β-catenin inhibitors ICG-001 and pyrvinium sensitize bortezomib-resistant multiple myeloma cells to bortezomib

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Abstract. Although bortezomib (BTZ) displays efficacy in treating multiple myeloma (MM), BTZ resistance in MM patients has been reported. Meanwhile, treating BTZ resistant MM cells with β-catenin inhibitors have demonstrated the ability to reserve BTZ resistance. Thus, the present study aimed to investigate the synergistic effect of the β-catenin inhibitors, ICG-001 and pyrvinium (PP), with BTZ in the treatment of BTZ-resistant MM cells. Different concentrations of ICG-001 (0-32 μM) or PP (0-32 nM) were used to treat the BTZ-resistant RPMI-8226 (RPMI-8226BR) and BTZ-resistant KMS-11 (KMS-11BR) cell lines, followed by a BTZ combination treatment. Subsequently, cell viability and apoptosis in these two cell lines were determined by CCK-8 assay and flow cytometry, respectively. The proteins involved in the Wnt/β-catenin signaling pathway were detected using western blotting. The Wnt/β-catenin signaling pathway was activated in the RPMI-8226BR and the KMS-11BR cells. In addition, the cell viability of RPMI-8226BR and KMS-11BR cells were decreased following β-catenin inhibitor (ICG-001 and PP) treatment alone. Furthermore, the β-catenin inhibitors, ICG-001 and PP, plus BTZ combination treatment revealed a notable decrease in cell viability and a marked increase in cell apoptosis rate, compared with that in cells treated with ICG-001, PP or BTZ alone in the RPMI-8226BR and KMS-11BR cell lines. In conclusion, the β-catenin inhibitors, ICG-001 and PP not only increased apoptosis, but also sensitized BTZ-resistant MM cells to BTZ, indicating their potential therapeutic application in MM.

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Abbreviations: MM, multiple myeloma; BTZ, bortezomib; PP, pyrvinium; KMS-11BR, BTZ-resistant KMS-11; RPMI-8226BR, BTZ-resistant RPMI-8226; CCK-8, Cell Counting Kit-8; NC, nitrocellulose; pGSK-3β, phosphorylated GSK-3β; OD, optical density; USP, ubiquitin-specific peptidase

Key words: MM, BTZ resistance, ICG-001, PP, β-catenin inhibitors

Introduction

Multiple myeloma (MM) is a mature B cell malignancy characterized by an excessive production of monoclonal antibodies in the bloodstream, which leads to end organ damage, such as renal failure and lytic bone lesions (1,2). It is worth noting that, due to improvements in the pharmaceutical industry, a number of novel therapies have recently been developed for the treatment of MM (3). Bortezomib (BTZ), one of the novel chemotherapy drugs, is widely used to treat MM (4-6). Previous studies found that BTZ inhibited proteasome degradation and lead to a favorable clinical outcome in patients with MM (4-6). However, some patients with MM develop BTZ resistance following BTZ administration for a prolonged period of time, partly due to dysregulated cellular metabolic activity and altered intracellular signaling pathways (7,8). Furthermore, a poor prognosis has been reported in MM cases with BTZ resistance, reflected by a shorter overall survival time and disease recurrence (9). Thus, relevant studies on the mechanism of BTZ resistance are required to reverse this resistance.

β-catenin, a key protein of the Wnt/β-catenin signaling pathway, plays a critical role in MM pathogenesis (10,11). Furthermore, from the existing evidence, β-catenin inhibitors have been shown to exert a positive effect in the treatment of MM. As a commonly used β-catenin inhibitor, ICG-001 induces MM cell apoptosis via the activation of pro-apoptotic proteins, phorbol-12-myristate-13-acetate-induced protein 1 and p53 upregulated modulator of apoptosis (12). Another β-catenin inhibitor, pyrvinium (PP), promotes intracellular β-catenin degradation and leads to enhanced cell apoptosis activity in MM cells (13). In addition, another *in vitro* study showed that PP enabled the suppression of mitochondrial respiratory complex I to inhibit cell proliferation in MM cells (14). Apart from ICG-001 and PP, other β-catenin inhibitors (such as C-82) are available; however, it has not been used to treat hematology-related diseases (15). Previous studies have investigated the direct therapeutic effect of ICG-001 and PP on MM; however, the synergistic effect of β-catenin inhibitors plus BTZ requires further investigation. Therefore, the aim of the present study was to investigate the therapeutic effect of β-catenin inhibitors administered with BTZ in BTZ-resistant MM cells.

Materials and methods

Cell culture and reagents. The RPMI-8226 (cat. no. JCRB00344), KMS-11 (cat. no. JCRB1179) and BTZ-resistant KMS-11 (KMS-11BR; cat. no. JCRB1642) human MM cell lines were purchased from the Japanese Collection of Research Bioresources Cell Bank and cultured with RPMI-1640 medium (cat. no. A3823) containing 10% FBS (cat. no. 26170035) (both from Thermo Fisher Scientific, Inc.). The BTZ-resistant RPMI-8226 (RPMI-8226BR) cell line was constructed by increasing the concentration of BTZ stepwise over a period of 3 months and was adapted to a final concentration of 20 nM BTZ, and the detailed procedure was adapted from a previous study (16). BTZ (cat. no. S1013), ICG-001 (cat. no. S262) and PP (cat. no. 5816) were purchased from Selleck Chemicals. The Cell Counting Kit (CCK)-8 (cat. no. C0037), Annexin V-FITC Apoptosis Detection kit (cat. no. C1067S), RIPA Lysis Buffer (cat. no. P0013C), SDS-PAGE sample loading buffer (cat. no. P0015), BCA Protein Assay kit (cat. no. P0012S), 4-20% precast PAGE (cat. no P0670) and BeyoECL Plus (cat. no. P0018FS) were purchased from Beyotime Institute of Biotechnology. The nitrocellulose (NC) filter membrane (cat. no. GSWP037000) was purchased from Merck KGaA.

