CANCER DIAGNOSIS & PROGNOSIS
5: 8-14 (2025) doi: 10.21873/cdp.10405

Elevated-c-MYC-expressing Fibrosarcoma Cells With Acquired Gemcitabine Resistance Remain Sensitive to Recombinant Methioninase: A Potential Clinical Strategy for a Recalcitrant Disease

SEI MORINAGA[1,2,3], QINGHONG HAN[1], KOHEI MIZUTA[1,2], BYUNG MO KANG[1,2], MICHAEL BOUVET[2], NORIO YAMAMOTO[3], KATSUHIRO HAYASHI[3], HIROAKI KIMURA[3], SHINJI MIWA[3], KENTARO IGARASHI[3], TAKASHI HIGUCHI[3], HIROYUKI TSUCHIYA[3], SATORU DEMURA[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 Orthopaedic Surgery, Graduate School of Medical Sciences, Kanazawa University, Kanazawa, Japan*

Abstract. *Background/Aim: For second-line chemotherapy of soft-tissue sarcoma, gemcitabine is administered in combination with docetaxel. However, more effective treatments are required for advanced soft-tissue sarcoma, where the efficacy is limited. The purpose of the present study was to compare the efficacy of rMETase and gemcitabine against HT1080 human fibrosarcoma cells and Hs27 normal fibroblasts, as well as to identify and effectively treat HT1080 cells that are resistant to gemcitabine associated with elevated c-MYC. Materials and Methods: Cell viability was measured with the WST-8 reagent. Four groups of in vitro tests were conducted involving HT1080 and Hs27 cells: gemcitabine alone; rMETase alone; and a combination of gemcitabine plus rMETase. Gemcitabine-resistant cells (GR-HT1080) were established by culturing HT-1080 cells in increasing concentrations of gemcitabine, ranging from 0.016 nM to 16 nM over five months. Western immunoblotting was performed to measure c-MYC levels in HT1080 and GR-HT1080 cells. Results: Gemcitabine had an IC_{50} of 12.8 nM against HT1080 cells; 30.8 nM against GR-HT1080 cells; and 4.48 nM against Hs27 cells. The rMETase IC_{50} value for HT1080 was 0.75 U/ml. The IC_{50} value of rMETase for GR-HT1080 cells was 0.85 U/ml. The IC_{50} value for rMETase on Hs27 cells was 0.93 U/ml. Gemcitabine and rMETase demonstrated synergy in killing fibrosarcoma cells, but no synergy was observed on normal fibroblasts. The c-MYC level was more than 5.1 times higher in GR-HT1080 cells compared to HT-1080 cells. Both the parental HT1080 cells and the GR-HT1080 cells had a similar high sensitivity to rMETase alone. Conclusion: rMETase may be used as a future clinical strategy to overcome gemcitabine resistance in sarcoma.*

Correspondence to: Robert M. Hoffman, Ph.D., AntiCancer Inc, 7917 Ostrow St, Suite B, San Diego, CA, 92111, U.S.A. Tel: +1 6198852284, e-mail: all@anticancer.com

Key Words: Methioninase, gemcitabine, HT1080, fibrosarcoma, normal fibroblast, gemcitabine-resistance, methionine restriction, methioninase, methionine addiction, efficacy, synergy, Hoffman effect, c-MYC.

Gemcitabine is an inhibitor of DNA polymerase (1). Since 2002, gemcitabine has been used in conjunction with docetaxel for the treatment of locally-advanced or metastatic soft-tissue sarcoma. Numerous small studies have investigated this combination therapy over many years, showing only moderate efficacy due to the development of gemcitabine resistance in patients (2-7).

The methioninase gene from *Pseudomonas putida* was cloned and over-expressed in *Escherichia coli*, enabling large-scale production of recombinant methioninase (rMETase) (8). Numerous studies have shown that combining chemotherapy with either rMETase, a methionine-free medium, or a diet that is deficient in methionine results in synergistic efficacy with many types of chemotherapy (9-19).

The objective of the present study was to determine the synergy between rMETase and gemcitabine on fibrosarcoma cells compared to normal fibroblasts, as well as investigate the potential of rMETase to reverse c-MYC-associated acquired gemcitabine resistance in fibrosarcoma cells.

Materials and Methods

Cell culture. The HT1080 and Hs27 cell lines were obtained from the American Type Culture Collection (Manassas, VA, USA). Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1 IU/ml of penicillin and streptomycin was used to cultivate the cells.

Reagents. Gemcitabine was obtained from BluePoint Laboratories (Cork, Ireland). rMETase was produced at AntiCancer Inc. (San Diego, CA, USA). The rMETase production process has been previously described (8).

Establishment of gemcitabine-resistant HT1080 (GR-HT1080) cells. HT1080 cells were cultured with DMEM in progressively higher concentrations of gemcitabine, ranging from 0.016 nM to 16 nM, stepwise over five months. This process was carried out in a stepwise manner.

