1003 TIM-3+ NATURAL KILLER CELL DYSFUNCTION IS DRIVEN BY GALECTIN-9 IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

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Background Natural killer (NK) cells are innate effector lymphocytes that play a central role in anti-tumor immunity. Their activation states are regulated by an interplay of activating and inhibitory surface receptors, including T-cell immunoglobulin and mucin-domain containing molecule 3 (TIM-3), an immune checkpoint receptor (ICR) that is expressed on terminally-differentiated NK cells. The role TIM-3 plays in the context of NK cell-mediated immunosurveillance remains evasive, partly because TIM-3 signaling is modulated by four ligands: galectin-9, phosphatidylserine, HMGB1 and CEACAM-1.

Methods Single-cell RNA sequencing and flow cytometry were implemented to study the prevalence, phenotypes and functional differences of TIM-3$^+$ NK cells in head and neck squamous cell carcinoma (HNSCC) patient tumors and blood. *In vitro* killing and proliferation assays were used to evaluate whether the four TIM-3 ligands differentially modulate TIM-3$^+$ NK cell functions, and whether disruption of TIM-3/ligand interaction can enhance NK cell-mediated anti-tumor effector mechanisms. Finally, TCGA survival analysis and digital spatial profiling were employed to study the potential impact of etiology-associated differences on HNSCC patient outcomes.

Results We demonstrate that TIM-3 is the dominant NK cell ICR, that it marks NK cells with enhanced effector potential in blood and tumors, and that galectin-9 is the only TIM-3 ligand that consistently suppresses NK cell cytotoxic and proliferative capacity. Galectin-9-induced effects on cytotoxicity can be abrogated using the clinical-grade anti-TIM-3 blocking antibody, MBG453. Clinically, high intratumoral TIM-3$^+$ NK cell gene signature associates with worse outcome in HPV$^+$, but not HPV$^-$ HNSCC patients. This may be due to higher intratumoral galectin-9 protein expression in HPV$^+$ HNSCC lesions.

Conclusions Our data stress the importance and complexity of TIM-3 in the context of NK cells and suggest that targeting the TIM-3/galectin-9 pathway may be a cogent immunotherapeutic strategy to reinvigorate NK cell effector function in HPV$^+$ HNSCC patients.

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Ethics Approval Peripheral blood and tumor tissues from treatment-naïve HNSCC patients were collected with their written consent in accordance with the Declaration of Helsinki, under the University of Pittsburgh Cancer Institute Review Board–approved protocol (99-069).

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