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

Kathleen M. Kokolus[1], Nataša Obermajer[2], Per Basse[1] and Pawel Kalinski[1,2]
[1]Roswell Park Comprehensive Cancer Center and [2]UPMC Hillman Cancer Center

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

Charles Hinzman[1], Shivani Bansal[1], Yaoxiang Li[1], Jose Trevino[2], Partha Banerjee[1] and Amrita Cheema[1]
[1]Georgetown University Medical Center and [2]University of Florida College of Medicine

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

Staci A. Weaver, Tatsuyoshi Kono, Farooq Syed, Robert Bone and Carmella Evans-Molina
Indiana University School of Medicine

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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