

Methionine Restriction Must Be Continuously Maintained to Selectively Inhibit Cancer Cells Co-cultured With Normal Cells

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Abstract

Background/Aim: Methionine addiction (Hoffman effect) is a fundamental and general cancer hallmark targetable by methionine restriction, using methionine-depleted media or diet, or recombinant methioninase (rMETase). Our previous studies showed differential sensitivity of HCT-116 colon-cancer cells and Hs27 normal fibroblasts to rMETase in co-culture. The present study aimed to demonstrate the rescue conditions of cancer cells by methionine replenishment in the co-cultures of HCT-116 and Hs27 cells after rMETase treatment.

Materials and Methods: Equal numbers of HCT-116 colon-cancer cells and Hs27 normal fibroblasts were co-cultured in 6-well plates in Dulbecco's modified Eagle's medium (DMEM). Two days after seeding, co-cultures were treated with rMETase at the HCT-116 IC₅₀ (0.46 U/ml) or left untreated as controls. Growth of each cell type in co-culture was evaluated by phase-contrast microscopy on days 2, 4, 6, 8, 10, and 12 after treatment to assess the response to rMETase. On day 12, the existing medium in all wells was replaced with fresh DMEM containing methionine (methionine replenishment). Regrowth of HCT-116 and Hs27 was then assessed by phase-contrast microscopy 3, 6, and 9 days later.

Results: In the untreated control group, HCT-116 cancer cells rapidly proliferated, and progressively overtook the Hs27 fibroblasts and predominated by day 12. In the rMETase-treated group, viable HCT-116 cells progressively decreased and were almost undetectable by day 12, whereas Hs27 cells remained viable throughout the observation period. After day-12 replenishment of methionine, previously rMETase-treated co-cultures showed reappearance of viable HCT-116 cells by day 3 and dominance over Hs27 cells by day 9.

Conclusion: Continuous treatment with rMETase is necessary to maintain inhibition of cancer cells and normal-cell dominance in co-culture with cancer cells. These results have clinical implications indicating that methionine restriction must be continually maintained to inhibit cancer.

Keywords: Cancer cells, methionine addiction, Hoffman effect, normal fibroblasts, co-culture, recombinant methioninase, selective cancer efficacy, methionine rescue.



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Introduction

Methionine addiction is a fundamental and general hallmark of cancer, known as the Hoffman effect (1-12). Cancer cells show an increased demand for methionine to maintain their proliferation and survival, whereas normal cells can still grow under methionine-restricted conditions (1, 3, 4, 11). The specific vulnerability of cancer cells to methionine restriction is a therapeutic target and methionine depletion strategies – including methionine-restricted media or diet, and treatment with the methionine-cleaving enzyme recombinant methioninase (rMETase)- have been widely studied to target methionine addiction.

In our earlier studies, we demonstrated that HCT-116 human colon-cancer cells are more sensitive to rMETase treatment than Hs27 normal fibroblasts, showing a significant decrease in viability of HCT-116 cells under methionine depletion, compared to the normal fibroblasts (13). We also showed that HCT-116 cells treated with rMETase underwent cell toxicity but were rescued and resumed growth following methionine replenishment (14).

To better model the tumor microenvironment, we developed a co-culture model of HCT-116 cancer cells with Hs27 normal fibroblasts. In this co-culture model, rMETase treatment selectively eliminated the cancer cells while sparing the normal fibroblasts (15).

Based on these findings, the present study investigated whether methionine replenishment after treatment with rMETase, at the HCT-116 IC₅₀ concentration, could rescue cancer cells in the co-culture model of HCT-116 and Hs27 cells. The present study will enable further understanding of the dynamics of methionine addiction in cancer cells and its potential therapeutic implications under more physiologically-relevant conditions.

Materials and Methods

Cell culture. HCT-116 human colorectal cancer cells and Hs27 normal fibroblasts were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Both

cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM; GIBCO, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; GIBCO) and 1 IU/ml of penicillin-streptomycin (GIBCO). Cells were cultured at 37°C in a humidified incubator under 5% CO₂ atmosphere.

Co-culture model. To establish the co-culture model, 3.75×10⁴ HCT-116 cells and 3.75×10⁴ Hs27 cells were seeded together per well in 6-well plates containing DMEM, for a total of 7.5×10⁴ cells per well.

