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Discovery of somatic mutations associated with clonally expanded T cells

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Motivation and Aim: Somatic mutations may occur during cell division, and intensively dividing cells are more susceptible to acquiring such mutations. Somatic mutagenesis has been actively studied in cancer, where mutations can act as oncogenic drivers, but their role in other diseases is not fully clear [1]. We hypothesized that somatic mutations can arise in T cells proliferating after activation during immune response, and some of such mutations may have functional consequences affecting cell survival, proliferation, and thus promoting persistence and/or dysregulation of T cell clones. Recent studies reported presence of somatic mutations in different subsets of T cells from patients with autoimmune diseases [2, 3]. However, these mutations have been detected in bulk CD4+ and CD8+ T cells, and a direct association of these mutations with specific clones has not been shown. Thereby, our research aims to investigate the presence of somatic mutations in persistent clonally expanded T cells associated with different types of immunity: autoreactive and antiviral response.

Methods and Algorithms: The experimental design included T cells isolated on the basis of their antigen-specificity from peripheral blood of 2 donors (D1, D2) with chronic viral infection – cytomegalovirus (CMV) and 2 donors (D3, D4) with autoimmune disease – ankylosing spondylitis (AS). Fractions of antigen-specific CD8+ T cells for AS (HLA-B*27:05-YeiH epitope) and CMV (HLA-A*02:01-NLV epitope), as well as control T cell samples were isolated from PBMC of each donor in multiple replicates using FACS and peptide-MHC multimer staining [4]. For each cell sample (60-200 cells per sample), T cell receptor (TCR) sequencing was performed followed by clonotype enrichment analysis using MiXCR software [5, 6]. Multimer-positive samples containing at least 50% of one AS/CMV-associated T cell clone were then used for whole genome amplification, followed by whole exome sequencing (WES) averaging ~100x read coverage per sample. Somatic mutations were identified from the WES data using Mutect2 and VarScan [7]. For further analysis, we considered variants detected by Mutect2 in both replicates.

Results: YeiH- and NLV-specific T cell fractions (AS-related and CMV pp65 protein epitopes, respectively) had 1 or 2 large T cell clones comprising at least 70% of all T cells in the sample. At the same time, none of these clones were present in the cell

fractions containing multimer-negative CD8+ T cells. The obtained NLV-specific clonotypes were highly homologous with CMV-specific clonotypes from VDJdb database [8]. And YeiH-specific clonotypes matched TCR beta chain motif previously identified in other researches [9].

For each cell sample (60-200 T cells per sample) we obtained at least $90*10^6$ paired reads, of which 99% were mapped to the human reference genome (hg38). Overall read coverage reached 111 and 116 in exonic regions on average for each sample.

For D1 we found 32 somatic mutations reproduced in both replicates and confirmed by Mutect2, for D2 – 13, for D3 – 48, and for D4 – 18. It is noteworthy that the mutation frequency roughly corresponds to the clone representation frequency in the repertoire. In the NLV+ sample from D1, there are two clusters of variants reproduced in both replicates and verified by Mutect2, with a fraction of reads with mutation around 0.4 and 0.1 (Fig. 1). This corresponds to the presence of two large clones in this sample, accounting for 80% and 20% based on the TCR sequencing results.

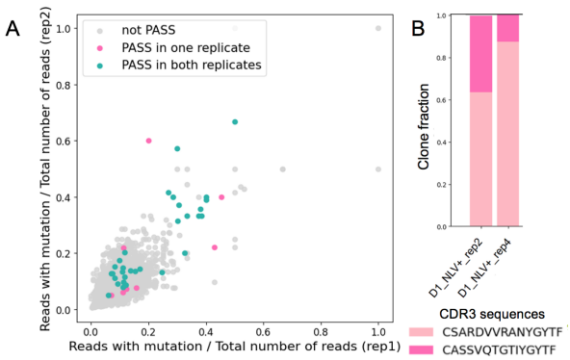

Fig. 1. A – Fraction of reads with a variant in each replicate for NLV+ samples of D1; B – Fractions of two large T cell clones based on the TCR sequencing results. PASS – variants verified by Mutect2; rep – replicate

Several mutations were found in genes involved in the regulation of cell proliferation, apoptosis, tumorigenesis, and tumor suppression. None of the detected mutations were shared at the gene level across different donors and between AS and CMV. However, some genes were grouped into common signaling pathways: MAPK, Ras, Rap1, PI3K-Akt signaling pathways; or had similar functions (Table 1).

Conclusion: Using peptide-MHC multimer cell sorting followed by whole exome sequencing of samples enriched for epitope-specific T cells, we discovered genomic variants associated with clonally expanded T cells involved in antiviral response or autoimmunity. The data obtained suggest that these variants represent somatic mutations that arise either during maturation or after activation and subsequent proliferation of naive precursors of the T cell clones. Despite the uniqueness of mutations between different donors and T cell types, some of the variants detected in different donors affect genes involved in processes of immune regulation and cell survival, implying their impact on the functional activity and/or persistence of the T cells.

Table 1. Identified somatic mutations for NLV- and YeiH-specific samples from WES data

Samples	Gene	Gene function	KEGG pathways*
NLV+	EPHA2	Involved in tumorigenesis and malignant progression	MAPK/Ras/Rap1/PI3K-Akt signaling pathways
	IGF1R	Regulates proliferation and apoptosis	MAPK/Ras/Rap1/FoxO/PI3K-Akt/AMPK/mTOR signaling pathways, Pathways in cancer, Transcriptional misregulation in cancer
YeiH+	CEBPB	Tumor suppressor, inhibits T cell proliferation	TNF/IL-17 signaling pathways, Transcriptional misregulation in cancer
	SLC8A2	Tumor suppressor	Calcium/cGMP-PKG signaling pathways
	AFDN	Inhibits cell proliferation	Ras/Rap1/cAMP signaling pathways
	DLG2	Knockout promotes cell proliferation	Hippo signaling pathway

*according to KEGG pathways database [10].

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