BTZ treatment. The RPMI-8226, RPMI-8226BR, KMS-11 and KMS-11BR cell lines were cultured with different concentrations (0, 1, 2, 3, 4, 8 and 16 nM) of BTZ for 24 h. The cell viability was then evaluated using CCK-8, according to the manufacturer's instructions.

Western blot analysis. To evaluate the protein expression levels of β-catenin, GSK-3β, phosphorylated (p)GSK-3β and c-Myc, western blot analysis was performed. The RPMI-8226, RPMI-8226BR, KMS-11 and KMS-11BR cell lines were lysed with RIPA Lysis Buffer. Following quantification with the BCA Protein Assay kit, 20 μg total protein was separated using 4-20% precast PAGE. The protein was then transferred to a NC membrane. The membrane was blocked with 5% BSA at 37˚C for 1 h and incubated with the primary antibodies at 4˚C overnight, then incubated with a secondary antibody at 37˚C for 1 h. Finally, the protein bands were visualized with BeyoECL Plus. The antibodies used for western blot analysis are listed in Table SI. For densitometry analysis, ImageJ v1.8.0 (National Institutes of Health) was used. The relative protein expression for β-catenin and c-Myc was calculated as the absolute protein density/GAPDH density, while the relative protein expression for pGSK-3β was calculated as the pGSK-3β density/GSK-3β density.

ICG-001 and PP treatment. Different concentrations of ICG-001 (0, 1, 2, 4, 8, 16 and 32 μM) or PP (0, 1, 2, 4, 8, 16 and 32 nM) were added to the RPMI-8226BR and KMS-11BR cell lines for 24 h. Next, CCK-8 was used to determine cell viability.

Combination treatment. he RPMI-8226BR cell line was cultured with 8 nM BTZ alone, 32 μM ICG-001 alone, 32 nM PP alone, 8 nM BTZ plus 32 μM ICG-001 or 8 nM BTZ plus 32 nM PP for 24 h, while the KMS-11BR cell line was cultured with 6 nM BTZ alone, 32 μM ICG-001 alone, 32 nM PP alone, 6 nM BTZ plus 32 μM ICG-001 or 6 nM BTZ plus 32 nM PP for 24 h. Following incubation, the CCK-8 and Annexin V-FITC Apoptosis Detection kits were used to assess cell viability and apoptosis, respectively. The RPMI-8226BR and KMS-11BR cells cultured in normal medium were used as a control. Furthermore, the Chou-Talalay method was used to calculate the combination index of the β-catenin inhibitors and BTZ, according to a previous study (17).

Cell viability. CCK-8 was used to determine relative cell viability. In brief, the cells were washed with PBS, and then 10 μl CCK-8 regent and 100 μl serum-free RPMI-1640 medium were added to cells. Next, the cells were incubated for 2 h, and their optical density (OD) value was recorded at 450 nm. Finally, the relative cell viability was calculated as the OD value in each tested group/OD value in the control group x100.

Cell apoptosis. The Annexin V-FITC Apoptosis Detection kit was used to assess the cell apoptosis rate. In brief, the cells were collected and suspended in PBS. Next, 5 μl Annexin V and 5 μl PI were added to the cell solution for a 15-min incubation in the dark, at room temperature. Finally, the apoptosis rates were detected using a BD FACSCelesta™ Flow Cytometer (BD Biosciences) and analyzed by FlowJo (v7.6.5; FlowJo LLC).

Statistical analysis. GraphPad Prism v7.02 (GraphPad Software Inc.) was used for data analysis and presentation. The data are presented as the mean ± standard deviation. Statistical significance was determined using one-way ANOVA, followed by Dunnett's or Tukey's multiple comparisons tests. Unpaired Student's t-test was used to compare two groups. All the experiments were repeated three times. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Validation of BTZ-resistant MM cells. Compared with RPMI-8226 cell line, the relative cell viability of the RPMI-8226BR cell line was increased under 2 ($P<0.05$), 4 ($P<0.05$), 8 ($P<0.01$) and 16 nM ($P<0.01$) BTZ treatment (Fig. 1A). The relative cell viability of the KMS-11BR cell line was increased following 2 ($P<0.05$), 4 ($P<0.05$), 8 ($P<0.01$) and 16 nM ($P<0.001$) BTZ treatment compared with that in the KMS-11 cell line (Fig. 1B).

Activation of the Wnt/β-catenin signaling pathway in BTZ-resistant MM cells. The protein expression levels of both β-catenin and c-Myc were increased in the BTZ-resistant cell lines compared with that in the normal MM cell lines (both $P<0.05$) (Fig. 2A and B). However, pGSK-3β expression level was decreased in the RPMI-8226BR cell line compared with that in the RPMI-8226 cell line ($P<0.01$) (Fig. 2A and B), as well as in the KMS-11BR cell line compared with that in the KMS-11 cell line ($P<0.05$) (Fig. 2A and B). These data suggested that the activation of the Wnt/β-catenin signaling pathway might be involved in the mechanism of BTZ resistance in MM.

