury. Our objective is to develop potent and selective inhibitors for this enzyme that can serve as chemical probes for elucidating the role of this enzymatic pathway in neuronal injury.

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Potential effect of serum from hypertensive donors on PP2A expression and activity in endothelial cells

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ABSTRACT IMPACT: Racial differences in the prevalence of hypertension and endothelial (dys)function are well established, yet research investigating the mechanism(s) underlying this disparity is still lacking. OBJECTIVES/GOALS: Investigate the influence of race and the effect of serum collected from hypertensive donors on Protein Phosphatase 2A (PP2A) and endothelial nitric oxide synthase (eNOS) expression and activity in human umbilical vein endothelial cells (HUVECs) from Caucasian (CA) and African American (AA) donors. METHODS/STUDY POPULATION: HUVECs from 3 CA & 3 AA donors were cultured in parallel. Experiments were conducted between passages 5-7. At ?90% confluency, cells were serum starved ~12hrs prior to incubating for 24 or 48 hours in one of the following conditions: 1) Control (Fetal Bovine Serum), 2) serum from normotensives (NT; 5 CA & 5 AA donors), or 3) serum from hypertensives (HT; 5 CA & 5 AA donors). NT and HT serum was pooled from donors with the following characteristics: Male, 30-50 years, nonsmokers, no comorbidities, and non-obese (BMI < 30 kg/m2). Western blotting was used to measure protein

https://doi.org/10.1017/cts.2021.432 Published online by Cambridge University Press