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Original Article

Silencing MAGE-C1/CT7 enhances the anticancer effects of bortezomib in multiple myeloma cell line

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Abstract: Multiple myeloma (MM) is a hematologic malignancy. The treatment of MM has remarkably improved over the past decade. The routine procedure of high-dose therapy followed by autologous stem cell transplantation and the application of novel drugs, such as bortezomib, lenalidomide, and thalidomide, have changed the treatment of patients with MM and become part of front-line therapy. However, given the persistence of minimal residual disease, relapse is inevitable for the majority of patients; thus, new therapeutic strategies are needed. MAGE-C1/CT7 as a cancer-testis antigen could inhibit growth and induce apoptosis of MM cells, and possess synergistic effects with bortezomib. This study assessed the expression of MAGE-C1/CT7 in myeloma cell lines U266 and RPMI-8226 by quantitative real-time PCR and western blot, and then examined the biological role of this gene in proliferation and apoptosis by using RNA interference to silence the gene. Finally, the effect of MAGE-C1/CT7 together with bortezomib was detected, and the expression of apoptosis related genes were also measured. We found that both U266 and RPMI-8226 had a high expression level of MAGE-C1/CT7. RPMI-8226 was selected and transfected for stable expression of small interfering RNA-MAGE-C1/CT7 (siRNA-MAGE-C1/CT7). The viability of cells in siRNA group (cells transfected with siRNA-MAGE-C1/CT7) significantly decreased and that of cells in siRNA+Bt group (cells transfected with siRNA-MAGE-C1/CT7 and treated with bortezomib) dramatically decreased. Furthermore, cells in siRNA, mock+Bt (cells transfected with negative control siRNA and treated with bortezomib) and siRNA+Bt groups showed an increased percentage of apoptosis compared with normal controls, and apoptosis was more significant in siRNA+Bt. Moreover, the expression level of apoptosis related genes were detected. The mRNA expression of Bax was upregulated to some extent in the siRNA or Bt groups compared with that in the mock and to a greater extent in the siRNA+Bt group. Similar changes were also observed by western blot. The protein level of cleaved caspase-9 was also upregulated in the group with MAGE-C1/CT7 knockdown or bortezomib treatment, however, it was nor further upregulated in siRNA+Bt group. Therefore, targeting MAGE-C1/CT7 could be a future therapy in MM, particularly in combination with proteasome inhibitors.

Keywords: MAGE-C1/CT7, bortezomib, myeloma cell lines

Introduction

Multiple myeloma (MM) is a clonal B cell malignancy with proliferation of plasma cells in the bone marrow; MM is characterized by the presence of monoclonal immunoglobulin in blood, lytic bone lesions, renal insufficiency, and increased risk of infection [1-3]. This malignancy accounts for 1.5% of all cancers and is the second most frequent type of hematological malignancy [4, 5]. In the United States, 24,050 new cases of MM were diagnosed with 11,090 deaths in 2014 [6]. High-dose therapy followed by autologous stem cell transplant and novel drugs, such as proteasome inhibitors (e.g., bortezomib) and immunomodulatory agents (e.g., thalidomide and lenalidomide), have been introduced to treat MM [7]. These treatments have improved the outcome of MM, and several patients achieved complete remission. However, relapse and death are still inevitable in a majority of patients because of the persistence of minimal residual disease, and MM remained an incurable disease with a median survival of 7-8 years, which was improved substantially from 3 years to 4 years [8, 9]. Therefore, therapeutic strategies that can target remaining myeloma cells more effectively and specifically are needed.

Tumor immunology is dependent on the identification of antigens that are overexpressed on

tumor cells, and it has been utilized for several decades in the treatment of malignancy. Cancer-testis antigens (CTAs) were first discovered in patients with malignant melanoma by Vander Bruggen and his colleagues in 1991 [10, 11]. CTAs are expressed in a variety of tumor cells, while their expression in normal tissues is restricted to germ cells of the testis, fetal ovary, and placental cells [12-14]. With the development of detection techniques, some CTAs are also found in the liver and spleen, but the level of expression is much lower than that in germ cells [15].

The CT antigen database describes more than 250 CTAs (http://www.cta.lncc.br) [16], and the majority of which are melanoma antigens (MAGE) [17]. CTAs are divided into two groups according to the location of CTAs on either the X or Y chromosome [18, 19]. The CT7 gene is located on the chromosomal region Xq26-27 and is identified through serological analysis of recombinant cDNA expression libraries. MAGE-C1 is identical to CT7, which is identified using representational difference analysis [20, 21]. MAGE-C1/CT7 is approximately 800 amino acids longer than other MAGE proteins and contains a large number of unique repetitive sequences in front of the MAGE homology domain [20].

