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**BLOCKING SIGLEC-7/9-SIALIC ACID INTERACTIONS INDUCES IMMUNE CELL-MEDIATED SUPPRESSION OF PROSTATE CANCER**

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**Background**

Prostate cancer is among the leading causes of male cancer death worldwide. Although immune checkpoint inhibitors have shown remarkable success in treating various cancers, their effectiveness in prostate cancer remains limited. Hypersialylation has been found in different malignancies and has been associated with tumor suppression through interactions with Siglec receptors in immune cells. The binding of Siglec-7/9 receptors to sialic acids on the surface of cancer cells suppresses the immune response in several cancer types, including melanomas, leukemias, and lung cancers.

**Methods**

Siglec-7/9 Fc chimera protein conjugated with FITC was used to evaluate Siglec-7/9 ligand expression levels by flow cytometry. Siglec-7/9 expression on immune cells was evaluated by flow cytometry. T cell-mediated cytotoxicity was used to evaluate the interactions of Siglec-7/9 and their ligands on prostate cancer cells in a co-culture system. CD59 knockout cells were generated by the CRISPR/cas9 system and validated by western blot. The blocking of Siglec-7/9-sialic acid interactions was investigated in vivo using a humanized mouse model.

**Results**

We observed high expression of Siglec-7/9 in immune cells, including T cells and myeloid cells by analyzing PRAD TCGA RNA expression profiles. Immunohistochemistry and flow cytometry analysis revealed high expression of Siglec-7/9 ligands in surgically resected prostate cancer tumor tissues, contrasting with little to no expression in adjacent normal tissues. Prostate cancer cell lines, particularly PC3 and 22Rv1 cells, displayed high expression of Siglec-7/9 ligands. Cell surface sialic acids were detected in multiple prostate cancer cell lines. Blocking of Siglec-7/9-sialic acid interactions in vitro using sialidase or anti-Siglec-7/9 antibodies promoted T cell-mediated cytotoxic killing of prostate cancer cells. Anti-Siglec-7/9 antibody treatment suppressed PC3 and 22Rv1 tumor growth in a humanized mouse model. Immunohistochemistry analysis of anti-Siglec-7/9-treated prostate xenografts revealed reduced expression of the proliferation marker Ki67, vascularization (CD31), and increased levels of apoptosis (cleaved caspase 3), and infiltrating immune cells (CD4, CD8, and CD68), compared with isotype controls. The CRISPR screen and mass spectrometry identified CD59 as a potential ligand for Siglec-9. Knockout of CD59 decreased Siglec-9-Fc binding activity and enhanced T cell-mediated cytotoxic killing of prostate cancer cells.

**Conclusions**

We demonstrated that blocking Siglec-7/9 and their ligands interactions promotes immune cell-mediated prostate cancer cell death both in vitro and in vivo. CD59 is identified as a potential sialylated ligand for Siglec-9. Targeting Siglec-7/9-sialic acid interactions could represent a novel strategy and lay the ground for the development of immune checkpoint inhibitor-based drugs for prostate cancer.

**Acknowledgements**

This work was supported by a Department of Defense (DoD) Young Investigator Award to RMW. (W81XWH2110195), ChEM-H postdoc seed grant to RMW and JCS, and NCI grants U01CA226051 to SJP, CRB and JDB. We thank the Stanford Shared FACS Facility NIH S10 Shared Instruments (grant no. S10RR025518–01) and Stanford Cell Sciences Imaging Facility (CSIF) and Neuroscience Microscopy Service Core.

**Consent**

Prostate cancer patients’ tissues were obtained from the Stanford Tissue Bank, while blood samples were obtained from the Stanford Blood Bank. All patients’ identities were de-identified for the study. The study received approval from the Institutional Review Board by Stanford University with eProtocol ID 59488. Written informed consent was obtained from all participants.

NSG (NOD/LtSz-SCID IL-2Rnull) mice were procured from the Jackson Laboratory and housed at the Veterinary Service Center of Stanford University. The animals were provided with unrestricted access to food and water throughout the duration of the experiments. The use of animals in the study was in compliance with the guidelines and regulations of the Institutional Animal Care and Use Committee (IACUC) of Stanford University, approved under protocol ID 12844.

http://dx.doi.org/10.1136/jitc-2023-SITC2023.0517