Classification of Acute Myeloid Leukemia and Myelodysplastic Syndrome Based on Molecular Profiling

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ABSTRACT

Introduction: Acute myeloid leukemia (AML) is currently distinguished from myelodysplastic syndrome (MDS) based on the presence of 20% blasts in bone marrow, an arbitrary cut-off adopted by the WHO classification and replacing the 30% cut-off required by the older FAB (French, American and British) classification. Patients with t(15;17), t(8;21), or inversion 16 cytogenetic abnormalities are classified as having AML irrespective of the percentage of blasts. We explored the possibility that currently defined molecular abnormalities can distinguish AML from MDS without relying on an arbitrary percentage of blasts in the bone marrow. We compared the molecular profiles obtained by next generation sequencing (NGS) from consecutive patients with a clinical diagnosis of AML or MDS by WHO criteria.

Methods: NGS data from 251 patients with the diagnosis of AML and 294 patients with the diagnosis of MDS was studied. All samples were analyzed using a panel of 25 genes including FLT3, NPM1 SF3B1, CBL, DNMT3A, ASXL1, BRAF, CEBPA, CSFR3, ETV6, EZH2, IDH1, IDH2, JAK2, c-KIT, KRAS, NRAS, PHF6, PTPN11, RUNX1, SETBP1, TET2, TP53, WT1, and ZRSR2. We compared the frequency of mutations in each gene between AML and MDS patients.

Results: Mutations in FLT3 and NPM1 were uniquely and commonly detected in AML (27% and 22%, respectively). In contrast, mutations in SF3B1 gene were uniquely dominant (22%) in MDS and FLT3 and NPM1 mutations were rare (2% and 3%, respectively). SF3B1 mutations were extremely rare in AML (1%). Overall, 102 (41%) of all AML patients had mutations in either FLT3 or NPM1 and 8% of AML patients had mutations in both FLT3 and NPM1. In addition, WT1 gene was mutated in 8% of AML cases, but none of the MDS cases showed WT1 mutation. TET2 gene was commonly mutated in both AML and MDS (25% and 36%, respectively), but the frequency was significantly higher in MDS (p=0.003). IDH1, IDH2, NRAS, and PTPN11 were mutated slightly more often in AML than in MDS, while ASXL1, EZH2, and ZRSR2 were more frequently mutated in MDS than in AML. There was no statistically significant difference in mutation frequency between AML and MDS for the other genes analyzed.

Conclusion: Mutations in FLT3, NPM1 and WT1 are molecular abnormalities characteristically detected in patients with AML and can be used as objective criteria for the classification of AML rather than blast count in bone marrow. These mutations are detected in 49% of AML patients. This suggests that approximately half of AML patients can be diagnosed based on the detection of molecular abnormalities, irrespective of bone marrow morphology. The presence of mutation in SF3B1 gene is also a characteristic molecular finding for MDS.

INTRODUCTION

Acute myeloid leukemia (AML) is currently distinguished from myelodysplastic syndrome (MDS) based on the presence of 20% blasts in bone marrow, an arbitrary cut-off adopted by the WHO classification and replacing the 30% cut-off required by the older FAB (French, American and British) classification.

Most of the recent studies suggest that clinical behavior of MDS and AML is dictated by the underlying molecular and cytogenetic abnormalities rather than the percentage of blasts in bone marrow. Patients with MDS and significant molecular cytogenetic and molecular abnormalities may die from their disease without ever transforming into acute myeloid leukemia. Recent study showed that no significant differences in the median overall survival of between patients with blast count from 10% to 20% and patients with blast count between 20% and 30%. Furthermore patients with AML and features of MDS (dysplastic changes) have molecular and clinical course similar to that seen in patients with MDS.

Blast count may vary dependent on the quality of bone marrow sample and can be subjective. In contrast, genetic composition can be more objective and more reliable.

In fact, even WHO classification allows patients with t(15;17), t(8;21) or inversion 16 cytogenetic abnormalities to be classified as AML irrespective of the percentage of blasts.

We explored whether molecular abnormalities can distinguish AML from MDS without relying on an arbitrary percentage of blasts in the bone marrow. We compared the molecular profiles obtained by next generation sequencing (NGS) from consecutive patients with a clinical diagnosis of AML or MDS by WHO criteria.

METHODS

Patients and samples

Bone marrow samples from 251 patients with the diagnosis of AML and 294 patients with MDS were used for testing by NGS. All work was performed with Institutional Review Board (IRB) approval.

DNA isolation

Total nucleic acid was isolated from plasma using QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions.

