Engineering CAR-T Cells to Combat Obesity: From Tumor Microenvironments to Metabolic Dysfunction

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Introduction

Obesity affects over 650 million adults globally and is a major risk factor for cardiovascular disease and type 2 diabetes.
Chimeric antigen receptor T-cell (CAR-T) therapy has transformed cancer treatment, especially for blood cancers, but struggles with solid tumors due to T-cell exhaustion and immunosuppressive environments.
The similarities between ***tumor-associated macrophages*** and ***adipose tissue macrophages (ATMs)*** in fostering inflammatory environments suggest that similar therapies could be effective for metabolic diseases. Both obese adipose tissue and tumor environments exhibit chronic inflammation, immune cell infiltration, and immunosuppressive factors.

Methodology

Development of Tumor Microenvironment Models:
- Generated Vascular Immune Organoids (VIOs) from induced pluripotent stem cells through optimized hematopoietic differentiation protocols
- Co-cultured VIOs with patient-derived lung adenocarcinoma organoids to create CIVA assembloids
- Characterized tissue architecture using immunofluorescence microscopy and histological analysis

CAR-T Cell Engineering and Tracking:
- Employed Larry barcode technology for individual CAR-T cell clone tracking over time
- Transduced primary human T cells with lentiviral vectors carrying unique DNA barcodes alongside CAR constructs
- Validated CAR expression using flow cytometry and optimized transduction protocols

Multi-omics Analysis Pipeline:
- Performed single-cell RNA sequencing at multiple time points (days 3, 6, and 12) using 10x Genomics platform
- Conducted spatial transcriptomics using VisiumHD platform to understand spatial organization of inflammatory niches
- Applied computational analysis for cell type annotation and trajectory analysis

Conclusions

This research demonstrates successful cross-disciplinary application of cancer immunotherapy insights to metabolic disease therapy. The ***development of CIVA organoids*** and i***dentification of IL-1β inflammatory niches*** as drivers of immune dysfunction provide actionable targets for combination therapies.
Key achievements include:
- Validation that mechanistic understanding in cancer can accelerate progress in metabolic diseases
- Establishment of scientific foundation for advancing obesity CAR-T therapy toward clinical testing
- Development of precision medicine platform using patient-specific organoid testing

The potential global health impact of successful obesity CAR-T therapy could be enormous, given worldwide metabolic disease prevalence and limited current treatment efficacy. This work establishes principles for expanding immunotherapy applications beyond traditional oncology contexts.

Objectives

Primary Research Question: How can understanding CAR-T cell exhaustion mechanisms in complex inflammatory microenvironments guide the development of CAR-T therapies targeting adipose tissue dysfunction?
Specific Objectives:
- Develop sophisticated model systems to study CAR-T cell behavior in inflammatory microenvironments
- Characterize factors leading to CAR-T cell exhaustion using Cancer Immunized Vascularized Assembloids (CIVA)
- Identify therapeutic targets for obesity-directed CAR-T therapy
- Establish translational framework for applying cancer immunotherapy insights to metabolic diseases

Key Results

Characterization of IL-1β+ Inflammatory Niches:
- Identified distinct IL-1β+ macrophage populations within CIVA assembloids expressing inflammatory markers (IL1B, CCL2, PTGES2)
- Spatial transcriptomics revealed IL-1β+ macrophages co-localized with immunosuppressive factors (PD-L1, IL-6)
- Validated clinical relevance through correlation with patient tumor samples from ALK-rearranged non-small cell lung cancer

CAR-T Cell Exhaustion Dynamics:
- Progressive T cell exhaustion observed with hierarchy of induction: T cells alone (LAG-3: ~20%) < VIO co-culture (~30%) < CIVA assembloids (~45%)
- Larry barcode tracing revealed heterogeneous clonal responses, with some clones showing extensive expansion before exhaustion
- Exhaustion marker upregulation began as early as day 3, with PD-1 showing earlier upregulation compared to LAG-3

Mechanistic Insights:
- Identified IL-1β-PGE2 axis as key mechanism driving CAR-T cell exhaustion
- IL-1β+ macrophages highly expressed PTGES2 (PGE2 synthesis enzyme), creating local immunosuppressive environments
- • IL-1β signaling induced differentiation of inflammatory cancer-associated fibroblasts (iCAFs) forming physical barriers around tumor regions

Discussion & Future Directions

Significance:
- systematic application of ***cancer immunotherapy*** insights to metabolic disease, opening new field of investigation
- CIVA assembloids provide broadly ***applicable*** platform for studying immune-microenvironment interactions

Limitations:
- In vitro model systems ***cannot fully recapitulate*** in vivo complexity including systemic immune responses
- Short timeframe limits assessment of ***long-term*** CAR-T persistence and memory formation