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Targeting isoform-specific PI3 kinase signaling for treatment of cutaneous T cell lymphoma

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OBJECTIVES/SPECIFIC AIMS: (1) Determine the anti-proliferative effect of Copanlisib (α/δ PI3 kinase inhibitor) in CTCL cell lines and synergy with other anti-tumor drugs such as HDAC inhibitors. (2) Determine the effect of Copanlisib treatment on downstream targets of the PI3K/AKT/mTOR pathway. METHODS/STUDY POPULATION: We will test the anti-proliferative effect of Copanlisib on the cell lines H9 and HH, which are well characterized for evaluating new therapies for CTCL (Netchiporouk *et al.*, 2017). We will also test the anti-proliferative effect of Copanlisib in combination with HDAC inhibitors on CTCL cell lines. Cell Titer Glo (Promega) will be used for the proliferation assay. Briefly, Cell-Titler Glo will be added after 24, 48, and 72 hours and luminescence will be measured as a % of maximal growth. Inhibitory effect will be determined by comparison to % growth of the control cultures without Copanlisib treatment. Our next objective is to determine the effect of Copanlisib treatment on downstream targets of the PI3K/AKT/mTOR pathway using Western blot analysis. In brief, 30 μg of each lysate will be subjected to 4%–12% gradient SDS-PAGE gel eletrophoresis. All primary antibodies were purchased from Cell Signaling Technology. Membranes will be washed with TBST and incubated with 1:10,000 dilution of IRDye-conjugated secondary antibody (Licor) for 1 hour. Results will be expressed as relative intensity: the intensity of each band adjusted to that of GAPDH. Experiments will be done in triplicate and 1-way analysis of variance followed by multiple comparison test will be applied to compare the cell proliferation between different treatment groups. RESULTS/ANTICIPATED RESULTS: Previous results from the Ai lab have demonstrated the importance of the PI3 kinase signaling cascade using high-throughput proliferation assays and siRNA knockdown of individual and double PI3 kinase isoforms (unpublished data). So far I have successfully established culture of HH and H9 cells in 96 well plates (100,000 cells/200 μL). We are titrating Copanlisib at doses from 20 nm to 20 μM. Initial results suggest a promising anti-proliferative effect and currently we are optimizing the most effective pharmacologic dose. Thus we anticipate that Copanlisib will exhibit a potent anti-proliferation activity in CTCL cell lines. H9 and HH cell lysates have been collected and preserved for Western blot analysis. We anticipate that Copanlisib treatment will significantly decrease the phosphorylation of AKT and 4EB-P1, both downstream targets of PI3 kinase signaling. DISCUSSION/SIGNIFICANCE OF IMPACT: These findings will elucidate the importance of the PI3 kinase/AKT/mTOR pathway in tumor proliferation in CTCL. Identifying the importance of specific isoforms of PI3 kinase in CTCL will allow for more targeted selection of treatment. Copanlisib targets α and δ isoforms of PI3K and is newly approved by the FDA for low grade B cell lymphoma. Our results seek to quantify the anti-proliferative effect of Copanlisib and determine an on-target mechanism of action by investigating the drug's effects on downstream signaling molecules of the PI3 kinase pathway. This project will elucidate the disease process of CTCL and provide important insight for its management.

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The cell-cell adhesion component PLEKHA7 regulates the pro-tumorigenic MIR17HG long non-coding RNA in colon epithelial cells

