Interclonal and Intraclonal Heterogeneity in Patients with Early Myelodysplastic Syndrome (MDS)

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ABSTRACT

Introduction: Recent data suggest that MDS evolves by accumulating mutations. Early mutations may involve genes that require additional mutations prior to clinical manifestation as MDS. We explored if mutant allele burden and the relative mutation of one gene to another gene could provide information on the interclonal and intraclonal progression of MDS using next generation sequencing (NGS) in patients with early MDS.

Methods: NGS data was generated from 96 patients diagnosed with MDS with marrow blast count <5% using a targeted sequencing covering mutations in the following genes: TET2, SF3B1, ASXL1, DNMT3A, SRSF2, RUNX1, NRAS, ZRSR2, EZH2, ETV6, TP53, CBL, NPM1, JAK2, U2AF1, IDH1, KRAS, IDH2, FLT3, PTPN11, SETBP1, and BCOR. The average depth of sequencing was 10,000X. Differences in mutant allele frequency between two genes in the same sample were considered significant if they were >10%. A difference of 10% to 20% was considered mild, 20%-30% moderate, and >30% severe. A heat map reflecting these differences in mutant allele frequency was generated.

Results: In this group of early MDS patients, 63 patients (66%) had more than one gene mutated and 38 (40%) had a significant (>10%) difference in allele frequency. The median number of genes mutated was 2 (range 1 to 5). Difference in mutant allele frequency was severe in 15 patients (16%), intermediate in 15 patients (16%), and mild in 13 patients (14%). TET2 was the most commonly mutated gene (43 patients, 45%) and was rarely the sole mutation with most cases exhibiting a mutation in a second gene (39 patients, 91%). The mutant allele burden was highest in TET2 in 26 of these 39 patients (67%), reflecting early event in the tumorigenic process. Of the 13 cases with TET2 mutation and allele burden less than the companion gene, 6 had a mutation in SF3B1, 3 had significant cytogenetic abnormalities (monosomy 5, del(7q), and trisomy 8), 2 had a mutation in SRSF2, 1 had a mutation in ZRSR2 and 1 had a mutation in ASXL1, which suggests that these abnormalities might be the initiating event. A second TET mutation (biallelic mutation) was detected in 16 of the 39 patients. SF3B1 was the most common gene having a solitary mutation (10% of all patients), although mutation in SF3B1 was detected in 27 patients (26% of all patients). All solitary SF3B1 mutations were associated with normal karyotypes, except for one patient with del(11q). JAK2 was mutated with SF3B1 in two cases diagnosed as RARS-T (refractory anemia with ring sideroblasts and thrombocytosis). In one case, the JAK2 and SF3B1 mutation allele frequencies were similar, but in the other, the JAK2 mutant allele frequency was 23% higher, suggesting that a myeloproliferative neoplasm was the initiating process. ASXL1 was mutated in 14 cases, 13 of which had additional mutations. DNMT3A gene was mutated in 18 cases, 5 of which were solitary; two of these five showed cytogenetic abnormalities. TP53 was mutated in 13 cases, but except for one case, all had either mutation in another gene or a cytogenetic abnormality.

Conclusion: These data suggest that in patients with clinically confirmed early MDS, TET2 mutations are most likely the initiating oncogenic event, but mutations in other genes or cytogenetic abnormalities most likely lead to clinically confirmed MDS. In contrast, patients with SF3B1 mutation can have clinical disease without additional mutations. Our data suggest that SRSF2, ZRSR2, and ASXL1 may initiate mutagenesis in patients with MDS.

INTRODUCTION

Myelodysplastic syndrome (MDS) is a heterogenous neoplastic disease that involves hematopoeitic stem cells. The current data suggests that in most patients, this disease starts with genomic abnormalities that accumulate with time until they lead to significant changes in hematopoietic cells resulting in clinical manifestation. Therefore, the disease is diagnosed mainly in elderly patients, most of the time as a chronic disease manifesting in peripheral cytopenia. Unlike the localized dysplastic preneoplastic lesions in epithelial tissue, hematopoietic pre-neoplastic cells are mixed and multiple clones can be detected in patients with MDS when high sensitivity assays, such as next generation sequencing, are used.

At the time of clinical manifestation, more than one clone is usually detected. The most aggressive clone is the dominant one, but this clone might be competing with other clones (interclonal) as well as with its own subclones (intraclonal) for dominance.

In this study, we studied the spectrum of interclonal and intraclonal hetrogeniety in early MDS (blast count <5%). Early MDS cases are chosen to avoid the potential that in more advanced cases, one dominant aggressive clone may suppress other less aggressive clones.

METHODS

Patients and samples

Bone marrow samples from 96 patients with MDS and marrow blasts <5% 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.

SUMMARY

- **In patients with clinically confirmed early MDS, TET2 mutations are most likely the initiating oncogenic event.**
- **Subsequent mutations in genes other than TET2 or cytogenetic abnormalities most likely lead to clinically confirmed MDS.**
- **SF3B1 mutation can induce clinical disease without additional mutations.**
- **SRSF2, ZRSR2, and ASXL1 may initiate clinically significant MDS.**

RESULTS

1. Mutations in multiple genes

- 63 of 96 patients (66%) had more than one gene mutated.

