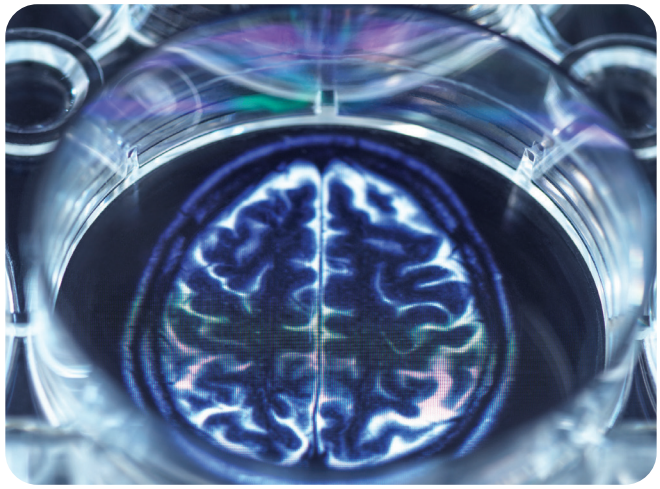
PUBLICATION REVIEW

Multifunctional mRNA-based CAR T-Cells show promising anti-tumor activity against glioblastoma

Glioblastoma is the most common type of malignant brain tumor in adults. It is usually very aggressive, with less than 10% of patients expected to survive five years following diagnosis. The standard of care for patients with glioblastoma is surgery followed by radiation and chemotherapy. Unfortunately, this treatment strategy is rarely curative, emphasizing the urgent need to discover more effective therapies for this type of cancer.

Chimeric Antigen Receptor (CAR) T cell therapies have delivered promising clinical responses in patients with hematological cancers, leading researchers to explore the application of this strategy to solid cancers. However, the anti-tumor activity of CAR T-cells in patients with glioblastoma has so far been modest. This is due to several factors, including the impact of the tumor immunosuppressive microenvironment on CAR T-cell activity, the challenge of identifying tumor-specific antigens due to tumor heterogeneity, and the limited persistence of CAR T-cell therapies.

To overcome these challenges, multi-targeting or tandem CARs hold potential; yet many of these approaches use vector-based systems which have limitations such as limited transgene capacity, lengthy manufacturing processes, and the risk for off-tumor toxicities. Researchers are addressing these limitations by exploring mRNA-based CAR-T strategies, which offer a safe, rapid, and cost-effective alternative to vector-based systems.



Summary

The present study evaluates the efficiency of mRNA-based multifunctional CAR T-cells against glioblastoma *in vitro* and *in vivo*. By using high-content screening and fluorescence molecular tomography in glioblastoma mouse models, the researchers found that samples with T cell co-expressing the CAR with the pro-inflammatory cytokines IL12 and IFNα2 had the highest number of active T cell and were most effective. The study provides a strong rationale for future clinical studies using mRNA-based multifunctional CAR T-cells to treat malignant brain tumors.

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Assessing the anti-tumor activity of multifunctional mRNA-based CAR T-Cells

In a recent study, Meister et al. developed multi-targeting mRNA-based CAR T-cells that demonstrated promising anti-glioma activity *in vitro* and *in vivo*.[1] The researchers co-expressed a CAR based on the natural killer group 2D (NKG2D) receptor with the pro-inflammatory cytokines IL12 and IFNα2. They say their findings provide a strong rationale for future clinical studies using mRNA-based multifunctional CAR T-cells to treat malignant brain tumors.

Methods and results

The researchers first assessed the anti-tumor activity of the multifunctional mRNA-based CAR T-cells in two murine glioma cell lines, GL-261 or CT-2A. Murine T cells were electroporated with mRNAs to express either NKG2D CAR, two pro-inflammatory cytokines (mIL12 and mIFNα2), or all three transgenes together. Overall, co-expression of the CAR and cytokines led to the highest level of cytolytic activity in both cell lines.

Next, Meister and colleagues determined the anti-tumor activity of the mRNA-based CAR T-cells in immunocompetent orthotopic glioma-bearing mouse models following intravenous (IV) and intratumoral administration. The team used fluorescence molecular tomography (FMT) imaging to demonstrate that the modified T cells accumulated in the tumors following IV administration. Notably, IV injection of the multifunctional CAR T-cells co-expressing mIL12 and mIFNα2 conferred a survival benefit compared to injection of T cells expressing the CAR or cytokines alone. Intratumoral administration further improved the therapeutic efficacy of NKG2D CAR T-cells co-expressing mIL12 and mIFNα2, with 84% of GL-261 glioma-bearing mice surviving after receiving two intratumoral injections.

To determine the translational potential of their approach the researchers generated PBMC-derived mRNA-modified T cells and used high-content imaging to assess their anti-tumor activity in glioblastoma patient samples. Patient tissue samples were dissociated, seeded into clear-bottom PhenoPlate™ 384-well microplates, and co-cultured with control or mRNA-modified T cells for 24 hours. The cell mixtures contained glioma cells along with cells from the tumor microenvironment. Cells were fixed, blocked, and stained with BioLegend® antibodies before being imaged using an Opera Phenix® high-content screening system to determine the anti-tumor activity of the engineered T cells at the single-cell level.

Image analysis revealed that the greatest reduction of cancer cells was induced by mRNA-based NKG2D

CAR T-cells co-expressing hIL12 and hIFNα2. In contrast, T cells that expressed either the CAR or the pro-inflammatory cytokines did not consistently reduce the number of glioma cells. To determine whether specific T-cell phenotypes were associated with increased anti-glioma activity, the researchers assessed T cell activation through deep learning-based morphological profiling. In six out of 10 patients, T cells that co-expressed the CAR and cytokines had the highest number of activated T cells. Of these, four were significantly higher than the NKG2D CAR T-cells alone, suggesting higher activation levels.

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Conclusion

CAR T-cell therapy has revolutionized the treatment of certain hematological cancers, but this success has yet to be translated to glioblastoma. Factors limiting their efficacy in solid cancers include tumor heterogeneity, the immunosuppressive microenvironment, and CAR T-cell persistence. mRNA-based T cell engineering is a promising approach to overcome these challenges and facilitate the clinical translation of innovative CAR-T strategies. In the present study, Meister et al. demonstrated the preclinical efficacy of multifunctional mRNA-based NKG2D CAR T cell co-expressing two pro-inflammatory cytokines and translated this strategy to glioblastoma patient samples. The team notes that the limitation of the mRNA-based T-cell modifications is the transient nature, which is advantageous in the case of off-tumor toxicities but would require repeated administrations. However, they conclude that their approach could be a promising treatment strategy for patients with glioblastoma.

Reference

1. Meister H, Look T, Roth P, Pascolo S, Sahin U, Lee S, *et al.* Multifunctional mRNA-based CAR T-cell display promising antitumor activity against glioblastoma. Clinical Cancer Research. 2022 Nov 1;28(21):4747–56.

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