Drug sensitivity assay 1: IC_{50} determination. The WST-8 reagent (Dojindo Laboratory, Kumamoto, Japan) was used to determine cell viability. Cells (HT1080, GR-HT1080, or Hs27) were cultured in DMEM (100 μl/well) in 96-well plates at a concentration of 3×10^3 cells per well. The plates were then incubated at 37˚C overnight. Gemcitabine, in concentrations ranging from 4 nM to 64 nM, or rMETase, in concentrations ranging from 0.5 U/ml to 8 U/ml, were administered to the cells for 72 h. After the culture period, 10 μl of the WST-8 solution was added to each well. The plates were them incubated for one hour at 37˚C. Absorption of WST-8-treated cells was measured at 450 nM in microplate reader (SUNRISE: TECAN, Mannedorf, Switzerland). The drug sensitivity curves were generated with Microsoft Excel for Mac 2016 version 15.52 (Microsoft, Redmond, WA, USA). Values for the half-maximal inhibitory concentration (IC_{50}) were determined with ImageJ version 1.53k (National Institutes of Health, Bethesda, MD, USA). Each experiment was carried out twice, in triplicate.

Drug sensitivity assay 2: Synergy. HT1080 or Hs27 cells (3×10^3) were seeded in 96-well plates. After 24 h, the cells were treated as follows: 1) Control (DMEM); 2) Gemcitabine (12.8 nM or 4.48 nM, respectively); 3) rMETase (0.75 U/ml or 0.93 U/ml, respectively); or 4) A combination of gemcitabine (12.8 nM or 4.48 nM, respectively) and rMETase (0.75 U/ml or 0.93 U/ml, respectively). The viability of the cells was evaluated after 72 h. Cells were assessed using the method of Drug Sensitivity Assay 1, which was performed three times.

Western immunoblotting. RIPA Lysis buffer and Extraction Buffer (Thermo Fisher Scientific, Waltham, MA, USA) and 1% Halt Protease Inhibitor Cocktail (Thermo Fisher Scientific) were used for protein extraction. Protein samples were loaded onto 10% SDS-PAGE gels. Subsequently, the samples were transferred onto 0.45 μm polyvinylidene difluoride (PVDF) membranes (GE Healthcare, Chicago, IL, USA). Bullet Blocking One for Western Blotting (Nakalai Tesque, Inc., Kyoto, Japan) was used for membrane blocking. Anti-c-MYC antibody (1:2,000, #10828-1-AP), and β-Actin (20536-1-AP, 1:1,000) were from Proteintech (Rosemont, IL, USA). Secondary antibodies were horseradish-peroxidase-conjugated anti-rabbit IgG (1:5,000, #SA00001-2, Proteintech). The UVP ChemStudio (Analytik Jena, Upland, CA, USA) and the Clarity Western ECL Substrate (Bio-Rad Laboratories, Hercules, CA, USA) were used to scan the western blots. The experiments were carried out three times to ensure accuracy.

Statistical analysis. EZR software (Jichi Medical University, Saitama, Japan) was used for statistical analyses (20). Tukey–Kramer analysis and the Welch's *t*-test were used to determine the relationship between variables. For statistical significance, p-values ≤ 0.05 were considered adequate.

Results

Generation of gemcitabine-resistant HT1080 cells. Gemcitabine-resistant cells (GR-HT1080) were established by cultivating HT1080 cells in DMEM in progressively increasing concentrations of gemcitabine, ranging from 0.016 nM to 16 nM. GR-HT1080 cells were 2.4 times more resistant to gemcitabine than the parental HT1080 cells.

Drug sensitivity assay 1: IC_{50} of gemcitabine alone and rMETase alone on HT1080, GR-HT1080, and Hs27 cells. The IC_{50} of gemcitabine for HT 1080 cells was 12.8 nM; and 30.8 nM for GR-HT1080 cells. The IC_{50} of gemcitabine for Hs27 cells was 4.48 nM. The IC_{50} of rMETase for HT1080 cells was 0.75 U/ml [data from (10)], and 0.85 U/ml for GR-HT1080 cells. The IC_{50} of rMETase for Hs27 cells was 0.93 U/ml [data from (10)] (Figure 1).

Drug sensitivity assay 2: Synergy of gemcitabine and rMETase. The combination of gemcitabine [12.8 nM (IC_{50})] plus rMETase [0.75 U/ml (IC_{50})] had synergistic efficacy on HT1080 cells ($p<0.05$). In contrast, gemcitabine [4.48 nM (IC_{50})] plus rMETase (0.93 U/ml [IC_{50}]) did not show synergy in Hs27 cells (Figure 2A and B).

Western blotting of c-MYC. There was a 5.1-fold increase in the expression of c-MYC in GR-HT1080 cells compared to HT1080 cells ($p<0.05$) (Figure 3 and Figure 4).

Discussion

Patients with metastatic soft-tissue sarcoma participated in a randomized phase II study, in which the median progression-free survival was 6.2 months for gemcitabine plus docetaxel and 3.0 months for gemcitabine alone. Additionally, the median overall survival was 17.9 months for gemcitabine-docetaxel and 11.5 months for gemcitabine alone (21). Among patients with previously-untreated advanced unresectable or metastatic soft-tissue sarcomas, a randomized controlled phase III trial demonstrated that the group receiving gemcitabine plus docetaxel had a median progression-free survival of only 5.9 months (22). Therefore, significantly improved therapy is necessary.

