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Mechanisms of G1 cell cycle arrest and apoptosis in myeloma cells induced by hybrid-compound histone deacetylase inhibitor

Seiko Fujii [a,b], Toshinori Okinaga [a,c], Wataru Ariyoshi [a,c], Osamu Takahashi [b], Kenjiro Iwanaga [b], Norikazu Nishino [c], Kazuhiro Tominaga [b], Tatsuji Nishihara [a,c,*]

[a] Division of Infections and Molecular Biology, Kyushu Dental University, Japan
[b] Division of Maxillofacial Surgery, Kyushu Dental University, Japan
[c] Oral Biology Research Center, Kyushu Dental University, Japan

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ABSTRACT

Objectives: Histone deacetylase (HDAC) inhibitors are new therapeutic agents, used to treat various types of malignant cancers. In the present study, we investigated the effects of Ky-2, a hybrid-compound HDAC inhibitor, on the growth of mouse myeloma cells.
Materials and methods: Myeloma cells, HS-72, P3U1, and mouse normal cells were used in this study. Effect of HDAC inhibitors on cell viability was determined by WST-assay and trypan blue assay. Cell cycle was analyzed using flow cytometer. The expression of cell cycle regulatory and the apoptosis associated proteins were examined by Western blot analysis. Hoechst's staining was used to detect apoptotic cells.
Results: Our findings showed that Ky-2 decreased the levels of HDACs, while it enhanced acetylation of histone H3. Myeloma cell proliferation was inhibited by Ky-2 treatment. Interestingly, Ky-2 had no cytotoxic effects on mouse normal cells. Ky-2 treatment induced G1-phase cell cycle arrest and accumulation of a sub-G1 phase population, while Western blotting analysis revealed that expressions of the cell cycle-associated proteins were up-regulated. Also, Ky-2 enhanced the cleavage of caspase-9 and -3 in myeloma cells, followed by DNA fragmentation. In addition, Ky-2 was not found to induce apoptosis in *bcl-2* over-expressing myeloma cells.
Conclusion: These findings suggest that Ky-2 induces apoptosis via a caspase-dependent cascade and Bcl-2-inhibitable mechanism in myeloma cells.

1. Introduction

Multiple myeloma is a hematological disorder characterized by unregulated proliferation of terminally differentiated mature B lymphoid cells. Despite several recent advances in understanding of its pathogenesis and therapeutic effects of new agents, such as thalidomide [1], proteasome inhibitors [2], and arsenic trioxide [3], multiple myeloma remains an incurable disease with a median survival period of 3–4 years [4]. Therefore, novel effective drugs and therapeutic strategies for multiple myeloma needed for affected patients.

Recently, a number of studies have concentrated on epigenetic dysregulation of DNA methylation or histone acetylation to clarify the mechanism of cancer prognosis. It is known that a variety of target genes, such as tumor suppressor, cell cycle, differentiation, and DNA repair genes, are silenced by epigenetic transcriptional repression [5–8]. The expression of several genes that regulate cell differentiation and proliferation can be controlled by changes in histone status through acetylation and deacetylation [9], which are catalyzed by histone acetyltransferases and histone deacetylases (HDACs) [10]. Notably, inhibition of HDACs has been reported to introduce cell differentiation, apoptosis, and cell cycle arrest in several cancer cell lines [11–13].

Ky-2, a cyclo(Asu(NAOH)-Aib-Phe-D-Pro) and a chlamydocin–hydroxamic acid analog, is a novel class I HDAC inhibitor produced by a combination of the HDAC inhibitors TSA and TPX [14,15]. In a previous report, TPX was shown to introduce selective changes in genes that control the cell cycle, followed by regulation of that cycle in lung carcinoma [16]. Although inhibition of oncogenic growth and apoptosis in multiple myeloma cells by HDAC inhibitors such as sodium butyrate and TSA have been reported [15,16], little is known regarding the mechanism by which hybrid HDAC inhibitors induce cell cycle arrest and apoptosis.

In the present study, we investigated the effectiveness of a low concentration of Ky-2 to induce cell cycle arrest in the G1-phase

* Corresponding author. Address: Department of Health Promotion, Division of Infections and Molecular Biology, Kyushu Dental University, 2-6-1 Manazuru, Kokurakita-ku, Kitakyushu 803-8580, Japan. Fax: +81 93 581 4984.
E-mail address: tatsujin@kyu-dent.ac.jp (T. Nishihara).

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and apoptosis in myeloma cells. In addition, we examined the signaling pathway related to the cell cycle and apoptosis in Ky-2-treated myeloma cells.

2. Materials and methods

2.1. Cell cultures

HS-72 and P3U1, mouse myeloma cell lines, were cultured in Iscove's modified Dulbecco's medium (IMDM, GIBCO-BRL, Gaithersburg, MD, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS), streptomycin (100 μg/ml), and penicillin (100 U/ml) at 37 °C in an atmosphere of 5% CO_2 [17,18]. HS-72 cells were transfected by electroporation with a human *bcl-2* expression plasmid (pCΔJ-SV2). HS-72 transfectants (HS-72 B-16) were selected by culturing with Geneticin® Selective Antibiotic (G418 Sulfate; GIBCO-BRL) at 450 μg/ml, then maintained in IMDM supplemented with 10% FBS and G418 (450 μg/ml) at 37 °C in an atmosphere of 5% CO_2 [18].

