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 assays 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.

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.
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.

**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-fat 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 in inflammation in normal and diabetic wound macrophages. We found that SETDB2 was dependent on IFNβ signaling 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 ****NFκB**** 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.