EUROPEAN
HEMATOLOGY
ASSOCIATION

haematologica

Journal of the European Hematology Association
Published by the Ferrata Storti Foundation

21st Congress of the European Hematology Association
Copenhagen, Denmark, June 9 - 12, 2016

ABSTRACT BOOK

ISSN 0390-6078

Volume 101
JUNE
2016|s1

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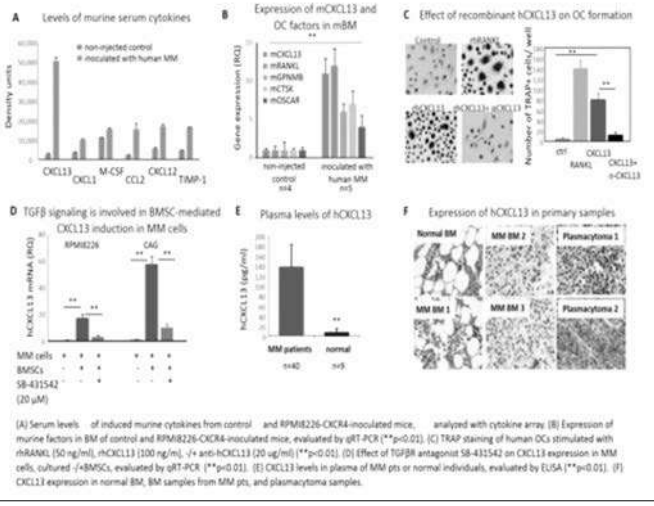
Methods: We utilized our recently established *in vivo* xenograft model of BM-disseminated human myeloma (using engineered CXCR4-expressing RPMI8226 cells), as well as analysis of MM cell lines, stromal components and primary samples from patients (pts) with MM.
Results: Evaluation of the cytokines in sera of MM-inoculated mice in comparison to control mice detected increased levels of the CXCL13 chemokine being the highest factor among the broad panel analyzed. Elevated mCXCL13 was also detected in bone marrow (BM) samples from the MM-bearing mice, and correlated with induced expression of murine factors associated with osteoclast (OC) activation (RANKL, NFATc, GPNMB, CTSK, OSCAR). IHC analysis of MM-occupied murine BM revealed myeloid cells being the main source of increased mCXCL13, while human RPMI8226 cells in murine BM milieu also expressed detectable levels of hCXCL13. In addition, hCXCL13 mRNA was found to be expressed by MM cell lines (n=8), BM stromal cell lines and peripheral-blood generated Mϕ. Strong induction of CXCL13 expression in both MM and stromal cells was detected upon their co-culture. Furthermore, CXCL13 expression in BMSCs and Mϕ was significantly induced following RANKL treatment; in turn, addition of CXCL13 up-regulated RANKL levels, demonstrating a positive regulation loop between CXCL13 and RANKL. Functional tests revealed the ability of CXCL13 to induce *in vitro* formation of TRAP+ OCs, while CXCL13 neutralizing antibodies blocked this effect. Furthermore, CXCL13 neutralization markedly decreased RANKL expression in BMSCs. Of note, CXCR5, cognate CXCL13 receptor, was expressed predominantly by stromal and myeloid cells, suggesting the paracrine effects of MM-generated CXCL13. Mechanistically, we found that TGFβ signaling was involved in CXCL13 induction in both MM and stromal cells. Addition of TGFβ receptor kinase inhibitor SB-431542 interfered with the activation triggered by the interaction between MM cells and stromal components and prevented the increase in CXCL13 expression. Finally, we evaluated the presence of hCXCL13 in primary MM samples. CXCL13 transcript was detected in BM aspirates from MM pts (n=20), its expression was significantly upregulated upon co-culture with BM stromal cells and correlated with expression of osteoclastogenic factors, including RANKL and MT1-MMP, an important component of OC fusion machinery. In addition, plasma level of CXCL13 was significantly higher in MM pts (n=44) (148 pg/ml±136) in comparison to normal individuals (n=9) (19 pg/ml±7.6) (p<0.001). Furthermore, IHC analysis of BM biopsies from MM pts (n=7) and plasmacytoma samples (n=6) demonstrated the expression of CXCL13 in malignant plasma cells. Importantly, CXCL13 showed markedly increased expression within plasmacytoma tissues, suggesting that elevated CXCL13 levels may be associated with extramedullary disease (Figure 1).



(A) Serum levels of induced murine cytokines from control and RPMI8226-CXCR4-inoculated mice, analyzed with cytokine array. (B) Expression of murine factors in BM of control and RPMI8226-CXCR4-inoculated mice, evaluated by qRT-PCR (**p<0.01). (C) TRAP staining of human OCs stimulated with rhRANKL (50 ng/ml), rhCXCL13 (100 ng/ml), -/+ anti-hCXCL13 (20 ug/ml) (**p<0.01). (D) Effect of TGFβR antagonist SB-431542 on CXCL13 expression in MM cells, cultured -/+BMSCs, evaluated by qRT-PCR (**p<0.01). (E) CXCL13 levels in plasma of MM pts or normal individuals, evaluated by ELISA (**p<0.01). (F) CXCL13 expression in normal BM, BM samples from MM pts, and plasmacytoma samples.

Figure 1.

Summary/Conclusions: Altogether, our data define a previously unrecognized role of CXCL13 in MM, unravel its involvement in the osteoclastogenic process and suggest CXCL13 as potential novel target for the diagnosis and treatment of MM.

