Arginine methyltransferase inhibitor-1 inhibits sarcoma viability in vitro and in vivo

BAOLAI ZHANG1,2*, XUE CHEN1,2*, SUYIN GE1,2, CAILI PENG3, SU ZHANG1,2, XU CHEN1,2, TAO LIU1,2 and WENKAI ZHANG1,2

1Department of Pharmacology, School of Basic Medical Sciences; 2Key Lab of Preclinical Study for New Drugs of Gansu Province, Lanzhou University; 3Day-Care Unit, Gansu Provincial People's Hospital, Lanzhou, Gansu 730000, P.R. China

Received July 19, 2017; Accepted April 16, 2018

DOI: 10.3892/ol.2018.8929

Correspondence to: Dr Baolai Zhang, Department of Pharmacology, School of Basic Medical Sciences, Lanzhou University, 199 Donggang West Road, Lanzhou, Gansu 730000, P.R. China
E-mail: zhangbl@lzu.edu.cn

*Contributed equally

Key words: arginine methyltransferase inhibitor 1, sarcoma, protein arginine methyltransferase 5, histone methylation, symmetric dimethylation of histone 4, symmetric dimethylation of histone 3

Abstract. Protein arginine methyltransferases (PRMTs) are a class of epigenetic modified enzymes that are overexpressed in a various types of cancer and serve pivotal functions in malignant transformation. Arginine methyltransferase inhibitor-1 (AMI-1) is a symmetrical sulfonated urea that inhibits the activity of type I PRMT in vitro. However, previous studies demonstrated that AMI-1 may also inhibit the activity of type II PRMT5 in vitro. To the best of our knowledge, the present study provides the first evidence that AMI-1 may significantly inhibit the viability of mouse sarcoma 180 (S180) and human osteosarcoma U2OS cells. Additionally, the results demonstrated that AMI-1 downregulated the activities of PRMT5, the symmetric dimethylation of histone 4 and histone 3 (a PRMT5-specific epigenetic mark) in a mouse xenograft model of S180 and induced apoptosis in S180 cells. Taken together, the results suggest that AMI-1 may exhibit antitumor effects against sarcoma cells by targeting PRMT5.

Introduction

Sarcoma is a rare type of cancer and is usually categorized into two types: Sarcomas that develop in soft tissues (including muscle, tendons, fat, blood vessels, lymph vessels, nerves and tissue around joints) and bone sarcomas. Sarcomas account for ~1% of malignancies in adults and 15% of malignancies in children (1). Chemotherapy using anthracyclines with or without ifosfamide has been widely used as the standard treatment for soft tissue sarcoma (2-6). However, these agents often fail to treat patients with soft tissue sarcoma and cause adverse side effects (7). Therefore, the available treatment options for patients with sarcoma are poor and the development of novel drugs is required.

The protein arginine methyltransferase 5 (PRMT5) is a type II arginine methyltransferase that catalyzes the symmetric dimethylation of arginine residues in histones 4 (H4R3me2s) and 3 (H3R8me2s) (8). PRMT5 is upregulated in various types of cancer (9-15) and small molecule inhibitors of PRMT5 may be attractive targets for the treatment of sarcoma (16-18). Arginine methyltransferase inhibitor-1 (AMI-1), also known as 7,7′-carbonylbis(azanediyl)bis(4-hydroxynaphthalene-2-sulfonic acid), was the first inhibitor of PRMTs to be identified (19). AMI-1 may also inhibit the activity of type I PRMT in vitro (20). A recent study demonstrated that AMI-1 significantly inhibited the activity of type II PRMT5 in vitro (15). The aim of the present study was to examine the effects of the AMI-1 in sarcoma in vitro and in vivo and investigate the underlying molecular mechanisms.

Materials and methods

Cell culture and reagent. Mouse sarcoma 180 (S180) and human osteosarcoma U2OS cells were obtained from the Chinese Academy of Science (Shanghai, China) and were cultured in RPMI-1640 medium (Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin sodium and 100 mg/ml streptomycin at 37°C in a humidified atmosphere containing 5% CO2. AMI-1 was synthesized in house, according to the method of Peng et al (21) and Ragno et al (22).