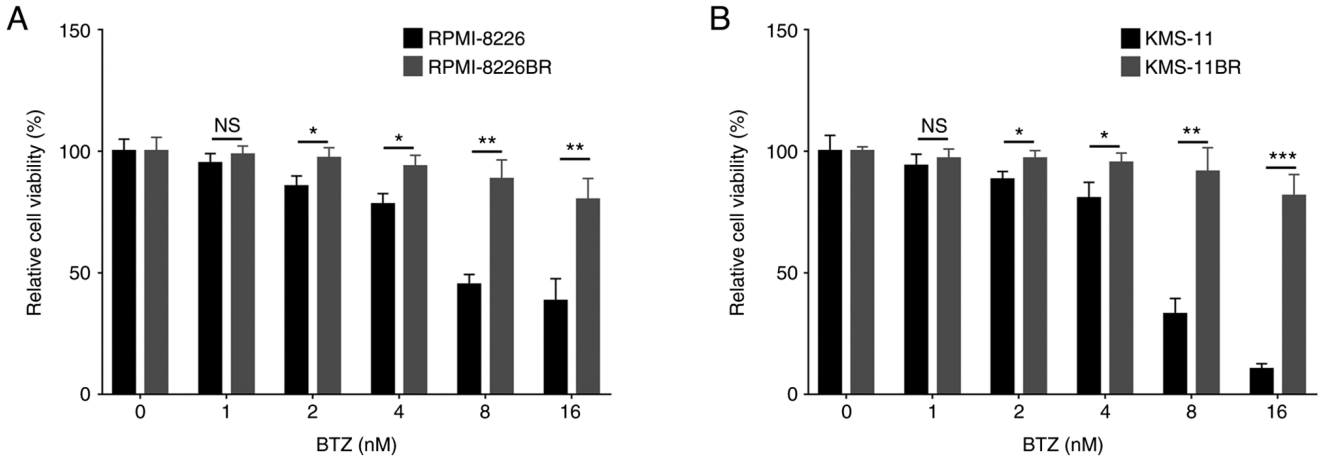

Figure 1. Difference in relative cell viability between BTZ-resistant and normal multiple myeloma cell lines. Difference in relative cell viability between (A) RPMI-8226 and RPMI-8226BR cell lines, and (B) KMS-11 and KMS-11BR cell lines. *P<0.05, **P<0.01 and ***P<0.001. Unpaired Student's t-test was used for statistical analysis. BTZ, bortezomib; BR, BTZ-resistant; NS, non-significant.

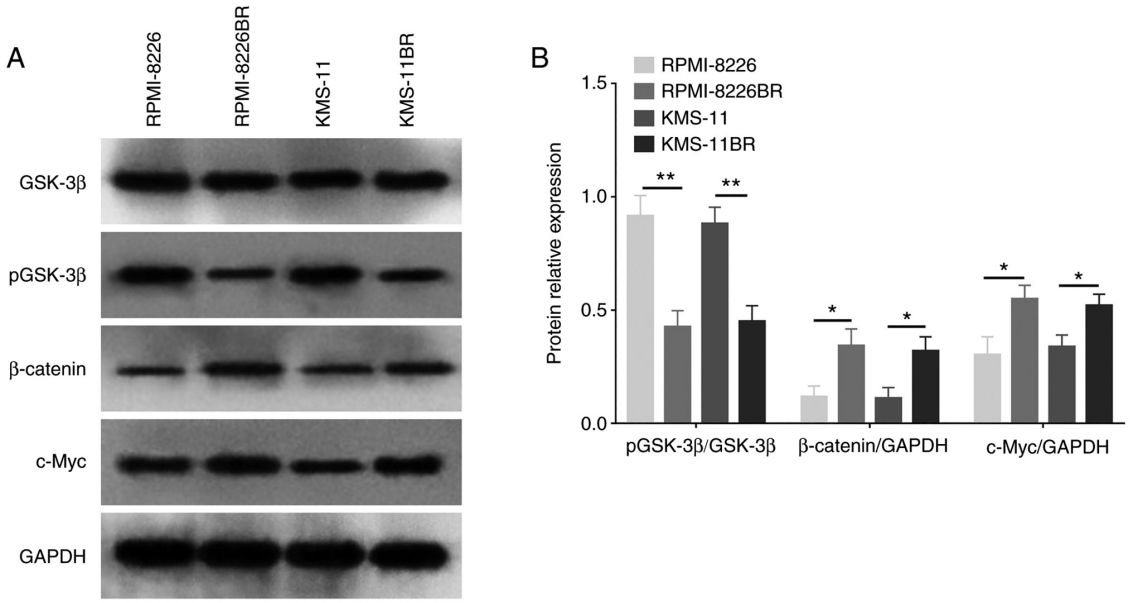

Figure 2. Effect of BTZ resistance on Wnt/β-catenin signaling-related proteins in MM cells. (A) Protein expression level of Wnt/β-catenin signaling-related proteins and (B) the densitometry analysis of western blot bands between BTZ resistance MM cells and normal MM cells. *P<0.05 and **P<0.01. Unpaired Student's t-test was used for statistical analysis. GAPDH was used as loading control for normalization to eliminate interference. NS: non-significant; p, phosphorylated.