Recombinant methioninase (rMETase) production. rMETase, a methionine-degrading enzyme originally derived from *Pseudomonas putida*, was produced by fermenting genetically engineered *Escherichia coli* carrying the *Pseudomonas putida methioninase gene*. rMETase production and purification were carried out at AntiCancer Inc. (San Diego, CA, USA) as previously described using a heat step, polyethylene-glycol precipitation, and DEAE Sepharose ion-exchange chromatography (16, 17).

Recombinant methioninase (rMETase) treatment of co-cultures. On day 2 after seeding, wells were designated as either a control group (no treatment) or a treatment group, which received rMETase at the HCT-116 IC₅₀ concentration of 0.46 U/ml, as determined in preliminary experiments (15). The co-cultures were observed and photographed using an IX71 inverted microscope under phase contrast (Olympus, Tokyo, Japan) on days 2, 4, 6, 8, 10, and 12 after rMETase treatment.

Methionine replenishment of rMETase-treated co-cultures. On day 12, when no viable cancer cells could be observed under phase-contrast microscopy in the treatment group, the culture medium in all wells (with or without rMETase) was replaced with standard DMEM containing methionine, to initiate methionine replenishment in the treatment group. Following methionine replenishment, the co-cultures were observed under phase-contrast microscopy

and documented on days 3, 6, and 9 after the medium was replaced with fresh DMEM.

Results

By day 2 of the experimental timeline (day 4 after seeding), HCT-116 cancer cells in the co-culture without rMETase treatment (control group) had already proliferated rapidly and become the dominant cell type, occupying a significant portion of the well surface. Subsequently, in the control group, the clusters of HCT-116 cells continued to expand and became increasingly dominant by day 12, while the Hs27 fibroblasts appeared less prominent, forming narrow streams between the expanding HCT-116 clusters as they were progressively displaced. In the rMETase-treated co-culture (treatment group), the Hs27 normal fibroblasts maintained their viability throughout the observation period. In contrast, HCT-116 cancer cells gradually decreased in number, with no viable cells detectable under phase-contrast microscopy by day 12 after rMETase treatment began (Figure 1). This observation was consistent with and fully reproduced our previous findings (15).

On day 12, the existing medium in both groups was replaced with fresh DMEM containing methionine (methionine replenishment). In the previously-treated rMETase group, viable HCT-116 cancer cells reappeared by day 3 after methionine replenishment (day 15 after initial treatment). By day 6, the proportion of rescued HCT-116 cells became comparable to Hs27 cells, and by day 9, HCT-116 cells had proliferated extensively, becoming dominant over the Hs27 fibroblasts. In the control group, HCT-116 cells continued to expand throughout the observation period, and by day 9 after methionine replenishment, Hs27 cells were no longer visible, with HCT-116 cells dominating the co-culture (Figure 2).

Discussion

The co-culture system modeling competitive interactions between HCT-116 colorectal cancer cells and Hs27 normal

fibroblasts highlights the large disparity in methionine requirements between cancer and normal cells. Treatment with rMETase at the HCT-116 IC_{50} (0.46 U/ml) shifted dominance toward the normal fibroblasts, such that by day 12 of treatment, no viable cancer cells were detectable by phase-contrast microscopy. However, when rMETase was discontinued and methionine was replenished with DMEM on day 12, HCT-116 cells reappeared by day 15 (3 days post-replenishment) and regained dominance by day 21 (9 days post-replenishment). These cellular kinetics indicate that methionine restriction can reverse the growth advantage of methionine-addicted cancer cells, whereas restoration of methionine permits rapid rescue and regrowth of the cancer population.

Our prior monoculture and co-culture studies established two consistent features: 1) HCT-116 cancer cells are much more sensitive to methionine depletion than Hs27 normal fibroblasts (13, 15); and 2) HCT-116 cells exposed to their IC_{50} dose of rMETase can be rescued by subsequent methionine repletion (14). The present study extends these observations by demonstrating that the same rescue phenomenon occurs in co-culture, where cell-cell competition and contact influence growth dynamics. Thus, the “Hoffman effect” (methionine addiction) is not only evident in isolated cancer cells but also determines cell-population outcomes during rMETase treatment in competition with normal fibroblasts.

