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Original Article

In vitro migratory aberrancies of mesenchymal stem cells derived from multiple myeloma patients only partially modulated by bortezomib

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Abstract: Recent studies indicated that bone marrow mesenchymal stem cells (BM-MSCs) derived from multiple myeloma (MM) patients were different from those of normal subjects in a variety of aspects. However, it is largely unknown whether BM-MSCs derived from MM patients display any aberrant chemotactic migration. To this aim, we compared the chemotactic migration of BM-MSCs derived from MM patients with those from normal subjects. Our results showed that BM-MSCs derived from MM patients migrated more vigorously to myeloma cell line. Furthermore, proteasome inhibitor bortezomib was showed to suppress chemotactic migration of BM-MSCs whatever their origins. However, although the chemotactic migration of BM-MSCs derived from MM patients to myeloma cell line was more significantly suppressed by bortezomib treatment, migration to SDF-1 or FBS of BM-MSCs was less compromised. Both SDF-1 and TNF-α enhanced phosphorylation of iκ-Bα in BM-MSCs. Although bortezomib significantly inhibited the iκ-Bα phosphorylation by SDF-1, it had little effect on iκ-Bα phosphorylation by TNF-α. Collectively, our results suggested that aberrant chemotactic migration of BM-MSCs derived from MM patients and the possible migration-regulatory role of bortezomib treatment.

Keywords: Multiple myeloma, bone marrow mesenchymal stem cells, chemotactic migration, bortezomib

Introduction

Multiple myeloma (MM) is a clonal plasma cell disorder characterized by the synthesis of an abnormal monoclonal immunoglobulin and/or light chain, bone destruction, immunodeficiency, and renal impairment. The genetic basis of the disease includes recurrent and complex genetic abnormalities in myeloma cells. In addition, the interaction of MM cells with the bone marrow (BM) microenvironment in the pathogenesis of this disorder is nowadays widely accepted [1].

Bone marrow mesenchymal stem cells (BM-MSCs) not only provide microenvironmental support for hematopoietic stem cells, but also can also differentiate into various mesodermal lineages [2]. Recently, several studies showed that BM-MSCs derived from MM patients displayed multiple aberrant characteristics such as certain cytokines production, abnormal proliferative capacity, and distinctive gene expression profile [3-6]. Moreover, in a variety of cancers, MSCs were showed to exhibit tropism for migrating to tumor sites [7-9]. Once MSCs were in close contact with tumor cells, tumor cells behaved more aggressively [9]. However, there is few study addressing chemotactic features of BM-MSCs in MM patients so far.

Aimed to investigate whether there is any difference in chemotactic migration of BM-MSCs between MM patients and normal subjects, we compared chemotactic migration of BM-MSCs between two cohorts. Bortezomib is the first proteasome inhibitor that has demonstrated efficacy in the treatment of MM [10]. In addition to inhibitory effects on MM cells, bortezomib was also indicated to exert positive effect on

Table 1. Primers used in RT-PCR

Name		Forward & reverse primers	Product size
SDF-1	Forward	5'-AGATGCCCTTGCCGATTC-3'	200 bp
	Reverse	5'-TTTGGCTGTTGTGCTTACTTG-3'	
MCP-1	Forward	5'-GCTCATAGCAGCCACCTTCATTC-3'	147 bp
	Reverse	5'-GGACACTTGCTGCTGGTGATTC-3'	
HGF	Forward	5'-GACCCTGGTGTTTCACAAGCAA-3'	132 bp
	Reverse	5'-TGCCTGATTCTGTATGATCCATGAG-3'	
VEGF	Forward	5'-GAGCCTTGCCTTGCTGCTCTAC-3'	148 bp
	Reverse	5'-CACCAGGGTCTCGATTGGATG-3'	
IGF-1R	Forward	5'-GGTCTCTGAGGCCAGAAATGGA-3'	124 bp
	Reverse	5'-TGGACGAACTTATTGGCGTTGA-3'	
PDGF-Rα	Forward	5'-GTGCGAAGACTGAGCCAGATTG-3'	121 bp
	Reverse	5'-CGATAAACAGAATGCTTGAGCTGTG-3'	
GAPDH	Forward	5'-TGGGTGGAATCATATTGGAAC-3'	136 bp
	Reverse	5'-TCAACGGATTTGGTCGTATTG-3'	

osteoblastic activity [11-13]. So the effects of bortezomib on BM-MSCs' migration were also investigated in this study.

