**Multi-Omics Profiling of JMML HSPCs Reveals Onco-Fetal Reprogramming in High-Risk JMML and Identifies Novel Prognostic Biomarkers and Therapeutic Targets**

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Juvenile myelomonocytic leukemia (JMML) is a myeloproliferative neoplasm of early childhood, characterized by heterogenous clinical outcomes ranging from spontaneous resolution to early relapse after hematopoietic stem cell transplantation (HSCT). Established clinical risk factors and characteristic mutational patterns do not fully explain this heterogeneity. Recently, we and others identified JMML DNA methylation subgroups (Lipka et al., Nat Commun 2017; Stieglitz et al., Nat Commun 2017; Muramatsu et al., Blood 2018) that were shown to be the only significant factor for predicting overall survival and thus might facilitate the design of risk-stratified clinical trials (Schönung et al., Clinical Cancer Research 2021). Together, these findings suggest a functional role for DNA methylation in the molecular pathogenesis of JMML.

To elucidate the molecular pathways altered in JMML subgroups and especially the impact of aberrant DNA methylation on disease pathogenesis and progression, we leveraged a multi-modal molecular analysis approach. We integrated flow cytometry data with single-cell and ultra-low input bulk RNA-seq and whole-genome bisulfite sequencing (WGBS) data, to dissect hematopoiesis in JMML patients across all risk groups (n=8), with a focus on hematopoietic stem and progenitor cells (HSPCs). With this approach, we aimed to identify novel candidate prognostic biomarkers and therapeutic targets for high-risk JMML patients.

Single-cell RNA-seq of hematopoietic cells, isolated from JMML patients, revealed disease-specific aberrations relative to the healthy hematopoiesis, which appeared to be most pronounced in terminally differentiated cell types and within the HSPC compartment. We observed conservation of epigenetic JMML subgroups already in the HSPC compartment, suggesting that epigenomic changes might be key to JMML pathogenesis and disease progression. Consequently, we focused our work on JMML HSPCs. In-depth analysis showed a high degree of immunophenotypic, transcriptomic, and methylomic heterogeneity within the HSPC compartment of JMML patients across different DNA methylation subgroups.

Subgroup-specific aberrations included upregulation of distinct developmental programs in HSPCs from high-risk JMML patients. This is of particular interest regarding the cell-of-origin in JMML, as mutations activating the RAS signaling pathway have been documented in newborn blood samples from children who later developed JMML (Behnert et al., Leukemia 2022). Comparison of the methylome data of highly purified HSPCs isolated from JMML patients with HSPCs isolated from healthy individuals at different developmental stages suggested progressive epigenomic reprogramming of JMML HSPCs towards high-risk JMML. As a consequence, we observed signatures of both accelerated aging and aberrant activation of oncofetal programs.

Systematic comparison of JMML HSPCs with their healthy nearest normal counterparts revealed disease-specific expression of several surface markers in JMML. The expression patterns appeared to be regulated in a subgroup-specific manner, which nominates these factors as novel prognostic biomarkers in JMML. Moreover, certain surface markers were not only differentially methylated but also known drug targets in other entities. Therefore, we selected these genes as high-confidence candidates for further functional validation. In a preclinical patient-derived xenograft (PDX) mouse model, targeted treatment with therapeutic antibodies efficiently depleted high-risk JMML HSPCs and improved the survival of JMML-PDX mice.

In conclusion, the personalized molecular analysis of JMML HSPCs disentangled disease-specific heterogeneity and enabled novel insights into tumorigenesis. The subgroup-specific epigenetic dysregulation, including oncofetal reprogramming of JMML HSPCs, revealed novel prognostic biomarkers and promising novel treatment options specific for high-risk JMML.

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