in genotype frequencies. Patients belonging to the IPSS good prognosis group showed a significantly lower incidence of GSTM1 null genotype as compared to the controls (OR = 0.60, 95%CI = 0.37–0.97, p = 0.037). The most commonly detected cytogenetic abnormalities found as sole changes were +8 (9.5%), −7/del(7q) (3.5%), −Y (2.8%), −5/del(5q) (1.8%), and del(20q) (1%). We observed higher frequencies of GSTT1 null genotype in the −Y group (37.5%) and of GSTM1 null in patients with del(7q)/−7 (60%). However, these differences were not statistically verified. Interestingly, all patients showing 5q abnormalities exhibited a GSTT1 positive genotype.

Discussion: Our results, representing the larger series of primary MDS cases tested for GSTT1 and GSTM1, suggest that these genotypes do not constitute independent risk factors in MDS susceptibility. The absence of GSTT1 null genotype in patients with 5q abnormalities could be explained by the fact that the functional enzyme, while generally protective, may increase the mutagenic risk of some genotoxic agents involved in the del(5q) occurrence.

P059 DNA instability in low-risk myelodysplastic syndromes – refractory anemia with or without ring sideroblasts

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Introduction: We verified whether the comet assay could provide a suitable tool for analysis of ineffective hematopoiesis in refractory anemia with or without ring sideroblasts (RARS and RA).

Methods: Erythroid and myeloid cell population from bone marrow aspirates of MDS patients and controls were separated by magnetic labeling with antibody against glycophorin A and subjected to comet assay. The extent of DNA migration was measured in single cells using special software.

Results: On average, RA showed a higher DNA instability than controls only in erythroid cells but the individual levels of DNA fragmentation in both glycophorin A positive and negative cells were closely associated with peripheral cytopenia. RARS exceeded significantly the control as well as RA values of DNA fragmentation in both analyzed fractions without any relationship to peripheral cytopenia. In contrast, acute myeloid leukemia and chronic myelomonocytic leukemia were characterized by an extremely low degree of DNA breakage in marrow cells.

Discussion: The results corresponded with the concept of increased apoptosis in low-risk MDS subtypes. An extreme damage in RARS patients reflected probably additional DNA breaks of non-apoptotic origin. These could be associated with an increased repair of oxidative damage in DNA arising due to mitochondrial iron deposits in ring sideroblasts.

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P060 Effect of azacitidine on PI-PLC-beta1 expression and Akt activation in a patient affected by high-risk myelodysplastic syndrome (MDS)

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Introduction: Lipid signaling pathways are involved in many processes, such as cell growth, differentiation and apoptosis. A connection has been suggested between signal transduction pathways and MDS, since it has been demonstrated that both PI-PLC-beta1 and p-Akt expression are altered in the disease, mainly in high-risk MDS patients. Here we report for the first time that azacitidine therapy affected the expression of both PI-PLC-beta1 and p-Akt in a patient with high-risk MDS.

Methods: FISH analysis was performed on mononuclear cells from peripheral blood (PBMC) or bone marrow (BMMC), in order to assess the presence of the PI-PLC-beta1 gene, whilst the Real-Time PCR analyses were carried out by using a TaqMan specific approach on both PBMCs and BMMCs. Furthermore, immunocytochemical analyses were performed by using a specific rabbit polyclonal antibody to PI-PLC-beta1; mouse monoclonal to PIP_2; rabbit polyclonal to Ser473 p-Akt.

Results: FISH analyses demonstrated that our patient did not bear the monoallelic deletion of the PI-PLC-beta1 gene, whereas Real-Time PCR analyses showed a low pre-treatment expression of both PI-PLC-beta1 mRNAs. Following treatment with azacitidine, the patient showed an increase in PI-PLC-beta1 mRNA levels, which could be related to his clinical outcome. In fact, the levels of PI-PLC-beta1 increased during the period in which the patient was maintaining a partial remission, whilst they lowered during the subsequent period, when peripheral blood counts decreased. These results prompted us to investigate the protein expression of PI-PLC-beta1, its substrate PIP_2 and p-Akt by means of an immunocytochemical approach. Our results showed fluctuating PIP_2 levels which followed the levels of PI-PLC-beta1 and p-Akt, indicating a possible correlation between these two molecules. In fact, while before the start of treatment we found high levels of p-Akt

and low levels of PI-PLC-beta1, during partial remission we observed the highest levels of PI-PLC-beta1, and the lowest expression of p-Akt.
Discussion: This is the first time that a correlation between azacitidine therapy and the lipid signaling pathways has been described; furthermore, this is the first report of a possible direct association between PI-PLC-beta1 and p-Akt levels. Future investigations are needed to fully understand the molecular mechanisms underlying the pathogenesis of the disease and the role of azacitidine on these signaling pathways.

