Am J Transl Res 2017;9(11):4994-5002
www.ajtr.org /ISSN:1943-8141/AJTR0058169

Original Article

Histone deacetylase inhibitor NaBut suppresses cell proliferation and induces apoptosis by targeting p21 in multiple myeloma

Ruosi Yao[1,2,3*], Danyang Han[1,3*], Xiaoyang Sun[1,3], Chunling Fu[1,2,3], Qingyun Wu[1,2,3], Yao Yao[1,2,3], Hujun Li[2], Zhenyu Li[2], Kailin Xu[1,2,3]

[1]Blood Diseases Institute, Xuzhou Medical University, Xuzhou, Jiangsu, China; [2]Department of Hematology, The Affliated Hospital of Xuzhou Medical University, Xuzhou, Jiangsu, China; [3]Key Laboratory of Bone Marrow Stem Cell, Xuzhou, Jiangsu, China. [*]Equal contributors.

Received May 24, 2017; Accepted October 30, 2017; Epub November 15, 2017; Published November 30, 2017

Abstract: Multiple myeloma (MM) is an extremely serious hematological malignancy that remains incurable due to chemotherapy resistance. Epigenetic regulation is closely associated with progression of MM. Histone deacetylase inhibitor NaBut functions in various physiologic processes, including inflammation and differentiation. Its' possible roles in MM progression have not been explored. In this report, NaBut decreased survival of several human MM cell lines in a dose- and time-dependent manner. NaBut could also lead to cell cycle arrest at the G2/M phase in a dose-dependent manner. NaBut inhibited bortezomib-resistant cell proliferation in dose- and time-dependent manners, and NaBut was likely to induce partly bortezomib-resistant MM cell death. Moreover, NaBut induced MM cell apoptosis via transcriptional activation of p21. Overall, our results implicate NaBut as a potential therapeutic drug for MM.

Keywords: NaBut, proliferation, apoptosis, multiple myeloma, p21

Introduction

Multiple myeloma (MM) is an extremely serious hematological malignancy that remains incurable. Most patients will ultimately relapse or become refractory to the chemotherapy [1]. It accounts for 1% of all tumors and more than 10% of all hematological malignancies [2]. Despite improved treatments, the major problem in MM is the resistance to therapy [3]. Many novel agents are currently developed for the treatment of relapsed/refractory MM, including immunomodulatory drugs (lenalidomide, pomalidomide), proteasome inhibitors (bortezomib [BTZ], carfilzomib), monoclonal antibodies (elotuzumab, daratumumab), cell signaling targeted therapies, and strategies targeting the tumor microenvironment [4-6]. Although the agents have demonstrated antitumor activity in relapsed/refractory MM, drug resistance is still a problem and MM continues to be mostly an incurable disease. Therefore, it is essential to identify novel therapeutic agents to overcome drug resistance and to understand the molecular mechanism of MM.

Post-translational modifications of histones occur via various mechanisms including acetylation, phosphorylation, methylation, and ubiquitination. All can alter interactions of DNA with histones. Among them, histone acetylation is regulated by histone acetyltransferases and histone deacetylases (HDACs) [7]. Depletion of HDAC activity promotes proliferation, cell cycle arrest, and apoptosis of tumor cells [1]. Currently, many HDAC inhibitors have emerged as novel therapeutic agents for tumors, including MM. These include vorinostat, rocilinostat, and panabinostat [8-10]. Mechanisms of action whereby HDAC inhibitors trigger anti-MM activities have not been fully characterized.

Apoptosis is a major mechanism for kinds of cancer cell elimination. The HDAC inhibitor sodium butyrate (NaBut) exhibits effective anticancer behavior [11]. A study using MCF-7 breast

cancer cells also demonstrated that NaBut has dose- and time-dependent anti-proliferative effects [12].

In this study, we confirm that the HDAC inhibitor NaBut decreases the survival of several human MM cell lines in dose- and time-dependent manners. NaBut leads to cell arrest at the G2/M phase of the cell cycle in a dose-dependent manner. NaBut induced apoptosis in multiple MM cell lines by up-regulation of p21 expression. These data provide detailed information concerning the cytotoxic effects of NaBut on MM cells and offers a basic foundation for the exploration of NaBut as a potential target for the therapeutic intervention in MM.

Materials and methods

Cell culture

RPMI-8226, IM9, U266, and HEK293T cell lines were purchased from American Type Culture Collection (Manassas, VA, USA). Cells were grown in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) at 37°C in a humidified 5% CO_2 incubator. RPMI-8226, BTZ-resistant cell lines (RPMI8226/BTZ7 and RPMI8226/BTZ100 [13]) were kindly provided by Dr. Jacqueline Cloos (VU University Medical Center, The Netherlands). HEK293T cells were cultured in DMEM medium with 10% FBS and antibiotics.

Cell viability assay

MM cell lines were plated at $1x10^4$ cells per well in a 96-well plate and treated with different concentrations of NaBut for 24 h or for different times in the presence of 5 mM NaBut. Cell viability was measured using the CCK8 cell proliferation kit according to manufacturer's instructions (Beyotime, China).

Flow cytometry analysis of cell cycle and apoptosis

For cell cycle assay, MM cells were treated with different concentrations of NaBut for 24 h, then incubated with 50 μg/ml propidium iodide (PI) and 20 units/ml RNase-A. DNA content was analyzed by flow cytometry. For apoptosis assay, MM cells were treated with indicated concentrations of NaBut for 24 h and cells were processed using an Annexin V-APC/7-AAD kit (KeyGEN, China). The rate of apoptosis was analyzed by flow cytometry.

