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POSTER ABSTRACTS

508.BONE MARROW FAILURE: ACQUIRED

Molecular Mechanisms Underlying Abnormal Activation of T Cells in Idiopathic Aplastic Anemia and Hypoplastic Myelodysplastic Neoplasms

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Idiopathic aplastic anemia (AA) and hypoplastic myelodysplastic neoplasms (MDS-h) are severe hematopoietic disorders characterized by pancytopenia and hypoplastic bone marrow. There is compelling evidence that these distinct clinical entities share a common pathophysiology based on the damage of hematopoietic stem and progenitor cells (HSPCs) by cytotoxic T cells. Expanded T cells overproduce proinflammatory cytokines, resulting in decreased proliferation and increased apoptosis of HSPCs. To uncover the molecular mechanisms underlying this abnormal immune response, we used RNA-Seq for the transcriptome analysis of T cells from patients with idiopathic AA and MDS-h.

CD3+ cells were isolated from peripheral blood of 15 patients with AA or MDS-h at diagnosis and 6 healthy blood donors. The RNA-Seq library was constructed by NEB Next Ultra II Directional RNA Library Prep Kit and the data were analysed by R software 4.0.2. Functional annotation of differently expressed genes was performed using DAVID database and Gene Set Enrichment Analysis (GSEA).

Principal component analysis-based clustering of RNA-Seq data defined the three major components for AA and MDS-h patients, and healthy individuals (Fig. 1A). As expected, the control samples formed a distinct group, meanwhile patient samples partially clustered together. The same results were obtained using unsupervised hierarchical clustering. The differential expression analysis (DEA) identified 256 significantly upregulated and 96 downregulated genes (|logFC|>1, FDR<0.05) in patients compared to controls. Notably, the DEA detected many long noncoding RNAs (lncRNAs) including upregulated lncRNAs associated with immunological disturbances (e.g. *CDC42-IT1* and *NEAT1*), and oncogenesis (e.g. *ACAP2-IT1*, and *FAM238A*). Particularly, *NEAT1* positively regulates differentiation of CD4+ T cells into Th17 cells through STAT3 protein. Autoreactive T cells may be further stimulated by increased expression of *S100A8/A9* detected in the patient cells.

Gene Ontology (GO) analysis annotated the upregulated genes in AA/MDS-h to biological processes associated with oxygen transport, B cell receptor signaling pathway, innate immune response, positive regulation of inflammatory response, and apoptotic process, etc. (adjusted p<0.05) (Fig. 1B). The downregulated genes were significantly enriched in processes related to adaptive immune response, cell surface receptor signaling pathway, mitochondrial ATP synthesis coupled proton transport, etc. (adjusted $p<0.05$) (Fig.1B). B cell receptor signaling pathway was enriched by upregulated genes such as *BANK1*, *MEF2C*, and *SYK* whose deregulation is associated with autoimmune disorders. Herein, the activation of apoptosis is likely driven via *AP-1* positive modulators that showed increased expressions in the patients. These modulators are essential for T cell activation and regulatory T cell differentiation.