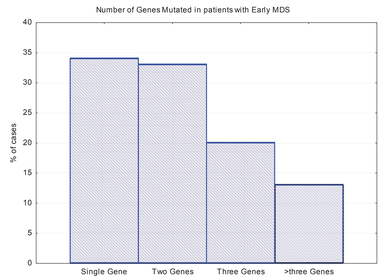

Figure 1. *Proportion of cases with mutations in one, two, three, and more than three genes.*

2. Multigene mutation is more common with normal cytogenetics (p=0.08)

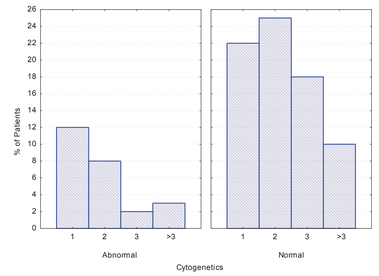

Figure 2. *Proportion of cases with mutations in one, two, three, or more than three genes in cases with normal and abnormal karyotypes.*

3. Variation in the mutant allele burden

3A. TET2

- Most commonly mutated gene (43 patients, 45%)
- Rarely the sole mutation with most cases exhibiting a mutation in a second gene (39 patients, 91%)
- The highest mutant allele burden in 26 of these 39 patients (67%), reflecting early event in the tumorigenic process.
- Of 13 cases with TET2 mutation and allele burden less than the companion gene, 6 had a mutation in SF3B1, 3 had significant cytogenetic abnormalities (monosomy 5, del(7q), and trisomy 8), 2 had a mutation in SRSF2, 1 had a mutation in ZRSR2 and 1 had a mutation in ASXL1, suggesting that these abnormalities, rather than the mutation in TET2 might be the initiating event.
- A second TET mutation (biallelic mutation) was detected in 16 of the 39 patients.

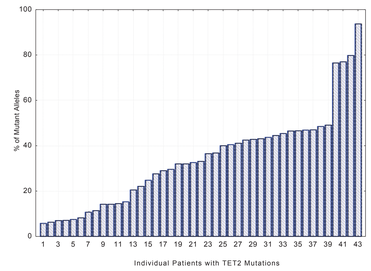

Figure 3a. *Profile of percent of mutant alleles involving TET2 gene.*

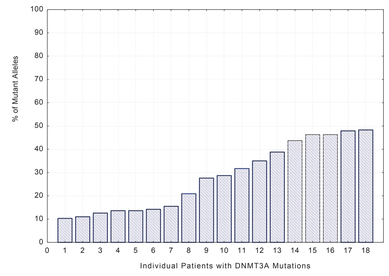

Figure 3c. *Profile of percent of mutant alleles involving DNMT3A gene.*

3B. SF3B1

- Mutation detected in 27 patients (26% of all patients).
- Most common gene having a solitary mutation (10% of all patients).
- All solitary SF3B1 mutations were associated with normal karyotypes, except for one patient with del(11q).

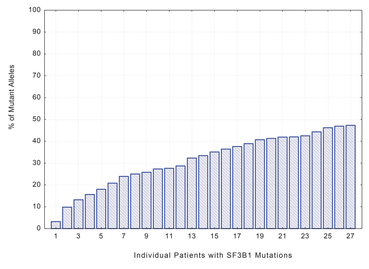

Figure 3b. *Profile of percent of mutant alleles involving SF3B1 gene.*

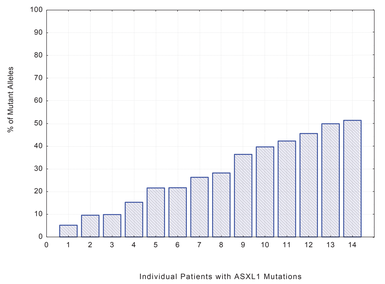

Figure 3d. *Profile of percent of mutant alleles involving ASXL1 gene.*

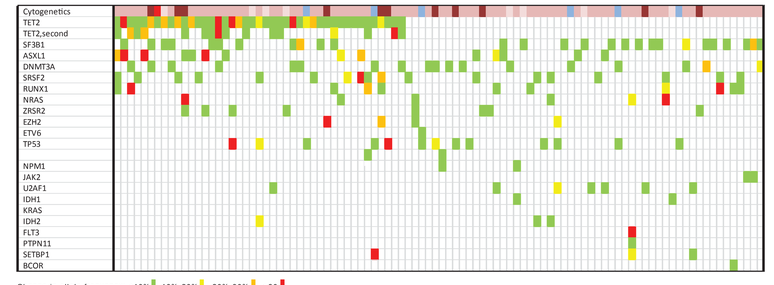

Figure 4. *Heat map reflecting the differences in mutant allele frequency and prognosis based on karyotype.*

Authors are employees of a diagnostic company offering NGS testing.