271

RASSF1A MUTATION IN MYELODYSPLASTIC SYNDROMES

F.D.S. Monteiro[1], P. Curi de Freitas[2], A.B. Carvalho-Salles[3], O. Ricci Junior[4], A.C. Fett-Conte[5]
[1]Biology Department, Instituto de Biociências- Letras e Ciências Exatas- Paulista State UniversityIBILCE/UNESP, São José do Rio Preto, Brazil; [2]Biology Department, Instituto de Biociências- Letras e Ciências Exatas- Paulista State UniversityIBILCE/UNESP-, São José do Rio Preto, Brazil; [3]Laboratory of Genetics- FUNFARME, Molecular Biology, São José Do Rio Preto, Brazil; [4]Medicine Department, Faculdade de Medicina de São José do Rio Preto., São José Do Rio Preto, Brazil; [5]Faculdade de Medicina de São José do Rio Preto FAMERP/FUNFARME, Molecular Biology, São José Do Rio Preto, Brazil

Myelodysplastic Syndrome (MDS) is a designation of a representative group of clonal diseases of hematopoietic cells characterized by cytopenias, dysplasia in myeloid cell lines, ineffective hematopoiesis and an increased risk of progression to acute myeloid leukemia. The MDS involve epigenetic alterations and mutations in genes that control cell events such as proliferation and apoptosis. The diagnosis and classifications includes molecular and cytogenetic profiles. With the advent of microarrays and sequencing technologies, a tremendous amount of progress has been made toward better understanding of MDS genetic and molecular changes. Recurring genetic abnormalities have been revealed in up to 80–90% of MDS patients and detection of mutation can provide additional diagnostic support. The Ras-Association Domain Family 1A (RASSF1A) protein, is consistently expressed in all hematopoietic cells and its methylation pattern was investigated in patients with acute AML and multiple myeloma (MM). The objective of this study was to investigate the presence of mutations in sequence of RASSF1A in 46 patients with MDS. Genomic DNA was extracted from bone marrow cells of patients and from peripheral blood of controls. After DNA extraction by the salting-out technique, exons 3, 4 and 5 were amplified by polymerase chain reaction (PCR) with sequences being determined by direct sequencing. We found the Ala133Ser polymorphism (A133S) in 4% of patients, which was not found in controls. Some studies try to establish an association between this polymorphism and predisposition to some types of cancer. However, limited sample size does not permit conclusions, our findings suggest that mutations in sequence of RASSF1A gene, particularly in exon 3, 4 and 5 are no biomarkers for myelodysplastic syndromes. MDS is a complex disease with genetic and environmental causes. Advances in the understanding of the molecular drivers of the disease can provide important information in its diagnosis, prognosis, and treatment.

272

BCL-2 INHIBITION BY ABT-199 POTENTLY INDUCES CELL DEATH IN MDS PROGENITORS DESPITE HIGH-RISK MUTATIONS IN ASXL1, RUNX1, TP53 OR EZH2

S. Jilg[1], V. Reidel[1], J. Kauschinger[1], C. Müller-Thomas[1], R. Hauch[1], J. Schauwecker[2], S. Burkhard[3], U. Höckendorf[1], C. Peschel[1], W. Kern[4], T. Haferlach[4], J. Slotta-Huspenina[5], K.S. Götze[5], P.J. Jost[1]
[1]TU Muenchen – Klinikum rechts der Isar, III. Medizinische Klinik, München, Germany; [2]TU Muenchen - Klinikum rechts der Isar, Klinik für Orthopädie und Sportorthopädie, München, Germany; [3]Gemeinschaftspraxis Hämato-Onkologie, Pasing, München, Germany; [4]Munich Leukemia Laboratory, MLL, Munich, Germany; [5]TU Muenchen – Klinikum rechts der Isar, Institut für Pathologie, München, Germany

A clinical key feature of Myelodysplastic Syndromes (MDS) is variable outcome. Recent findings emphasize the prognostic impact of specific somatic mutations within the MDS clone. Especially alterations in ASXL1, RUNX1, TP53 or EZH2 are associated with a significantly shorten overall survival despite well-established therapeutic approaches.

The induction of clinical remission in higher-risk MDS critically depends on the capacity of MDS progenitor cells for sustained pro-survival signals. BCL-2 provides protection against various pro-apoptotic stresses. Pharmacologic inhibition of BCL-2 therefore serves as a potent cell death-inducing strategy in MDS. Specifically higher-risk MDS patients present with de-regulated expression of proteins from the BCL-2 family effectively blocking cell death induction at the mitochondria. Our previous work has shown that ABT-199 has a positive therapeutic index in higher-risk MDS when compared to healthy age-matched progenitor cell survival.

Murine models demonstrate an interaction between ASXL1, RUNX1, EZH2 and BCL-2. We therefore tested the susceptibility of patients bearing a mutational adverse profile to ABT-199. Analyzing BCL-2 gene expression in a large cohort of MDS patients (n = 106) and healthy controls (n = 110) we did not detect any differences in dependence on the mutational status. These findings were confirmed by immunohistochemistry for BCL-2 in an independent set of bone marrow biopsies of MDS/sAML patients. Our results are in contrast to previous data from murine Asxl1$^{-/-}$ Lin^{-}c-Kit^{+} cells where loss of ASXL1 led to a significant down-regulation of Bcl-2. Thus, highlighting the impact of primary human samples in MDS.

Based on these data we tested the therapeutic efficacy of ABT-199 in a number of primary BM samples of MDS/sAML patients presenting with one or two of the indicated mutations *in vitro*. Controls consisted of MDS/sAML patients bearing no mutation and additionally age-matched healthy controls. Within the high-risk MDS/sAML cohort induction of apoptosis by ABT-199 was similarly effective in mutated and non-mutated samples. Even the accumulation of more than one adverse mutation did not impair the broad apoptotic effect of ABT-199. Healthy controls, low- or intermediate-risk samples were only slightly affected. Treatment response clearly correlated with BCL-2 expression as measured by intracellular flow cytometry. Also colony forming capacity was reduced by ABT-199 treatment in high-risk MDS/sAML irrespective of the mutational status.

In summary our data demonstrate that ABT-199 effectively induces apoptosis in the stem/progenitor cells of high-risk MDS/sAML samples despite the presence of adverse genetic aberrations offering promising therapeutic options.

273

RELEVANCE OF NEXT GENERATION SEQUENCING IN HELPING DIAGNOSIS OF UNEXPLAINED CYTOPENIAS

P.Y. Juvin[1], S. Laclau[2], N. Prade[1], T. Comont[3], E. Delabesse[1], O. Beyne Rauzy[3]
[1]Toulouse hospital, Laboratory of Hematology, Toulouse, France; [2]Rheumatology, Toulouse Hospital, Toulouse, France; [3]Interne Medicine, Toulouse Hospital, Toulouse, France

Introduction: Establishing a diagnosis in patients suspected of having a myelodysplastic syndrome (MDS) can be challenging. Approximately 20% of bone marrow analysis performed lead to MDS diagnosis according to WHO criteria. Additionally, targeted sequencing of 40 or so genes can identify 1 or more somatic mutations in over 80% of MDS cases. Unexplained cytopenia could be therefore informed by the identification of somatic mutations.

Objective: Assessment of the relevance of NGS in helping diagnosis of unexplained cytopenia.

Materials and methods: Since October 2015, we are performing a prospective study to examine the frequency and types of mutations encountered in patients with unexplained cytopenias. Patients