75 METHODS TO IMPROVE CO-EXPRESSION EFFICACY OF GENES MODULATING PROMOTER AND LINKER

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Background Adoptive T cell therapy is an important modality of cancer immunotherapy. Engineering of various genes associated with improved T cell function, such as viability, sustainability, migration potential, and target specificity is important. Therefore, we need to identify effective methods for multigene transfer to T cells. The selection of appropriate promoters or linkers in the production of virus vectors is critical for efficacy of multigene expression.

Methods In order to optimize vectors that can increase the expression efficiency of multigenes, we intended to compare the expression amount of each gene with different promoters and linkers. The expression of each gene was compared when 1G4 TCR (alpha and beta chains are linked by furin-SGSG-P2A) and CCR10 were linked using IRES, T2A, or furin-SGSG-T2A. Three cloning vectors were produced using one EF1a, one EFS, or two EFS to express 1G4 and CCR10 genes simultaneously with T2A. Transfection was performed on Lenti-x 293T and transduction was performed in Jurkat cells to measure the difference in expression of the 1G4 with mouse TCR constant antibody and CCR10 protein by flow cytometry. Finally, 1G4 and CCR10 dual expressing cells were sorted and cultured to see the persistence of expression on the day of culture, day 6, and day 12.

Results Since CCR10 was not expressed with the vector using IRES, T2A was selected. In addition, differences in protein expression between T2A and furin-SGSG-T2A were not observed. Using two EFS promotors, 1G4 and CCR10 were expressed at 4.3% and 33.4%, respectively, so they were not studied further. When using one EFS promotor, 1G4 was expressed at 97.6%, but CCR10 (downstream of T2A) was expressed significantly lower at 26.1% on the transduction day. When using the EF1a, the expression of 1G4 was decreased to 74.2%, but CCR10 expression was increased to 37.4%. After sorting of dual expressing cells, the expression of 1G4 and CCR10 with the EFS decreased from 100% and 86.1% (day 0) to 89.5% and 2.66%, respectively (day 12). However, with the EF1a, the expression of 1G4 was almost the same from 99.2% (day 0) to 99.7% (day 12), and CCR10 expression decreased slightly from 94.6% (day 0) to 73.5% (day 12).

Conclusions T2A showed better gene expression efficiency for downstream gene than IRES. EF1a was better than EFS and it was associated durable transduction efficacy. Comparison of linkers and promoters are necessary to improve multigene transfer to T cells for adoptive T cell therapy.

http://dx.doi.org/10.1136/jitc-2022-SITC2022.0075