CAR-T Cell Therapy in the Treatment of Glioblastoma

Luke Isham, Jeeban Bhattarai, Yan Li

FSU UNDERGRADUATE RESEARCH OPPORTUNITY PROGRAM
CENTER FOR UNDERGRADUATE RESEARCH & ACADEMIC ENGAGEMENT

FSU | FLORIDA STATE UNIVERSITY

FAMU-FSU College of Engineering

Introduction

Glioblastoma multiforme (GBM) is an aggressive and deadly form of brain cancer characterized by rapid proliferation and extensive infiltration into surrounding neural tissue. These features make GBM particularly difficult to treat, and existing therapeutic strategies have shown limited long-term effectiveness.

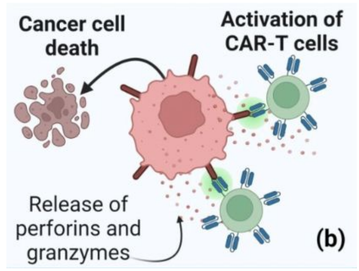
Targeted cellular approaches have emerged as a potential strategy to better understand and modulate glioblastoma behavior. Chimeric antigen receptors (CARs) represent one such approach, enabling engineered cells to interact more specifically with tumor-associated signals. In this study, human stem cell–derived neural tissues were engineered under controlled culture conditions to examine interactions between CAR-expressing neural environments and extracellular vesicles (EVs) derived from glioblastoma cells.

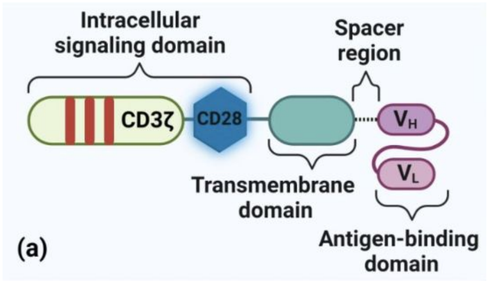
Extracellular vesicles play a significant role in tumor–microenvironment communication and may influence tumor progression within neural tissue. By utilizing engineered neural models and controlled EV exposure, this study aims to investigate how CAR expression within neural tissue affects glioblastoma-associated signaling dynamics.

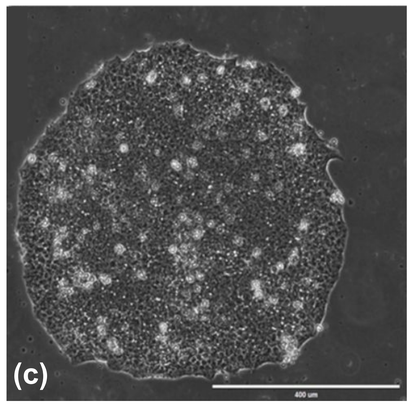
Methods

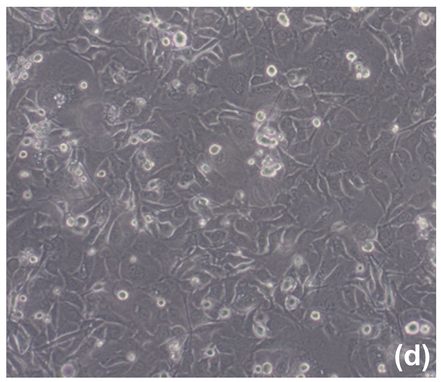
Human pluripotent stem cells (hPSCs) were first genetically engineered using CRISPR-Cas9 to insert a chimeric antigen receptor into a safe genomic locus. These CAR-modified hPSCs were then differentiated under chemically defined conditions through hematopoietic and myeloid progenitor stages to generate mature neutrophils.

Following differentiation, culture media from CAR-expressing neutrophils was collected and processed via ultracentrifugation to isolate neutrophil-derived extracellular vesicles . The resulting CAR-expressing EVs were subsequently applied to an in vitro glioblastoma model to evaluate CAR-mediated antigen recognition and downstream effects on tumor cell behavior, including apoptosis and inhibition of mitosis.









a) CAR-T cell signaling pathway (“Research on” 2024).

b) CAR-T cell activation and effect on glioblastoma cells (“Research on” 2024).

c) Culture of hPSCs (Krasnova et al., 2022).

d) Glioblastoma cell culture.

Conclusion/Results

The findings demonstrate that EVs released from CAR-engineered neutrophils contain a diverse set of biomolecules with established anti-cancer functions, including proteins and microRNAs involved in apoptosis, inflammatory signaling, tumor suppression, and regulation of cell proliferation. These results indicate that CAR expression influences EV cargo composition as neutrophils mature.

CAR-neutrophil–derived EVs were efficiently internalized by glioblastoma cells in both two-dimensional and three-dimensional in vitro models and exhibited consistent cytotoxic activity across both systems. These functional outcomes align with proteomic and genomic analyses, supporting the biological relevance of the EV cargo identified.

Future work will focus on quantifying the anti-proliferative effects of CAR-neutrophil EVs and validating their impact on key apoptotic, inflammatory, and cell-cycle pathways using targeted molecular analyses. Collectively, these results support the potential of CAR-derived neutrophil EVs as a novel and scalable therapeutic platform for glioblastoma.

Acknowledgements

I would like to express my sincere gratitude to Dr. Yan Li for providing the opportunity to conduct this research within her laboratory. Special thanks to Jeeban Bhattarai for his dedicated technical guidance, patience, and support in mastering the experimental protocols and data analysis used in this study.

References