Materials and methods

Patients

We studied 13 newly diagnosed patients with symptomatic myeloma defined by International Myeloma Working Group (IMWG). The median age in patient cohort is 56-year-old (range from 48 to 73). All patients' BM samples were obtained before any anti-myeloma treatment. As a control, 11 normal donors' BM samples were also obtained. These control subjects had the marched age range as the MM patients. Informed consent was obtained from all patients and all protocols were in accordance with ethical standards and approved by local institutional ethics committee.

Cell culture

To obtain human BM-MSCs, marrow mononucleated cells were isolated by centrifugation at 400 g on Ficoll-Hypaque (1.077 g/mL; StemCell, BC, Canada) and resuspended in DMEM medium supplemented with 10% FBS (Gibico, Carlsbad, CA, USA). After 24 h incubation, non-adherent cells were removed. The medium was changed every 3-4 days thereafter. After a mean 10 to 14-day culture, all adherent cells were digested with 0.25% trypsin-EDTA and split into new flasks. Subculture of BM-MSCs was performed in the same way when they reached 90% confluence. Myeloma cell line U266 was brought from ATCC and maintained in 10% FBS RPMI 1640.

Bortezomib was purchased from Millennium Pharmaceuticals Inc. (Cambridge, Massachusetts, USA). It was reconstituted in dimethylsulfoxide (DMSO, Sigma-Aldrich, St. Louis, Missouri, USA) at a stock concentration of 50 mM. For the treatment with either bortezomib or TNF-α (Prospec, Ness-Ziona, Israel), BM-MSCs were first cultured in the medium containing 2.5 nmol/L bortezomib or 1 ng/mL TNF-α for 24 hours, then the cells were harvested for further assays.

MTT assay

The MTT (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma-Aldrich, St. Louis, Missouri, USA) test was used for assessment of cell viability following bortezomib treatment. P3-P4 BM-MSCs were seeded into 96-well plates at the concentration of 3×10^3 cells/well and incubated for at 37°C and 5% CO_2 until 50% confluence. Then the medium of each well was replaced with 10 μl of 0.5 mg/ml MTT stock solution diluted in 90 μl PBS. After 2 h of incubation, isopropanol with 0.04 M HCl was added (100 μl/well). The absorbance was determined using a multiwell scanning spectrophotometer at 570 nm (GeneQuantpro, Biochrom Ltd, England). Growth inhibition was expressed as a percentage of viable cells under bortezomib treatment relative to control cells treated by an identical sequence of protocol steps except for bortezomib treatment.

Chemotactic migration assays

Chemotactic migration assays were performed in transwell dishes (Corning Costar, Lowell, MA, USA) with 8-μm pore filters. The upper side of the transwell filter was coated for 1 hour at 37°C with 0.1% (wt/vol) bovine gelatin (Sigma-Aldrich, St. Louis, Missouri, USA) in phosphate-buffered saline (PBS). Untreated or treated

Table 2. Proliferation inhibitory rates of BM-MSCs at a range of bortezomib concentration

Bortezomib (nmol/L)	BM-MSCs		
	24 h (n = 5)	48 h (n = 5)	96 h (n = 3)
1.25	0.0606 ± 0.05009	0.0972 ± 0.10805	0.1709 ± 0.25959
2.5	0.0711 ± 0.05596	0.1115 ± 0.10149	0.2158 ± 0.23668
5	0.1183 ± 0.06824	0.1871 ± 0.14086	0.3427 ± 0.19321
10	0.1720 ± 0.08421	0.2856 ± 0.12359	0.4239 ± 0.14305
25	0.2424 ± 0.07828	0.3229 ± 0.11671	0.5378 ± 0.1355
50	0.3309 ± 0.07628	0.4052 ± 0.14212	0.6427 ± 0.11057
100	0.4186 ± 0.12718	0.478 ± 0.10371	0.6619 ± 0.09383

P3-P4 BM-MSCs (2×10^5 cells) were added to the upper chamber, and 600 μL of migration medium containing either SDF-1 (Prospec, Ness-Ziona, Israel), or 30% FBS, or 2×10^5 myeloma cell line U266 cells was added to the bottom chamber. After overnight incubation at 37°C, the upper side of the filters was carefully washed with PBS, and cells remaining on the upper face of the filters were removed with a cotton wool swab. Transwell filters were stained using Wright's staining, cut out with a scalpel, and mounted onto glass slides, putting the lower face on the top. Ten random fields were selected for microscopic count at × 100 magnification by Nikon TE300 inverted microscope (Nikon Instrument Inc, Melville, NY, USA). Each experiment was performed in duplicate.

