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Base Editing Unlocks NK Cell Potential for Cancer Immunotherapy

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Natural killer (NK) cells are critical components of the innate immune system, capable of targeting tumor and virus-infected cells. Their function is regulated by a balance of activating and inhibitory receptors. Among these, NKG2A—encoded by the KLRC1 gene—recognizes the non-classical MHC molecule HLA-E on target cells, delivering inhibitory signals that suppress NK cell cytotoxicity and contribute to immune evasion by tumors. CRISPR/Cas9-mediated disruption of KLRC1 has been explored to enhance NK cell function, but its clinical application is constrained by suboptimal efficiency and risks associated with DNA double-strand breaks (DSBs), including genotoxicity. In this study, we employed adenine base editing (ABE) to achieve precise and safe KLRC1 knockout in primary human NK cells. A panel of guide RNAs (gRNAs) was tested, and one highly effective gRNA/ABE combination was selected for direct comparison with CRISPR/Cas9. ABE editing achieved >95% knockout efficiency at both genomic and protein levels without being dependent on DSBs. In contrast, Cas9 editing with the same gRNA yielded lower efficiency and detectable off-target activity. In contrast, evaluation of specificity using UCAST-Seq confirmed minimal off-target effects in ABE-edited NK cells. Functional assays confirmed complete loss of NKG2A expression and significantly enhanced cytotoxicity against HLA-E–positive tumor cells. These results highlight ABE as a highly efficient and precise platform for KLRC1 knockout in NK cells, offering a safe alternative to conventional gene editing for the development of next-generation NK cell–based immunotherapies.

Preferred type of presentation

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