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MESENCHYMAL STEM CELLS (MSC) PROMOTES TUMOR MICROENVIRONMENT TRANSFORMATION DRIVING GRANULOCYTE-LIKE MYELOID DERIVED SUPPRESSOR CELLS (G-MDSC) ACTIVATION IN SMOLDERING AND MULTIPLE MYELOMA PATIENTS

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Background: A well-recognized feature of multiple myeloma (MM) is the intimate relationship between plasma cells (PC) and BM microenvironment, which is mainly composed of mesenchymal stromal cells (MSC), endothelial cells, immune cells and extracellular matrix. Granulocytic-Myeloid-derived suppressor cells (G-MDSC) accumulate in the tumor microenvironment during tumor development. MDSC promote tumor growth and invasion, immunosuppression and host immune evasion by suppressing lymphocyte activation and antigen recognition. Even though it has been demonstrated that G-MDSC are increased in MM microenvironment, the role of MSC in promoting immunosuppressive microenvironment through activation of G-MDSC remains unexplored.
Aims: Analyzing MSC from MGUS, Smoldering myeloma (SMM) and MM patients in promoting tumor microenvironment transformation.
Methods: Human peripheral blood mononucleated cells (PBMC) isolated from healthy subjects (HS) were cultured alone, with HS- (n=8), MGUS- (n=6), SMM- (n=4) or MM-MSC (n=12, 8 patients at diagnosis and 4 relapsed) at 1:100 ratio. After one week, PBMC were collected. G-MDSC were isolated using anti-CD66b magnetic microbeads and the phenotype (CD11b+CD33+CD14-HLADR-) was confirmed by cytofluorimetric analysis. Immunosuppression was analyzed after incubation with autologous T cells CFSE+ stimulated by phytohaemagglutinin (PHA-P).
Results: G-MDSC educated by SMM- and MM-MSC co-cultures (MSCed-G-MDSC) exhibited suppressive effect with a reduction of T cell proliferation (p<0.001) compared to G-MDSC control (isolated from PBMC cultured in medium alone). Notably, neither MDSC control nor HS- or MGUS-MSCed-G-MDSC showed suppressive ability. Before incubation with T cells, the expression of immunomodulatory factors was investigated by real-time PCR in SMM- and MM-MSCed-G-MDSC compared to MGUS-MSCed-G-MDSC. SMM- and MM-MSCed-G-MDSC up-regulated Arg1 (56.4±18.2 and 24.9±13, p<0.001), NOS2 (82±35 and 21±18, p<0.001), TNFα (10±3 and 45.7±28.8, p<0.05) and CEBPA (90±23 and 65±19, p<0.001), a transcription factor promoting suppressive phenotype. Adding Bortezomib (5 nM) to co-culture of SMM- and MM-MSC with PBMC, isolated G-MDSC lost immunosuppressive ability. Analysis of MM-MSC from 4 patients revaluated after 3 bortezomib-based therapy followed by autologous stem cell transplantation showed that their immunological dysfunction was reverted after therapy. Since it has been reported that neutrophils can acquire monocytic characteristics in response to inflammatory signals, G-MDSC control and MSCed-G-MDSC were plated onto dentine disks (DDs) for 3 days. A significant digestive activity was observed only in DDs with MM-MSCed-G-MDSC (p=0.002) and was lost by MM-MSCed-G-MDSC isolated from co-culture with Bortezomib. Moreover, compared to MGUS-MSCed-G-MDSC, SMM- and MM-MSCed-G-MDSC up-regulated PROK2 expression (5.2±1.2 and 7.6±2, p<0.05), a chemotactic and pro-angiogenic factor. Investigating effect on angiogenesis *in vitro*, MM-MSCed-G-MDSC induced tube formation. On the contrary, this effect was not observed in the condition with MM-MSCed-G-MDSC isolated from co-culture with Bortezomib.
Summary/Conclusions: MSC from SMM and MM but not MGUS patients are able to activate G-MDSC favoring indirectly transformation of microenvironment in a "tumor" milieu with consequent immune escape and PC growth and survival. Their immunological dysfunction can be reverted by bortezomib exposure.

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TARGETING OF BMI-1 WITH PTC-209 AFFECTS MYELOMA CELL GROWTH & SURVIVAL AND IMPAIRS THE TUMOUR MICROENVIRONMENT

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Background: The polycomb complex protein BMI-1 was previously reported to be overexpressed in multiple myeloma (MM) and silencing of BMI-1 was shown to impair the proliferation and survival of MM cells. However, therapeutic agents specifically targeting BMI-1 are not available so far.
Aims: Here we investigated PTC-209, a novel transcriptional repressor of BMI-1, for its activity in MM.
Methods: *BMI-1* expression was analysed by using publically available gene expression (GEP) data. The anti-MM activity of PTC-209 was examined by viability testing, cell cycle analysis, annexin V and 7-AAD staining, quantification of cleaved PARP, JC-1 as well as colony formation assays. Gene and protein expression was studied by quantitative PCR and flow cytometry, respectively. The impact of PTC-209 on osteoclast, osteoblast and tube formation was analysed *in vitro* by using cell type specific differentiation assays.
Results: Overexpression of *BMI-1* in MGUS, SMM and MM patients was confirmed by using publically available GEP-datasets. Of note, *BMI-1* expression was further increased at relapse which translated into significantly shorter overall survival in relapsed/refractory patients treated with bortezomib or dexamethasone (median OS 22.2 months *vs* 13.7 months, *P*=0.003).
Treatment with PTC-209 induced downregulation of BMI-1 protein levels and significantly impaired viability of all HMCLs analysed with IC50 values <2 μM in 6 of 8 MM cell lines (range: 0.21-5.68 μM). Mechanistically, PTC-209 led to an accumulation of MM cells in the G1 phase of the cell cycle and induced apoptosis. The latter was confirmed by annexin V/7-AAD staining, detection of cleaved PARP and depolarization of the mitochondrial membrane. These alterations were