Western blotting analysis

Cells were lysed with SDS sample buffer containing 20 mM Tris-HCl (pH 7.6), 250 mM NaCl, 0.5% NP-40, 3 mM ethylenediaminetetraacetic acid, and 1.5 mM ethylene glycoltetraacetic acid with 10 mg/mL aprotinin, 10 mg/mL leupeptin, 1 mM DTT, 1 mM paranitrophenyl phosphate, and 0.1 mM Na3V04 as protease and phosphatase inhibitor (all from Sigma-Aldrich, St. Louis, Missouri, USA). Cell lysates were separated by sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) electrophoresis and transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). Blots were probed by anti-phospho-iκ-Bα, anti-iκ-Bα, or anti-GAPDH antibody (Cell Signaling Technology, Danvers, Massachusetts, USA) before visualizing with horseradish peroxidase-conjugated secondary antibodies (Cell Signaling Technology, Danvers, Massachusetts, USA) followed by development with FluorChem FC2 System (Alpha Innotech Corporation, San Leandro, CA, USA).

Real-time RT-PCR assays of chemotaxis associated genes

Total RNA was isolated from cultured BM-MSCs using single-step method with TRIzol (Invitrogen A/S, Taastrup, Denmark) according to the manufacturer's instructions. Total RNA (1 μg) was then reverse transcribed into DNA using cDNA synthesis kit (Promega, Fitchburg, Wisconsin, USA). Quantitative PCR was done with an SYBR Premix Ex Taq TM detection system (Takara Bio, Otsu, Shiga, Japan) according to manufacturer's instructions. After initial denaturation at 95°C for 2 minutes, 40 cycles of denaturation at 95°C for 10 seconds, annealing at 62°C for 15 seconds, and extension at 72°C for 27 seconds were carried out on Mx3000P PCR machine (Agilent Technologies Co., Santa Clara, CA, USA) and then analyzed by MxPRO-Mx3000P version 2.0 software. Each reaction was run in triplet. Primer sequences are listed in **Table 1**.

To adjust for differences in PCR efficiency, relative standard curves were obtained from 8-fold dilutions of pooled cDNA from pooled control BM-MSCs for either target or reference genes described by Standal et al. [14].

Flow cytometry

Flow cytometry (FCM) was performed as previously described [15]. Briefly, the cells were incubated for 20 min at 4°C with the following MoAb: CD10, CD13, CD14, CD15, CD34, CD45, CD73, CD90, CD19 and HLA-DR (Immunotech, Marseulle Cedex, France). As a control, corresponding cells labeled with isotype IgG were used. After washing with PBS, they were analyzed on ELITE Coulter flow cytometer (Coulter Electronics, Fullerton, CA, USA) using Epics list-mode software by the acquisition of 10,000 events for each sample.

