Background Natural Killer (NK) cells can detect and eliminate virally-infected and malignant cells. As such, major efforts are underway to identify pathways that regulate these protective immune functions and exploit these for novel anti-cancer therapies. NK cells are dependent on IL-15 for their differentiation, survival and function within solid tumors, and negative regulators of this axis represent attractive drug targets. However, functional genomic screening in NK cells has been hampered by their resistance to viral transduction and efficient genome editing.

Methods We developed a CRISPR-based framework for large-scale screening in primary human NK cells and derived lines as well as rapid target validation in vitro and in vivo. Human NK92 cells with Cas9 (KO) or dCas9-VP64 (activation) were transduced with a genome-wide guide library and cultured under low IL-15 or optimal growth conditions and sequenced after an extended culture period for comparison against the initial library. A druggable sub-library was also designed for screening in multiple primary human NK donors under similar limiting IL-15 conditions.

Results Amongst the top enriched hits in human NK cells under limiting IL-15 availability were Cish (cytokine-induced SH2 containing protein) and oNKo-036-040. The genetic knockout of these targets identified within the multiple CRISPR screens led to enhanced NK cell proliferation assessed by EdU uptake and release of key pro-inflammatory cytokines and chemokines. Under in vitro serial killing pressure where tumor targets are replenished at regular intervals, Cish and oNKo-036-040 KO dramatically enhanced primary human NK cell cytotoxicity compared with control AAVS1 KO, which undergo functional exhaustion with repeated challenges. Furthermore, oNKo36-40 deletion alleviated NK cell immunosuppression in the presence of adenosine (NECA), prostaglandin (PGE2) and TGF-beta. oNKo-036-040 deletion further enhanced anti-BCMA CAR-NK cell function in vitro under conditions of serial tumor challenge. Target deletion in murine NK cells recapitulates human data with oNKo-037 KO enhancing IL-15 signalling and supports murine NK cell anti-metastatic function in vivo. Mechanistically, bulk RNAseq data from primary human NK cells modified with AAVS1 or oNKo-037 sgRNA indicates enhanced metabolic profile with target KO necessary for NK cell survival, persistence and effector function.

Conclusions Functional genomic screens in primary human NK cells have revealed potent negative regulators of IL-15 signalling. Ablating IL-15 checkpoints in NK cells results in enhanced IL-15 signalling which translates to improved NK cell fitness and anti-tumor immunity. Cytokine checkpoints are orthogonal to traditional immune checkpoints and warrant drug discovery efforts for mono- and combination cancer immunotherapy trials.

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