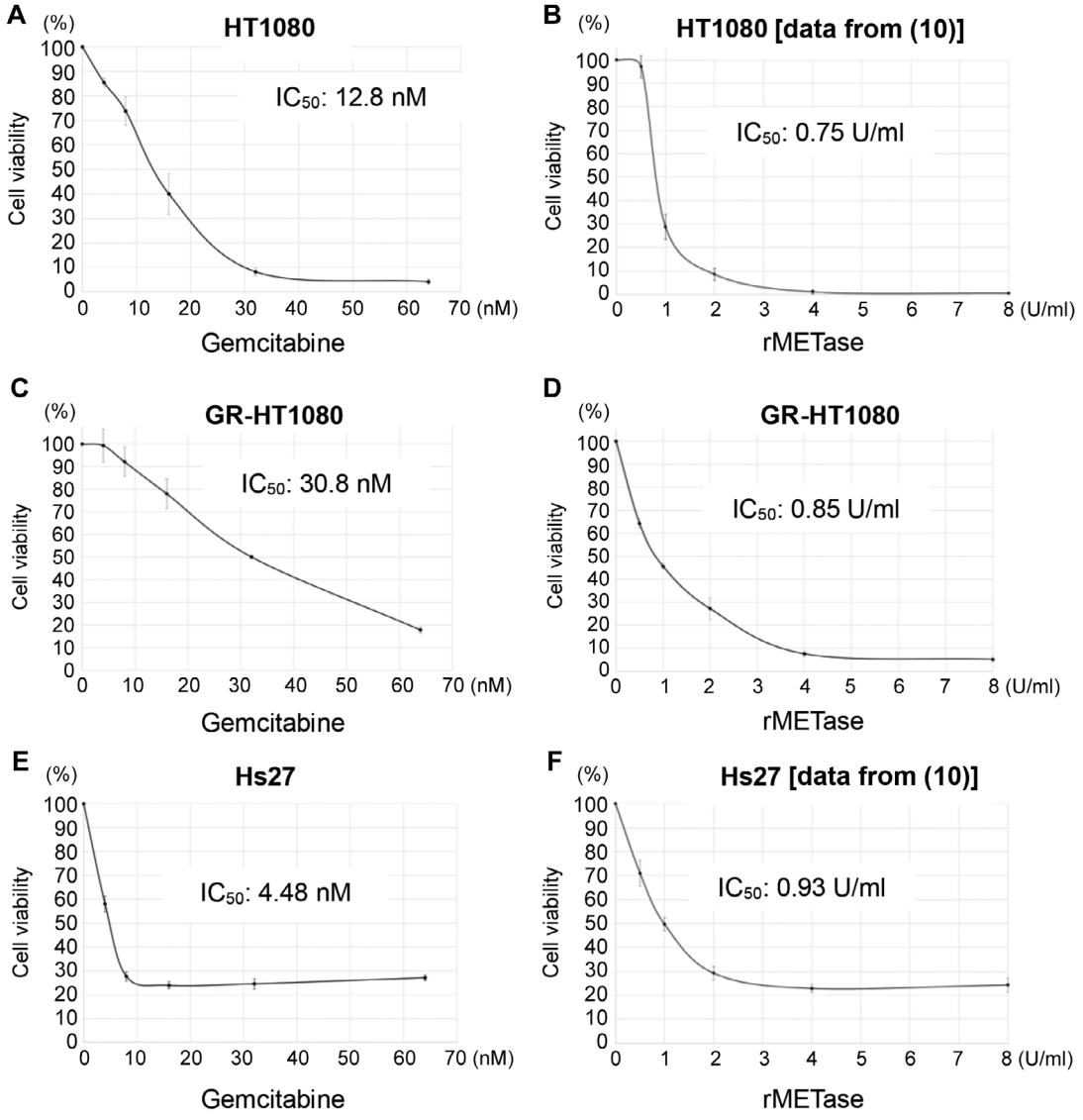

Figure 1. *Gemcitabine and rMETase sensitivity of HT1080 fibrosarcoma cells, gemcitabine-resistant HT1080 (GR-HT1080) cells, and Hs27 normal fibroblasts (mean±standard deviation). A) Sensitivity to gemcitabine of HT1080 cells. B) Sensitivity to rMETase of HT1080 cells. C) Sensitivity to gemcitabine of GR-HT1080 cells. D) Sensitivity to rMETase of GR-HT1080 cells. E) Sensitivity to gemcitabine of Hs27 cells. F) Sensitivity to rMETase of Hs27 cells.*

Methionine addiction of cancer was first identified by one of the authors (RMH) in 1976 (23-26) and is termed the Hoffman effect (26). To target methionine addiction, our research group developed rMETase. rMETase was found to be effective by oral administration (27).