Western blot

Experiments were carried out as described previously [14]. Antibodies used were directed against β-actin (Santa Cruz Biotechnology, Santa Cruz, CA, USA), Caspase 3, Caspase 8, and Caspase 9 (Cell Signaling Technology, Beverley, MA, USA), Cyclin D1, CDK2, PARP1, and p21 (Proteintech, Chicago, IL, USA).

RNA extraction, reverse transcription, and real-time RT-PCR

Experiments were performed as we described previously [15].

Luciferase reporter assay

The experiments were performed as described previously [14]. The pGL4.20-p21 reporter gene vectors were constructed by inserting the PCR amplification products from the human genome.

Statistical analysis

Data are presented as mean ± SD as indicated. The Student t test (two-tailed) was used to determine statistical significance of differences between groups. $P < 0.05$ was considered statistically significant. All statistical analyses were carried out using the GraphPad Prism 5 software (GraphPad, La Jolla, CA, USA).

Results

NaBut reduces viability of MM cells

To investigate the effect of HDAC inhibitor NaBut on human MM cell growth, RPMI-8226 cells were treated with different concentrations of NaBut (0, 0.5, 1, 5 and 10 mM). Inhibition of cell viability was observed following treatment with NaBut at 0.5 mM, but viability of RPMI-8226 cells did not decrease continually after 24 h of exposure to NaBut at 1, 5 and 10 mM (**Figure 1A**). Additionally, we observed that NaBut clearly decreased survival of RPMI-8226 cell line in a time-dependent manner (**Figure 1B**). Subsequently, we evaluated the impact of NaBut on other MM cells. The results showed that NaBut treatment induced significant suppression of IM9 cell viability at low concentra-

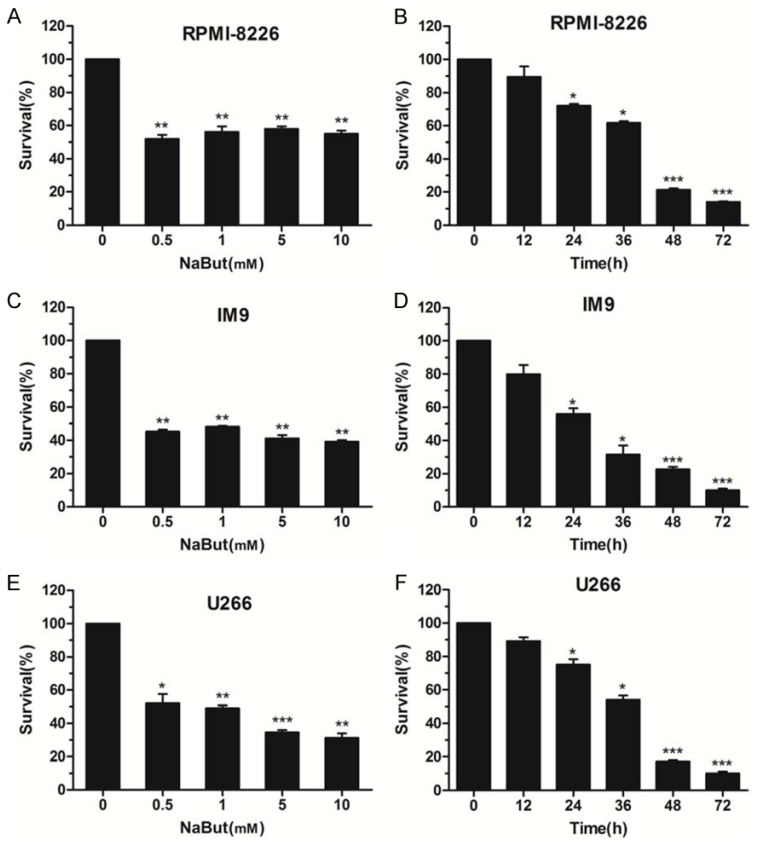

Figure 1. NaBut induces cytotoxicity in MM cells. Viability of cultured cells treated with different concentrations (0, 0.5, 1, 5, 10 mM) of NaBut for 24 h was assessed by the CCK8 assay in RPMI-8226 cells (A), IM9 cells (C), and U266 cells (E). Viability of cultured cells exposed to 5 mM NaBut at 12, 24, 36, 48, and 72h was assessed by CCK8 assay in RPMI-8226 cells (B), IM9 cells (D), and U266 cells (F). All experiments were repeated independently at least three times. Error bars, mean ± SD. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

tions (**Figure 1C**). Conversely, NaBut treatment inhibited U266 cell proliferation in a dose-dependent manner (**Figure 1E**). The viability of IM9 and U266 cells was markedly decreased in a time-dependent manner (**Figure 1D** and **1F**).

NaBut leads to cell cycle arrest at G2/M phase

Since NaBut inhibited MM cell proliferation, and cell survival was closely associated with cell cycle, we conducted flow cytometry analysis to examine the effect of NaBut on MM cell cycle distribution. NaBut treatment increased the percentage of IM9 cells in G2/M phase in a dose-dependent manner, and reached a maximal peak at 5 mM. Meanwhile, a lower percentage of IM9 cells were present in the S phase compared with the untreated group (**Figure 2A** and **2B**). However, the proportion of RPMI-8226 and U266 cells treated by NaBut in G2/M phase did not increase in a concentration-dependent manner (data not shown). As expected, cyclin D1 and cyclin-dependent kinase 2 (CDK2), which are important regulators of G1-S phase transition, exhibited a lower level expression in a concentration-dependent manner (**Figure 2C**).

