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BONE MARROW-MEDIATED DRUG RESISTANCE IS PROMOTED BY JAGGED-INDUCED NOTCH SIGNALING IN MULTIPLE MYELOMA

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Background. Multiple myeloma (MM) is an hematologic tumor caused by the accumulation of malignant plasma cells in the bone marrow (BM). The dysregulated expression of two Notch ligands, Jagged1 and Jagged2, hyperactivates the Notch pathway both in MM cells and in BM stromal cell (BMSC). Several Notch downstream mediators are involved in MM cell survival and proliferation, *i.e.* IL6, SDF1α, CXCR4, NF-kB, VEGF and IGF. Although treatments with new drugs, such as alkylating agents, proteasome inhibitors and immunomodulatory agents, increased patients'survival, MM remains incurable, principally due to the development of endogenous or BM-mediated drug resistance (DR). Therefore it is crucial to find new therapeutic targets. The aim of this study was to investigate the role of Notch signaling in endogenous and BMSC-promoted DR in MM. *Materials and Methods*. U266 and OPM2 cell lines were maintained in complete RPMI-1640 medium. The BMSC lines NIH3T3 (murine) and HS5 (human) were maintained in complete DMEM medium. MM cells were cultured alone or on a BMSC monolayer for 24h, and subsequently treated with Mitoxantrone, Bortezomib, Melphalan or the vehicle in the presence or the absence of the CXCR4 antagonist AMD3100 for additional 24 hours. BMSCs were previously stained with PKH26 (Sigma-Aldrich) to discriminate them from MM by flow cytometry. Apoptosis was determined by Annexin V-FITC staining. qRT-PCR reactions were carried out in a 7500 Fast Real-time PCR system (Applied Biosystems) using the Maxima™ SYBR Green/ROX qPCR Master Mix (ThermoScientific Inc) using murine or human primer sets to discriminate the source of the RNA molecules. Silencing of Jagged1 and 2 was obtained by transient expression of specific siRNAs. *Results*. The possible role of Notch withdrawal in DR was investigated by silencing Jagged 1 and 2 ligands in MM cell lines OPM-2 and U266. Results showed an increased sensitivity to Bortezomib, Mitoxantrone and Melphalan associated to a decrease in the expression of SDF1α, CXCR4, Bcl-XL, Bcl-2, Survivin and ABCC1. When co-cultured with murine and human BMSCs, MM cells showed increased DR due to: i) increased expression of anti-apoptotic genes in MM cells, *i.e.* Bcl-XL, Bcl-2, Survivin and ABCC1; ii) BMSC release of soluble mediators relevant to MM cells, *i.e.* SDF1α and VEGF. We suggest that these effects may be driven by the reciprocal activation of Notch signaling observed in both cell types and consistently we demonstrated that DR may be significantly reduced by silencing Jagged1 and 2 in MM cells. Finally, the evidence that CXCR4 blockade significantly reduced MM cells resistance to Bortezomib induced by BMCSs, indicates that CXCR4/SDF1α chemokine axis is a key mediator of Notch in MM-associated DR. *Conclusions*. The evidence that Jagged1/2 silencing affects endogenous and BMSC-induced DR in MM cells supports the use of a Jagged-targeted approach in MM therapy alone or in combination with common drugs.

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ROLE OF BENDAMUSTINE IN RELAPSED AND REFRACTORY MULTIPLE MYELOMA

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Efficacy and tolerance of bendamustine in combination with bortezomib-dexamethasone was evaluated in patients with relapsed and refractory multiple myeloma (rrMM), whose prognosis is severe, so that there is a strong need for new options for the management of these patients. 24 patients, 13 males, 11 females, with rrMM, who had been treated with a schedule Bendamustine-based, were retrospectively analyzed. Median age at diagnosis was 63.2 years (range 39-82) while age at start of treatment was 66 years (range 48-83), and median number of prior lines of treatment was 6.3 (range 4-8). ISS was equally distributed, and cytogenetic characteristics were evaluable in 9 patients, only two of whom had cytogenetic abnormalities, and in particular one of them had del13q and in the other one was observed t(11;14). All the patients had previously been treated with schedule containing bortezomib and lenalidomide, while 90% of them had been treated with melphalan, 77% with cyclophosphamide and 34% with anthracyclines, and 30% had also received radiotherapy. 58% of patients had undergone at least to a single autologous stem cell transplantation. Last treatment before bendamustine was a bortezomib-based regimen in 39%, an IMIDs-based regimen in 49% (a combined bortezomib/IMIDs-based regimen in 27%), while 12% of patients had received other chemotherapies. All patients were relapsed and refractory to last therapies received. Only patients completing at least two two courses of Bendamustine were considered. A total of 87 cycles was administered (median 4.3, range 2-9). In 91% of patients bendamustine was variously associated to bortezomib (66%), or IMIDs (25%) and only in 8% it was combined only with dexametasone. In our schedule, Bendamustine was given, at a median dose of 90 mg/sqm (range total dose: 120-180 mg) on day +1 and +2 every 28 days. After a median follow-up of 6.1 months, median OS from diagnosis was 57.3 months, while median OS from start of Bendamustine was 6.7 months (range 2-19 months). 11/24 patients died for progressive disease. 2/24 patients died for other causes (one for cardiovascular disease and the other one had a gastric cancer). Grade 3 transfusion-dependent anemia occurred in 36% while in 53% grade 3 neutropenia occurred. We observed no severe extrahematologic toxicity, only grade 1 gastrointestinal side effect (nausea), treated by common antiemetic drugs. According to IMWG uniform response criteria, 15 out of 24 evaluable patients achieved a partial response after a median time of 2.4 months with an overall response rate of 62.5%. In particular, for 3 patients of this study. In conclusion, Bendamustine has shown significant efficacy in a particularly severe setting of patients, relapsed and refractory to almost all available therapeutic resources, and in particular cases it could be considered as a bridge to a second autologous or to allogeneic BMT.