CANCER GENOMICS & PROTEOMICS *20*: 679-685 (2023)
doi:10.21873/cgp.20415

The Combination of Methioninase and Ethionine Exploits Methionine Addiction to Selectively Eradicate Osteosarcoma Cells and Not Normal Cells and Synergistically Down-regulates the Expression of *C-MYC*

YUSUKE AOKI[1,2,3], YUTARO KUBOTA[1,2], QINGHONG HAN[1], NORIYUKI MASAKI[1,2], KOYA OBARA[1,2], MICHAEL BOUVET[2], SANT P. CHAWLA[4], YASUNORI TOME[3], KOTARO NISHIDA[3] and ROBERT M. HOFFMAN[1,2]

[1]*AntiCancer Inc, San Diego, CA, U.S.A.;*
[2]*Department of Surgery, University of California, San Diego, CA, U.S.A.;*
[3]*Department of Orthopedic Surgery, Graduate School of Medicine, University of the Ryukyus, Okinawa, Japan;*
[4]*Sarcoma Oncology Center, Santa Monica, CA, U.S.A.*

Abstract. *Background/Aim: The fundamental and general hallmark of cancer cells, methionine addiction, termed the Hoffman effect, is due to overuse of methionine for highly-increased transmethylation reactions. In the present study, we tested if the combination efficacy of recombinant methioninase (rMETase) and a methionine analogue, ethionine, could eradicate osteosarcoma cells and down-regulate the expression of c-MYC. Materials and Methods: 143B osteosarcoma cells and Hs27 normal human fibroblasts were tested. The efficacy of rMETase alone and ethionine, alone and in their combination, on cell viability was determined with the WST-8 assay on 143B cells and Hs27 cells. c-MYC expression was examined with western immunoblotting and compared in 143B cells treated with/without rMETase, ethionine, or the combination of both rMETase and ethionine. Results: 143B cells were more sensitive to both rMETase and ethionine than Hs 27 cells, with the following IC_{50}s: rMETase (143B: 0.22 U/ml; Hs27: 0.82 U/ml); ethionine (143B: 0.24 mg/ml; Hs27: 0.42 mg/ml). The combination of rMETase and ethionine synergistically eradicated 143B cells, lowering the IC50 for ethionine 14-fold compared to ethionine alone ($p<0.001$). In contrast, Hs27 fibroblasts were relatively resistant to the combination. The expression of c-MYC was significantly down-regulated only by the combination of rMETase and ethionine in 143B cells ($p<0.001$). Conclusion: In the present study, we showed, for the first time, the synergistic combination efficacy of rMETase and ethionine on osteosarcoma cells in contrast to normal fibroblasts, which were relatively resistant. The combination of rMETase and ethionine down-regulated c-MYC expression in the cancer cells. The present results indicate the combination of rMETase and ethionine may reduce the malignancy of osteosarcoma cells and can be a potential future clinical strategy.*

Correspondence to: Robert M. Hoffman, AntiCancer Inc, 7917 Ostrow St, San Diego, CA, 92111, U.S.A. Tel: +1 6198852284, e-mail: all@anticancer.com and Yasunori Tome, Department of Orthopedic Surgery, Graduate School of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa, 903-0125, Japan. Tel: +81 988953331, e-mail: yastome@med.u-ryukyu.ac.jp and Yusuke Aoki, Department of Orthopedic Surgery, Graduate School of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa, 903-0125, Japan. Tel: +81 988953331, e-mail: yaoki0630@gmail.com

Key Words: Methioninase, ethionine, methionine addiction, Hoffman effect, osteosarcoma, eradication, synergy, *c-MYC*, down-regulation.

Osteosarcoma is a rare cancer type, although it is the most common primary malignant bone tumor. Currently with the combination of surgery and neo-adjuvant/adjuvant chemo-therapy, including doxorubicin (DOX), cisplatinum (CDDP), and ifosfamide (IFO), the 5-year survival of osteosarcoma patients is 60% to 80%. However, the 5-year survival of metastatic osteosarcoma is only 20% to 30% and has not improved in three decades (1-7).

The fundamental and general hallmark of cancer is methionine addiction, reported almost 50 years ago (8) by one of us (RMH) and is termed the Hoffman effect (8-13).

Methionine addiction of cancer is due to overuse of exogenous methionine for highly-increased transmethylation reactions (14-23). We have previously reported that all types of cancer cells, including osteosarcoma, are addicted to methionine (18, 21-25).

In the present study, we targeted methionine addiction of osteosarcoma cells with the combination of rMETase and the methionine-analogue ethionine (Figure 1), a competitive inhibitor of reactions involving methionine (26), on cell viability of osteosarcoma cells in comparison with normal fibroblasts, and *c-MYC* expression in the osteosarcoma cells.

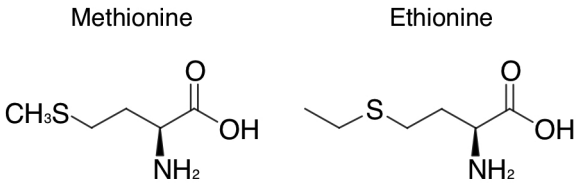

Figure 1. *Structural formulas of methionine and ethionine.*

Materials and Methods

Cell culture. The 143B human osteosarcoma cell line and Hs27 normal human foreskin fibroblasts were purchased from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Corning Inc., Corning, NY, USA), supplemented with 1 IU/ml penicillin/streptomycin and 10 % fetal bovine serum (FBS).