It has been demonstrated that a wide range of combinations of rMETase and chemotherapy drugs are synergistic (9-19). The present study demonstrated that the combination of rMETase and gemcitabine was synergistic on HT1080 cells and reversed the high gemcitabine resistance of GR-HT1080 cells (Figure 1,2). Methionine depletion causes cancer cells to enter a reversible arrest in the late S/G_2-phase of the cell cycle (28), which is the phase that gemcitabine targets (1). Our previous research demonstrated that gemcitabine and rMETase were synergistic in a mouse model of an orthotopic pancreatic-cancer cell-line and a patient-derived orthotopic xenograft (PDOX) mouse model (29, 30). In contrast, Hs27 normal fibroblasts did not demonstrate synergy with the combination of gemcitabine and rMETase (Figure 2). This is due to the fact that rMETase does not arrest normal cells in the S-phase of the cell cycle (31, 32).

c-MYC is one of the most frequently-activated oncogenes in human cancer (33, 34). It is responsible for promoting cell proliferation and growth. A correlation has been observed

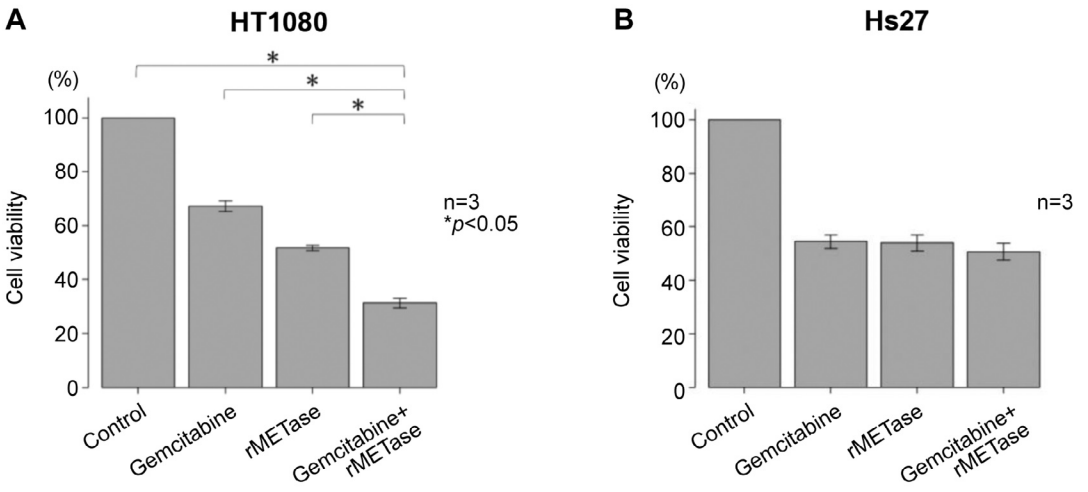

Figure 2. *Efficacy of the combination of gemcitabine and rMETase on HT1080 fibrosarcoma cells and Hs27 normal human fibroblasts. A) HT1080 cells. 1: control (DMEM); 2: gemcitabine [12.8 nM (IC_{50})]; 3: rMETase [0.75 U/ml (IC_{50})]; 4: gemcitabine [12.8 nM (IC_{50})] plus rMETase [0.75 U/ml (IC_{50})]. B) Hs27 normal human fibroblasts. 1: control (DMEM); 2: gemcitabine [4.48 nM (IC_{50})]; 3: rMETase [0.93 U/ml (IC_{50})]; 4: gemcitabine [4.48 nM (IC_{50})] plus rMETase [0.93 U/ml (IC_{50})].*

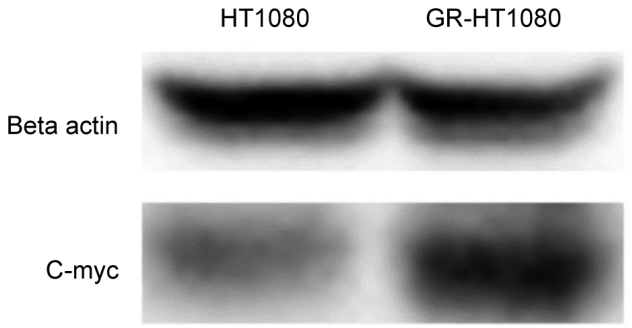

Figure 3. *Expression of c-MYC in HT1080 and gemcitabine-resistant HT1080 (GR-HT1080) fibrosarcoma cells. Figure 3 is representative of three different experiments.*

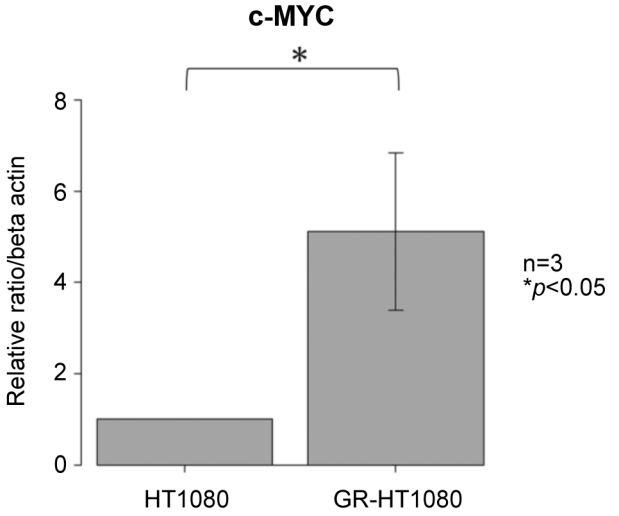

Figure 4. *Quantitative comparison of c-MYC expression in HT1080 and gemcitabine-resistant HT1080 (GR-HT1080) fibrosarcoma cells. Data are shown as mean±standard deviation. *p<0.05.*

between increased c-MYC expression and elevated chemoresistance (35-37). c-MYC levels were shown to be elevated in methotrexate-resistant osteosarcoma cells in our previous study (37). In the present study, the expression of c-MYC was 5.1 fold higher in GR-HT1080 cells than in parental HT1080 cells (Figure 3,4).

rMETase is effective because it targets the fundamental cancer hallmark of methionine addiction (23, 24-26, 28, 31, 32, 38-60). The present study suggests that rMETase and gemcitabine synergy has the potential to be used in the treatment of drug-resistant soft tissue sarcoma in future clinical trials.

rMETase as an oral dietary supplement (o-rMETase) is showing clinical promise (12, 27, 61-68).

