34380

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.

37161

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.

38189

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

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expression of total eNOS, p-eNOSS1177, total PP2A, and p-PP2AY307. For activity p-eNOSS1177/total eNOS and p-PP2AY307/ total PP2A ratio was used. A two-way ANOVA was used for statistical analysis. RESULTS/ANTICIPATED RESULTS: Irrespective of the donors' race, there was no influence of serum treatment or interaction effect in any of the measured proteins of interest. Moreover, compared to CA, HUVECs from AA had lower expression of eNOS irrespective of condition (race p=0.01). Compared to CA, HUVECs from AA tended to have lower expression of p-eNOSS1177 irrespective of condition (race p=0.07). However, there was no racial differences in eNOS activity (p=0.68). There was no racial difference in the expression of PP2A (p=0.35), p-PP2AY307 (p=0.30), or PP2A activity (p=0.97) in all conditions. DISCUSSION/SIGNIFICANCE OF FINDINGS: Our preliminary results suggest no influence serum constituents from hypertensive donors or race on PP2A or eNOS expression and activity in HUVECS. Future research should consider conducting proteomics profiling to compare NT and HT serum.

39800

Immune Checkpoint Blockade during Periprosthetic Joint Infection

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ABSTRACT IMPACT: If immune checkpoint blockade increases bacterial clearance with or without antibiotics in vitro, clinical application would be almost immediate and dramatic creating a seismic shift in the current therapeutic paradigm of periprosthetic joint infection. OBJECTIVES/GOALS: Periprosthetic joint infection (PJI) is a major cause of failure after joint replacement. Currently, the treatment of PJI relies on removing biofilm contaminated implants. Some of the bacteria within biofilm undergo a phenotypic shift becoming small colony variants (SCVs). SCVs induce local immunosuppression through PD-1/L1 signaling. METHODS/STUDY POPULATION: We will infect cultured human macrophages and bone marrow aspirate with stable Staphylococcus aureus SVCs and treat with anti-PD-1 or anti-PD-L1 monoclonal antibodies with and without antibiotics (e.g., gentamycin, cefazolin, vancomycin, rifampicin) and assess the residual bacterial viability. We will utilize multiplexed ion beam imaging to quantify PD-1/L1 expression in human tissue from patients with a chronic PJI and compare those to patients undergoing an aseptic revision. Patients with a chronic PJI are likely to have increased expression of PD-1/L1 as their tissue samples are prospectively screened. RESULTS/ANTICIPATED RESULTS: SCVs reduce the phagocytic activity of macrophages and can survive intracellularly. SCVs also induce anti-inflammatory M2-macrophage polarization and recruit a heterogeneous group of immature monocytes and granulocytes called myeloid-derived suppressor cells (MDSC) to the periprosthetic microenvironment. M2-macrophages and MDSCs then produce an immunosuppressive cytokine milieu characterized by increased IL-10 and decreased TNF-α. Clinically isolated SCVs up-regulate the expression of PD-L1 and PD-L2 on the surface of macrophages, representing a mechanism by which SCVs induce host immunosuppression and survive immune clearance. Our preliminary data show PD-L1 expression during septic PJI, but not in aseptic revisions. DISCUSSION/SIGNIFICANCE OF FINDINGS: If immune checkpoint blockade is shown to increase bacterial clearance with or without antibiotics, host immunomodulation would represent a novel class of therapeutic adjuvants to assist surgical debridement and antibiotic administration that could be superimposed on existing treatment algorithms to improve PJI related outcomes.

41224

REDUCED FRONTOSTRIATAL FUNCTIONAL CONNECTIVITY IN 41- TO 70-YEAR-OLD ADULTS WITH HIV*

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ABSTRACT IMPACT: The knowledge acquired from my research can inform the development of early diagnostic methods for HIV-associated neurocognitive disorders. OBJECTIVES/GOALS: In the era of combination antiretroviral therapy (cART), the prevalence of HIV-associated neurocognitive disorders (HAND) remains high but the neural mechanisms are unclear. We examined whether older people with HIV (PWH) with minimal cognitive impairment have reduced functional connectivity in frontostriatal circuits compared to controls. METHODS/STUDY POPULATION: 99 PWH (mean age 56.6 years, 75% male, 62% Black, mean duration of HIV-infection 26.2 years ±9.3, 90% viral load <50 copies, 98% on stable cART) and 38 demographically-comparable controls (mean age 54.5 years, 71% male, 58% Black) participated in a cross-sectional study. A 7-domain neuropsychological battery and an Activities of Daily Living index were used to determine HAND diagnoses: 32 PWH met criteria for asymptomatic to mild HAND. Motor skill was assessed using the Grooved Pegboard Test by measuring performance speed. Structural MRI and resting-state functional MRI were collected. Seed-to-voxel analyses were conducted using 4 distinct regions in the striatum as seed regions. We used a voxel threshold of $p<0.001$ and cluster threshold of $p<0.05$ (FDR-corrected) after controlling for demographic variables. RESULTS/ANTICIPATED RESULTS: Compared to controls, PWH had lower resting state functional connectivity between the default mode region of the striatum (i.e., medial caudate) and bilateral superior frontal gyrus, supplementary motor cortex and paracingulate gyrus ($p<0.05$; cluster size: 567 voxels). Also, compared to controls, PWH had reduced resting state functional connectivity between the motor division of the striatum (i.e., posterior putamen) and anterior cingulate cortex and left supplementary motor cortex ($p<0.05$, cluster size: 405 voxels). Performance speed on the Grooved Pegboard motor test negatively correlated with functional connectivity between the motor region of the striatum and supplementary motor frontal regions in all participants (Spearman's rho=-0.18, p=0.04). DISCUSSION/SIGNIFICANCE OF FINDINGS: Our results support the hypothesis that frontostriatal abnormalities are widely present in PWH and might play a key role in HAND development. Our data suggest that dysfunction within the frontostriatal circuits may be involved in motor impairment in PWH, and ongoing inflammation may contribute to motor impairment and frontostriatal injury.

45724

VC2 Oncolytic Virotherapy Induces Robust Systemic Anti-Tumor Immunity and Increases Survival in an Immunocompetent B16F10-derived Mouse Melanoma Model

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ABSTRACT IMPACT: Our data demonstrate that VC2 oncolytic virotherapy has significant clinical potential. OBJECTIVES/

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