In the present study, under IC_{50} -level rMETase, HCT-116 became undetectable and subsequently recovered after methionine repletion, indicating complete functional rescue in the competitive context. The present findings demonstrate both the strong dependence of cancer cells on methionine and their selective depletion by rMETase as well as the rapid rescue and re-expansion that occur upon restoration of methionine. The present findings are consistent with rMETase-induced interruption of methionine supply causing a reversible cell-cycle arrest that can return to active cell cycling upon methionine replenishment (18, 19). In co-culture, rMETase selectively inhibits cancer-cell proliferation and survival while sparing normal fibroblasts (15).

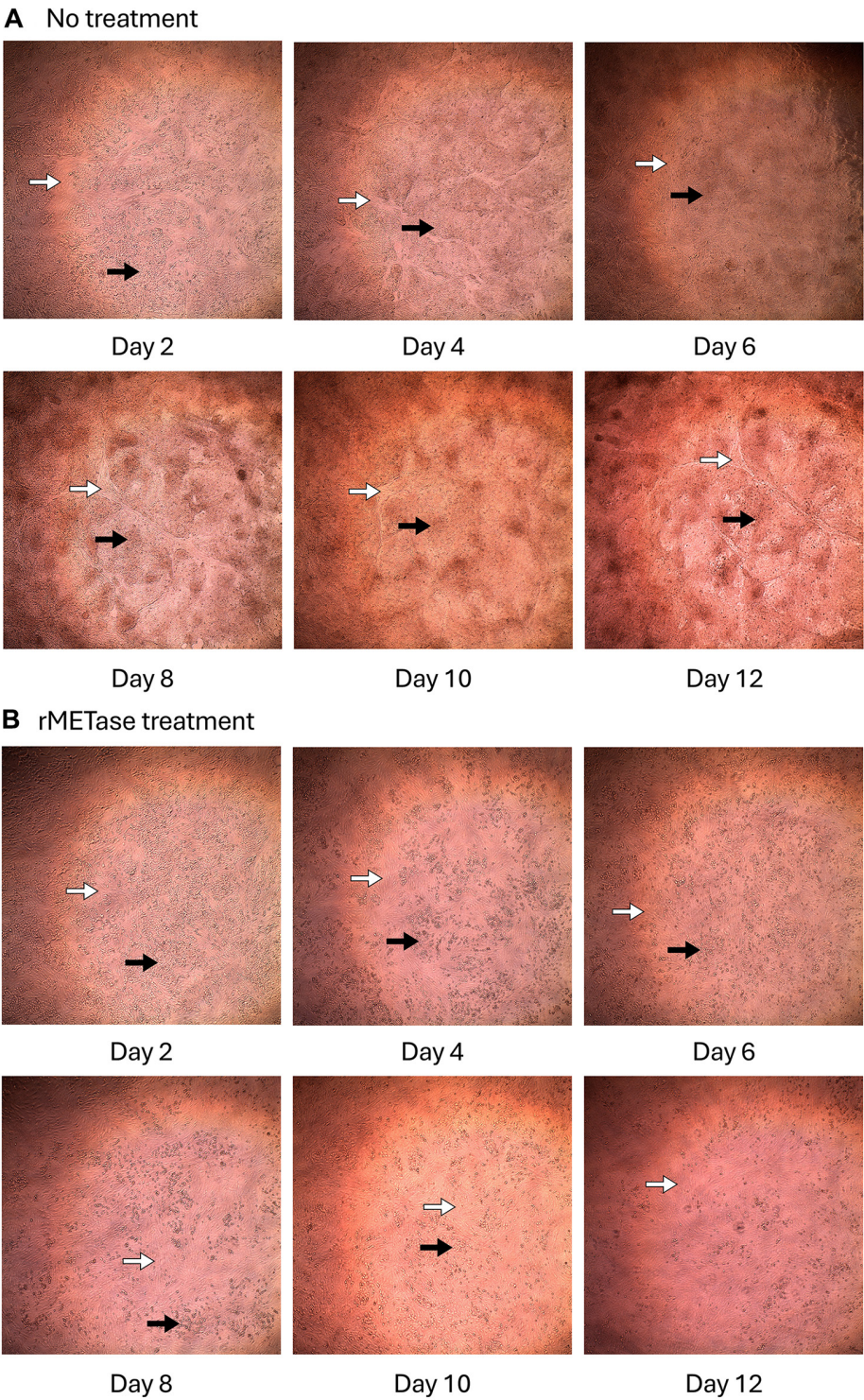


Figure 1. Time-course imaging of co-cultures of HCT-116 colon-cancer cells with Hs27 normal fibroblasts (40× microscopy). (A) Control (No rMETase treatment) group. (B) rMETase-treatment group. Black arrows indicate HCT-116 cancer cells; white arrows indicate Hs27 normal fibroblasts. Please see Materials and Methods for details.