Conflicts of Interest

The Authors declare no competing interests in relation to this study.

Authors' Contributions

SM, HQ, KM, BMK, MB, NY, KH, HK, SM, KI, TH, HT SD, and RMH designed the study. SM performed experiments. SM was a major contributor to writing the manuscript and RMH revised the paper. All Authors read and approved the final manuscript.

References

1 Huang P, Chubb S, Hertel LW, Grindey GB, Plunkett W: Action of 2',2'-difluorodeoxycytidine on DNA synthesis. Cancer Res 51: 6110-6117, 1991.

2 Hensley ML, Maki R, Venkatraman E, Geller G, Lovegren M, Aghajanian C, Sabbatini P, Tong W, Barakat R, Spriggs DR: Gemcitabine and docetaxel in patients with unresectable leiomyosarcoma: results of a phase II trial. J Clin Oncol 20(12): 2824-2831, 2002. DOI: 10.1200/JCO.2002.11.050

3 Leu KM, Ostruszka LJ, Shewach D, Zalupski M, Sondak V, Biermann JS, Lee JS, Couwlier C, Palazzolo K, Baker LH: Laboratory and clinical evidence of synergistic cytotoxicity of sequential treament with gemcitabine followed by docetaxel in the treatment of sarcoma. J Clin Oncol 22(9): 1706-1712, 2004. DOI: 10.1200/JCO.2004.08.043

4 Bay JO, Ray-Coquard I, Fayette J, Leyvraz S, Cherix S, Piperno-Neumann S, Chevreau C, Isambert N, Brain E, Emile G, Le Cesne A, Cioffi A, Kwiatkowski F, Coindre JM, Bui NB, Peyrade F, Penel N, Blay JY, Groupe Sarcome Français: Docetaxel and gemcitabine combination in 133 advanced soft-tissue sarcomas: A retrospective analysis. Int J Cancer 119(3): 706-711, 2006. DOI: 10.1002/ijc.21867

5 Hensley ML, Blessing JA, Degeest K, Abulafia O, Rose PG, Homesley HD: Fixed-dose rate gemcitabine plus docetaxel as second-line therapy for metastatic uterine leiomyosarcoma: a Gynecologic Oncology Group phase II study. Gynecol Oncol 109(3): 323-328, 2008. DOI: 10.1016/j.ygyno.2008.02.024

6 Hensley ML, Blessing JA, Mannel R, Rose PG: Fixed-dose rate gemcitabine plus docetaxel as first-line therapy for metastatic uterine leiomyosarcoma: a Gynecologic Oncology Group phase II trial. Gynecol Oncol 109(3): 329-334, 2008. DOI: 10.1016/j.ygyno.2008.03.010

7 Seddon B, Scurr M, Jones RL, Wood Z, Propert-Lewis C, Fisher C, Flanagan A, Sunkersing J, A'Hern R, Whelan J, Judson I: A phase II trial to assess the activity of gemcitabine and docetaxel as first line chemotherapy treatment in patients with unresectable leiomyosarcoma. Clin Sarcoma Res 5: 13, 2015. DOI: 10.1186/s13569-015-0029-8

8 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

9 Choobin BB, Kubota Y, Han Q, Ardjmand D, Morinaga S, Mizuta K, Bouvet M, Tsunoda T, Hoffman RM: Recombinant methioninase lowers the effective dose of regorafenib against colon-cancer cells: a strategy for widespread clinical use of a toxic drug. Cancer Diagn Progn 3(6): 655-659, 2023. DOI: 10.21873/cdp.10268

10 Morinaga S, Han Q, Kubota Y, Mizuta K, Kang BM, Sato M, Bouvet M, Yamamoto N, Hayashi K, Kimura H, Miwa S, Igarashi K, Higuchi T, Tsuchiya H, Hoffman RM: Extensive synergy between recombinant methioninase and eribulin against fibrosarcoma cells but not normal fibroblasts. Anticancer Res 44(3): 921-928, 2024. DOI: 10.21873/anticanres.16886

11 Ardjmand D, Kubota Y, Sato M, Han Q, Mizuta K, Morinaga S, Hoffman RM: Selective synergy of rapamycin combined with methioninase on cancer cells compared to normal cells. Anticancer Res 44(3): 929-933, 2024. DOI: 10.21873/anticanres.16887

12 Kubota Y, Han Q, Morinaga S, Tsunoda T, Hoffman RM: Rapid reduction of CEA and stable metastasis in an NRAS-mutant rectal-cancer patient treated with FOLFIRI and bevacizumab combined with oral recombinant methioninase and a low-methionine diet upon metastatic recurrence after FOLFIRI and bevacizumab treatment alone. In Vivo 37(5): 2134-2138, 2023. DOI: 10.21873/invivo.13310

