Comprehensive analysis of TCR function using a novel system reveals the multiple unconventional tumor-reactive T cells in breast cancer-infiltrating lymphocytes

T細胞受容体の網羅的機能解析法の開発と乳癌腫瘍浸潤リンパ球中の非典型的T細胞の発見

Background: T cell receptor (TCR)-T cell therapy is drawing attention as a promising next-generation immunological cancer therapy. To achieve a significant therapeutic effect in TCR-T cell therapy, highly functional tumor-specific TCRs are prerequisite. Tumor-infiltrating lymphocytes (TILs) are a potent source for obtaining tumor-reactive TCRs. Although comprehensive methods to analyze the TCR repertoire in TILs have been reported, tumor-reactivity of TCRs in TILs has not been comprehensively analyzed. The function of only five to ten TCR clones from fifty to thousands of obtained TCRs have been conventionally analyzed to obtain tumor-reactive TCRs. This is because examination of dual TCRα- or β-expression in a single T cell and their functional combination, preparation of TCR-expression vectors and their assessment of DNA sequences is extremely complicated and laborious. The aim of this study is to develop the method for comprehensive analysis of the tumor-reactivity of TCRs derived from TILs.

Methods: We developed a novel, efficient, and comprehensive evaluation system for TCR-function and designated it c-FIT (comprehensive functional investigation of TCRs). In the c-FIT system, we prepare retroviral TCR-expression vectors without cloning and sequencing. In detail, we insert cDNAs of TCRβ and α into retrovirus vector using homologous recombination and connect TCRβ, TCRα fragments, and the blasticidin S-resistant gene (Bsr) with a nucleotide sequence encoding self-cleaving 2A peptides. We named the vector TCR-2A-Bsr. The resultant plasmids contained not only functional TCR-expression vectors but also unfunctional ones. We transfect the mixture of functional and unfunctional TCR-expression vectors into a BW5147.3 reporter T cell line, which exhibits strong sensitivity to stimuli from antigen presenting tumor

cells. We then culture the transfectants in the presence of blasticidin S to enrich the transfectants with functional TCR-expression vectors, in which TCRβ and TCRα fragments are connected to Bsr in frame. We then coculture TCR-transduced BW5147.3 reporter cells with target cells and assess the reactivity of TCRs to the target cells by analyzing IL-2-secretion with ELISA. When analyzing the cytotoxicity-inducibility of TCRs, we transfect TCR-expression vector into peripheral blood T cells and coculture them with target cells expressing luciferase, whose activity corresponds to live cell number.

Results: To assess the c-FIT system using clinical samples, we single cell-sorted PD-1^+ $CD137^+$ $CD8^+$ TILs of two breast cancer patients, analyzed the TCR repertoire and amplified the clones of TCRβ and TCRα cDNA. We then prepared the bulk TCR-2A-Bsr vector for each TIL clone, retrovirally transferred the vectors into BW5147.3 reporter cells expressing human CD8αβ and cultured them in the presence of blasticidin S. As target cells, we used breast cancer cell lines (MCF7) in which the patient's HLA molecules were exogenously expressed. We analyzed 70 TCRs obtained from $CD8^+$ TILs of two breast cancer patients and found 23 TCRs that reacted to a breast tumor cell line. Surprisingly, two TCRs were patient's HLA class I-restricted and conventional TCRs, while the remaining 21 TCRs were non-HLA-restricted unconventional TCRs. Representative two obtained TCRs responded and killed MCF7 cells when expressed on human peripheral blood T cells.

Conclusions: We established a novel method, c-FIT, that is suitable for comprehensive assays for the function of TCRs. c-FIT does not require cloning and verification of TCR-expression vectors or the troublesome dual expression of TCRα or β in a single T cell. Furthermore, the c-FIT protocol uses BW5147.3 reporter cells, the most sensitive reporter mouse T cell line, for the functional assay. c-FIT system enables us to analyze up to 90 TCRs for their reactivity to tumor cells by a single assay within a month. c-FIT can be applied for monitoring multiple conventional and unconventional antigen-specific killer T cells in TILs, leading to the development of new designs for more effective T-cell-based immunotherapies.