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CORRESPONDENCE

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Evasion of immunosurveillance by the upregulation of Siglec15 in bladder cancer

Dingshan Deng[1,2], Jiatong Xiao[1,2], Jinhui Liu[1,2], Huihuang Li[1,2], Minghui Hu[1,2], Bohan Zhou[1,2], Haisu Liang[1,2], Benyi Fan[1,2], Jinbo Chen[1,2*], Xiaogen Kuang[1,3*], Zhenyu Nie[1,2*], Jiao Hu[1,2*] and Xiongbing Zu[1,4,5*]

Abstract

Immunotherapy resistance in bladder cancer (BLCA) is associated with elevated levels of sialic acid–binding immunoglobulin-like lectin (Siglec15). This protein plays a crucial role in fostering a noninflammatory tumor microenvironment (TME), which is conducive to cancer progression. Our study confirmed that the overexpression of Siglec15 led to a reduction in $CD8^+$ T cell infiltration. This effect was mediated by the downregulation of pro-inflammatory cytokines and chemokines, which in turn exacerbated BLCA malignancy. Furthermore, Siglec15 inhibited the cytotoxicity of effector T cell, contributing to immune evasion. An in vivo study demonstrated that Siglec15 overexpression induced a non-inflammatory TME and promoted resistance to immunotherapy. These findings highlight Siglec15 as a potential therapeutic target for BLCA. By modulating inflammation in the TME and $CD8^+$ T cell function, targeting Siglec15 may offer a novel strategy for overcoming immunotherapy resistance and improving patient outcomes.

Keywords Siglec15, Bladder cancer, Immunotherapy, Noninflamed tumor microenvironment

†Jiao Hu is the lead contact among the three co-Corresponding Authors for all communication or inquiries relating to this manuscript.

*Correspondence:
Jinbo Chen
chenjinbo@csu.edu.cn
Xiaogen Kuang
zndxkxg@csu.edu.cn
Zhenyu Nie
22023160@csu.edu.cn
Jiao Hu
hujiao@csu.edu.cn
Xiongbing Zu
zuxbxy@csu.edu.cn
[1] Department of Urology, Xiangya Hospital, Central South University, Changsha, China
[2] National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Central South University, Changsha, China
[3] Department of Urology, The First Affiliated Hospital, Hengyang Medical School, University of South China, University of South China, Hengyang, China
[4] Department of Urology, Hunan Provincial People's Hospital, Changsha, China
[5] Department of Urology, The First Affiliated Hospital of Hunan Normal University, Hunan Normal University, Changsha, China

To editor

Immunotherapy has shown promise in bladder cancer (BLCA) [1, 2], due to its high immunogenicity and tumor mutation burden, however, most patients develop resistance [3–5]. Siglec15, a sialic acid-binding protein [6], promotes tumor growth by inhibiting $CD8^+$ T cells and facilitating immune evasion [7–9]. We previously reported that Siglec15 levels inversely correlate with tumor immune status and predict response to immune checkpoint inhibitors in BLCA [10]. Further validation is required, prompting our investigation into the role of Siglec15 in the BLCA immune microenvironment.

Results and discussion

Siglec15 is overexpressed in BLCA cells, but not in immune or stromal cells within the TME

We evaluated Siglec15 expression in two single-cell cohorts, showing enrichment in BLCA epithelial cells and negligible expression in other TME components

(Supplementary Fig. 1A, B). Multicolor immunofluorescence confirmed Siglec15 colocalization with CK19 in tumors, but not adjacent tissues (Supplementary Fig. 1C). Cancer tissues showed increased $CK19^+$, $Siglec15^+$, and double-positive cells compared to normal tissues, with no significant differences in other immune subsets (Supplementary Fig. 1D). Further analysis confirmed Siglec15 expression in $CK19^+$ tumor cells (Supplementary Fig. 1E). In a tissue microarray of 45 BLCA samples, Siglec15 was positive in 50.37% of cases, with 31.44% of cells both $Siglec15^+$ and $CK19^+$ (Supplementary Fig. 1F), indicating a specific Siglec15 expression pattern in tumor cells.

Siglec15 regulates chemokine expression and lymphocyte migration in BLCA

We overexpressed Siglec15 in human T24 cells and mouse MB49 and MBT2 BLCA cells and knocked down in T24 cells (Supplementary Fig. 2A–D). GO and KEGG analyses revealed disrupted leukocyte migration and immune pathways following Siglec15 modulation (Supplementary Fig. 2E–F). High-throughput liquid-phase protein chip detection showed Siglec15 suppressed cytokine/chemokine secretion by T24 cells, whereas Siglec15 knockdown increased secretion (Fig. 1A). Cytokines levels varied in the supernatants of Siglec15-modulated T24 cells (Supplementary Fig. 2G), with decreasing in Siglec15-overexpressing cells and increasing in knockdown cells at both mRNA and protein levels (Fig. 1B, Supplementary Fig. 2H). These findings were validated in MB49 and MBT2 cells (Supplementary Fig. 3A–F), confirming the role of Siglec15 in modulating cytokine/chemokine secretion in BLCA.