Several studies demonstrated that CTA overexpression is rare in most hematologic malignancies including non-Hodgkin's B-cell lymphoma and leukemia [22, 23]. Nevertheless, the overexpression is common in MM, and it was considered to be the malignancy with the richest CTA expression. MAGE-C1/CT7 is the most frequently expressed gene in newly diagnosed and relapsed patients [5], and high-frequency MAGE-C1/CT7 expression is observed in patients with advanced stage [16]. Atanackovic et al. suggested that MAGE-C1/CT7 appears to play the "gatekeeper" role for other CTAs [24] and protect myeloma cells against spontaneous as well as drug-induced apoptosis [25].

Bortezomib is a reversible proteasome inhibitor that has become a cornerstone treatment in both newly diagnosed and relapsed patients with MM, and it has improved the overall survival of patients [26-28]. Small fractions of myeloma cells can escape chemotherapy and remain in the patient's bone marrow, and these cells are responsible for the disease recurrence [17] MAGE-C1/CT7 is speculated to be resistant to chemotherapy.

To elucidate the biological role of MAGE-C1/CT7 in the tumor genesis of MM, we initially evaluated the expression of MAGE-C1/CT7 in MM cell lines U266 and RPMI-8226. Subsequently, myeloma cell line RPMI-8226 was transfected for stable expression of small interfering RNA-MAGE-C1/CT7 (siRNA-MAGE-C1/CT7). Finally, the effect of novel drug bortezomib and silencing MAGE-C1/CT7 gene on the proliferation and apoptosis of MM cells was measured and apoptosis related genes were also detected.

Materials and methods

Materials

Human MM cell lines RPMI-8226 and U266 as well as mantle cell lymphoma cell line Mino were provided by Suzhou Institute of Hematology. The bone marrow samples used as healthy controls were obtained from three healthy volunteers. This study was approved by the Ethics Committee of Nanjing Drum Tower Hospital.

Cells and cell culture

The MM cell lines RPMI-8226 and U266 as well as mantle cell lymphoma cell line Mino were cultured in RPMI 1640 supplemented with 10% fetal bovine serum and penicillin/streptomycin at 37°C in a humidified atmosphere of 5% CO_2. Cells were passed every 2-3 days, and the cells in exponential growth phase were collected for further experiment.

siRNA transfection

The siRNA targeting the MAGE-C1/CT7 was purchased from Sigma, and a negative control siRNA from Sigma was used as a negative control. Transfections were performed according to the recommended protocol. In brief, exponentially growing RPMI-8226 and U266 were seeded into six-well plates and then 9 μl of lipofectamine 2000 transfection reagent was diluted with 150 μl of Opti-MEM. At the same time, 3 μl of 10 μM siRNA was diluted with 150 μl of Opti-MEM media. The diluted lipofectamine 2000 solution and diluted siRNA solution were mixed and incubated at 25°C for

Table 1. Detailed groups and treatment schedule

Group	Name	Description
I	Normal control	Cells without treatment
II	mock	Cells transfected with negative control siRNA
III	siRNA	Cells transfected with siRNA-MAGE-C1/CT7
IV	mock+Bt	Cells transfected with negative control siRNA and treated with bortezomib
V	siRNA+Bt	Cells transfected with siRNA-MAGE-C1/CT7 and treated with bortezomib

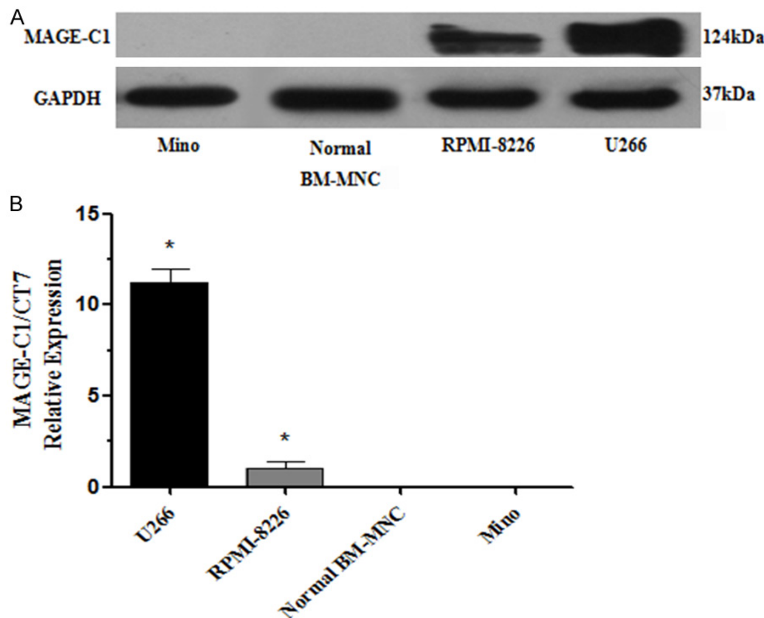

Figure 1. MAGE-C1/CT7 expression in four cell lines. A. The protein expression is higher in myeloma cell lines RPMI-8226 and U266 when compared with mantle cell lymphoma cell line Mino and normal bone marrow mononuclear cells (BM-MNC), and U266 exhibits the highest level. B. The relative expression of mRNA in four cell lines and the consequence is in consistent with protein. GAPDH protein is used as an internal control. *Statistic significant was detected when compared with BM-MNC and Mino (P<0.05).