Animals. A total of 22 male Kunming mice (age, 6-7 weeks old; body weight, 18-22 g) were purchased from Lanzhou University (Gansu, China). The mice were acclimated to laboratory conditions (25°C, 12/12 h light/dark, 50% humidity and ad libitum access to food and water) for 3 days prior to experimentation. The present study was approved by the Institutional Animal Care and Treatment Committee of Lanzhou University (Gansu, China). On day 7, mice were...
euthanized prior to cervical dislocation with an intraperitoneal injection of 50 mg/kg pentobarbital sodium.

**In vitro cytotoxicity assay.** Briefly, S180 or U2OS cells were seeded at 2x10^4 cells/well in 96-well plates. Following 24 h of culture, cells were treated with various concentrations of AMI-1 (0.6, 1.2 and 2.4 mM) and the control group was treated with the vehicle control (PBS). Cytotoxicity was evaluated using the Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Inc., Kumamoto, Japan), according to the manufacturer's protocol. S180 cells were seeded at 7.5x10^4 cells/well in 24-well plates were incubated at 37°C at indicated timepoints (48, 72 or 96 h). The cell morphology and numbers were observed under a light inverted microscope (Olympus CK40; Olympus Corporation, Tokyo, Japan; magnification, x100). Cytotoxicity was determined by measuring the absorbance at a wavelength of 450 nm using a plate reader. IC_{50} values were evaluated using CurveExpert 1.3 software (Hyams Development, Mississippi, MS, USA).

**Colony formation assay.** U2OS cells were seeded at a density of 300 cells in 60 mm dishes and incubated for 24 h. S180 cells were not used due to them being suspended cells and not suitable for colony formation assay. RPMI medium with FBS was replaced with 5 ml fresh medium, containing AMI-1 (0.3 or 0.6 mM) or PBS (control) and incubated at 37°C for 18 days. Colonies were fixed with a 7:1 ratio of methanol to glacial acetic acid for 25 min at 25°C and then stained with 0.1% crystal violet (in 20% methanol and PBS) for 25 min at 25°C.

**Flow cytometric analysis of apoptosis.** S180 cells were seeded at a density of 1.2x10^4 cells/well in 6-well plates and treated with AMI-1 (1.2 and 2.4 mM) or vehicle (PBS) for 48 and 72 h. The cells were harvested, washed twice and resuspended in 1X binding buffer. A total of 500 µl S180 cells (1x10^6 cells/ml) were incubated with 5 µl annexin V-fluorescein isothiocyanate and 5 µl propidium iodide for 15 min at room temperature in dark. The samples were then analyzed using a flow cytometer equipped with FCSDiva 6.2 software (LSR Fortessa™; BD Biosciences, Franklin Lakes, NJ, USA).

**Tumor implantation and treatment.** A total of 2x10^6 S180 cells (in 0.2 ml 0.9% NaCl in PBS) were subcutaneously inoculated into the right axillary region of Kunming mice. Following 3 days of implantation with S180 cells, mice were divided into two groups (11 animals/group): AMI-1-treated (0.5 mg in 200 µl 0.9% NaCl) or vehicle treated (200 µl 0.9% NaCl). The treatments were administered intratumorally (200 µl per mouse, once daily for a total of 7 days). The weight of the mice was determined daily. On day 7, mice were sacrificed by cervical dislocation and tumors were removed and weighed. The inhibition rate of tumor viability (IR) was calculated as: (1-the average tumor weight of treated group/tumor weight of vehicle group) x100%. The dose of AMI-1 was chosen in vivo experiments based on our preliminary experiments and previous literatures (23-26).

**Western blot analysis.** Western blot analysis was performed as described previously (15). Briefly, tumor tissues were lysed using RIPA buffer (cat no. P0013B; Beyotime Institute of Biotechnology, Jiangsu, China) containing phenylmethylosulfonyl fluoride (Beyotime Institute of Biotechnology) for 30 min at 4°C. The extract was centrifuged at 12,000 x g for 15 min at 4°C to clear insoluble debris. The protein concentration was assayed using Quick Start™ Bradford (cat no. 500-0205; Bio-Rad Laboratories, Inc., Hercules, CA, USA). Equal amounts of protein (40 µg per lane) were separated by SDS-PAGE (12% gel) and then transferred onto polyvinylidene difluoride membranes (Bio-Rad Laboratories, Inc.). The membranes were then blocked with 5% non-fat milk in Tris-buffered saline with 0.1% Tween-20 for 1 h at 25°C. Following blocking, membranes were incubated overnight at 4°C with the following primary antibodies: Anti-PRMT5 (1:500; cat no. sc-376937; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), anti-PRMT7 (1:1,000; cat no. 14762; Cell Signaling Technology, Inc., Danvers, MA, USA), anti-H4R3me2s (1:2,000; cat no. HW027; Signalway Antibody LLC, College Park, MD, USA), anti-H3R8me2s (1:1,000; cat no. HW015; Signalway Antibody LLC), anti-p53 (1:1,000; cat no. 9282; Cell Signaling Technology, Inc.) or anti-β-actin (1:2,000, cat no. 4970; Cell Signaling Technology, Inc.). Membranes were then incubated with horseradish peroxidase-conjugated secondary antibodies (1:5,000, cat nos. ZB-2301 and ZB-2305; OriGene Technologies, Inc., Beijing, China) for 1.5 h at 25°C. The protein bands were visualized by BeyoECL Plus kit (Beyotime Institute of Biotechnology). The densitometry was performed using Gel-Pro Analyzer 4.0 software (Media Cybernetics, Inc., Rockville, MD, USA).