2.2. Reagents and antibodies

Ky-2 [cyclo(Asu(NAOH)-Aib-Phe-D-Pro)], a hybrid-compound consisting of the cyclic tetrapeptide of TPX B and hydroxamic acid of TSA, and a class I HDAC inhibitor was synthesized for the present study (Fig. 1A) [14]. TSA was purchased from Sigma Chemical Co. (St. Louis, MOS, USA) and stock solutions were prepared in dimethyl sulfoxide (DMSO) at a concentration of 1 μM. Antibodies against the following proteins were used; acetyl-histone H3 (Lys14), HDAC1, HDAC2, and HDAC3 (Cell Signaling Technology, Beverly, MA, USA), p21, cyclin-dependent kinase 4 (CDK4), and CDK6 (Santa Cruz Biotechnology, CA, USA), retinoblastoma protein (Rb) (BD Biosciences, San Jose, CA, USA), caspase-3 and -9 (Medical & Biological Laboratories, Woburn, MA, USA), and β-actin (Sigma). In some experiments, the caspase-3 inhibitor Z-Asp-Glu-Val-Asp-FMK (Z-DEVD-FMK; MBL, Nagoya, Japan) and caspase-9 inhibitor Z-Leu-Glu-His-Asp-FMK (Z-LEHD-FMK; Medical & Biological Laboratories) were utilized.

2.3. HDAC inhibitor treatment and assessment of cell proliferation

Cell proliferation was analyzed using colorimetric 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate (WST-1) assay (Dojindo, Kumamoto, Japan). Mouse myeloma cells (1×10^4 cells/ml) were seeded in 96-well plates, then incubated with HDAC inhibitors. After treatment with an HDAC inhibitor, WST-1 reagent was added to each well followed by incubation for 4 h at 37 °C. Absorbance at 450 nm against a reference wavelength of 650 nm was measured using a Multiskan JX microplate reader (Thermo Scientific, Rockford, IL, USA).

2.4. Flow cytometric analysis

To analyze the cell cycle, mouse myeloma cells (1×10^6 cells/well) were suspended in hypotonic solution [0.1% Triton X-100, 1 mM Tris–HCl (pH 8.0), 3.4 mM sodium citrate, 0.1 mM EDTA] and stained with 5 μg/ml of propidium iodide. DNA contents were analyzed using an EPICS XL (Beckman Coulter, Fullerton, CA, USA). The percentage of cells in each cell cycle phase was determined with MultiCycle for Windows (Phoenix Flow Systems, San Diego, CA, USA).

2.5. Cell morphology

Apoptotic cells were analyzed by morphological change after nuclear DNA staining with Hoechst 33342 (Dojindo). Cells were fixed in 1% glutaraldehyde in phosphate buffered saline (PBS; pH 7.2) for 30 min, then washed with PBS and stained with Hoechst 33342 (1 mM). Nucleus morphology was observed with a fluorescent microscope (Olympus, Melville, NY, USA).

2.6. Western blotting

Ky-2-treated cells were lysed in SDS lysis buffer (50 mM Tris–HCl, 2% SDS; pH 6.8), then protein in the samples was determined using a protein assay reagent (Bio-Rad Laboratories, Hercules, CA, USA). Next, the samples (20 μg of protein) were electrophoresed on 7.5% or 15% SDS–polyacrylamide gels and transferred to PVDF membranes (Millipore Corp., Billerica, MA, USA). After incubation with 5% non-fat skim milk in PBS for 1 h, the membranes were reacted with primary antibodies overnight at 4 °C. Following reaction with secondary antibodies, immunodetection was performed using a Chemi Lumi One Superwestern blotting detection system (Nacalai tesque, Kyoto, Japan) and band densities were measured with a Molecular Imager® Chemi Doc TM XRS Plus system (Bio-Rad Laboratories, Hercules, CA, USA).

2.7. Measurement of cell viability by trypan blue dye exclusion

Cell viability was examined using a trypan blue dye exclusion test. Ky-2-treated cells were suspended in PBS and mixed with an equal amount of 0.5% trypan blue stain (Nacalai). The number of cells excluding trypan blue, representing viable cells, was counted using an Eosinophil counter (SLGC, Tokyo, Japan).

2.8. Isolation of spleen, B, and T cells from mice

Female BALB/c mice (6–7 weeks old, Kyudo, Saga, Japan) were used, and handled according to guidelines of the institutional animal care and use committee of Kyushu Dental University under an approved protocol. Spleen cells were isolated from the mice and red blood cells were removed using RBC lysis Buffer (BioLegend Inc., San Diego, USA), then B and T cell populations were depleted using Dynabeads® Mouse pan T (Thy 1.2) and B (B220) (Dynal Biotech, Oslo, Norway) enriched according to the manufacturer's instructions. Cells were cultured in Roswell Park Memorial Institute 1640 (RPMI1640, GIBCO-BRL) supplemented with 10% heat-inactivated FBS, streptomycin (100 μg/ml), and penicillin (100 U/ml) at 37 °C in an atmosphere of 5% CO_2. Isolated cells were then incubated for 3 h, after which adherent cells, such as macrophages and NK cells were removed from the cell suspension.

2.9. Statistical analysis

Quantitative data are presented as the mean ± standard deviation (S.D.) from triplicate experiments. Statistical analyses of apoptotic nuclei and trypan blue stained cells were conducted using the JMP® 9.0.1 software package (SAS Institute Inc., Cary, NC, USA). Statistical differences were determined using an unpaired Student's *t*-test. *P* values less than 0.05 were considered to be significant.