Effect of β-catenin inhibitors (ICG-001 and PP) on BTZ-resistant MM cells. The relative cell viability was significantly reduced following 4, 8, 16 and 32 μM ICG-001 treatment (all P<0.05) compared with that in cells treated with 0 μM ICG-001 in the RPMI-8226BR cell line (Fig. 3A). The relative cell viability was significantly decreased following 8, 16 and 32 μM ICG-001 treatment (all P<0.05) compared with that in cells treated with 0 μM ICG-001 in the KMS-11BR cell line (Fig. 3B). Furthermore, the relative cell viability was decreased following 16 and 32 nM PP treatment (both P<0.05) compared with that in cells treated with 0 nM PP in the RPMI-8226BR cell line (Fig. 3C). The relative cell viability was reduced following 8, 16 and 32 nM (all P<0.05) PP treatment compared with that in cells treated with 0 nM PP in the KMS-11BR cell line (Fig. 3D).

β-catenin inhibitors sensitize BTZ-resistant MM cells to BTZ. The combination of BTZ with ICG-001 significantly decreased the relative cell viability compared with that in cells treated with ICG-001 or BTZ alone in the RPMI-8226BR (all P<0.05; Fig. 4A) and KMS-11BR cell lines (all P<0.01; Fig. 4B). In addition, BTZ combined with PP significantly reduced the relative cell viability compared with that in cells treated with PP or BTZ alone, in the RPMI-8226BR (all P<0.05; Fig. 4C) and KMS-11BR cell lines (all P<0.05; Fig. 4D).

Furthermore, BTZ combined with ICG-001 (all P<0.01) and BTZ combined with PP (all P<0.05) significantly increased the cell apoptosis rate compared with that in cells treated with ICG-001, PP or BTZ alone in the RPMI-8226BR cell line (Fig. 5A-C). In addition, BTZ combined with ICG-001 (all P<0.05) and BTZ combined with PP (all P<0.01) also

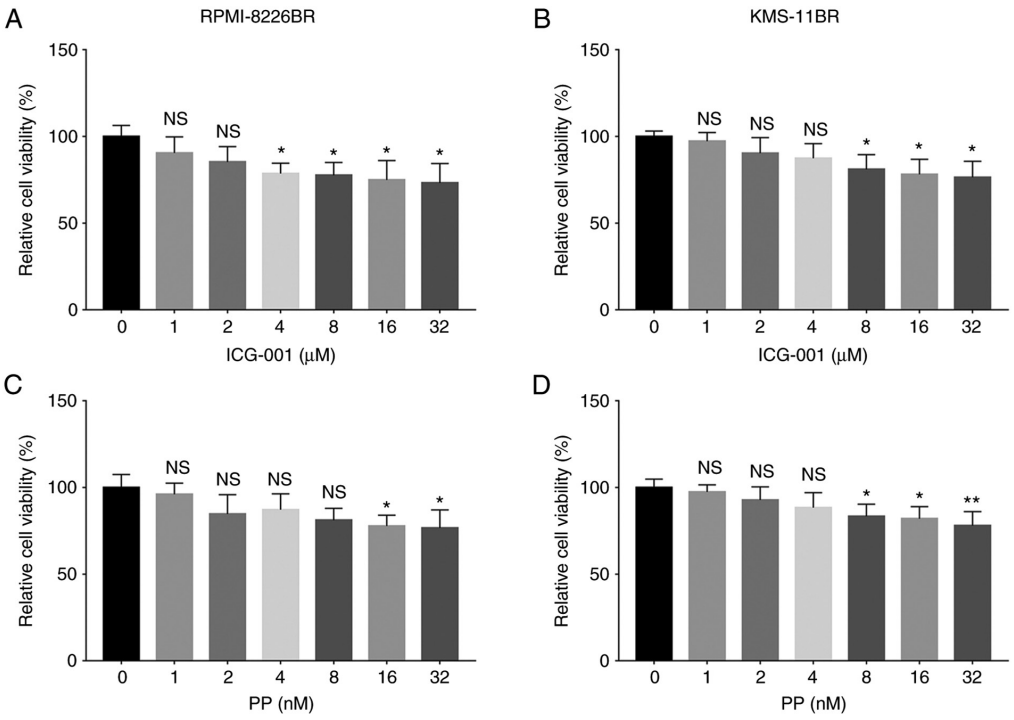

Figure 3. Effect of different concentrations of β-catenin inhibitors on BTZ-resistant multiple myeloma cells. Effect of different concentrations of ICG-001 on (A) RPMI-8226BR and (B) KMS-11BR cell viability. *P<0.05 vs. 0 μM. Effect of different concentrations of PP on (C) RPMI-8226BR and (D) KMS-11BR cell viability. *P<0.05 and **P<0.01 vs. 0 nM. For statistical analysis, one-way ANOVA followed by Dunnett's test was used. PP, pyrvinium; BTZ, bortezomib; NS, non-significant; BR, BTZ-resistant.

