Chapter 1

Introduction

1.1 Background

A novel kind of immunotherapy called chimeric antigen receptor (CAR) T-cell therapy genetically modifies a patient's T-cells so that they express CARs, artificial receptors made to identify particular antigens in cancerous cells (Sadelain et al., 2014). There are now several approved CAR T-cell therapies for patients with regressed lymphoma, and a majority of those treatments focus on targeting the CD19 antigen (Turtle et al., 2016; Vairy et al., 2018). While CAR T-cell therapy has proven to be successful in treating specific blood-based cancers, there have been cases of disease relapse after initial treatment due to a loss of target antigens (Xie et al., 2022).

With the exploration of different types of multiple antigen-expressing CARs, the coexpression of multiple proteins is an important factor. This can be done by incorporating a P2A self-cleaving site, which enables "ribosomal skipping" (Yang et al., 2017). In addition, P2A peptides are compact and smaller in size, which helps preserve the proteins' functionality and integrity (Shibuta et al., 2019). Typically, the insertion of P2A peptide sites allows for high expression levels of both target proteins, while also reducing the development of uncleaved fusion proteins due to its high cleavage efficiency. These properties of the P2A cleavage sites are favorable in various applications of gene therapy (Liu et al., 2017).

Khaniya et al. (2024) previously investigated promoter-driven CAR constructs involving P2A peptide sites. The researchers found that the unidirectional dual CAR resulted in a lower second gene expression (CD19 CAR) compared to the single-directional dual CAR tested. From these results it can be hypothesized that the lack of gene expression was caused by insufficient P2A cleavage. Notably, only the second gene was considerably lower in expression, as the first P2A-linked gene showed a

relatively high expression. Other studies have also observed inefficient cleavage of P2A constructs; however, it is still inconclusive as to whether or not P2A specifically causes these lowered gene expressions (Amendola et al., 2005; Sun et al., 2017). To explore further, the creation of multiple constructs involving P2A sites is required. Moreover, it is also necessary to infer a trend by analyzing the gene expression levels of the proteins of interest and reporter genes that P2A separates.

1.2 Objective

This study aims to determine whether the splitting peptide (P2A) interferes with the second gene expression in multiple antigen-expressing CAR T-cells through specific objectives as follows:

1. Create different CAR constructs involving P2A sites by switching the positions of GFP and CD19-CAR
2. Observe CAR T-cells expansion, viability, and efficiency through cell counting and reading expression levels by flow cytometry
3. Carry out a cytotoxic assay to establish CAR T-cells functionality

1.3 Hypothesis

This study hypothesizes that the inclusion of P2A self-cleaving peptide in multiple antigen-expressing CAR T-cells will cause a decrease in the second gene expression.