Overexpression of Yin Yang 1 in bone marrow-derived human multiple myeloma and its clinical significance

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Abstract. Multiple myeloma (MM) patients initially respond to conventional therapy, however, many develop resistance and have recurrences. We have reported in other tumors that the transcription factor Yin Yang 1 (YY1) is a resistant factor and, thus, we hypothesized that YY1 may be over-expressed in MM. Significantly, higher expression (staining intensity and cell frequency) of YY1 in MM cell lines and in bone marrow-derived (BM) MM from 22 MM patients was observed as compared to expression in normal BM. Higher nuclear YY1 staining was associated with disease progression. Bioinformatic analyses of mRNA in data sets corroborated the above findings and showed significant overexpression of YY1 in MM compared to normal tissues and other hematopoietic disorders. The role of YY1 expression in the regulation of drug resistance was exemplified in a drug-resistant MM cell line transfected with YY1 siRNA and which was shown to be sensitized to bortezomib-induced apoptosis. These findings highlight the potential prognostic significance of YY1 expression level in MM patients and as a therapeutic target.

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Introduction

Multiple myeloma (MM) is a fatal plasma cell (PC) malignancy characterized by an accumulation of malignant PCs within the bone marrow (1). MM is responsible for 15,000 new cases per year both in Europe and in the US (2) and represents 10-15% of hematological malignancies in Caucasian and 20% in the Afro-American populations. Several risk factors are associated with this malignant disease, such as male gender, black race, positive family history and monoclonal gammopathy of undetermined significance (MGUS), another clinical picture that often precedes multiple myeloma (3).

The introduction of several novel and active treatments and improvements in the supportive care of myeloma patients has resulted in the prolongation of the survival of these patients, although some patients may be cured with an allogeneic stem cell transplantation, and the median survival remains around 3-4 years (4). In the past decade, there have been major advances in the treatment of MM. The proteasome inhibitor bortezomib has emerged as a highly active agent in the treatment of MM (5). The response rates with relapsed diseases are approximately 50% with the combination of bortezomib with thalidomide and steroids and 65% with a 3-drug combination of thalidomide, steroids and cyclophosphamide (6). Patients who are not transplant candidates are treated with standard alkylating agents therapy, namely, melphalan, prednisone and thalidomide (MPT); melphalan, prednisone and bortezomib (MPB); and melphalan, prednisone and lenalidomide (MPR).

Although the initial clinical responses to drug therapies are achieved, a significant percentage of MM patients relapses and no longer responds to single or combined treatments (7). Therefore, the resistance of MM to current therapeutic regimens remains an unsolved problem in the management of patients with MM. The main problem in MM patient treatments is the development of cross-resistance to conventional therapies and this resistance causes this disease to remain incurable. Therefore, the identification of novel biomarkers for unresponsiveness is urgently needed in

order to predict the outcomes of various treatment regimens of MM and to apply an appropriate customized and more beneficial treatment regimen.

Ying Yan 1 (YY1) is a ubiquitous and multifunctional transcription factor that can act as a transcriptional repressor, activator, or initiator element-binding protein, depending on the context in which it acts (8). We and others have reported of the role of YY1 as a prognostic marker in different cancer diseases, such as prostate cancer and cervical carcinoma (9,10). Recent studies suggest that elevated YY1 expression and/or its transcriptional activity might contribute to tumor formation and/or progression (11-13). In addition, YY1 has been identified as a potential repressor factor for several genes involved in immuno- and chemo-resistance. We reported that YY1 can act as a transcription repressor for both the Fas and the TRAIL DR5 receptors in prostate carcinoma and lymphoma cell lines (14,15). YY1 also mediates the regulation of tumor cell resistance to cytotoxic chemotherapy (16).

The objective of the present study was to identify a biomarker of prognostic significance in MM and investigate its clinical significance. We hypothesized that high YY1 expression in MM might be directly associated in both the pathogenesis of MM and its resistance to cytotoxic chemotherapy. To test this hypothesis, we have investigated the following: i) the expression levels of YY1 in MM cell lines and bone marrow-derived tissues from MM patients; ii) the association between the cell frequency and the intensity of YY1 expression as a function of disease progression; iii) bioinformatic analyses of YY1 transcript levels in publicly available datasets containing gene expression profiles of MM and normal B cells and correlation with both clinical parameters and patient's outcome; and iv) the role of YY1 in the regulation of MM resistance to cytotoxic drugs such as bortezomib. The findings reported herein supported the above hypothesis.

Materials and methods

Cells and culture conditions. The human MM cell lines (MM1s, 8266, IM-9 and U266) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were maintained in culture dishes in RPMI-1640 (Life Technologies, Bethesda, MD, USA), supplemented with 5% heat-inactivated fetal bovine serum (FBS) (Life Technologies) (to ensure the absence of complement), 1% (v/v) penicillin (100 U/ml), 1% (v/v) streptomycin (100 U/ml), 1% (v/v) L-glutamine, 1% (v/v) pyruvate, and 1% non-essential amino acids (Invitrogen Life Technologies, Carlsbad, CA, USA). The cell cultures were incubated at 37˚C and 5% CO_2.