20 min. The medium was refreshed with complete growth medium after incubation for 6 h. Bortezomib (100 ng/ml) was added to the cells after successful transfection for 72 h. The groups were divided as follows: normal controls (cells without treatment), mock (cells transfected with negative control siRNA), siRNA (cells transfected with siRNA-MAGE-C1/CT7), mock+Bt (cells transfected with negative control siRNA and treated with bortezomib), siRNA+Bt (cells transfected with siRNA-MAGE-C1/CT7 and treated with bortezomib) (**Table 1**).

Viability assays

Cell viability of cells with different treatments was measured using a Cell Counting Kit-8 (CCk-8). The cells in logarithmic phase were seeded in 96-well plates with a density of 2 × 10^4 cells/well and incubated at 37°C in a humidified atmosphere of 5% CO_2 for 96 h after the different treatments. The medium was incubated for another 4 h after 10 μl/well CCK-8 was added, and viability activity was measured at 450 nm by reading the optical density (OD) using a microplate reader. Cell viability (%) was calculated as follows: OD of test group/OD of control group × 100%. Each sample was analyzed in triplicate.

Measurement of apoptosis

Cells in the exponential growth phase with different treatments were seeded into a 24-well plate at a density of 1 × 10^5 cells/well and incubated at 37°C in the medium with a humidity of 5% CO_2 atmosphere. The cells were washed three times with PBS and centrifuged at 1,000 rpm for 5 min. The pellet was then resuspended in 500 μl of binging buffer and incubated at room temperature with 5 μl of Annexin V-FITC in the dark for 15 min. Afterward, 10 μl of propidium iodide (PI) was added and mixed. The apoptosis of cells was measured by flow cytometry after incubation in an ice bath for 15 min in the dark.

Western blot assay

Lysis buffer was added to the total protein of 50 μg, and the mixture was incubated at 70°C for 10 min. Protein lysates were separated on 10% polyacrylamide gels and electrotrans-

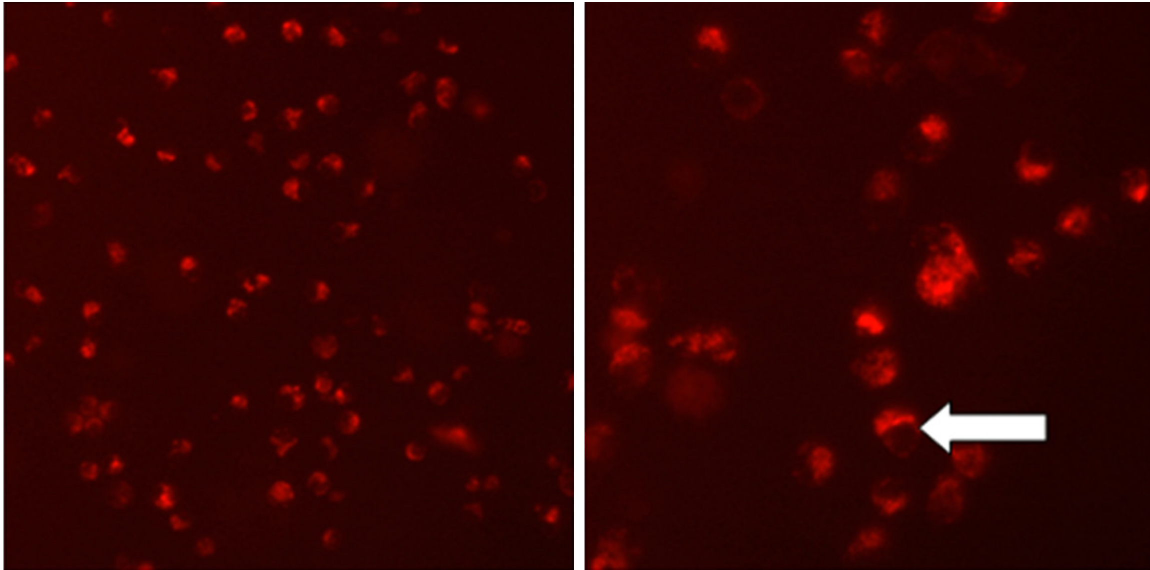

Figure 2. The efficacy of transfection detected by fluorescence microscope.

ferred to polyvinylidene fluoride membrane difluoride. Membranes were blocked by 5% non-fat dry milk and then incubated at room temperature for 4-6 h with MAGE-C1/CT7 antibody (dilution of 1:500). After incubation of overnight at 4°C, the membranes were washed three times with TBST for 10 min each and incubated at room temperature for an additional of 1 h together with peroxidase-conjugated anti-mouse IgG (dilution of 1:5000). Assays were carried out in triplicates.