Siglec15 promotes a noninflamed immune microenvironment in BLCA

Transwell chemotaxis assays (Fig. 1C) revealed that Siglec15 overexpression reduced immune cell recruitment (Fig. 1D), while knockdown increased it (Supplementary Fig. 4A). Panoramic scanning (Fig. 1E) correlated $Siglec15^+$ tumors and $CD8^+$ T cell infiltration negatively (Fig. 1F, Supplementary Fig. 4B–C). Siglec15 overexpression hindered $CD8^+$ T cell infiltration, while other immune cells showed different patterns. $CD8^+$ T cell infiltration increased with distance from $Siglec15^+$/$CK19^+$ cells (Supplementary Fig. 4D), highlighting the role of Siglec15 in modulating $CD8^+$ T cell infiltration. These results were validated in three independent cohorts: E-MTAB-4321, GSE48075, and GSE69795 (Supplementary Fig. 5–7).

Siglec15 promotes tumor growth, immune escape and immunotherapy resistance in vitro *and* in vivo

Siglec15 enhanced proliferation, invasion, and migration of BLCA cells whereas knockdown suppressed these processes (Supplementary Fig. 8A–E). Coculture assays (Fig. 1G) showed that Siglec15-knockdown cells were more susceptible to T cell killing (Fig. 1H), whereas Siglec15 overexpression inhibited cytotoxicity of T cell by suppressing TNF-α and IFN-γ (Fig. 1I).

In the allografted BLCA mouse model, Siglec15 overexpression led to larger tumors and immunotherapy resistance (Fig. 1J–N and Supplementary Fig. 8F). Flow cytometry and immunofluorescent staining showed that Siglec15 overexpression suppressed $CD8^+$ T cell infiltration and function, which was partially restored by a PD-1 inhibitor (Fig. 1O–R, Supplementary Fig. 8G).

Siglec15 overexpression correlated with shorter survival time in patients with nasopharyngeal adenocarcinoma and glioblastoma, receiving immunotherapy, and showed a trend in urothelial cancer (Supplementary Fig. 8H–J). These findings highlight the role of Siglec15 in immune evasion and $CD8^+$ T cell suppression in immunotherapy resistance.

Siglec15 levels correlate with a non-inflammatory TME and immunotherapy resistance in BLCA [10]. Siglec15 overexpression inhibits cytokines, hinders $CD8^+$ T cell recruitment and inhibits $CD8^+$ T cells cytotoxicity

(See figure on next page.)

Fig. 1 Evasion of immunosurveillance by the upregulation of Siglec15 in BLCA. **A** High-throughput liquid-phase protein chip detection shows the effects of different Siglec15 expression levels on the concentrations of 34 cytokines. **B** The effects of different Siglec15 expression levels on CCL2, CCL3, CCL4, CCL5, CXCL9, and CXCL10 were determined by reverse transcription—quantitative polymerase chain reaction and enzyme-linked immunosorbent assays. **C** Transwell chemotaxis assay process diagram. **D** Natural killer cell, $CD4^+$ T cell, and $CD8^+$ T cell recruitment decreased with Siglec15 overexpression. **E** Panoramic scanning was used to analyze the co-expression and spatial distance between each cell. **F** Spatial distribution of $CD8^+$ T cells and $Siglec15^+$/$CK19^+$ cells. **G** Schematic diagram of the $CD8^+$ T cell and tumor cell coculture killing assay. **H** Residual tumor cells were stained with crystal violet. **I** Flow cytometry analysis of the expression levels of TNF-α and IFN-γ in $CD8^+$ T cells. **J** Schematic diagram of the in vivo experimental process. **K** Tumor tissue photos at the endpoint of treatment. **L** Tumor growth curves of the mice in the different treatment groups. **M** Tumor volume statistics at the endpoint of treatment. **N** Survival curves of the mice in the different treatment groups. **O** Flow cytometry analysis of the expression levels of TNF-α, GZMB and Ki67 in different groups of tumor tissues. **P** Expression of cytokines in $CD8^+$ T cells within the tumor tissues of different treatment groups. **Q** Immunofluorescence staining revealed different levels of $CD8^+$ T-cell infiltration in the tumor tissues of different treatment groups. **R** Quantitative statistical analysis of the number of $CD8^+$ T cells in **Q**