**Statistical analysis.** The relevant data are expressed as the mean ± standard deviation (SD). Statistical analysis was performed using SPSS 17.0 (SPSS, Inc., Chicago, IL, USA). The difference between two groups was analyzed using Student's t-test. For comparison of multiple groups, one-way analysis of variance followed by Dunnett's post hoc test was used. P<0.05 was considered to indicate a statistically significant difference.

**Results**

**AMI-1 inhibits viability of sarcoma cells in vitro.** Cytotoxicity in response to AMI-1 treatment was determined using CCK-8 assay. At 72 h, IC_{50} values for S180 and U2OS cells were 0.31±0.01 and 0.75±0.02 mM, respectively (data not shown). As presented in Fig. 1A-C, AMI-1 treatment inhibited the cell viability of sarcoma in S180 and U2OS cells in a time-dependent and dose-dependent manner in vitro. AMI-1 treatment significantly inhibited the viability of sarcoma S180 and U2OS cells in response to treatment of AMI-1 (0.6, 1.2 and 2.4 mM) for 48, 72 and 96 h (Fig. 1A and B).

**AMI-1 induces S180 cell apoptosis in vitro.** To evaluate whether AMI-1 may inhibit cell viability by regulating cell apoptosis, S180 cells were treated with AMI-1 (1.2 and 2.4 mM) or vehicle for 48 and 72 h. Cellular apoptosis was evaluated using flow cytometry. The results demonstrated that AMI-1 may increase the percentages of cells undergoing apoptosis, compared with that in the vehicle group (Fig. 2).
Table I. Evaluation of body and tumor weight of mice on d 7, following AMI-1 treatment.

| Group | Body weight at d 0 (g) | Body weight at d 7 (g) | Tumor weight at d 7 (g) | IR (%) |
|-------|------------------------|------------------------|-------------------------|--------|
| Control | 24.87±1.19            | 31.97±2.63            | 1.8170±0.41             |        |
| AMI-1   | 24.73±1.66            | 31.06±2.32            | 1.0684±0.27             | 41.42±6.34 |

AMI-1, arginine methyltransferase inhibitor-1; d, day; IR, inhibition rate of tumor viability. *P<0.05 vs. Control. Mean ± standard deviation; n=11.

Figure 1. AMI-1 inhibits sarcoma cell viability in vitro. (A) S180 or U2OS cells were treated with vehicle or AMI-1 at indicated doses and timepoints as indicated. The cytotoxicity of AMI-1 on sarcoma cells was assessed using a Cell Counting Kit-8. (B) Cell numbers were evaluated using a light inverted microscope (magnification, x100). (C) The effect of AMI-1 on colony formation of U2OS cells. Three individual experiments were performed. *P<0.05, **P<0.01, ***P<0.001 vs. Control; AMI-1, arginine methyltransferase inhibitor-1; OD, optical density.

Figure 2. AMI-1 induces apoptosis in S180 cells in vitro. (A) S180 cells were treated with vehicle or AMI-1, and apoptosis was evaluated by flow cytometry using Annexin V-FITC/PI double staining. (B) The bar graph is a quantitative presentation of the flow cytometric data. Three individual experiments were performed. *P<0.05, **P<0.01, ***P<0.001 vs. control. AMI-1, arginine methyltransferase inhibitor-1; PI, propidium iodine; FITC, fluorescein isothiocyanate.
This indicated that AMI-1 reduces S180 cell viability through the induction of cell apoptosis.