Quantitative real-time PCR (qPCR)

Total RNA was prepared using TRIzol as described previously [29]. cDNA was synthesized with RNAiso Plus reagent in accordance to the manufacturer's instruction. qPCR was carried out with ABI Prism 7500 PVR master, and thermal cycling was as follows: 95°C for 5 s, 40 cycles of denaturation at 95°C for 30 s, and annealing at 60°C for 34 s. The relative quantity was analyzed with the 2-ΔΔCt method. GAPDH mRNA was used as a control, and each experiment was performed in triplicate.

Statistical analysis

Data are expressed as mean ± standard deviation. Statistical analyses were performed with a parametric test (Student's *t*-test) by using SPSS software. $P<0.05$ was considered significant.

Results

Expression of MAGE-C1/CT7 in four cell lines

qPCR and western blot were carried out to confirm whether the expression of MAGE-C1/CT7 was much higher in the cell lines of MM. As illustrated in **Figure 1A**, the protein levels of MAGE-C1/CT7 in RPMI-8226 and U266 were higher than those in the other two groups, which corresponded to the consequence of qPCR (**Figure 1B**). In addition, the expression level of MAGE-C1/CT7 in U266 was the highest, with 10-fold to RPMI-8226, 353-fold to bone marrow mononuclear cells (BM-MNC), and 330-fold to Mino. These results indicate that a close relationship existed between MAGE-C1/CT7 and MM.

Effects of siRNA transfection on the expression of MM cells

The siRNA labeled with red flour Cy5 was successfully transfected into U266 and RPMI-8226 cells, which can be detected under a fluorescence microscope (**Figure 2**). As shown in **Figure 3A**, siRNA transfection resulted in declined mRNA expression of MAGE-C1/CT7 in both U266 and RPMI-8266. This finding confirms the effect of siRNA on the reduction of mRNA levels. Next, we analyzed the effect of introducing siRNA at the protein level by using the western blot assay. As demonstrated in

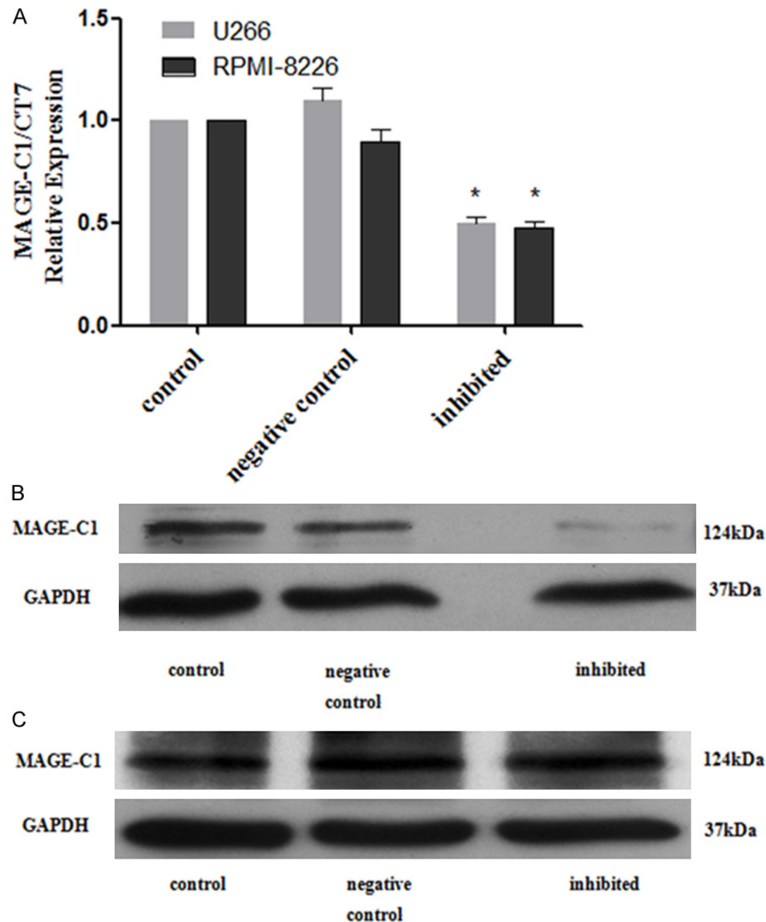

Figure 3. A. The relative mRNA expression of MAGE-C1/CT7 in U266 and RPMI-8226 after transfection for 72 h, and the decrease was significantly in both cell lines. B. The protein expression of MAGE-C1/CT7 in RPMI-8226, and it exhibited a dramatic decrease when compared with normal control and mock group. C. The protein expression of MAGE-C1/CT7 in U266, no significant difference was detected in comparison with normal control and mock group. (normal control: cells without treatment; mock: cells transfected with negative control siRNA; siRNA: cells transfected with siRNA-MAGE-C1/CT7). GAPDH protein is used as an internal control. *Statistic significant was detected when compared with normal control and mock group ($P<0.05$).