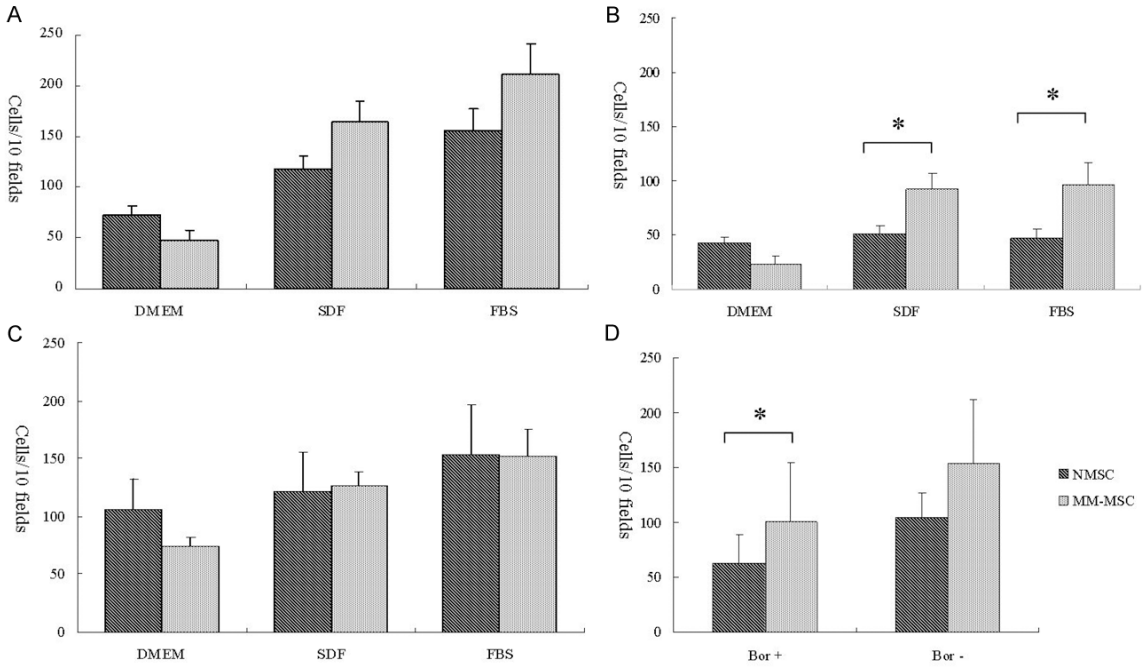

Figure 1. Transwell chemotactic migration of BM-MSCs. (A) Spontaneous transwell migration or chemotaxis of BM-MSCs to SDF-1 or 30% FBS. BM-MSCs were first treated with 2.5 nmol/l bortezomib (B) or 1 ng/ml TNF-α (C) for 24 hours, then spontaneous transwell migration or chemotaxis of BM-MSCs to SDF-1 or 30% FBS were assayed. D. Chemotactic migration of BM-MSCs to myeloma cell line U266 in the presence (Bor+) or absence (Bor-) of bortezomib treatment. NM-MSC: BM-MSCs derived from control cohort (n = 11). MM-MSCs: BM-MSCs derived from MM patients (n = 13). *Indicated there was statistic difference between two cohorts (independent *t*-test, $P < 0.05$).

Statistical analysis

Student's independent *t*-test or Paired *t*-test for data was used to test the probability of significant differences between samples. A value of $P < 0.05$ was used to define statistical significance.

Results

BM-MSCs immunophenotypic characteristics

As our previous results [15], the immunophenotype of normal BM-MSCs is positive for CD13, CD73 and CD90, but lacked CD45, CD14, CD15, CD34, CD10 and HLA-DR. In consistent with other reports [4, 16], the immunophenotype of BM-MSCs of MM patients was similar to that of control subjects (data did not show).

High concentrations of bortezomib inhibited BM-MSCs in vitro proliferation

It was previously reported that 2.5 nmol/L of bortezomib did not significantly inhibit proliferation and survival of osteoblastic cells [11]. In this study, we had the similar finding of BM-MSCs. However, if the treatment period is longer than 24 hours, the inhibitory effect on BM-MSCs would be more pronounced (**Table 2**). So we employed 24-hour incubation for all the subsequent assays experiments.

Chemotactic migration of MM BM-MSCs to SDF-1 or FBS less compromised by bortezomib

As shown in **Figure 1**, for both MM and control BM-MSCs, there is "spontaneous" transwell migration (48.00 ± 9.76 *vs.* 73.17 ± 8.55 cells/10 fields) when cultured in serum-free medium. BM-MSCs' migration was enhanced by either SDF-1(164.78 ± 19.97 *vs.* 117.92 ± 12.63 cells/10 fields) or 30% FBS (211.67 ± 30.03 *vs.* 155.67 ± 21.77 cells/10 fields) (**Figure 1A**). However, there was no statistical difference in migration between two cohorts (Independent *t*-test, $P = 0.052$).

After a 24-hour bortezomib treatment, the spontaneous migration (26.67 ± 7.28 *vs.* 42.58 ± 5.74 cells/10 fields), migration to either SDF-1 (92.00 ± 15.54 *vs.* 51.58 ± 7.10 cells/10 fields) or 30% FBS (96.22 ± 19.92 *vs.* 47.11 ± 8.55