AMI-1 inhibits tumor viability of S180 cells in vivo. To assess the antitumor activity of AMI-1 in vivo, S180 cells were subcutaneously inoculated into the right axillary of mice. Mice were divided into two groups (11 animals/group): AMI-1-treated (0.5 mg in 0.9% NaCl) or vehicle-treated (0.9% NaCl). At the end of treatment tumors were dissected and weighted. ***P<0.001 vs. Control. AMI-1, arginine methyltransferase inhibitor-1.

AMI-1 decreases the levels of H4R3me2s and H3R8me2s in a tumor xenograft model. PRMT5 is a major type II arginine methyltransferase that catalyzes ω-NG, N’G-symmetric dimethylarginine (H4R3me2s and H3R8me2s) (8,27,28). Western blot analysis was employed to investigate the molecular mechanism by which AMI-1 may inhibit the viability of S180 cells in vivo. The results demonstrated that AMI-1 treatment significantly decreased the levels of H4R3me2s and H3R8me2s compared with those in the control group (Fig. 4).

Discussion
AMI-1 is a symmetrical sulfonated urea that inhibits type I PRMT activity in vitro (19,20). Investigation of the molecular mechanisms that lead to the inhibition of viability and induction of apoptosis of cancer cells may contribute to the design of novel therapeutic strategies and drugs (29,30). Therefore, in the present study, the possible antitumor effects of AMI-1 on S180 and U2OS cell were evaluated in vitro. The results demonstrated that AMI-1 significantly inhibited sarcoma cell viability using a CCK-8 assay. Additionally, the 2.4-mM dose of AMI-1 exhibited the highest antitumor activity. Next, the molecular mechanisms underlying the viability inhibitory activity of AMI-1 were investigated. Flow cytometric analysis demonstrated that treatment with AMI-1 induced apoptosis in S180 cells. Furthermore, the in vivo antitumor effects of AMI-1 were evaluated using S180-bearing mouse models. The
results demonstrated that AMI-1 significantly inhibited the viability of S180-implanted tumors in vivo.

PRMTs are classified into three groups of enzymes (type I, II and III) depending on their catalytic activity. Type II PRMT (PRMT5) catalyzes the transfer of methyl groups to the guanidino nitrogen atoms of arginine, resulting in ω-NG, N'-symmetric dimethylarginine, whereas PRMT7 is the only type III PRMT catalyzing the formation of ω-NG-monomethylarginine (27,31,32). PRMT5 expression or activity is upregulated in various types of cancer and modulation of its expression regulates the viability of cancer cells. Therefore, PRMT5 may be a potential therapeutic target in cancer (33-35). Additionally, a previous study demonstrated that AMI-1 inhibited the activity of PRMT5 and suppressed the viability of colorectal cancer cells by targeting PRMT5 (15). Nevertheless, the molecular mechanism underlying the anticancer effect of AMI-1 in S180 remains unclear. In the present study, the levels of PRMT5 and PRMT7 following AMI-1 treatment were evaluated using a tumor-bearing mouse model implanted with S180 cells. The results demonstrated that AMI-1 treatment significantly decreased the expression levels of PRMT5 but did not affect the expression of PRMT7. These results suggest that AMI-1 may suppress the viability of S180 cells by downregulating the expression of PRMT5.

Similar to PRMT5, PRMT7 catalyzes the symmetrical methylation of arginine 3 of histone H4. However, PRMT7 does not catalyze the formation of H3R8me2s (a PRMT5-specific target) (36-38). In the present study, it was demonstrated that AMI-1 was able to decrease the levels of H4R3me2s and H3R8me2s in a tumor-bearing mouse model implanted with S180 cells. These results confirm that AMI-1 may inhibit the viability of S180 cells by targeting PRMT5 but not PRMT7.

The tumor suppressor p53 is an extensively studied gene in human cancer. PRMT5 is responsible for methylating p53 and PRMT7 depletion triggers p53-dependent apoptosis (39-42). Thus, in the present study, the expression of p53 expression was evaluated in a S180 tumor xenograft model treated with AMI-1. The results demonstrated that AMI-1 did not affect the expression of p53 in vivo. Additional studies are required to further elucidate the function of p53 in mediating the anti-tumor efficacy of AMI-1 in sarcoma.