13 Sato M, Han Q, Kubota Y, Baranov A, Ardjmand D, Mizuta K, Morinaga S, Kang BM, Kobayashi N, Bouvet M, Ichikawa Y, Nakajima A, Hoffman RM: Recombinant methioninase decreased the effective dose of irinotecan by 15-fold against colon cancer cells: a strategy for effective low-toxicity treatment of colon cancer. Anticancer Res 44(1): 31-35, 2024. DOI: 10.21873/anticanres.16785

14 Kubota Y, Aoki Y, Masaki N, Obara K, Hamada K, Han Q, Bouvet M, Tsunoda T, Hoffman RM: Methionine restriction of glioma does not induce MGMT and greatly improves temozolomide efficacy in an orthotopic nude-mouse model: A potential curable approach to a clinically-incurable disease. Biochem Biophys Res Commun 695: 149418, 2024. DOI: 10.1016/j.bbrc.2023.149418

15 Morinaga S, Han Q, Kubota Y, Mizuta K, Kang BM, Sato M, Bouvet M, Yamamoto N, Hayashi K, Kimura H, Miwa S, Igarashi K, Higuchi T, Tsuchiya H, Demura S, Hoffman RM: DNA-binding agent trabectedin combined with recombinant methioninase is synergistic to decrease fibrosarcoma cell viability and induce nuclear fragmentation but not synergistic on normal fibroblasts. Anticancer Res 44(6): 2359-2367, 2024. DOI: 10.21873/anticanres.17043

16 Morinaga S, Han Q, Mizuta K, Kang BM, Sato M, Bouvet M, Yamamoto N, Hayashi K, Kimura H, Miwa S, Igarashi K, Higuchi T, Tsuchiya H, Demura S, Hoffman RM: Recombinant methioninase is selectively synergistic with doxorubicin against wild-type fibrosarcoma cells compared to normal cells and overcomes highly-doxorubicin-resistant fibrosarcoma. Anticancer Res 44(8): 3261-3268, 2024. DOI: 10.21873/anticanres.17144

17 Kubota Y, Han Q, Aoki Y, Masaki N, Obara K, Hamada K, Hozumi C, Wong ACW, Bouvet M, Tsunoda T, Hoffman RM: Synergy of combining methionine restriction and chemotherapy: the disruptive next generation of cancer treatment. Cancer Diagn Progn 3(3): 272-281, 2023. DOI: 10.21873/cdp.10212

18 Morinaga S, Han Q, Mizuta K, Kang BM, Sato M, Bouvet M, Yamamoto N, Hayashi K, Kimura H, Miwa S, Igarashi K, Higuchi T, Tsuchiya H, Demura S, Hoffman RM: Recombinant methioninase increases eribulin efficacy 16-fold in highly eribulin-resistant HT1080 fibrosarcoma cells, demonstrating potential to overcome the clinical challenge of drug-resistant soft-tissue sarcoma. Anticancer Res 44(9): 3777-3783, 2024. DOI: 10.21873/anticanres.17202

19 Morinaga S, Han Q, Mizuta K, Kang BM, Sato M, Bouvet M, Yamamoto N, Hayashi K, Kimura H, Miwa S, Igarashi K, Higuchi T, Tsuchiya H, Demura S, Hoffman RM: Overcoming high trabectedin resistance of soft-tissue sarcoma with recombinant methioninase: a potential solution of a recalcitrant clinical problem. Anticancer Res 44(9): 3785-3791, 2024. DOI:10.21873/anticanres.17203

20 Kanda Y: Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant 48(3): 452-458, 2013. DOI: 10.1038/bmt.2012.244

21 Maki RG, Wathen JK, Patel SR, Priebat DA, Okuno SH, Samuels B, Fanucchi M, Harmon DC, Schuetze SM, Reinke D, Thall PF, Benjamin RS, Baker LH, Hensley ML: Randomized phase II study of gemcitabine and docetaxel compared with gemcitabine alone in patients with metastatic soft tissue sarcomas: results of Sarcoma Alliance for Research Through

Collaboration Study 002. J Clin Oncol 25(19): 2755-2763, 2007. DOI: 10.1200/JCO.2006.10.4117

22 Seddon B, Strauss SJ, Whelan J, Leahy M, Woll PJ, Cowie F, Rothermundt C, Wood Z, Benson C, Ali N, Marples M, Veal GJ, Jamieson D, Küver K, Tirabosco R, Forsyth S, Nash S, Dehbi HM, Beare S: Gemcitabine and docetaxel versus doxorubicin as first-line treatment in previously untreated advanced unresectable or metastatic soft-tissue sarcomas (GeDDiS): a randomised controlled phase 3 trial. Lancet Oncol 18(10): 1397-1410, 2017. DOI: 10.1016/S1470-2045(17)30622-8

23 Hoffman RM: Development of recombinant methioninase to target the general cancer-specific metabolic defect of methionine dependence: a 40-year odyssey. Expert Opin Biol Ther 15(1): 21-31, 2015. DOI: 10.1517/14712598.2015.963050

24 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 U S A 73(5): 1523-1527, 1976. DOI: 10.1073/pnas.73.5.1523

25 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

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

27 Han Q, Tan Y, Hoffman RM: Oral dosing of recombinant methioninase is associated with a 70% drop in PSA in a patient with bone-metastatic prostate cancer and 50% reduction in circulating methionine in a high-stage ovarian cancer patient. Anticancer Res 40(5): 2813-2819, 2020. DOI: 10.21873/anticanres.14254

