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Synergy of oral recombinant methioninase (rMETase) and 5-fluorouracil on poorly differentiated gastric cancer

Masuyo Miyake [a, b, c, 1], Kentaro Miyake [a, b, c, 1, *], Qinghong Han [a], Kentaro Igarashi [a, b], Kei Kawaguchi [a, b], Maryam Barangi [a], Tasuku Kiyuna [a, b], Norihiko Sugisawa [a, b], Takashi Higuchi [a, b], Hiromichi Oshiro [a, b], Zhiying Zhang [a, b], Sahar Razmjooei [a], Michael Bouvet [b], Itaru Endo [c, **], Robert M. Hoffman [a, b, ***]

[a] AntiCancer, Inc., San Diego, CA, USA
[b] Department of Surgery, University of California, San Diego, CA, USA
[c] Department of Gastroenterological Surgery, Yokohama City University Graduate School of Medicine, Yokohama, Japan

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ABSTRACT

Gastric cancer is highly malignant and recalcitrant to first line chemotherapies that include 5-fluorouracil (5-FU). Cancer cells are addicted to methionine for their proliferation and survival. Methionine addiction of cancer is known as the Hoffman effect. Methionine restriction with recombinant methioninase (rMETase) has been shown to selectively starve cancer cells and has shown synergy with cytotoxic chemotherapy including 5-FU. The present study aimed to investigate the efficacy of rMETase alone and the combination with 5-FU on poorly differentiated human gastric cancer cell lines (MKN45, NUGC3, and NUGC4) in vitro and vivo. rMETase suppressed the tumor growth of 3 kinds of poorly differentiated gastric cancer cells in vitro. The fluorescence ubiquitination-based cell cycle indicator (FUCCI) demonstrated cancer cells treated with rMETase were selectively trapped in the S/G_2 phase of the cell cycle. In the present study, subcutaneous MKN45 gastric cancer models were randomized into four groups when the tumor volume reached 100 mm^3: G1: untreated control; G2: 5-FU (i.p., 50 mg/kg, weekly, three weeks); G3: oral-rMETase (o-rMETase) (p.o., 100 units/body, daily, three weeks); G4: 5-FU with o-rMETase (5-FU; i.p., 50 mg/kg, weekly, three weeks o-rMETase; p.o., 100 units/body, daily, three weeks). All mice were sacrificed on day 22. Body weight and estimated tumor volume were measured twice a week. 5-FU and o-rMETase suppressed tumor growth as monotherapies on day 18 ($p = 0.044$ and $p = 0.044$). However, 5-FU combined with o-rMETase was significantly superior to each monotherapy ($p < 0.001$ and $p < 0.001$, respectively) and induced extensive necrosis compared to other groups. The combination of 5-FU and o-rMETase shows promise for transformative therapy for poorly differentiated gastric cancer in the clinic.

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1. Introduction

The prognosis of gastric cancer is improving with advances in endoscopic therapy and the advent of new treatment options such as immune-checkpoint inhibitors [1]. However, gastric cancer remains one of the leading causes of death worldwide and developing new and effective treatments is imperative [2]. In particular, poorly-differentiated gastric cancer has a worse prognosis than highly differentiated tumors and is recalcitrant to first-line chemotherapy, including 5-FU [3].

We discovered that cancer cells are addicted to methionine (MET), an essential amino acid in humans [4–6]. All cancers have been shown to be methionine addicted due to methionine overuse for high levels of aberrant transmethylation [7]. This fundamental and general hallmark of cancer is termed the “Hoffman effect” [8]. Methionine restriction (MR) arrests the cell cycle of cancer cells in

* Corresponding author. Department of Gastroenterological Surgery, Yokohama City University Graduate School of Medicine, Yokohama, Japan.
** Corresponding author.
*** Corresponding author. Department of Surgery, University of California, San Diego, CA, USA.
E-mail addresses: kmkmkm@yokohama-cu.ac.jp (K. Miyake), endoit@med.yokohama-cu.ac.jp (I. Endo), all@anticancer.com (R.M. Hoffman).
[1] Equal contributors.

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the S/G2 phase and suppresses tumor growth [9,10]. In addition, MR enhances the efficacy of cytotoxic chemotherapy drugs due to the S/G_2-phase cell cycle trap of the cancer cells [11–15]. However, methionine is contained in all foods and is difficult to limit by diet alone.

Thus, we developed recombinant methioninase (rMETase), a methionine-degrading enzyme, and reported the anti-tumor efficacy of methioninase in many malignant cancers in vitro and in vivo [16–21]. Furthermore, we found that rMETase exhibits anti-tumor efficacy when administered orally (oral-rMETase [o-rMETase]) [21–27].

In the present study, we tested the efficacy of o-rMETase on poorly differentiated gastric cancer cell lines in vitro and in vivo, focusing on oral administration, and combination with 5-FU.

2. Materials and methods

2.1. Cell lines and cell culture

MKN45, NUGC3 and NUGC4, human poorly differentiated gastric cancer cell lines were used for in vitro experiments. Cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 100 IU/ml penicillin/streptomycin in a humidified atmosphere (5% CO_2 at 37 °C).

2.2. Recombinant methioninase (rMETase) production

We cloned recombinant L-methionine α-deamino-γ-mercapto-methane lyase (recombinant methionase, [rMETase]) [EC 4.4.1.11] from Pseudomonas putida. rMETase was produced in *Escherichia coli* (AntiCancer, Inc., San Diego, CA), as described previously [16,28,29].

2.3. Cell viability assay

Cell viability of gastric cancer cell lines was assessed by the Cell Counting Kit-8 (CCK-8; Dojindo, Kumamoto, Japan). Each gastric cancer cell line was treated with rMETase at concentrations between 0 and 30 units/ml, and 5-FU at concentrations between 0 and 300 mg/ml. Absorption at 450 nm was measured by a microprocessor-controlled microplate reader (Sunrise™; TECAN, San Jose, CA, USA) to determine cell viability with the CCK-8, as previously reported [27].

