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617.ACUTE MYELOID LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS

Presence of Recurrent Somatic Mutations in Mesenchymal Stromal Cell Fractions Isolated from Acute Myeloid Leukemia As an Evidence of Clonality

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Introduction: Acute myeloid leukemia (AML) involves somatic mutations in hematopoietic stem cells (HSCs). Recent evidence suggests the bone marrow microenvironment, particularly mesenchymal stromal cells (MSCs), also influences AML development. Abnormalities in MSCs from AML patients have been observed (von der Heide et al., 2016), potentially impacting leukemia progression. This study aims to identify MSC somatic mutations and their consequences, further exploring their role in AML. Understanding MSCs' impact on the leukemic microenvironment may shed light on AML pathophysiology and guide targeted therapies, particularly benefiting patients with adverse risk disease.
Methods: A total of 28 bone marrow cases were collected from AML patients with adverse risk, including those with complex karyotype, monosomy karyotype, chromosome 5/7/17 abnormalities, TP53 mutation, or relapsed/refractory AML. From these cases, 14 paired MSC and leukemic cell (LC) fractions were analyzed and are presented here. Magnetic bead sorting was used to isolate CD33+ LCs, while the plastic adherence method was employed for MSC culture. Following magnetic isolation, CD33- cells were plated in DMEM-LG with FBS and pen/strep, and MSC colonies formed within 48 hours. Contaminating cells were removed through media changes, and passaging was performed at 70-80% confluency. Flow cytometry at passage 3 characterized MSCs based on cell surface expression of CD105, CD73, and CD90, in accordance with the ISCT guideline.
DNA and RNA were extracted from MSCs at passage 3 and subjected to whole exome sequencing (WES) using the Illumina platform, along with the LC fractions. WES aimed for 200X depth for the paired DNA samples. A standard bioinformatics pipeline was used for sequence alignment and variant calling, with variants requiring sufficient read depth (≥30x) and variant allele frequency in the case (>5%) and in the control (<5%). Synonymous variants were filtered out, and gene ontology and pathway enrichment analyses explored the biological significance and potential involvement in leukemogenesis of the identified mutations. Whole transcriptomics for gene expression analyses was also conducted for both MSC and LC fractions.
Results: In 14 analyzed cases, a total of 548 somatic variants were identified in the MSC fraction, affecting 480 genes, including 518 nonsynonymous single nucleotide variants (SNVs), 7 stop-gain SNVs, 1 stoploss, 1 non-frameshift insertion, 2 frameshift deletions, 1 frameshift insertion, and 2 non-frameshift deletions, with 16 unknown variants. The average read depth was 75 (range: 15 - 887), and the average variant allele frequency was 50% (range: 11% - 90%). On average, 38 genes were mutated per MSC case, with 8 genes detected in at least 3 cases, 25 in exactly 2 cases, and 447 genes found in only one case.
In the leukemic fraction, 686 somatic variants in 595 genes were identified, with an average read depth of approximately 75 (range: 10 - 2596) and an average variant allele frequency of 47% (range: 8% - 85%). On average, 48 mutations were detected per leukemic case, with 12 mutations found in at least 3 cases, 40 in 2 cases, and 543 in only one case. Among the 14 cases,

10 cases (71%) had mutations in known driver genes for AML (*FLT3, NPM1, IDH1, IDH2, DNMT3A, RUNX1, TP53*) exclusively in the leukemic fraction.

As seen in Figure 1., of the 480 genes mutated in the MSC fraction, 69 overlapped with the 595 genes mutated in the leukemic fraction, while 411 genes were uniquely mutated in the MSC fraction. Further analysis is ongoing, and pathway analysis will be conducted once the remaining 14 cases are processed.

Conclusions: This study identifies distinct somatic mutations in AML patients' MSC and leukemic cell fractions, revealing genomic complexity and crosstalk impacting leukemia progression. Understanding the functional implications of these mutations is crucial for unraveling their roles in leukemogenesis and developing personalized therapeutic interventions targeting MSC somatic mutations.