Figure 3B, RPMI-8226 cells exhibited a decreased level of MAGE-C1/CT7 compared with the normal controls and mock group. However, a significant decrease of protein expression was not detected in U266 cells (**Figure 3C**); thus, RPMI-8226 was selected for the subsequent function analysis.

Effects of MAGE-C1/CT7 and bortezomib on cell proliferation and cell apoptosis

After transfection for 72 h, cells in the mock group performed better compared with those in the siRNA group as observed clearly under a microscope (**Figure 4**). This observation indicates that MAGE-C1/CT7 was involved in the proliferation of MM cell line RPMI-8226.

Bortezomib (100 ng/ml) was added to MM cells after successful transfection for 72 h and then incubated for 24 h. To quantitatively determine the inhibition rate induced by MAGE-C1/CT7 silencing and bortezomib, we conducted a CCK-8 assay. As shown in **Figure 5**, cells in siRNA, mock+Bt, and siRNA+Bt groups exhibited a remarkable decrease in viability. The decrease was more prominent in siRNA+Bt group, and no evident difference was observed in the normal control and mock groups.

Subsequently, we evaluated the effect of silencing MAGE-C1/CT7 and bortezomib on the apoptosis of RPMI-8226. As shown in **Figure 6**, the total apoptosis rate in the mock group was 22.7% after incubation for 96 h, with early apoptosis and late apoptosis rates accounting for 14.3% and 8.4%, respectively. These rates were not significantly different from the normal control group. In the siRNA group, the total apoptosis rate increased to 35.7%, which was significant compared with the normal control. The early apoptosis rate in this group was 27% and exhibited a statistical significant difference compared with the normal control. The late apoptosis rate was 8.7% and showed no significant increase. These results revealed that silencing of MAGE-C1/CT7 induced spontaneous apoptosis in myeloma cells compared with untreated cells and cells transfected with negative siRNA, and it was dominant in early apoptosis. In mock+Bt group, a total apoptosis rate of approximately 48% was observed, with the early apoptosis and late apoptosis rates increased to 22.3%

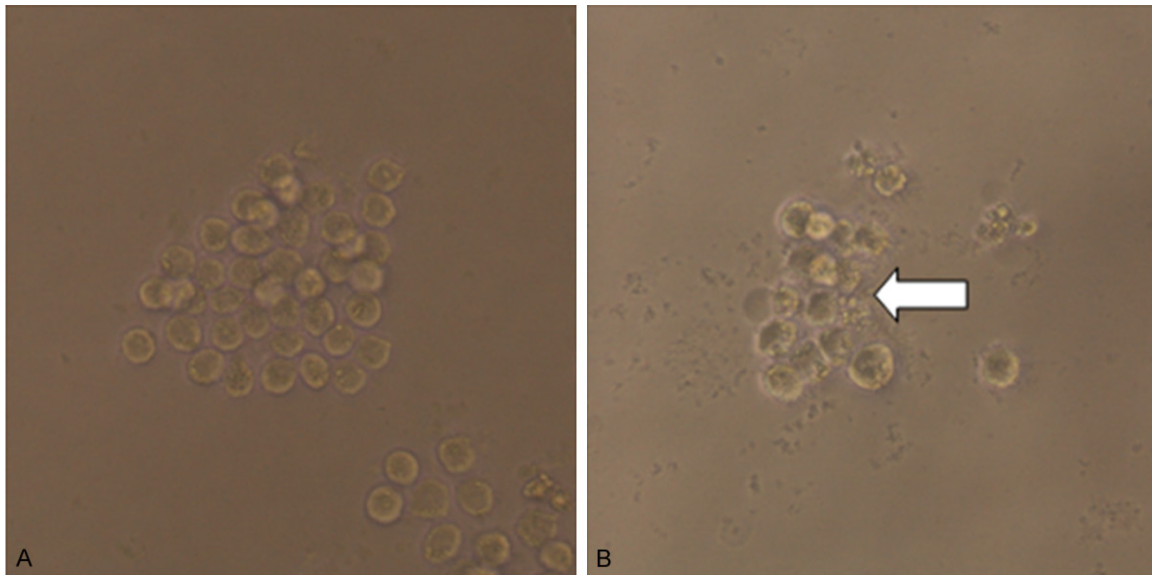

Figure 4. The morphology of RPMI-8226 cells under microscope after transfection for 72 h (× 200). A. Mock (cells transfected with negative control siRNA). B. siRNA (cells transfected with siRNA-MAGE-C1/CT7).