2.4. Establishment of FUCCI-expressing MKN-45 cells

Previously, two plasmids were utilized for establishment of FUCCI-expressing MKN-45 cells:

mKO2-hCdt1 containing an orange-red fluorescent protein and mAG-hGem, containing a green fluorescent protein (Medical and Biological Laboratory, Nagoya, Japan) which were sequentially transfected into MKN-45 cells with the use of Lipofectamine™ LTX (Invitrogen, Carlsbad, CA) [30]. After transfection with each plasmid, the cells were cultured for appropriate periods of time and sorted for the fluorescence color corresponding to plasmids used as described before [31].

2.5. Imaging of FUCCI-MKN45 cells

After seeding FUCCI-MKN45 cells on 35 mm glass dishes and cultured overnight, cells were treated with rMETase, at 0, 0.25, and 0,50 units/ml for 48 h. The FV1000 confocal laser scanning microscope (Olympus TOKYO, Japan) which contains 473 nm and 559 nm lasers, was used to visualize the cell cycle of single FUCCI-expressing MKN-45 cells [32].

2.6. Animal studies

Athymic nu/nu female nude mice (AntiCancer, Inc., San Diego, CA) which were 4–6 weeks old, were used in this study. All mice were maintained in a barrier facility on a high efficiency particulate arrestance (HEPA)-filtered rack under standard conditions of 12-h light/dark cycles. The animals were fed an autoclaved laboratory rodent diet. All animal experiments were conducted with an AntiCancer Institutional Animal Care and Use Committee (IACUC)-protocol specifically approved for this study and in accordance with the principals and procedures outlined in the National Institutes of Health Guide for the Care and Use of Animals under Assurance Number A3873-1. Subcutaneous injection of an anesthesia mixture (a 0.02 ml solution of 20 mg/kg ketamine, 15.2 mg/kg xylazine, and 0.48 mg/kg acepromazine maleate) was used for all mice to avoid suffering of the mice as previously reported [33].

2.7. In vivo study using the MKN45 subcutaneous model

MKN45 (2×10^6 cells) were injected subcutaneously into the flanks of nude mice.

After 3–4 weeks harvest, the grown subcutaneous tumor was cut into 2–3 mm tumor pieces and implanted under the right flanks of nude mice. The subcutaneous models were divided randomly into 4 groups below when tumor volume reached 100 mm^3; G1: Control group; G2: 5-FU group (50 mg/kg, i.p., weekly, 3 weeks); G3: o-rMETase group (50 U, p.o., twice a day, 3 weeks); G4: Combination group (5-FU: 50 mg/kg, i.p., weekly, 3 weeks, o-rMETase: 50 U, p.o., twice a day, 3 weeks). Each group consisted of 6 nude mice (Fig. 1). We determined these dosages from previous reports [25,34]. Tumor size and body weight were evaluated twice a week. Estimated tumor volume was calculated by the following predictive formula: tumor volume (mm^3) = length (mm) × width (mm) × width (mm) × 1/2. All mice were humanly sacrificed after 3 weeks, or the estimated tumor volume exceed 2000 mm^3.

2.8. H & E staining and immunohistochemistry

Hematoxylin and eosin (H&E) staining and immunohistochemical staining were performed as previously described. In instances where there were discrepancies, a multiheaded microscope was used for consensus among researchers.

2.9. Statistical analysis

All statistical analyses were analyzed by free statistical software EZR (Saitama Medical Center, Jichi Medical University), which is a graphical user interface for R (The R Foundation for Statistical Computing, version 3. 4. 1.). It is a modified version of R commander (version 2. 4-0) including statistical functions for biostatistics. Kruskal-Wallis with Steel-Dwass for post hoc analysis was used for a non-parametric test to compare intragroup. A probability value of $P < 0.05$ was defined as statistical significance.

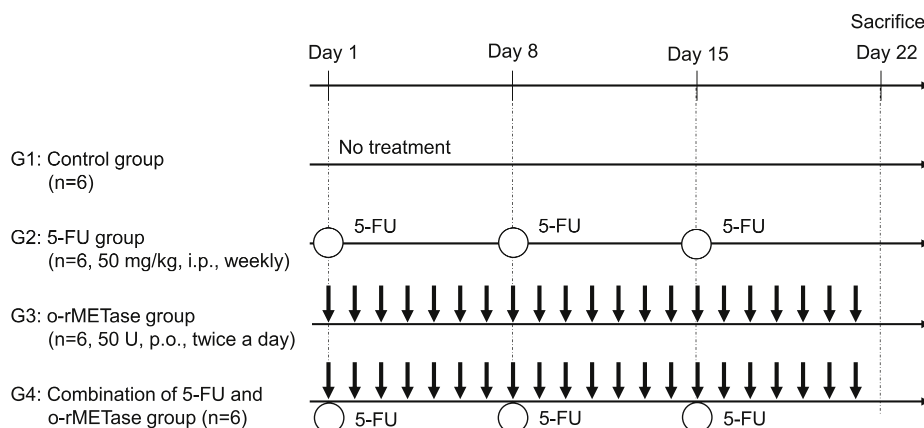
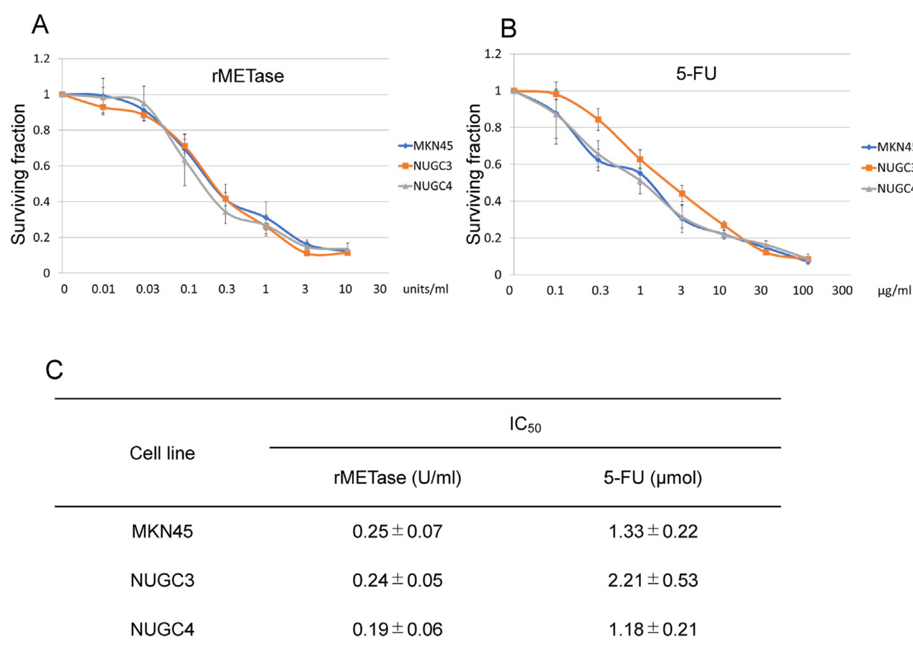


