IMPACT OF NK RECEPTOR AND LIGAND GENE EXPRESSION IN ACUTE MYELOID LEUKEMIA: A COMPUTATIONAL APPROACH

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Acute Myeloid Leukemia (AML) alters immune profiles of T and NK cells, suppressing cell-mediated responses and potentially aiding tumor evasion. NK cells are crucial in anti-tumor defense by eliminating malignant cells without specific antigens. However, immunosuppressive cytokines in the tumor microenvironment can impair NK cell function, allowing neoplastic cell survival and progression. NK cell activity is regulated by a complex network of receptors and ligands (NKRLs). NK receptors interact with HLA class I molecules on target cells, providing inhibitory signals or allowing tumor cell elimination in their absence. The prognostic impact of NKRL expression in AML blasts and the combined effect of NKRLs still require thorough investigation. We aimed to investigate how receptor and ligand expression influences NK cell function in AML pathogenesis. *In silico* approaches were used to analyze immune cell transcriptomic signatures with bulk RNA-seq data from BeatAML 1.0. The analysis focused on mononuclear cell transcriptomic profiles from AML patient samples at diagnosis, emphasizing gene modulation. Statistical analysis was performed on the R platform using the limma-voom algorithm with Benjamini-Hochberg adjustment. The analysis revealed that variable expression of NKRLs significantly influenced critical clinical outcomes in AML. In the overall survival analysis, high expression levels of ULBP1 were associated with improved survival compared to lower levels ($p = 0.016$). The interaction between ULBP1 and receptors such as NKG2D on NK cells and T cells can trigger anti-tumoral immune responses, enhancing the elimination of tumor cells. Additionally, some tumor cells express HLA class I ligands like HLA-C, binding inhibitory receptors such as KIR2DL on NK cells, inhibiting their anti-neoplastic activity. Decreased NKG2D ligand expression or increased HLA class I ligand expression may promote leukemogenesis and disease aggressiveness, aiding tumor survival. These findings highlight the complexity and adverse effects of immune response modulation in AML, stressing the importance of understanding NKRLs' mechanisms and clinical implications for personalized treatment. The study identified high expression of genes like HLA-E, HLA-C, MICAB, KLRK1, KLRC1, KIR2DL2, CD226, MICA, and PVRL2, associated with inflammation-related gene enrichment in AML samples. A coordinated pattern of inflammatory gene expression was often seen in AML samples with high gene expression, suggesting a potential causal relationship that needs further functional investigation. Heatmap analysis indicated that samples with high MICA and PVRL2 gene expression showed negative enrichment relative to genes positively enriched in other samples, implying complex molecular interactions influencing NKRL expression in AML. These results show how inflammatory gene expression patterns influence NK cell behavior and the immune response against AML, offering valuable insights for understanding this hematological disease. The findings suggest that assessing NKRL expression may serve as a prognostic biomarker in AML. Understanding this interaction is crucial for developing therapies to reverse immune escape and improve outcomes in myeloid leukemias.

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A NOVEL IN VITRO BONE MARROW NICHE PLATFORM FOR INVESTIGATING THERAPEUTIC STRATEGIES FOR ACUTE LEUKEMIAS

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Aims: Acute lymphoblastic leukemia (ALL), the most prevalent childhood cancer, disrupts the hematopoietic bone marrow (BM) niche, a specialized microenvironment that supports normal hematopoiesis. During ALL development, this niche undergoes remodeling and reprogramming to support tumor growth, hindering blood cell formation. Mesenchymal stromal cells (MSCs), particularly those located in the perivascular niche, play a pivotal role by shielding leukemic lymphoblasts from external stressors, impacting drug responses and potentially contributing to relapse. Understanding this complex interplay between ALL cells and the BM niche is vital for developing effective drug screening models. Therefore, our study aimed to develop an *in vitro* coculture system that mimics the ALL BM tumor microenvironment. **Methods:** T-ALL (Jurkat), B-ALL (RS4-11), and MSCs (HS5) cell lines were maintained in RPMI-1640 supplemented with 10% FBS. Tridimensional cocultures were established using hemostatic sponges (Gelfoam, Pfizer) and spheroids (Hanging Drop or Liquid Overlay Technique containing 0, 5, 10 and 20% Matrigel). These cocultures combined normal MSCs (HS5) and ALL cells to mimic different cellular environments within the tumor microenvironment. Different cell ratios (MSC to T-ALL proportion: 1:1, 1:2, 1:5, 2:5) were tested to simulate different cellular environments. To evaluate the system's applicability,