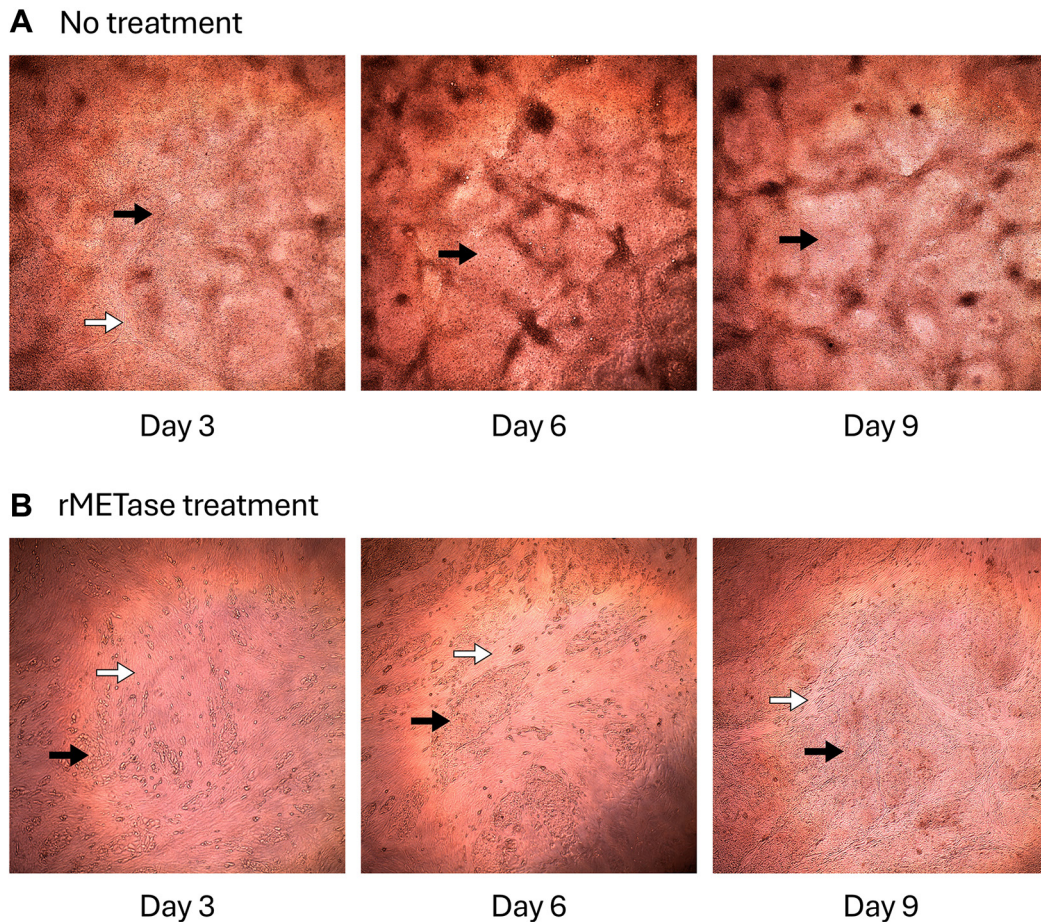


Figure 2. Time-course imaging of co-cultures of HCT-116 colon-cancer cells and Hs27 normal fibroblasts after methionine replenishment (40× microscopy). (A) Control (No rMETase treatment) group. (B) Co-culture previously treated with rMETase with subsequent methionine rescue. Black arrows indicate HCT-116 cancer cells; white arrows indicate Hs27 normal fibroblasts. Days indicate time after methionine replenishment. Please see Materials and Methods for details.

Methionine addiction reflects increased transmethylation demand and dependence on sustained methyl-group flux in cancer cells (5, 9, 11, 12, 22). Therefore, when methionine is depleted by rMETase, the insufficient methyl-donor supply is expected to induce a reversible cell-cycle arrest, and this is supported by the observed “rescue phenomenon” following restoration of methionine as seen in the present study. This is consistent with studies indicating that depletion of extracellular methionine impairs methyl-dependent epigenetic regulation in methionine-addicted cancer cells, causing them to stop

proliferating, whereas normal cells are mostly spared (9-11, 20-25).