Fig. 1. Treatment protocol. G1: Control group; G2: 5-FU group (50 mg/kg, i.p., weekly, 3 weeks); G3: rMETase group (50 U, p.o., twice a day, 3 weeks); G4: Combination group (5-FU: 50 mg/kg, i.p., weekly, 3 weeks, o-rMETase: 50 U, p.o., twice a day, 3 weeks). Each group consisted of 6 mice. Tumor size and body weight were measured 2 times per week. All mice were sacrificed on day 22.



Cell line	IC_{50}	
	rMETase (U/ml)	5-FU (μmol)
MKN45	0.25 ± 0.07	1.33 ± 0.22
NUGC3	0.24 ± 0.05	2.21 ± 0.53
NUGC4	0.19 ± 0.06	1.18 ± 0.21

Fig. 2. rMETase and 5-FU IC_{50} for the poorly-differentiated gastric cancer cells. A: Dose response curve of rMETase for each poorly-differentiated gastric cancer cells (MKN45, NUGC3, and NUGC4). B: Dose response curve of 5-FU for each poorly-differentiated gastric cancer cell line. C: IC50 of rMETase and 5-FU for each gastric cancer cell line.

3. Results

3.1. IC_{50} values for rMETase and 5-FU on gastric cancer cell lines

We first evaluated the efficacy of rMETase and 5-FU, which is a first-line drug for gastric cancer on three poorly-differentiated gastric cancer cell lines: MKN45, NUGC3, and NUGC4. Fig. 2-A, B show the dose response curve of rMETase and 5-FU for each gastric cancer cell line. The IC_{50} value of rMETase for MKN45 was 0.25 ± 0.07 units/ml, for NUGC3 was 0.24 ± 0.05 units/ml, for NUGC4 was 0.19 ± 0.06 units/ml. The IC_{50} value of 5-FU for MKN45 was 1.33 ± 0.22 μmol, for NUGC3 was 2.21 ± 0.53 μmol, and NUGC4 was 1.18 ± 0.21 μmol (Fig. 2-C).

3.2. FUCCI imaging shows rMETase traps MKN45 cancer cells in S/G_2 phase of the cell cycle

We chose MKN45 to demonstrate the cell cycle trap by rMETase. FUCCI imaging after 48 h revealed a large shift of cancer-cell population from G_0/G_1 (red color) to S/G_2/M (green color) (Fig. 3-A). There was a significant cell cycle trap effected by rMETase. The control had approximately 40% of the cells in S/G_2, and the rMETase-treated cells had approximately 80% in the S/G_2 phase (Fig. 3-B).

3.3. Synergy of o-rMETase and 5-FU on MKN45 tumor growth

We tested the efficacy of 5-FU and o-rMETase alone, and their combination on MKN45 grown in nude mice. 5-FU and o-rMETase significantly suppressed the tumor growth at day 18 ($p < 0.05$) (Fig. 4). All mice in the control group were humanly sacrificed on day 18 due to tumor size over 2000 mm^3. The combination of o-rMETase with 5-FU was more effective compared to each monotherapy ($p < 0.001$, respectively). The final estimated tumor volume (mm^3) was as follows: the untreated control group (G1) (2319 ± 812) (day 18); 5-FU-treated (G2) (1332 ± 455); o-rMETase-

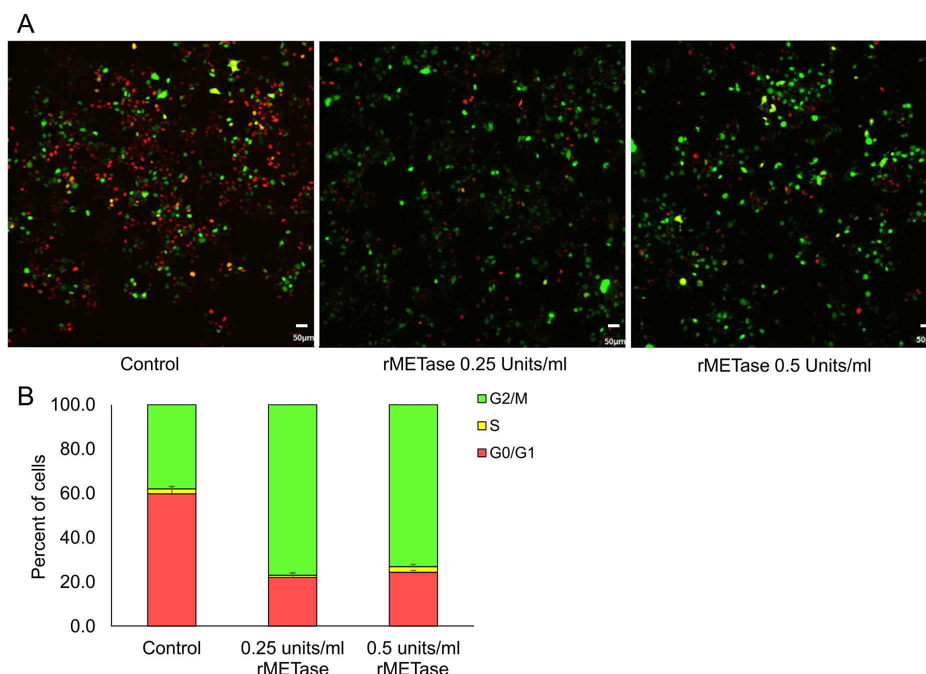


Fig. 3. FUCCI cell-cycle imaging of rMETase treated MKN45 gastric cancer cell line. A: Representative images of MK45N–FUCCI cells treated with rMETase at the indicated doses. The histogram shows the percentages of cells in G_1 (red), early S (yellow), or late G_2/M (green). FUCCI imaging after 48 h showed that rMETase induced a significant shift in the cancer-cell population from G_0/G_1 to S/G_2/M period. Experiments were repeated 5 times. Scale bars: 50 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