P061 Methylation status of the p15^{INK4b} and p16^{INK4a} genes in pediatric primary myelodysplastic syndrome

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MDS are rare hematological malignancies in childhood, they appear with distinct clinical and laboratory characteristics. The high frequency of chromosomal loss suggests that tumor suppressor genes are likely to play an important role in the development of MDS. Epigenetic alterations, such as the aberrant methylation of CpG island in the promoter regions have been frequently observed in tumor suppressor genes. Nowadays, genes including p15 and p16 have been reported to undergo methylation control in MDS, however the majority of these studies were focused in the adult disease. The aim of this study is to analyze the p15^{INK4b} and p16^{INK4a} gene methylation pattern in children MDS, the correlation with FAB subtype and the role of p15^{INK4b} and p16^{INK4a} gene methylation in the progression of MDS toward acute myeloid leukemia (AML). We analyzed bone marrow sample from 45 primary MDS patients with age from 5 month to 18 years old. The patients were distributed in accordance with FAB classification. To investigate the role of p15^{INK4b} and p16^{INK4a} gene methylation we used the methyl-specific polymerase chain reaction (MSP) method and sequencing. Aberrant methylation of p15 was detected in 16 (35.5%) of 45 children with de novo MDS, whereas only 4 patients (8.8%) demonstrated hypermethylation of p16 gene. The distribution of p15 gene methylation according to FAB classification was: RA (5/26); RAEB (5/9), RAEB-t (5/8) and JMML (1/2). The methylation of p16 gene was present in RAEB (2/9) and RAEB-t (2/8). Therefore p15 and p16 genes were more frequently hypermethylated in advanced subtypes of MDS compared with early MDS. Thirteen of the forty-five patients showed transformation to AML. Nine of these patients who showed progression of disease had p15 gene methylation (75%) and three had p16 gene methylation (25%). Our results showed that methylation, mainly in p15^{INK4b}, is correlated with disease evolution toward AML. Stem cell transplantation (SCT) is recognized as only curative treatment for patients with MDS, however relapse after SCT is a major cause of treatment failure. By this way, other therapies like demethylating agents have been used in the treatment of adults SMD. Studies showing the frequency of methylation in children with MDS are very important because likely children with relapse or resistant MDS may be benefited from demethylation agents, as well as adults.
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P062 An increase in aberrant p15^{INK4b} gene methylation in myelodysplastic syndromes correlates with the number of bone marrow blasts and with the progression of the disease

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Introduction: Epigenetic de novo methylation of CpG islands is thought to be one of the crucial moments in malignant transformation of the cell because of its capability to silence tumor suppressor genes. Our study was focused on evaluation of p15^{INK4b} gene methylation in patients with untreated primary myelodysplastic syndromes (MDS) and acute myelogenous leukemia (AML) arising from MDS and its effect on progression of MDS towards overt leukemia.
Methods: 29 MDS patients were divided according to WHO classification as follows: 4 RA, 1 RARS, 12 RCMD, 3 5q– syndrome, 2 RAEB I, 4 RAEB II and 3 AML from MDS. Methylation specific PCR (MSP) monitored the methylation status at bone marrow mononuclear cells after sodium bisulfite modification (CpG WIZ^{TM} p15 Amplification and CpGenome^{TM} Modification kits). During bisulfite treatment are all unmethylated cytosines deaminated and sulfonated, converting them to uracils, while 5-methylcytosines remain unaltered, hence primers for MSP are designed to amplify unmethylated v. methylated CpG sites distinctively. Methylation status (indices in the range 0.0–1.0) was correlated with the