Immunotherapy

Major Finding: Antigen density and tumor control are reduced after CAR-NK cell therapy by CAR-activated trogocytosis.

Concept: Design of NK cells that express both an activating and an inhibitory CAR prevents CAR-NK fratricide and exhaustion.

Impact: These results suggest targetable mechanisms that can improve antitumor activity of these therapies.

INHIBITORY CARs IMPEDE CAR-NK CELL TROGOCYTOSIS–INDUCED TUMOR ESCAPE

Trogocytosis, the receptor antigen ligation–induced transfer of cell-surface proteins between cells, has been observed in T cells and natural killer (NK) cells, with preclinical studies also demonstrating the occurrence of this process between chimeric antigen receptor (CAR) T cells and tumor cells, where it leads to antigen reduction and tumor relapse. Li and colleagues sought to determine if trogocytosis can also alter the efficacy of CAR-NK cell therapy and showed CAR activation promotes trogocytosis leading to reduced antigen density on tumor cells as well as loss of tumor control by CAR-NK cell therapy. Evaluation of the effects of trogocytosis on the effector functions of CD19-targeting CAR-NK cells revealed their initial increased activity, but this was accompanied by an increased susceptibility to fratricide and lack of a sustained antitumor response. Additional studies revealed that those CAR-NK cells that did not succumb to fratricide and underwent continuous antigen exposure acquired an exhausted phenotype due to antigen-induced self-engagement. Moreover, trogocytic antigen (TROG-antigen) expression reduced the persistence of CAR-NK cells, which was attributable to fratricide and led to development of a functional model following TROG-antigen acquisition on CAR-NK cells that involves activation first, followed by fratricide, functional exhaustion, and eventually tumor control failure. Clinically, a higher probability of relapse was observed in patients with highly expressing TROG-antigen CAR-NK cells, which supports that reduced antitumor efficacy is induced by CAR-mediated trogocytosis. Furthermore, use of a dual CAR system that includes an NK self-recognizing inhibitory CAR along with an activating CAR against a tumor antigen (AI-CAR system) reduced the occurrence of fratricide and improved the antitumor activity of the CAR-NK cells in *in vivo* models of both liquid and solid tumors. In summary, this study shows a mechanism of tumor escape after CAR-NK cell therapy through trogocytosis and suggests that use of the AI-CAR system can improve persistence of these cells as well as their antitumor activity, indicating its useful application moving forward. ■

Li Y, Basar R, Wang G, Liu E, Moyes JS, Li L, et al. KIR-based inhibitory CARs overcome CAR-NK cell trogocytosis-mediated fratricide and tumor escape. Nat Med 2022;28:2133–44.

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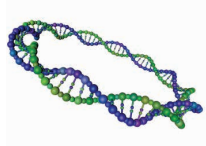
Extrachromosomal DNA

Major Finding: CRISPR-CATCH allows for improved isolation of megabase-sized ecDNA from cancer cells and patient tissue.

Concept: EcDNA genesis, epigenomic landscapes, and amplicon structures can all be evaluated using this method.

Impact: This improved insight into ecDNA structure and diversity can enhance the understanding of ecDNA regulation.

CRISPR-CATCH IMPROVES HUMAN ecDNA PROFILING IN TUMOR TISSUE



Extrachromosomal DNA (ecDNA) frequently contributes to oncogene amplification, with current techniques for ecDNA isolation and targeted profiling having limitations. Hung and colleagues sought to improve upon these previous methods by adapting CRISPR-CATCH (Cas9-assisted targeting of chromosome segments), previously developed for bacterial chromosome segments, to enrich for megabase-sized ecDNA. This single cut technique was found to successfully isolate megabase-sized ecDNA and the corresponding chromosomal locus from the same cancer cell sample as well as from archived patient tissues, with a 30-fold enrichment of target ecDNA being observed, which was demonstrated in *EGFR*, *FGFR2*, *MYC*, and *NRAS* ecDNA. Additionally, the *EGFR* locus in human glioblastoma cells was used to identify structural variants from short-read sequencing data, which revealed predominance of the *EGFRvIII* mutation on ecDNA, while full-length EGFR was seen on the chromosomal locus, suggesting ecDNA can harbor unique genetic alterations. Moreover, single-nucleotide variants were significantly divergent on ecDNA compared to chromosomal DNA, which indicates origination from different parental alleles and together contributes to the evidence in support of an excision model of ecDNA genesis. Examination of the feasibility of analyzing epigenomic profiles using this technique, specifically through investigation of DNA cytosine methylation profiles of the *EGFR* chromosomal locus, demonstrated reduced DNA methylation at regulatory elements on ecDNA, which supports that altered gene regulation can be revealed using CRISPR-CATCH. Furthermore, CRISPR-CATCH was used to directly estimate and reconstruct molecule size and amplicon-phased structural information, establishing the utility of these data to ascertain and provide insight into ecDNA structural and regulatory landscapes. In conclusion, the results of this study show that ecDNA isolated from tumor cells using this CRISPR-CATCH method can be analyzed for both genomic and epigenomic features that will, in turn, allow for greater insight into ecDNA origin, structure, diversity, and regulation in cancer. ■

Hung KL, Luebeck J, Dehkordi SR, Colón CI, Li R, Wong IT, et al. Targeted profiling of human extrachromosomal DNA by CRISPR-CATCH. Nat Genet 2022 Oct 17 [Epub ahead of print].

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