Pathway enrichment analysis determined the following signaling pathways (adjusted $p<0.05$): B cell receptor signaling pathway, osteoclast differentiation, oxidative phosphorylation, chemical carcinogenesis - reactive oxygen species, and cytokine-cytokine receptor interaction.
The GSEA using the Hallmark gene sets showed that DNA Repair and Oxidative Phosphorylation were significantly negatively correlated with patient phenotype (Fig. 1C). Both GSEA and pathway analysis revealed that oxidative phosphorylation was supressed in patient-derived T cells. This is consistent with previous metabolic findings demonstrating that a transition from oxidative phosphorylation to glycolysis is a sign of T cell activation and it is critical for support of rapid cell growth.
In conclusion, our study suggests the possible mechanisms of aberrant T cellular-immunity in idiopathic AA and MDS-h. Consistent with abnormal biological features of T cells, a large number of deregulated genes in the patients was involved in immune and inflammatory responses. Furthermore, our analyses revealed novel pathways, such as oxidative phosphorylation, as well as candidates (*TLR2*, and *CDC42-IT1)* for functional studies. The deregulation of multiple lncRNAs in the patients indicates their implication in the molecular pathogenesis and thus their roles in bone marrow failure need to be explored.
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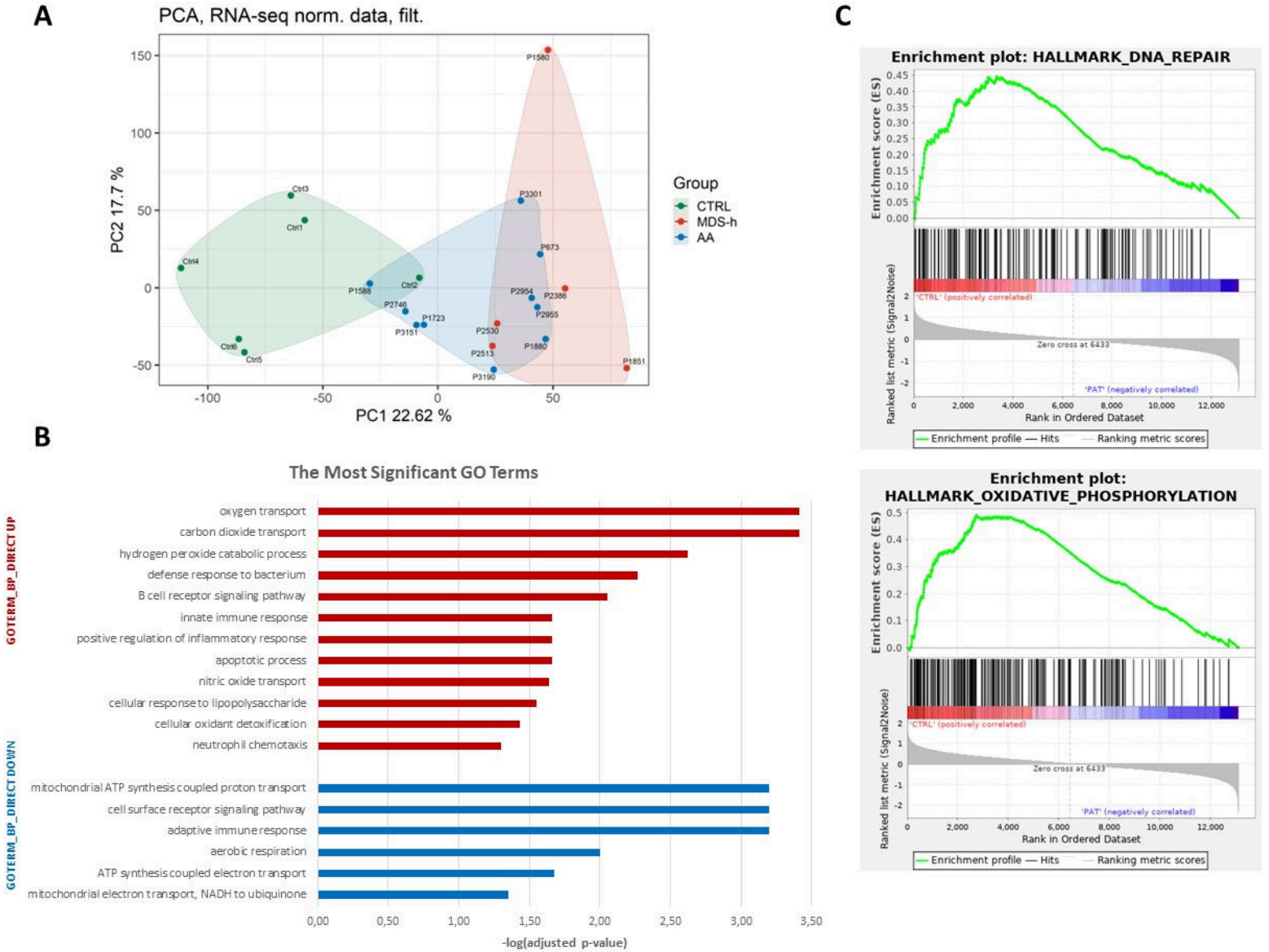

Figure 1: A) Principal component analysis of AA, MDS-h, and control RNA-Seq data. B) Gene Ontology (GO) functional analysis of significantly upregulated (red) and downregulated (blue) genes in T cells from patients with AA and MDS-h. C) GSEA plots for HALLMARK gene sets: DNA Repair and Oxidative Phosphorylation (controls vs. patients).

Figure 1