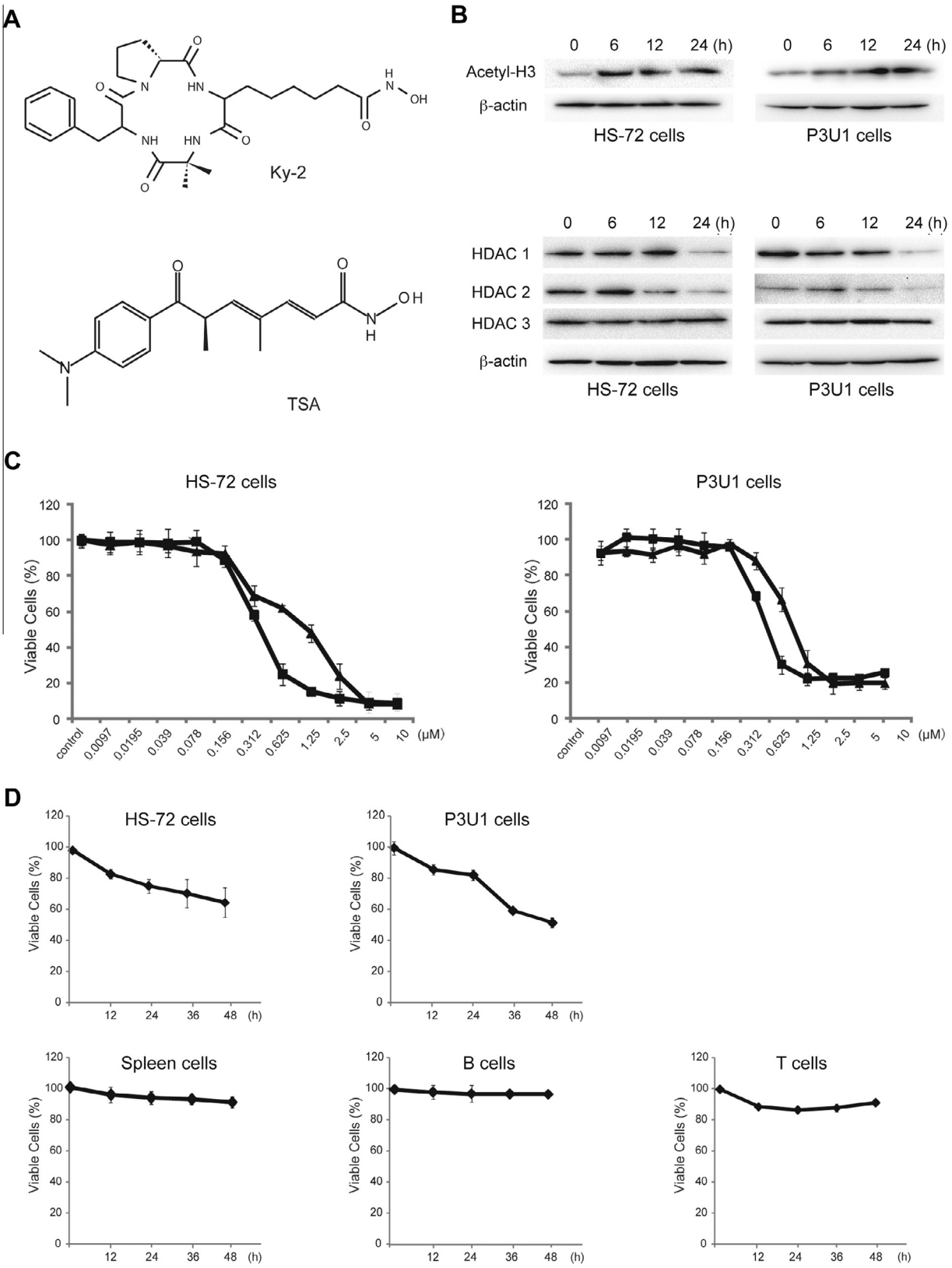

Fig. 1. Chemical structures and biological activities of HDAC inhibitors. (A) Chemical structures of Ky-2 and TSA. (B) Myeloma cells were treated with Ky-2 (1 μM) for the indicated times, then whole lysates were analyzed by SDS–PAGE and Western blotting analyses as described in Section 2. (C) Cells were treated with Ky-2 (■) and TSA (▲) (0–10 μM) for 48 h, and then cell proliferation was assessed using a WST-1 assay. Data are shown as the percentage of cell growth of triplicate samples. (D) Mouse spleen cells were isolated from female BALB/c mice. B and T cells were separated using Dynabeads® Mouse pan B or Dynabeads® Mouse pan T as described in Section 2. Cells were treated with Ky-2 (1 μM) for the indicated times, then live cells were counted after staining with trypan blue dye. Data are shown as the percentage of viable cells of triplicate samples.

3. Results

3.1. Ky-2, a novel HDAC inhibitor, remarkably inhibited myeloma cell proliferation

In order to verify that Ky-2 functions as an HDAC inhibitor, mouse myeloma cells were treated with Ky-2 (1 μM) for various times, and the expressions of histone H3 and HDACs were examined using Western blotting analysis. The expression of histone H3 acetylation was increased in Ky-2-treated mouse myeloma cells after 6 h (Fig. 1B). In addition, Ky-2 inhibited the expressions of HDAC1 and HDAC2 proteins after 24 h. Inhibition of HDAC3 protein was not detected among the Ky-2-treated cells (Fig. 1B). On the other hand, we did not detect a clear expression of histone H3 acetylation or HDACs in normal cells (data not shown). As shown in Fig. 1C, cell proliferation was inhibited by Ky-2 and TSA in a dose-dependent manner, indicating that Ky-2 effectively inhibited cell proliferation compared with TSA treatment. We examined the cytotoxic effects of Ky-2 on primary isolated cells using a trypan blue assay. After 48 h, 45% and 50% of the HS-72 and P3U1 cells, showed cytotoxicity respectively, whereas no cytotoxicity was seen among mouse spleen cells, B and T cells (Fig. 1D).

