GOALS: Use our novel oncolytic herpes simplex virus type I (HSV-1), VC2, to understand how oncolytic virotherapy affects the immunosuppressive tumor microenvironment as a mechanism of efficacy. METHODS/STUDY POPULATION: We tested the efficacy of VC2 as an oncolytic virotherapy (OVT) in a syngeneic B16F10-derived mouse model of melanoma. We modified the B16F10 to express nectin-1 (B16F10n-1), the major receptor for HSV-1. Engrafted B16F10n-1 tumors were intratumorally treated with either phosphate-buffered saline (PBS) or 1x10^6 pfu VC2. At indicated time points, treated tumors were excised and processed for immunohistochemistry or flow cytometry analysis. For our experimental metastasis studies, mice were intravenously challenged with B16F10n-1 cells. For our depletion studies, CD4+ and CD8+ T cells were depleted in mice by treatment with mouse anti-CD4 and anti-CD8 monoclonal antibodies respectively, while the control mice were given Rat IgG2b isotype. RESULTS/ANTICIPATED RESULTS: We found that VC2 slowed tumor growth rates and significantly enhanced survival times over control treated mice. VC2-treated mice that survived initial tumor engraftment were able to reject a second tumor challenge and were also resistant to lung colonization (experimental metastasis) of tumor cells. Furthermore, VC2 treatment promoted increased intratumoral T cell infiltration and induced a strong antitumor effect that decreased growth rates of distant, untreated tumors. DISCUSSION/SIGNIFICANCE OF FINDINGS: Our data demonstrate that VC2 OVT has significant clinical potential. Furthermore, due to the increased survival rates and CD8+ T cells dependence, our model will enable study of the immunological correlates of protection for VC2 OVT and OVT in general, as well as to inform the rational design of future OVs with improved therapeutic potentials.

47461

Regulation of the immune response in the tumor microenvironment of lung adenocarcinoma

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ABSTRACT IMPACT: This work will provide a rational approach to improve the efficacy of current immunotherapy approaches in patients that have historically responded poorly to immune checkpoint inhibitors. OBJECTIVES/GOALS: Recent evidence of immunogenic cell death as a predictor of response to therapy has increased the interest in monitoring the presence of damage-associated molecular pattern protein (DAMPs). By regulating DAMP expression, our lab is interested in discovering new ways to improve the patient response rate to immune checkpoint inhibition. METHODS/STUDY POPULATION: Using cultured cell, and a limited number of patient tumors and serum (n=4), we measured intracellular and extracellular levels of DAMP molecule, high mobility group box 1 (HMGB1) using enzyme-linked immunosorbent assays and immunoblots. Immunological assayed were compared to the expression of immune checkpoint molecules PD-1/PDL1 on patient tumors as presented in pathology reports. RESULTS/ANTICIPATED RESULTS: HMGB1 release was associated with increased levels of PD-L1 on tumor cells. Targeted inhibition of HMGB1 altered the expression of programmed death-ligand 1 (PD-L1), a target for immune checkpoint inhibition therapy. Patients with higher levels of PD-L1 possessed increased levels of HMGB1 in serum. DISCUSSION/SIGNIFICANCE OF FINDINGS: This implies that regulating the expression of HMGB1 could have an effect on the response of patients to immunotherapy. The main objective of the work is to determine the potential benefit of targeting HMGB1 to improve the efficacy of current therapeutic approaches to treating lung cancer.

48019

Create a mouse model of chronic sleep deprivation by specific-neuron targeted ablation

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ABSTRACT IMPACT: A mouse model of minimally-invasive chronic sleep deprivation is essential for elucidating the impact of sleep deprivation on various health issues, and it would lead to the possibility of sleep as a therapeutic target. OBJECTIVES/GOALS: The lack of sleep has been associated with various health conditions. In mice, sleep deprivation has been achieved mainly by physical disturbances, which raises concern about confounding effects by stresses. Without physical disturbance, targeted neuron ablation can address this methodological flaw. METHODS/STUDY POPULATION: AdultVgat-IRES-cre mice undergo a stereotaxic injection of adeno-associated virus (AAV) vector containing mCherry-dtA to bilateral parafacial zone (PZ) to perform GABAergic neuron-specific cell ablation. Control mice receive an injection of AAV vector containing hSyn-DIO-mCherry. All mice are implanted with electroencephalogram and electromyogram (EEG/EMG) electrodes for sleep-wake analysis. After 7-10 days of the postoperative recovery period, mice are kept individually in a cage for sleep-wake state recording. EEG/EMG and video recording are used to measure total wake time, total sleep time, percent of rapid eye movement (REM) and non-REM sleep, and detailed characterization with spectral analysis. RESULTS/ANTICIPATED RESULTS: We anticipate that the ablation of GABAergic neurons in bilateral PZ decreases the fraction of sleep state in mice, especially non-REM sleep. In the Vgat-IRES-cre mice that received the injection of AAV vector containing mCherry-dtA, total sleep time is expected to be decreased constantly during the 8-week observation period. Sleep-wake staging by video activity recording is anticipated to be closely correlated with the gold standard staging by EEG/EMG. Possible stresses caused by the restriction of physical activity and handling of mice for EEG/EMG recording can be further minimized by the sleep-wake staging performed with the video activity recording. DISCUSSION/SIGNIFICANCE OF FINDINGS: The lack of sleep has been associated with negatively affecting overall health and is implicated in major health conditions including obesity, diabetes, and cardiovascular diseases. This chronic sleep deprivation mouse model can be used to understand the mechanisms of such detrimental effects on health, and would improve many health conditions.