Reagents. DL-Ethionine was purchased from Sigma-Aldrich, Inc. (#5139-5G, St. Louis, MO, USA). Recombinant methioninase (rMETase) (AntiCancer Inc., San Diego, CA, USA) was produced by fermentation of *E.coli*, engineered with the *Pseudomonas putida methioninase* gene, as previously reported (27).

Drug sensitivity assay. Cell viability was determined with the WST-8 reagent (Dojindo Laboratory, Kumamoto, Japan). Cells were cultured on 96-well plates (143B: 7.5×10^2 cells/well; Hs27: 2×10^3 cells/well) in DMEM (100 μl/well), incubated overnight at 37˚C. Cell culture medium was then replaced with medium containing increasing concentrations of rMETase (0; 0.025; 0.05; 0.1; 0.2; 0.4;

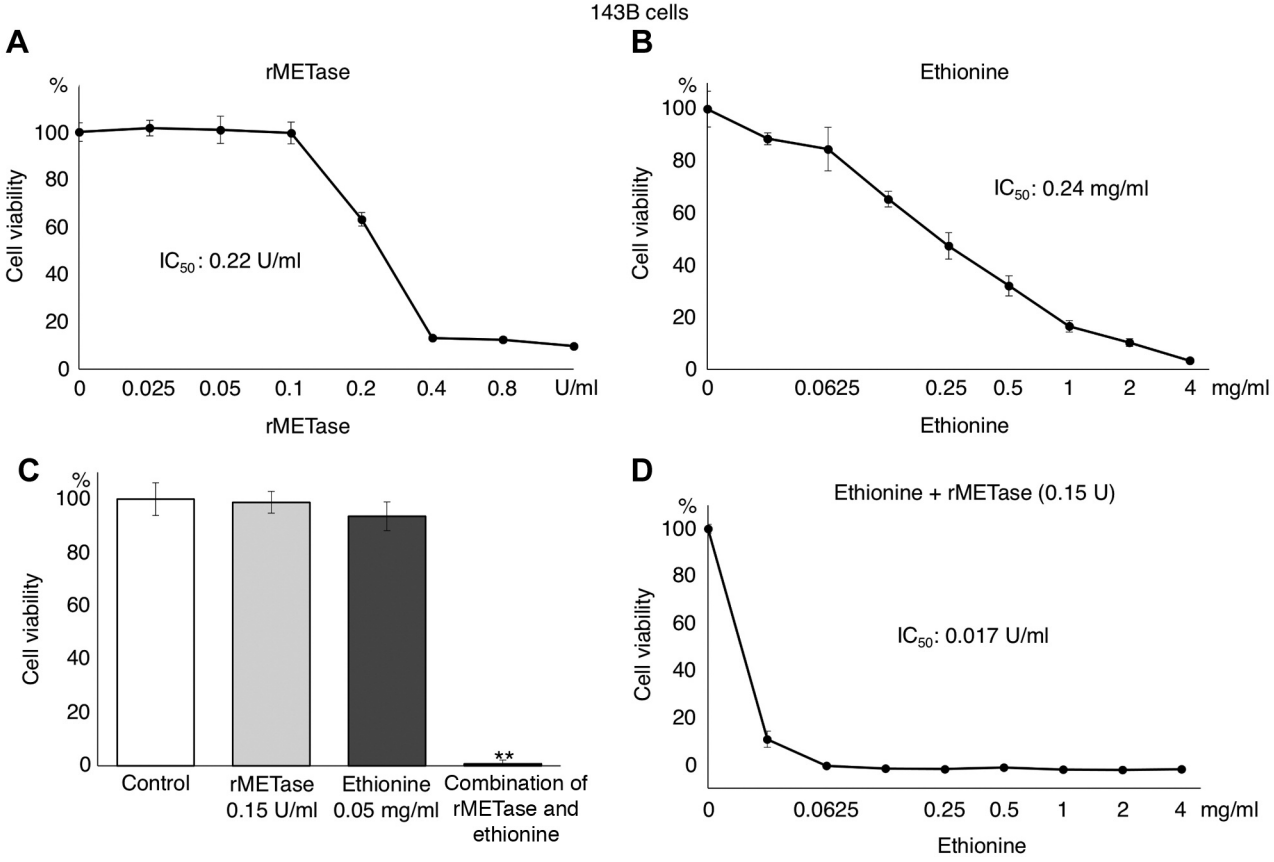

Figure 2. *rMETase and ethionine sensitivity of 143B osteosarcoma cells (mean±SD, n=3). (A) Sensitivity to rMETase. (B) ethionine Sensitivity to ethionine. (C) Comparison of the efficacy of control, rMETase (0.15 U/ml), ethionine (0.05 mg/ml), and the combination of rMETase (0.15 U/ml) and ethionine (0.05 mg/ml). (D) Sensitivity to ethionine in combination with 0.15 U/ml rMETase. **p<0.001. IC_{50}: Half-maximal inhibitory concentration.*