Reagents. The anti-β-actin and the anti-tubulin monoclonal antibodies were purchased from Biosource International (Camarrillo, CA, USA) and from Calbiochem (San Francisco, CA, USA), respectively. Anti-YY1 antibody was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Phycoerythrin (PE)-conjugated anti-active caspase 3 and PE-conjugated IgG were purchased from BD Pharmingen (San Diego, CA, USA). SureSilencing™ siRNA kits were purchased from SuperArray Bioscience Corporation (Frederick, MD, USA). Melphalan was purchased from Sigma-Aldrich (St. Louis, MO, USA). Bortezomib was available commercially (Millennium Pharmaceuticals, Inc, Cambridge, MA, USA).

Table I. The patient cohort.

No of patient	Stage	Treatment response	Disease
1011	IA/I	PR	Non-progressive
1020	IA/II	PR	Non-progressive
1022	IIIA/II	NR	Progressive
1029	NA	NR	Progressive
1033	IIA/II	PR	Non-progressive
1034	IIA/II	PR	Non-progressive
1036	IIIA	NR	Progressive
1058	IA/I	NR	Non-progressive
1059	IA/I	NR	Non-progressive
1101	IA/I	NR	Progressive
1111	NA	PR	Non-progressive
1115	IA/I	NR	Progressive
1185	IA/I	PR	Non-progressive
3494	IA	NR	Progressive
6581	IIIA	NA	Progressive
7720	IA	PR	Non-progressive
8480	NA	NR	Progressive
8579	IIIA	NR	Progressive
9662	IA	NR	Progressive
11110	IA	NR	Progressive

PR, positive response; NR, negative response; NA, not available.

Patient population. Eligible patients had a prior confirmed diagnosis of MM based on the Durie criteria (17). All patients had been pretreated, showing either relapsed disease at any point in time following stabilization or a response to at least one prior anti-myeloma regimen or refractory disease (progressed while receiving an anti-myeloma treatment) (Table I).

Tissues used for analysis. Peripheral blood (PB) and bone marrow (BM) aspirates were obtained from patients with MM and age and gender-matched healthy control subjects. The study was approved by the Institutional Review Board (Western IRB BIO 001) and informed consent was obtained in accordance with the Declaration of Helsinki. Patients were defined as having indolent MM or symptomatic disease. The treated patients were determined by showing a progressive or a responsive disease [partial response (PR), very good (VG), or complete response (CR)] according to the International Myeloma Working Group (IMWG) criteria (18). Individual patients with multiple myeloma were analyzed from the time of their first assessment. PB and BM aspirates were collected in heparinized tubes and mononuclear cells (MCs) were isolated using density-gradient centrifugation with Histopaque-1077 (Sigma-Aldrich). Cells were cultured in RPMI-1640 medium (Omega Scientific, Tarzana, CA, USA) supplemented with 10% fetal bovine serum, non-essential amino acids, 2 mmol/l glutamine, 1 mmol/l sodium pyruvate,

25 mmol/l HEPES, 200 U/ml penicillin and streptomycin at 37˚C and 5% CO_2.

Cell treatment. The log-phase culture of the U266 cell line was seeded into 6-well plates at approximately $6x10^5$ cells/ml and grown in 1 ml of medium, as described above in 5% FBS for 24 h to approximately 70% confluence. The U266 cells were synchronized by treatment with 1% FBS for 18 h prior to each experiment.

Semiquantitative reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was extracted and purified from $1x10^6$ cells by a single-step monophasic solution of phenol and guanidine isothiocyanate-chloroform using TRIzol® reagent (Life Technologies) as previously described (19).

Western blot analysis. MM cells were cultured at a low FBS concentration (1%) for 18 h prior to each treatment and then lysates were prepared and analyzed by western blot analysis as described (20).

Immunocytochemistry. The expression level of YY1 was determined using the antibody directed against YY1 in MM cell lines or bone marrow samples derived from MM patients. Analysis and quantification were done as previously described (21).

Scoring of immunocytochemistry of bone marrow cells derived from MM patients. A semiquantitative assessment of antibody staining on the bone marrow cells derived from MM patients was done by a study pathologist (M.C.-M.) blinded to the clinopathologic variables. The stained slides were checked by a second pathologist for consistency of scoring. YY1 cytoplasmic and nuclear expressions were scored based on the positive staining in the nucleus and cytoplasm or both. Data are presented as either positively stained target cells per 200 cells (range 10-100% positive), per region on each spot (4 regions in each spot), or density (quantitative assessment), where four 100-μm^2 regions per spot were selected randomly and the density of the staining intensity in each region was analyzed using the Image Pro-plus 6.4 software (Media Cybernetics, Bethesda, MD, USA). To represent expression within cases, the mean pooled integrated intensity of the bone marrow cells from MM patients or normal bone marrow was used.