NaBut induces apoptosis in human MM cells

To elucidate the cytotoxic mechanism of NaBut on MM cells, we performed flow cytometry analysis and detected proteins involved in programmed cell death by western blot. Following a 24 h exposure to NaBut at 5 mM, RPMI-8226 cells exhibited typical apoptotic alterations, including cell shrinkage and loss of normal nuclear architecture. Annexin V/7-AAD staining showed a markedly higher percentage of apoptosis in RPMI-8226 cells (**Figure 3A**). Similarly, NaBut treatment increased apoptosis of IM9 and U266 cells (**Figure 3B** and **3C**).

Increased proteolytic cleavage of PARP1, and Caspase 9, but not Caspase 8, was evident in RPMI-8226, IM9, and U266 cell lines (**Figure 3D**), indicating cell apoptosis. Surprisingly, western blot analysis revealed increased activated Caspase 3 in RPMI-8226 and IM9 cell lines, but not in U266 cell lines (**Figure 3D**). Collectively, the above results indicate that NaBut induces apoptosis in multiple MM cell lines.

NaBut inhibited BTZ-resistant MM cell proliferation

Currently, the first-in-class proteasome inhibitor BTZ has demonstrated favorable anti-MM activity. Unfortunately, BTZ resistance is present in many patients with relapsed/refractory MM [16, 17]. Herein, we wanted to clarify

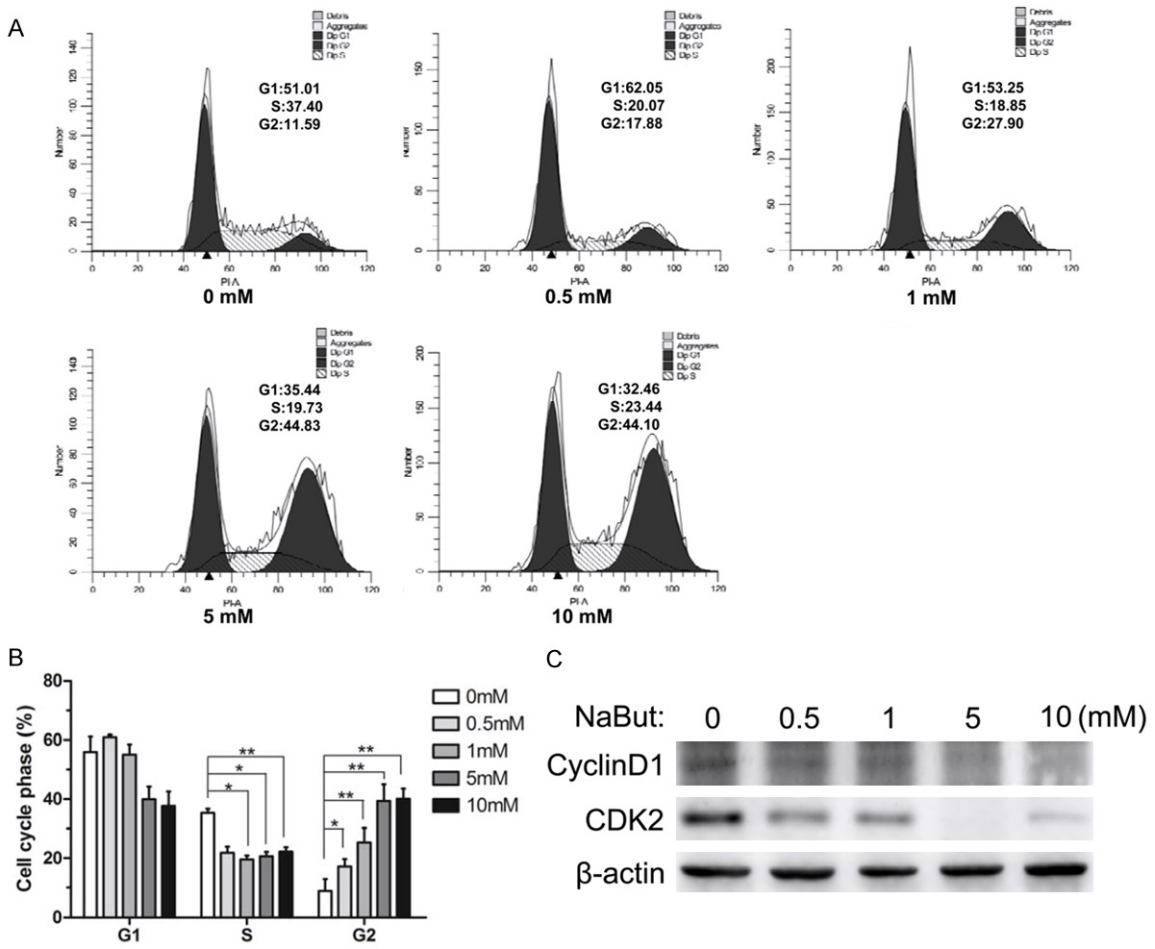

Figure 2. NaBut treatment leads to MM cell cycle arrest. A. Cell cycle analysis of IM9 cell lines treated with different concentrations (0, 0.5, 1, 5, 10 mM) of NaBut for 24 h. B. Percentages of subpopulation of cells at different cell cycle phases based on three independent experiments. Error bars, mean ± SD. *, $P < 0.05$; **, $P < 0.01$. C. Expression of cell cycle-associated proteins (Cyclin D1, CDK2) was analyzed by western blot.

whether HDAC inhibitor NaBut could overcome BTZ resistance. BTZ at the specified concentrations failed to reduce growth of RPMI-8226/BTZ7 or RPMI-8226/BTZ100 cells (data not shown). Nevertheless, NaBut treatment decreased the RPMI-8226/BTZ7 cell viability in dose- and time-dependent manners (**Figure 4A** and **4B**). The HDAC inhibitor NaBut still reduced cell survival in 100 nM BTZ-resistant MM cell lines in a dose- and time-dependentt manner (**Figure 4C** and **4D**).

