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

CAB Garcia [a], M Medeiros [b], AG Carvalho [b], LS Binelli [b], LO Marani [b], BGS Macedo [a], GRFN Soares [b], LL Figueiredo-Pontes [b]

[a] *Fundação Hemocentro de Ribeirão Preto (FUNDHERP), Faculdade de Medicina de Ribeirão Preto (FMRP), Universidade de São Paulo (USP), Ribeirão Preto, Brazil*
[b] *Hematology, Hemotherapy and Cell Therapy Division, Department of Medical Imaging, Hematology, and Clinical Oncology, Faculdade de Medicina de Ribeirão Preto (FMRP), Universidade de São Paulo (USP), Ribeirão Preto, Brazil*

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

DP Echevarria [a,b,c], MLP Bueno [a], I Santos [a], NG Amôr [a], FM Roversi [a,d], AN Bruno [c], STO Saad [a]

[a] *Centro de Hematologia e Medicina Transfusional (Hemocentro), Universidade Estadual de Campinas (UNICAMP), Campinas, Brazil*
[b] *Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil*
[c] *Instituto Federal de Educação Ciência e Tecnologia do Rio Grande do Sul (IFRS), Porto Alegre, Brazil*
[d] *Department of Surgery Division of Transplantation, Emory University, Atlanta, United States*

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,

the cocultures were treated for 72h with a BCL2 inhibitor, Venetoclax, or an MCL1 inhibitor, AZD5991, as single agents or in combination. Both agents were used at their 10% maximal response concentration (IC10 - 20 nM). Following the treatment, the 3D systems were dissociated using collagenase (for Gelfoam) or trypsin (for spheroids), and cell populations were analyzed by flow cytometry. **Results:** In comparison to monocultures, T-ALL cell viability was enhanced in cocultures with MSCs in Gelfoam 3D system, with˜80% of viable ALL cells in all cell ratios tested. Conversely, proliferation of MSCs was diminished compared to monocultures. Although spheroid formation was obtained using hanging drop technique, increasing Matrigel concentrations impaired spheroid formation, except at the highest cell seeding ratio (2:5), which still resulted in highly irregular spheroids with a near-equal distribution of ALL cells and MSCs (1:1). Thus, considering the bone marrow microenvironment, Gelfoam (1:5 cell ratio) was the 3D system that most closely resembled the cellular composition observed in ALL. Cocultures in Gelfoam were treated with Venetoclax and/or AZD5991. The combination therapy effectively inhibited the proliferation of ALL cells in monoculture. However, the combined treatment was not enough to overcome the protective niche action. Importantly, the treatments did not affect MSCs proliferation. **Conclusions:** Our findings demonstrate an innovative 3D coculture system that successfully replicates the BM stromal niche. This novel platform holds promise for the development of new therapeutic strategies for ALL. **Apoio:** FAPESP 2017/21801-2, 2019/25247-5, 2021/05320-0; 2023/13850-4; CNPq 303405/2018-0.

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HIGH LEUKEMIC STEM CELL FREQUENCY IN FLT3- MUTATED ACUTE MYELOID LEUKEMIA PATIENTS WITH NORMAL KARYOTYPE: IMPLICATIONS FOR PROGNOSIS

CAB Garcia [a], LO Marani [a], M Medeiros [a], MIA Madeira [a], K Pagnano [b], E Nunes [c], ABF Glória [d], EM Rego [e], AG Carvalho [a], F Traina [a], LL Figueiredo-Pontes [a]

