NKG2A AND HLA-E DEFINE A NOVEL ALTERNATIVE IMMUNE CHECKPOINT AXIS IN BLADDER CANCER

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Background Bladder cancer is characterized by a poor prognosis, with muscle-invasive cases harboring a 34–76% 10-year recurrence-free survival rate.¹ Neoadjuvant PD-1/PD-L1 blockade strategies have recently been approved by the US Food and Drug Administration for bladder cancer treatment, yet only achieving a complete response rate of 31–37%, thereby suggesting additional mechanisms of resistance.² HLA-E is a known inhibitor of NKG2A+ CD8 T cells and NK cell responses. A monoclonal antibody binding to the NKG2A receptor has been developed and proven to restore CD8 T cell and NK cell responses in head and neck cancer, with ongoing clinical trials across multiple tumor indications.^{3–4} We evaluated the potential role of the HLA-E/NKG2A inhibitory pathway in modulating T cell immunity in bladder cancer.

Methods CyTOF was performed on CD8+ T cells from fresh bladder tumors (n=6), as well as on expanded CD8+ T cells from bladder-draining lymph nodes (n=11) and tumors (n=8). Flow cytometry (n=25) and single-cell RNA-sequencing (scRNAseq) (n=13) were performed on cells from fresh bladder tumors.

Results Mechanisms of tumor escape from CD8+ T cell recognition include impairment of antigen presentation. Accordingly, we found a significant reduction of HLA class I expression on tumors. However, expression of DNAM-1-activating ligands (e.g. CD112, CD155) on bladder tumors was retained, indicating a possible role for TCR-independent activation pathways traditionally ascribed to natural killer (NK) cells. Using mass cytometry and scRNAseq, we observed that acquisition of NKG2A on tumor-derived PD-1+ CD8+ T cells promotes tissue-resident memory features alongside diminished CD28 expression and significantly weaker sensitivity to CD3/CD28-signaling. However, NKG2A+ CD8 T cells possess a proliferative advantage with enhanced expression of DNAM-1 and cytolytic machinery. Strikingly, we found that NKG2A+PD-1+ CD8 T cells are strongly activated in response to HLA class I-deficient tumors compared to their NKG2A- PD-1+ CD8 T cell counterparts. TCR-independent NK-like function by NKG2A+ CD8 T cell is partly mediated by the DNAM-1 pathway and inhibited by HLA-E. NKG2A+ CD8 T cell functions are restored upon NKG2A blockade, where efficiency positively correlates with HLA-E expression on bladder tumors.

Conclusions Collectively, our data indicate that NKG2A+ CD8 T cells display a strong capacity for TCR-independent activation that enables them to circumvent bladder tumor evasion mechanisms. NKG2A+ CD8 T cells lack expression of CD28 suggesting a lower susceptibility to PD-1-mediated inhibition. Our data suggest a need for thorough reappraisal of current protocols that assess CD8 T cell exhaustion and for strategies to restore their antitumor functions.

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