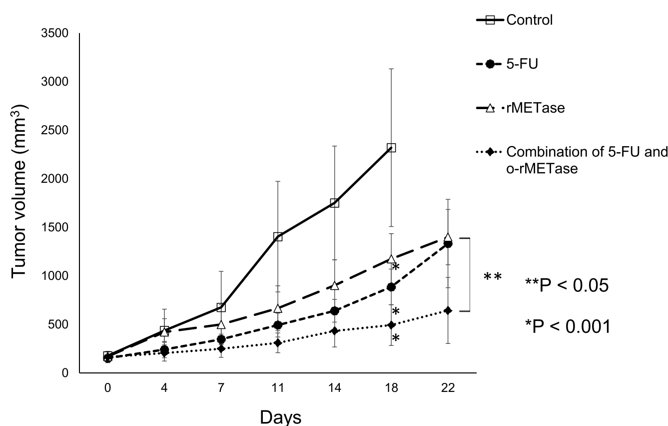


Fig. 4. Efficacy of o-rMETase and 5-FU alone and in combination on MKN45 tumors in nude mice. Line graphs show the tumor volume (mm^3) throughout the treatment protocol. 5-FU and o-rMETase inhibited tumor growth significantly compared with the untreated group respectively on day18 ($P < 0.05$). 5-FU combined with o-rMETase was more effective treatment than either monotherapy on day 22 ($P < 0.001$). Error bars: ±SD. *P < 0.05, **P < 0.001.

treated (G3) (1399 ± 284); combination of 5-FU and rMETase (G4) (642 ± 340). There was no significant decrease in body weight in any groups, and no statistical difference in body weight between each group (**Supplemental Figure**).

3.4. Synergy of o-rMETase and 5-FU to induce necrosis of MKN45

5-FU resulted in slight necrosis compared to the untreated group ($p < 0.001$) (Fig. 5). o-rMETase did not induce significant necrosis compared to the control group. The combination of rMETase and 5-FU showed extent necrosis compared to 5-FU or rMETase treatment alone ($p < 0.001$).

3.5. Synergy of o-rMETase and 5-FU to inhibit gastric cancer cell proliferation in vivo

Ki-67 immunohistochemical staining for evaluation of the proliferative capacity of MKN45 cells showed that tumors treated with combination therapy of 5-FU and o-rMETase had a significantly lower Ki-67 index than the control or 5-FU treated tumors ($p < 0.001$, respectively) (Fig. 6). The Ki-67 labelling index (%) in each group was as follows: the untreated control group (G1) (69.6 ± 10.1); 5-FU-treated (G2) (45.3 ± 6.7); o-rMETase-treated (G3) (66.7 ± 5.1); combination therapy-treated (G4) (28.3 ± 8.6).

4. Discussion

In the present study, we demonstrated that rMETase suppressed the proliferation of poorly-differentiated gastric cancer cells in vitro by trapping them in S/G_2 phase of the cell cycle as previously shown for other cancer cell type [9,10,34]. The S/G_2 phase is generally the most drug-sensitive phase for cancer cells and is the probable basis of the synergy of the combination therapy of 5-FU with o-rMETase in the MKN45 subcutaneous tumor mouse model tested in the present report.

Cancer cells require more methionine for their proliferation than normal cells [35]. Cancer cells can biosynthesize methionine from homocysteine but still require extensive methionine for transmethylation reactions compared to normal cells [4,36,37]. Methionine addiction of cancer cells is termed the “Hoffman effect” [8]. The Warburg effect of glucose addiction of cancer is less than the Hoffman effect as [^{11}C]-MET positron emission tomography (PET) shows a stronger signal than 2-[fluorine-18] fluoro-2- deoxy-D-glucose-PET in cancer patients [38,39].