However, our prior studies have shown that prolonged, high-concentration rMETase can drive HCT-116 cells beyond a recoverable state, so that restoration of methionine can no longer rescue cancer cells (methionine-depletion catastrophe) (14). High concentrations of rMETase can also compromise the growth and division of normal fibroblasts (13). The present findings identify a therapeutic window in which rMETase influences cell-population outcomes by selectively inhibiting methionine-

addicted cells while preserving normal cells co-cultured with the cancer cells. Accordingly, we used the HCT-116 IC₅₀ dose (0.46 U/ml) to inhibit the growth of the cancer population without impairing normal-cell growth. Under these conditions, we evaluated how methionine depletion and replenishment affected co-culture dynamics.

The present results highlight two key translational implications. First, maintaining methionine restriction is crucial: interruption, even once cancer cells are undetectable, can rapidly rescue the rare cancer cells in the co-culture and restore their competitive dominance within days. Second, methionine restriction *via* diet and/or rMETase may serve as a platform that targets cancer-cell-specific vulnerabilities revealed under methionine restricted conditions (1).

Methionine restriction induces the cancer cells to be more sensitive to chemotherapy including: 1) inhibitors of folate and purine metabolism (5-fluorouracil with or without folinic acid, methotrexate); 2) agents targeting methylation-dependent pathways (azacitidine, decitabine); 3) cytotoxic cell-cycle agents (doxorubicin, cisplatin, gemcitabine, eribulin, paclitaxel); and 4) mTOR-pathway targeted agents (rapamycin) (21).

Conclusion

Treatment of HCT-116/Hs27 co-cultures with rMETase at the HCT-116 IC₅₀ (0.46 U/ml) eradicated cancer cells to undetectable levels by day 12. However, methionine replenishment led to cancer-cell reappearance within three days and renewed dominance by nine days. These findings indicate that continuous methionine restriction is required to maintain normal-cell dominance and to prevent cancer-cell rescue and regrowth. Clinically, the growth kinetics of the methionine-rescued, rMETase-treated cancer cells suggest that methionine-restriction in cancer patients should be maintained without interruption to prevent cancer regrowth, since restoration of methionine availability may result in cancer progression. On the basis of the present findings, future studies will evaluate strategies with methionine restriction to prevent cancer-cell regrowth under clinically-relevant conditions.

rMETase is effective because it targets the fundamental hallmark of cancer, methionine addiction (1-19, 22-42). rMETase is showing clinical promise (43-47).

The Hoffman effect of methionine addiction is stronger than the Warburg effect of glucose addiction, as shown by comparison of methionine-based and glucose-based PET imaging, respectively (47, 48).

Conflicts of Interest

All Authors have no conflicts of interest or financial ties to disclose related to this study.

Authors' Contributions

BMK and RMH conceived the project and designed the study. QH and SL produced rMETase. BMK conducted all experiments and prepared the initial draft. RMH made substantive revisions. JSK, KM, YA, YM, and MB critically reviewed the final manuscript.

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Artificial Intelligence (AI) Disclosure

No artificial intelligence (AI) tools, including large language models or machine learning software, were used in the preparation, analysis, or presentation of this manuscript.