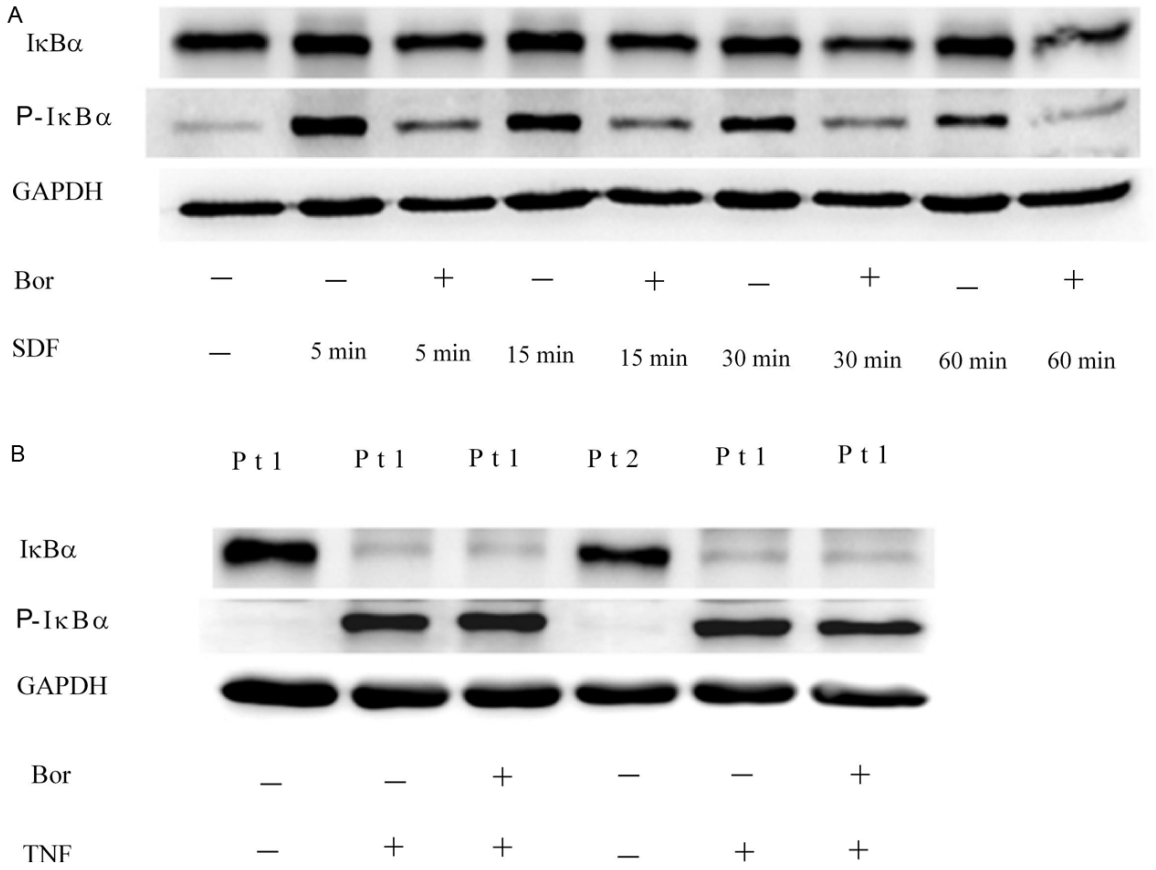

Figure 2. Bortezomib suppressed NF-κB pathway activation in BM-MSCs by SDF-1. A. Firtstly, BM-MSCs were stimulated by SDF-1 for 5, 15, 30 and 60 minutes, respectively. Then iκ-Bα or phospho-iκ-Bα (P-iκ-Bα) in BM-MSCs were detected by Western blot. To test the effect of bortezomib on iκ-Bα or P-iκ-Bα, BM-MSCs were pre-treated by bortezomib for 24 hours before SDF-1 stimulation. B. Firstly, BM-MSCs were stimulated by TNF-α, iκ-Bα or phospho-iκ-Bα (P-iκ-Bα) in BM-MSCs was detected by Western blot 5 minutes later. To test the effect of bortezomib on iκ-Bα or P-iκ-Bα, BM-MSCs were pre-treated by bortezomib for 24 hours before TNF-α stimulation. Results are shown in two representative samples (Pt1 & Pt2).

cells/10 fields) were suppressed in both MM and control cohorts. Interestingly, although there was no statistical difference for spontaneous migration between two cohorts (Independent *t*-test, $P > 0.05$), the migration of MM BM-MSCs toward SDF-1 (independent *t*-test, $P = 0.016$) or 30% FBS (independent *t*-test, $P = 0.038$) was less compromised than that of control by bortezomib treatment (**Figure 1B**).

MM and control BM-MSCs show no difference in transwell migratory capacity in the presence of TNF-α

TNF-α was previously reported to enhance in vitro migration of MSCs [17]. After BM-MSCs were treated with TNF-α for 24 hours, the spontaneous transwell migration, migration to SDF-1 or 30% FBS of either MM or control BM-MSCs were 74.00 ± 7.68 vs. 106.18 ± 26.67cells/10 fields, 126.67 ± 11.76 vs. 121.64 ± 33.08 cells/10 fields, 152.00 ± 20.60 vs. 153.25 ± 43.67 cells/10 fields, respectively. However, there was no statistical difference in transwell migration between MM and control cohorts no matter spontaneous or migration to SDF-1 or 30% FBS (**Figure 1C**; independent *t*-test, all $P > 0.05$).