Determination of apoptosis. After each treatment, the cells were recovered following centrifugation at 1,800 rpm for 8 min. The cells were washed and resuspended in 100 μl of the cytofix/cytoperm solution (BD Pharmigen) for 20 min. Thereafter, the samples were washed and were stained with PE-labeled anti-active caspase 3 mAb for 30 min. The samples were subsequently washed and analyzed on a flow cytometer EPICSR XL-MCL (Beckman Coulter, Co., Miami, FL, USA), with the System II™ Software and the percent positive cells was recorded. As a negative control, the cells were stained with the isotype control (PE-IgG) under the conditions described above.

siRNA transfection. U266 cells were cultured in 1 ml of RPMI medium supplemented with 5% FBS. Transfection was performed by using the Lipofectamine 2000 CD Reagent supplied by Invitrogen (Invitrogen Life Technologies) and the SureSilencin siRNA kit supplied by SuperArray Bioscience Corporation according to the manufacturer's instructions. To determine the expression of YY1, the cells were harvested 48 h after transfection and RT-PCR and western blot analyses were performed. To determine the MM sensitization to bortezomib-mediated apoptosis, 48 h after transfection the cells were treated for 18 h with bortezomib (2.5 and 5 nM) or melphalan (5, 10 and 20 μM) and then levels of apoptosis were evaluated.

Oncomine data analysis. The web-based human cancer microarray database Oncomine (https://www.oncomine.com) was used to analyze the mRNA expression associated with MM (22,23). Details of standardized normalization techniques and statistical calculations can be found on the Oncomine website (https://www.oncomine.com).

Study description. A total of 74 multiple myeloma samples, 7 multiple myeloma cell lines, 5 monoclonal gammopathy of undetermined significance samples, and 45 normal samples were analyzed on Affymetrix HumanGeneFL microarrays.

Statistical analysis. The experimental values were expressed as the mean ± SD for the number of separate experiments indicated in each case. One-way ANOVA was used to compare variance within and among different groups. When necessary, Student's t-test was used for comparison between two groups. Significant differences were considered for probabilities <5% ($p<0.05$).

Results

Overexpression of YY1 in MM BM cells. Given the reported role of YY1 as a negative regulator of apoptosis in various tumors including prostate, ovarian carcinoma and NHL (15,24,25), we have hypothesized that YY1 may also be playing an important role in the resistance of MM to chemotherapy-induced apoptosis. We tested this hypothesis by examining first the expression levels of YY1 in four different MM cell lines and bone marrow-derived MM tissues from MM patients by western blot and immunocytochemistry analyses. All of the tested MM cell lines, MM1.S, RPMI-8826, IM9 and U266, expressed significantly higher levels of YY1 compared to PBMC cells used as a normal control (Fig. 1A). These findings were further verified using immunocytochemistry whereby the expression of YY1 was significantly higher in the entire MM cell lines tested and was predominantly expressed in the nucleus. In contrast, the expression of YY1 in PBMC was very low and predominantly in the cytoplasm (Fig. 1B).

The above findings in cell lines were corroborated in bone marrow-derived MM from patients. Fig. 2A shows the western blot analysis results of two representative MM samples from two patients whereby the expression of YY1 was significantly higher than the expression in a normal PBMC. These findings were further verified using immunocytochemistry staining which demonstrated an increased expression of YY1 in MM compared to a normal BM sample (Fig. 2B). Similar findings were observed when the YY1 expression in a few representative MM samples was analyzed by flow cytometry. Fig. 2C shows a significantly higher expression of YY1 in BM-derived MM samples from three patients as compared to normal

