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Single-cell Approach to Generate Functional TCR-Ts: A Potential Accelerator of TCR-T Cell Therapy for Infectious Diseases

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Abstract

T cell-mediated immune response is essential for host defense against viruses, including the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which has caused a global pandemic. Genetically engineered T cells with antigen-specific T cell receptors (TCR-Ts) are redirected to eliminate target cells via TCRs recognizing peptides bound with major histocompatibility complexes (MHCs). TCR-T cell therapy has proved effective in human trials involving solid tumors, while it is considered promising treatment for infectious diseases. To expedite the identification of functional TCRs and development of TCR-T cell therapy, we established an experimental pipeline to generate functional virus-specific TCR-Ts through coupled analysis of single-cell transcriptomic data and single-cell full length TCR V(D)J sequences. We validated this approach in selecting functional TCRs specific to a cytomegalovirus (CMV) pp65 epitope, $NLV_{495-503}$, and published the results recently. In this commentary, we summarized the single-cell work flow and discussed its potential application in developing TCR-T cell therapy against SARS-CoV-2.

Keywords: TCR-T, Single-cell RNA sequencing, Single-cell TCR V(D)J sequencing, SARS-CoV-2

Introduction

Acute or chronic infectious diseases caused by human transmissible viruses (such as cytomegalovirus (CMV), severe acute respiratory syndrome coronavirus (SARS-CoV), middle east respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2 causing the ongoing COVID-19 pandemic) have posed tremendous threats to human health [1-4]. During viral infection, immune systems are activated to defend against viruses, including the innate immune responses, which respond rapidly as the first line of defense, and followed by the adaptive immune responses, which are composed of humoral immunity and cell-mediated immunity. In humoral immune responses, antibody-secreting effector B cells secrete neutralizing antibodies, which bind to viruses by the antigenic domain and mainly interfere with the process that virus particles entering into the host cells [5,6]. Besides, cell-mediated immune responses are also crucial to both acute and chronic viral infection. Both cytolytic activity against viral infected cells and production of immune inflammatory factors by $CD4^+/CD8^+$ T cells contribute to the clearance of viral infection. However, it was found that functional exhaustion of $CD4^+/CD8^+$ T cells help viruses to escape

