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POSTER ABSTRACTS

203.LYMPHOCYTES AND ACQUIRED OR CONGENITAL IMMUNODEFICIENCY DISORDERS

Multi-Omic Insights into the Mutational Landscape and Dysregulated Transcriptional Programs of Autoimmune B-Cells in Systemic Lupus Erythematosus

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Systemic Lupus Erythematosus (SLE) is an autoimmune disease with diverse manifestations, from mild and sporadic to severe and life-threatening. SLE heterogeneity arises from various factors, including genetic, epigenetic, and immune niche-related features (Kaul et al., 2016; Jenks et al., 2018). However, the exact interactions and hierarchy between these features remain unclear. Recent studies have found somatic mutations in genes typically mutated in B-cell lymphomas, in subsets of B-cells that are associated with SLE pathogenesis (Piotrowski et al., 2015; Pullabhatla et al., 2018; Singh et al., 2020; Brown et al., 2022). These mutations may support B-cell clonal bursts, but direct information from clinical specimens on the impact of these mutations on B-cell lineages and their influence on SLE progression or malignant transformation is missing.

To define the somatic mutational landscape of B-cells in SLE, we first profiled peripheral blood mononuclear cells obtained from a pilot cohort of four SLE patients using whole-exome sequencing (WES; 200X depth). Following GATK best practices (Van der Auwera and O'Connor, 2020), we identified somatic alterations in *DDX11* [variant allele frequency (VAF): 10.2%], *DNMT3A* [VAF: 3.4%]; *PMS2* [VAF: 47.9%], and *BICD1* [VAF: 47.3%]; these genes have been linked to lymphoid malignancies and SLE pathogenesis (Piotrowski et al., 2015; Pullabhatla et al., 2018; Niroula et al., 2021; Saeed et al., 2021). We validated the presence of the top-identified mutations using droplet digital PCR technology on sorted B-cell subsets (naïve, memory, and double-negative [DN; CD19 ^{hi}IgD ^{lo}CD27 ^{lo}]) from these SLE patients. DN cells are a heterogeneous subtype of autoreactive memory B-cells that play a crucial role in SLE pathogenesis being the precursors of autoantibody producing plasma cells (Wei et al., 2007; Jenks et al., 2018).

To investigate the transcriptional and epigenetic alterations in the mutation-harboring B-cells, we then conducted single-cell multi-omic sequencing. We profiled sorted activated B-cells (CD19 ^{hi}IgD ^{lo}) from the four SLE cases, to largely exclude non-autoreactive naïve B-cells. To maintain a comparison with naïve B cells, we included a small proportion of these cells in the input. In total, we analyzed 35,339 cells, and identified different B-cell subsets with distinct gene expression patterns, including DN cells (n = 1,437 cells; ZEB2 ^{hi}TRAF5 ^{lo}, Jenks et al., 2018). To uncover, in an unbiased way, transcriptional differences between B-cell subsets, we catalogued variation in transcriptomic signatures derived from the scRNA data using consensus non-negative matrix factorization. We found a unique gene expression program exclusively present in DN cells (**Panel A**), which includes key genes implicated in immune regulation and the development of autoimmunity, such as *PTPN22* (Chung & Criswell, 2007; Tizaoui et al., 2021) . Pathway analysis of the DN-specific program revealed an enrichment of complement system genes (FDR = 0.074; Hallmark), which are crucial for immune regulation and tolerance, such as *CBLB* (Tang et al., 2019). Notably, in DN cells from one of the SLE samples carrying lymphoma/SLE-associated mutations in *BICD1*, *PMS2*, *DDX11*, we observed an altered expression of this gene program compared to DN cells from other samples (P-value = 3.6x10 $^{-11}$; **Panel A, *inset***), suggesting that a malfunctioning complement system in DN cells may lead to an overly active immune response (Weinstein et al., 2021). In line with this notion, using chromatin accessibility profiles as a measure of transcription factor (TF) regulatory activities, we showed increased activity of TFs involved in interferon-dependent innate immune response and in B-cell activation and differentiation (RFX and OCT-1, respectively; FDR < 0.05), in the DN B-cells of this sample (**Panel B**).

In summary, we uncovered a unique transcriptional program associated with autoimmune DN B-cells. This program showed enrichment in key genes and pathways involved in immune response regulation. The disruption of this program, accompanied

by the somatic SLE-associated mutations, may lead to overactivation of DN B cells, ultimately contributing to autoimmunity. Further analyses involving a larger cohort of clinical specimens are needed to directly link B-cell genotypes with their transcriptional and epigenetic programs and to elucidate the role of the identified mutations in SLE pathobiology.

Disclosures No relevant conflicts of interest to declare.

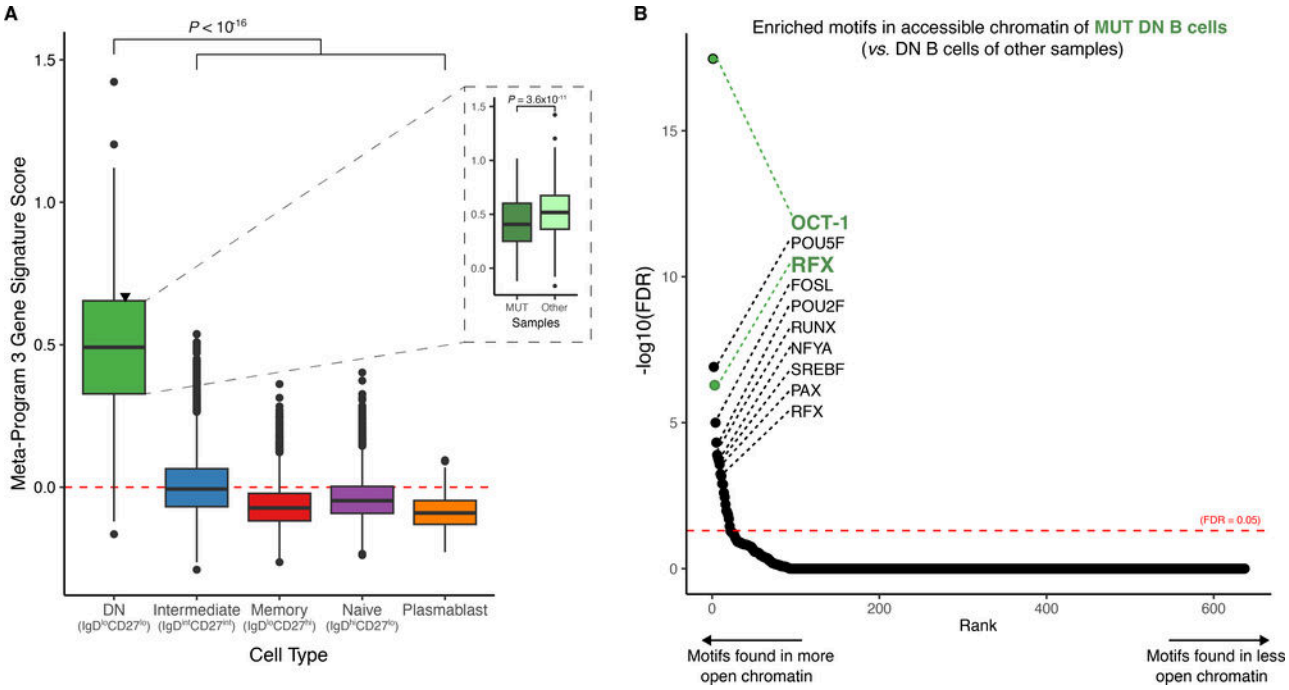

Figure 1

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