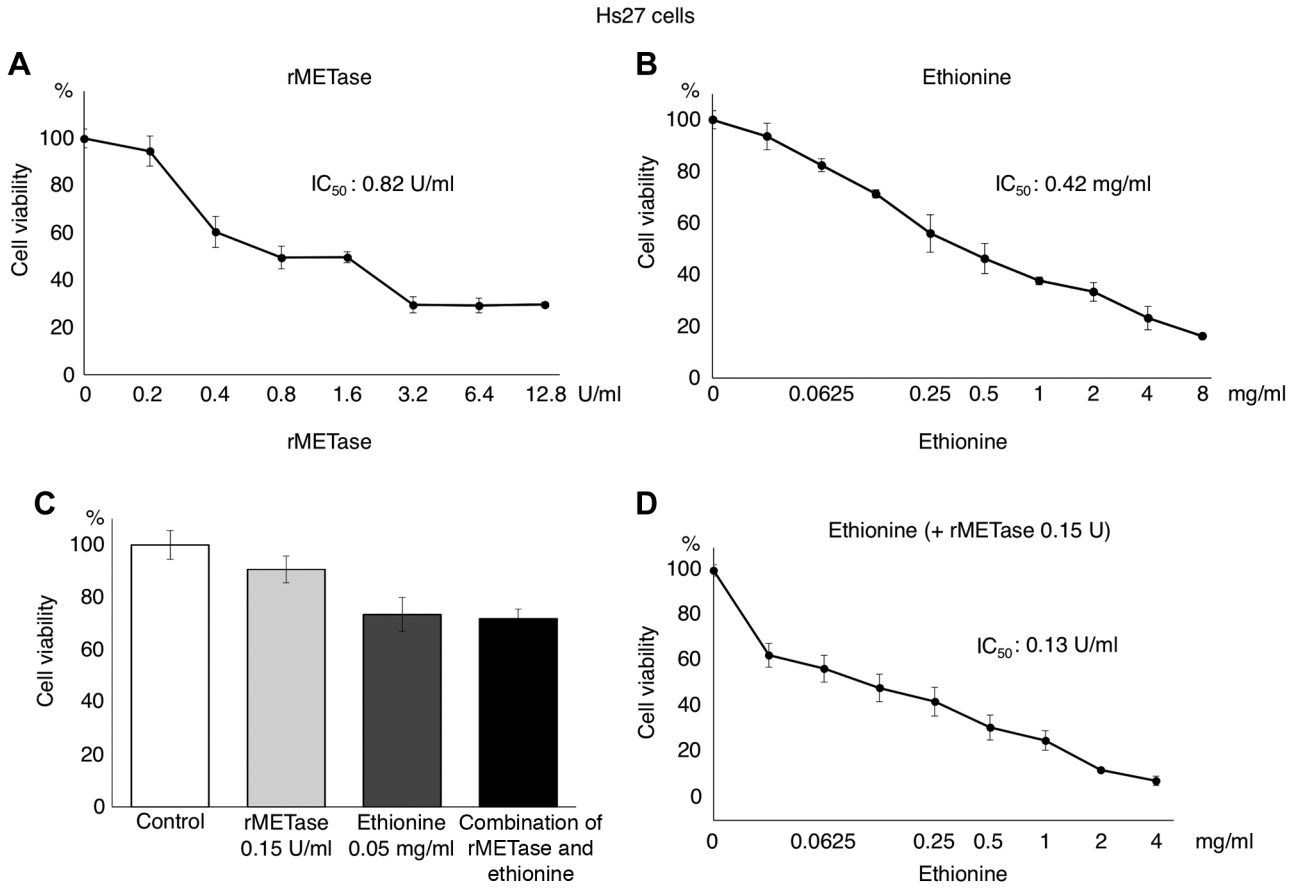

Figure 3. *rMETase and ethionine sensitivity of Hs27 normal-human fibroblasts (mean±SD, n=3). (A) Sensitivity to rMETase. (B) Sensitivity to ethionine. (C) Comparison of the efficacy of control, rMETase (0.15 U/ml), ethionine (0.05 mg/ml), and the combination of rMETase (0.15 U/ml) and ethionine (0.05 mg/ml). (D) Sensitivity to ethionine combined with 0.15 U/ml rMETase. IC_{50}: Half-maximal inhibitory concentration.*

0.8; 1.6; 3.2; 6.4; 12.8 U/ml), ethionine (0; 0.03125; 0.0625; 0.125; 0.25; 0.5; 1; 2; 4; 8 mg/ml), or both of rMETase and ethionine. The cells were incubated at 37˚C for 72 h. After the 72 h incubation period, 10 μl of the WST-8 solution was added to each well, followed by an additional 1 h incubation at 37˚C. Absorbance was measured with a microplate reader (SUNRISE: TECAN, Männedorf, Switzerland) at 450 nm. Drug-sensitivity curves were constructed with Microsoft Excel for Mac ver. 16.74 (Microsoft, Redmond, WA, USA) and half-maximal inhibitory concentration (IC_{50}) values were calculated with ImageJ ver. 1.53k (National Institutes of Health, Bethesda, MD, USA). Experiments were performed in triplicate, repeated at least twice.