3.2. Cell cycle arrest in myeloma cells induced by Ky-2

In Ky-2-treated HS-72 cells, the percentage of cells in the G1-phase population increased from 44.9% to 66.9% after 24 h, while that of the sub G1-phase population increased from 6.7% to 21.5% after 36 h. As for P3U1 cells, the G1-phase population increased from 36.8% to 49.1% after 24 h and the sub G1-phase population from 2.1% to 17.5% after 36 h (Fig. 2A). Fig. 2B shows that the expressions of p21 and pRb (hypophosphorylated form of Rb) were up-regulated. The expression of CDK6 protein was down-regulated in HS-72 cells at 24 h after Ky-2 treatment, but not in CDK4. In Ky-2-treated P3U1 cells, the expression levels of p21 and pRb proteins were similar to the levels in HS-72 cells, while the expression level of CDK6 protein was not changed after Ky-2-treatment (Fig. 2B).

3.3. Apoptosis in myeloma cells induced by Ky-2

Hoechst staining revealed that Ky-2 treatment for 24 h caused apoptotic cell morphology, such as characteristic chromatin condensation and degradation of nuclei in myeloma cells (Fig. 3A, red arrowheads). To clarify the molecules involved in apoptosis,

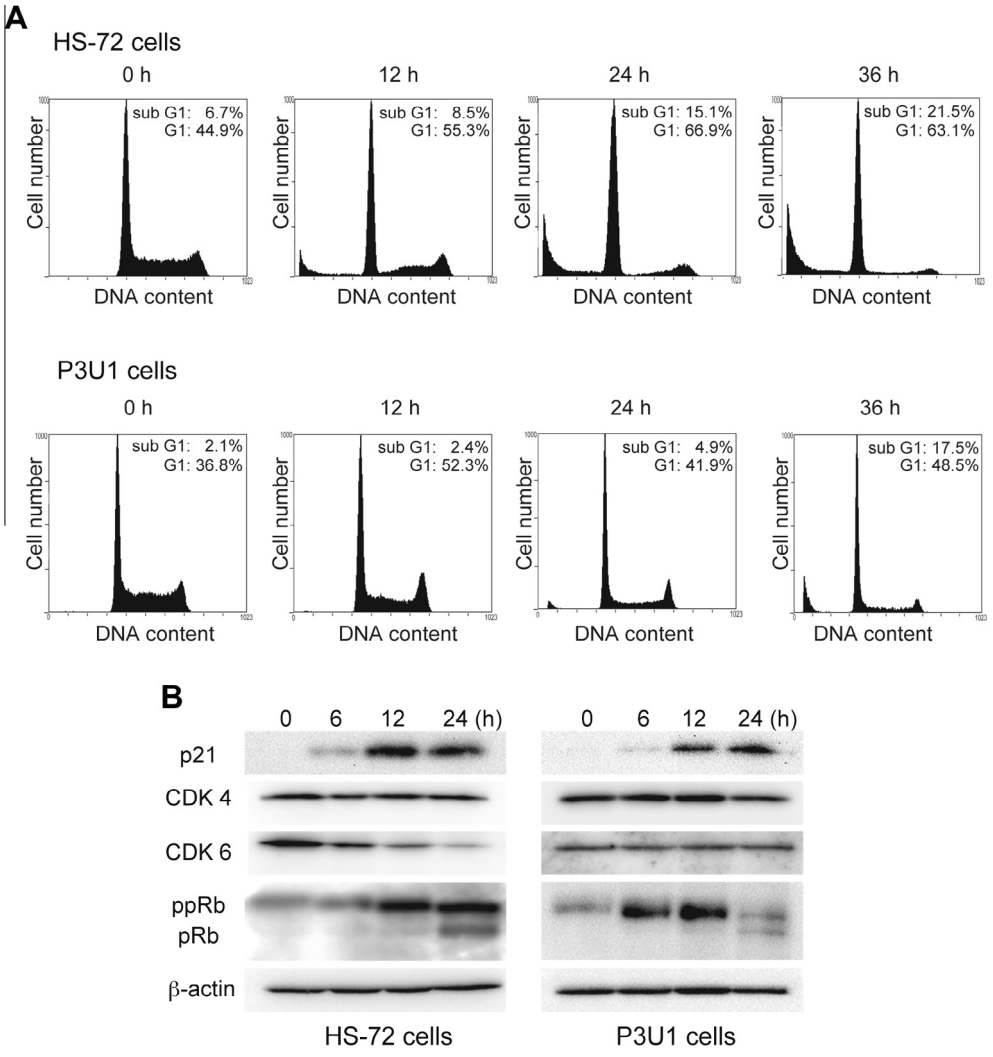

Fig. 2. Effects of Ky-2 on cell cycle arrest in myeloma cells. (A) Myeloma cells were treated with Ky-2 (1 μM) for the indicated times, then stained with propidium iodine to determine distribution during each phase of the cell cycle. Data shown are representative of three independent experiments, with similar results obtained in each. (B) Cells were treated with Ky-2 (1 μM) for the indicated times, then whole lysates were analyzed by SDS–PAGE and Western blotting analyses as described in Section 2.