We further explored whether NaBut treatment regulated BTZ-resistant MM cells programmed cell death by flow cytometry and western blot analysis. The proportion of apoptotic cells was not remarkable in NaBut-treated RPMI-8226/BTZ7 cell lines compared with control group, and we did not observe increased proteolytic cleavage of PARP1 and Caspase 9 (**Figure 4E**). Despite Annexin V/7-AAD staining assay showed that NaBut did not induce RPMI-8226/BTZ100 cell death, western blot analysis revealed markedly increased levels of apoptosis-related proteins, cleaved PARP1 and Caspase 9 in NaBut-treated RPMI-8226/BTZ100 cell lines (**Figure 4F**). This implies that NaBut likely at least partially induces BTZ-resistant MM cell death.

NaBut induces MM cell death via p21 upregulation

We next determined the molecular mechanism by which NaBut induces myeloma cell death. Firstly, we detected the expression level of apoptosis-associated factors in three MM cell lines. The mRNA levels of the pro-apoptotic fac-

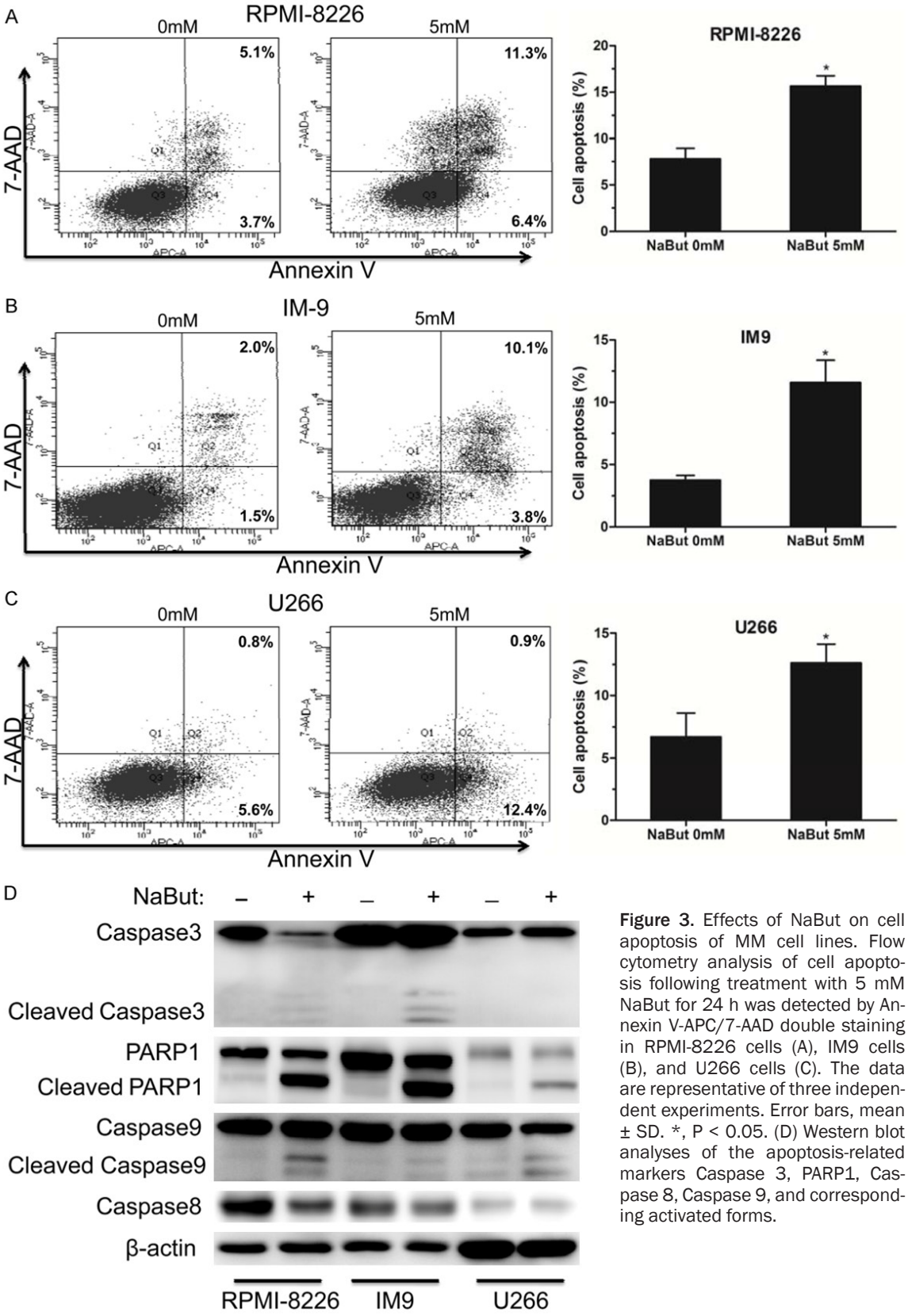

Figure 3. Effects of NaBut on cell apoptosis of MM cell lines. Flow cytometry analysis of cell apoptosis following treatment with 5 mM NaBut for 24 h was detected by Annexin V-APC/7-AAD double staining in RPMI-8226 cells (A), IM9 cells (B), and U266 cells (C). The data are representative of three independent experiments. Error bars, mean ± SD. *, $P < 0.05$. (D) Western blot analyses of the apoptosis-related markers Caspase 3, PARP1, Caspase 8, Caspase 9, and corresponding activated forms.