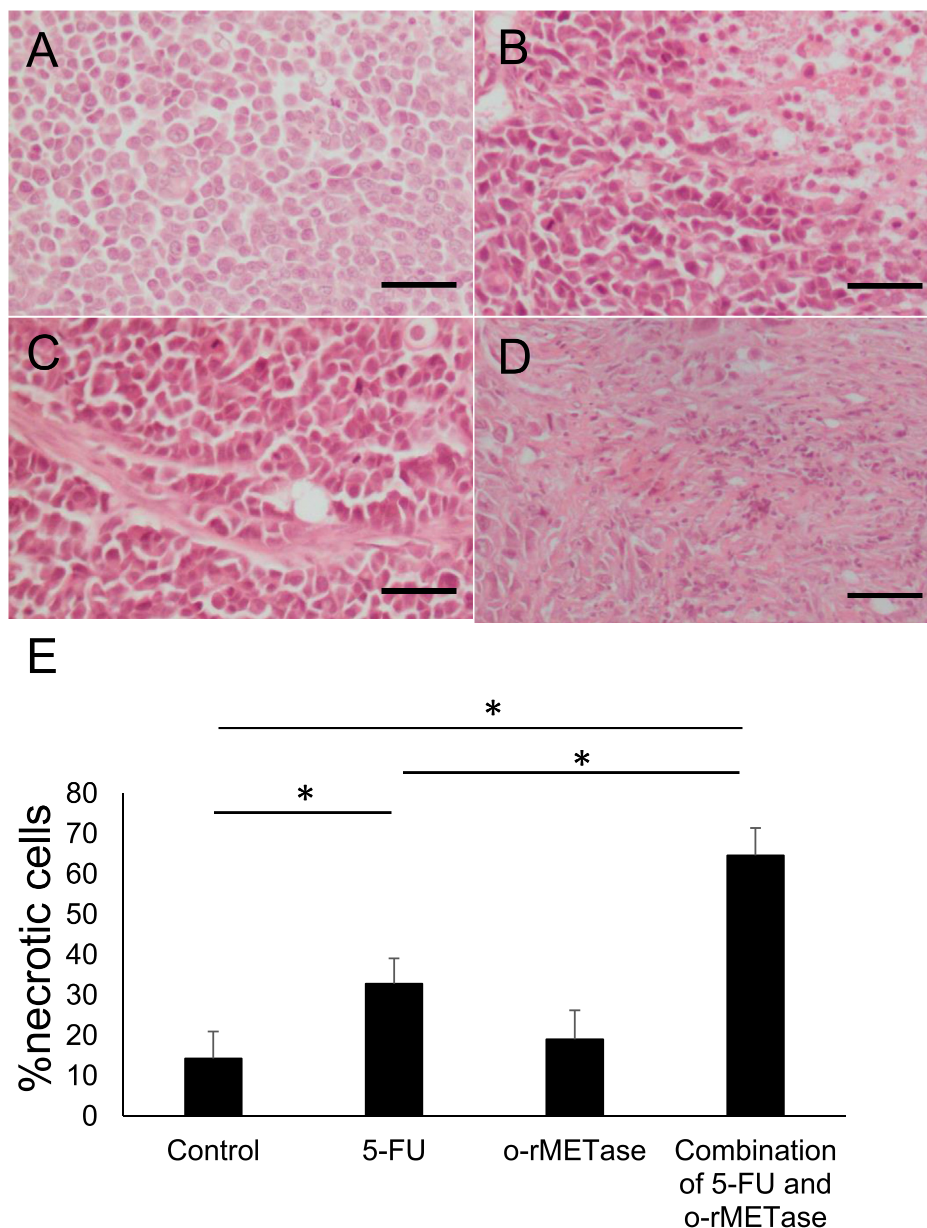


Fig. 5. Effect of treatment on tumor histology. A: Control. B: 5-FU. C: o-rMETase. D: combination. E: Necrosis extent of the tumor. The combination of 5FU + rMETase induced significant necrosis compared to other groups. Scale bars: 100 μm *P < 0.001. Error bars: ±SD.

Although its mechanism is not fully understood, methionine restriction induces apoptosis of cancer cells and reversible arrest of the cell cycle in S/G_2 [7,8]. Since methionine is found in a variety of foods, it is difficult to limit it completely through diet, which can lead to a decrease in the patient's quality of life. For this reason, we have developed an enzyme, rMETase, which degrades methionine. rMETase inhibits cancer growth by decreasing methionine levels in blood and tumors [17]. rMETase has synergy with cytotoxic chemotherapy as originally shown by us in 1986 [7]. Recently, we reported that combination therapy of rMETase with TNF-related apoptosis-induced ligand receptor-2 (TRAIL-R2) agonist tigatuzumab, a molecular targeting agent, inhibited pancreatic cancer by increasing TRAIL-R2 expression [27].

Oral administration of rMETase has been developed recently and does not induce anaphylaxis. It has been shown to reduce blood methionine levels for up to 6 h after oral administration, making it more realistic than intraperitoneal administration [23]. o-rMETase has also shown efficacy in mouse models of various cancers and has shown synergy in combination with chemotherapy including gemcitabine, 5-FU, etc. [25,26,34,40,41].

We have already started to use rMETase in clinical practice: rMETase was administered every two weeks in combination with the combination of 5-fluorouracil/leucovorin, irinotecan, and oxaliplatinum for Stage IV advanced pancreatic cancer and in combination with a low methionine diet [42]. As a result, the patient has maintained tumor shrinkage for 19 months. rMETase was administered for a case with local recurrence of rectal cancer. rMETase and methionine restriction with a low methionine diet have maintained tumor shrinkage and normalization of tumor markers for 1.5 years [43]. o-rMETase has also shown efficacy in prostate cancer patients and metastatic breast cancer patients [44–47], and metastatic breast cancer patient [48].

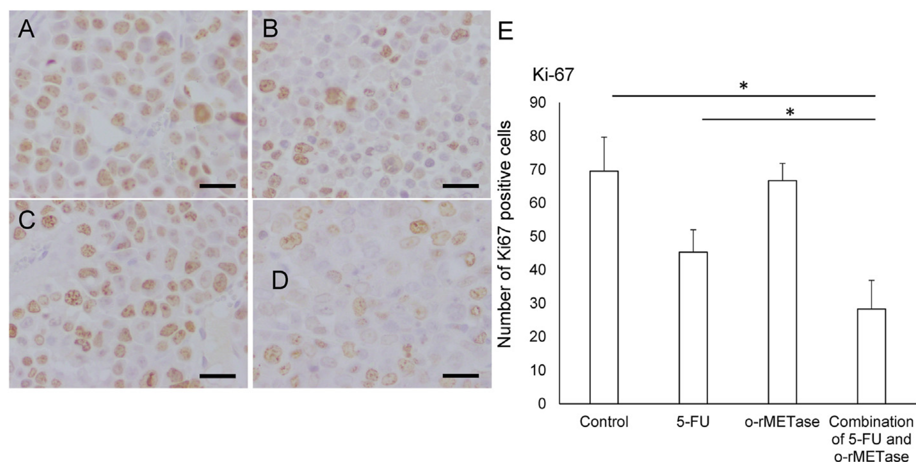


Fig. 6. Effect of treatment on Ki-67 expression. A: Control. B: 5-FU. C: o-rMETase. D: Combination of 5-FU and rMETase. E: Percentage of ki-67 positive tumor cells in each group. Scale bars: 50 μm. *P < 0.001. Error bars: ±SD.

5. Conclusions

The present results indicate that the combination of 5-FU with o-rMETase is a promising future clinical therapy for poorly differentiated gastric cancer.

Dedication

This paper is dedicated to the memory of A. R. Moossa, M.D., Sun Lee, M.D., Masaki Kitajima, M.D., Shigeo Yagi, Ph.D., and Jack Geller, M.D.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix ASupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2022.12.062.

