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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we also ran a pilot study using a tissue clearing and 3D immunolabeling method combined with light sheet microscopy. RESULTS/ANTICIPATED RESULTS: We would expect to see higher cFos activation for brain areas in the reward pathway [including the Nucleus Accumbens (NAc), Ventral Tegmental Area (VTA), Prefrontal Cortex (PFC)] in heroin animals compared to saline animals. We can also expect higher activation in more novel areas like the lateral hypothalamus. DISCUSSION/SIGNIFICANCE OF FINDINGS: If we are able to track OUD effects through imaging in mice and rats, this can help us find better diagnostics, therapeutics, and procedures to treat the disorder. We can also eventually have a human brain atlas that outlines these affected areas as well in order to gain a better understanding on OUD particularly in the human population.

61892

Systemic TLR3-targeting Combinatorial Chemokine Modulation Sensitizes Murine Tumors to PD-1 Blockade

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ABSTRACT IMPACT: This work will lead to improved efficacy of immunotherapy directly impacting the survival of patients with hard to treat cancers. OBJECTIVES/GOALS: Immune checkpoint inhibitors (ICI) are most effective against 'hot' tumors highly infiltrated with cytotoxic T lymphocytes (CTLs) but have not worked well in poorly infiltrated 'cold' tumors. Thus, we are working to achieve a pretreatment regimen that will create a favorable immune profile allowing more effective ?PD-1 therapy. METHODS/STUDY POPULATION: BALB/c or C57BL/6 mice were inoculated with CRC murine cells CT26 or MC38, respectively. Mice were inoculated by two injection types: subcutaneous (SC), for systemic therapy, or intraperitoneal (IP), for local therapy. Tumor-bearing mice were given a two dose course of CKM consisting of IFN-? and rintatolimod via IP injection. Following CKM administration, mice were treated with three doses of ?PD-1 via IP injection. Mice were monitored for the kinetics of tumor growth and survival following treatment. The tumor microenvironment of treated mice was analyzed for production of chemokines, inflammatory cytokines and immune cell infiltration. RESULTS/ANTICIPATED RESULTS: CKM consisting of combination IFN-? and rintatolimod, but neither monotherapy alone, sensitized murine CRC tumors to subsequent ?PD-1 treatment. In both CT26 and MC38 tumor-bearing mice, tumor growth was hindered by CKM plus ?PD-1 treatment, independently on the route of treatment (local or systemic). Mice which experienced complete tumor regression were protected from re-challenge with a dose of tumor cells double that of the initial inoculation. Sensitizing tumors to ?PD-1 did not require intratumoral CKM administration and was observed with systemic application at distant sites. In accordance with these observations we expect that systemic CKM will induce strong increases of total and tumor-specific CTL counts in the tumor tissues as measured by both PCR and flow cytometry. DISCUSSION/SIGNIFICANCE OF FINDINGS: CKM sensitizing cold tumors to ?PD-1 indicates that intratumoral CTLs are an important factor dictating therapeutic effectiveness, independent of other factors such as tumor mutational load. The benefit of the sequential short-term CKM followed by routine ?PD-1 make this strategy feasible for rapid inclusion of into routine immunotherapy plans.

68477

Pancreatic cancer cell extracellular vesicles drive the unfolded protein response in recipient normal pancreatic cells

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ABSTRACT IMPACT: This study advances our understanding of potentially key drivers in the early formation of pancreatic cancer, a disease with few treatment options and poor patient outcomes. OBJECTIVES/GOALS: Patients diagnosed with pancreatic ductal adenocarcinoma (PDAC) have a 5-year survival rate of ~9%. A key driver of poor patient outcomes is late-stage diagnosis. A better understanding of PDAC onset is needed. This study was developed to understand how extracellular vesicles may be involved in the early formation of PDAC. METHODS/STUDY POPULATION: Extracellular vesicles (EVs) were isolated from several human PDAC and normal pancreatic cell lines, using ultracentrifugation with filtration or size exclusion chromatography. We next treated normal pancreatic cell lines with cancer cell EVs (cEVs). Next generation sequencing was used to measure global gene expression changes after treatment. Validations were performed using qPCR and luciferase activity assays. Multi-omics characterization of EVs was accomplished using mass spectrometry based proteomics, metabolomics and lipidomics analysis. RESULTS/ANTICIPATED RESULTS: We found that normal cells upregulated a variety of stress response pathways in response to cEVs. Lipid synthesis was also severely downregulated in these cells. We further validated activation of the unfolded protein response (UPR) in normal cells treated with cEVs. Multi-omics characterization of cEVs identified several enriched proteins, lipids and metabolites which may play a role in the activation of the UPR. DISCUSSION/SIGNIFICANCE OF FINDINGS: Our results indicate that cEVs induce stress, and in particular the UPR, in normal pancreatic cells. Long-term UPR can impact a variety of cancer hallmarks. The UPR can mediate progression of pancreatic intraepithelial neoplasia (PanIN) to PDAC. Our results highlight a potential role for cEVs to alter the function of normal cells, aiding disease onset.

68722

Role of ER calcium in beta cell senescence and diabetes pathophysiology

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ABSTRACT IMPACT: The proposed study has the potential to inform new paradigms of type 1 diabetes prevention and therapy with the overall goal of improving β cell health during autoimmunity. OBJECTIVES/GOALS: Type 1 diabetes (T1D) results from immune-mediated destruction of pancreatic βcells. Recent data suggest that activation of senescence and acquisition of a senescence associated secretory phenotype (SASP) by βcells may contribute to T1D pathogenesis. However, the molecular mechanisms responsible for this phenotype are not well understood. METHODS/STUDY POPULATION: We hypothesize that loss of endoplasmic reticulum (ER) Ca2+ induces βcell senescence, SASP as well as mitochondrial dysfunction which drive T1D development. The current study utilizes SERCA2 KO INS-1 βcells (S2KO) exhibiting loss of ER Ca2+ and a SERCA2 haploinsufficient mice on a non-obese diabetic

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