**INTRODUCTION**

DNA methylation in AML/MDS plays a major role in the pathogenesis of acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). The major genes involved in DNA methylation in AML/MDS are IDH1 and IDH2, TET2 and DNMT3A. Mutations in IDH1/2 result in the production of an aberrant metabolite, 2-hydroxyglutarate, which acts as a competitive inhibitor of alpha-ketoglutarate and inhibits TET2 oxidation of 5-methylcytosine to 5-hydroxymethylcytosine (5hmC). Mutations in TET2 or IDH1/2 are associated with reduced levels of 5hmC and genomic hypermethylation. TET2 mutations were detected in 15 (7.5%) of the 198 patients. IDH1/2 mutations were detected in 201 of the 1182 (17%). IDH1 was detected in 87 (7.4%). IDH2 was detected in 112 (10.1%), including 6 patients with mutations in both IDH1 and IDH2.

**OBJECTIVE**

Toward better understanding of interaction between these genes, the mutation profile of these genes was analyzed in patients with AML/MDS.

**SAMPLES AND METHODS**

**Samples**

1182 bone marrow aspirates

**DNA extraction**

DNA was extracted from bone marrow aspirate using the QIamp DNA Mini Kit

**Sequencing**

TruSight Myeloid Next Generation Sequencing Panel (Illumina, San Diego, CA) covering hot spot mutations in 54 genes

Average depth of sequencing of 10,000X

**RESULTS**

1. Co-Occurrence of IDH1 & IDH2 Mutations
   - IDH2 mutations were detected in 201 of the 1182 (17%).
   - IDH1 was detected in 87 (7.4%).
   - IDH2 was detected in 112 (10.1%), including 6 patients with mutations in both IDH1 and IDH2.

2. Co-Occurrence of TET2 & IDH1/2 Mutations
   - TET2 mutations were detected in 15 (7.5%) of the patients with IDH1/2 mutations.
   - There was significant difference (P=0.03) in VAF between IDH1/2 and TET2.
   - Nine patients showed comparable VAF while 6 patients showed completely different VAF, suggesting subclonal heterogeneity.

3. Co-Occurrence of DNMT3A & IDH1/2 Mutations
   - DNMT3A mutations were detected in 3 patients.
   - There was no significant difference in VAF between IDH1/2 and DNMT3A.
   - While there was no significant difference in VAF between IDH1 and DNMT3A, VAF in IDH1 was <50% in 6 of these patients and in DNMT3A in 3 patients.

4. Co-Occurrence of ASXL1 & IDH1/2 Mutations
   - ASXL1 mutation was detected in 35 patients.
   - High Frequency of IDH1/2 and ASXL1 mutations: 5 (IDH1/2 mutations and ASXL1 mutation were detected in 25 patients with IDH1/2 mutations (7%). The VAFs of the two mutations were overall similar without statistical difference.

5. Co-Occurrence of TP53 & IDH1/2 Mutations
   - TP53 mutation was detected in 24 patients.
   - Twenty four patients had TP53 mutation, of which 16 had IDH1 mutation and 8 had IDH2 mutation, which is disproportional with the prevalence of IDH1 mutation.
   - There was no statistically significant difference in VAF between TP53 and IDH1/2, but 4 of these patients had both DNMT3 and IDH2 mutations and one had both IDH1 and IDH2 mutations.
   - None of the patients with TP53 mutation had TET2 mutation.

**CONCLUSIONS**

- IDH1 mutations may coexist with IDH1 and TET2 mutations. This co-mutation appears to be in the same clone in some patients and in a separate clone in others.
- The presence of VAF>50% in 6.5% of patients, suggesting homozygosity, along with co-presence of IDH1 and IDH2 and TET2 mutations suggests possible dosage effects in the biology of MDS/AML.
- The high rate (29%) of co-presence of DNMT3A with IDH1/2 mutations suggests cooperation between the two mechanisms in influencing DNA methylation and leukemogenesis.
- The relatively high incidence of TP53 mutation in IDH1 patients suggests that IDH1 mutation might be associated with more aggressive disease than IDH2.
- This data suggests that there is interaction and significant interclonal and intraclonal heterogeneity in DNA methylation genes in AML/MDS.
- Complete profiling of these genes is necessary for better understanding and proper prediction of clinical behavior particularly when patients are treated with DNA methylation inhibitors.

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- DNA was extracted from bone marrow aspirate using the QIamp DNA Mini Kit
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