Western immunoblotting. Protein extraction of 143B osteosarcoma cells, treated with/without rMETase (0.15 U/ml), ethionine (0.05 mg/ml), or the combination of rMETase (0.15 U/ml) and ethionine (0.05 mg/ml) for 72 h, was performed as previously reported (22), as follows: RIPA Lysis and Extraction buffer (Thermo Fisher Scientific, Waltham, MA, USA) with 1% Halt Protease Inhibitor Cocktail (Thermo Fisher Scientific) were used for extraction of total protein from cells. 10% SDS-PAGE gels were used for electrophoresis, and 0.45 μm polyvinylidene difluoride (PVDF) membranes (GE Healthcare, Chicago, IL, USA) were used for transfer of proteins after gel electrophoresis. Bullet Blocking One for Western Blotting (Nakalai Tesque, Inc., Kyoto, Japan) was used for blocking membranes. Anti-*c-MYC* antibody (1:2,000, #10828-1-AP, Proteintech, Rosemont, IL, USA) and anti-β actin antibody (1:1,500, #20536-1-AP, Proteintech) were used. β-actin was used as internal loading control. For the secondary antibody, horseradish-peroxidase-conjugated anti-rabbit IgG (1:5,000, #SA00001-2, Proteintech) was used. A UVP ChemStudio (Analytik Jena, Upland, CA, USA) was used to image the western blot, using the Clarity Western ECL Substrate (Bio-Rad Laboratories, Hercules, CA, USA). Quantification of western immunoblotting was performed with ImageJ ver. 1.53k (National Institutes of Health).

Statistical analysis. Tukey's honest significant difference (Tukey's HSD) test was performed to statistically compare the means of four treatment groups: control; rMETase; ethionine; combination of rMETase and ethionine, with JMP pro ver. 15.0.0 (SAS Institute, Cary, NC, USA). Bar graphs indicate the mean, error bars indicate standard deviation of the mean. A statistically-significant difference was defined with a probability value ≤0.05.