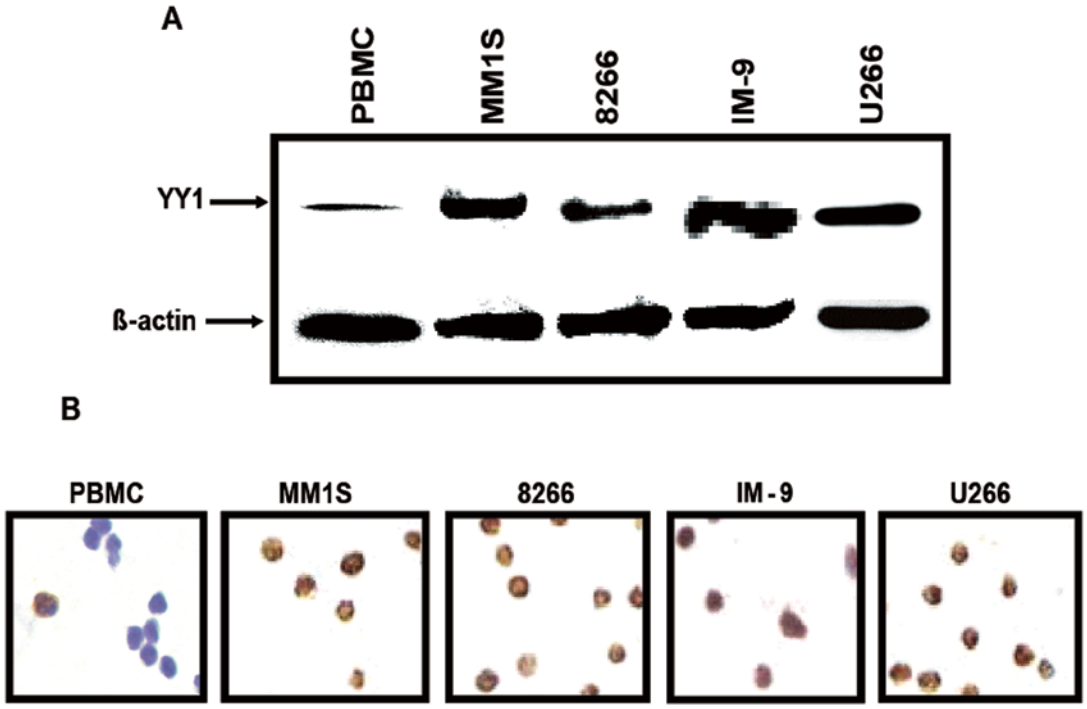

Figure 1. Overexpression of YY1 in MM cell lines. (A) Western blot analysis. The MM cell lines (MM1S, 8266, IM-9 and U266) and PBMC were cultured and total lysates were prepared and examined by western blot analysis using anti-YY1 antibody and anti-β-actin antibodies. β-actin was used as a loading control. All blots are representative of one of three separate experiments. (B) Immunohistochemistry. The expression of YY1 in MM cell lines and PBMC was assessed by immunocytochemistry. Magnification, x40.

BM. The MM 8226 cell line was used as a positive control for YY1 staining. The frequency of cells stained for YY1 in BM-derived MM tissues varied from one patient to another. Altogether, the above findings demonstrated that MM cells (cell lines and patient samples) expressed higher levels of YY1 in comparison with normal BM or PBMC.

The overexpression of YY1 in MM is associated with progressive disease

Analysis by immunocytochemistry. We examined a total of 20 BM-derived MM tissue samples and separated the non-progressive from the progressive patients (Table I). There were no differences on the total density of YY1 between the two groups (data not shown). Analysis of the cell frequencies for total expression (cytoplasmic and nuclear) of YY1 did not demonstrate any significant differences between the progressive and non-progressive groups (Fig. 3A). Likewise, there were no significant differences for the cytoplasmic expression of YY1 between the two groups (Fig. 3B). However, in contrast, there was a significant overexpression of nuclear YY1 in the progressive group versus the non-progressive group (Fig. 3C) ($p=0.035$). These findings suggested that the overexpression of YY1 in the nucleus (presumably it is transcriptionally active) correlated with a poor prognosis.

Bioinformatic analyses. In order to corroborate the above findings, we used computational analysis of YY1 mRNA levels in different groups of MM patients, as described in Materials and methods, and the findings are summarized in Fig. 4. In Fig. 4A, we show the Oncomine analysis of YY1 mRNA expression in MM. Box plot diagrams were analyzed to compare the YY1 mRNA levels in normal plasma cells, tonsillar lymphoid tumors and monoclonal gammopathy. Analysis of studies reported by Zhan *et al* (22,23) revealed that the YY1 expression was higher in MM than in normal plasma cells, tonsillar lymphoid tissue and monoclonal gammopathy, suggesting that YY1 may play a crucial role in the pathogenesis of MM development. The vertical axis represents the log2 median value. The upper (75%) and lower (25%) quartiles are represented by the upper and lower borders of the boxes, respectively ($p<0.05$). In Fig. 4B, YY1 gene expression is shown as a function of clinical stages and shows that YY1 is significantly overexpressed in stage II and stage III as compared with stage I ($p<0.001$).

The direct role of YY1 expression in an MM cell line in the regulation of resistance to bortezomib-induced apoptosis. The above findings demonstrated that the overexpressed YY1 in BM correlated with poor prognosis. We have recently reported that YY1 regulates chemoresistance in solid tumor cells (25). Thus, we hypothesized that the overexpression of YY1 in MM may be involved in the chemoresistance to cytotoxic chemotherapeutic drugs. The MM cell line U266 was used as a representative cell line for analysis. Transfection of U266 cells with YY1 siRNA or control siRNA was performed as described in Materials and methods. Fig. 5A demonstrates that treatment of U266 cells with YY1 siRNA, but not control siRNA, inhibited the expression of mRNA and protein for YY1 as assessed by both western blot analysis (Fig. 5B) and immunocytochemistry (Fig. 5C). The YY1 siRNA transfected cells or control siRNA transfected cells were treated with two different concentrations of bortezomib (2.5 and 5 nM) or different concentrations of melphalan (5, 10 and 20 μM) for 18 h and analyzed for