28 Hoffman RM, Jacobsen SJ: Reversible growth arrest in simian virus 40-transformed human fibroblasts. Proc Natl Acad Sci USA 77(12): 7306-7310, 1980. DOI: 10.1073/pnas.77.12.7306

29 Kawaguchi K, Miyake K, Han Q, Li S, Tan Y, Igarashi K, Lwin TM, Higuchi T, Kiyuna T, Miyake M, Oshiro H, Bouvet M, Unno M, Hoffman RM: Targeting altered cancer methionine metabolism with recombinant methioninase (rMETase) overcomes partial gemcitabine-resistance and regresses a patient-derived orthotopic xenograft (PDOX) nude mouse model of pancreatic cancer. Cell Cycle 17(7): 868-873, 2018. DOI: 10.1080/15384101.2018.1445907

30 Kawaguchi K, Miyake K, Han Q, Li S, Tan Y, Igarashi K, Kiyuna T, Miyake M, Higuchi T, Oshiro H, Zhang Z, Razmjooei S, Wangsiricharoen S, Bouvet M, Singh SR, Unno M, Hoffman RM: Oral recombinant methioninase (o-rMETase) is superior to injectable rMETase and overcomes acquired gemcitabine resistance in pancreatic cancer. Cancer Lett 432: 251-259, 2018. DOI: 10.1016/j.canlet.2018.06.016

31 Miki K, Xu M, An Z, Wang X, Yang M, Al-Refaie W, Sun X, Baranov E, Tan Y, Chishima T, Shimada H, Moossa AR, Hoffman RM: Survival efficacy of the combination of the methioninase gene and methioninase in a lung cancer orthotopic model. Cancer Gene Ther 7(2): 332-338, 2000. DOI: 10.1038/sj.cgt.7700103

32 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

33 Dang CV: c-Myc target genes involved in cell growth, apoptosis, and metabolism. Mol Cell Biol 19(1): 1-11, 1999. DOI: 10.1128/MCB.19.1.1

34 Dhanasekaran R, Deutzmann A, Mahauad-Fernandez WD, Hansen AS, Gouw AM, Felsher DW: The MYC oncogene - the grand orchestrator of cancer growth and immune evasion. Nat Rev Clin Oncol 19(1): 23-36, 2022. DOI: 10.1038/s41571-021-00549-2

35 Ganesan S: MYC, PARP1, and chemoresistance: BIN there, done that? Sci Signal 4(166): pe15, 2011. DOI: 10.1126/scisignal.2001946

36 Scionti I, Michelacci F, Pasello M, Hattinger CM, Alberghini M, Manara MC, Bacci G, Ferrari S, Scotlandi K, Picci P, Serra M: Clinical impact of the methotrexate resistance-associated genes C-MYC and dihydrofolate reductase (DHFR) in high-grade osteosarcoma. Ann Oncol 19(8): 1500-1508, 2008. DOI: 10.1093/annonc/mdn148

37 Aoki Y, Kubota Y, Masaki N, Obara K, Tome Y, Bouvet M, Nishida K, Hoffman RM: Reduced malignancy of super methotrexate-resistant osteosarcoma cells with dihydrofolate reductase amplification despite paradoxical gain of oncogenic PI3K/AKT/mTOR and c-MYC expression. Anticancer Res 44(7): 2787-2792, 2024. DOI: 10.21873/anticanres.17090

38 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 USA 79(14): 4248-4251, 1982. DOI: 10.1073/pnas.79.14.4248

39 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

40 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

41 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

42 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

43 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

44 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

45 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

46 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

47 Hoffman RM, Jacobsen SJ, Erbe RW: Reversion to methionine independence in simian virus 40-transformed human and malignant rat fibroblasts is associated with altered ploidy and altered properties of transformation. Proc Natl Acad Sci USA 76(3): 1313-1317, 1979. DOI: 10.1073/pnas.76.3.1313

48 Hoffman RM, Jacobsen SJ, Erbe RW: Reversion to methionine independence by malignant rat and SV40-transformed human fibroblasts. Biochem Biophys Res Commun 82(1): 228-234, 1978. DOI: 10.1016/0006-291X(78)90600-9

49 Yamamoto J, Aoki Y, Han Q, Sugisawa N, Sun Y, Hamada K, Nishino H, Inubushi S, Miyake K, Matsuyama R, Bouvet M, Endo I, Hoffman RM: Reversion from methionine addiction to methionine independence results in loss of tumorigenic potential of highly-malignant lung-cancer cells. Anticancer Res 41(2): 641-643, 2021. DOI: 10.21873/anticanres.14815

50 Abo Qoura L, Balakin KV, Hoffman RM, Pokrovsky VS: The potential of methioninase for cancer treatment. Biochim Biophys Acta Rev Cancer 1879(4): 189122, 2024. DOI: 10.1016/j.bbcan.2024.189122

51 Hoffman RM: Altered methionine metabolism, DNA methylation and oncogene expression in carcinogenesis. A review and synthesis. Biochim Biophys Acta 738: 49-87, 1984. DOI: 10.1016/0304-419x(84)90019-2

