P262

HIGH-THROUGHPUT SEQUENCING FOR THE IDENTIFICATION OF DIS3 MUTATIONS IN MULTIPLE MYELOMA

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DIS3 is a catalytic subunit of the human exosome complex, containing exonucleolytic (RNB) and endonucleolytic (PIN) domains and recently found mutated in about 10% of patients with multiple myeloma (MM). To analyze DIS3 mutation in untreated MM, we investigated by next generation sequencing (NGS) highly purified plasma cells (PC) of a retrospective cohort of 130 cases at onset, 14 of whom were also tested at relapse. Moreover, we examined 10 patients with secondary PC leukemia (sPCL). Deep sequencing of the PIN and RNB domains was performed by Roche 454 pyrosequencing on the Genome Sequencer Junior instrument. Mutations were validated in an independent PCR product by conventional Sanger sequencing or NGS. Median depth of coverage was 264x (range: 102-870). The analysis revealed the presence of 30 different tumor-specific non-synonymous variants, identified in 27 patients: of these, 23 mutations were missense, five introduced a frameshift, and two an in-frame deletion. Seven of these variants have been already reported by others, also specifically in MM patients, while 23 were novel. Mutations were predominantly localized in the RNB domain; the most recurrently affected residues were R780 and D488, mutated in five and four cases, respectively. Globally, 18.5% of MM and 30% of sPCL patients were found mutated. DIS3 mutations were preferentially carried by IGH-translocated patients, and were often detected at low variant allele frequency (VAF): in particular, a VAF of around 100% was exclusively observed in a fraction of patients with 13q deletion. In the rest of the cases, i.e. the remaining del(13q14) samples and all the patients disomic for chr 13, the VAFs were respectively suggestive of a mutation present in hemizygosis in a tumor subclone (0.8%<VAF<57%), or in heterozygosis either in all MM cells (if around 50%) or in a tumor subclone (0.5%<VAF<40%). Sequential analysis highlighted a few instances of increase of DIS3 mutation burden during disease progression. By means of NGS of DIS3 cDNA in mutated cases, we found that the majority of variants were comparably detectable also at transcript level. Furthermore, gene expression profiling analysis identified in DIS3-mutated patients a transcriptional phenotype apparently compatible with impaired RNA exosome function. In conclusion, these data demonstrate the relevance of DIS3 mutations and suggest it as a potential tumor suppressor in PC dyscrasias.

P263

OSTEONECROSIS OF THE JAWS IN MULTIPLE MYELOMA PATIENTS TREATED WITH ZOLEDRONIC ACID: A SINGLE-CENTER EXPERIENCE

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It has been demonstrated that bisphosphonate-based supportive therapy reduces skeletal events (onset or progression of osteolytic lesions) both in patients with multiple myeloma and in cancer patients with bone metastasis. Bisphosphonates are generally well tolerated and associated with minimal adverse effects: fever, renal function impairment, myalgias and hypocalcemia. In recent years, several cases of jaw bone necrosis associated with long-term use of bisphosphonates have been reported. The estimated incidence varies from 1.8% to 12.8% (Hematology 2006). The pathogenesis of this complication is unknown; however, several predisposing factors have been identified: poor oral hygiene, periodontal disease, dentoalveolar surgery, corticosteroid therapy, immune-compromised state predisposing to increased risk of infection. We performed a retrospective study on osteonecrosis of the jaws in 34 multiple myeloma patients with a history of chronic zoledronic acid therapy. Between January 2008 and December 2014 we observed four patients with osteonecrosis of the jaws (14.7%). Diagnosis was radiological and clinical. CT scan confirmed the presence of an osteolityc area with signs of periosteal reaction. Microbiology showed actinomycetes and mixed bacteria. The characteristic of the patients were the following: median age=75 years (43-84), M/F=3/2, IgG/IgA=3/2 kappa/lambda=3/2. Steroids use=5 patients. Thalidomide use=3 patients. Autologous stem cell transplantation=1 patient. Previous dental extraction=1 patients. Median time of exposure to zoledronic acid=5 months (3-13). Aminobisphosponates exert several antitumor effect, including induction of tumor cell apoptosis, inhibition of tumor cell adhesion to the extracellular matrix, and inhibition of tumor invasion. Zoledronic acid also have antiangiogenesis properties and can activate g-d T cells. It has resulted in a statistically significant reduction in skeletal complication. We have initiated the following guidelines in an effort to ameliorate the incidence of jaw bone necrosis. Patients have a screening dental examination and an appropriate radiographic study before the administration of zoledronic acid. They are encouraged to practice good dental hygiene and see a dentist promptly if oral or dental symptoms appear. In addition, zoledronic acid are held for a period of 3 months prior to invasive dental procedures to allow for the osteoclastic recovery. Following the dental procedure we re-introduce bisphosphonates only after the healing process is complete.

P264

MULTIPLE MYELOMA-ASSOCIATED DRUG RESISTANCE: TARGETING THE NOTCH PATHWAY

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Multiple myeloma (MM) represents 11% of hematological malignancies and is caused by the accumulation of malignant plasma cells in the bone marrow (BM). Although treatments with new drugs, such as immunomodulators and proteasome inhibitors, are increasing patients' survival, MM is still incurable due to the development of endogenous or BM mediated drug resistance. Therefore it is important to find new therapeutic targets. The dysregulated expression of two Notch ligands, Jagged1 and 2, causes the hyperactivation of the Notch signaling both in MM cells and in bone marrow stromal cell (BMSC). The aim of this study was to investigate the role of Notch signaling in endogenous and BMSC-induced drug resistance in MM. At this purpose, we silenced two Notch ligands, Jagged1 and 2, in the MM cell lines OPM-2 and U266. Jagged1/2 silencing causes a reduction in the expression of anti-apoptotic genes, i.e. SDF-1a, Bcl-XL, Bcl-2, Survivin and ABCC1. In accordance, MM cells with reduced levels of Jagged1 and 2 showed an increased sensitivity to different drugs commonly used in MM therapy such as Bortezomib, Mitoxantrone and Melphalan. In addition, Jagged1/2 knockdown affects the pathological interaction between MM and BMSCs resulting in the activation of Notch signaling in both cell types. Indeed, when co-cultured with human BMSCs, MM cells displayed an higher level of drug resistance due to: 1) an increased expression of anti-apoptotic genes in MM cells, i.e. Bcl-XL, Bcl-2, Survivin and ABCC1; 2) the BMSC-mediated release of soluble factors, i.e. SDF-1a and VEGF, relevant for MM cell growth and survival. Jagged1 and 2 silencing in MM cells could reverse all these effects. These in vitro results were confirmed in co-culture experiments performed with primary human CD138+ multiple myeloma cells and BMSCs isolated from patient's bone marrow aspirates. The evidence that Jagged-1/2 silencing affects endogenous and BMSC-induced drug resistance in MM cells supports the use of a Jagged-targeted approach in MM therapy alone or in a combination with standard of care drugs.

P265

OUTCOME OF NEWLY DIAGNOSED SYMPTOMATIC MULTIPLE MYELOMA IN VERY ELDERLY PATIENTS (AGED 80 YEARS OR MORE)

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