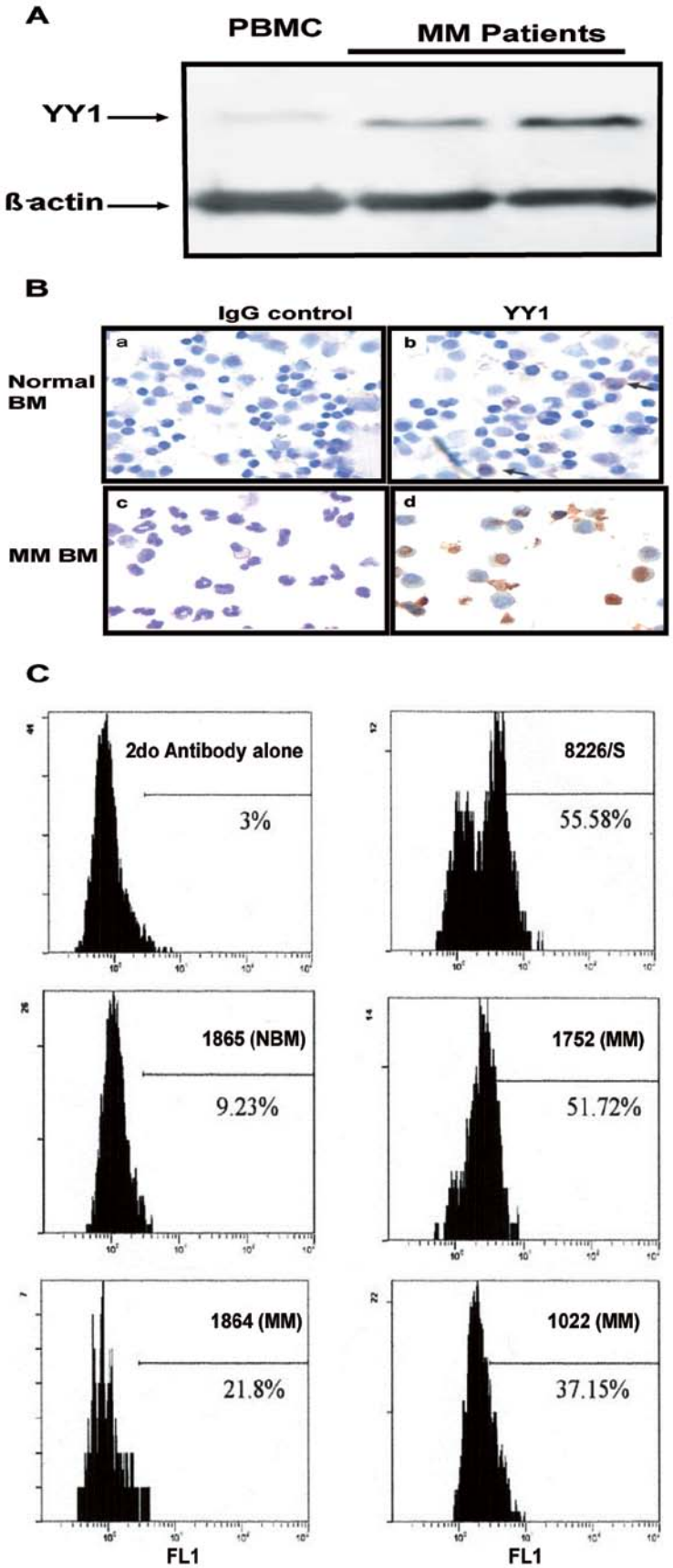

Figure 2. Overexpression of YY1 in BM cells derived from MM patients. (A) Western blot analysis. The bone marrow (BM) cells derived from two representative MM patients and normal PBMC were cultured and total lysates were prepared and examined for YY1 by western blot analysis. β-actin was used as a loading control. All blots are representative of one of two separate experiments. (B) Analysis of YY1 expression by immunocytochemistry. Representative samples of a normal BM and a BM from an MM patient were examined for cytoplasmic and nuclear expression of YY1 (panels b and d). Non-immune pooled rabbit IgG served as a negative control (panels a and c). Magnification, x100. (C) Analysis of YY1 expression in MM cell lines and BM from different MM patients by flow cytometry. A secondary antibody alone was used as negative control. Data are representative of three independent experiments.

