309

Measurement of agonist-induced calcium and mechanical signals in human airway smooth muscle cells

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OBJECTIVES/GOALS: The goal of this proposal is to develop a technology that combines calcium imaging via confocal microscopy, and force measurement via monolayer stress microscopy to perform simultaneous quantitative measurements of agonist-induced Ca2+ and mechanical signals in HASMCs. METHODS/STUDY POPULATION: The methods by which second messenger signals and changes in mechanical forces determine specific physiological responses are complex. Recent studies point to the importance of temporal and spatial encoding in determining signal specificity. Hence, approaches that probe both chemical and mechanical signals are needed. We combine hyperspectral imaging for second messenger signal measurements, monolayer stress microscopy for mechanical force measurements, and S8 analysis software for quantifying localized signals. Imaging was performed using an excitation-scanning hyperspectral microscope. Hyperspectral images were unmixed to identify signals from fluorescent labels and microparticles. Images were analyzed to quantify localized force dynamics through monolayer stress microscopy. RESULTS/ANTICIPATED RESULTS: Results indicate that localized and transient cellular signals can be quantified and mapped within cell populations. Importantly, these results establish a method for simultaneous interrogation of cellular signals and mechanical forces that may play synergistic roles in regulating downstream cellular physiology in confluent monolayers. DISCUSSION/SIGNIFICANCE: We will measure the distribution of chemical and mechanical signals within cells, providing insight into the dynamics of cell signaling. Studies will have implication in the understanding of infections, drug delivery in which non-uniform distributions of drugs are a certainty, and in understanding coordinated responses in cellular systems.

310

Mechanisms of progression of myelodysplastic syndrome to fatal secondary acute myeloid leukemia

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OBJECTIVES/GOALS: Aim 1: Define the genetic events that cooperate with Trp53 mutations to mediate the transformation of MDS to sAML at the stem cell level. Aim 2: Use Bayesian networks to model the signaling pathway activation states identify aberrant regulators of self-renewal in LSCs. METHODS/STUDY POPULATION: To model the transformation of MDS, I will utilize a mouse model of MDS and sAML. The Trp53 mutated mouse mimics the genetics of human MDS and develops MDS. To discover how additional mutations contribute to disease progression, I use the Sleeping Beauty transposon system which induces random mutations. I propose to use this mouse model to define the molecular mechanisms of transformation of MDS to sAML. I will also measure levels of activated signaling intermediates in sAML using CyTOF. CyTOF is a method of flow cytometry that measures up to 40 proteins simultaneously at single-cell resolution. I will also use Bayesian network to model the signaling architecture of the MDS/sAML stem cells. RESULTS/ANTICIPATED RESULTS: I will identify putative mediators of self-renewal in gene expression data and the corresponding Bayesian networks model. I will prioritize genes and signaling intermediates that act in pathways that rank highly in both methods and those that have previously published roles in self-renewal. Based on prior work, I will focus on EZH2, NFi «B, and mTOR pathways in addition to candidate pathways revealed by these studies. Small molecule inhibitors that target candidate signaling intermediates will be used for experimental validation. I will test the impact of these small molecule inhibitors on LSC self-renewal using serial colony forming assays as well as in vivo mouse reconstitution assays. DISCUSSION/SIGNIFICANCE: We focus on the role of stem cells in the transformation of MDS to sAML. Understanding the mutations and signaling relationships that mediate self-renewal in Trp53 mutant stem cells will allow for precise therapeutic target of this disease and improve outcomes for these patients.

311

Mental Illness and the Development of Postoperative Atrial Fibrillation in Transcatheter Aortic Valve Replacement Patients: Trends over Time

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OBJECTIVES/GOALS: The purpose of this retrospective cohort study was to evaluate the impact of mental illness on first-time transcatheter aortic valve replacement (TAVR) and repeat TAVR (viv-AVR) outcomes including postoperative atrial fibrillation (POAF/AFL), as well as trends over time. METHODS/STUDY POPULATION: Using de-identified data reports from the New York Statewide Planning and Research Cooperative System (SPARCS) database from 2005-2018, multivariate logistics models were used to predict endpoints including POAF, the Society of Cardiothoracic surgeon (STS) endpoint (MM), and 30-day readmission (READMIT) in patients with and without mental illness. The TAVR procedure was approved for high-risk patients after 2012, and intermediate-risk patients after 2016, indicting a need to analyze the two populations separately. Multivariate analysis was only conducted on the first-time TAVR patients because of the small n in the viv-TAVR population. RESULTS/ANTICIPATED RESULTS: After 2012, 13.05% (1,810/13,870) of patients undergoing TAVR and 20.83% (15/72) undergoing viv-TAVR were diagnosed with a mental illness before the procedure. After 2016, 15.59% (1,485/9,524) TAVR patients and 20.00% (11/55) viv-TAVR patients had a preoperative diagnosis of mental illness. Multivariate analysis showed that mentally ill patients did not have significant differences in rates of POAF, 30-day readmission, and 30-day composite outcomes when compared to patients without mental illnesses following TAVR procedures after 2012 and 2016. Patients with POAF after both 2012 and 2016 were significantly less likely to be mentally ill, Black, and Hispanic. DISCUSSION/SIGNIFICANCE: Of the mentally ill patients who underwent TAVR, there was no significant difference in short-term outcomes after 2012 vs. 2016, compared to patients without mental

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