References

- 1 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
- 2 Sugimura T, Birnbaum SM, Winitz M, Greenstein JP: Quantitative nutritional studies with water-soluble, chemically defined diets. VIII. The forced feeding of diets each lacking in one essential amino acid. *Arch Biochem Biophys* 81(2): 448-455, 1959. DOI: 10.1016/0003-9861(59)90225-5
- 3 Halpern BC, Clark BR, Hardy DN, Halpern RM, Smith RA: The effect of replacement of methionine by homocystine on survival of malignant and normal adult mammalian cells in culture. *Proc Natl Acad Sci U S A* 71(4): 1133-1136, 1974. DOI: 10.1073/pnas.71.4.1133
- 4 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
- 5 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
- 6 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
- 7 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 U S A* 76(3): 1313-1317, 1979. DOI: 10.1073/pnas.76.3.1313
- 8 Yamamoto J, Aoki Y, Han Q, Sugisawa N, Sun YU, 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/anticancer.14815
- 9 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
- 10 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
- 11 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
- 12 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
- 13 Kang BM, Han Q, Mizuta K, Morinaga S, Bouvet M, Hoffman RM: Comparison of cell-death kinetics of recombinant methioninase (rMETase)-treated cancer and normal cells: only cancer cells undergo methionine-depletion catastrophe at low rMETase concentrations. *Anticancer Res* 45(1): 105-111, 2025. DOI: 10.21873/anticancer.17397
- 14 Kang BM, Han Q, Li S, Mizuta K, Kim JS, Asano Y, Bouvet M, Hoffman RM: The conditions of non-rescuability of methioninase-treated cancer cells by methionine replenishment: the point of no return. *Anticancer Res* 45(7): 2817-2823, 2025. DOI: 10.21873/anticancer.17650
- 15 Kang BM, Han Q, Li S, Kim JS, Mizuta K, Asano Y, Miyashi Y, Bouvet M, Hoffman RM: Recombinant methioninase selectively eliminates cancer cells co-cultured with normal fibroblasts indicating the high-precision efficacy of targeting methionine addiction of cancer. *Anticancer Res* 45(10): 4193-4200, 2025. DOI: 10.21873/anticancer.17771
- 16 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 recombinant methionine- α -deamino- γ -mercaptomethane-lyase for novel anticancer therapy. *Protein Expr Purif* 9(2): 233-245, 1997. DOI: 10.1006/prep.1996.0700
- 17 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
- 18 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
- 19 Yano S, Li S, Han Q, Tan Y, Bouvet M, Fujiwara T, Hoffman RM: Selective methioninase-induced trap of cancer cells in S/G2 phase visualized by Fucci imaging confers chemosensitivity. *Oncotarget* 5(18): 8729-8736, 2014. DOI: 10.18632/oncotarget.2369
- 20 Dai Z, Mentch SJ, Gao X, Nichenametla SN, Locasale JW: Methionine metabolism influences genomic architecture and gene expression through H3K4me3 peak width. *Nat Commun* 9(1): 1955, 2018. DOI: 10.1038/s41467-018-04426-y
- 21 Kubota Y, Han Q, Aoki Y, Masaki N, Obara K, Hamada K, Hozumi C, Wong ACW, Bouvet M, Tsunoda T, Hoffman RM: Synergy of

- p>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
- 22 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
 - 23 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
 - 24 Raboni S, Montalbano S, Stransky S, Garcia BA, Buschini A, Bettati S, Sidoli S, Mozzarelli A: A key silencing histone mark on chromatin is lost when colorectal adenocarcinoma cells are depleted of methionine by methionine γ -Lyase. *Front Mol Biosci* 8: 735303, 2021. DOI: 10.3389/fmolb.2021.735303
 - 25 Montalbano S, Raboni S, Sidoli S, Mozzarelli A, Bettati S, Buschini A: Post-translational modifications of histone variants in the absence and presence of a methionine-depleting enzyme in normal and cancer cells. *Cancers (Basel)* 15(2): 527, 2023. DOI: 10.3390/cancers15020527
 - 26 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
 - 27 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
 - 28 Lin DW, Carranza FG, Borrego S, Lauinger L, Dantas de Paula L, Pulipelli HR, Andronicos A, Hertel KJ, Kaiser P: Nutrient control of splice site selection contributes to methionine addiction of cancer. *Mol Metab* 93: 102103, 2025. DOI: 10.1016/j.molmet.2025.102103
 - 29 Andronicos A, Yoneda KC, Lin D-W, Law FV, Bae H, Basirattalab A, Graham NA, Jang C, Kaiser P: Carboxy-methylation of the catalytic subunit of protein phosphatase 2A (PP2Ac) integrates methionine availability with methionine addicted cancer cell proliferation. *Biomolecules* 15(9): 1210, 2025. DOI: 10.3390/biom15091210
 - 30 Kaiser P: Methionine dependence of cancer. *Biomolecules* 10(4): 568, 2020. DOI: 10.3390/biom10040568
 - 31 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.
 - 32 Kubota Y, Sato T, Hozumi C, Han Q, Aoki Y, Masaki N, Obara K, Tsunoda T, Hoffman RM: Superiority of [(11)C]methionine over [(18)F]deoxyglucose for PET imaging of multiple cancer types due to the methionine addiction of cancer. *Int J Mol Sci* 24(3): 1935, 2023. DOI: 10.3390/ijms24031935
 - 33 Stern PH, Hoffman RM: Enhanced in vitro selective toxicity of chemotherapeutic agents for human cancer cells based on a metabolic defect. *J Natl Cancer Inst* 76(4): 629-639, 1986. DOI: 10.1093/jnci/76.4.629
 - 34 Tisdale MJ: Utilization of preformed and endogenously synthesized methionine by cells in tissue culture. *Br J Cancer* 49(3): 315-320, 1984. DOI: 10.1038/bjc.1984.49
 - 35 Breillout F, Antoine E, Poupon MF: Methionine dependency of malignant tumors: a possible approach for therapy. *J Natl Cancer Inst* 82(20): 1628-1632, 1990. DOI: 10.1093/jnci/82.20.1628
 - 36 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.
 - 37 Hoffman RM: Altered methionine metabolism, DNA methylation and oncogene expression in carcinogenesis. A review and synthesis. *Biochim Biophys Acta* 738(1-2): 49-87, 1984. DOI: 10.1016/0304-419x(84)90019-2
 - 38 Gao X, Sanderson SM, Dai Z, Reid MA, Cooper DE, Lu M, Richie JP Jr, Ciccarella A, Calcagnotto A, Mikhael PG, Mentch SJ, Liu J, Ables G, Kirsch DG, Hsu DS, Nichenametla SN, Locasale JW: Dietary methionine influences therapy in mouse cancer models and alters human metabolism. *Nature* 572(7769): 397-401, 2019. DOI: 10.1038/s41586-019-1437-3
 - 39 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
 - 40 Mecham JO, Rowitch D, Wallace CD, 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
 - 41 Tisdale MJ: Effect of methionine deprivation on methylation and synthesis of macromolecules. *Br J Cancer* 42(1): 121-128, 1980. DOI: 10.1038/bjc.1980.210
 - 42 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
 - 43 Asano Y, Han Q, Li S, Mizuta K, Kang BM, Kim JS, Miyashi Y, Yamamoto N, Hayashi K, Kimura H, Miwa S, Igarashi K, Higuchi T, Morinaga S, Tsuchiya H, Demura S, Hoffman RM: Very rapid eradication of a large squamous-cell carcinoma of the head and neck treated with first-line combination chemotherapy, a low-methionine diet, and oral recombinant methioninase. *Anticancer Res* 45(11): 5225-5231, 2025. DOI: 10.21873/anticancer.17862