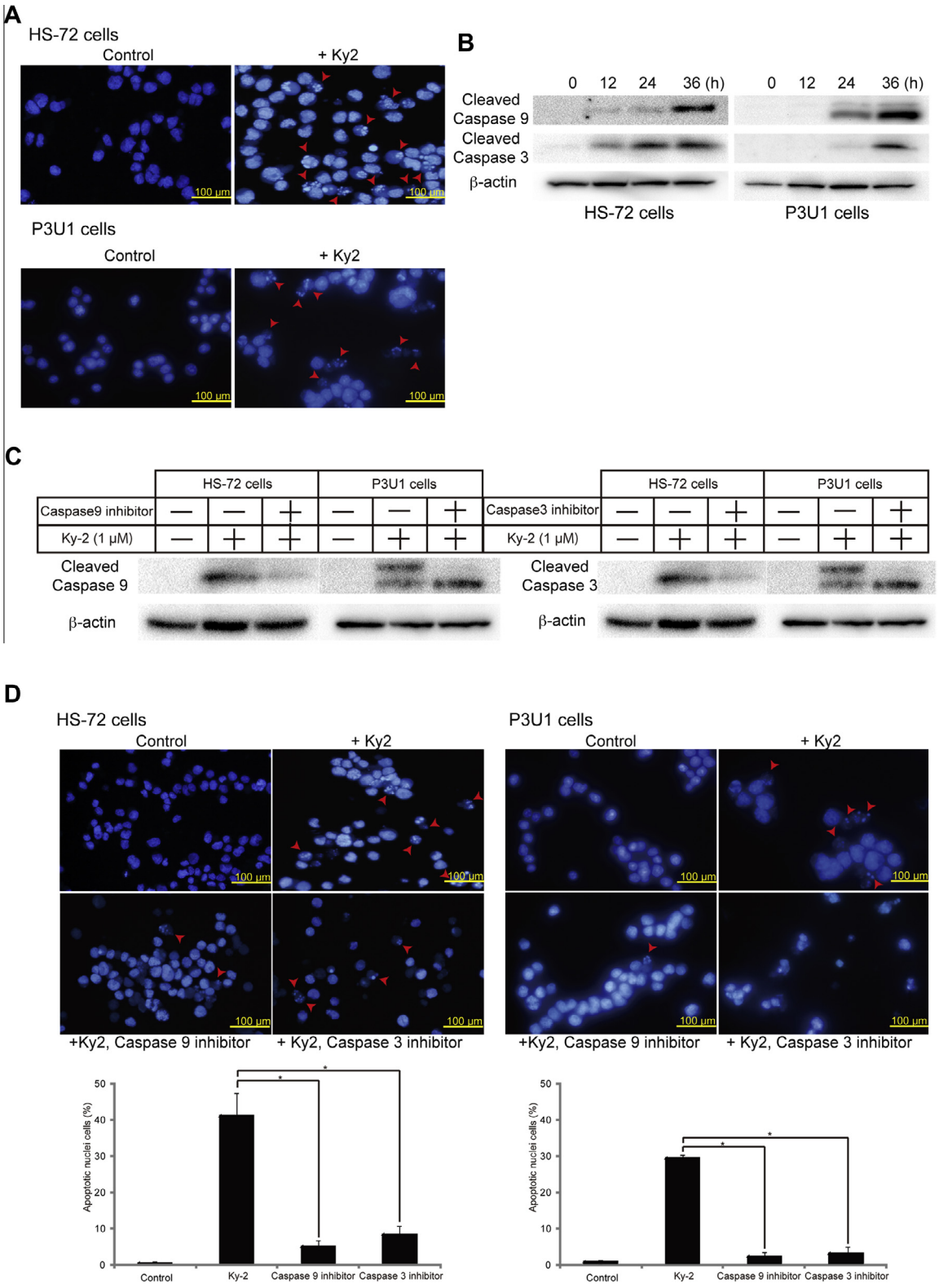

Fig. 3. Effects of Ky-2 on apoptosis in myeloma cells. (A) Apoptosis induction by Ky-2 was analyzed by fluorescence microscopy (400×). Myeloma cells were incubated with Ky-2 (1 μM) for 24 h, then stained with Hoechst 33242. Cells exhibiting chromatin condensation and nuclear fragmentation are indicated by red arrowheads. Data shown are representative of three independent experiments, with similar results obtained in each. (B) Cells were treated with Ky-2 (1 μM) for the indicated times. Each sample (20 μg of protein) was separated on a 15% polyacrylamide gel and was electro-blotted onto PVDF membranes and analyzed by immunoblotting, as described in Section 2. (C) Cells were treated with Ky-2 in the presence of a caspase-9 (Z-LEHD-FMK; 100 μM) or -3 (Z-DEVD-FMK; 100 μM) inhibitor for 24 h, then whole lysates were analyzed by SDS–PAGE and Western blotting analyses. Data shown are representative of three independent experiments, with similar results obtained in each. (D) HS-72 and P3U1 cells were treated with Ky-2 (1 μM) in the presence of Z-LEHD-FMK (100 μM) or Z-DEVD-FMK (100 μM) for 24 h, then stained with Hoechst 33342. Cells exhibiting chromatin condensation and nuclear fragmentation are indicated by red arrowheads. $^{*}P < 0.05$ as compared with control group.

we investigated the induction of apoptosis-associated proteins on Ky-2 treatment. Ky-2 induced cleavage of caspase-9 and -3 in HS-72 cells after 12 h of treatment, while cleavage of those was seen after 24 h in P3U1 cells (Fig. 3B). We confirmed that the Ky-2-induced apoptotic pathway is dependent on the caspase cascade using caspase-9 and -3 inhibitors. As shown in Fig. 3C, those inhibitors completely blocked the expressions of cleaved caspase-9 and -3 in myeloma cells at 24 h after Ky-2 treatment. Furthermore, Hoechst staining revealed that both inhibitors significantly prevented about 80% of Ky-2-induced apoptosis (Fig. 3D).

3.4. Overexpression of Bcl-2 protected against Ky-2-mediated cytotoxicity in HS-72 cells

FACS analysis revealed that there was no increase in the sub-G1-phase population when HS-72 B-16 cells were treated with Ky-2 (Fig. 4A). Furthermore, no expression of the cleaved forms of caspase-9 and -3 was detected in HS-72 B-16 cells after 24 h (data not shown). Hoechst staining revealed that approximately 41% and 9.3% of nuclei in Ky-2-treated HS-72 and HS-72 B-16 cells, were apoptotic after respectively for 24 h (Fig. 4B).