In summary, the present study investigated the antitumor effects of AMI-1 on sarcoma cells in vitro and in vivo. To the best of our knowledge, the present study provides the first evidence that the effective antitumor activity of AMI-1 in S180-bearing mice was mainly due to the inhibition of the activity of PRMT5. Therefore, AMI-1 may be a potential therapeutic target for patients with sarcomas.

Acknowledgements

Not applicable.

Funding

This research work was supported in part by Fundamental Research Funds of the Central Universities (grant no. lzujbky-2013-169).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

BZ and XueC conceived and designed the experiments. CP, SZ and XuC performed the experiments, TL and WZ analyzed the data. XueC and SG contributed to the acquisition of data, writing and revision of the manuscript. All authors read and approved final manuscript.

Ethical approval and consent to participate

Animal experiments were performed in accordance with the Institutional Animal Care and Treatment Committee of Lanzhou University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Demetri GD, Antonia S, Benjamin RS, Bui MM, Casper ES, Conrad EU III, DeLaney TF, Ganjoo KN, Heslin MJ, Hutchinson RJ, et al: Soft tissue sarcoma. J Natl Compr Canc Netw 8: 630-674, 2010.
2. Lima AP, Pereira FC, Almeida MA, Mello FM, Pires WC, Pinto TM, Delella FK, Felisbino SL, Moreno V, Batista AA and de Paula Silveira-Lacerda E: Cytoxicity and apoptotic mechanism of ruthenium(II) amino acid complexes in sarcoma-180 tumor cells. PLoS One 9: e105865, 2014.
3. Paiva GS, Taft CA, Carvalho MC, de Souza IA, da Silva EC, Cavalcanti KP, L RF Jr and De la Cruz NM: A comparative study of the effects of vitamins C and E in the development of sarcoma 180 in mice. J Cancer 4: 724-726, 2013.
4. Yi J, Qu H, Wu Y, Wang Z and Wang L: Study on anti-tumor, antioxidant and immunoregulatory activities of the purified polyphenols from pinecone of Pinus koraiensis on tumor-bearing S180 mice in vivo. Int J Biol Macromol 94: 735-744, 2017.
5. Mo L, Chen Y, Li W, Guo S, Wang X, An H and Zhan Y: Anti-tumor effects of (1→3)-β-d-glucan from Saccharomyces cerevisiae in S180 tumor-bearing mice. Int J Biol Macromol 95: 385-392, 2017.
6. Seddon B, Strauss SJ, Whelan J, Leahy M, Woll PI, Cowie F, Rothermundt C, Wood Z, Benson C, Ali N, et al: Gemcitabine and docetaxel versus doxorubicin as first-line treatment in previously untreated advanced unresectable or metastatic soft-tissue sarcomas (GeDDSS): A randomised controlled phase 3 trial. Lancet Oncol 18: 1397-1407, 2017.
7. Tap WD, Papai Z, Van Tine BA, Attia S, Ganjoo KN, Jones RL, Schuetze S, Reed D, Chawla SP, Riedel RF, et al: Doxorubicin plus evofosfamide versus doxorubicin alone in locally advanced, unresectable or metastatic soft-tissue sarcoma (TH CR-406/SARC02): An international, multicentre, open-label, randomised phase 3 trial. Lancet Oncol 18: 1098-1103, 2017.
8. Yang Y and Bedford MT: Protein arginine methyltransferases and cancer. Nat Rev Cancer 13: 37-50, 2013.
9. Bao X, Zhao S, Liu T, Liu Y and Yang X: Overexpression of PRMT5 promotes tumor cell growth and is associated with poor disease prognosis in epithelial ovarian cancer. J Histochem Cytochem 61: 206-217, 2013.
10. Dong Y, Song C, Wang Y, Lei Z, Xu F, Guan H, Chen A and Li F: Inhibition of PRMT5 suppresses osteoclast differentiation and partially protects against ovariectomy-induced bone loss through downregulation of CXCL10 and RSAD2. Cell Signal 34: 55-65, 2017.

11. Han X, Li R, Zhang W, Yang X, Wheeler CG, Friedman GK, Province P, Ding Q, You Z, Fathallah-Shaykh HM, et al: Expression of PRMT5 correlates with malignant grade in gliomas and plays a pivotal role in tumor growth in vitro. J Neurooncol 118: 61-72, 2014.

12. Ibrahim R, Matsubara D, Osman W, Morikawa T, Goto A, Morita S, Ishikawa A, Aburatani H, Takeda N, Nakajima J, et al: Expression of PRMT5 in lung adenocarcinoma and its significance in epithelial-mesenchymal transition. Hum Pathol 45: 1397-1405, 2014.