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OBJECTIVES/SPECIFIC AIMS: The goal of this study is to test the hypothesis that the adherens junctions of colon epithelial cells regulate lncRNAs levels and function via the microprocessor and RISC complexes to suppress expression of pro-tumorigenic markers and aberrant cell behavior. METHODS/STUDY POPULATION: To test this hypothesis, we used colon epithelial cancer cell lines. We performed RNA-seq following knockdown of PLEKHA7, a key component of the adherens junctions, to identify changes in lncRNA expression and downstream mRNA levels. We confirmed junctional localization of affected lncRNAs from the RNA-seq and those that we found in our preliminary study by using in situ hybridization (ISH). RESULTS/ANTICIPATED RESULTS: RNA-seq identified junction-associated lncRNAs whose expression levels are regulated by PLEKHA7. The top upregulated lncRNA upon PLEKHA7 depletion was MIR17HG, an oncogenic host transcript of a cluster of miRNAs. These mature miRNAs also co-precipitate with PLEKHA7. PLEKHA7 knockdown results in increased levels of MIR17HG, but only a subset of its hosted miRNAs (miR-19a,b). Notably, miR-19a and mir-19b are highly upregulated in colon cancer. Our data suggest that 2 PLEKHA7-associated miRNAs, miR-203a and miR-372, mediate suppression MIR17HG. Re-expression of PLEKHA7 in aggressive colon cancer cells that lack PLEKHA7 suppressed expression of MIR17HG, as well as anchorage independent growth of these cells. DISCUSSION/SIGNIFICANCE OF IMPACT: Our data point towards a novel mechanism of lncRNA regulation that tethers epithelial tissue integrity with pro-tumorigenic cell transformation. Reducing elevated MIR17HG levels, is a potential therapeutic approach to suppress the tumorigenic behavior of cells that have lost their junctional integrity and homeostasis. identify a network of miRNA-mRNA-lncRNA interactions that could be exploited for further mechanistic studies, as well as for diagnostic and therapeutic purposes in the future.

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The direct effect of trimethylamine *N*-oxide (TMAO) on cardiac muscle contractile mechanics

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OBJECTIVES/SPECIFIC AIMS: The objective of this study was to determine if trimethylamine *N*-oxide (TMAO) alone could acutely alter cardiac contractile function on a beat-to-beat basis. METHODS/STUDY POPULATION: CD1 adult mouse hearts were extracted, attached to a force transducer, oxygenated, and paced within an organ bath. Changes in contractility were measured after pipetting or reverse perfusing TMAO through the aorta via a modified Langendorff apparatus to facilitate TMAO delivery into the myocardium. To determine if our findings translated to the human heart, we performed contractility experiments using human right atrial appendage biopsy tissue retrieved during cardiopulmonary bypass procedures. To investigate whether TMAO alters contractile rate, in a separate series of experiments, the atria and sinoatrial node of isolated hearts were kept intact to allow for spontaneous beating without artificial pacing and were treated with TMAO or vehicle. In addition, intracellular calcium measurements were performed on spontaneously beating embryonic rat cardiomyocytes after TMAO or vehicle treatment. RESULTS/ANTICIPATED RESULTS: We found acute exposure to TMAO, diluted into the organ bath, increased average contraction amplitude 20% and 41% at 300 μM and 3000 μM, respectively ($p < 0.05$, $n = 6$). Langendorff reverse perfusion of mouse hearts ex vivo with 300 μM TMAO generated an even greater response than nonperfusion peripheral exposure and increased isometric force 32% ($p < 0.05$, $n = 3$). Consistent with what we observed in mouse hearts, incubation of human atrial muscle tissue with TMAO at 3000 μM increased isometric tension 31% compared with vehicle ($p < 0.05$, $n = 4$). TMAO treatment (3000 μM) also increased average beating frequency of ex vivo mouse hearts by 27% compared with vehicle ($p < 0.05$, $n = 3$) and increased the spontaneous beating frequency of primary rat cardiomyocytes by 13% compared with vehicle treatment ($p < 0.05$, $n = 4$). DISCUSSION/SIGNIFICANCE OF IMPACT: TMAO, at pathological concentrations, directly increases the force and rate of cardiac contractility. Initially, these inotropic and chronotropic effects may be adaptive during CKD; however, chronic increases in isometric tension and beating frequency can promote cardiac remodeling and heart failure. Further translational studies are needed to understand the intricate relationship between the microbiome, kidneys, and heart and to examine if TMAO represents a therapeutic target for reducing cardiovascular mortality in CKD patients.

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The effect of antipyretics and fever on the mortality of mechanically ventilated patients

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OBJECTIVES/SPECIFIC AIMS: (1) To evaluate clinical outcomes in mechanically ventilated patients with and without fever. We hypothesize that, after adjusting for confounding factors such as age and severity of illness: (a) In septic patients, fever will be associated with improved clinical outcomes. (b) In nonseptic patients, fever