Highlights in myelodysplastic syndromes

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SUMMARY

The past years enormous progress has been made in the understanding of the pathogenesis of myelodysplastic syndromes (MDS).[1] The introduction of next-generation sequencing (NGS) and whole-exome sequencing (WES) has revealed that about 90% of patients with MDS harbor mutations in pathways such as spliceosome machinery, in epigenetic regulators and other genes previously unknown to be involved in MDS. At this year's conference, the focus was on these mutations. There have been no major breakthroughs on the therapeutic side.

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ARID2 –MEDIATED MDS

A collaborative study of Seishi Ogawa's lab in Tokyo with investigators of the Cleveland Clinic in the USA identified a role of *"At rich interactive domain 2" (ARID2)* mutations in patients with MDS and other myeloid neoplasms. *ARID2* is located on chromosome 12q and encodes a component of the SWI/SNF complex, involved in chromatin remodeling. About 10% of patients with MDS demonstrate decreased *ARID2* expression due to multiple kinds of genetic lesions (mutations, deletions). Decreased expression of *ARID2* was also shown to be the result of alternative splicing by *U2AF1* mutations. Morphologically, decreased expression of *ARID2* leads to hypo-lobulated megakaryocytes. Functional assays demonstrate that decreased expression of *ARID2* leads to more myeloid and erythroid differentiation and more apoptosis, resulting in reduced cell populations without a reduction in the proliferative capacity of hematopoietic progenitor cells.[2] These results have recently been published.[3]

In summary, *ARID2* expression is abnormal in 10% of cases with MDS and results in morphologic (hypolobulated megakaryocytes) and functional aberrancies

IMPACT OF SPLICEOSOME MUTATIONS

Mutations in spliceosomal genes (including *SF3B1, SRSF2* and *U2AF1*) occur in more than 50% of patients with MDS. Andrea Pellagatti from the University of Oxford presented the results of a large collaborative investigation.[4] The aim was to identify which pathways are deregulated as a result of aberrant splicing due to spliceosome mutations and to gain new insights into how these mutations impact cellular processes. They therefore performed RNA sequencing on bone marrow $CD34^+$ cells of 91 MDS patients (including 42 cases with spliceosome mutations). The aberrant splicing events associated with each mutated splicing factor tended to affect different sets of genes, although some overlap was observed. Gene ontology analysis showed a marked convergence of aberrantly spliced genes associated with *SF3B1, SRSF2* and *U2AF1* mutations, including RNA splicing and translation. *SF3B1* mutations resulted in aberrant splicing of iron transporter *ABCB7*.[5]

RNA-sequencing was also performed on $CD34^+$ and different erythroid, granulocytic and monocytic cell populations isolated from bone marrow of *SF3B1* mutant cases. Pathway analysis revealed that several pathways were deregulated in specific cell populations (e.g. 'mTOR signaling" in erythroid cells), whereas transcription factor *E2F1* signaling, involved in protein synthesis initiation were deregulated in all cell populations studied.

In summary, different spliceosome mutations lead to lineage specific RNA splicing deregulation. Although mutations in different spliceosome genes lead to a specific set of aberrantly spliced genes, they share markedly convergent pathways.

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PROGNOSIS OF GENE EXPRESSION PROFILING

Seishi Ogawa's group of Tokyo University determined whether gene expression profiling (GEP) could add prognostic information in patients with MDS.[6] Transcriptome sequencing was performed on bone marrow mononuclear cells (BM MNC) and CD34$^+$ cells of 100 patients (training cohort) and of 183 patients in a validation cohort. Unsupervised clustering of gene expression data identified two subgroups (Class-I and Class-II). Patients in the Class-II cluster had higher BM blasts and many signaling pathways were upregulated. The erythroid signature was rather suppressed in the Class-II subtype and was characterized by an increased expression of genes related to progenitor cells. The Class-II subtype was associated with a significantly shorter survival in both univariate and multivariate analysis. There was a higher frequency of leukemic transformation in the Class-II subgroup (38%) compared to no leukemic transformation in the Class-I subgroup. The prognostic significance of the classification was validated in an independent cohort of 183 patients. The model was also used to predict the subgroups using GEP of BM MNCs. Again, the association was more pronounced for leukemic transformation (HR[95%CI]: 18[4.2-80], p< 0.001). A multivariate analysis also demonstrated that the predicted Class-II subgroup was independently associated with leukemic transformation (HR[95% CI]: 7.3[1.3-41], p=0.024).

