Manipulation of MAP3K Signaling to Improve CAR-T Cell Therapy

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Introduction

- Chimeric Antigen Receptor (CAR)-T cell therapy has made remarkable strides in the treatment of patients with B-cell malignancies and certain hematological cancers.
- Although CAR-T cells confer tumor antigen specificity, success with solid tumors has been limited, partly due to the metabolically hostile tumor microenvironment (TME).1
- Mitogen Activated Protein (MAP) kinase kinase (MAP3K) is a crucial triple kinase that mediates CAR-T cell efficacy and is also a promising target for improving the effectiveness of CAR-T cell therapy in solid tumors.2
- MAP3K is a highly labile protein because its degradation is ubiquitin-dependent. This, however, can be prevented by the deletion of its N-terminal region.3 Due to MAP3K’s essential role in CAR-T cell therapy, there is a clear rationale for further investigating its mechanistic role as well as developing methods to increase its stability.
- This study aims to construct a CAR-T cell that expresses a stable form of MAP3K lacking its N-terminal region (MAP3KΔN) with the hope of further improving the antitumor efficacy of CAR-T cell therapy in solid tumors. We hypothesize that MAP3KΔN expression will promote CAR-T cell activation and metabolic fitness in the TME of solid tumors.

Methods and Experimental Design

1. Construct anti-human CD19 CAR expressing MAP3KΔN through molecular cloning

2. Test expression by transient expression (transfection) in HEK293T cells and by infection (transduction) in OTI CD8+ T cells

3. Generate mouse CAR-T cells by infecting CD8+ T cells with anti-hCD19 CAR-MAP3KΔN and examine tumor rejection using B16F10-hCD19 melanoma mouse models

Results

- Restriction Enzyme Digestion of hCD19-MAP3KΔN-CAR

- Immunoblotting Analysis of HA tag in hCD19-MAP3KΔN-CAR

- GFP Expression after OTI Mouse CD8+ T Cells Transduction

- MAP3KΔN Shows Similar Response to Control CAR, but MAP3KWT Dramatically Reduces Tumor Burden

Discussion and Conclusion

- We have found evidence to reject our hypothesis that MAP3KΔN will promote CAR-T cell efficacy due to avoidance of degradation. However, MAP3KWT leads to a notable decrease in tumor growth in vivo consistent with our previous observations. Several points remain to be addressed to define cause-effect relationships and we propose further directions to enhance this promising immunotherapy.
- We have concluded that expression of MAP3KWT, but not MAP3KΔN, profoundly improves the antitumor function of CAR-T cells.

Future directions

- Perform flow cytometric analysis of the tumor microenvironment after injection of MAP3KΔN-CAR-T, MAP3KWT-CAR-T and control CAR-T.
- Analyze for potential cytokine storm or organ inflammation.
- Develop an inducible MAP3K expression system to manipulate the metabolic activity and function of CAR-T cells based on temporary MAP3KWT expression.
- Apply MAP3KWT expression to tumor models beyond melanoma to test its universality.

Acknowledgements

This project was funded by the RP170067 CPRIT Research Training Grant. I want to thank Drs. Meidi Gu and Xiaofei Zhou for their excellent and patient mentorship and guidance throughout the project. I also want to thank Dr. Shao-Cong Sun for his remarkable advice and enthusiasm throughout my time conducting this study.

References