4. Discussion

Mutations and chromosomal translocation are promoted in several cancers, resulting in repression of transcription through abnormal recruitment and activation of HDACs. Among of the four HDACs groups, class I HDACs have been found to be overexpressed in various types of malignant cancers [19–22]. It is generally accepted that class I HDACs are involved in cell growth and differentiation, and are up-regulated in malignant cancer cells including myeloma cells [23–25]. Ky-2 is a class I HDAC inhibitor, and described as a hybrid compound consisting of TSA and TPX, which are known potent inhibitors of HDACs that do not show sufficient antitumor activity, probably because of their instability *in vivo* [26]. First, we verified the function of Ky-2 as HDAC inhibitor in mouse myeloma cells (Fig. 1B). These results suggest that Ky-2 has activity to inhibit HDACs in myeloma cells, resulting in acetylation of histone H3. In WST-1 assay, Ky-2 prevented myeloma cell proliferation at lower level of exposure as compared with TSA (Fig. 1C). Fig. 1D also found that Ky-2 had no effect on the cytotoxicity of B and T cells isolated from mouse spleen cells, suggesting that Ky-2 has a cytotoxic effect on myeloma cells, but not normal cells. To explain this novel phenomenon, we examined the molecular mechanisms used by Ky-2 in mouse myeloma cells.

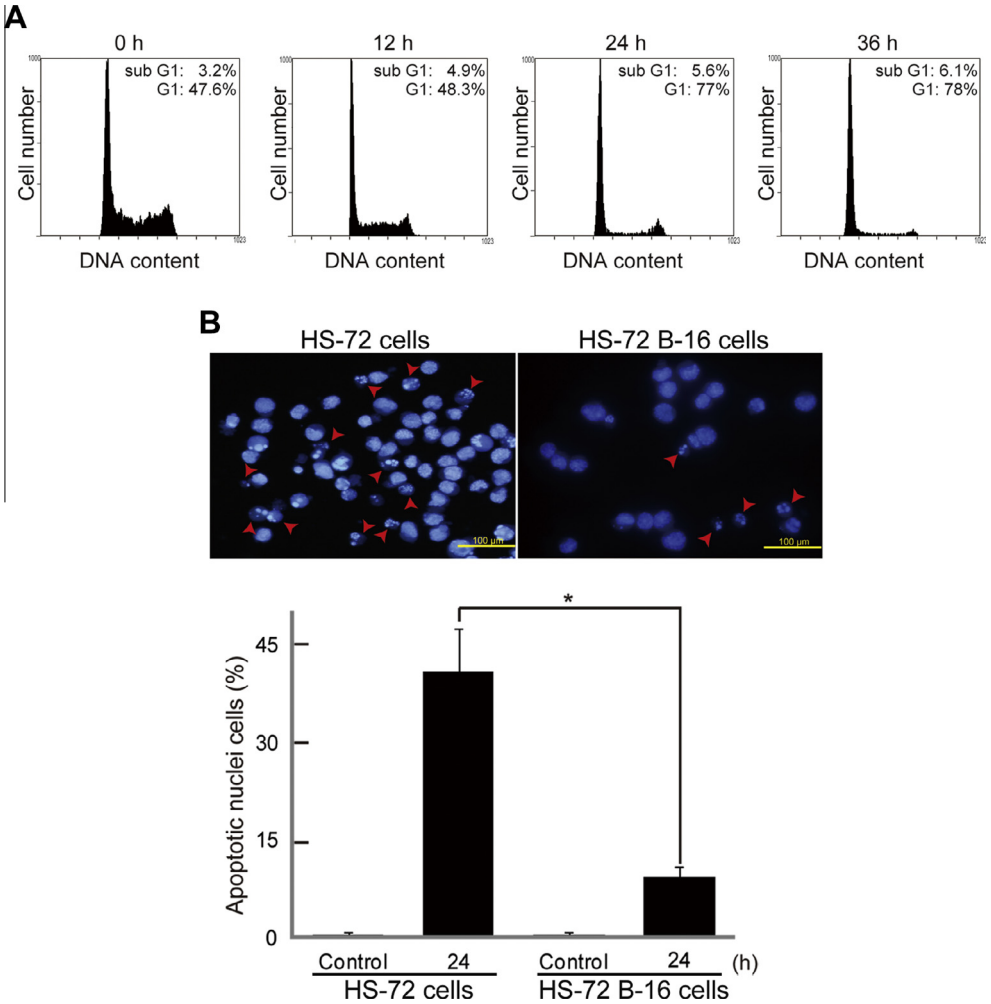

Fig. 4. Bcl-2 overexpression suppressed apoptosis induced by Ky-2 (A) HS-72 B-16 cells were treated with Ky-2 (1 μM) for the indicated times, then distribution was examined during each phase of the cell cycle. Data shown are representative of 3 independent experiments, with similar results obtained in each. (B) Apoptosis induction by Ky-2 was analyzed by fluorescence microscopy (400×). HS-72 and HS-72 B-16 cells were incubated with Ky-2 (1 μM) for 24 h, then stained with Hoechst 33242. Cells exhibiting chromatin condensation and nuclear fragmentation are indicated by red arrowheads. $^{*}P < 0.05$ as compared with control group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

HDAC inhibitors reactivate epigenetically silenced genes in malignant cells through the control of growth arrest [27]. We found that Ky-2 treatment remarkably inhibited G1 cell cycle arrest, as well as the expressions of p21 and pRb (Fig. 2). Rb controls cell cycle progression during the G1 to S transition in response to extracellular signals for growth inhibition [28], while its phosphorylation is induced by catalytic subunits of CDKs [29]. These results are in agreement with previous findings of up-regulation of p21 as well as dephosphorylation of Rb by HDAC inhibitors, TSA, and suberoylanilide hydroxamic acid (SAHA) [16,30,31].