- 44 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/anticancer.16089
- 45 Asano Y, Sato T, Han Q, Li S, Hozumi C, Mizuta K, Kang BM, Kim JS, Miyashi Y, Yamamoto N, Hayashi K, Kimura H, Miwa S, Igarashi K, Higuchi T, Morinaga S, Tsuchiya H, Demura S, Hoffman RM: Eradication of extensive lymph-node, bone and pleural metastases of a breast-cancer patient treated with radiation, immunotherapy and oral recombinant methioninase. *Cancer Diagn Progn* 5(5): 614-619, 2025. DOI: 10.21873/cdp.10476
- 46 Asano Y, Han Q, Li S, Sato T, Hozumi C, Kang BM, Kim JS, Miyashi Y, Yamamoto N, Hayashi K, Kimura H, Miwa S, Igarashi K, Higuchi T, Morinaga S, Tsuchiya H, Demura S, Hoffman RM: Rapid eradication of extensive spinal metastases in a prostate-cancer patient taking androgen-deprivation therapy, chemotherapy, and oral recombinant methioninase on a low-methionine diet. *Anticancer Res* 45(12): 5799-5805, 2025. DOI: 10.21873/anticancer.17912
- 47 Asano Y, Sato T, Hozumi C, Han Q, Li S, Kang BM, Kim JS, Miyashi Y, Yamamoto N, Hayashi K, Kimura H, Miwa S, Igarashi K, Higuchi T, Morinaga S, Tsuchiya H, Demura S, Hoffman RM: FDG- and MET-PET imaging reveal glucose and methionine addiction in a primary endometrial cancer and methionine addiction only in a para-aortic lymph-node metastasis in a 58-year-old patient. *Anticancer Res* 45(12): 5819-5824, 2025. DOI: 10.21873/anticancer.17915
- 48 Sato M, Sato T, Hozumi C, Han Q, Mizuta K, Morinaga S, Kang BM, Kobayashi N, Ichikawa Y, Nakajima A, Hoffman RM: [11C] Methionine PET vs. [18F] Fluorodeoxyglucose PET wholebody imaging to determine the extent of methionine addiction compared to glucose-addiction of primary and metastatic cancer of the trunk in patients. *Anticancer Res* 44(9): 3891-3898, 2024. DOI: 10.21873/anticancer.17216