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POSTER ABSTRACTS

636.MYELODYSPLASTIC SYNDROMES-BASIC AND TRANSLATIONAL

Somatic Mutations and DNA Hypermethylation at Enhancers and Promoters Identify Distinct Subtypes within Lower-Risk Myelodysplastic Syndromes

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Myelodysplastic syndromes (MDS) are a group of heterogeneous disorders caused by the accumulation of somatic mutations in the hematopoietic stem and progenitor compartment. Besides del(5q), *SF3B1* or *TP53* mutations, referred to as defining genetic abnormalities, mutation patterning hardly structured the classification of MDS. Mutations in epigenetic factors occur early in the development of clonal hematopoiesis leading to precocious alterations of DNA methylation implicated in oncogenesis. Convergent DNA methylation patterns related to both mutations and microenvironment imprinting may induce specific gene expression profiles and contribute to phenotypic variations. To refine a pathophysiological classification of lower-risk MDS, we combined genetic profiling to DNA methylation and transcriptome data.

Methods: We enrolled 543 lower-risk treatment-naïve MDS patients at diagnosis and performed DNA-sequencing of 24 genes. DNA methylation and RNA-sequencing data were generated in a representative subset of 175 cases with available material (75 MDS-SLD/MLD, 40 MDS-EB1, 53 MDS-RS-SLD/MLD, 5 MDS del(5q), 2 MDS-U) and 7 age-matched healthy controls. IPSS-R was very low, low or intermediate in 87.4%. During a median follow-up of 2 years, 16% of MDS cases progressed to AML (MDSp). Infinium EPIC 850K array allowed after filtering the examination of methylation of 723,612 CpG sites with enrichment at enhancers and promoters. Differentially methylated regions with differentially methylated CpG sites (DMR-CpG) between cases and controls were defined with a minimum of 2 probes, a Benjamini-Hochberg-adjusted *P*-value cut-off of 0.05, a false discovery rate of 0.001 and a mean $\Delta\beta$-value >20% at CpG sites. Consensus motifs for transcription factors were identified using Homer.

Results: Unsupervised clustering on genetic profiling of 543 patients identified distinct subsets of lower-risk MDS with different clinical features: one cluster (group A) was enriched in del(5q), three clusters were characterized by *SF3B1* mutations, either isolated (group B) or associated with *DNMT3A* (group E) or *TET2* (group F), while the two remaining clusters were enriched in either complex pattern of mutations (group C) or *SRSF2* mutations (group D). Genetic clusters showed significantly different clinical outcomes, clusters C, D and E having lower overall survival and higher risk of progression to high risk MDS or AML compared to other clusters. In the subset of 175 cases, a total of 2,953 unique DMR-CpGs allowed a robust repartition of patient samples in 4 groups using the k-means method. The 4 methylation groups were clinically distinct in terms of age, hemoglobin, neutrophils, monocytes, platelets, BM blasts, WHO classification, IPSS-R, and mutation pattern. A significant correlation was found between genetic and methylation groups ($P<0.001$). Hypomethylated CpGs defined group 4 which was enriched in MDS-RSwith low blast count and few co-mutations, while groups 1 and 3 demonstrated hypermethylated profiles driven by *TET2/ IDH1-2* mutations and *TET2/ SRSF2* mutations, respectively. *TET2*-mutated cases exhibited an increased proportion of hypermethylated DMR-CpGs at enhancers (DME) significantly enriched in C/EBP and ETS family transcription factor motifs. In MDS without evidence of progression, we identified 1,418 DME and their gene targets of which those significantly deregulated in RNA-seq were involved in the control of translation and response to TGF-β. By contrast, in MDS with rapid progression to AML, DMR-CpGs were significantly enriched at CpG islands and promoters ($P<0.0001$) and a set of 10-20 genes downstream of these promoters were specifically deregulated.

Conclusion: Somatic mutations and DNA methylation at enhancers and promoters identify distinct subsets of lower-risk MDS with different clinical behavior. Hypermethylation at enhancers with C/EBP or ETS family motifs is a hallmark of MDS and may account for changes in transcription factor recruitment and cell fate. Hypermethylation of promoters with downstream effects on gene expression may indicate a propensity to rapid evolution to AML.

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