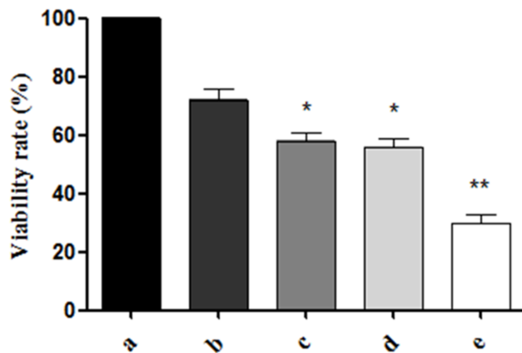

Figure 5. The viability of RPMI-8226 cells with different treatment (normal control: cells without treatment; mock: cells transfected with negative control siRNA; siRNA: cells transfected with siRNA-MAGE-C1/CT7; mock+Bt: cells transfected with negative control siRNA and treated with bortezomib; siRNA+Bt: cells transfected with siRNA-MAGE-C1/CT7 and treated with bortezomib). *Statistic significant was detected when compared with normal control and mock ($P<0.05$). **Statistic significant was detected when compared with ssiRNA and mock+Bt group ($P<0.05$).

and 25.7%, respectively. The total rate of apoptosis was significant in both siRNA and mock+Bt groups compared with the normal control and mock group. In the group of siRNA, the increase of early apoptosis was distinct, but it was not statistically significant in late apoptosis. In contrast to siRNA group, the late apoptosis rate was greatly increased in mock+Bt group compared with the normal controls, and no significant difference existed in the early apoptosis. To confirm whether silencing of MAGE-C1/CT7 could potentiate apoptosis induced by bortezomib in RPMI-8226, we introduced bortezomib to the siRNA-MAGE-C1/CT7 transfected cells. The total apoptosis rate increased with the presence of bortezomib, and the early apoptosis and late apoptosis rates were significantly increased compared with the siRNA and mock-Bt groups. These findings demonstrate that silencing MAGE-C1/CT7 could enhance the sensitivity of MM cells to bortezomib-induced apoptosis.

Expression levels of Bax and cleaved caspase-9

As shown in **Figure 7**, the mRNA expression of Bax was upregulated in the group of siRNA compared with that in the mock and to a greater extent in the group with bortezomib treatment. In the group of siRNA+Bt, the expression level was further upregulated. Similar changes of Bax were also observed by western blot (**Figure 8**). The protein level of cleaved caspase-9 was elevated after MAGE-C1/CT7 knockdown or bortezomib treatment, however, enhanced elevated was not detected in the group with both MAGE-C1/CT7 knockdown and bortezomib treatment.

Discussion

Development in tumor immunology and immunotherapy has been partially attributed to the successful identification of CTAs, which are

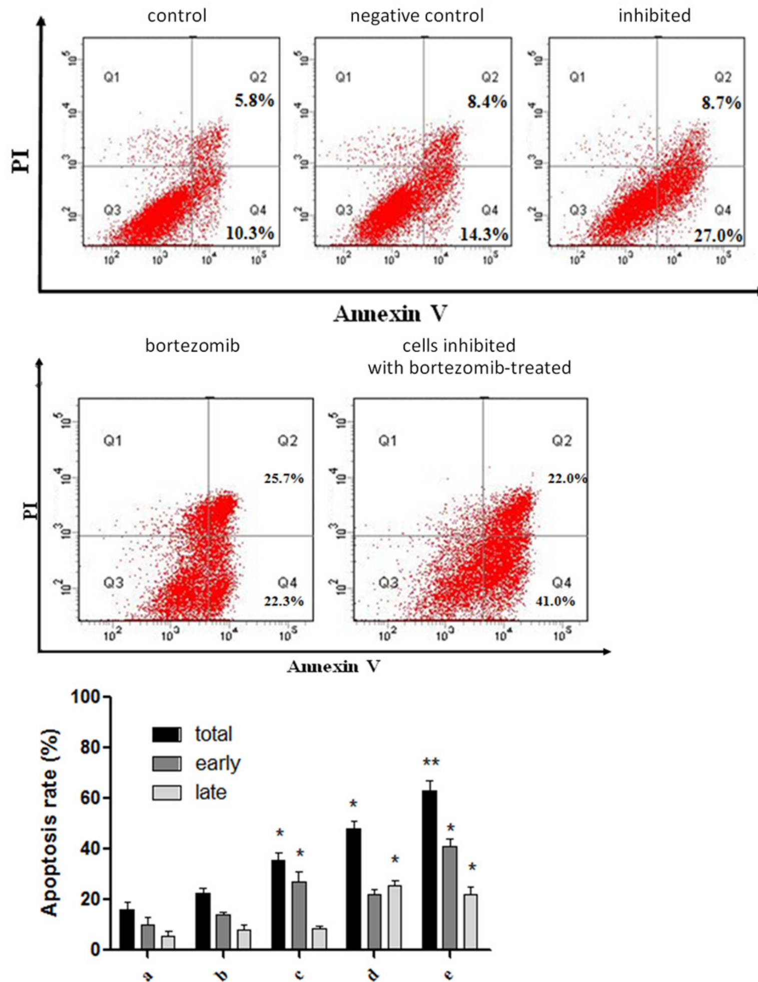

Figure 6. The apoptosis rate of RPMI-8226 cells with different treatment. (normal control: cells without treatment; mock: cells transfected with negative control siRNA; siRNA: cells transfected with siRNA-MAGE-C1/CT7; mock+Bt: cells transfected with negative control siRNA and treated with bortezomib; siRNA+Bt: cells transfected with siRNA-MAGE-C1/CT7 and treated with bortezomib). *Statistic significant was detected when compared with normal control and mock ($P<0.05$). **Statistic significant was detected when compared with siRNA and mock+Bt group ($P<0.05$).