References

[1] D.A. Norwood, E.E. Montalvan, R.L. Dominguez, D.R. Morgan, Gastric cancer: emerging trends in prevention, diagnosis, and treatment, Gastroenterol. Clin. N. Am. 51 (2022) 501–518, https://doi.org/10.1016/j.gtc.2022.05.001.

[2] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA A Cancer J. Clin. 68 (2018) 394–424, https://doi.org/10.3322/caac.21492.

[3] R. Xiang, Y. Rong, Y. Ge, W. Song, J. Ren, T. Fu, Cell differentiation trajectory predicts patient potential immunotherapy response and prognosis in gastric cancer, Aging (Albany NY) 13 (2021) 5928–5945, https://doi.org/10.18632/aging.202515.

[4] D.W. Coalson, J.O. Mecham, P.H. Stern, R.M. Hoffman, Reduced availability of endogenously synthesized methionine for S-adenosylmethionine formation in methionine-dependent cancer cells, Proc. Natl. Acad. Sci. U. S. A. 79 (1982) 4248–4251, https://doi.org/10.1073/pnas.79.14.4248.

[5] R.M. Hoffman, R.W. Erbe, 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 (1976) 1523–1527, https://doi.org/10.1073/pnas.73.5.1523.

[6] P.H. Stern, J.O. Mecham, C.D. Wallace, R.M. Hoffman, Reduced free-methionine in methionine-dependent SV40-transformed human fibroblasts synthesizing apparently normal amounts of methionine, J. Cell. Physiol. 117 (1983) 9–14, https://doi.org/10.1002/jcp.1041170103.

[7] P.H. Stern, R.M. Hoffman, Elevated overall rates of transmethylation in cell lines from diverse human tumors, In Vitro 20 (1984) 663–670, https://doi.org/10.1007/bf02619617.

[8] P. Kaiser, Methionine dependence of cancer, Biomolecules 10 (2020), https://doi.org/10.3390/biom10040568.

[9] R.M. Hoffman, S.J. Jacobsen, Reversible growth arrest in simian virus 40-transformed human fibroblasts, Proc. Natl. Acad. Sci. U. S. A. 77 (1980) 7306–7310, https://doi.org/10.1073/pnas.77.12.7306.

[10] S. Yano, S. Li, Q. Han, Y. Tan, M. Bouvet, T. Fujiwara, R.M. Hoffman, Selective methioninase-induced trap of cancer cells in S/G2 phase visualized by FUCCI imaging confers chemosensitivity, Oncotarget 5 (2014) 8729–8736, https://doi.org/10.18632/oncotarget.2369.

[11] P.H. Stern, R.M. Hoffman, Enhanced in vitro selective toxicity of chemotherapeutic agents for human cancer cells based on a metabolic defect, J. National Cancer Institute 76 (1986) 629–639, https://doi.org/10.1093/jnci/76.4.629.

[12] Y. Tan, X. Sun, M. Xu, X. Tan, A. Sasson, B. Rashidi, Q. Han, X. Tan, X. Wang, Z. An, F.X. Sun, R.M. Hoffman, Efficacy of recombinant methioninase in combination with cisplatin on human colon tumors in nude mice, Clin. Cancer Res. 5 (1999) 2157–2163.

[13] T. Yoshioka, T. Wada, N. Uchida, H. Maki, H. Yoshida, N. Ide, H. Kasai, K. Hojo, K. Shono, R. Maekawa, S. Yagi, R.M. Hoffman, K. Sugita, Anticancer efficacy in vivo and in vitro, synergy with 5-fluorouracil, and safety of recombinant methioninase, Cancer Res. 58 (1998) 2583–2587.

[14] Y. Hoshiya, T. Kubota, T. Inada, M. Kitajima, R.M. Hoffman, Methionine-depletion modulates the efficacy of 5-fluorouracil in human gastric cancer in nude mice, Anticancer Res. 17 (1997) 4371–4375.

[15] Y. Hoshiya, T. Kubota, S.W. Matsuzaki, M. Kitajima, R.M. Hoffman, Methionine starvation modulates the efficacy of cisplatin on human breast cancer in nude mice, Anticancer Res. 16 (1996) 3515–3517.

[16] Y. Tan, M. Xu, X. Tan, X. Tan, X. Wang, Y. Saikawa, T. Nagahama, X. Sun, M. Lenz, R.M. Hoffman, Overexpression and large-scale production of recombinant L-methionine-alpha-deamino-gamma-mercaptomethane-lyase for novel anticancer therapy, Protein Expr. Purif. 9 (1997) 233–245, https://doi.org/10.1006/prep.1996.0700.

[17] Y. Tan, Sr J. Zavala, M. Xu, J. Zavala Jr., R.M. Hoffman, Serum methionine depletion without side effects by methioninase in metastatic breast cancer patients, Anticancer Res. 16 (1996) 3937–3942.

[18] K. Igarashi, K. Kawaguchi, T. Kiyuna, K. Miyake, T. Murakami, N. Yamamoto, K. Hayashi, H. Kimura, S. Miwa, H. Tsuchiya, R.M. Hoffman, Effective metabolic targeting of human osteosarcoma cells in vitro and in orthotopic nude-mouse models with recombinant methioninase, Anticancer Res. 37 (2017) 4807–4812, https://doi.org/10.21873/anticanres.11887.

[19] K. Kawaguchi, K. Igarashi, S. Li, Q. Han, Y. Tan, T. Kiyuna, K. Miyake, T. Murakami, B. Chmielowski, S.D. Nelson, T.A. Russell, S.M. Dry, Y. Li, M. Unno, F.C. Eilber, R.M. Hoffman, Combination treatment with recombinant methioninase enables temozolomide to arrest a BRAF V600E melanoma in a patient-derived orthotopic xenograft (PDOX) mouse model, Oncotarget 8 (2017) 85516–85525, https://doi.org/10.18632/oncotarget.20231.