In summary, it was demonstrated that comprehensive transcriptome analysis identifies two subgroups of MDS with biological and clinical relevance, which could improve risk prediction and treatment stratification of MDS.

IMMUNE SYSTEM IN MDS

A specific session was dedicated to the role of the immune system in MDS. Of special interest was the presentation of Grobaek.[7] In contrast to solid tumors, the total mutational burden in MDS is low. This is predicted to result in a lower number of neoantigens that are presented through MHC class I molecules to T cells. Hypomethylating agents have been shown to demethylate constitutively silenced endogenous retroviruses (ERVs).[8] ERVs and genes in the viral defense pathway are upregulated *in vivo* during treatment with DNMT inhibitors. This expression may be enhanced by raising the vitamin C level to the normal level. T cells directed against ERV derived oligopeptides are upregulated in patients during treatment with DNMT inhibitors.

USEFULNESS OF NGS TO DIAGNOSE MDS

Hematologists are often confronted with patients with cytopenias that do not fulfill the criteria for MDS. A Danish study looked at survival patterns of patients with idiopathic cytopenia of undetermined significance (ICUS) and clonal cytopenia of undetermined significance (CCUS).[9] A gene panel including the 20 most commonly mutated genes was analyzed in these patients. The risk of evolution to MDS was restricted to patients with patients with detectable mutations at enrolment. These results confirm the recently published results of the Pavia group.[10]

Constance Baer presented the results of the usefulness of panel sequencing in routine diagnostics in patients with suspected myeloid malignancies.[11] The samples underwent morphologic analysis, NextSeq of MiSeq sequencing (Illumina). FLT3-ITD and KMT2A-PTD data was obtained according to standard protocols. Analyzing 39 genes, they were able to detect at least 1 mutation in 90% (500/556) patients with a definitive diagnosis. In the MDS cases, 79% (124/157) showed mutations, of which 108 had at least 1 prognostic relevant change (according to *Bejar et al.*).[12] Overall *TET2* mutations were most frequent (25%). Using the panel sequencing in cases with possible MDS, unclear or reactive morphology revealed at least one molecular marker for clonal disease in 47% (91/199), 36% (43/118) or 17% (36/221) of cases respectively (excluding sole *ASXL1, DNMT3A* and *TET2* mutations with < 10% allele burden).

In summary, these studies show that NGS can be useful in routine clinical practice to support diagnosis of samples with borderline morphology.

CHECKPOINT INHIBITORS IN MDS

Checkpoint inhibitors have found their way in the treatment in solid tumors and lymphomas. The rationale for their use in MDS is the observation that PD-1, PDL-1 and CTLA-4 are upregulated in MDS CD34$^+$ cells. *Montalban-Bravo et al*. presented the results of an ongoing phase II study of nivolumab and ipilimumab in patients with previously treated and untreated MDS patients, with or without azacitidine (AZA). Results of 54 patients were presented: 21 treated with frontline nivolumab and AZA; 15 with nivolumab and 18 with ipilimumab after HMA (hypomethylating agents) failure. The ORR was highest in the AZA-nivolumab cohort (80%) including 6 patients with a complete response. The preliminary results indicate that PD-1 blockade with nivolumab in combination with AZA in untreated higher-risk MDS patients is associated with a tolerable safety profile and clinical activity.[13]

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KEY MESSAGES FOR CLINICAL PRACTICE

1 *ARID2* expression is abnormal in 10% of cases with MDS and results in morphologic (hypolobulated megakaryocytes) and functional aberrancies.

2 Different spliceosome mutations lead to lineage specific RNA splicing deregulation. Although mutations in different spliceosome genes lead to a specific set of aberrantly spliced genes, they markedly share convergent pathways.

3 Comprehensive transcriptome analysis identifies two subgroups of MDS with biological and clinical relevance, which could improve risk prediction and treatment stratification of MDS.

4 NGS is useful in routine clinical practice to support diagnosis of samples with borderline morphology.

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