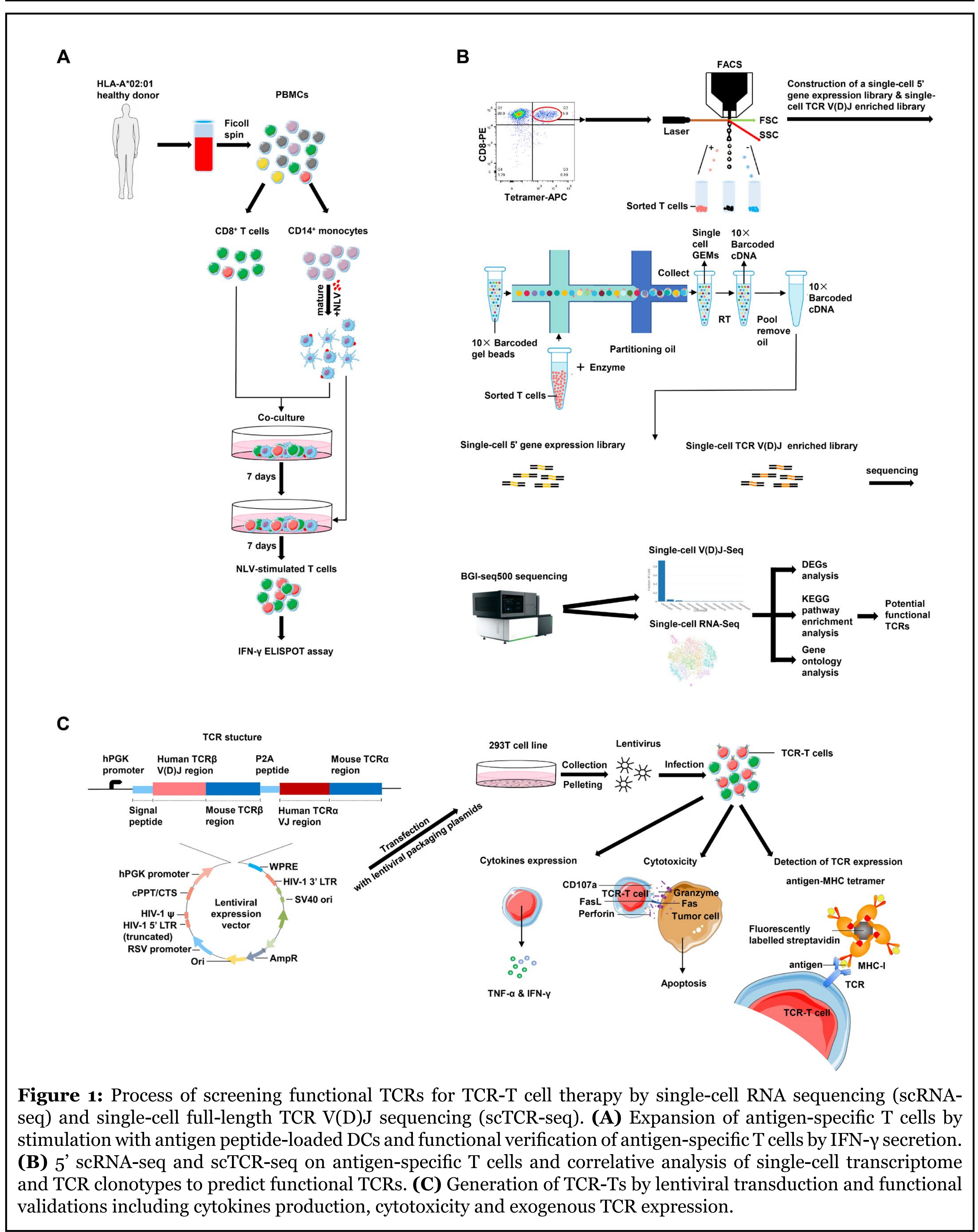

Figure 1: Process of screening functional TCRs for TCR-T cell therapy by single-cell RNA sequencing (scRNA-seq) and single-cell full-length TCR V(D)J sequencing (scTCR-seq). **(A)** Expansion of antigen-specific T cells by stimulation with antigen peptide-loaded DCs and functional verification of antigen-specific T cells by IFN-γ secretion. **(B)** 5' scRNA-seq and scTCR-seq on antigen-specific T cells and correlative analysis of single-cell transcriptome and TCR clonotypes to predict functional TCRs. **(C)** Generation of TCR-Ts by lentiviral transduction and functional validations including cytokines production, cytotoxicity and exogenous TCR expression.

from T cell responses in chronic and acute viral infection [7,8]. As a consequence, effective and specific tools targeting to viruses are needed to strengthen our antivirus capabilities.

Immunotherapies, including adoptive T cell therapy, have emerged to the front line of treatment for cancers, and they are also feasible and promising as treatment options for infectious diseases [9,10]. T cell receptor engineered T cells (TCR-Ts) are T cells transferred with exogenous antigen-specific TCRs, which are redirected to recognize target cells via TCR-CD3 cluster binding to the peptide-MHC. After recognition, downstream signaling pathways are activated to release cytokines and granzymes (such as IFN-γ, TNF-α, granzyme A and granzyme B) to execute cytotoxicity to target cells and activate other immune cells [11]. In essence, TCR-T cell therapy harnesses our immune system to control viral duplication and attack infected cells or tumors in patients.

Single-cell Approach to Generate Functional TCR-Ts

A major technical problem in the development of TCR-T cell therapy comes from accurate identification of functional TCRs with paired α and β chains [12]. Advances of next-generation sequencing technology enables high-throughput parallel sequencing of millions of DNA fragments, however, sequencing from bulk materials usually lost the information on the pairing of TCR α and β chains. Single-cell full-length TCR sequencing, along with single-cell RNA sequencing opens up a new path to map the TCR repertoire with paired α and β sequences [13,14], which can be achieved through several technologies and platforms, such as microdroplet-based microfluidics technology [15], Smart-seq2 technology [16], nested PCR [17], VDJ Puzzle [18], and Single-cell TCR seq [19]. Besides the TCR repertoire profiling, the correlated analysis of the single-cell transcriptome and TCR information allows for functional TCR selection based on transcription profiles, which have not been fully explored yet.

