

IDENTIFICATION OF THE ROLE OF ONCOMETABOLITES ON NATURAL KILLER CELL IN THE TUMOR MICROENVIRONMENT BY CRISPR SCREENING

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Background Natural killer (NK) cells are part of innate immunity with high cytolytic activity against tumor cells, along with the ability to secrete cytokines and chemokines. However, solid tumors have been largely resistant to treatment, owing to unfavorable interactions between immune cells and tumors driven by an immunosuppressive tumor microenvironment (TME). The TME of solid tumors is characterized by elevated levels of oncometabolites. However, how their presence affects the activity of NK cells against solid tumors is not known. We have found that sodium succinate, one of the metabolites of the TCA cycle, can alter NK cell behavior in the TME aspects via different mechanisms, including by reducing the cells' ability to produce IFN- γ . To define and understand these mechanisms of oncometabolite-driven NK cell suppression, we performed a lentiviral CRISPR screen in NK and cancer cell systems.

Methods NK cells were isolated from lung cancer patients and healthy adult donors. To study the effect of oncometabolites, NK cells were treated with various concentrations of oncometabolites for 24 hours, then viability of NK cells was measured via the CCK8 assay. Lung adenocarcinoma (A549) cells were then cocultured with oncometabolite-treated NK cells in the absence or presence of oncometabolites to measure the cytotoxicity and IFN- γ production.

Separately, oncometabolite-treated NK cells were used in a co-culture setting to identify drivers of response of cancer cells to NK-mediated killing via a genome-based CRISPR screen. A549 cells were transduced with the CRISPR pooled library and positively selected with puromycin. After co-culture with NK cells, DNA genome was isolated and sequenced.

Results We tested a panel of oncometabolites for the effects on NK cell responses. Our data showed that metabolites negatively impact NK cell effector responses. Interestingly, we found that sodium succinate heterogeneously modulate the killing ability of NK cells and their ability to produce cytokines, suggesting that context- and receptor-specific activation – such as the continued presence of cytokines and metabolites – drives NK cell responses to metabolic factors in the TME. Specifically, among oncometabolites, sodium succinate could drastically decrease IFN- γ levels without affecting the NK cell viability. We also identified genetic markers of response of cancer cells to killing by NK cells in the presence of oncometabolites.

Conclusions Taken together, our data show the potentially significant effects of oncometabolites present in the TME and suggest possible genetic targets that can promote NK cell activation in these contexts.

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