[20] D. Machover, J. Zittoun, R. Saffroy, P. Broët, S. Giraudier, T. Magnaldo, E. Goldschmidt, B. Debuire, M. Orrico, Y. Tan, Z. Mishal, O. Chevallier, C. Tonetti, H. Jouault, A. Ulusakarya, M.L. Tanguy, G. Metzger, R.M. Hoffman, Treatment of cancer cells with methioninase produces DNA hypomethylation and increases DNA synthesis, Cancer Res. 62 (2002) 4685–4689.

[21] K. Miyake, T. Kiyuna, S. Li, Q. Han, Y. Tan, M. Zhao, H. Oshiro, K. Kawaguchi, T. Higuchi, Z. Zhang, S. Razmjooei, M. Barangi, S. Wangsiricharoen, T. Murakami, A.S. Singh, Y. Li, S.D. Nelson, F.C. Eilber, M. Bouvet, Y. Hiroshima, T. Chishima, R. Matsuyama, S.R. Singh, I. Endo, R.M. Hoffman, Combining tumor-selective bacterial therapy with Salmonella typhimurium A1-R and cancer metabolism targeting with oral recombinant methioninase regressed

an ewing's sarcoma in a patient-derived orthotopic xenograft model, Chemotherapy 63 (2018) 278–283, https://doi.org/10.1159/000495574.
[22] Y. Aoki, Y. Tome, Q. Han, J. Yamamoto, K. Hamada, N. Masaki, Y. Kubota, M. Bouvet, K. Nishida, R.M. Hoffman, Oral-recombinant methioninase converts an osteosarcoma from methotrexate-resistant to -sensitive in a patient-derived orthotopic-xenograft (PDOX) mouse model, Anticancer Res. 42 (2022) 731–737, https://doi.org/10.21873/anticanres.15531.
[23] R.M. Hoffman, Q. Han, K. Kawaguchi, S. Li, Y. Tan, Afterword: oral methioninase-answer to cancer and fountain of youth? Methods Mol. Biol. 1866 (2019) 311–322, https://doi.org/10.1007/978-1-4939-8796-2_24.
[24] H. Oshiro, Y. Tome, T. Kiyuna, S.N. Yoon, T.M. Lwin, Q. Han, Y. Tan, K. Miyake, T. Higuchi, N. Sugisawa, Y. Katsuya, J.H. Park, Z. Zang, S. Razmjooei, M. Bouvet, B. Clary, S.R. Singh, F. Kanaya, K. Nishida, R.M. Hoffman, Oral recombinant methioninase overcomes colorectal-cancer liver metastasis resistance to the combination of 5-fluorouracil and oxaliplatinum in a patient-derived orthotopic xenograft mouse model, Anticancer Res. 39 (2019) 4667–4671, https://doi.org/10.21873/anticanres.13648.
[25] J.H. Park, M. Zhao, Q. Han, Y. Sun, T. Higuchi, N. Sugisawa, J. Yamamoto, S.R. Singh, B. Clary, M. Bouvet, R.M. Hoffman, Efficacy of oral recombinant methioninase combined with oxaliplatinum and 5-fluorouracil on primary colon cancer in a patient-derived orthotopic xenograft mouse model, Biochem. Biophys. Res. Commun. 518 (2019) 306–310, https://doi.org/10.1016/j.bbrc.2019.08.051.
[26] N. Sugisawa, T. Higuchi, Q. Han, C. Hozumi, J. Yamamoto, Y. Tashiro, H. Nishino, K. Kawaguchi, M. Bouvet, T. Murata, M. Unno, R.M. Hoffman, Oral recombinant methioninase combined with paclitaxel arrests recalcitrant ovarian clear cell carcinoma growth in a patient-derived orthotopic xenograft (PDOX) nude-mouse model, Cancer Chemother. Pharmacol. 88 (2021) 61–67, https://doi.org/10.1007/s00280-021-04261-x.
[27] J. Yamamoto, K. Miyake, Q. Han, Y. Tan, S. Inubushi, N. Sugisawa, T. Higuchi, Y. Tashiro, H. Nishino, Y. Homma, R. Matsuyama, S.P. Chawla, M. Bouvet, S.R. Singh, I. Endo, R.M. Hoffman, Oral recombinant methioninase increases TRAIL receptor-2 expression to regress pancreatic cancer in combination with agonist tigatuzumab in an orthotopic mouse model, Cancer Lett. 492 (2020) 174–184, https://doi.org/10.1016/j.canlet.2020.07.034.
[28] R.M. Hoffman, Y. Tan, S. Li, Q. Han, S. Yagi, T. Takakura, A. Takimoto, K. Inagaki, D. Kudou, Development of recombinant methioninase for cancer treatment, methods in molecular biology, Clifton, N.J.) 1866 (2019) 107–131, https://doi.org/10.1007/978-1-4939-8796-2_10.
[29] R.M. Hoffman, Development of recombinant methioninase to target the general cancer-specific metabolic defect of methionine dependence: a 40-year odyssey, Expert Opin. Biol. Ther. 15 (2015) 21–31, https://doi.org/10.1517/14712598.2015.963050.
[30] S. Yano, K. Takehara, H. Tazawa, H. Kishimoto, Y. Urata, S. Kagawa, T. Fujiwara, R.M. Hoffman, Efficacy of a cell-cycle decoying killer adenovirus on 3-D gelfoam®-histoculture and tumor-sphere models of chemo-resistant stomach carcinomatosis visualized by FUCCI imaging, PLoS One 11 (2016), e0162991, https://doi.org/10.1371/journal.pone.0162991.
[31] S. Yano, S. Miwa, S. Mii, Y. Hiroshima, F. Uehara, M. Yamamoto, H. Kishimoto, H. Tazawa, M. Bouvet, T. Fujiwara, R.M. Hoffman, Invading cancer cells are predominantly in G0/G1 resulting in chemoresistance demonstrated by real-time FUCCI imaging, Cell Cycle 13 (2014) 953–960, https://doi.org/10.4161/cc.27818.
[32] K. Yamauchi, M. Yang, P. Jiang, M. Xu, N. Yamamoto, H. Tsuchiya, K. Tomita, A.R. Moossa, M. Bouvet, R.M. Hoffman, Development of real-time subcellular dynamic multicolor imaging of cancer-cell trafficking in live mice with a variable-magnification whole-mouse imaging system, Cancer Res. 66 (2006) 4208–4214, https://doi.org/10.1158/0008-5472.Can-05-3927.