tors Bax, Bid, Bad, p21, p16, and PTEN mRNA were upregulated in RPMI-8226 cells, while the anti-apoptotic factor Bcl-XL was downregulated following treatment with 5 mM NaBut for 24 h

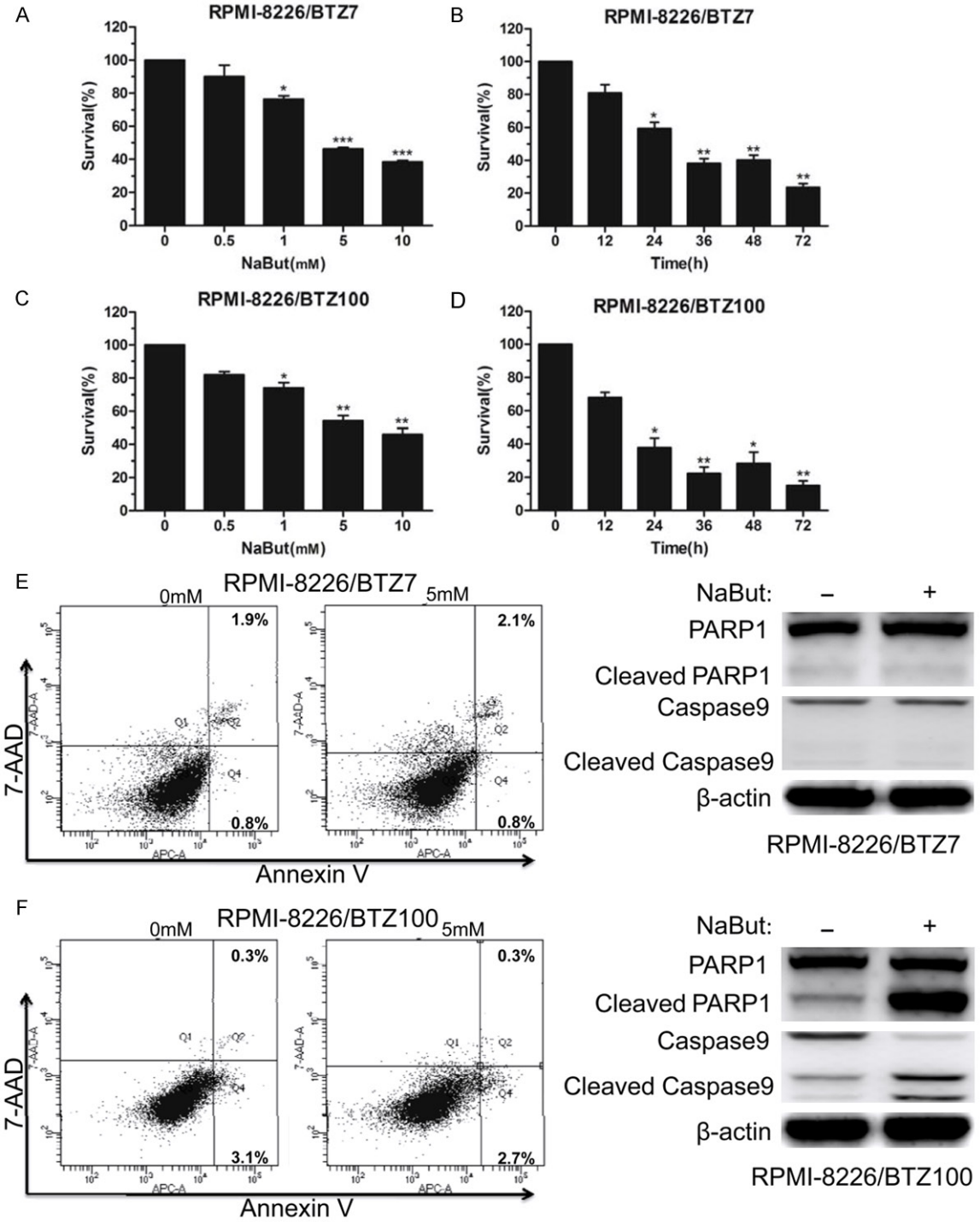

Figure 4. Effects of NaBut on cell viability and apoptosis of BTZ-resistant MM cells. Viability of cultured cells treated with different concentrations (0, 0.5, 1, 5, 10 mM) of NaBut for 24 h was assessed by CCK8 assay in RPMI-8226/BTZ7 cells (A) and RPMI-8226/BTZ100 cells (C). Viability of cultured cells exposed to 5 mM NaBut for 12, 24, 36, 48 and 72 h was assessed by CCK8 assay in RPMI-8226/BTZ7 cells (B) and RPMI-8226/BTZ100 cells (D). Assessment of cell apoptosis by Annexin V-APC/7-AAD double staining in RPMI-8226/BTZ7 cells (E) and RPMI-8226/BTZ100 cells (F). Western blot analyses of PARP1, Caspase 9 and corresponding activated forms. All are representative data from three independent experiments. Error bars, mean ± SD. *, $P < 0.05$; **, $P < 0.01$;***, $P < 0.001$.