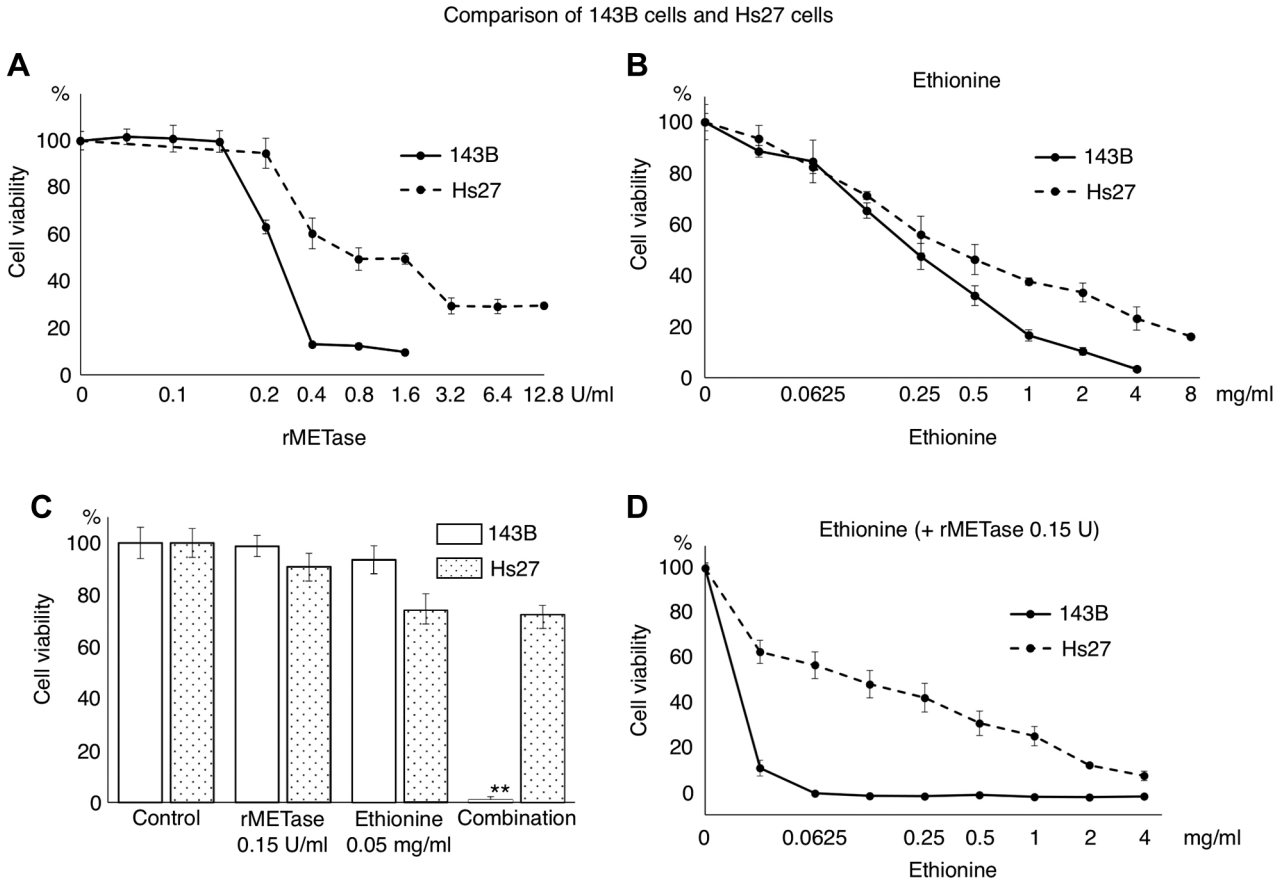

Figure 4. *Merged results of rMETase and ethionine sensitivity of 143B osteosarcoma cells and Hs27 fibroblasts shown in Figure 2 and Figure 3. (A) Sensitivity to rMETase. (B) Sensitivity to ethionine. (C) Comparison of the efficacy of control, rMETase (0.15 U/ml), ethionine (0.05 mg/ml), and the combination of rMETase (0.15 U/ml) and ethionine (0.05 mg/ml). (D) Sensitivity to ethionine combined with 0.15 U/ml rMETase. **p<0.001.*

Results

Combination of recombinant methioninase and ethionine synergistically eradicated 143B osteosarcoma cell, but Hs27 normal-human-foreskin fibroblasts were relatively resistant. 143B osteosarcoma cells were more sensitive to both rMETase alone and ethionine alone than Hs 27 fibroblasts, with the following IC_{50}s: rMETase (143B: 0.22 U/ml; Hs27: 0.82 U/ml) (Figure 2A, Figure 3A, and Figure 4A); ethionine (143B: 0.24 mg/ml; Hs27: 0.42 mg/ml) (Figure 2B, Figure 3B, and Figure 4B). The combination of rMETase and ethionine eradicated 143B cells with the IC_{50} for ethionine lowered to 0.017 from 0.24 for ethionine alone, 14-fold reduction ($p<0.001$). In contrast, the Hs27 fibroblasts were relatively resistant to the combination treatment (Figure 2C and D, Figure 3C and D, Figure 4C and D).

Combination of recombinant methioninase and ethionine synergistically down-regulates c-MYC expression. The protein expression of *c-MYC* was evaluated in 143B osteosarcoma cells treated with/without rMETase, ethionine, or the combination of rMETase and ethionine, with western immunoblotting, at the 15% inhibitory concentrations (IC_{15}) of rMETase (0.15 U/ml) and ethionine (0.05 mg/ml). This combination of rMETase and ethionine significantly down-regulated *c-MYC* expression (compared to control: $p<0.001$; compared to rMETase alone: $p=0.0057$; compared to ethionine alone: $p=0.0065$). rMETase alone (IC_{15}) or ethionine alone (IC_{15}) did not decrease the expression of *c-MYC* ($p=0.11$, 0.37, respectively) (Figure 5).

Discussion

In the present study, the combination of rMETase (0.15 U/ml) and ethionine (0.05 mg/ml) eradicated osteosarcoma cells and down-regulated *c-MYC* oncogene expression. In contrast, this combination of rMETase and ethionine, at the same concentrations, inhibited Hs27 normal-fibroblast viability by only 30%.