Ky-2 treatment of mouse myeloma cells induced caspase-3 and -9 cleavage after 24 h (Fig 3B), not caspase-8 (data not shown). There are two classical pathways for functionally separate molecularly linked apoptosis, caspase-3 processing mediated by caspase-9 (the mitochondrial intrinsic pathway) and that by caspase-8 (the death receptor extrinsic pathway) [32]. The present results clarified that specific caspase inhibitor, Z-DEVD-FMK and Z-LEHD-FMK remarkably abolish nuclear fragmentation in Ky-2-treated myeloma cells (Fig. 3D), clearly indicating Ky-2 induced apoptosis via a caspase-3 and -9 mediated pathway. In addition, they suggest involvement of the mitochondrial intrinsic pathway in induction of apoptosis in myeloma cells treated with this agent.

Anti-apoptotic Bcl-2 family members, such as Bcl-2 and Bcl-X_L, may act directly to prevent the release of cytochrome *c*, a molecule that binds Apaf-1 [33–35]. In the present study, we produced *bcl-2* overexpressing HS-72 cells (HS-72 B-16), and used them to analyze apoptotic morphological changes and caspase cleavage following Ky-2 treatment. Bcl-2 overexpression protected the cells from Ky-2-induced apoptosis, resulting in no increase in the sub-G1-phase population, and cleavage of caspase-9 and -3 (data not shown), as well as inhibition of nuclei fragmentation in HS-72 B-16 cells induced by Ky-2 treatment (Fig. 4A, B). These findings indicated that Ky-2-induced apoptosis was completely blocked by overexpression of Bcl-2, suggesting the importance of mitochondrial-dependent apoptosis signaling in Ky-2 treated myeloma cells.

In summary, our results showed that novel HDAC inhibitor Ky-2 significantly inhibited myeloma cell proliferation, while it demonstrated no cytotoxicity against normal cells. Furthermore, Ky-2 induced cell cycle arrest, resulting in regulation of cell cycle-associated proteins, p21, and hypophosphorylation of Rb proteins. Interestingly, we also found that Ky-2 induced apoptosis in myeloma cells via a caspase-mediated mitochondrial pathway, which promoted cleavage of caspase-9 and -3. We think that the most important finding would be the lack of cytotoxic effects on normal lymphocytes by Ky-2. At present, we have no ready precise explanation for this phenomenon, but we speculate that the reason for this difference in viability may be due to the function of Bcl-2 family. Further research is planned to these points. Together, these findings suggest that Ky-2, a novel class I HDAC inhibitor, may be an effective therapeutic agent against multiple myeloma in view of its target specificity and antitumor activities.

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References

[1] L. Mileshkin, J.J. Biagi, P. Mitchell, C. Underhil, A. Grigg, R. Bell, et al., Multicenter phase 2 trial of thalidomide in relapsed/refractory multiple myeloma: adverse prognostic impact of advanced age, Blood 102 (2003) 69–77.

[2] P.G. Richardson, B. Barlogie, J. Berenson, S. Singhal, S. Jagannath, D. Irwin, et al., A phase 2 study of bortezomib in relapsed, refractory myeloma, N. Engl. J. Med. 348 (2003) 2609–2617.

[3] N.C. Munshi, G. Tricot, R. Desikan, A. Badros, M. Zangari, A. Toor, et al., Clinical activity of arsenic trioxide for the treatment of multiple myeloma, Leukemia 16 (2002) 1835–1837.

[4] P.P. Pandolfi, Histone deacetylases and transcriptional therapy with their inhibitors, Cancer Chemother. Pharmacol. 48 (2001) 17–19.

[5] O.H. Kramer, M. Gottlicher, T. Heinzel, Histone deacetylase as a therapeutic target, Trends Endocrinol. Metab. 12 (2001) 294–300.

[6] P. Marks, R.A. Rifkind, V.M. Richon, R. Breslow, T. Miller, W.K. Kelly, Histone deacetylases and cancer: causes and therapies, Nat. Rev. Cancer 1 (2001) 194–202.

[7] R. Brown, G. Strathdee, Epigenomics and epigenetic therapy of cancer, Trends Mol. Med. 8 (2002) 43–48.

[8] R.X. Luo, D.C. Dean, Chromatin remodeling and transcriptional regulation, J. Natl. Cancer Inst. 91 (1999) 1288–1294.

[9] R. Furumai, Y. Komatsu, N. Nishino, S. Khochbin, M. Yoshida, S. Horinouchi, Potent histone deacetylase inhibitors built from trichostatin A and cyclic tetrapeptide antibiotics including trapoxin, Proc. Natl. Acad. Sci. USA 98 (2001) 87–92.

[10] A. Peserico, C. Simone, Physical and functional HAT/HDAC interplay regulates protein acetylation balance, J. Biomed. Biotechnol. (2011), http://dx.doi.org/10.11155/2011/371832.

[11] K.B. Glaser, Defining the role of gene regulation in resistance to HDAC inhibitors-mechanisms beyond P-glycoprotein, Leuk. Res. 30 (2006) 651–652.

[12] C.Y. Gui, L. Ngo, W.S. Xu, V.M. Richon, P.A. Marks, Histone deacetylase (HDAC) inhibitor activation of p21WAF1 involves changes in promoter-associated proteins, including HDAC1, Proc. Natl. Acad. Sci. USA 101 (2004) 1241–1246.

[13] R.W. Johnstone, Histone-deacetylase inhibitors: novel drugs for the treatment of cancer, Nat. Rev. Drug Discov. 1 (2002) 287–299.

[14] N. Nishino, B. Jose, R. Shinta, T. Kato, Y. Komatsu, M. Yoshida, Chlamydocin-hydroxamic acid analogues as histone deacetylase inhibitors, Bioorg. Med. Chem. 12 (2004) 5777–5784.