MM BM-MSCs display more vigorous migration to MM cell line

We then investigate BM-MSCs' transwell migration to myeloma cell line U266. On the contrary to the results with SDF-1 or 30% FBS, there was statistical difference in migration between MM and control cohorts (153.56 ± 58.47 *vs.* 104.72 ± 21.90 cells/10 fields), with the MM

BM-MSCs migrated more vigorously than the control cells (**Figure 1D**; independent *t*-test, $P = 0.04$). Similar to the results with SDF-1 or 30% FBS, the transwell migration of BM-MSCs from both MM and control cohorts was suppressed by bortezomib treatment (100.83 ± 53.55 *vs.* 62.67 ± 25.98 cells/10 fields; paired *t*-test, $P < 0.00$). However, there was no statistical difference in transwell migration of BM-MSCs between two cohorts (**Figure 1D**; independent *t*-test, $P > 0.05$).

Bortezomib suppressed NF-κB pathway activation by SDF-1

When unstimulated, the level of phospho-iκ-B α in BM-MSCs was undetectable or minimal although there was an evident accumulation of iκ-Bα in BM-MSCs, (**Figure 2A**, **2B**). After SDF-1 stimulation, the level of phospho-iκ-Bα in BM-MSCs increased markedly, achieving its highest level at 5 minutes post-stimulation and gradually decreasing thereafter. However, if BM-MSCs were pre-treated by bortezomib for 24 hours before SDF-1 stimulation, the levels of phospho-iκ-Bα for each time point in BM-MSCs were lower than control ones (**Figure 2A**). As another NF-κB agonist, TNF-α significantly increased the level of phospho-iκ-Bα and decreased the level of iκ-Bα in BM-MSCs. However, in contrast to the results with SDF-1, pre-treatment of BM-MSCs by bortezomib couldn't effectively suppress the phosphorylation of iκ-Bα (**Figure 2B**).

Levels of mRNAs for chemotaxix associated genes differentially expressed between MM and control BM-MSCs

As shown in **Figure 3**, the relative copies of SDF-1 (1.83 ± 0.91 *vs.* 3.12 ± 1.38), VEGF (1.05 ± 0.58 *vs.* 0.55 ± 0.17) and HGF (0.40 ± 0.21 *vs.* 0.20 ± 0.18) in BM-MSCs were statistically different between two cohorts (Independent *t*-test, $P < 0.05$), with the mRNA level of SDF-1 in MM cohort was lower, while the levels of VEGF and HGF were higher than those of control cohort. However, there was no difference in the mRNA levels of MCP-1 (1.92 ± 1.22 *vs.* 1.55 ± 1.46), IGF-1R (0.53 ± 0.36 *vs.* 0.34 ± 0.18) and PDGF-Rα (1.18 ± 0.83 *vs.* 0.85 ± 0.57) between two cohorts (**Figure 3A**).

Next, we investigated the effects of bortezomib on mRNA levels of these genes in BM-MSCs. As shown in **Figure 3B**, **3C**, bortezomib down-regulated expression of all these investigated genes in BM-MSCs whatever their origins (Paired t-test, all $P < 0.05$). Interestingly, it seemed that bortezomib down-regulated mRNA levels of tested genes in the control cohort more markedly than those in the MM cohort (**Figure 3D**).

Discussion

The pivotal role of the BM microenvironment in MM pathogenesis is now well established. Specifically, the balanced homeostasis among the cellular, extracellular and liquid compartments within the BM is disrupted [1]. Bortezomib is a novel approach to treat MM and it acts on a unique target in cells, the proteasome. These effects are partly mediated through inhibition of the NF-κB pathway. In addition to target on myeloma cells directly, bortezomib was also showed to exert positive effect on abnormal marrow environment of MM. De Matteo et al. [18] reported that bortezomib up-regulated the osterix transcription. In another study by Giuliani et al. [11], they reported that a stimulatory effect of bortezomib on bone nodule formation in osteoblastic progenitors.

In our previous study, transwell migration of BM-MSCs was shown to be inhibited after 48-hour incubation with bortezomib [19]. However, it is unclear whether bortezomib could modulate the chemotactic migration of BM-MSCs derived from MM or normal subjects differentially. This study showed that BM-MSCs derived from MM patients exhibited more vigorous chemotactic migration to U266 than that of control. Besides, after a 24-hour bortezomib treatment, the migration of BM-MSCs derived from MM subjects to 30% FBS or SDF-1 seemed to be less affected. All those findings implied that the chemotactic capacity of BM-MSCs derived from MM patients was different of that of normal individuals. Furthermore, bortezomib treatment was showed to reverse the migratory difference to U266 between two cohorts. These finding implied that bortezomib only partially modulate MM BM-MSCs' aberrant chemotactic migration .

