GAPDH knock-in of high-affinity CD16 and mbIL-15 in iPSC-derived NK cells drives high-level expression and increased anti-tumor function

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OBJECTIVE
To evaluate the level of cytotoxicity and persistence against tumor cells using CRISPR-AsCas12a-mediated knock-in of CD16 and membrane-bound interleukin-15 (mbIL-15) in natural killer (NK) cells derived from induced pluripotent stem cells (iPSCs).

RESULTS

1. CD16+/−/mbIL-15− biNK cells had increased CD16 and IL-15Ra expression compared with unedited iNK cells

2. CD16+/−/mbIL-15− biNK cells demonstrated increased ADCC compared with unedited iNK cells in a 2D cell killing assay

3. CD16+/−/mbIL-15− biNK cells elicited greater reductions in tumor spheroid size compared with unedited iNK cells in a 3D tumor spheroid killing assay

METHODS

- iPSCs were edited at the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) locus with an engineered CRISPR-AsCas12a to knock-in CD16 and mbIL-15 using the SLEEK method. iPSC clones were then differentiated into iPSC-derived NK (iNK) cells.
- Flow cytometry was used to detect CD16 and the IL-15 receptor alpha (IL-15Ra) chain.
- A fluorescent activated cell sorter assay using the ovarian cancer cell line SKOV-3 was used to assess 2D cell killing. CD16 shielding was analyzed by extracting iNK cells from the 2D killing assay after 48 hours and running flow cytometry.
- A 3D tumor spheroid killing assay using Incucyte® imaging of NucLight® Red-tagged SKOV-3 cells was used to assess NK cell cytotoxicity.
- In vitro persistence was measured by culturing unedited and edited iNK cells in basal media without supporting cytokines for 21 days.

CONCLUSIONS

- CRISPR-AsCas12a-mediated SLEEK knock-in of CD16 and mbIL-15 at the GAPDH locus in iNK cells increased expression of CD16 and mbIL-15 on the surface of iNK cells.
- CD16+/−/mbIL-15− iNK cells demonstrated enhanced cytotoxicity (due to increased and maintained CD16 expression) and increased persistence (due to mbIL-15 expression) versus unedited iNK cells.

REFERENCES


DISCLOSURES


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