ABSTRACT:

Myelodysplastic syndrome (MDS) is a heterogeneous group of clonal hematopoietic stem cell disorders with ineffective hematopoiesis. It is characterized by morphological dysplasia, peripheral cytopenias affecting one or more cell lineages and an increased risk of transformation into acute myeloid leukemia (AML). The early stages of MDS can be considered a premalignant disease. The pathogenesis of MDS has not been fully explained yet, but due to the development of molecular genetic and cytogenetic methods, the origin and development of the disease is gradually being elucidated.

In addition to the cytogenetic changes that are part of the prognostic system (IPSS-R), the somatic mutations found in different genes come to the forefront of interest. However, they are not routinely used in clinical practice. One of the objectives of this study was monitoring of mutations in *TP53* gene in lower-risk MDS patients who generally have a good prognosis and for whom these findings have a particularly relevant prognostic significance. We investigated a total of 154 patients with lower-risk MDS, and 13% of them had a mutation. After dividing patients according to the presence of del(5q), we observed significant differences in the incidence of the mutations. The mutations were detected in 23.6% of patients with deletion compared to 3.8% of patients without deletion. Using multivariate analysis, we determined that the mutation in *TP53* gene (HR 3.7) is the strongest prognostic factor for both overall survival (OS) and progression-free survival (PFS). We also found that small *TP53*-mutated subclones did not have the same unfavorable prognostic impact on OS and PFS in patients with low-risk MDS as the clones with high mutational burden. A high correlation in the size of the mutated clone was found between cells isolated from peripheral blood and bone marrow. Based on these findings, we assume that the *TP53* mutations should be routinely detected at the time of diagnosis, during the course of the disease, and prior to initiation of treatment, in patients with lower-risk MDS.

Azacitidine (AZA) hypomethylation therapy is currently used for the treatment of patients with advanced stages of MDS who are not indicated for hematopoietic stem cell transplantation. Approximately only half of the patients respond positively to the AZA therapy and the clinical patient outcome after AZA treatment failure is very poor. Therefore, we focused on the identification of markers that could predict the response to AZA therapy. We detected an increased expression of several ribosomal genes in non-responsive patients before AZA treatment that likely reflected an intensive proteosynthesis in proliferative/neoplastic cells. We suppose that a possible treatment failure results from a high rate of proliferation and advanced state of the disease cannot be reversed by demethylation treatment.

Single-nucleotide polymorphisms (SNPs) in the DNA sequence are the most common polymorphisms in the human genome, and can serve to detect genetic predispositions to disease in association studies. We attempted to identify SNPs that could be related to MDS either directly or were in linkage disequilibrium with the actual causal allele. We found nine point polymorphisms that were associated with MDS phenotype. Of these, three SNPs are located in DNA repair genes (*LIG1, RAD52,* and *MSH3*) and one in the gene protecting the cells from oxidative damage (*GPX*3). In addition, two SNPs (*ROS1, STK6*), whose genotype was associated with overall survival of the patients, were identified. We hypothesize that these genes may be involved in the pathogenesis of MDS.

The results of the presented dissertation provide further information on the molecular background of MDS and possible mechanisms leading to the onset or progression of the disease and also represent the basis for further studies aimed at applying these findings to clinical practice.

Key words: Myelodysplastic syndrome; mutations in *TP53* gene; hypomethylation therapy; association study; single nucleotide polymorphisms