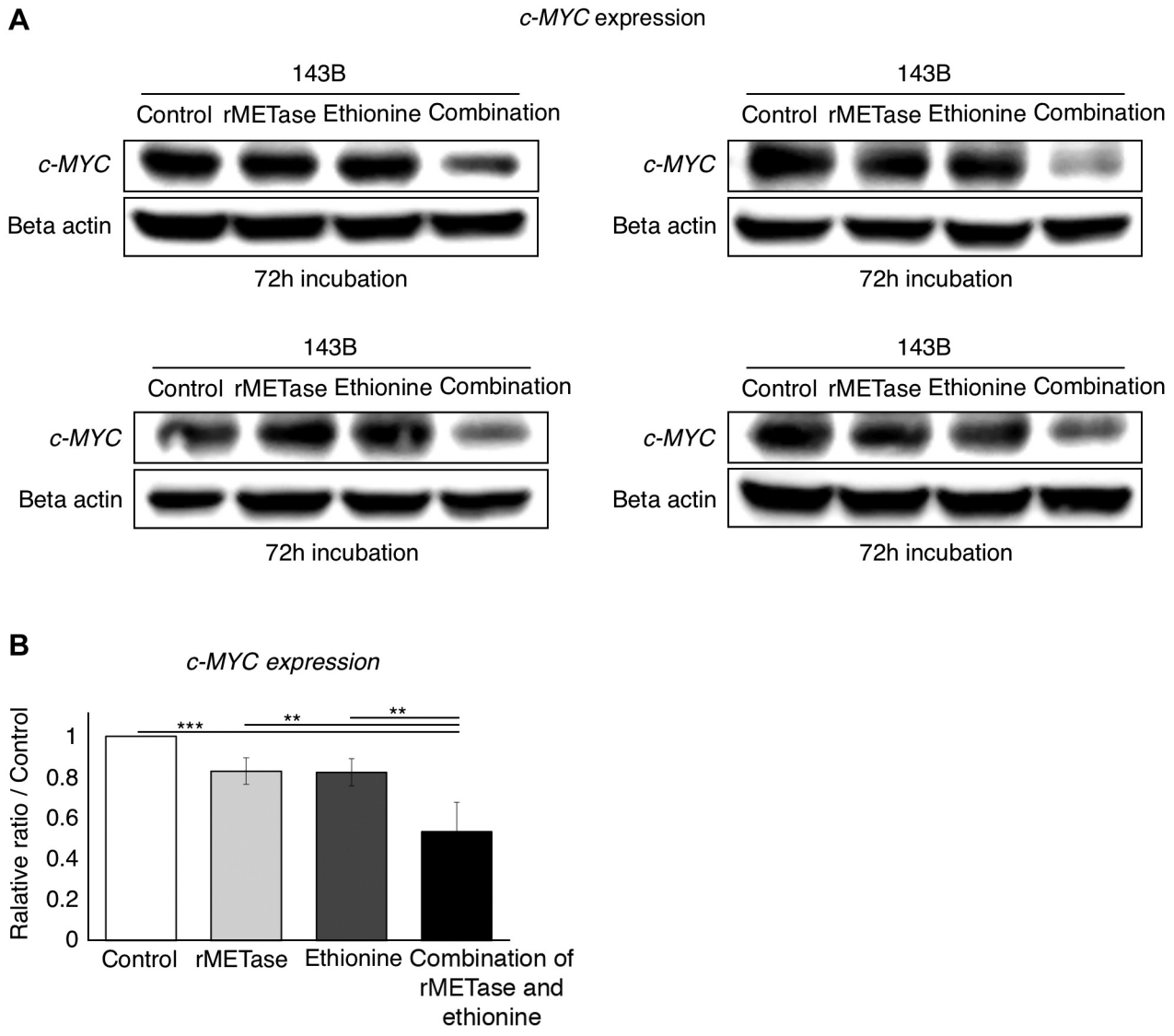

Figure 5. *Expression of c-MYC in 143B cells treated with rMETase (0.15 U/ml), ethionine (0.05 mg/ml), or the combination of rMETase (0.15 U/ml) and ethionine (0.05 mg/ml), analyzed with western immunoblotting. (A) Western immunoblotting of c-MYC of 143B cells treated with rMETase, ethionine, or combination of rMETase and ethionine. Four replicate western blots are shown. (B) Quantitative Comparison of c-MYC expression of 143B cells treated with rMETase, ethionine, or the combination of rMETase and ethionine. **p<0.01, ***p<0.001.*

Ethionine is a structural analog of methionine, with a methyl group replaced by an ethyl group. Ethionine is an anti-metabolite of methionine and cells synthesize S-adenosylethionine from ethionine, instead of S-adenosyl-methionine, which is the main methyl donor for proteins, DNA, and RNA (26). The synergistic efficacy of rMETase and ethionine on cell viability may be due to the combination of the mimetic effect of ethionine on methionine and depletion of exogenous methionine itself by rMETase. The synergy was strong: rMETase lowered the IC50 for ethionine 14-fold compared to ethionine alone.

The results of the present study are consistent with previous reports showing that the combination of methionine starvation and ethionine inhibited the proliferation of prostate cancer cells (28) and glioma cells (29), and the growth of the Yoshida sarcoma in mice (30). The present results show that the combination of rMETase and ethionine is highly effective for osteosarcoma, and may be highly effective on other types of sarcomas and carcinomas.

The combination of rMETase and ethionine is much more toxic to cancer cells than normal cells, because it attacks methionine addiction, the fundamental basis of cancer (8-13).

c-MYC is one of the most commonly activated oncogenes in osteosarcoma. *c-MYC* is related to cancer-cell proliferation and tumor growth. Expression of the *c-MYC* has been reported to be closely related to prognosis of osteosarcoma patients (31-34). *c-MYC* is linked to both osteosarcoma malignancy and methionine addiction (23).

In the present study, the combination of rMETase and ethionine synergistically down-regulated *c-MYC* expression. These results indicate the combination of rMETase and ethionine has reduced osteosarcoma cell viability, at least in part, by down-regulating of *c-MYC*.

In the present study, we showed for the first time, the combination of rMETase and ethionine selectively eradicated osteosarcoma cells, while down-regulating *c-MYC* expression, indicating the combination of rMETase and ethionine is a potential future clinical strategy for osteosarcoma.

Conflicts of Interest

The Authors declare that there are no conflicts of interest to declare in relation to this study.

Authors' Contributions

YA, YT and RMH were involved in study conception and design. YA, YK, NM and KO were involved in acquisition of data. YA, YK, NM, KO, and RMH analyzed and interpreted data. YA, YT and RMH wrote the manuscript. All Authors reviewed and approved the manuscript.

Funding

This study was funded in part by the Robert M. Hoffman Foundation for Cancer Research and Fukuoka Public Health Promotion Organization Cancer Research Fund.

Acknowledgements

This article is dedicated to the memory of A. R. Moossa, MD, Sun Lee, MD, Gordon H. Sato, PhD, Professor Li Jiaxi, Masaki Kitajima, MD, Joseph R. Bertino, MD, Shigeo Yagi, PhD, and J. A. R. Mead, PhD.