52 Ghergurovich JM, Xu X, Wang JZ, Yang L, Ryseck RP, Wang L, Rabinowitz JD: Methionine synthase supports tumour tetrahydrofolate pools. Nat Metab 3(11): 1512-1520, 2021. DOI: 10.1038/s42255-021-00465-w

53 Sullivan MR, Darnell AM, Reilly MF, Kunchok T, Joesch-Cohen L, Rosenberg D, Ali A, Rees MG, Roth JA, Lewis CA, Vander Heiden MG: Methionine synthase is essential for cancer cell proliferation in physiological folate environments. Nat Metab 3(11): 1500-1511, 2021. DOI: 10.1038/s42255-021-00486-5

54 Mecham JO, Rowitch D, Wallace C, Stern PH, Hoffman RM: The metabolic defect of methionine dependence occurs frequently in human tumor cell lines. Biochem Biophys Res Commun 117(2): 429-434, 1983. DOI: 10.1016/0006-291x(83)91218-4

55 Tan Y, Xu M, Hoffman RM: Broad selective efficacy of recombinant methioninase and polyethylene glycol-modified recombinant methioninase on cancer cells In Vitro. Anticancer Res 30(4): 1041-1046, 2010

56 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

57 Jacobsen SJ, Hoffman RM, Erbe RW: Regulation of methionine adenosyltransferase in normal diploid and simian virus 40-transformed human fibroblasts. J Natl Cancer Inst 65(6): 1237-44, 1980

58 Oden KL, Carson K, Mecham JO, Hoffman RM, Clarke S: S-adenosylmethionine synthetase in cultured normal and oncogenically-transformed human and rat cells. Biochim Biophys Acta 760(2): 270-277, 2983. DOI: 10.1016/0304-4165(83)90173-3

59 Rubnitz JE, Jacobsen SJ, Hoffman RM: Constitutive behavior of methionyl-tRNA synthetase compared to repressible behavior of methionine adenosyltransferase in mammalian cells. Biochim Biophys Acta 677(2): 269-273, 1981. DOI: 10.1016/0304-4165(81)90095-7

60 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

61 Kubota Y, Han Q, Hozumi C, Masaki N, Yamamoto J, Aoki Y, Tsunoda T, Hoffman RM: Stage IV pancreatic cancer patient treated with FOLFIRINOX combined with oral methioninase: a highly-rare case with long-term stable disease. Anticancer Res 42(5): 2567-2572, 2022. DOI: 10.21873/anticanres.15734

62 Kubota Y, Han Q, Masaki N, Hozumi C, Hamada K, Aoki Y, Obara K, Tsunoda T, Hoffman RM: Elimination of axillary-lymph-node metastases in a patient with invasive lobular breast cancer treated by first-line neo-adjuvant chemotherapy combined with methionine restriction. Anticancer Res 42(12): 5819-5823, 2022. DOI: 10.21873/anticanres.16089

63 Kubota Y, Han Q, Hamada K, Aoki Y, Masaki N, Obara K, Tsunoda T, Hoffman RM: Long-term stable disease in a rectal-cancer patient treated by methionine restriction with oral recombinant methioninase and a low-methionine diet. Anticancer Res 42(8): 3857-3861, 2022. DOI: 10.21873/anticanres.15877

64 Sato M, Han Q, Mori R, Mizuta K, Kang BM, Morinaga S, Kobayashi N, Ichikawa Y, Nakajima A, Hoffman RM: Reduction of Tumor Biomarkers from very High to Normal and Extensive Metastatic Lesions to Undetectability in a Patient With Stage IV HER2-positive Breast Cancer Treated With Low-dose Trastuzumab Deruxtecan in Combination With Oral Recombinant Methioninase and a Low-methionine Diet. Anticancer Res 44(4): 1499-1504, 2024. DOI: 10.21873/anticanres.16946

65 Sato M, Han Q, Hozumi C, Kujiraoka H, Mizuta K, Morinaga S, Kang BM, Kobayashi N, Ichikawa Y, Nakajima A, Hoffman RM: First-line chemotherapy in combination with oral recombinant methioninase and a low-methionine diet for a stage IV inoperable pancreatic-cancer patient resulted in 40% tumor reduction and an 86% CA19-9 biomarker decrease. Anticancer Res 44(9): 3885-3889, 2024. DOI: 10.21873/anticanres.17215

66 Sato M, Han Q, Mizuta K, Mori R, Kang BM, Morinaga S, Kobayashi N, Ichikawa Y, Nakajima A, Hoffman RM: Extensive Shrinkage and Long-term Stable Disease in a Teenage Female Patient With High-grade Glioma Treated With Temozolomide and Radiation in Combination With Oral Recombinant Methioninase and a Low-methionine Diet. In Vivo 38(3): 1459-1464, 2024. DOI: 10.21873/invivo.13591

67 Han Q, Hoffman RM: Chronic treatment of an advanced prostate-cancer patient with oral methioninase resulted in long-term stabilization of rapidly rising PSA levels. In Vivo 35(4): 2171-2176, 2021. DOI: 10.21873/invivo.12488

68 Han Q, Hoffman RM: Lowering and stabilizing PSA levels in advanced-prostate cancer patients with oral methioninase. Anticancer Res 41(4): 1921-1926, 2021. DOI: 10.21873/anticanres.14958

Received October 7, 2024
Revised October 22, 2024
Accepted October 23, 2024