Molecular profiling in determining diagnosis and prognosis of "early" myelodysplastic syndrome

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ABSTRACT

Cytogenetic abnormalities, used to confirm a diagnosis of MDS, are detected by conventional cytogenetics and FISH in approximately 50% of cases. We report the use of a panel of 14 genes (ASXL1, ETV6, EZH2, IDH1, IDH2, NRAS, CBL, RUNX1, SF3B1, SRSF2, TET2, TP53, U2AF1 and ZRSR2) in a prospective fashion as an objective means for confirming the presence of MDS rather than a reactive process in cases with a normal karyotype. Fifty one of the 135 patients (38%), all with less than 5% blasts, had mutations in 1 to 3 genes. The results suggest that utilizing an appropriately designed, relatively small molecular panel can provide valuable objective diagnostic means for the diagnosis of MDS in patients without cytogenetic abnormalities. Such a panel may also provide important prognostic information that can be used for stratifying patients and determining specific therapeutic approach.
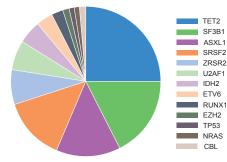
INTRODUCTION

Cytogenetic abnormalities have been the confirmatory biomarkers for MDS and are an important component in the diagnosis of the disorder. However, these abnormalities are identified only in approximately 50% of cases. Sequencing studies have identified driver mutations in RNA splicing, DNA methylation, chromatin modification, transcription regulation, DNA repair, signal transduction, and cohesin complex genes in MDS, in both cases with and without cytogenetic abnormalities. Confirming a diagnosis of MDS can be very difficult, especially in the early stages of the disease and in cases without apparent cytogenetic abnormalities. Most studies of gene mutations in MDS have been retrospective and included cases with and without cytogenetic abnormalities.

OBJECTIVE: Investigate the utility of using molecular profiling of driver genes in the diagnosis and confirmation of early MDS cases with <5% blasts.

SAMPLES AND METHOD

- 135 patients referred from community practice with a questionable diagnosis of MDS, with cytopenia involving at least one lineage, no evidence of cytogenetic abnormalities and blast count less than 5%

- DNA was extracted from bone marrow or peripheral blood specimens

- Genes investigated: DNA methylation genes (TET2 and IDH1/IDH2), RNA splicing genes (SF3B1, SRSF2, U2AF1 and ZRSR2), chromatin modification genes (ASXL1 and EZH2), transcription gene (RUNX1), DNA repair control gene (TP53), RAS pathway genes (NRAS, CBL) and transcription factor gene (ETV6)

- Bidirectional Sanger sequencing

RESULTS:

- Mutations in 1 to 3 genes were observed in 51 of 135 patients (38%).

- TET2 was most frequently mutated followed by SF3B1, ASXL1 and SRSF2 (Figure 1).

- In 16 of 20 cases with TET2 mutation, one or more additional genes were mutated (Figure 2).

- SF3B1 mutation detected alone (10 cases) or along with TET2 (4 cases) was observed in 14 of 51 cases (28%) of cases with mutations (Figure 2).

- 28 of 51 cases (55%) had a mutation in only one of the genes included in the panel (Figure 2).

- 23 of the 51 cases (45%) with mutations had a mutation in one or more additional genes included in the panel (TET2, SF3B1, SRSF2, ASXL1, ZRSR2, ETV6, EZH2 or IDH2) (Figure 2).
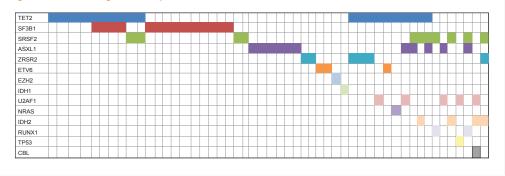
Figure 1. Frequency of mutations by gene

TET2, SF3B1, ASXL1, SRSF2, ZRSR2, U2AF1, IDH2, ETV6, RUNX1, EZH2, TP53, NRAS, CBL

Table. Comparison of frequency of genes mutated in the current series and in reports in the literature

Mutated Genes	Number of cases in this study (%)	Cazzola et al. 2013 (%)	Papemmanuil et al. 2012 (%)	Malcovati et al. 2011 (%)	Haferlach et al. (%)
TET2	20 (14.8)	20-30	22	20-25	30-35
SF3B1	14 (10.4)	15-30	24	25-30	30-35
ASXL1	11 (8.1)	15-20	~12	10-15	20-25
SRSF2	11 (8.1)	10-20	14	10-15	15-20
ZRSR2	6 (4.4)	<10	<5	5	5-10
U2AF1	5 (3.7)	<10	<10	5-10	5-10
IDH2	4 (3.0)	~5		2-3	0-5
ETV6	3 (2.2)			2	<5
RUNX1	2 (1.5)	~10		10-20	1-15
EZH2	1 (0.7)	~5		5	10
TP53	1 (0.7)	~5		5-10	5-10
NRAS	1 (0.7)			5-10	<5
CBL	1 (0.7)			1-2	~5

Figure 2. Cases with single and multiple mutations

DISCUSSION:

An appropriately designed, relatively small molecular panel is a valuable and objective diagnostic means for the diagnosis of MDS patients without cytogenetic abnormalities. Mutations were observed most frequently in TET2, SF3B1, ASXL1 and SRSF2. Analysis of only these genes would have identified abnormal clones in 43 of 135 (32%) cases compared to 38% with a panel of 14 genes.

The genes listed above are likely adequate to initiate MDS but may require additional genetic changes for clinically overt neoplastic changes as illustrated by TET2, with additional genes mutated in the majority of cases with mutation of TET2 (10 of 16; 63%).

The relative frequency of specific genes mutated in this series of MDS, with less than 5% blasts, is comparable to that reported in the literature of confirmed MDS cases[1,2,3,4], indicating the importance of the genes included in this panel in the biology and etiology of MDS.

Genes mentioned above were not mutated in eight (8) cases, suggesting the importance of genes other than TET2, SF3B1, ASXL1 and SRSF2 in the etiology of MDS. Comparative analysis of cases with and without mutations of TET2, SF3B1, ASXL1 and SRSF2 genes may help determine if the two groups are biologically and clinically different.

CONCLUSION

Mutations in the same genes in "early" MDS (with apparently normal karyotype and <5% blast), as in confirmed cases of MDS, reflects the significance of these genes in the etiology of the disorder. Hence, evaluating these genes can be informative in the diagnosis of "early" MDS.

In the absence of cytogenetic markers, genetic characterization of MDS at an "early" stage may be important to benefit from specific therapeutic intervention and better clinical outcome.

While mutations in SF3B1 are associated with good overall survival, mutations in SRSF2, U2AF1, IDH1/IDH2, ASXL1, EZH2, RUNX1 and TP53 have been reported to be unfavorable. Hence characterization at the gene level is an important level of classification of MDS and should be considered in the diagnostic work up of MDS.

Next generation sequencing should enhance the ability to obtain information on a larger cohort of genes that could aid in better diagnosis and classification of MDS.

Limitation of this study: Longer follow up is needed to rule out MDS and confirm a reactive process in patients without a mutation.

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