Recently, there were reports that once tumor cells were in close contact with MSCs, they showed more aggressive behavior. For example, Kurtova et al. [20] reported that MSCs protect Mantle cell lymphoma (MCL) cells from

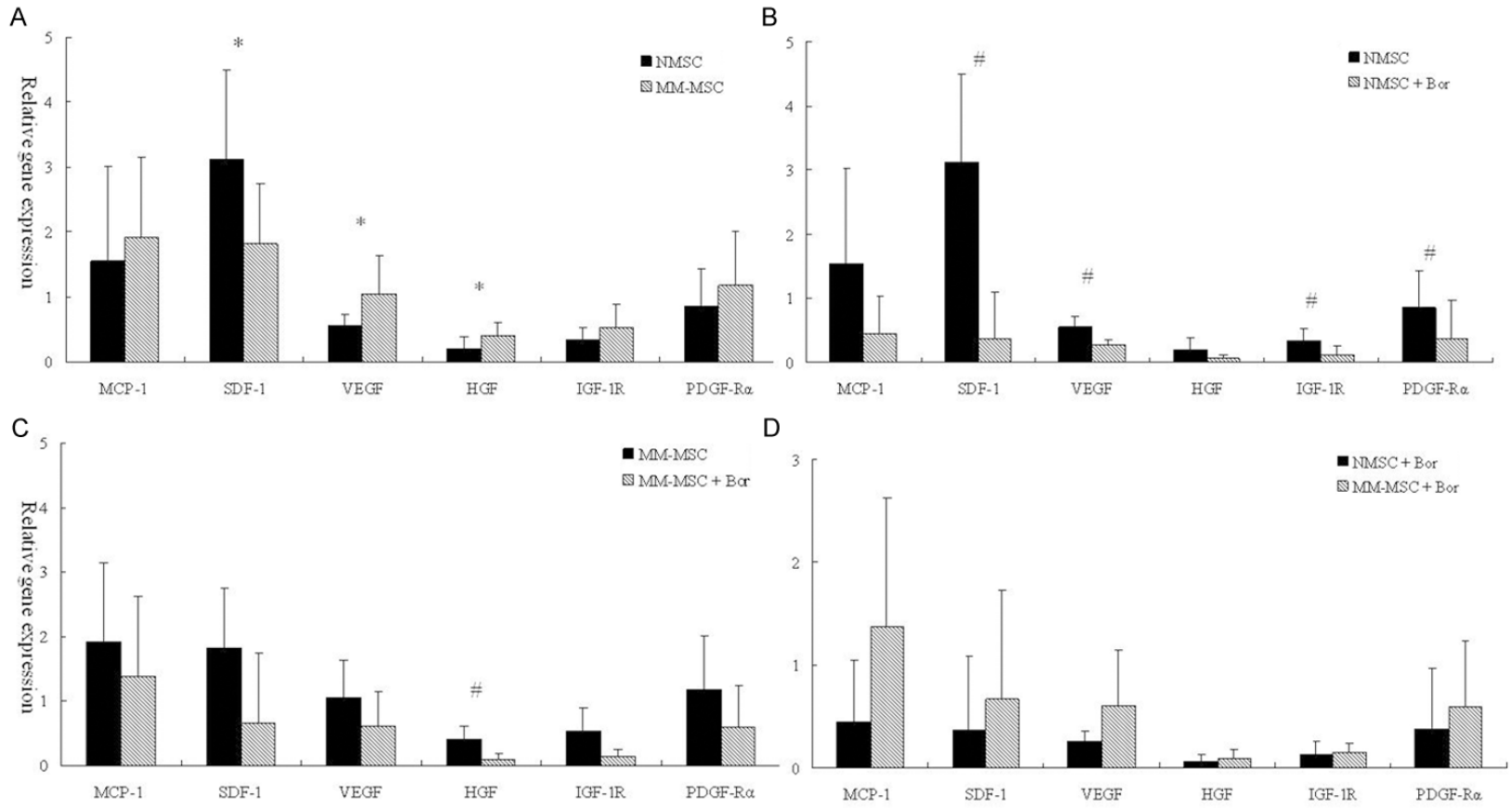

Figure 3. Real-time PCR assays of BM-MSCs pre- or post-bortezomib treatment. MCP-1, SDF-1, VEGF, HGF, IGF-1R and PDGF-Rα mRNA levels of BM-MSCs derived from MM or control cohorts (A). mRNA levels of BM-MSCs in control cohort pre- or post-bortezomib treatment (B). mRNA levels of BM-MSCs in MM cohort pre- or post-bortezomib treatment (C). Comparison of mRNA levels of MM and control cohorts' post-bortezomib treatment (D). *There was statistical difference between MM and control BM-MSCs (Independent *t*-test, $P < 0.05$). #Indicated that there was significant statistical difference between non-treated and bortezomib-treated BM-MSCs derived from either control or MM subjects (Paired *t*-test, $P < 0.01$). NMSC, control BM-MSCs.