References

1 Meyers PA, Schwartz CL, Krailo M, Kleinerman ES, Betcher D, Bernstein ML, Conrad E, Ferguson W, Gebhardt M, Goorin AM, Harris MB, Healey J, Huvos A, Link M, Montebello J, Nadel H, Nieder M, Sato J, Siegal G, Weiner M, Wells R, Wold L, Womer R, Grier H: Osteosarcoma: a randomized, prospective trial of the addition of ifosfamide and/or muramyl tripeptide to cisplatin, doxorubicin, and high-dose methotrexate. J Clin Oncol 23(9): 2004-2011, 2005. DOI: 10.1200/JCO.2005.06.031

2 Messerschmitt PJ, Garcia RM, Abdul-Karim FW, Greenfield EM, Getty PJ: Osteosarcoma. J Am Acad Orthop Surg 17(8): 515-527, 2009. DOI: 10.5435/00124635-200908000-00005

3 Mirabello L, Troisi RJ, Savage SA: Osteosarcoma incidence and survival rates from 1973 to 2004: data from the Surveillance, Epidemiology, and End Results Program. Cancer 115(7): 1531-1543, 2009. DOI: 10.1002/cncr.24121

4 Gorlick R, Khanna C: Osteosarcoma. J Bone Miner Res 25(4): 683-691, 2010. DOI: 10.1002/jbmr.77

5 Ritter J, Bielack SS: Osteosarcoma. Ann Oncol 21 Suppl 21: vii320-vii325, 2010. DOI: 10.1093/annonc/mdq276

6 Saraf AJ, Fenger JM, Roberts RD: Osteosarcoma: Accelerating progress makes for a hopeful future. Front Oncol 8: 4, 2018. DOI: 10.3389/fonc.2018.00004

7 Gill J, Gorlick R: Advancing therapy for osteosarcoma. Nat Rev Clin Oncol 18(10): 609-624, 2021. DOI: 10.1038/s41571-021-00519-8

8 Hoffman RM, Erbe RW: High *in vivo* rates of methionine biosynthesis in transformed human and malignant rat cells auxotrophic for methionine. Proc Natl Acad Sci USA 73(5): 1523-1527, 1976. DOI: 10.1073/pnas.73.5.1523

9 Hoffman RM, Coalson DW, Jacobsen SJ, Erbe RW: Folate polyglutamate and monoglutamate accumulation in normal and SV40-transformed human fibroblasts. J Cell Physiol 109(3): 497-505, 1981. DOI: 10.1002/jcp.1041090316

10 Coalson DW, Mecham JO, Stern PH, Hoffman RM: Reduced availability of endogenously synthesized methionine for S-adenosylmethionine formation in methionine-dependent cancer cells. Proc Natl Acad Sci U S A 79(14): 4248-4251, 1982. DOI: 10.1073/pnas.79.14.4248

11 Stern PH, Mecham JO, Wallace CD, Hoffman RM: Reduced free-methionine in methionine-dependent SV40-transformed human fibroblasts synthesizing apparently normal amounts of methionine. J Cell Physiol 117(1): 9-14, 1983. DOI: 10.1002/jcp.1041170103

12 Stern PH, Wallace CD, Hoffman RM: Altered methionine metabolism occurs in all members of a set of diverse human tumor cell lines. J Cell Physiol 119(1): 29-34, 1984. DOI: 10.1002/jcp.1041190106

13 Kaiser P: Methionine dependence of cancer. Biomolecules 10(4): 568, 2020. DOI: 10.3390/biom10040568

14 Stern PH, Hoffman RM: Elevated overall rates of transmethylation in cell lines from diverse human tumors. In Vitro 20(8): 663-670, 1984. DOI: 10.1007/BF02619617

15 Judde JG, Ellis M, Frost P: Biochemical analysis of the role of transmethylation in the methionine dependence of tumor cells. Cancer Res 49(17): 4859-4865, 1989.

16 Wang Z, Yip LY, Lee JHJ, Wu Z, Chew HY, Chong PKW, Teo CC, Ang HY, Peh KLE, Yuan J, Ma S, Choo LSK, Basri N, Jiang X, Yu Q, Hillmer AM, Lim WT, Lim TKH, Takano A, Tan EH, Tan DSW, Ho YS, Lim B, Tam WL: Methionine is a metabolic dependency of tumor-initiating cells. Nat Med 25(5): 825-837, 2019. DOI: 10.1038/s41591-019-0423-5

17 Yamamoto J, Han Q, Inubushi S, Sugisawa N, Hamada K, Nishino H, Miyake K, Kumamoto T, Matsuyama R, Bouvet M, Endo I, Hoffman RM: Histone methylation status of H3K4me3 and H3K9me3 under methionine restriction is unstable in methionine-addicted cancer cells, but stable in normal cells. Biochem Biophys Res Commun 533(4): 1034-1038, 2020. DOI: 10.1016/j.bbrc.2020.09.108

18 Aoki Y, Tome Y, Han Q, Yamamoto J, Hamada K, Masaki N, Bouvet M, Nishida K, Hoffman RM: Histone H3 lysine-trimethylation markers are decreased by recombinant methioninase and increased by methotrexate at concentrations which inhibit methionine-addicted osteosarcoma cell proliferation.

