HARNESSING GENETICALLY ENGINEERED FEEDER CELL LINE FOR EX VIVO EXPANSION OF HUMAN NATURAL KILLER CELLS WITH INCREASED PRODUCTION OF IFN-γ

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Objective

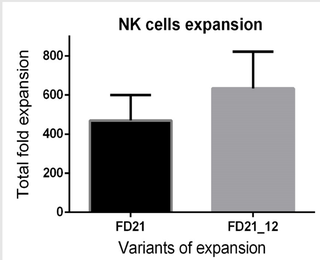
Natural killer (NK) cells belong to the group of innate immune cells. Numerous clinical studies are currently underway on the use of NK cells in anticancer immunotherapy. Interferon-γ (IFN-γ) is a cytokine that has antitumor activity, and can be effectively used in immunotherapy of oncological diseases. Various cell types are responsible for the production of IFN-γ, including activated NK cells. However, the level of IFN-γ production by NK cells can be altered by various cytokines. We have previously shown that the use of a K562-mbIL21-41BBL feeder line (further FD21) leads to a significant expansion and activation of NK cells.

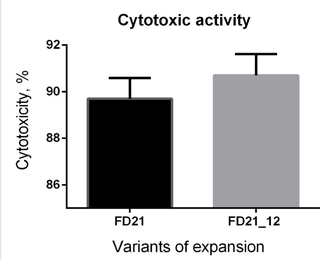
Thus, the aim of the study is to obtain genetically engineered K562-mbIL21-mbIL12-41BBL (further FD21_12) feeder cell line based on FD21 with the expression of a membrane-bound recombinant variant of human IL-12 for the expansion of natural killer cells with increased production of IFN-γ and antitumor activity.

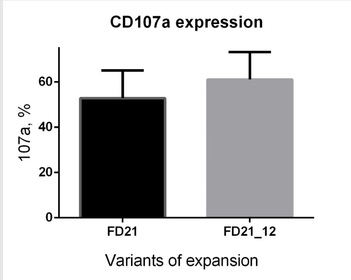
Methods

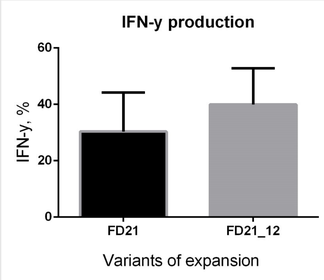
Whole blood of 5 healthy donors was used in the work. Mononuclear cells were isolated from the donor's peripheral blood on a density gradient by centrifugation and the number of isolated cells was counted.

The expansion of NK cells was performed by culturing peripheral blood mononuclear cells of donors in the presence of irradiated (100 Gy) feeder cells and IL-2 (50 IU/ml) in complete RPMI-1640 medium for 12 days in G-Rex 24-well plate. On the 12th day, the number of NK cells were determined and the level of their activation, as well as the number of NK cells, producing IFN-γ, expression CD107a and cytotoxic activity. Two variants of feeder cell lines were tested. First – FD21; the second – FD21_12.









Results

Previously received line FD21 was transduced with lentiviral particles to obtain stably modified variants expressing the recombinant cytokine IL-12. Expression of the introduced transgenes was confirmed at the mRNA level by quantitative PCR and at the level of protein products by flow cytometry.

- The level of expansion of NK cells through 12 days of cultivation, when using the obtained feeder lines, was 392 (193-843) and 528 (234-1227) for FD21 and FD21_12 lines.
- Cytotoxic activity in a ratio 10:1 against for K562 cells for the first line was 89.7% (87.7 – 91.6 %) and the second line 90.7% (82.2 – 91.1%).
- The percentage of CD107a+ cells was 64.3 % (52.5 – 72.4 %), 71.6 % (56.0 – 84.6 %), accordingly.
- The number of cells producing IFN-γ - 20.6% (9.3-62.2%), 31.7% (29-63.1%), accordingly.

Key words

Natural Killer cells, Interferon-γ, feeder cell line, cytokines, antitumor activity.

Conclusions

As a result of the work, a new feeder cell line FD21_12 was obtained, which makes it possible to obtain a NK cell with high level of expansion, cytotoxic activity, IFN-γ production and expression CD107a.