49483

Evaluating the Role of IFNLR1 Receptor Dynamics and Plasticity in Regulating Cellular Response to Interferons

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ABSTRACT IMPACT: We hope to provide a more nuanced understanding of the type-III IFN system, thereby exploring its therapeutic

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potential in the realm of infectious diseases. OBJECTIVES/GOALS: The role of IFNLR1 receptor dynamics and plasticity in regulating the type-III IFN response is largely unknown. As a specific, powerful component of innate immunity, understanding how the type-III IFN system is regulated could lead to the development of novel therapeutic targets and strategies to face a multitude of viral illnesses. METHODS/STUDY POPULATION: To facilitate our investigation, we will generate doxycycline-inducible FLAG-tagged IFNLR1-expression plasmids representing all known transcriptional variants. These plasmids will allow us to: 1) Evaluate the effect of IFNLR1 surface abundance on the type-III IFN transcriptional profile and 2) Assess the extent of IFNLR1-FLAG co-localization with several notable intracellular structures using immunofluorescence, before and after stimulation with IFNL3. RESULTS/ANTICIPATED RESULTS: We have successfully generated three IFNLR1-FLAG transcriptional variants and confirmed inducible-expression and function in vitro. We are currently assessing the role of surface abundance, internalization, differential isoform expression, and trafficking. DISCUSSION/SIGNIFICANCE OF FINDINGS: By completing this study, we hope to provide a more nuanced understanding of the type-III IFN system, thereby exploring its therapeutic potential in the realm of infectious diseases.

55270

The Histone Methyltransferase SETDB2 Regulates Inflammation in Normal and Diabetic Wound Repair

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ABSTRACT IMPACT: Our data reveal a histone modifying enzyme involved in regulating inflammation that may be a novel target for treating non-healing diabetic wounds. OBJECTIVES/GOALS: We investigate molecular mechanisms that regulate the inflammatory phenotype of macrophages in normal and diabetic wound healing. Our goal is to identify novel pathways that may be used to better treat diabetic patients with non-healing wounds. METHODS/STUDY POPULATION: We utilize normal and transgenic murine models on standard chow or high-diet to identify chromatin modifying enzymes involved in regulating macrophage function during wound healing. We validate our murine studies with human blood monocytes or wound macrophages from diabetic patients undergoing limb amputation surgery. RESULTS/ANTICIPATED RESULTS: We have identified the histone methyltransferase SETDB2 as a regulator inflammation in normal and diabetic wound macrophages. We found that SETDB2 was dependent on IFNβ singaling and that both IFNβ and Setdb2 expression were impaired in diabetic wound macrophages. Further, we show that SETDB2 regulates inflammatory response and immune cell trafficking pathways. We also show that SETDB2 genomic localization is dependent on ****NFÎºÎ’ deposition of the promoter. DISCUSSION/SIGNIFICANCE OF FINDINGS: Our results indicate that SETDB2 is a regulator of macrophage plasticity and that SETDB2 expression is impaired in diabetic wound macrophages leading to hyper-inflammatory response and delayed wound healing. These data provide a novel potential therapeutic pathway for treating non-healing diabetic wounds.

56371

The Signaling Axis of Tumor Suppressor LKB1 in Triple Negative Breast Cancer*

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ABSTRACT IMPACT: Identifying an important pathway in treatment resistant TNBC will allow for the future development of clinical therapeutics specific for this disease. OBJECTIVES/GOALS: Triple Negative Breast Cancer (TNBC) is a subtype of breast cancer characterized by negative expression of estrogen receptor, progesterone receptor, and HER2/neu amplification. It resists therapies and has a high recurrence rate after resection. The goal of my research is to identify & characterize a TNBC pathway for future development of therapies. METHODS/STUDY POPULATION: The project uses a combination of cell lines, patient derived xenograft (PDX) models, as well as patient databases. Standard cellular and molecular biology techniques will be used including: Cell culture, qPCR, western blotting, and flow cytometry. RESULTS/ANTICIPATED RESULTS: LKB1 is a master kinase that activates 14 possible downstream kinases. The signaling pathway has been demonstrated to play a role in energy homeostasis and metabolism. Mutation of LKB1 signaling results in Peutz-Jeghers Syndrome and is associated with neoplasias of the lung, pancreas, and breast. Based on preliminary analysis, overexpression of LKB1 by shRNA in TNBC cell lines results in suppression of EMT and reduction of the cancer stem cell population. Additional studies show that LKB1 overexpression has no effect on growth rate in 2D culture while significant reduction in 3D mammosphere formations can be seen. Downstream studies using commercially available SIK1 inhibitor HG-9-91-01 is able to induce a larger fraction of CSC from reduced LKB1 overexpression as well as from baseline levels. DISCUSSION/SIGNIFICANCE OF FINDINGS: Overall, our results suggest that LKB1 acts through SIK1 to suppress EMT and the generation of cancer stem cells. This results in reduced cancer functionality, as evidenced by inhibition of mammosphere formation. These results establishes a foundation for future mechanistic studies on the LKB1 axis and its mechanisms in TNBC.

59821

Brain Mapping Addiction

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ABSTRACT IMPACT: Gaining a better understanding on the role of opioids in opioid use disorder (OUD) can help us find better diagnostics, treatments, and procedures to treat the disorder. OBJECTIVES/GOALS: While we are familiar with brain areas and pathways that are implicated in opioid use disorder (OUD), we do not have a full understanding of the neural circuits activated upon drug exposure. METHODS/STUDY POPULATION: In order to identify areas of the brain most activated by opioids, we ran a pilot study using transgenic cFos-GFP mice that were injected with saline or heroin and examined the brain-wide activity patterns using a quantitative high-resolution mapping method. We observed many brain regions highly activated upon drug exposure. To examine cFos based brain activation in rats,

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