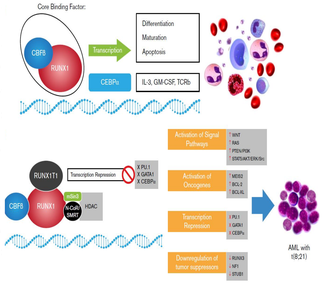
Analysis of the Genomic Heterogeneity of CBF-AML Using High-throughput Sequencing

E. Motyko[1], A. Kirienko[1], D. Kustova[1], M. Bakai[1], O. Uspenskaya[2], E. Karyagina[3], V. Shuvaev[1], S. Voloshin[1], S. Sidorkevich[1], I. Martynkevich[1]
[1]Russian Research Institute of Hematology and Transfusiology, St. Petersburg, Russia;
[2]Leningrad Oblast Clinical Hospital, St. Petersburg, Russia;
[3]Municipal Clinical Hospital No. 15, St. Petersburg, Russia

Background, objective

A subgroup of acute myeloid leukemia (AML) with chromosomal rearrangements of t(8;21) and inv(16)/t(16;16) is referred to as CBF-AML. Despite the fact that CBF leukemia in different classifications is referred to the group with a favorable prognosis, in 30-50% of patients there is relapse of the disease.

The group is rather heterogeneous with respect to the duration of complete remissions (CR), which may be due to the presence of additional risk factors - advanced age, hyperleukocytosis, changes in karyotype, and the presence of a wide range of gene mutations found in over 90% of CBF-AML patients.



Al-Harbi, Blood advances, 2020

Objective: To characterize the mutational profile of patients with CBF-AML using high-throughput sequencing.

Materials & Methods

This study was performed using the information about patients observed in hematology clinics from 2015-2021 . A total of 28 CBF-AML patients: 13 patients with inv(16) and 15 patients with t(8;21), all with de novo AML, 18 (64.2%) male and 10 (35.8%) female, median age was 39 years (18-74). For all patients, sequencing was performed using a panel of 121 genes on a Miseq sequencer (Illumina). A 3% allele frequency threshold (VAF) was used to analyze of the data obtained.

Results

74 mutations were detected in 39 of 121 target genes: 48 mutations in patients with t(8;21) and 26 mutations in those with inv(16). Mutations were found repeatedly in 20 genes (≥2 times). At least 1 mutation was found in 93% of patients with t(8;21) and 92% of patients with inv(16). On average, significantly more mutations were found in patients with t(8;21) than in patients with inv(16), 3.2 versus 2 mutations, respectively (p=0.001). In AML patients with t(8;21), 31 different genes were mutated; with inv(16), 17 genes contained mutations.

Of the 39 affected genes, mutations in 9 genes were common in both CBF-AML subgroups, whereas patients with t(8;21) had a significantly higher proportion of private mutations (70% (22) versus 47% (8), respectively; p=0.012).

Regarding the type of mutations detected, these were point mutations and insertions/deletions (in the *KIT, ASXL1, EZH2, CBL, KMT2C, MYC, FAT1, RAD21, MGA* genes). Mutations in RTK/RAS signaling pathways were the most common events in the two types of CBF-AML, with the highest incidence in *NRAS+KRAS* (18%; t(8;21), 20%; inv(16), 15%), *KIT* (21%; t(8;21), 20%; inv(16), 23%) and *FLT3* (7%; t(8;21), 0%; inv(16), 15%).

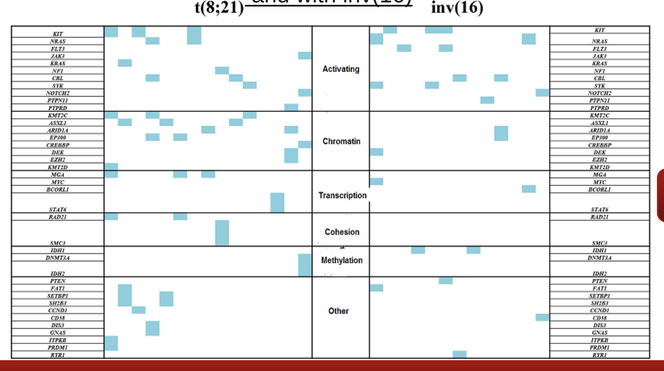
Gene mutations in CBF-OML patients



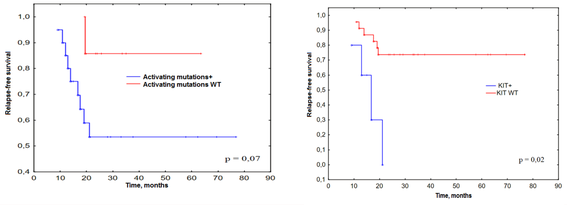
We characterized the molecular genetic profile of patients with t(8;21): mutations were found in epigenetic regulation genes - *ASXL1* (20%) and *EZH2* (7%), DNA methylation - *DNMT3A* (7%), cohesion complex - *RAD21* (13%) and *SMC3* (7%), *MGA* (20%), involved in MYC signaling pathway, *KMT2C* (27%), *SETBP1* (13%), which were absent in AML with inv(16). Molecular genetic profile of patients with inv(16) AML: aberrations of genes involved in chromatin modification were rare events (in the *ASXL1* gene - 0%), as were mutations related to the cohesion complex, which were absent in all cases. A mutation in the transcriptional corepressor BCORL1 was detected in one patient.

When analyzing the prognostic effect of mutations in genes that activate signaling pathways, patients with such mutations tended to have an increased risk of relapse compared to patients without them (p=0.07). Patients with mutations in the *KIT* gene had a significantly worse recurrence-free survival rate than patients without mutations (p=0.02).

Molecular-genetic profile of patients with AML with t(8;21) and with inv(16)



Prognostic significance of mutations involved in activation of signaling pathways and in KIT gene



Summary

1. The molecular genetic profile of patients with CBF-AML is highly heterogeneous.
2. In spite of belonging of AML with t(8;21) and inv(16) to the same CBF-AML group, each subgroup has its own characteristic biological and clinical features.
3. Activating mutations in tyrosine kinase receptor genes (*KIT, RAS*) are frequent events in both subgroups of CBF-AML.
4. Mutations in genes involved in the activation of signaling pathways and in particular in *KIT* gene increase the risk of relapse in CBF-AML patients.
5. The use of high-throughput sequencing is reasonable for the diagnosis of CBF-AML because of the ability to analyze a massive volume of data and obtain a broad characterization of the mutational profile for each patient.

References

1. Litzow M.R. The increasing complexity of the management of core-binding factor AML. Haematologica. 2020; 105(6): 1475-77
2. El Achi H., Kanagal-Shamanna R. Biomarkers in Acute Myeloid Leukemia: Leveraging Next Generation Sequencing Data for Optimal Therapeutic Strategies. Front. in oncology.2021;11: 74825