[a] *Hematology, Hemotherapy and Cell Therapy Division, Department of Medical Imaging, Hematology, and Clinical Oncology, Faculdade de Medicina de Ribeirão Preto (FMRP), Universidade de São Paulo (USP), Ribeirão Preto, Brazil*
[b] *Centro de Hematologia e Medicina Transfusional (Hemocentro), Universidade Estadual de Campinas (UNICAMP), Campinas, Brazil*
[c] *Hematology Division, Complexo Hospital de Clínicas (CHC), Universidade Federal do Paraná (UFPR), Curitiba, Brazil*
[d] *Hematology Division, Hospital das Clínicas da Universidade Federal de Minas Gerais (HC-UFMG), Belo Horizonte, Brazil*
[e] *Internal Medicine, Faculdade de Medicina, Universidade de São Paulo (FMUSP), São Paulo, Brazil*

Normal karyotype in Acute Myeloid Leukemia (NK-AML) occurs in 40-50% of patients. Leukemic Stem Cells (LSC) persistence is known to trigger relapse, but the prognostic impact of their identification is understudied. We correlated LSC frequency at diagnosis with mutational profile and clinical outcomes in a Brazilian NK-AML cohort. Bone marrow samples (n = 93) were submitted to LSC quantification by multiparametric flow cytometry (MPFC). Patients were categorized based on LSC percentage as lower (LSClow) or higher (LSChigh) than 0.1%. Regarding risk stratification, the LSClow group was composed by 19 favorable, 13 intermediate and no adverse-risk. In the LSChigh group, the patients were classified as 25 favorable, 28 intermediate, and 6 adverse, thus suggesting that lower LSC frequency is associated with favorable risk, although statistical significance was not reached. In the LSClow group, 12.5%had the *FLT3* mutation whereas in 33.3% of the LSChigh samples *FLT3* mutation was detected. Of note, LSChigh samples presented lower FLT3allelic ratio as compared to LSClow (0.47 ± 0.28 vs 0.87 ± 0.53). When associating LSC frequency with *NPM1* gene status, 65.62% of LSClow group and 40% of LSChigh group were *NPM1* mutated. When the co-mutational status was considered, among individuals with LSClow, 81.8% (n = 18) was *FLT3* wt*NPM1* mut, 13.64% (n = 3) was *FLT3* mut*NPM1* mut, and 4.56% (n = 1) *FLT3* mut*NPM1* wt. In the LSChigh group, 37.5% (n = 12) was *FLT3* wt*NPM1* mut,37.5% (n = 12) was *FLT3* mut*NPM1* mut, and 25% (n = 8) *FLT3* mut*NPM1* mut. Overall, complete remission (CR/CRi) rate was 64.5% (n = 60) after 2 cycles of chemotherapy. No significant difference was observed between groups:59.4% (n = 19) in the LSClow and 67.2% (n = 41) in the LSChigh group achieved CR. After remission, 15.6% (n = 5) of the patients relapsed in the LSClow group as compared to 16.2% (n = 16) of the LSChigh patients. The LSClow group had a mean overall survival (OS) and event-free survival (EFS) of 568 and 541days, respectively, whereas the LSChigh group had both mean OS and EFS of466 days. We also assessed outcomes according to ELN2017 risk stratification. Favorable-risk patients with low LSC levels had a mean OS of 130 days, compared to 182 days for those with high LSC levels. Intermediate-risk patients with low LSC levels had a mean OS of 174 days, while the high LSC group had a mean OS of 228 days. Adverse-risk patients with low LSC levels had no measurable mean OS, whereas those with high LSC levels had a mean OS of 216 days. No significant differences in survival distributions between the groups. Our data indicated that *FLT3* mutation was more prevalent in patients with higher frequency of LSCs indicating that cases with high LSC content at the flow cytometry initial examination should be quickly assessed for *FLT3* mutation, especially considering that target therapy with FLT3 inhibitors can be included in the induction regimen. In agreement, ELN2017 favorable-risk patients had lower LSC quantification at diagnosis. The implication of LSC quantification in survival was not statistically demonstrated due to technical limitations including the number of patients with available data, but remission and survival rates suggested that LSChigh group present worse outcomes. The findings suggest that LSC identification is a potential biomarker that can guide clinicians, particularly for assessment of AML with out karyotypic