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Fas-Reporter Cells As A Tool For TCR Antigen Identification

Takahiko Miyama is a postdoctoral fellow in the Department of Genomic Medicine at MD Anderson Cancer Center. He earned his M.D. from Hiroshima University, Japan in 2006 and his Ph.D. in 2017. After many years of working as a hematologist specializing in hematopoietic cell transplantation, he has made researches that aim to elucidate the mechanism by which specific T-cell clonotypes are selected for effective immune responses against virus, tumor, and alloantigens. Recently, he reported that highly shared TCR chronotypes against cytomegalovirus prevailed in the T cell repertoire. Takahiko is currently working on the development of a TCR antigen screening platform.

Abstract: [Background] Recent advances in high-throughput sequencing technology have led to understanding of the landscape of T cell receptor (TCR) repertoire against life-threatening viral infections and malignant diseases. However, the identification of antigens for orphan TCRs remains a challenge. The purpose of this study is to establish a cell-based reporter system for screening TCR antigens. We developed reporter cells by engineering antigen-presenting cells (APCs) to express Fas-tumor necrosis factor receptor 2 (TNFR2) chimeric receptor that can be specifically activated by antigen-specific T cells. [Method] We constructed a chimeric receptor composed of the extracellular domain of Fas receptor and the cytoplasmic domain of TNFR2. 293T cells which are HLA-A2 positive were used as APCs and lentivirally transduced with the chimeric receptor gene followed by NF-kB-GFP reporter gene. To test antigen-specific activity, HLA-A2 restricted epitopes derived from human papillomavirus 16 (HPV16) and cytomegalovirus pp65 (CMV pp65) were chosen as model antigens and expressed on APCs by peptide or gene transfer. Cell-based reporter assays were performed by co-culturing reporter APCs with CD8 positive Jurkat cells expressing cognate TCR. Reporter activity was assessed by measuring GFP expression levels using flow cytometry. [Result] Treatment of reporter cells with anti-Fas monoclonal antibody increased GFP expression levels, verifying that NF-kB activation was induced by the extracellular Fas signal via the chimeric receptor. Both HPV16 and CMV pp65 antigen-specific Jurkat cells specifically activated cognate antigen-presenting reporter

cells with particularly high GFP expression in the antigen-mediated aggregate fraction. Antigen-specific activation was also achieved in different antigen-expressing cell mixtures, indicating cognate reporter cells were distinguishable from non-cognate cells by antigen-reactive T cells. [Conclusion] Our results indicate Fas-TNFR2 chimeric receptor can medicate activation of reporter cells by antigen-specific T cells. This technology may be applied to screening TCR antigens using peptide libraries as well as cDNA libraries from target cells.