(**Figure 5A**). An increase in the endogenous mRNA levels of Bid, Bad, and p21 was evident with NaBut treatment in U266 and IM9 cells (**Figure 5B** and **5C**). Among the apoptosis-asso-

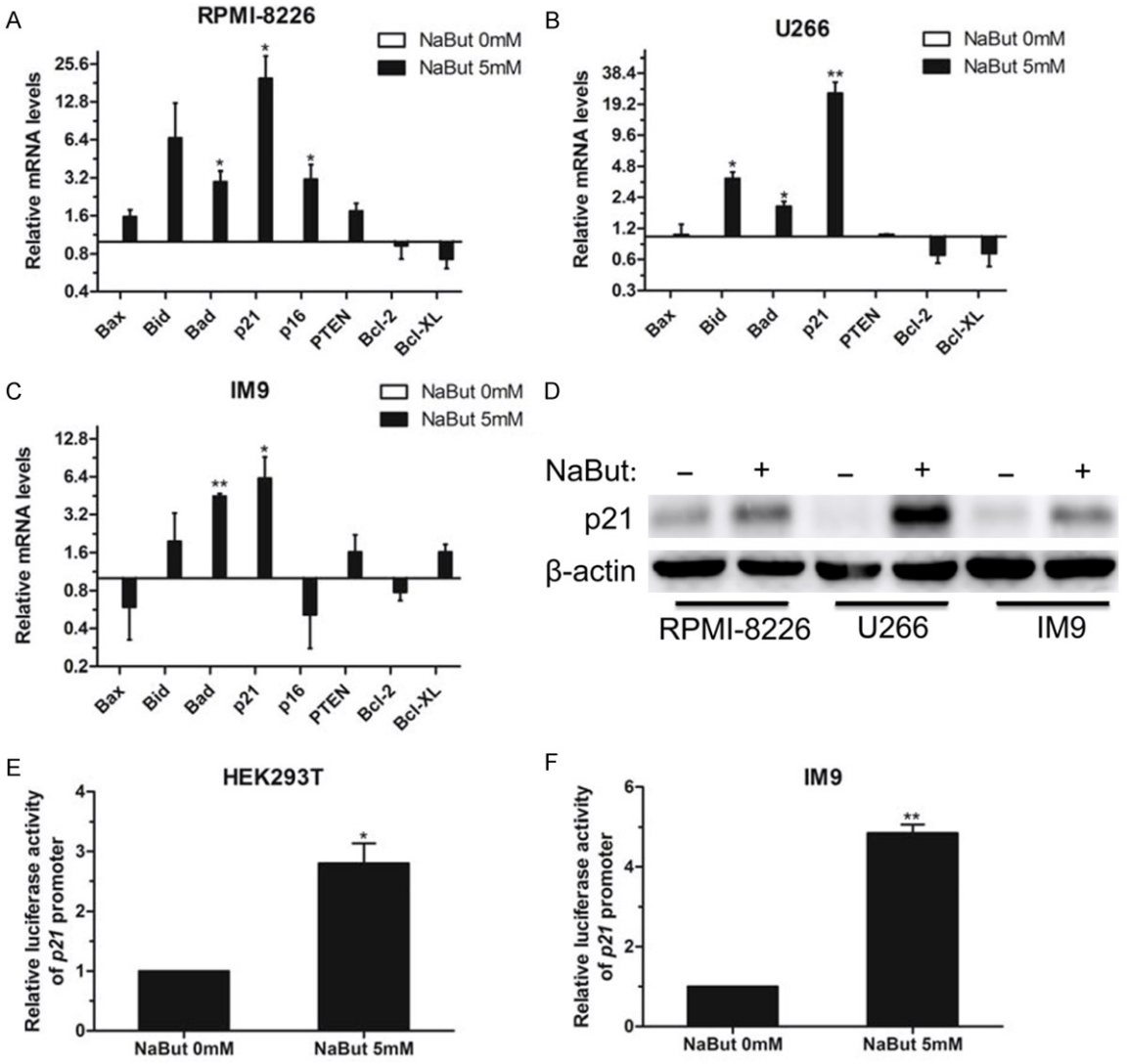

Figure 5. NaBut induces MM cell death via transcriptional activation of p21. Expression of apoptosis-associated markers mRNAs were assessed by real-time PCR in RPMI-8226 cells (A), U266 cells (B), and IM9 cells (C). (D) Western blot analyses of p21 expression in three MM cell lines treated by NaBut. Luciferase reporter assay of p21 promoter activities in HEK293T cells treated by NaBut (E), and in IM9 cells treated by NaBut (F). All data are representatives of three independent experiments. *, $P < 0.05$; **, $P < 0.01$.

ciated factors detected above, the HDAC inhibitor NaBut stimulated p21 high-level expression in RPMI-8226, U266, and IM9 cells. Specifically, a significant increase was evident in the endogenous protein levels of p21, along with NaBut treatment in RPMI-8226, U266, and IM9 cells (**Figure 5D**). We then tested whether NaBut could enhance the promoter activity of p21. NaBut alone was able to facilitate the transcription of p21 promoter-driven luciferase reporter in HEK293T and IM9 cell lines (**Figure 5E** and **5F**). These data indicate that the HDAC inhibitor NaBut suppresses MM cell proliferation and induced apoptosis by targeting p21.

