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ABSTRACT BOOK

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ed mice, with an average size of tumors of 530.03±69.91 $mm^{3 vs. 301.41 \pm 51.25 mm3 after 4 weeks (} p<0.05$). Besides, immunohistochemistry and Western blot results showed that pre-treatment with MDSCs increased Ki-67, Bcl-2 and Cyclin D1 expression in MM cells (Figure 1).

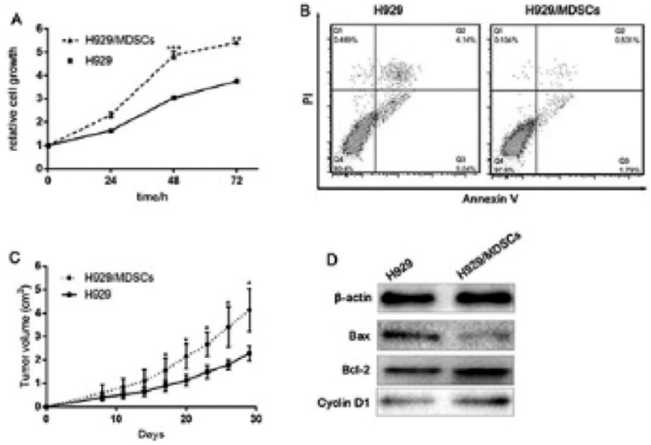

Figure 1. A. Promoting effect of MDSCs on NIH929 cells proliferation after 24h co-culture. B. Apoptotic effects of MDSCs on NIH929 cells as determined by flow cytometry. C. MDSCs co-culture accumulated tumor growth in the myeloma xenografted BALB/c nude mice model. D. Effects of mogrol on the expression of Bax, Bcl-2, and Cyclin D1.

Summary and Conclusions: Our results showed elevated MDSCs levels in MM patients and revealed the positive association between MDSCs and ISS staging in MM. Thus, we concluded that MDSCs might grow to be an important biomarker for MM diagnosis and prognosis. Furthermore, the study *in vitro* and *in vivo* both validate the promoting effects of MDSCs on MM proliferation and progression. These findings suggested that targeting MDSCs may become a new therapeutic strategy to improve MM patients' survival.

PF541

TARGETING THE INTERACTION OF MULTIPLE MYELOMA AND THE BONE MARROW MICROENVIRONMENT THROUGH NOVEL SMALL MOLECULES DIRECTED TO NOTCH PATHWAY

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Background: Multiple myeloma (MM) is an incurable hematological cancer characterized by plasma cells accumulation in the bone marrow (BM), where they shape the nearby BM milieu, inducing it to support tumor progression and acquisition of drug resistance. Despite recent advances, poor clinical response and relapse remain major problems. The oncogenic Notch pathway consists of 4 receptors (Notch1-4) activated upon binding to two families of ligands, Jag (Jag1 and 2) and Dll (Dll1, 3 and 4) and plays a crucial role in the pathological interaction between MM and BM cells. In MM the aberrant expression of Notch receptors and ligands on tumor cell results in homotypical interactions, which affect myeloma cell biology and heterotypical interactions with the BM cells, that favor tumor progression, osteoclastogenesis and drug resistance. In particular, aberrant Notch2 activation and overexpression of Jag2 ligand in MM cells play an important role in MM progression by stimulating osteoclast differentiation, release of pro-tumor cytokines by BM cells and MM cell self-renewal. Therefore uncoupling the interaction between Notch2 and Jag2 is critical to affect MM cell growth along with their pathological interaction with the BM niche. Currently, indirect approaches to inhibit Notch signaling are mainly based on inhibition of γ-Secretase, an enzyme that catalyzes Notch activation and the cleavage of several other γ-Secretase substrates. Moreover, γ-Secretase-mediated inhibition of all four Notch receptors is associated with gut toxicity, that might be avoided by selectively blocking of Notch signaling triggered by only one of the two family of ligands, Jag or Dll.
Aims: These lines of evidence prompted us to develop a therapeutic tool to selectively inhibit Notch2 signaling triggered by Jag2 using an unprecedented approach based on drug-like small molecules.
Methods: To select the small molecules, we performed *in silico* a protein-protein docking and virtual high-throughput screening (HTS) of an Asinex chemoteque of small molecules. The biological efficacy was validated through Notch responsive gene reporter and viability assays.
Results: We have set-up a strategy to exclusively uncouple Notch2::Jag2, leaving unaltered the interaction with Dll. The lack of crystallographic structures for Notch2::Jag2 was overcome by exploiting the differences in the surfaces of the Notch2::Jag2 and Notch2::Dll4 complexes, modeled by protein::protein docking on the bases of the crystallographic structures of Notch1::Jag1 and Notch1::Dll4, respectively. It allowed us to select *in silico* 100 top-scoring compounds supposed to be exclusively directed to Notch2::Jag2 surface by HTS of the small molecules chemoteque . Initially, 2 of 100 compounds were validated *in vitro*. A Notch responsive reporter assay on HEK293T cells showed that the compounds were able to significantly reduce Notch transcriptional activity. A viability assay of MM cell lines showed a dose-dependent cell growth inhibition in the presence of the compounds. Finally a Notch responsive reporter assay on co-culture systems allowed us to measure Notch2 activation triggered either by Dll4 or Jag2 ligands and to demonstrate that one of the two tested compounds specifically inhibited Notch2::Jag2 but not Notch2::Dll4 interactions.
Summary and Conclusions: Our integrated pipeline represents a successful strategy to identify compounds that directly and selectively antagonize Notch activation and lays a basis for the development of an entirely novel class of drugs to inhibit Notch signaling in cancer.

