Targeting Siglec-9 immune checkpoint with CD59 as a potential ligand to inhibit prostate cancer progression

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Background:

Prostate cancer is a leading cause of death in men worldwide. Cancer immunotherapy has made tremendous progress in improving patients' quality and quantity of life. However, despite high immune cell infiltration in tumors, immune-checkpoint inhibitor-based therapy is largely ineffective for prostate cancer. Sialic acid-binding immunoglobulin-type lectins (Siglecs), which bind to sialic acids, are expressed in immune cells. Siglec-7 and Siglec-9, expressed in immune cells including dendritic cells and T cells, have been identified as potential immune checkpoints in various cancers. It is suggested that blocking the interactions between Siglec-7/9 and sialic acids can enhance immune responses and effectively inhibit tumor growth in several cancers. Siglec-9 ligands have been detected in prostate cancer tumor tissues. Despite these findings, the understanding of Siglec-7/9-sialic acid interactions in prostate cancer remains limited.

Methods:

Single-cell RNA (sc-RNA) sequencing was used to assess the Siglec-7/9 transcript levels in immune cells within prostate cancer tumor tissues. Flow cytometry was utilized to assess the expression levels of Siglec-7/9 on immune cells and their ligands on prostate cancer cells. T cell-mediated cytotoxicity was evaluated using a coculture system with GFP labeling on prostate cancer cells. Sialidase and anti-Siglec-7/9 antibodies were used to block the interactions between Siglec-7/9 and their ligands. A CRISPRi screen and mass spectrometry were used to identify Siglec-9 ligands. An in vivo humanized mouse model was further used to examine the inhibition of Siglec-7/9 and their ligand interactions. Immunohistochemistry and immunofluorescence were used to assess the expression levels of CD59 and immune cell markers. The CRISPR/Cas9 sgRNA system was employed to generate CD59 knockout prostate cancer cells.

Results:

High expression of Siglec-7 and Siglec-9 was found on myeloid cells, particularly macrophages, in human prostate tumors using sc-RNA sequencing. Elevated levels of Siglec-7 and Siglec-9 ligands were found in prostate cancer tissues compared to adjacent normal tissues. Further, high expression levels of these ligands and sialic acids were observed in prostate cancer cells. Immunofluorescence analysis confirmed the co-expression of Siglec-7 and Siglec-9 in macrophages within human metastatic prostate cancer bone tissues. Disrupting Siglec-7 and Siglec-9 interactions using anti-Siglec-7 and -9 antibodies inhibited PC3 and 22Rv1 tumor growth in a humanized mouse model. Immunohistochemistry analysis showed reduced proliferation, increased apoptosis, and enhanced immune cell infiltration in tumors treated with anti-Siglec-7/9 antibodies. A genome-wide CRISPR screen and mass spectrometry suggested that CD59 is a potential Siglec-9 ligand candidate. The CD59 protein band was observed in prostate cancer cell protein lysates by Siglec-9 Fc pull-down assay. Knocking out CD59 reduced Siglec-9-Fc binding capacity and enhanced T cell-mediated cytotoxicity against prostate cancer cells. The expression of CD59 was detected in tumor cells

through sc-RNA sequencing of metastatic prostate tumor tissues. Immunofluorescence analysis further validated CD59 expression levels in human prostate cancer tumor tissues.

Conclusion:

Disrupting the interactions between Siglec-7/9 and their ligands inhibits prostate cancer progression, and CD59 is a potential ligand for Siglec-9. These findings provide insight into targeting Siglec-7 and Siglec-9 immune checkpoints and pave the way for the development of novel immune checkpoint inhibitor-based drugs for prostate cancer.

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Conflict of Interests

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