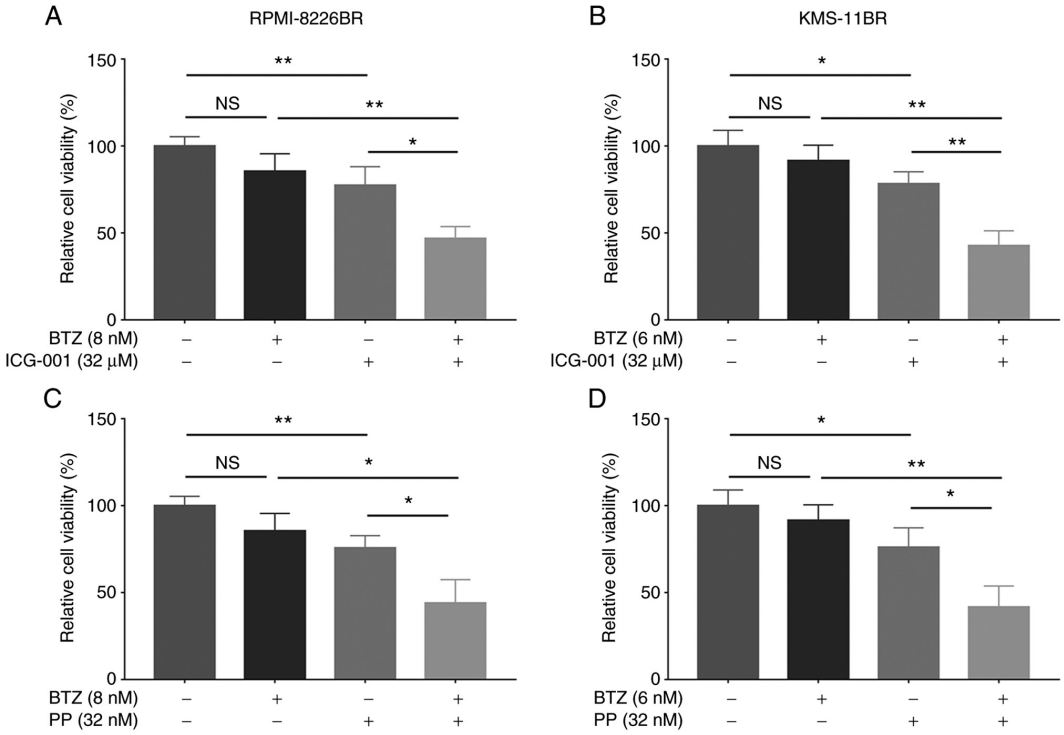

Figure 4. Effect of β-catenin inhibitors combined with BTZ in BTZ-resistant multiple myeloma cell viability. Effect of ICG-001 plus BTZ on (A) RPMI-8226BR and (B) KMS-11BR cell viability. Effect of PP plus BTZ on (C) RPMI-8226BR and (D) KMS-11BR cell viability. *P<0.05 and **P<0.01. For statistical analysis, one-way ANOVA followed by Tukey's multiple comparison test was used. PP, pyrvinium; BTZ, bortezomib; NS, non-significant; BR, BTZ-resistant.

significantly increased the cell apoptosis rate, compared with that in cells treated with ICG-001, PP or BTZ alone in the KMS-11BR cell line (Fig. 5D-F).

Wnt/β-catenin pathway-related molecular alteration under ICG-001 or PP treatment. In the RPMI-8226BR cell line, ICG-001 decreased the protein expression level of β-catenin

