56ASM-0135 | Molecular design of novel NKG2D chimeric antigen receptor and its ligands

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Background: Despite the success of chimeric antigen receptor (CAR)-T therapy in hematological malignancies, for solid tumors results remain limited. For CAR-T therapy searching for universal but highly specific tumor antigen is the key to effectiveness and safety. A promising candidate is a pair of NKG2D receptor and its ligands (MICA, MICB, ULBP2-6) as NKG2D is expressed on NK-cells and some populations of CD8+ T-cells, providing recognition and elimination of cancer cells, and NKG2DLs gets dramatically increased due to cancer transformation or treatment. In this study, we performed the design of NKG2D-CAR and NKG2DLs, followed by the demonstration of high expression of NKG2DLs using in vitro cell-based models.

Materials and Methods: Lentiviral plasmid vector pLVT was used for CAR. The following constructs were cloned into this vector: protein-coding sequence of NKG2D fused to CD3z-signaling domain, protein-coding sequence of Dap10 adapter protein, and sequence of suicide cassette based on truncated EGFR. For NKG2DLs constructs were based on third-generation lentiviral plasmid vector pUltra-hot plasmid #24130 (Addgene) and mCherry reporter protein was replaced with selective blasticidin resistance gene by molecular cloning. The validity of the resulting vectors was verified by restriction. Obtained vectors were used for lentiviral transduction of target cells (primary T-cells for NKG2D-CAR and HeLa for NKG2DLs) with confirmation of expression by flow cytometry (NKG2D-CAR) and qPCR (HeLa).

Results: We have successfully obtained a plasmid vector encoding NKG2D-CAR and generated NKG2D-CAR-T-cells (19,58% transduction efficacy). We also designed a series of NKG2DLs plasmids and generated a modified HeLa cell line overexpressing NKG2DLs: MICA (200x higher than control), ULBP2 (200x higher than control), ULBP6 (77000x higher than control).

Conclusions: Further experiments will evaluate the effectiveness of these NKG2D-CAR-T-cells against tumor cells overexpressing NKG2D ligands.

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56ASM-0137 | Functional assessment of cytotoxic activity of NKG2D CAR-T cells

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Background: NKG2D is an activating transmembrane receptor presented on the surface of NK or T cells. NKG2D CAR recognizes not only tumor cells, but also immunosuppressive cells such as myeloid suppressor cells and regulatory T cells, as well as endothelial cells in the tumor microenvironment. NKG2D CAR-T cells eradicate tumor cells and produce numerous cytokines. The aim of this study was to study the cytotoxic activity of CAR-T cells in real-time dynamics using RTCA methodology, as well as to assess the release of interferon-gamma during co-cultivation of tumor cells and CAR-T cells.

Materials and Methods: The cytotoxic activity of CAR-T cells was assessed in real time during co-cultivation with the HeLa line using the RTCA xCelligence (ACEA Biosciences). The evaluation of cytotoxic effect of CAR-T cells was carried out within 72 hours of co-cultivation at a ratio of E:T = 1:1. Effector CAR-T cells were seeded after 24 hours of target tumor cell culture. The functional cytotoxic activity of CAR-T cells was also assessed using ELISA (Vector-Best) to identify the concentration of interferon-gamma in co-cultivation supernatant.

Results: The results revealed that NKG2D CAR-T cells possess a significant cytotoxic effect against HeLa cells overexpressing NKG2D ligands. According to ELISA, co-cultivation of NKG2D CAR-T effectors with HeLa cells leads to increased levels of interferon-gamma. However, these levels are lower than for anti-CD19 CAR-T cells co-cultivated with HeLaCD19+.

Conclusions: ELISA results are in good agreement with RTCA data and indicate a significant NKG2D CAR-T-mediated antitumor cytotoxic effect.

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