PF542

EXPRESSION LEVELS OF THE THREE GENES CRBN, IKZF1, AND IKZF3 IN PRIMARY MULTIPLE MYELOMA CELLS AT PRE- AND POST- LENALIDOMIDE TREATMENT

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Background: Lenalidomide (Len) binds to cereblon and alters its substrate specificity, which results in immunomodulatory and anti-tumor effects. Among the substrates ubiquitinated by cereblon by Len exposure, the degradation of the two transcription factors IKZF1 and IKZF3 is considered critical in anti-tumor effect of Len. Although expression levels of *CRBN* and its related genes have been analyzed in association with treatment outcome of Len, their results are still controversial. In addition, the expression levels of these genes after Len treatment have not been fully investigated.
Aims: This study was conducted to explore if expression levels of *CRBN*, *IKZF1* and *IKZF3* mRNAs before treatment with Len plus dexamethasone (Ld) in primary multiple myeloma (MM) cells were associated with its treatment outcome and to investigate their alteration at post-Ld treatment.
Methods: A total of 83 patients with relapsed MM were treated with Ld therapy in our hospital between July 2010 and May 2017 and their samples and data were retrospectively analyzed. Forty-eight bone marrow (BM) specimens were collected just prior to Ld therapy. Among these, 25 paired BM samples were obtained at pre- and post- Ld therapy. After purification of CD138 positive cells from mononuclear cell fraction, mRNA levels of *CRBN*, *IKZF1* and *IKZF3* were quantified using real-time RT-PCR. Next, their expression levels were analyzed in association with the following outcomes such as response levels, progression-free survival (PFS) and overall survival (OS). Alteration of the expression levels of these genes in primary MM cells were compared between pre- and post-Ld treatment.
Results: Out of 48 patients who provided BM specimens with their informed consents, 47 patients were evaluated for the efficacy of Ld therapy. Expression levels of any of the three genes, *CRBN*, *IKZF1* and *IKZF3*, were not significantly associated with the PFS or OS. When tested using the ratio of *IKZF1* divided by *CRBN* expression, poor responders (SD+PD, n=15) to Ld therapy showed a significantly lower ratio than good responders (CR+VGPR+PR, n=32) (P=0.01). In addition, when the median value of the ratio of *IKZF1/CRBN* was set as a cut-off value, the patients with lower ratio of *IKZF1/CRBN* showed a significantly shorter OS than those with higher ratio (Figure 1a, median OS: 17 *vs* 38 months, P=0.034). There was no association of the ratio of *IKZF3* to *CRBN* with the efficacy of the Ld therapy. Of the 25 paired BM samples collected at both pre- and post-Ld therapy, 22 post-Ld samples were obtained when the patients became refractory to Ld. The expression levels of *CRBN* were reduced in 17 patients (17/25, 68%) and increased in 8 patients (8/25, 32%) at post-Ld compared to pre-Ld (Figure 1b). In the analysis of 22 refractory MM