Discussion

Epigenetic regulation is closely associated with progression of MM. The methylation of cytosine in the CpG island is an epigenetic mechanism. Studies in MM have shown variable DNA methylation patterns with identification of hypermethylation patterns in aggressive subtypes [18]. Vorinostat and panabinostat are the most widely studied HDAC inhibitors in MM [8]. Inhibition of HDAC activity by vorinostat results in alteration of gene expression in various cancer cell lines, including MM [19]. In this study, the HDAC inhibitor NaBut repressed the prolif-

eration of various MM cell lines at lower concentration. The viabilities of MM cells at higher concentrations were not changed significantly, compared with the low concentration. To our knowledge, this is the first study to report the biological function of NaBut in MM, which improve the knowledge of MM progression and development.

Most MM patients are sensitive to initial chemotherapy, but relapse often occurs because of acquired drug resistance [20, 21]. The introduction of BTZ represented an important breakthrough in treatment of MM [22]. The present study showed that BTZ at specified concentrations failed to reduce cell growth in BTZ-resistant cell lines (data not shown). However, HDAC inhibitor NaBut could inhibit BTZ-resistant cell proliferation in dose- and time-dependent manners (**Figure 4**). Surprisingly, flow cytometry and western blot analyses demonstrated that NaBut treatment did not induce lower concentration BTZ-resistant MM cell programmed cell death. On the contrary, significantly increased proteolytic cleavage of PARP1 and Caspase 9 was observed in RPMI-8226/BTZ100 cell lines. This indicates that NaBut is more sensitive to high concentration BTZ-resistant MM cells and is likely to induce partly BTZ-resistant MM cell death.

p21 is currently accepted as a potent universal CDK inhibitor [23]. p21 inhibits the kinase activity of cyclin A/CDK1 and 2, resulting in cell cycle inhibition through and into S phase [24, 25]. We confirmed that NaBut induces IM9 cell cycle arrest at the G2-M phase and that CDK2 exhibits a lower level of expression in a dose-dependent manner. p21 has a key function in carcinogenesis and apoptosis [26]. Presently, NaBut induced MM cell death by transcriptional activation of p21. p21 serves as a downstream mediator of p53 and cooperates with p53 [27, 28]. So it is strange that tumor suppressor p53 was downregulated in MM cell lines treated by NaBut (data not shown). Therefore, we speculate that p21 participates in NaBut-induced MM cell death in a p53-independent manner.

In summary, the present study is the first investigation of the biological functions of HDAC inhibitor NaBut in MM. NaBut reduces the viability of MM cells, leads to cell cycle in the G2/M phase, and induces cell apoptosis by the transcriptional activation of p21. These findings imply the potential value of NaBut as a potential therapeutic drug for MM.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (81600173, 81400127); Natural Science Foundation of Jiangsu Province (BK20160230); Postdoctoral Science Foundation of China (2016M601895) and Postdoctoral Science Foundation of Jiangsu Province (1601092B); Science and Technology Special Project in Clinical Medicine of Jiangsu Province (BL2013010).

Disclosure of conflict of interest

None.

Address correspondence to: Kailin Xu, Department of Hematology, The Affliated Hospital of Xuzhou Medical University, Xuzhou, Jiangsu, China. E-mail: lihmd@163.com

References

[1] Richardson PG, Sonneveld P, Schuster MW, Irwin D, Stadtmauer EA, Facon T, Harousseau JL, Ben-Yehuda D, Lonial S, Goldschmidt H, Reece D, San-Miguel JF, Bladé J, Boccadoro M, Cavenagh J, Dalton WS, Boral AL, Esseltine DL, Porter JB, Schenkein D, Anderson KC; Assessment of Proteasome Inhibition for Extending Remissions (APEX) Investigators. Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. N Engl J Med 2005; 352: 2487-98.

[2] Palumbo A and Anderson K. Multiple myeloma. N Engl J Med 2011; 364: 1046-1060.

[3] Kyle RA, Gertz MA, Witzig TE, Lust JA, Lacy MQ, Dispenzieri A, Fonseca R, Rajkumar SV, Offord JR, Larson DR, Plevak ME, Therneau TM and Greipp PR. Review of 1027 patients with newly diagnosed multiple myeloma. Mayo Clin Proc 2003; 78: 21-33.

[4] Mimura N, Hideshima T and Anderson KC. Novel therapeutic strategies for multiple myeloma. Exp Hematol 2015; 43: 732-741.

[5] Leleu X, Karlin L, Macro M, Hulin C, Garderet L, Roussel M, Arnulf B, Pegourie B, Kolb B, Stoppa AM, Brechiniac S, Marit G, Thielemans B, Onraed B, Mathiot C, Banos A, Lacotte L, Tiab M, Dib M, Fuzibet JG, Petillon MO, Rodon P, Wetterwald M, Royer B, Legros L, Benboubker L, Decaux O, Escoffre-Barbe M, Caillot D, Fermand JP, Moreau P, Attal M, Avet-Loiseau H, Facon T and Intergroupe Francophone du M. Pomalidomide plus low-dose dexamethasone in multiple myeloma with deletion 17p and/or translocation (4;14): IFM 2010-02 trial results. Blood 2015; 125: 1411-1417.