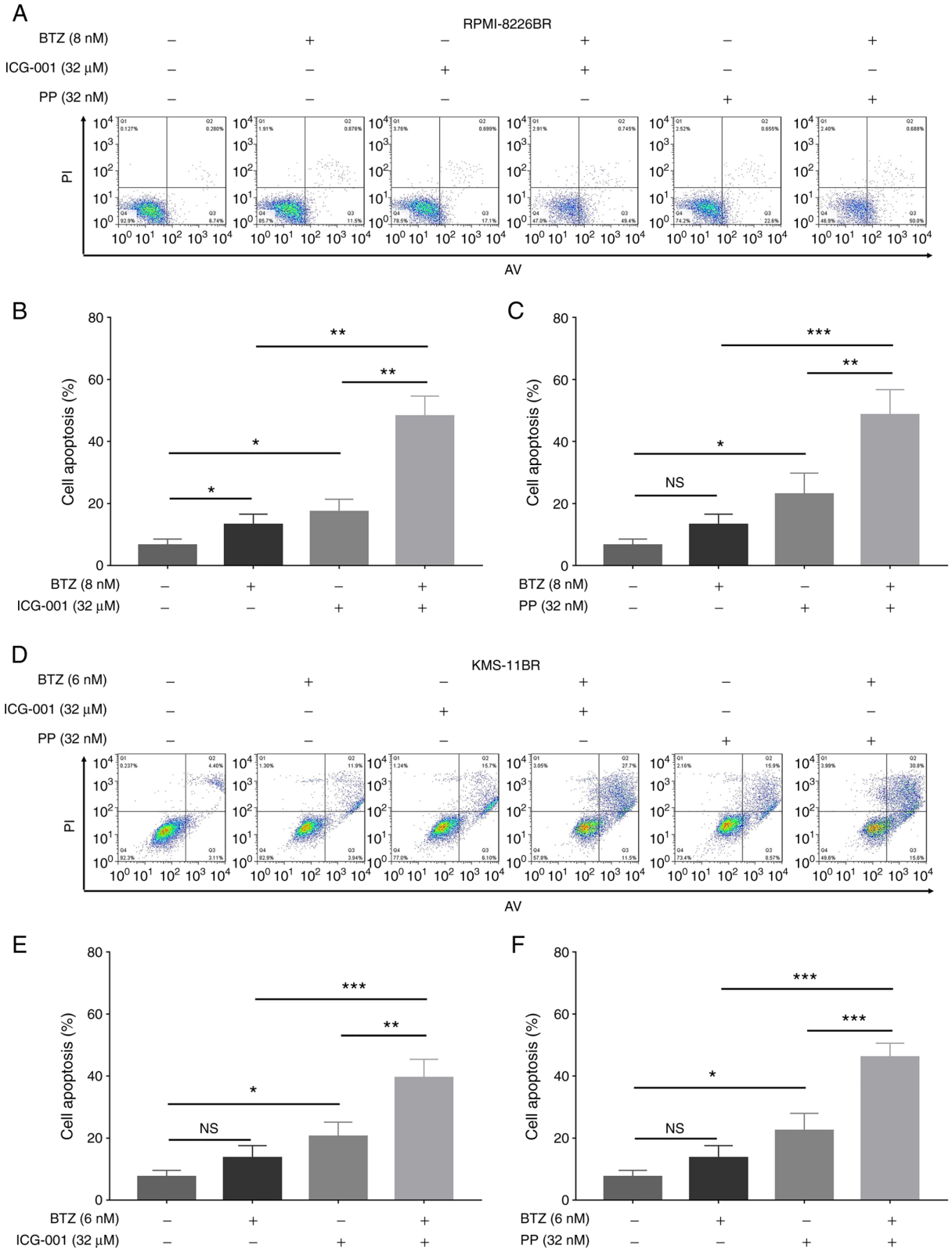

Figure 5. Effect of β-catenin inhibitors combined with BTZ on the apoptotic rate of BTZ-resistant multiple myeloma cells. (A) The representative flow cytometry plots of effect of (B) ICG-001 plus BTZ and (C) PP plus BTZ on the apoptotic rates of RPMI-8226BR cells. and (C). (D) The representative flow cytometry plots of effect of (E) ICG-001 plus BTZ and (F) PP plus BTZ on the apoptotic rates of the KMS-11BR cell line. *P<0.05, **P<0.01 and ***P<0.001. For statistical analysis, one-way ANOVA followed by Tukey's multiple comparison test was used. PP, pyrvinium; BTZ, bortezomib; NS, non-significant; BR, BTZ-resistant.

and c-Myc compared with that in cells receiving no treatment (both P<0.05; Fig. 6A and C). Furthermore, PP reduced the pGSK-3β, β-catenin and c-Myc protein expression levels (all P<0.05; Fig. 6A and C) compared with those in cells receiving no treatment. Furthermore, ICG-001 plus PP also significantly decreased the pGSK-3β protein expression level compared with that in cells treated with ICG-001 alone (P<0.001; Fig. 6A and C). In addition, ICG-001 plus PP also significantly reduced