restrictedly expressed in tissues. To delineate the role of MAGE-C1/CT7 in MM, we initially investigated the expression of mRNA and protein in cell lines U266 and RPMI-8226 by using qPCR and western blot. High expression level of MAGE-C1/CT7 was detected in both cell lines, indicating a close relationship between MAGE-C1/CT7 and MM. This finding is in agreement with that of Fabricio de Carvalho who reported that MAGE-C1/CT7 is expressed in 67% of the 46 MM analyzed patients [16]. Moreover, the level of MAGE-C1/CT7 expression is considerably low in MM patients treated with autologous stem cell transplantation, which was in favor of our results.

RNA interference (RNAi) is a useful approach to identify a function of a specific gene by suppressing its expression. This mechanism is triggered in the cell cytoplasm by endogenous microRNAs (miRNAs) and exogenous siRNAs via specific mRNA sequence matching and leads to the blockade of protein translation [30, 31]. SiRNA is a suitable matched sequence that can be designed to a specific mRNA and cause mRNA cleavage and degradation [32] to silence the expression of the target gene. After tranfection, the protein expression of MAGE-C1/CT7 in U266 was not significantly decreased. We hypothesized that the expression of MAGE-C1/CT7 was extremely high in U266; therefore, even half of mRNA was inhibited, and a high expression of protein was observed. In apparent contrast to U266, RPMI-8226 showed a high efficiency in transfection. Given this result, we selected RPMI-8226 cell line for further study.

Bortezomib, a boronic acid dipeptide of the 26S proteasome inhibitor, is formed by a 20S core complex and a 19S regulatory particle. The 26S proteasome is fundamental for the ubiquitin/proteasome pathway [33]. This pathway is implicated in regulating cell proliferation, cell adhesion, differentiation, transcription, angiogenesis of cancer cells, and apoptosis [34-36]. Therefore, bortezomib could induce the cell cycle arrest, apoptosis, and inhibition of angiogenesis by disrupting various intracellular signaling pathways. With the clinical application, the approach of MM treatment has considerably changed. However, several studies showed that the failure of achieving complete remission is related to the small fractions of

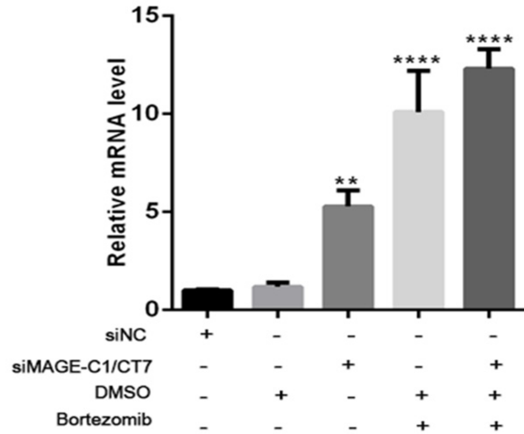

Figure 7. At 48 h post siRNAs transfection, RPMI-8226 cells were harvested; or cells were treated with 100 nM bortezomib for 24 h, then lysed for total RNA extraction to examine apoptosis related proteins Bax by Q-PCR. Data are representative of at least two independent experiments, with each determination performed in triplicate, and are shown as the mean ± SEM of the results analyzed using unpaired student's t test (**, significant differences at P<0.01; ****, significant differences at P<0.001).

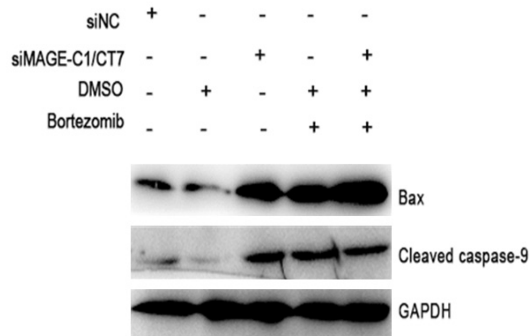

Figure 8. At 48 h post siRNAs transfection, RPMI-8226 cells were harvested; or cells were treated with 100 nM bortezomib for 24 h, then lysed for testing apoptosis related proteins Bax and cleaved caspase-9 by western blotting. The results demonstrate that protein level of Bax and cleaved caspase-9 was elevated after MAGE-C1/CT7 knockdown or bortezomib treatment; moreover, both MAGE-C1/CT7 knockdown and bortezomib treatment can synergically enhance Bax expression, while cleaved caspase-9 was not further elevated.