[15] L.C. Sambucetti, D.D. Fischer, S. Zabludoff, P.O. Kwon, H. Chamberlin, N. Trogani, et al., Histone deacetylase inhibition selectively alters the activity and expression of cell cycle proteins leading to specific chromatin acetylation and antiproliferative effects, J. Biol. Chem. 274 (1999) 34940–34947.

[16] D. Lavelle, Y.H. Chen, M. Hankewych, J. DeSimone, Histone deacetylase inhibitors increase p21 (WAF1) and induce apoptosis of human myeloma cell lines independent of decreased IL-6 receptor expression, Am. J. Hematol. 68 (2001) 170–178.

[17] T. Koseki, K. Yamato, S. Krajewski, J.C. Reed, Y. Tsujimoto, T. Nishihara, Activin A-induced apoptosis is suppressed by BCL-2, FEBS Lett. 376 (1995) 247–250.

[18] T. Nishihara, Y. Ohsaki, N. Ueda, T. Koseki, Y. Eto, Induction of apoptosis in B lineage cells by activin A derived from macrophages, J. Interferon Cytokine Res. 15 (1995) 509–516.

[19] K. Halkidou, L. Gaughan, S. Cook, H.Y. Leung, D.E. Neal, C.N. Robson, Upregulation and nuclear recruitment of HDAC1 in hormone refractory prostate cancer, Prostate 59 (2004) 177–189.

[20] B.H. Huang, M. Laban, C.H. Leung, L. Lee, C.K. Lee, M. Salto-Tellez, et al., Inhibition of histone deacetylase 2 increases apoptosis and p21Cip1/WAF1 expression, independent of histone deacetylase 1, Cell Death Differ. 12 (2005) 395–404.

[21] J. Song, J.H. Noh, J.H. Lee, J.W. Eun, Y.M. Ahn, S.Y. Kim, et al., Increased expression of histone deacetylase 2 is found in human gastric cancer, APMIS 113 (2005) 264–268.

[22] A. Hrzenjak, F. Moinfar, M.L. Kremser, B. Strohmeier, P.B. Staber, K. Zatloukal, et al., Valproate inhibition of histone deacetylase 2 affects differentiation and decreases proliferation of endometrial stromal sarcoma cells, Mol. Cancer Ther. 5 (2006) 2203–2210.

[23] K. Kitazoe, M. Abe, M. Hiasa, A. Oda, H. Amou, T. Harada, et al., Valproic acid exerts anti-tumor as well as anti-angiogenic effects on myeloma, Int. J. Hematol. 89 (2009) 45–57.

[24] R. Somech, S. Izraeli, S.J. Simon, Histone deacetylase inhibitors–a new tool to treat cancer, Cancer Treat. Rev. 30 (2004) 461–472.

[25] J. Kikuchi, T. Wada, R. Shimizu, T. Izumi, M. Akutsu, K. Mitsunaga, et al., Histone deacetylases are critical targets of bortezomib-induced cytotoxicity in multiple myeloma, Blood 116 (2010) 406–417.

[26] Y. Komatsu, K.Y. Tomizaki, M. Tsukamoto, T. Kato, N. Nishino, S. Sato, et al., Cyclic hydroxamic-acid-containing peptide 31, a potent synthetic histone deacetylase inhibitor with antitumor activity, Cancer Res. 61 (2001) 4459–4466.

[27] F. McLaughlin, N.B. La Thangue, Histone deacetylase inhibitors open new doors in cancer therapy, Biochem. Pharmacol. 68 (2004) 1139–1144.

[28] R.A. Weinberg, The retinoblastoma protein and cell cycle control, Cell 81 (1995) 323–330.

[29] H. Matsushime, M.F. Roussel, R.A. Ashmun, C.J. Sherr, Colony-stimulating factor 1 regulates novel cyclins during the G1 phase of the cell cycle, Cell 65 (1991) 701–713.

[30] V. Sandor, A. Senderowicz, S. Mertins, D. Sackett, E. Sausville, M.V. Blagosklonny, et al., P21-dependent G1 arrest with downregulation of cyclin D1 and upregulation of cyclin E by the histone deacetylase inhibitor FR901228, Br. J. Cancer 83 (2000) 817–825.

[31] T.E. Fandy, S. Shankar, D.D. Ross, E. Sausville, R.K. Srivastava, Interactive effects of HDAC inhibitors and TRAIL on apoptosis are associated with changes in mitochondrial functions and expressions of cell cycle regulatory genes in multiple myeloma, Neoplasia 7 (2005) 646–657.

[32] E.A. Slee, M.T. Harte, R.M. Kluck, B.B. Wolf, C.A. Casiano, D.D. Newmeyer, et al., Ordering the cytochrome c-initiated caspase cascade: hierarchical activation of caspases-2, -3, -6, -7, -8, and -10 in a caspase-9-dependent manner, J. Cell Biol. 144 (1999) 281–292.
[33] E.H. Cheng, M.C. Wei, S. Weiler, R.A. Flavell, T.W. Mak, T. Lindsten, et al., BCL-2, BCL-X(L) sequester BH3 domain-only molecules preventing BAX- and BAK-mediated mitochondrial apoptosis, Mol. Cell 8 (2001) 705–711.
[34] H. Zou, Y. Li, X. Liu, X. Wang, An APAF-1.cytochrome c multimeric complex is a functional apoptosome that activates procaspase-9, J. Biol. Chem. 274 (1999) 11549–11556.
[35] J. Yang, X. Liu, K. Bhalla, C.N. Kim, A.M. Ibrado, J. Cai, T.I. Peng, D.P. Jones, X. Wang, Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked, Science 275 (1997) 1129–1132.