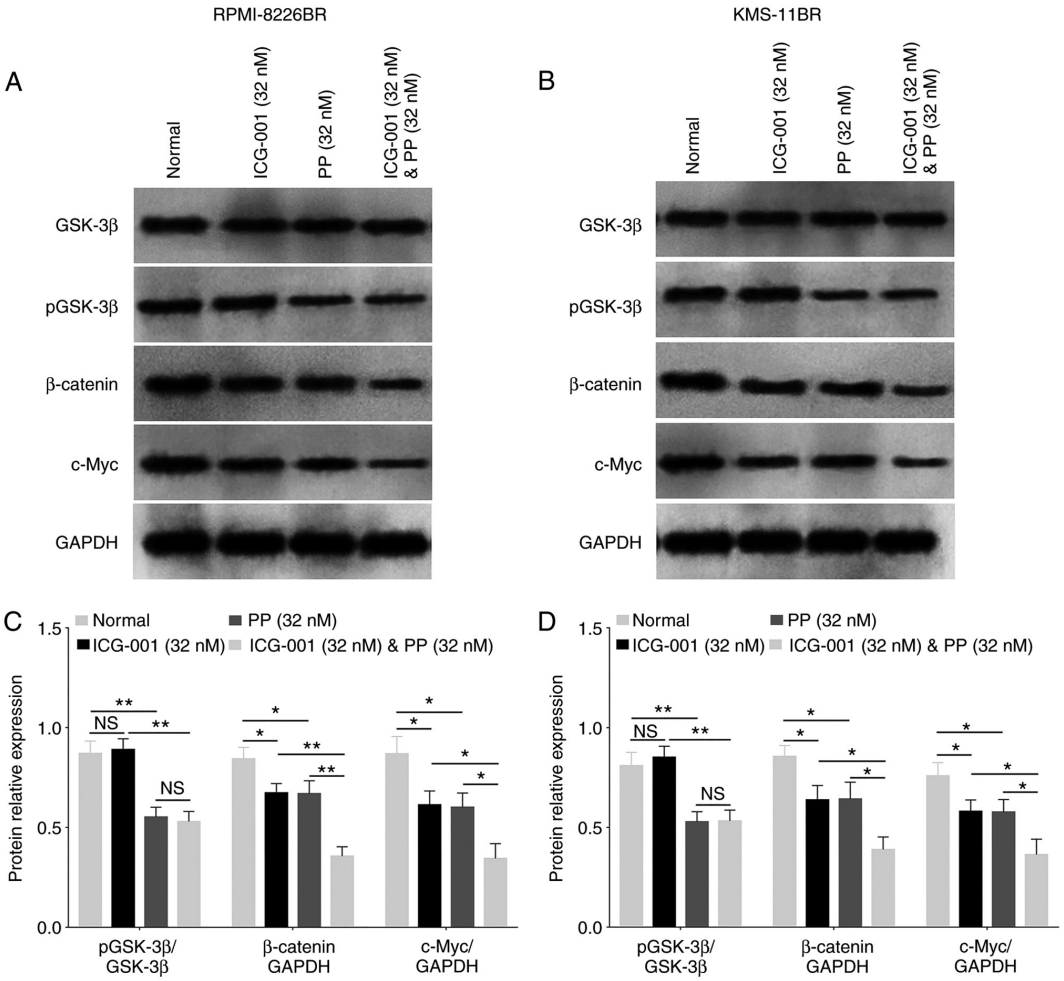

Figure 6. Effect of β-catenin inhibitors on the expression level Wnt/β-catenin signaling-related molecules. Effect of ICG-001 or PP on the protein expression level of pGSK-3β, β-catenin and c-Myc in the (A) RPMI-8226BR and (B) KMS-11BR cell lines. Subsequent data analysis of western blot bands in (C) the RPMI-8226BR and in (D) KMS-11BR cell lines. *P<0.05 and **P<0.01. For statistical analysis, one-way ANOVA followed by Tukey's multiple comparison test was used. GAPDH was used as loading control for normalization to eliminate interference. PP, pyrvinium; BTZ, bortezomib; NS, non-significant; p, phosphorylated; BR, BTZ-resistant.

the β-catenin and c-Myc protein expression level compared with that in the cells treated with ICG-001 or PP alone (both P<0.05; Fig. 6A and C).

In the KMS-11BR cell line, ICG-001 decreased the β-catenin and c-Myc protein expression level compared with that in cells receiving no treatment (both P<0.01; Fig. 6B and D). In addition, PP reduced pGSK-3β, β-catenin and c-Myc protein expression level (all P<0.01; Fig. 6B and D) compared with that in cells receiving no treatment. Furthermore, ICG-001 plus PP also significantly decreased pGSK-3β protein expression level compared with that in cells treated with ICG-001 alone (P<0.001; Fig. 6B and D). Lastly, ICG-001 plus PP also reduced β-catenin and c-Myc protein expression level compared with that in cells treated with ICG-001 or PP alone (all P<0.05; Fig. 6B and D). The data suggested that ICG-001 and PP could downregulate β-catenin and c-Myc protein expression level.

Combination index of β-catenin inhibitors and BTZ in BTZ-resistant MM cells. The combination index of BTZ plus ICG-001 in the RPMI-8226BR and KMS-11BR cell lines was 0.7081 and 0.5538, respectively. Furthermore, the combination index of BTZ plus PP in the RPMI-8226BR and KMS-11BR cell lines was 0.5950 and 0.6593, respectively. These combination values were all <1, which suggested a synergistic effect of the β-catenin inhibitors and BTZ in the BTZ-resistant MM cell lines.

Discussion

BTZ has been classified as a peptide boronate, based on its chemical properties (18); it has also been shown to be more potent and stable than proteasome inhibitor peptide aldehydes, which were invented first (18). With respect to its mode of action, BTZ reversibly inhibits the autophagic-lysosomal pathway and suppresses protein degradation in MM (6,19). BTZ suppresses MM cell proliferation via multiple signaling pathways, such as the NF-κβ and Wnt/β-catenin signaling pathways (6,20); it also promotes reactive oxygen species formation, which further leads to mitochondrial damage and cell apoptosis (21). Notably, it has been approved for MM treatment in patients with refractory MM since 2003 and in newly diagnosed patients with MM since 2008 by the US Food and Drug Administration (6). More importantly, BTZ

combined with dexamethasone can achieve improved clinical outcomes, including an improved 3-year survival rate and lower hematological toxicity incidence rate as compared with conventional therapy in patients with MM, as the first phase of pharmacological therapy (22).

A previous study found that BTZ induced cytotoxicity in MM cells via multiple signaling pathways (18,21). For example, BTZ has been shown to decrease β-catenin and c-Myc protein expression levels in MM cells, indicating the involvement of the Wnt/β-catenin signaling pathway in its cytotoxicity (20). The same study also revealed that the activation of the Wnt/β-catenin signaling pathway led to a decrease in cell proliferation and an increase in apoptosis (20). However, limited research has been performed on the role of the Wnt/β-catenin signaling pathway in BTZ-resistant MM cells; therefore, the present study aimed to investigate this area, and it was discovered that the activation of the Wnt/β-catenin signaling pathway (characterized by the upregulation of β-catenin and c-Myc and downregulation of pGSK-3β) might participate in the mechanism of BTZ resistance using western blot analysis. A possible reason for this could be due to the binding of the Wnt ligand to its receptor (Frizzled and LRP 5/6), which causes the phosphorylation of disheveled, the disassociation of β-catenin from the destruction complex and the phosphorylation of GSK-3β into its inactive form, pGSK-3β (23). As a result of the high intracellular level of β-catenin, the accumulated β-catenin is translocated and binds to TCF/LEF, as a promotor for target gene transcription (23,24). Meanwhile, c-Myc is one of the target genes of β-catenin, which is responsible for cell proliferation (23). Thus, the intracellular elevation of β-catenin was a result of GSK-3β phosphorylation, suggesting the activation of the Wnt/β-catenin signaling pathway in BTZ-resistant MM cells.