Our recent work performed the single-cell transcriptome and single-cell TCR analysis in selecting functional TCRs specific to CMV immunodominant epitope $NLV_{495-503}$, and established a comprehensive pipeline to generate functional TCR-Ts [20]. Most individuals infected by CMV usually remain asymptomatic due to their robust immune response [21]. However, CMV reactivation occurs frequently in immunocompromised patients or immunocompetent patients with critical illness [22,23]. Reconstruction of a functional CMV-specific T cell repertoire has been shown to effectively control CMV-related diseases [9,24,25]. In our research, $NLV_{495-503}$-specific T cells were preferentially expanded *in vitro* by co-culture and stimulation of primary $CD8^+$ T cells from a healthy donor with $NLV_{495-503}$ peptide-loaded autologous dendritic cells (DCs) (Figure 1A). The specific reactivity to the antigen was measured by the IFN-γ enzyme-linked immunospot (ELISPOT) assay. $NLV_{495-503}$-specific $CD8^+$ T cells were then stained by the fluorescently labeled $NLV_{495-503}$-MHC tetramer and sorted (Figure 1B). Single cells were isolated by microdroplet-based microfluidics, followed by construction of the single-cell 5' gene expression library and the single-cell TCR V(D)J enriched library, and sequencing on a BGI-seq 500 platform. Single-cell RNA sequencing of T cells allowed grouping of cells with similar transcriptomic features into clusters by unsupervised clustering. Meanwhile, paired, full-length TCR sequences were obtained from single-cell TCR sequencing and TCR clonotypes were mapped onto the clusters according to the transcriptomic data. As a result, 2 clusters with distinct transcriptomic features were defined, and further bioinformatic analysis suggested that cluster 1 were potentially functional T cells with high expression of cytotoxic effector molecules (*GZMH* and *NKG7*), proinflammatory cytokine *IFNG*, exhaustion marker *TIGIT* and 40 highly-expressed functional genes involved in cytokine receptor–ligand interactions as well as lymphocyte activation, while cluster 2 had features of naïve populations with high expression of naïve cell-related markers (*CD27* and *LEF1*). Representative TCR clonotypes were selected from both clusters and corresponding TCR-Ts were generated from primary T cells infected with lentiviral vectors carrying TCR expression cassettes (Figure 1C). *In vitro* assays were carried out to verify TCR expression, cytokine production and cytotoxic activity of TCR-Ts. In accordance with bioinformatic predictions, the TCR from cluster 1 was highly effective in functional assays and preliminarily potential for TCR-T cell therapy, while the TCR from cluster 2 was nonfunctional. Thus, we provided a single-cell approach to select functional TCRs, which is promising to be used in effective screening of antigen-specific TCRs and valid construction of functional TCR-Ts.

T cell Immune Response to SARS-CoV-2

The pandemic of COVID-19, caused by a novel coronavirus, SARS-CoV-2, has led to a human health crisis globally. As of July 22, 2020, there have been more than 14 million people infected, including over 612 thousand deaths reported to WHO throughout the world (data from WHO, https://covid19.who.int/). Understanding the host immune response and immunopathology of COVID-19 is critical for the proper use of clinical care, interpretation of clinical outcomes, development of effective therapies, and calibration of prevention measures. With rapidly emerging research work on this matter, we are now getting more profound knowledge of the innate and adaptive immune responses involved in COVID-19 [26].

A growing body of evidence suggested that apart from the humoral immune response, T cell-mediated immune response plays essential roles in the defense against SARS-CoV-2. A study of non-severe COVID-19 reported that similar to antibody secreting cells, the frequency of activated CD38$^+$HLA-DR$^+$ T cells in the patient's blood increased rapidly before the symptoms resolved, and remained higher than healthy controls days during convalescence, which demonstrated the induction of adaptive immune response by viral infection [27]. Characterization of bronchoalveolar lavage fluid (BALF) immune cells from COVID-19 patients by single-cell RNA sequencing revealed that a larger proportion of CD8$^+$ T cells were present in BALFs from patients with moderate disease compared to patients with severe COVID-19 [28]. Furthermore, CD8$^+$ T cells in moderate cases contained much more highly expanded TCR clones, indicating that they might function in controlling the disease progression. A longitudinal study also observed expanded or contracted T cell clones in PBMCs from two patients with mild COVID-19 and suggested that these clones were associated with the infection, most of which developed memory phenotypes by day 30 after the symptom onset [29].

T cells reactive to the components of SARS-CoV-2, especially the spike protein epitopes have been identified in PBMCs from COVID-19 patients [30-32]. Peptide megapools were generated for stimulation of CD8$^+$ or CD4$^+$ T cells from the patients, corresponding to HLA class I and HLA class II epitopes predicted by bioinformatic tools covering the entire viral proteome [31]. T cell activation markers, including CD137, CD69, and OX40, were analyzed by flow cytometry to identify specific T-cell response after stimulation. 100% of the convalescent COVID-19 patients displayed CD4$^+$ T cells reactive to the peptide megapools while the majority of them had specific response in CD8$^+$ T cells, albeit the frequency of reactive T cells was low, generally below 1% of the CD4$^+$ or CD8$^+$ population. The frequency of spike-reactive CD4$^+$ T cells was corelated with the spike-specific antibody titer in COVID-19 patients, in line with the functional role of CD4$^+$ T cells in promoting B cell maturation and antibody production [33]. Surprisingly, CD4$^+$ and CD8$^+$ T cells from a portion of healthy donors showed cross-reactivity to the predicted SARS-CoV-2 epitopes, which might result from their previous contact with other human coronaviruses [30,31]. Whether this pre-existing cross-reactive T cells helped in protecting from SARS-CoV-2 infection remains to be validated in a larger number of subjects.