[33] K. Miyake, T. Higuchi, H. Oshiro, Z. Zhang, N. Sugisawa, J.H. Park, S. Razmjooei, Y. Katsuya, M. Barangi, Y. Li, S.D. Nelson, T. Murakami, Y. Homma, Y. Hiroshima, R. Matsuyama, M. Bouvet, S.P. Chawla, S.R. Singh, I. Endo, R.M. Hoffman, The combination of gemcitabine and docetaxel arrests a doxorubicin-resistant dedifferentiated liposarcoma in a patient-derived orthotopic xenograft model, Biomed. Pharmacother. = Biomed. Pharmacotherapie 117 (2019), 109093, https://doi.org/10.1016/j.biopha.2019.109093.
[34] J.H. Park, Q. Han, M. Zhao, Y. Tan, T. Higuchi, S.N. Yoon, N. Sugisawa, J. Yamamoto, M. Bouvet, B. Clary, S.R. Singh, R.M. Hoffman, Oral recombinant methioninase combined with oxaliplatinum and 5-fluorouracil regressed a colon cancer growing on the peritoneal surface in a patient-derived orthotopic xenograft mouse model, Tissue Cell 61 (2019) 109–114, https://doi.org/10.1016/j.tice.2019.09.006.
[35] F. Breillout, E. Antoine, M.F. Poupon, Methionine dependency of malignant tumors: a possible approach for therapy, J. National Cancer Institute 82 (1990) 1628–1632, https://doi.org/10.1093/jnci/82.20.1628.
[36] J. Yamamoto, Y. Aoki, S. Inubushi, Q. Han, K. Hamada, Y. Tashiro, K. Miyake, R. Matsuyama, M. Bouvet, S.G. Clarke, I. Endo, R.M. Hoffman, Extent and instability of trimethylation of histone H3 lysine increases with degree of malignancy and methionine addiction, Cancer Genomics Proteomics 19 (2022) 12–18, https://doi.org/10.21873/cgp.20299.
[37] J. Yamamoto, S. Inubushi, Q. Han, Y. Tashiro, N. Sugisawa, K. Hamada, Y. Aoki, K. Miyake, R. Matsuyama, M. Bouvet, S.G. Clarke, I. Endo, R.M. Hoffman, Linkage of methionine addiction, histone lysine hypermethylation, and malignancy, iScience 25 (2022) 104162, https://doi.org/10.1016/j.isci.2022.104162.
[38] O. Warburg, On the origin of cancer cells, Science (New York, N.Y.) 123 (1956) 309–314, https://doi.org/10.1126/science.123.3191.309.
[39] K. Mitamura, Y. Yamamoto, T. Norikane, T. Hatakeyama, M. Okada, Y. Nishiyama, Correlation of (18)F-FDG and (11)C-methionine uptake on PET/CT with Ki-67 immunohistochemistry in newly diagnosed intracranial meningiomas, Ann. Nucl. Med. 32 (2018) 627–633, https://doi.org/10.1007/s12149-018-1284-6.
[40] T. Higuchi, Q. Han, K. Miyake, H. Oshiro, N. Sugisawa, Y. Tan, N. Yamamoto, K. Hayashi, H. Kimura, S. Miwa, K. Igarashi, M. Bouvet, S.R. Singh, H. Tsuchiya, R.M. Hoffman, Combination of oral recombinant methioninase and decitabine arrests a chemotherapy-resistant undifferentiated soft-tissue sarcoma patient-derived orthotopic xenograft mouse model, Biochem. Biophys. Res. Commun. 523 (2020) 135–139, https://doi.org/10.1016/j.bbrc.2019.12.024.
[41] H.I. Lim, Y.U. Sun, Q. Han, J. Yamamoto, R.M. Hoffman, Efficacy of oral recombinant methioninase and eribulin on a PDOX model of triple-negative breast cancer (TNBC) liver metastasis, In Vivo 35 (2021) 2531–2534, https://doi.org/10.21873/invivo.12534.
[42] Y. Kubota, Q. Han, C. Hozumi, N. Masaki, J. Yamamoto, Y. Aoki, T. Tsunoda, R.M. Hoffman, Stage IV pancreatic cancer patient treated with folfirinox combined with oral methioninase: a highly-rare case with long-term stable disease, Anticancer Res. 42 (2022) 2567–2572, https://doi.org/10.21873/anticanres.15734.
[43] Y. Kubota, Q. Han, K. Hamada, Y. Aoki, N. Masaki, K. Obara, T. Tsunoda, R.M. Hoffman, 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 (2022) 3857–3861, https://doi.org/10.21873/anticanres.15877.
[44] Q. Han, Y. Tan, R.M. Hoffman, 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 (2020) 2813–2819, https://doi.org/10.21873/anticanres.14254.
[45] Q. Han, R.M. Hoffman, Lowering and stabilizing PSA levels in advanced-prostate cancer patients with oral methioninase, Anticancer Res. 41 (2021) 1921–1926, https://doi.org/10.21873/anticanres.14958.
[46] Q. Han, R.M. Hoffman, Chronic treatment of an advanced prostate-cancer patient with oral methioninase resulted in long-term stabilization of rapidly rising PSA levels, In vivo (Athens, Greece) 35 (2021) 2171–2176, https://doi.org/10.21873/invivo.12488.
[47] J. Yamamoto, Q. Han, M. Simon, D. Thomas, R.M. Hoffman, Methionine restriction: ready for prime time in the cancer clinic? Anticancer Res. 42 (2022) 641–644, https://doi.org/10.21873/anticanres.15521.
[48] Y. Kubota, Q. Han, N. Masaki, C. Hozumi, K. Hamada, Y. Aoki, K. Obara, T. Tsunoda, R.M. Hoffman, 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 (2022) 5819–5823, https://doi.org/10.21873/anticanres.16089.