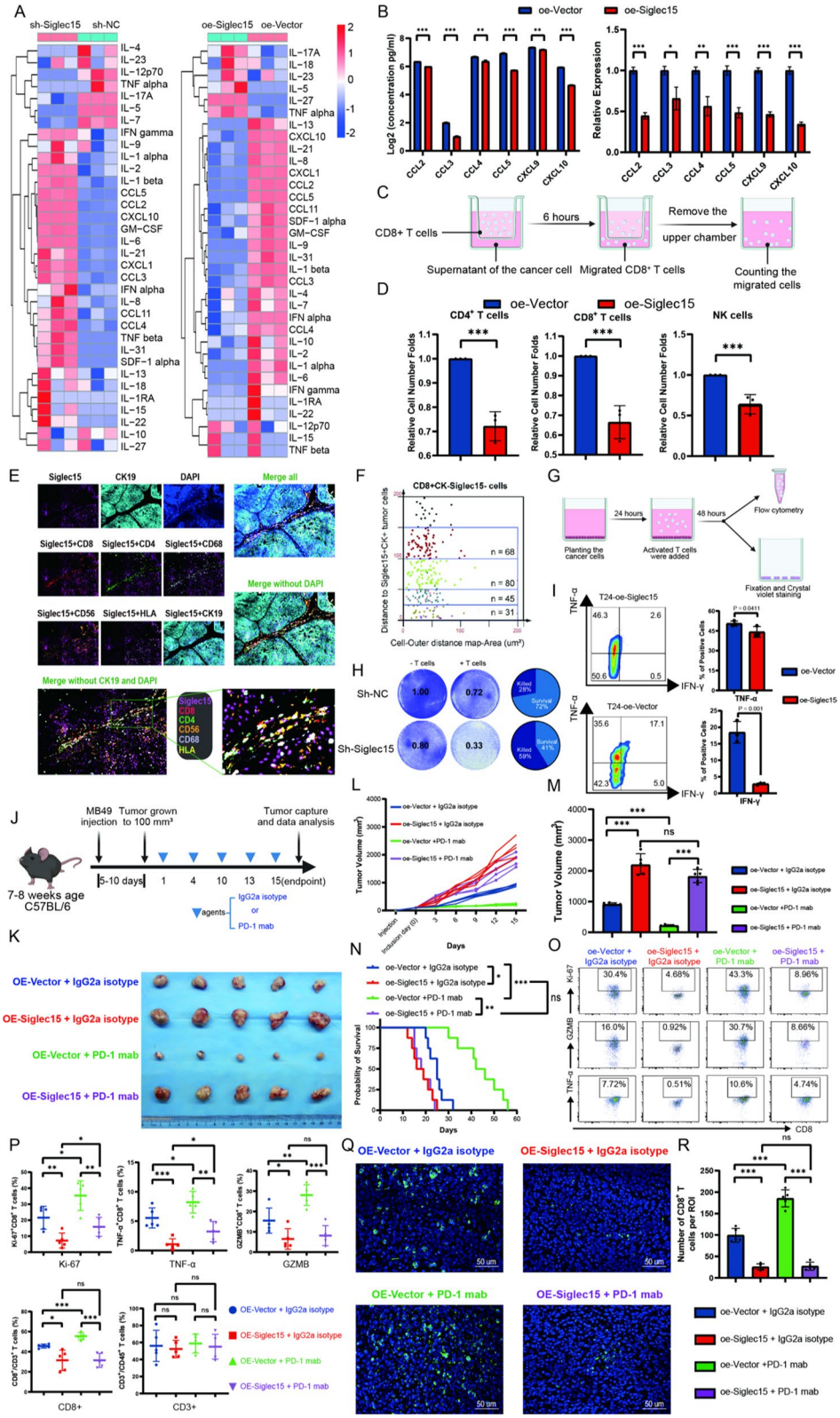

Fig. 1 (See legend on previous page.)

[10–12]. It promotes tumor progression and drives resistance to PD-1 inhibitors. Anti-Siglec15 therapy may enhance $CD8^+$ T cell infiltration and improve immune checkpoint inhibitor efficacy.

Conclusions

This study confirms Siglec15 as a novel immunotherapeutic target for BLCA, inhibiting $CD8^+$ T cell immunity in the TME and promoting immune escape and immunotherapy resistance.

Abbreviations

BLCA	Bladder urothelial carcinoma
DC	Dendritic cell
ELISA	Enzyme linked immunosorbent assay
FC	Fold change
GO	Gene Ontology
ICI	Immune checkpoint inhibitors
IL	Interleukin
KD	Knockdown
KEGG	Kyoto Encyclopedia of Genes and Genomes
NC	Negative control
NK	Nature killer cell
Oe	Overexpressed
PD-L1	Programmed death ligand 1
PD-1	Programmed death 1
Sh	Short hairpin
TME	Tumor microenvironment
Siglec15	Sialic acid binding Ig-like lectin 15

Supplementary Information

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Additional file1 (DOCX 31 KB)

Additional file2 (TIF 38132 KB)

Additional file3 (TIF 46023 KB)

Additional file4 (TIF 34372 KB)

Additional file5 (TIF 36853 KB)

Additional file6 (TIF 37674 KB)

Additional file7 (TIF 35669 KB)

Additional file8 (TIF 36214 KB)

Additional file9 (TIF 38936 KB)

Additional file10 (DOCX 14 KB)

Author contributions

Experiments and data collection were performed by DD, ZN, and JH. Data analysis were carried out by DD, ZN, JH, JC XK JX, JL, H-Li, MH, BZ, HH, BF and H-Liang. The study was designed by JH, ZN, XK and XZ. The manuscript was written and by DD, ZN, and JH. The manuscript was revised by ZN, JH, and JC. All authors revised the previous versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study and all experiments on mice were reviewed and approved by the Ethics Committees (ethical number: 202112241). The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Consent for publication

All data presented in this publication are de-identifed and do not contain individual information.

Competing interests

The authors declare no competing interests.

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