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THE HEMATOPOIETIC STEM CELLS (HSCS) IN SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) REPROGRAM THEIR TRANSCRIPTOME: IMPLICATIONS FOR THE PATHOGENESIS OF THE DISEASE

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Background:

- Hematopoietic stem cells (HSCs) consist the most primitive blood population giving rise to terminally differentiated blood cells.
- All such cells in System Lupus Erythematosus (SLE) exhibit aberrant phenotype by being activated, producing autoantibodies or driven to destruction.
- Characterization of HSCs under the influence of SLE inflammatory milieu and whether this phenotype is reflected to the whole hematopoietic tree are ill-defined.
- Until recently, a significant number of patients undergo autologous stem cell transplantation with long-term efficacy, so the transcriptional output of HSCs through the disease course could be very informative.

Objective: We hypothesize that some of the blood related clinical manifestations can be traced back to patient HSCs. To answer this, we describe the transcriptional alterations of CD34⁺ cells in the bone marrow of SLE patients.

Methods:

- CD34⁺ cells were isolated from BM aspirates and PB of SLE patients (n=8 females) and healthy subjects (n=2 females) with magnetic separation (Stem Cell Technologies).
- Total RNA was extracted and libraries for mRNA-seq were prepared.
- Sequencing performed in NextSeq Illumina Platform (SE 75 bp reads). Alignment in human genome (hg38) was performed by Star and differential expression analysis by edgeR algorithm.
- Genes with FDR≤0.05 and FC≥1.5 or ≤-1.5 were considered statistically significantly up- and down-regulated, respectively.
- Heatmaps of expression were constructed in R and Gene Ontology/Pathway Analysis were performed in ClueGo, RNEA and GSEA.

Table 1 - Clinical and demographical characteristics of SLE patients	
Sex, female/male	8/2
Age, mean ± SD	47.3 ± 16.02
SLEDAI (mean ± SD)	
High SLEDAI≥12	14 ± 1.41
Low SLEDAI<12	4.5 ± 2.34
Severity pattern	
Moderate SLE	4/10
Severe SLE	6/10
Activity pattern	
Active SLE	8/10
Inactive SLE	2/10
Nephritis	4 /10
NPSLE	1/10
Serositis	3/10
Arthritis	8/10
*Active SLE was defined based on PGA and/or SLEDAI. Controls included 3 healthy BM donors, 24–50 years of age.	

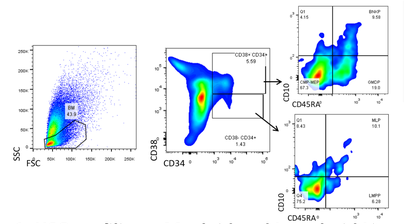
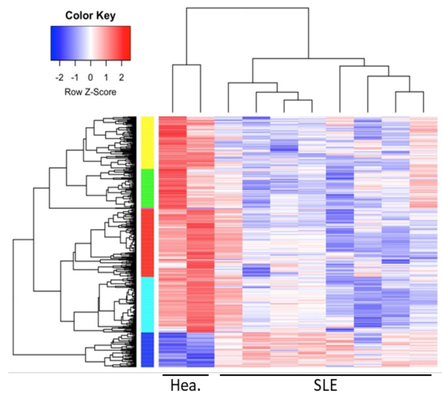


Fig 1. HSC profiling – Myeloid and Lymphoid Lineage



Differentially Expressed Genes	Up-regulated	Down-regulated
2364	832	1532

Fig 2. Heatmap of Differentially Expressed Genes (DEGs) in HSCs of SLE patients and Healthy Subjects

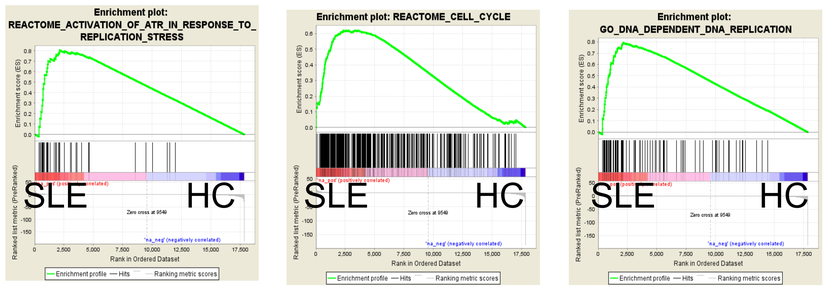


Fig 3. Gene enrichment analysis in SLE HSCs shows involvement in DNA damage response, cell cycle and replication stress

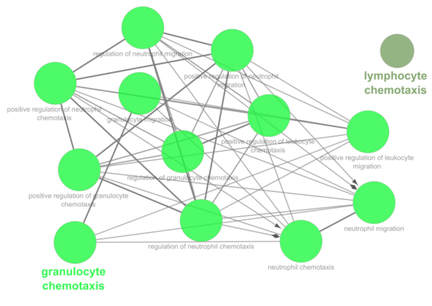


Fig 4. Gene Ontology and Pathway Analysis of DEGs in human lupus HSCs

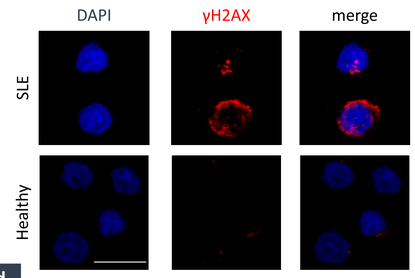


Fig 5. Monitoring of DNA damage by γH2AX staining with confocal microscopy

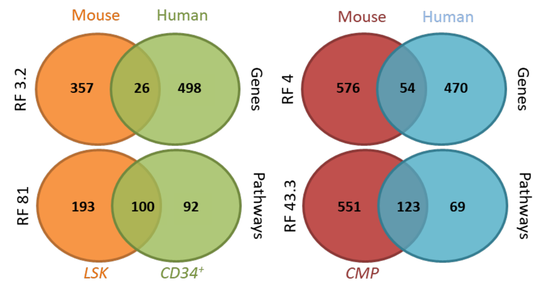


Fig 6. Comparative analysis of genes contributing in HSC phenotype in human and mouse lupus

Results – Conclusions:

- Comparing transcriptomes of BM-derived CD34⁺ of SLE patients and healthy subjects (Fig. 1), **we identified 2364 DEGs (Fig 2).**
- DEGs participate in **hematopoietic cell lineage fate, stem cell differentiation, DNA damage and cell cycle regulation (Fig 3).**
- Comparison of CD34⁺ profile between severe-moderate SLE reveals a **prominent neutrophilic signature in severe disease (Fig 4).**
- We also found evidence for cell cycle checkpoints signature which drives HSCs to experience **replication stress and activate ATR pathway, resulting to enhanced DNA damage (Fig 5).**
- **Comparative transcriptomic analysis of human vs murine SLE revealed a panel of common genes** again related to cell proliferation, differentiation and platelet activation **(Fig 6)**.

HSCs in SLE patients reprogram their transcriptome in response to inflammatory milieu within the BM, thus exiting from dormancy, differentiating to myeloid lineage and mounting a DNA damage response to replication stress.

References: 1) Mitroulis, Ioannis, et al. "Modulation of myelopoiesis progenitors is an integral component of trained immunity." Cell 172.1 (2018): 147-161.
2) Kaufmann, Eva, et al. "BCG educates hematopoietic stem cells to generate protective innate immunity against tuberculosis." Cell 172.1 (2018): 176-190.
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