

CRISPR Screening Identifies SLC7A5 as a Therapeutic Target to Enhance NK-Mediated Cytotoxicity in Prostate Cancer

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Background:

Immunotherapy remains largely ineffective in immune-cold solid tumors like prostate cancer. Dysfunction of cytotoxic CD8⁺ T cell responses, including impaired antigen presentation through reduced or aberrant expression of major histocompatibility complex class I (MHC-I), contributes to immune resistance. Natural killer (NK) cells eliminate tumor cells independently of antigen presentation and represent a promising strategy for targeting resistant tumors. In addition, NK cells contribute to responses to immune checkpoint blockade and cellular immunotherapies, including CAR-T therapies. However, many prostate tumors remain resistant to NK-mediated killing, suggesting additional tumor-intrinsic mechanisms of immune evasion.

Methods:

We performed pooled CRISPR knockout screening using a pan-cancer surfaceome library across 12 cell line models from six solid tumor types, co-cultured with NK92 cells under multiple effector-to-target conditions. Candidate genes were prioritized based on enrichment or depletion following NK selection pressure. Top hits were validated using individual knockout models, Incucyte based co-culture killing assays, flow based degranulation assays, in vivo xenograft studies, and mechanistic analyses. Metabolic mass spectrometry and RNA-seq were used to characterize immune-related surface markers and stress-response signaling.

Results:

The screen identified both known and novel regulators of NK sensitivity. Among the top hits, loss of SLC7A5, a key large neutral amino acid transporter, markedly enhanced tumor susceptibility to NK-mediated killing across multiple prostate cancer models. Genetic or pharmacologic inhibition of SLC7A5 reduced tumor growth under NK co-culture conditions. Mechanistically, SLC7A5 inhibition induced amino acid stress and activated the GCN2-eIF2 α -ATF4 integrated stress response, leading to transcriptional reprogramming, upregulation of NKG2D ligands, and enhanced NK-mediated cytotoxicity.

Conclusions:

Our study identifies SLC7A5 as a novel therapeutic vulnerability for enhancing NK sensitivity in prostate cancer. Targeting SLC7A5 using clinical-grade inhibitors may represent a promising strategy to overcome immune resistance and improve NK-based immunotherapy in immune-cold tumors.

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Conflicts of Interest

The authors declare no competing interests.