Gene sequencing

NGS was performed using an Illumina MiSeq system (San Diego, CA); NGS, amplification, and indexing were performed as recommended by the manufacturer. Amplicons were confirmed for each sample by running an agarose gel. Samples were pooled and the experiment sheet was generated using Illumina Experiment Manager. MiSeq Reporter was used for analysis and Variant Studio was used for calling. For confirmation of variant calling, NextGene software (SoftGenetics, State College, PA) was used. Average sequencing coverage across the entire coding regions was 10,000 in 90% of the sequenced amplicons.

RESULTS

1. AML unique molecular abnormalities

Mutations in FLT3 and NPM1 were uniquely and commonly detected in AML (27% and 22%, respectively). In addition, mutations in WT1 were uniquely detected in patients with AML, but were not as common as in MDS. IDH1, IDH2, NRAS, and PTPN11 were mutated slightly more often in AML than in MDS.

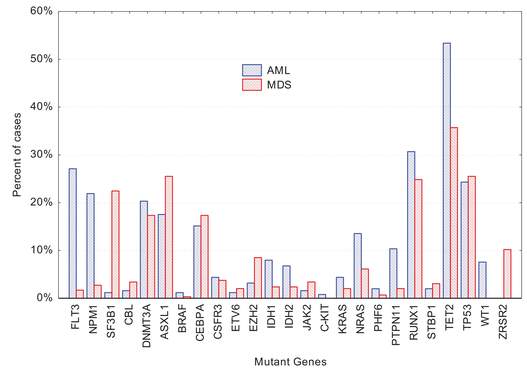

Figure 1. *Percentage of cases of AML (n=251) and MDS (n=294) with mutations*

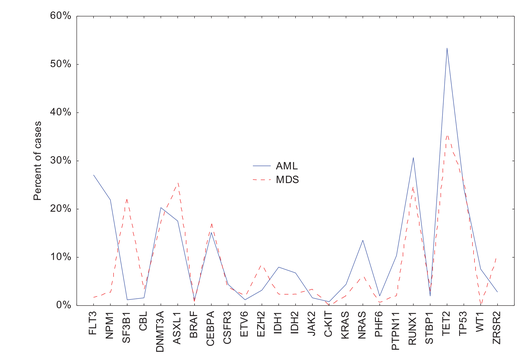

Figure 2. *Percentage of cases of AML (n=251) and MDS (n=294) with mutations*

Gene	AML (No=251)		MDS (NO=294)		P-Value
	No	%	No	%	
FLT3	68	27	5	2	0.00001
NPM1	55	22	8	3	0.0001
SF3B1	3	1	66	22	0.00006
CBL	4	2	10	3	NS
DNMT3A	51	20	51	17	0.07
ASXL1	44	18	75	26	0.01
BRAF	3	1	1	0	NS
CEBPA	38	15	51	17	NS
CSFR3	11	4	11	4	NS
ETV6	3	1	6	2	NS
EZH2	8	3	25	9	0.03
IDH1	20	8	7	2	0.03
IDH2	17	7	7	2	0.04
JAK2	4	2	10	3	NS
KIT	2	1	0	0	NS
KRAS	11	4	6	2	NS
NRAS	34	14	18	6	0.01
PHF6	5	2	2	1	NS
PTPN11	26	10	6	2	0.01
RUNX1	31	12	33	11	NS
SETBP1	5	2	9	3	NS
TET2	64	25	105	36	0.003
TP53	61	24	75	26	NS
WT1	19	8	0	0	0.01
ZRSR2	7	3	30	10	0.02

Table 1. *Mutation frequency and comparison in AML vs. MDS.*

2. MDS unique molecular abnormalities

Mutations in SF3B1 were uniquely detected in patients with MDS. Mutations in ZRSR2 as well as in EZH2 were more common in MDS than in AML (Figure 1).

3. Molecular abnormalities common in both AML & MDS

The TET2 gene was commonly mutated in both AML and MDS (25% and 36%, respectively), but the frequency was significantly higher in MDS (p=0.003) (Table 1).

There was no statistically significant difference in mutation frequency between AML and MDS for CBL or CEBPA. The rest of the tested genes had very low frequency and statistically were not different between AML and MDS (Table 1).

SUMMARY

1. **Mutations in FLT3, NPM1 and WT1 can be used as molecular markers for the diagnosis of AML and provide objective criteria for the classification of AML.**
2. **Since FLT3, NPM1, and WT1 mutations are detected in approximately half of AML patients, the diagnosis of AML can be made based on these findings, irrespective of bone marrow morphology or blast count.**
3. **The presence of mutation in SF3B1 gene is a characteristic molecular finding for MDS.**
4. **Correlation with clinical outcome and therapy is needed to determine the clinical relevance of these findings.**

Authors are employees of a diagnostic company offering NGS testing.