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An investigation of transcription factor PU.1 in renal cell carcinoma tumor-associated macrophages

John Echols, Victoria Ordonez, Kathleen A. Moratto, Arthur W. Meehan, Sunil Sudarshan, Sooryanarayana Varambally and Blake E. Hildreth III

University of Alabama at Birmingham

OBJECTIVES/GOALS: This study evaluates the significance of PU.1 upregulation and the PU.1-mediated regulation of the anti-inflammatory miR-146a on pro-inflammatory NF- κ B signaling in RCC-derived macrophages. The downstream effects of PU.1 targeting on key phenotypes related to RCC immunity and how this impacts RCC progression will also be examined. **METHODS/STUDY POPULATION:** Bioinformatics data identifying PU.1 upregulation within RCC tumors, correlation with prognostic clear cell RCC markers, and survival analysis were obtained using The Cancer Genome Atlas (TCGA) RNA-sequencing data. Murine RCC cell line Renca possessing a Vhl deletion are used to recapitulate a common deletion in human clear cell RCC tumors. This study utilizes two methods to target PU.1 in orthotopic RCC, a conditional PU.1 deletion model and a pharmacologic PU.1 inhibitor model. Quantitative real-time PCR, western blotting, and immunofluorescent staining will evaluate NF- κ B signaling in PU.1-targeted macrophages and murine RCC tumors. Flow cytometry and single-cell RNA-sequencing will reveal the effects of PU.1 targeting on key immune cell phenotypes and key metrics of RCC outcomes. **RESULTS/ANTICIPATED RESULTS:** PU.1 mRNA and protein expression are upregulated in human clear cell RCC tumors compared to normal kidney tissue. A 10-year overall survival analysis in clear cell RCC tumors indicates that increased PU.1 mRNA expression is unfavorable ($p = 0.0298$; HR = 1.24; N = 133/133). PU.1 inhibition in orthotopic RCC reduces tumor volume, compared to control ($p = 0.0328$; N = 5/5). NF- κ B signaling effectors are upregulated in PU.1-inhibited macrophages, including canonical miR-146a targets. We anticipate that PU.1-targeted macrophages and RCC tumors will yield significant increases in NF- κ B signaling, pro-inflammatory macrophage phenotypes, increased antitumor immunity, and reduced tumor volume. **DISCUSSION/SIGNIFICANCE OF IMPACT:** This is the first study to address PU.1 upregulation in RCC and use multiple methods to examine a potential function of PU.1 in immunosuppressive macrophages and RCC immunity. This work also assesses the relevance of miR-146a on canonical, pro-inflammatory NF- κ B signaling in the novel context of RCC tumor immunity.

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Investigating the B-cell response in patients with HPV+ head and neck cancer

Jacob Conarty, Ilham Bahhar and Andreas Wieland
The Ohio State University

OBJECTIVES/GOALS: Intratumoral B-cells are associated with better survival, but their antigenic targets are unclear. Here, I will

delineate B-cell responses in human papillomavirus (HPV)-positive head and neck cancer (HNC) patients against all major HPV E proteins to identify immunodominant responses. **METHODS/STUDY POPULATION:** To profile tumor-infiltrating antibody-secreting cells (ASCs), I will analyze freshly prepared single-cell suspensions of lymphocytes from HPV+ HNC tumors using ELISpot assays. To profile circulating memory B-cells in circulation, peripheral blood mononuclear cells obtained at time of surgery will be differentiated into ASCs, followed by ELISpot analysis. To assess systemic antibody titers, ELISAs will be performed on matched plasma samples. Due to the reliance on fresh tumor samples, our study population consists mostly of stage I HPV+ HNC patients, for which surgical resection is often the primary treatment option. Notably, HPV+ HNC is known to disproportionately affect males. **RESULTS/ANTICIPATED RESULTS:** I will identify the immunodominant targets of the HPV-specific B-cell response in HPV+ HNC. Based on my preliminary data, I expect that the HPV-specific responses will primarily target E1, E2, and E4, as opposed to the classical oncoproteins E6 and E7. I expect the magnitude of the intratumoral HPV-specific ASC response to be reflected in the systemic antibody level, with systemic antibodies, thus serving as viable surrogates for intratumoral HPV-specific responses. Additionally, I expect minimal presence of intratumoral B-cell response against antigens unrelated to the tumor, such as influenza or Epstein-Barr virus, in contrast to abundant systemic antibody titers and memory B-cells. **DISCUSSION/SIGNIFICANCE OF IMPACT:** The majority of current immunotherapeutic treatment options for this malignancy focus solely on targeting E6 and E7. However, if other HPV E proteins are shown to be more immunogenic, targeting these antigens could enable more efficient immunotherapies.

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Associations between baseline anhedonia severity and clinical response to transcranial magnetic stimulation in adolescents with major depressive disorder

Jacquelin Hecker¹, Paul A. Nakonezny², Cicek N. Bakir¹, Irem Azamet³, Magdalena Romanowicz⁴, Julia Shekunov⁴, Jennifer L. Vande Voort⁴, J. Luis Lujan⁵ and Paul E. Croarkin⁴

¹Mayo Clinic Graduate School of Biomedical Sciences; ²Department of Population and Data Sciences, UT Southwestern Medical Center;

³Mayo Clinic School of Graduate Education, Mayo Clinic;

⁴Department of Psychiatry and Psychology, Mayo Clinic and

⁵Departments of Neurologic Surgery, Biomedical Engineering and Physiology, Mayo Clinic

OBJECTIVES/GOALS: Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive neuromodulation therapy FDA-cleared for adjunct treatment of major depressive disorder (MDD) in adolescents. Here, we describe a double-blind, randomized study that explores the use of anhedonia severity as a predictor of treatment response. **METHODS/STUDY POPULATION:** This study included 41 participants (25 females) aged 12–18 years who had a Children's Depressed Rating Scale-Revised (CDRS-R) score