13. Tanaka H, Hoshikawa Y, Oh-hara T, Koike S, Naito M, Noda T, AraI H, Tsuruo T and Fujita N: PRMT5, a novel TRAIL receptor-binding protein, inhibits TRAIL-induced apoptosis via nuclear factor-kappaB activation. Mol Cancer Res 7: 557-569, 2009.

14. Zhang B, Dong S, Li Z, Lu L, Zhang S, Chen X, Cen X and Wu Y: Targeting protein arginine methyltransferase 5 inhibits human hepatocellular carcinoma growth via the downregulation of beta-catenin. J Transl Med 13: 349, 2015.

15. Zhang B, Dong S, Zhu R, Hu C, Hou J, Li Y, Zhao Q, Shao X, Bu Q, Li H, et al: Targeting protein arginine methyltransferase 5 inhibits colorectal cancer growth by decreasing arginineylation of eIF4E and FGFR3. Oncotarget 6: 22799-22811, 2015.

16. Deng X, Von Keudell G, Suzuki T, Dohmae N, Nakaduki M, Piao L, Yoshioka Y, Nakamura Y and Hamamoto R: PRMT1 promotes mitosis of cancer cells through arginine methylation of INCENP. Oncotarget 6: 35173-35182, 2015.

17. Greenblatt SM, Liu F and Nimer SD: Arginine methyltransferases in normal and malignant hematopoiesis. Exp Hematol 44: 435-441, 2016.

18. Sonohara F, Inokawa Y, Hayashi M, Koderaya Y and Nomoto S: Epi genetic modulation associated with carcinogenesis and prognosis of human gastric cancer. Oncol Lett 13: 3363-3368, 2017.

19. Cheng D, Yadav N, King RW, Swanson MS, Weinstein EJ and Bedford MT: Small molecule regulators of protein arginine methyltransferases. J Biol Chem 283: 23892-23899, 2004.

20. Castellano S, Milite C, Ragno R, Simeoni S, Mai A, Limongelli V, Novellino E, Bauer I, Brosch G, Spannhoff A, et al: Design, synthesis and biological evaluation of carboxy analogues of arginine methyltransferase inhibitor 1 (AM1). Chem Med Chem 5: 398-414, 2010.

21. Peng X, Yu H, Hang Y and Wang J: N,N'-phospho-225. 24. 23. 20. 19. 18. 16. 15. 14. 13. 12. 11. 10. 9. 8. 7. 6. 5. 4. 3. 2. 1. 0. Inhibition of PRMT5 suppresses osteoclast differentiation and partially protects against ovariectomy-induced bone loss through downregulation of CXCL10 and RSAD2. Cell Signal 34: 55-65, 2017.

22. Han X, Li R, Zhang W, Yang X, Wheeler CG, Friedman GK, Province P, Ding Q, You Z, Fathallah-Shaykh HM, et al: Expression of PRMT5 correlates with malignant grade in gliomas and plays a pivotal role in tumor growth in vitro. J Neurooncol 118: 61-72, 2014.

23. Ibrahim R, Matsubara D, Osman W, Morikawa T, Goto A, Morita S, Ishikawa A, Aburatani H, Takeda N, Nakajima J, et al: Expression of PRMT5 in lung adenocarcinoma and its significance in epithelial-mesenchymal transition. Hum Pathol 45: 1397-1405, 2014.

24. Tanaka H, Hoshikawa Y, Oh-hara T, Koike S, Naito M, Noda T, AraI H, Tsuruo T and Fujita N: PRMT5, a novel TRAIL receptor-binding protein, inhibits TRAIL-induced apoptosis via nuclear factor-kappaB activation. Mol Cancer Res 7: 557-569, 2009.

25. Zhang B, Dong S, Li Z, Lu L, Zhang S, Chen X, Cen X and Wu Y: Targeting protein arginine methyltransferase 5 inhibits human hepatocellular carcinoma growth via the downregulation of beta-catenin. J Transl Med 13: 349, 2015.

26. Zhang B, Zhang S, Zhu L, Chen X, Zhao Y, Chao L, Zhou J, Wang X, Zhang X and Ma N: Arginine methyltransferase inhibitor 1 inhibits gastric cancer by downregulating eIF4E and targeting PRMT5. Toxicol Appl Pharmacol 336: 1-7, 2017.