Alternatively, the intrinsic increase in β-catenin might have led to the overexpression of a deubiquitination enzyme, such as ubiquitin-specific peptidase (USP)-47, resulting in the accumulation of a reduced unfolded protein, which decreased cellular stress and enhanced cell survival, thus leading to BTZ resistance (25,26). However, the present study did not detect USP-7 expression level in BTZ-resistant MM cells, which could be performed in future studies. Overall, the Wnt/β-catenin signaling pathway plays a critical role in both BTZ normal function and its resistance acquisition in MM cells.

However, BTZ resistance has become a serious issue, as it worsens the clinical outcome of patients with MM (6,19). Therefore, the identification of alternative treatments is required to improve prognosis. As aforementioned, it was discovered that the activation of the Wnt/β-catenin signaling pathway might be involved in BTZ resistance in MM cells. Thus, it was suggested that the inhibition of β-catenin might reverse BTZ resistance and sensitize BTZ-resistant MM cells to BTZ. Several previous studies have shown that β-catenin suppression plays a role in reversing drug resistance (27,28). For example, in one study, β-catenin knockdown increased apoptosis and autophagy activity, which was characterized by an increase in the expression level of apoptotic proteins, including Bcl-2 and capase-3, and autophagy-related proteins, including beclin-1 and microtube-associated protein 1 light chain 3, in BTZ-resistant MM cells (27). Another study found that the suppression of β-catenin sensitized lenalidomide-resistant MM cells to lenalidomide through the use of short hairpin RNAs (28). Apart from β-catenin inhibition using molecular techniques, certain β-catenin inhibitors have been used to reverse the established drug resistance to therapeutic drugs.

ICG-001, a commonly used β-catenin inhibitor, reduced cancer stem cell stemness, which is characterized by decreased *in vitro* tumor sphere formation, to reverse chemosensitivity in both gastric cancer and nasopharyngeal carcinoma cells with acquired resistance (29,30). In addition, ICG-001, combined with tamoxifen treatment, reversed endocrine resistance in breast cancer cells (31). ICG-001, combined with cisplatin therapy, also reserved cisplatin resistance in nasopharyngeal carcinoma cells, manifesting as a suppression of proliferation both *in vitro* and *in vivo* (30). However, few studies have reported the role of ICG-001 in BTZ-resistant MM cells. In the present study, ICG-001 was found to decrease cell viability and sensitize BTZ-resistant MM cells to BTZ. The possible reasons for this are as follows: i) ICG-001 might inhibit the interaction between CREB binding protein and β-catenin, thereby leading to cell differentiation in the BTZ-resistant MM cells and further resulting in antibody production by mature B cells to eliminate MM cells (32); and ii) ICG-001 might downregulate several cancer stem cell markers, such as SRY-box 2, octamer-binding transcription factor 4 and cluster of differentiation 44, and inhibit tumor sphere formation to suppress BTZ-resistant MM cell stemness, eventually resulting in enhanced BTZ chemosensitivity (29,30). In combination, ICG-001 might induce apoptosis and enhance BTZ cytotoxicity in BTZ-resistant MM.

PP, another β-catenin inhibitor, was first identified as an anthelmintic drug and showed tumor suppression activity in ovarian cancer and MM (10,33). Preclinical experiments showed that PP targeted casein kinase 1α to induce a paclitaxel chemotherapy response in renal carcinoma cells (34). In addition, PP inhibited signal transducer and activator of transcription 3 to selectively target reactive oxygen species activation and suppress aerobic glycolysis in Kirsten rat sarcoma viral oncogene homolog-mutant lung cancer (35). However, few studies report the effect of PP on MM. One previous study revealed that PP inhibited MM cell proliferation through suppression of mitochondrial respiratory complex I and STAT3 (14). In addition, another study discovered that PP promoted MM cell apoptosis and destabilized β-catenin by downregulating the Wnt/β-catenin pathway in MM cells (13). In the present study, several experiments were performed to discover the effect of PP combined with BTZ in BTZ-resistant MM cell lines. It was found that PP induced MM cell apoptosis and inhibited MM cell viability. Furthermore, PP reversed sensitivity to BTZ in BTZ-resistant MM cells. The possible reasons for this could be as follows: i) PP might dysregulate the cellular lipid anabolism process in triple-negative breast cancer stem cells, which could further inhibit the lipid biosyncretic pathway in cancer stem cells and reduce BTZ resistance in BTZ-resistant MM cells (36); and ii) PP might suppress the transcriptional activity of glucose-regulated protein 78 to promote unfolded protein response in the endoplasmic reticulum, thus leading to an enhanced BTZ effect on, and chemosensitivity in, BTZ-resistant MM cells (37). In combination, these results suggested that PP might induce cell apoptosis and enhance BTZ chemosensitivity in BTZ-resistant MM. These findings

could be important in the clinical setting and could be used by clinicians to develop a treatment for patients with MM and BTZ resistance in the future.

There are some limitations in the present study. Firstly, the CalcuSyn software was not used to calculate the combination effect of the β-catenin inhibitors with BTZ in MM cells. Secondly, further molecular experiments are required to reveal the detailed mechanism of ICG-001 on cancer stemness. Thirdly, further investigation is required to detect cell behaviours at multiple time points. Finally, normal cells, as negative controls, were not used in the present study.

In conclusion, the β-catenin inhibitors, ICG-001 and PP not only increased apoptosis, but also sensitized BTZ-resistant MM cells to BTZ, indicating its potential therapeutic application in MM.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

CW designed this study and completed the data collection, interpretation and article writing. CW has read and approved the final manuscript. CW confirms the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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