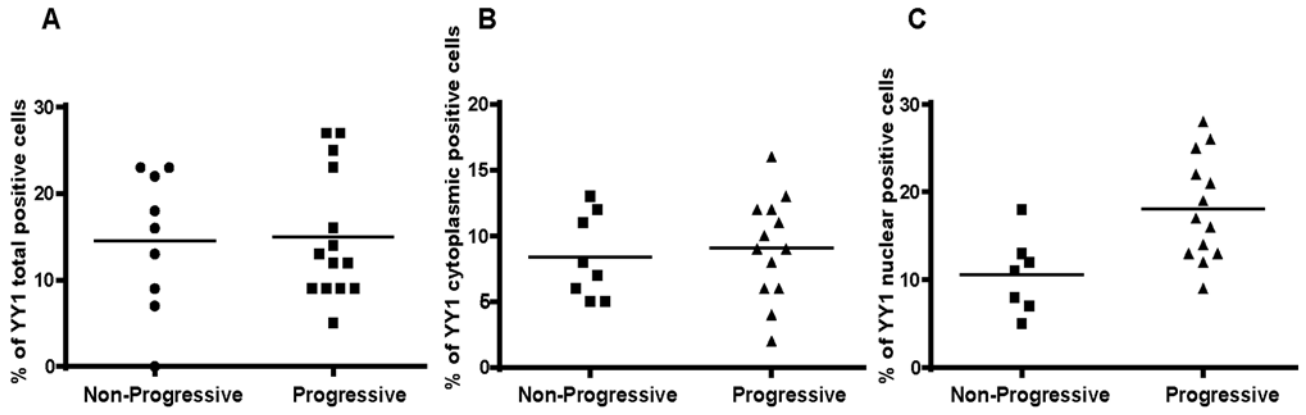

Figure 3. Frequency of YY1 positive cells in BM cells derived from MM patients. (A) The frequency of YY1 positive cells was calculated from BM derived from 20 MM patients. Following immunocytochemistry, the percentages of YY1 expression in cells (total cytoplasmic and nuclear) and the nuclear expression were calculated by counting 200 YY1 positive cells in each slide. The data were analyzed based on the frequencies of stained cells for non-progressive and progressive patients. (B) The frequencies of YY1 positive cells in the cytoplasm and C in the nucleus are represented for progressive and non-progressive MM patients (*p=0.035, progressive vs. non-progressive).

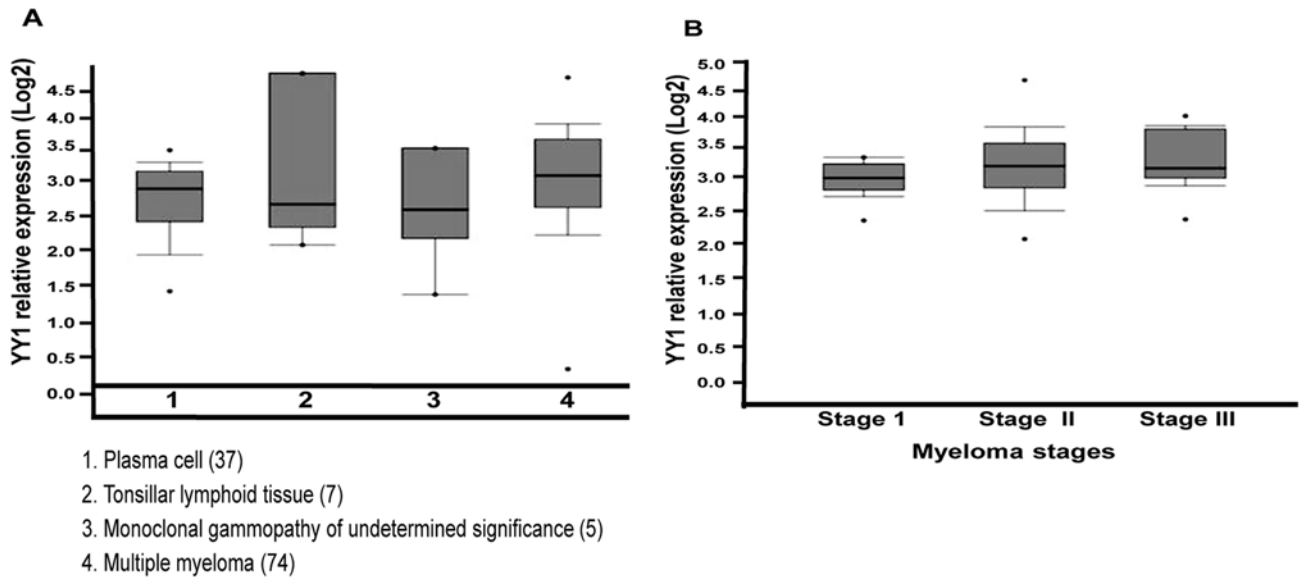

Figure 4. Bioinformatics analysis. The analysis of YY1 mRNA expression levels in MM was performed using a public dataset of microarrays retrieved from the Oncomine database and gene expression Omnibus as described in Materials and methods. (A) Relative YY1 expression in normal and malignant cells. The Oncomine™ boxed plot of YY1 expression levels are showed as boxed quartiles (median, 25th and 75th percentile) and whiskers (minimum and maximum). $p<0.05$ by one-way ANOVA. (B) Relative YY1 expression as a function of stage of MM disease (*$p<0.001$).

apoptosis as described in Materials and methods. There was significant apoptosis in YY1 siRNA-transfected cells treated with both concentrations of bortezomib as compared with control siRNA-transfected cells (Fig. 5D). Similar results were found when YY1 siRNA-transfected cells were treated with different concentrations of melphalan (Fig. 5E). The above findings demonstrated the participation of YY1 in the regulation of resistance of MM to drug-induced apoptosis.