cytotoxic effect of fludarabine and 4-hydroperoxycyclophosphamide when MCL cells were co-cultured with MSCs. In another study, otherwise weakly metastatic human breast carcinoma cells exhibited highly metastatic behavior when mixed with BM-MSCs [8]. Since the chemotactic migration of MSCs to MM cells will bring them into a closer proximity, it probably enhances the cross-talk between MM cells and BM-MSCs, and then favors the survival of MM cells [21]. Because bortezomib was shown to disrupt the chemotactic migration of BM-MSCs, so it could also inhibit the direct interaction between BM-MSCs and MM cells, which makes MM cells more vulnerable to the stress and therapy.

As one of important non-hemapoietic stem cells in BM, BM-MSCs were recently also showed to be able to modulate B-cell function such as immunoglobulin production, chemokine receptor expression [22]. SDF-1 (CXCL12) is the ligand for CXCR4, which was reported to be expressed in normal B-cells, plasma cells and myeloma cells [22-24]. SDF-1-expressing bone marrow stromal cells were indicated to be essential for the survival of long-lived plasma cells [25]. In MM, the SDF-1\CXCR4 axis was also believed to be important for the survival of MM cells [26, 27]. In this study, one of interesting findings is that the mRNA levels of SDF-1, VEGF and HGF in BM-MSCs were differentially expressed between MM and control cohorts ($P < 0.05$). It was previously reported that the concentrations of VEGF and HGF were higher in the BM of MM patients [28-31]. In contrast to VEGF and HGF, the mRNA level of SDF-1 was lower in the MM BM-MSCs. Since SDF-1 is a critical soluble factor for MM cells survival, it is surprised to find so. However, we assumed that low SDF-1 level in marrow might do more harm to the surviving niche of normal long-lived plasma cells [25], because SDF-1 could also be abundantly produced by myeloma cells themselves [32].

When BM-MSCs were treated by bortezomib for 24 hours, all the mRNA levels of chemotaxis-associated factors/receptors in BM-MSCs were down-regulated. According to published researches, MCP-1, SDF-1, VEGF and HGF were involved in pro-myeloma cell activity and/or marrow microenvironment abnormality in myeloma patients [21, 29, 31-34). Besides, IGF-1R and PDGF-Rα were reported to be associated with the chemotactic migration of human BM-MSCs [17, 35]. The suppression of those tested gene by bortezomib may be one of mechanisms that bortezomib inhibited migration of BM-MSCs.

Bortezomib was showed to induce myeloma cell apoptosis and interfere with NF-κB-dependent induction of cytokine secretion [10]. In addition to direct anti-myeloma effects, it was also showed to suppress the NF-κB-dependent transcription in bone marrow stromal cells [36]. Our study demonstrated that pre-treatment of BM-MSCs with bortezomib significantly inhibited phosphorylation of iκBα by SDF-1. For cells such as microglia and neural progenitor cell, SDF-1 was showed to up-regulate NF-κB pathway activity [37, 38]. Besides, the migration of some types of cells was partially regulated by NF-κB pathway [38, 39]. Since NF-κB pathway regulates a large panel of target genes (*http://www.bu.edu/nf-kb/gene-resources/target-genes/*) and some of them are involved in cell migration, it is reasonable to believe that bortezomib inhibit BM-MSCs chemotactic migration to SDF-1 at least partially via NF-κB pathway. As for TNF-α, it was also reported to be a NF-κB pathway agonist for MSCs [39, 40]. In consistent with those reports, we observed that TNF-α activated NF-κB pathway in BM-MSCs as well. However, unlike the results with SDF-1, pre-treatment of BM-MSCs with bortezomib was not enough to inhibit phosphorylation of iκBα by TNF-α. It may imply that bortezomib pre-treatment does not antagonize NF-κB pathway agonists TNF-α as effectively as SDF-1 in BM-MSCs.

In summary, our study showed that there is difference in migratory capacity of BM-MSCs between MM patients and normal subjects, and bortezomib was showed to be able to partially reverse the differences. Besides, bortezomib was also showed to inhibit NF-κB pathway activation by SDF-1 in BM-MSCs. Our finding implied that bortezomib might exert its anti-myeloma activities partially by both depriving extracellular survival signals for myeloma cells and re-adjustment of abnormal marrow microenvironment which used to favor the survival for myeloma cells. However, bortezomib itself may not be able to reverse all the abnormalities in BM microenvironment and more effective treatment should be developed for the correction of aberrant BM microenvironment.

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Disclosure of conflict of interest

None.

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