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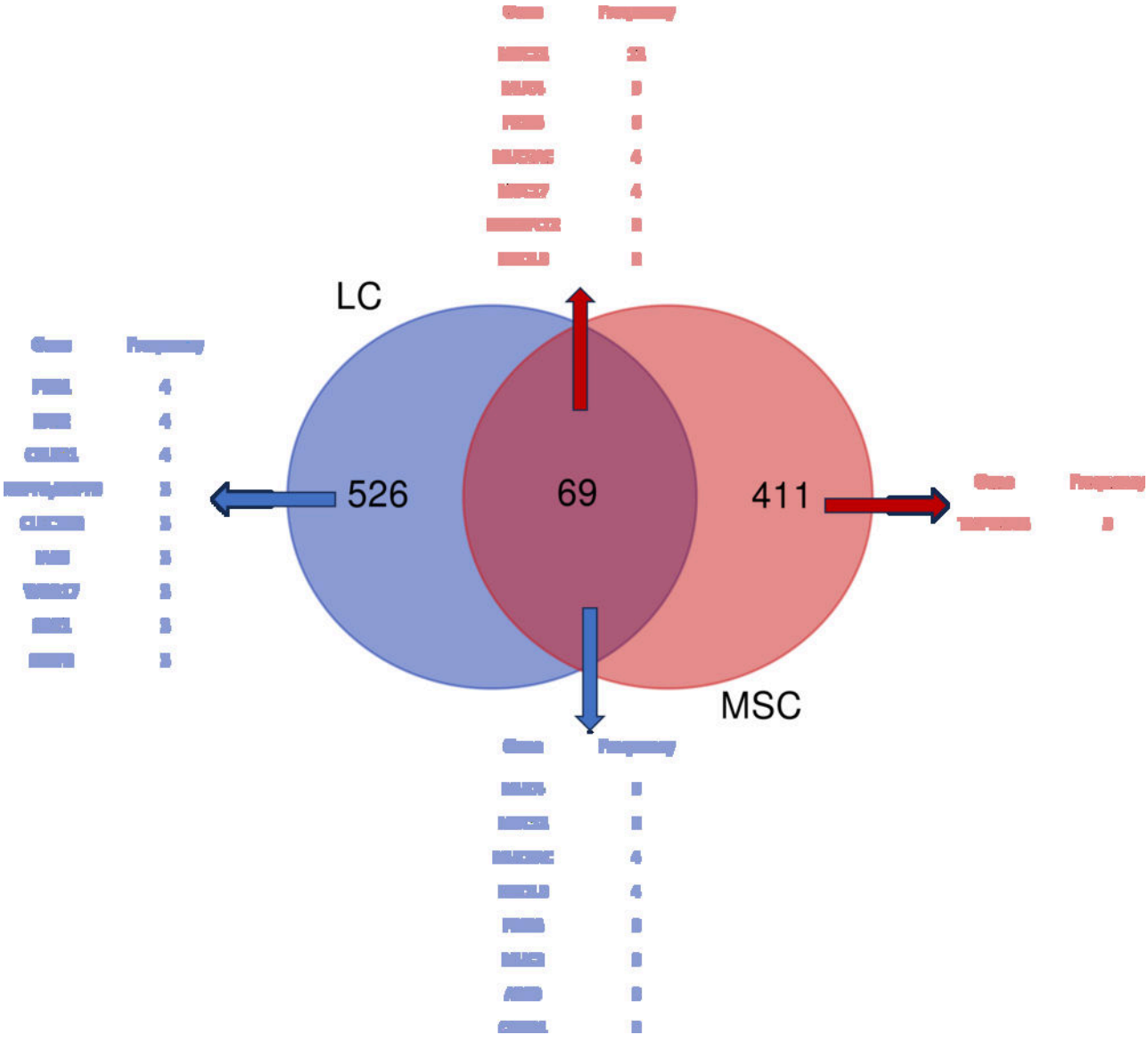

Figure 1

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