

**TARGETING THE IMMUNOMODULATORY ROLES OF T-CELL IMMUNOGLOBULIN- AND MUCIN DOMAIN-CONTAINING (TIM)-3 ON NATURAL KILLER CELLS IN GLIOBLASTOMA**

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**Background** Natural killer (NK) cells have emerged as a viable alternative to T cells in adoptive cell transfer for cancer treatment. NK cell activity is driven by the balance between inhibitory and activating receptors, many of which remain elusive. In addition, NK cell metabolism is also a driver of NK cell fitness in tumor settings, where changes in NK metabolic states with the tumor microenvironment *in vivo*, or with stimulants *ex vivo*, further confounds the NK cells' cytotoxic function in cancer settings. One receptor that lies at the intersection between NK cell function and metabolism is TIM-3, with its expression having consequences on NK cell cytokine production and glucose metabolism. However, the contribution of TIM-3 to NK cell anti-tumor immunity is unclear and its role in driving NK cell function so far not fully defined.

**Methods** NK cells were isolated from healthy adult peripheral blood and expanded in feeder-cell media. NK cell metabolism and function were evaluated by different flow cytometric assays to measure glucose uptake, cytotoxicity, degranulation, and cytokine production. TIM-3 knock-out cells were generated using the CRISPR-Cas9 system. Patient samples, including whole blood and tumor, were also processed and phenotyped to compare expression level with healthy donor samples.

**Results** Previously, we discovered that TIM-3 downregulation was associated with decreased cytokine production and target cytotoxicity, and that maintenance of expression above a certain threshold was needed for NK cell function. As cytokine production reflects immune cell metabolic state, we hypothesized that TIM-3 participates in regulation of *ex vivo*-activated NK cell metabolism, which in turn affect the production of the cytokine IFN- $\gamma$  to sensitize cancer targets to NK cell-mediated lysis. Here, we report the consequences of glucose starvation on TIM-3 expression, and how knock-out of TIM-3 on human NK cells affects NK cell metabolism and functionalities against glioblastoma targets. We also cross-reference TIM-3 expression level with glioblastoma patient samples, which provide clinical context for microenvironmental cues and nutrient deprivation.

**Conclusions** Our findings suggest that TIM-3 expression is associated with both *ex vivo*-activated NK cell glucose metabolism and cytotoxic function against glioblastoma. As *ex vivo*-activated NK cells are considered to be highly glycolytic, and as such associated with higher cytotoxicity, TIM-3's involvement with glucose uptake could prove crucial in sustaining NK cytotoxic phenotype in the tumor microenvironment. This information is shedding further light on the immunomodulatory roles of TIM-3, and aiding in leveraging this receptor usage in future NK cell-based immunotherapies.

**Ethics Approval** All procedures performed in studies involving human participants were approved by Purdue University's Institutional Review Board (IRB) in August 2018 (#1804020540). All institutional safety and biosecurity procedures were adhered to.

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