Biochem Biophys Rep 28: 101177, 2021. DOI: 10.1016/j.bbrep.2021.101177
19 Yamamoto J, Aoki Y, Inubushi S, Han Q, Hamada K, Tashiro Y, Miyake K, Matsuyama R, Bouvet M, Clarke SG, Endo I, Hoffman RM: Extent and instability of trimethylation of histone H3 lysine increases with degree of malignancy and methionine addiction. Cancer Genomics Proteomics 19(1): 12-18, 2022. DOI: 10.21873/cgp.20299
20 Yamamoto J, Inubushi S, Han Q, Tashiro Y, Sugisawa N, Hamada K, Aoki Y, Miyake K, Matsuyama R, Bouvet M, Clarke SG, Endo I, Hoffman RM: Linkage of methionine addiction, histone lysine hypermethylation, and malignancy. iScience 25(4): 104162, 2022. DOI: 10.1016/j.isci.2022.104162
21 Aoki Y, Tome Y, Han Q, Yamamoto J, Hamada K, Masaki N, Kubota Y, Bouvet M, Nishida K, Hoffman RM: Deletion of MTAP highly sensitizes osteosarcoma cells to methionine restriction with recombinant methioninase. Cancer Genomics Proteomics 19(3): 299-304, 2022. DOI: 10.21873/cgp.20321
22 Aoki Y, Han Q, Tome Y, Yamamoto J, Kubota Y, Masaki N, Obara K, Hamada K, Wang JD, Inubushi S, Bouvet M, Clarke SG, Nishida K, Hoffman RM: Reversion of methionine addiction of osteosarcoma cells to methionine independence results in loss of malignancy, modulation of the epithelial-mesenchymal phenotype and alteration of histone-H3 lysine-methylation. Front Oncol 12: 1009548, 2022. DOI: 10.3389/fonc.2022.1009548
23 Aoki Y, Han Q, Kubota Y, Masaki N, Obara K, Tome Y, Bouvet M, Nishida K, Hoffman RM: Oncogenes and methionine addiction of cancer: Role of c-MYC. Cancer Genomics Proteomics 20(2): 165-170, 2023. DOI: 10.21873/cgp.20371
24 Tan Y, Xu M, Hoffman RM: Broad selective efficacy of rmetase and PEG-rMETase on cancer cells *in vitro*. Anticancer Res 30(3): 793-798, 2010.
25 Aoki Y, Yamamoto J, Tome Y, Hamada K, Masaki N, Inubushi S, Tashiro Y, Bouvet M, Endo I, Nishida K, Hoffman RM: Over-methylation of histone H3 lysines is a common molecular change among the three major types of soft-tissue sarcoma in patient-derived xenograft (PDX) mouse models. Cancer Genomics Proteomics 18(6): 715-721, 2021. DOI: 10.21873/cgp.20292
26 Khupse R, Sarkar AB: Ethionine. Reference Module in Biomedical Sciences, 2023. DOI: 10.1016/B978-0-12-824315-2.00340-7
27 Tan Y, Xu M, Tan X, Tan X, Wang X, Saikawa Y, Nagahama T, Sun X, Lenz M, Hoffman RM: Overexpression and large-scale production of recombinantl-methionine-α-deamino-γ-mercapto methane-lyase for novel anticancer therapy. Protein Expr Purif 9(2): 233-245, 1997. DOI: 10.1006/prep.1996.0700
28 Poirson-Bichat F, Gonfalone G, Bras-Gonçalves RA, Dutrillaux B, Poupon MF: Growth of methionine-dependent human prostate cancer (PC-3) is inhibited by ethionine combined with methionine starvation. Br J Cancer 75(11): 1605-1612, 1997. DOI: 10.1038/bjc.1997.274
29 Poirson-Bichat F, Lopez R, Gonçoives R, Miccohi L, Bourgeois Y, Demerseman P, Poisson M, Dutrillaux B, Poupon MF: Methionine deprivation and methionine analogs inhibit cell proliferation and growth of human xenografted gliomas. Life Sci 60(12): 919-931, 1997. DOI: 10.1016/s0024-3205(96)00672-8
30 Guo H, Tan Y, Kubota T, Moossa AR, Hoffman RM: Methionine depletion modulates the antitumor and antimetastatic efficacy of ethionine. Anticancer Res 16(5a): 2719-2723, 1996.
31 Ladanyi M, Park CK, Lewis R, Jhanwar SC, Healey JH, Huvos AG: Sporadic amplification of the myc gene in human osteosarcomas. Diagn Mol Pathol 2(3): 163-167, 1993.
32 Fuchs B, Pritchard DJ: Etiology of osteosarcoma. Clin Orthop Relat Res 397: 40-52, 2002. DOI: 10.1097/00003086-200204000-00007
33 Wu X, Cai ZD, Lou LM, Zhu YB: Expressions of p53, c-MYC, BCL-2 and apoptotic index in human osteosarcoma and their correlations with prognosis of patients. Cancer Epidemiol 36(2): 212-216, 2012. DOI: 10.1016/j.canep.2011.08.002
34 Chen D, Zhao Z, Huang Z, Chen DC, Zhu XX, Wang YZ, Yan YW, Tang S, Madhavan S, Ni W, Huang ZP, Li W, Ji W, Shen H, Lin S, Jiang YZ: Super enhancer inhibitors suppress MYC driven transcriptional amplification and tumor progression in osteosarcoma. Bone Res 6: 11, 2018. DOI: 10.1038/s41413-018-0009-8

Received August 9, 2023
Revised October 15, 2023
Accepted October 26, 2023