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ORAL ABSTRACTS

509.BONE MARROW FAILURE AND CANCER PREDISPOSITION SYNDROMES: CONGENITAL

Interplay between Inflammatory Microenvironment and RUNX1-Mediated Transcriptomic Changes Drives Defective Hematopoiesis in Familial Platelet Disorder

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Background: Familial platelet disorder (FPD) is caused by germline loss-of-function mutations in *RUNX1* an essential transcription factor regulating hematopoiesis. The absence of functional RUNX1 leads to mild-to-moderate thrombocytopenia, platelet dysfunction, and increased bleeding in FPD patients. In ~40% of cases, the patients progress to overt leukemia at median age of 33 years through unknown mechanisms. FPD patients also present with skin allergy and eczema which are associated with systematic elevation of cytokines. Given the well-established connection between inflammation and leukemia initiation and progression, we sought to investigate whether inflammatory stress play a role in regulating abnormal hematopoiesis and eventually cause clonal evolution in FPD.

Methods: We acquired bone marrow cells from FPD patients who had not developed leukemia yet. We then compared FPD bone marrow hematopoietic stem and progenitors (HSPCs) with healthy progenitors using *in vitro* differentiation and colony formation assays. For comprehensive mechanistic evaluation, we performed 10x single-cell RNA sequencing (scRNA-seq) on FPD (n=10) and healthy (n=4) bone marrow cells, analyzing >100,000 cells using Seruat package. We evaluated cytokine levels in bone marrow fluid and peripheral blood using 65-plex Luminex assay. The impact of the inflammatory cytokines and intervention strategies were tested using *in vitro* differentiation and colony formation assays. The results were validated *in vivo* using mice with germline *Runx1* mutation.

Results: We identified that FPD progenitors are impaired in megakaryocytic (>2.0-fold, $p<0.05$) and erythroid differentiation with increased myeloid differentiation (>1.7-fold, $p<0.05$) compared to healthy control. In addition, FPD HSPCs show increased myeloid colony formation (>1.6-fold, $p<0.05$) and serial replating ability. scRNA-seq identified 18 cell clusters and pseudotime analysis revealed higher percentage of FPD cells differentiating towards monocytes, supporting enhanced myeloid differentiation. The pathway enrichment analysis of differentially expressed genes in HSC cluster revealed upregulation of inflammatory (TNFα via NF-kB) and pro-survival pathways (PI3K/AKT/mTOR). Accordingly, cytokine profiling revealed

upregulation of numerous cytokines and chemokines in FPD compared to healthy samples including TNFα, CXCL8, CCL24, CCL2, and IL1β (>2.5 to 1000-fold, p<0.05), secreted by progenitors, monocytes, and MSCs. Further, under inflammatory stress mediated by TNFα, CXCL8, CCL24, CCL2, and IL1β, FPD HSPCs exhibited resistant to growth suppression and have increased myeloid colony formation. However, pharmacological inhibition of individual cytokines only partially rescued the differentiation defects, possibly due to compensatory effects of each cytokine. Phospho-flow analysis revealed that increased cytokine levels leads to the activation of PI3K/mTOR and JAK signaling that we also found upregulated using scRNA-seq analysis. Interestingly, inhibition of mTORi (rapamycin and AZD2014), PI3Ki (idelalisib), and JAKi (ruxolitinib) showed an increase in percentage of megakaryocytes (up to 2-fold, p<0.05) and a decrease in monocytes (up to 0.6-fold change, p<0.05). Also, inhibition of these pathways in FPD reduced cytokines levels. In line with human FPD data, *in vivo* treatment of *Runx1* $^{R188Q/+}$ mouse with ruxolitinib suppressed myeloid differentiation with no effects on megakaryocytes, while rapamycin resulted in significantly increased platelet activation compared to untreated mice (>1.7-fold, p<0.05) and a reduction of monocytes (<0.5-fold, p<0.05), suggesting mTOR inhibition might be an effective intervention strategy.

Conclusion: FPD HSPCs manifest defective megakaryopoiesis with increased myelopoiesis. The elevated autocrine and paracrine inflammatory stress is an early event in FPD, driving myeloid differentiation. This inflammatory stress mediates activation of PI3K/mTOR and JAK signaling, leading to augmented myeloid differentiation. Consequently, the observed FPD phenotypes are the result of combined transcriptional changes and inflammatory stress. Targeting inflammatory and pro-survival signaling at an early stage might mitigate hematopoietic defects and delay disease evolution in FPD. Our results advance mechanistic understanding and hold significant clinical implications.

Disclosures No relevant conflicts of interest to declare.

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