Abstract

The development of multi-directional promoter-driven chimeric antigen receptors (CARs) has great potential to enhance the effectiveness of antigen-targeting CAR-T therapies. In order to express two proteins or genes simultaneously, a P2A peptide site is added between the target proteins. However, research has shown that an inserted P2A site between two genes seemingly leads to a lower gene expression of the second gene. Therefore, it is worth investigating the P2A site further by observing whether P2A can interfere with the second gene expression. Genetic cloning, ligation, and transformation methods allow for the generation of specific sequences involving P2A sites. These constructs can then be transfected into human T-cells, which will determine the expression profile of P2A-linked proteins and genes. The CAR efficiency assessment shows a decreased second gene expression for both P2A-invovled constructs, which aligns with the initial hypothesis. Moreover, by conducting a cytotoxicity assay through co-culturing CAR T-cells with $CD19^{+}$ Nalm-6 cells, it was also observed that the configuration of the CAR constructs did not affect the overall cytotoxicity of the CAR T-cells. However, this project design did not allow for the study on the effects on other types of genes in combination with P2A in CAR construct design, and therefore, future exploration on more complex combinations of genes could be beneficial to understanding the underlying mechanisms of P2A.

Keywords: P2A, CAR T-cell, gene expression