[6] de la Puente P, Muz B, Azab F, Luderer M and Azab AK. Molecularly targeted therapies in multiple myeloma. Leuk Res Treatment 2014; 2014: 976567.
[7] Kouzarides T. Chromatin modifications and their function. Cell 2007; 128: 693-705.
[8] Hideshima T and Anderson KC. Histone deacetylase inhibitors in the treatment for multiple myeloma. Int J Hematol 2013; 97: 324-332.
[9] Panobinostat approved for multiple myeloma. Cancer Discov 2015; 5: OF4.
[10] Hideshima T, Bradner JE, Wong J, Chauhan D, Richardson P, Schreiber SL and Anderson KC. Small-molecule inhibition of proteasome and aggresome function induces synergistic antitumor activity in multiple myeloma. Proc Natl Acad Sci U S A 2005; 102: 8567-8572.
[11] Xiong F, Mou YZ and Xiang XY. Inhibition of mouse B16 melanoma by sodium butyrate correlated to tumor associated macrophages differentiation suppression. Int J Clin Exp Med 2015; 8: 4170-4174.
[12] Li L, Sun Y, Liu J, Wu X, Chen L, Ma L and Wu P. Histone deacetylase inhibitor sodium butyrate suppresses DNA double strand break repair induced by etoposide more effectively in MCF-7 cells than in HEK293 cells. BMC Biochem 2015; 16: 2.
[13] Franke NE, Niewerth D, Assaraf YG, van Meerloo J, Vojtekova K, van Zantwijk CH, Zweegman S, Chan ET, Kirk CJ, Geerke DP, Schimmer AD, Kaspers GJ, Jansen G and Cloos J. Impaired bortezomib binding to mutant beta5 subunit of the proteasome is the underlying basis for bortezomib resistance in leukemia cells. Leukemia 2012; 26: 757-768.
[14] Yao R, Jiang H, Ma Y, Wang L, Wang L, Du J, Hou P, Gao Y, Zhao L, Wang G, Zhang Y, Liu DX, Huang B and Lu J. PRMT7 induces epithelial-to-mesenchymal transition and promotes metastasis in breast cancer. Cancer Res 2014; 74: 5656-5667.
[15] Hou P, Zhao Y, Li Z, Yao R, Ma M, Gao Y, Zhao L, Zhang Y, Huang B and Lu J. LincRNA-ROR induces epithelial-to-mesenchymal transition and contributes to breast cancer tumorigenesis and metastasis. Cell Death Dis 2014; 5: e1287.
[16] Chen D, Frezza M, Schmitt S, Kanwar J and Dou QP. Bortezomib as the first proteasome inhibitor anticancer drug: current status and future perspectives. Curr Cancer Drug Targets 2011; 11: 239-253.
[17] Skrott Z and Cvek B. Linking the activity of bortezomib in multiple myeloma and autoimmune diseases. Crit Rev Oncol Hematol 2014; 92: 61-70.
[18] Zhang W, Wang YE, Zhang Y, Leleu X, Reagan M, Zhang Y, Mishima Y, Glavey S, Manier S, Sacco A, Jiang B, Roccaro AM and Ghobrial IM. Global epigenetic regulation of microRNAs in multiple myeloma. PLoS One 2014; 9: e110973.
[19] Mitsiades CS, Mitsiades NS, McMullan CJ, Poulaki V, Shringarpure R, Hideshima T, Akiyama M, Chauhan D, Munshi N, Gu X, Bailey C, Joseph M, Libermann TA, Richon VM, Marks PA and Anderson KC. Transcriptional signature of histone deacetylase inhibition in multiple myeloma: biological and clinical implications. Proc Natl Acad Sci U S A 2004; 101: 540-545.
[20] Barlogie B, Shaughnessy J, Tricot G, Jacobson J, Zangari M, Anaissie E, Walker R and Crowley J. Treatment of multiple myeloma. Blood 2004; 103: 20-32.
[21] Anderson KC. Therapeutic advances in relapsed or refractory multiple myeloma. J Natl Compr Canc Netw 2013; 11: 676-679.
[22] Abidi MH, Gul Z, Abrams J, Ayash L, Deol A, Ventimiglia M, Lum L, Mellon-Reppen S, Al-Kadhimi Z, Ratanatharathorn V, Zonder J and Uberti J. Phase I trial of bortezomib during maintenance phase after high dose melphalan and autologous stem cell transplantation in patients with multiple myeloma. J Chemother 2012; 24: 167-172.
[23] Xiong Y, Hannon GJ, Zhang H, Casso D, Kobayashi R and Beach D. p21 is a universal inhibitor of cyclin kinases. Nature 1993; 366: 701-704.
[24] Bertoli C, Skotheim JM and de Bruin RA. Control of cell cycle transcription during G1 and S phases. Nat Rev Mol Cell Biol 2013; 14: 518-528.
[25] Choi WI, Kim MY, Jeon BN, Koh DI, Yun CO, Li Y, Lee CE, Oh J, Kim K and Hur MW. Role of promyelocytic leukemia zinc finger (PLZF) in cell proliferation and cyclin-dependent kinase inhibitor 1A (p21WAF/CDKN1A) gene repression. J Biol Chem 2014; 289: 18625-18640.
[26] Karimian A, Ahmadi Y and Yousefi B. Multiple functions of p21 in cell cycle, apoptosis and transcriptional regulation after DNA damage. DNA Repair (Amst) 2016; 42: 63-71.
[27] Kim J, Bae S, An S, Park JK, Kim EM, Hwang SG, Kim WJ and Um HD. Cooperative actions of p21WAF1 and p53 induce Slug protein degradation and suppress cell invasion. EMBO Rep 2014; 15: 1062-1068.
[28] Antognelli C, Ferri I, Bellezza G, Siccu P, Love HD, Talesa VN and Sidoni A. Glyoxalase 2 drives tumorigenesis in human prostate cells in a mechanism involving androgen receptor and p53-p21 axis. Mol Carcinog 2017; 56: 2112-2126.