Concurrent Identification of True Neoantigens and Neoantigen-Specific T Cells by SCRNA-seq and SCTCR-seq

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Background Next-generation sequencing has revolutionized the identification of specific gene alterations in patients, which has enabled the prediction of mutation-associated neoantigens (neoAgs) that are unique to each individual. However, the recognition of these candidate neoAgs by patients’ T cells is limited, with only a small fraction capable of eliciting T cell responses. Additionally, a significant proportion of tumor-infiltrating lymphocytes (TILs) within tumors are bystanders that do not target tumor antigens. These bystander CD8+ TILs exhibit diverse phenotypes that overlap with tumor-specific cells, making it challenging to identify ‘true neoAgs’ and neoAg-specific T cells. To overcome these hurdles, we have developed a method for the concurrent identification of genuine neoAgs and neoAg-specific T cell receptors (TCRs) using genetically-engineered TCR-T reporter cells.

Methods To determine the HLA types of the patients and estimate the binding affinity of mutated peptides to their corresponding MHC class I molecules, we utilized WES and RNA-Seq data, along with MHCflurry and netMHCpan4.0. Based on high MHC binding affinity and gene expression levels, candidate neoAgs were selected. T cell assays were performed using synthesized mutated peptides. Tumor tissues were enzymatically digested, generating a single-cell suspension that was cryopreserved as Fresh Tumor Digest (FTD). Simultaneously, tissue fragments were cultured in an IL-2-rich medium to obtain cultured TILs (cTIL). CD8+ T cells were isolated from FTD samples, designated as ex vivo TIL. We conducted scTCR-seq and scRNA-seq on ex vivo TILs and cTILs. Candidate neoAg-specific TCRs, including expanded clones and exhausted clones, were selected. The TCR α and β genes of these clones were subsequently cloned into a retrovirus vector. These TCR genes were genetically introduced into reporter cells lacking endogenous TCR. To assess their reactivity, candidate neoAg peptides were incubated with autologous B cell antigen-presenting cells from patients, along with co-cultured TCR-T reporter cells.

Results We identified TCRs reactive to neoAg in patients with lung cancer and myxofibrosarcoma. TCRs detected from lung cancer patients were exclusively found in FTD samples, indicating an exhausted state and low frequencies of neoAg-specific T cells. Conversely, TCRs detected from myxofibrosarcoma patients were derived from various phenotypes of expanded clones. The combination of scTCR-seq with scRNA-seq will enhance the accuracy of predicting neoAg-specific TCRs.

Conclusions We have successfully established a methodology to simultaneously identify true neoAgs and their corresponding TCRs. These neoAg-specific TCRs hold potential for application in TCR-T cell therapy.

Ethics Approval This study was approved by the University of Tokyo’s Ethics Board; approval number G10129.

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