27. Pouillard C, Corbo L and Le Romancer M: Protein arginine methylation/demethylation and cancer. Oncotarget 7: 67532-67550, 2016.

28. Bedford MT and Clarke SG: Protein arginine methylation in mammals: Who, what, and why. Mol Cell 33: 1-13, 2009.

29. Andrade AF, Borges KS, Castro-Gamero AM, Silveira VS, Suazo VK, Oliveira JC, Moreno DA, de Paula Queiroz RG, Scirdeli CA and Tone LG: Zebularine induces chemosensitization to methotrexate and efficiently decreases AhR gene methylation in childhood acute lymphoblastic leukemia cells. Anticancer Drugs 25: 72-81, 2014.

30. Herr I and Debatin KM: Cellular stress response and apoptosis in cancer therapy. Blood 98: 2603-2614, 2001.

31. Zurita-Lopez CL, Sandberg T, Kelly R and Clarke SG: Human protein arginine methyltransferase 7 (PRMT7) is a type III enzyme forming omega-NG-monomethylated arginine residues. J Biol Chem 287: 7859-7870, 2012.

32. Peng Y, Maity R, Whitelegge JP, Hadjikyriacou A, Li Z, Zurita-Lopez C, Al-Hadid Q, Clark AT, Bedford MT, Masson JY and Clarke SG: Mammalian protein arginine methyltransferase 7 (PRMT7) specifically targets RXRα sites in hystone- and arginine-rich regions. J Biol Chem 288: 37010-37025, 2013.

33. Cha B and Jho EH: Protein arginine methyltransferases (PRMTs) as therapeutic targets. Expert Opin Ther Targets 16: 651-664, 2012.

34. Blaumukh SR, Smith E and Shilatifard A: Covalent modifications of histones during development and disease pathogenesis. Nat Struct Mol Biol 14: 1008-1016, 2007.

35. Balint BL, Szanto A, Madi A, Bauer UM, Gabor P, Benko S, Puskás LG, Davies PJ and Nagy L: Arginine methylation provides epigenetic transcription memory for retinoid-induced differentiation in myeloid cells. Mol Cell Biol 25: 5648-5663, 2005.

36. Litt M, Qiu Y and Huang S: Histone arginine methylations: Their roles in chromatin dynamics and transcriptional regulation. Biosci Rep 29: 131-141, 2009.

37. Bedford MT and Richard S: Arginine methylation an emerging regulator of protein function. Mol Cell 18: 263-272, 2005.

38. Ma WL, Wang L, Liu LX, Wang XL: Effect of phosphorylation and methylation on the function of the p16INK4a protein in cervical carcinoma cells. Mol Biol Rep 29: 131-141, 2002.

39. Jenkins D, Durant ST, Cho EC, Sheahan S, Edelmann M, Kessler B and La Thangue NB: Arginine methylation regulates p53/p73 response. Nat Cell Biol 10: 1431-1439, 2008.

40. Li Y, Chitinis N, Nakagawa H, Kita Y, Natsugoe S, Yang Y, Li Z, Wasik M, Klein-Szanto AJ, Rustgi AK and Diehl JA: PRMT5 is required for lymphomagenesis triggered by multiple oncogenic drivers. Cancer Discov 5: 288-303, 2015.

41. Muller PA and Vousden KH: Arginine methylation provides an emerging role in myeloid cells. Mol Cell Biol 25: 5648-5663, 2005.

42. Muller PA and Vousden KH: Arginine methylation provides an emerging role in myeloid cells. Mol Cell Biol 25: 5648-5663, 2005.

43. Poulard C, Corbo L and Le Romancer M: Protein arginine methylation/demethylation and cancer. Oncotarget 7: 67532-67550, 2016.

44. Bedford MT and Clarke SG: Protein arginine methylation in mammals: Who, what, and why. Mol Cell 33: 1-13, 2009.

45. Andrade AF, Borges KS, Castro-Gamero AM, Silveira VS, Suazo VK, Oliveira JC, Moreno DA, de Paula Queiroz RG, Scirdeli CA and Tone LG: Zebularine induces chemosensitization to methotrexate and efficiently decreases AhR gene methylation in childhood acute lymphoblastic leukemia cells. Anticancer Drugs 25: 72-81, 2014.

46. Herr I and Debatin KM: Cellular stress response and apoptosis in cancer therapy. Blood 98: 2603-2614, 2001.