Discussion

The present findings revealed that the expression of the transcription factor YY1 is upregulated in MM cell lines and in patients bone marrow-derived MM tissues as compared to its expression in normal blood cells and normal bone marrow-derived tissues. The total expression of YY1 (cytoplasmic and nuclear) was the same in patients who experienced progressive and non-progressive diseases. However, patients who experienced a progressive disease showed a significantly higher nuclear expression of YY1 as compared to patients who experienced a non-progressive disease. Bioinformatic analysis of public mRNA data sets corroborated the recent findings and revealed that the expression of YY1 in MM was significantly elevated compared to the expression in plasma cells and monoclonal gammopathy of undetermined significance. Further, the expression of YY1 was increased as a function of disease stage. Since we have reported that YY1 regulates resistance of different tumors to chemotherapeutic drugs, we examined its

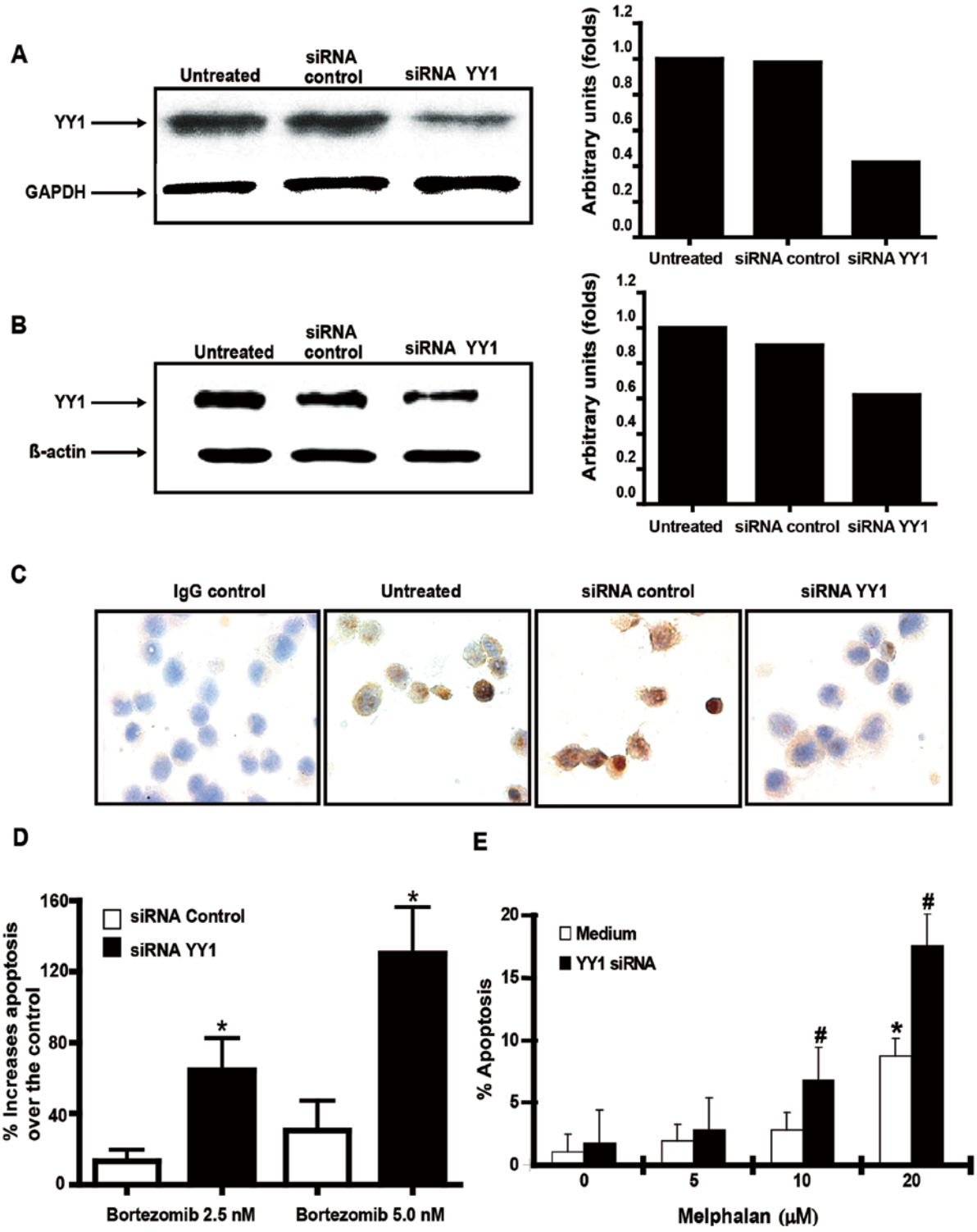

Figure 5. Inhibition of YY1 expression in MM cell lines by siRNA sensitizes the cells to bortezomib-mediated apoptosis. (A) Transfection of U266 by siRNA. U266 cells were treated with YY1 siRNA or control siRNA for 48 h and RNA was extracted and the expression of mRNA and protein for YY1 was analyzed. GAPDH was used as an internal control for RT-PCR. (B) Protein was extracted and the YY1 expression of was analyzed western blot. β-actin for the expression of YY1. Densitometric analyses were determined and represented next to the RT-PCR and western blot figures. Cell lysates were used by western for the expression of YY1. (C) The expression of YY1 after transfection with YY1 siRNA or control siRNA was also tested by immunocytochemistry. (D) Direct role of YY1 inhibition in the sensitization of U266 cells to apoptosis by bortezomib. U266 cells were transfected with either YY1 siRNA or control siRNA for 48 h and then treated with various concentrations of bortezomib (2.5 and 5 nM) or (E) various concentrations of melphalan (5, 10 and 20 μM) for an additional 18 h and the cells were then tested for apoptosis as described in Materials and methods ($^{*}p<0.05$).

role in the resistance of MM cells. Treatment of MM cells that are resistant to bortezomib-induced cytotoxicity with siRNA YY1 sensitized the MM cells to apoptosis by bortezomib. The above findings demonstrated that the nuclear expression of YY1 in MM may be considered as a novel prognostic biomarker for disease progression in a subset of patients and

these patients may benefit from different treatment regimens. We also suggest that YY1 is a potential therapeutic target.