myeloma cells that escaped chemotherapy and remained in the bone marrow. MAGE-C1/CT7 is speculated to resist chemotherapy. In the present study, MAGE-C1/CT7 silencing cells with bortezomib treatment manifested a dramatic decrease in viability in contrast to the normal controls. When compared with mock+Bt group, more proliferation inhibition was also observed, indicating that MAGE-C1/CT7 gene could enhance the proliferation inhibition of myeloma cells induced by bortezomib. In addition, the viability of myeloma cells was decreased in the siRNA group compared with the normal controls and mock group. The decreased viability suggests the role of MAGE-C1/CT7 gene in promoting the growth and survival of cells. This result agrees with that of Yang et al. Yang et al. reported that silencing of MAGE genes could inhibit the proliferation of neoplastic mast cells in vivo and in vitro [37], which verified that MAGE gene is involved in cell proliferation. Nevertheless, Fabricio de Carvalho et al. found only moderate effects on cell proliferation in silencing myeloma cells compared with the controls [16].

Proliferation and apoptosis are significant during tumor growth [38]. To further determine whether MAGE-C1/CT7 and bortezomib merely inhibited cell growth or induced cell death, cell apoptosis was observed. More apoptosis cells were found in the siRNA group compared with the normal control and mock groups. The abundance of these cells demonstrates that MAGE-C1/CT7 acted as an anti-apoptosis gene to protect myeloma cells from apoptosis. This result is in line with several studies. More apoptosis of cells was also detected in mock+Bt and siRNA+Bt groups compared with the normal control and mock group. Apoptosis was distinct in the siRNA+Bt group. These results proved that bortezomib could significantly increase the percentage of apoptosis. Suppression of MAGE-C1/CT7 expression could enhance the apoptosis of myeloma cells induced by bortezomib, suggesting that silencing MAGE-C1/CT7 could increase the sensitivity of myeloma cells to toxicity drugs.

Analysis of cell apoptosis revealed that silencing MAGE-C1/CT7 leads to more apoptosis of cells in the early stage. The cells treated with bortezomib exhibited a significant increase in the late stage, and the cells with both treatments showed a dominant increase in early apoptosis and late apoptosis. The apoptosis effect of MAGE-C1/CT7 primarily depended on p53 blockade instead of the caspase pathway, whereas according to Djordje Atanackovic, apoptosis is related to caspase-12 and caspase-9. The main mechanism of bortezomib's

anticancer activity is initially speculated to inhibit nuclear factor-κB, which acted as a transcription factor for anti-apoptosis proteins. Stabilization of p53, p21, and p27 proteins was also confirmed to be involved in its anticancer activity [39-42]. In this study, we evaluated the expression level of Bax and cleaved caspase-9. Bax is a pro-apoptotic protein which belonged to Bcl-2 family. In the groups with MAGE-C1/CT7 knockdown or bortezomib treatment, mRNA and protein expression of Bax were clearly upregulated and it was more dramatic in bortezomib treatment group, moreover, both MAGE-C1/CT7 knockdown and bortezomib treatment can synergically enhance Bax expression. Caspase-9 plays a vital role in the intrinsic apoptosis pathway. When caspase-9 is activated, effector caspases are cleaved and activated, leading to lysis of numerous cellular substrates and cell death [43]. In our study, protein level of cleaved caspase-9 was upregulated after MAGE-C1/CT7 knockdown or bortezomib treatment, while it was not further upregulated in siRNA+Bt group. Thus, the mechanism of MAGE-C1/CT7 knockdown and bortezomib in promoting apoptosis and inhibiting proliferation is related to Bax and cleaved caspase-9, moreover, MAGE-C1/CT7 knockdown and bortezomib treatment can synergically enhance Bax expression, while cleaved caspase-9 was not further upregulated, which is interesting and needed further study.

Conclusion

This study confirmed that MAGE-C1/CT7 was overexpressed in myeloma cell lines U266 and RPMI-8226. Both cell lines were involved in inhibiting cell proliferation and promoting cell apoptosis. This finding was detected when a stable silencing of MAGE-C1/CT7 gene was achieved. In addition, overexpression of the cell lines could increase the inhibition and apoptosis induced by bortezomib, indicating that MAGE-C1/CT7 played a biological role in protecting myeloma cells from spontaneous apoptosis and in enhancing the cytotoxic effects of anti-myeloma agents. In conclusion, MAGE-C1/CT7 gene may represent a valuable therapeutic strategy for myeloma, particularly in combination with bortezomib and other proteasome inhibitors. Accordingly, targeting MAGE-C1/CT7 gene may open a new therapeutic method for myeloma patients, especially those patients who are resistant to chemotherapy.

Acknowledgements

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Disclosure of conflict of interest

None.

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