Potential of TCR-T cell Therapy against SARS-CoV-2

Great efforts have been made to develop therapeutics and vaccines against SARS-CoV-2. For example, neutralizing antibodies targeting the spike protein on the viral surface have shown strong efficacies in prevention of viral infection *in vitro* and in mouse models, making them promising drug candidates [34,35]. As for vaccine approaches, studies showed that vaccines induced protective immunity including potent neutralizing antibodies and CD8$^+$ T cell responses, providing efficient protection against SARS-CoV-2 in mice and rhesus macaques [36,37]. Clinical trials are being held worldwide to evaluate the safety and efficacy of the drug candidates and vaccines.

A study showed that scientists identified memory T cell clones reactive to SARS-CoV-1, a prior coronavirus closely related to SARS-CoV-2, from individuals at 6 years post infection, and generated TCR-Ts transduced with the most potent TCR, which preserved the functions of the parent T cell clone and responded to viral antigen stimulation *in vitro* [38]. Though functional TCR-Ts have not yet been reported for SARS-CoV-2, TCR-T cell therapy holds the potential to become a new prophylactic or therapeutic treatment option to fight the current coronavirus outbreak [39].

There are two main strategies to identify TCRs for SARS-CoV-2 and assess their functions. First, one could monitor the dynamics of T cell clones through longitudinal TCR repertoire profiling and identify TCR clones that are expanded or contracted after SARS-CoV-2 infection [29]. For hundreds of TCR clones significantly expanded or contracted, computational analysis was performed to cluster TCR sequences, with each cluster containing highly similar clonotypes. Characteristic TCR motifs were identified, some of which were shared between different donors with COVID-19, suggesting that there might be public TCRs of diagnostic or therapeutic value. Yet the analysis is basically *in silico*, generation of TCR-Ts and functional assessment is necessary to determine whether these TCRs are truly reactive to SARS-CoV-2.

The second approach is to identify T cell clones reactive to SARS-CoV-2 by stimulation with viral immunogenic peptides [31,32]. It could be conveniently adapted to our functional TCR-T generation pipeline (Figure 1). Since virus-reactive T cell clones are also present in healthy donors [30,31], it should be possible to make use of CD8$^+$ T cells sorted from patient-derived PBMC or from healthy donors. *In-vitro* priming of T cells with autologous DCs loaded with immunogenic viral peptides allows for efficient expansion of virus-specific T cell clones [40], which would be detected by the corresponding tetramer staining and isolated for single-cell TCR sequencing and RNA sequencing. The concurrent analysis of single-cell transcriptome and TCR clonotypes would provide more thorough information on selection of functional TCRs than a few conventional activation markers [30-32], and

thereby streamline the downstream experiments including generation of TCR-Ts and functional assessment.

Limitations of TCR-T cell Therapy in Infectious Diseases

Several major challenges remain for the applications of TCR-T cell therapy in infectious diseases. Firstly, TCR recognition of the peptide-MHC is HLA-restricted, therefore one TCR-T cell therapy could not fit all, but has to target the HLA-matched patients. Secondly, safety concerns of TCR-T cell therapy need to be taken considerations, including excessive lyse of the infected cells which might be part of the essential organs and cytokine storms which might be life-threatening [41]. Lastly, TCR-T cell therapy, like other gene-modified cell therapies, requires specialized facilities and complicated production processes, which might be a big hurdle for its widely applications [42].

Nonetheless, the emerging new techniques such as high-throughput functional TCR screening [20,43], TCR-Ts with safety switches and universal TCR-Ts [44] as well as evolving manufactural technologies [45] will ultimately enhance our abilities to design and produce better TCR-T products for clinical use.

Conclusion

T cells are natural essential effector cells against virus infection. Adoptive cell therapy using virus-specific TCR-Ts has clinical applications in treating CMV-related diseases, and holds the potential to become a new prophylactic or therapeutic option for acute infectious diseases such as COVID-19. However, TCR-T cell therapy is being held back by various obstacles including lack of efficient methods for identifying functional TCRs. In our pilot study, we devised a simple and rapid approach to generate functional TCR-Ts based on the correlative analysis of the transcriptome and TCR clonotypes from single-cell RNA and full-length TCR sequencing data. Further investigations are in progress to validate its applications in functional TCR-T generation for other immunogens.

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Declaration of Interests

The authors declare no competing interests.

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