Analysis of MM cell lines for YY1 expression revealed a significant overexpression in both the cytoplasm and nucleus when compared to its expression in normal PBMC and normal BM tissues. The overexpression of YY1 was shown by various methods, namely, IHC, RT-PCR, flow cytometry and western blot analyses. The findings observed in MM cell lines were corroborated in patients bone marrow-derived MM tissues as assessed by both IHC and western blot analyses. The clinical history of the examined MM patients consisted of one subset who experienced disease progression and one subset who did not experience disease progression. Analysis of the IHC staining data with patient-derived MM tissues revealed that the frequency of YY1 positively stained MM cells as well as the frequency of positively stained cytoplasmic YY1 in cells was not significantly different between the progressive and non-progressive subsets. However, noteworthy, when the frequency of YY1 positively stained nuclei in the cells was analyzed, there was a significantly higher frequency in the progressive subset as compared to the frequency in the non-progressive subset. These findings suggest that in the progressive subset, the translocation of YY1 in the nucleus, whereby its transcriptional activity takes place, may be functionally consistent and by being involved in the regulation of gene products that regulate disease progression and unresponsiveness to treatment (26). These preliminary findings, however, need to be validated in a larger cohort of MM patients.

Our findings in MM were also observed in lymphoma and the prognostic role of YY1 in lymphoma was reported by various investigators. Sakhinia *et al* (27) reported the upregulation of YY1 mRNA in neoplastic lymph nodes in patients with follicular or DLBCL compared with reactive lymph nodes. A high level of YY1 was associated with poor outcome in both FL and DLBCL. In contrast, Naidoo *et al* (28) reported the association of high protein expression levels of YY1 with improved survival. These results were opposite to the YY1 mRNA levels. Both investigators used the same cohort of patients and, therefore, suggesting the presence of a negative feedback loop controlling YY1 mRNA and protein levels. The role of YY1 in the development of DLBCL in public microarray data was analyzed for the possible association of YY1 with other genes. The positive expression of Bcl-6 protein in tumors was associated significantly with high levels of YY1 gene transcripts in DLBCL (29). Further analysis of data sets for DLBCL identified the transcription factor paired box (PAX)-5 amongst the top 50 gene proteins correlating with YY1. These data suggested the involvement of YY1 in B cell transformation and resulting in the development of high grade lymphoma. PAX-5 downregulates p53 expression while YY1 overexpression promotes p53 degradation. YY1 and PAX-5 may, therefore, act to transform B cells via upregulation of the cell cycle.

Conflicting data also exist on the survival outcome and their relationship to YY1 expression. In some cases, high YY1 expression correlated with poor prognosis of prostate, breast and bone cancers (30,31). In other cases, YY1 expression correlated with positive outcomes (ovarian cancer, colon cancer and follicular lymphoma) (28,32-34).

The mechanism by which YY1 is overexpressed in MM cells is not clear. Different mechanisms have been shown to regulate the transcription of YY1 (35), YY1 regulates multiple genes including itself. Kim *et al* (36) reported that YY1 is able to regulate through its own DNA-binding sites in its first intron. Exogenous YY1 inhibits the expression of endogenous YY1 gene suggesting a feedback negative loop. YY1 expression is enhanced by NF-κB which directly binds the YY1 promoter through its heterodimer subunit p50/p65 (37). YY1 regulates post-translationally the modification of multiple proteins. YY1 is a signal modified with phosphorylation, acetylation, sumoylation and ubiquitination (35). YY1 is also regulated by growth-factors such as insulin-like growth factor 1 (38). YY1 can be suppressed by miRs. For example, miR-29 targets the U'-UTR of the YY1 mRNA and blocks its translation (39). AKT and PTEN also regulate YY1 expression (40). PTEN antagonizes PI3K/AKT signaling leading to YY1 downregulation.

Overall, the present findings demonstrated that YY1 expression is upregulated in MM cell lines and patient-derived MM in bone marrow tissues. The findings were corroborated by bioinformatic analysis. The role of YY1 in the progression of MM was suggested by comparing the nuclear expression of YY1 in the various MM tumor tissues. A correlation was found between the nuclear expression of YY1 and disease progression. The disease progression was, in part, the result of patients unresponsiveness to drug therapy and correlated with the overexpression of YY1 and its role in drug resistance. We suggest that patients with MM tumors that overexpress YY1 in the nucleus may benefit from different treatment regimens. We also suggest that YY1 may be considered as a novel prognostic biomarker and a therapeutic target for intervention. Clearly, the validation of our present findings should be examined with a large cohort of MM patients.

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