Review

# Can Dietary Methionine Restriction Increase the Effectiveness of Chemotherapy in Treatment of Advanced Cancer?

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Most metastatic tumors, such as those originating in the prostate, lung, and gastrointestinal tract, respond poorly to conventional chemotherapy. Novel treatment strategies for advanced cancer are therefore desperately needed. Dietary restriction of the essential amino acid methionine offers promise as such a strategy, either alone or in combination with chemotherapy or other treatments. Numerous in vitro and animal studies demonstrate the effectiveness of dietary methionine restriction in inhibiting growth and eventually causing death of cancer cells. In contrast, normal host tissues are relatively resistant to methionine restriction. These preclinical observations led to a phase I clinical trial of dietary methionine restriction for adults with advanced cancer. Preliminary findings from this trial indicate that dietary methionine restriction is safe and feasible for the treatment of patients with advanced cancer. In addition, the trial has yielded some preliminary evidence of antitumor activity. One patient with hormone-independent prostate cancer experienced a 25% reduction in serum prostate-specific antigen (PSA) after 12 weeks on the diet, and a second patient with renal cell cancer experienced an objective radiographic response. The possibility that methionine restriction may act synergistically with other cancer treatments such as chemotherapy is being explored. Findings to date support further investigation of dietary methionine restriction as a novel treatment strategy for advanced cancer.

**Key teaching points**

- Methionine is an essential amino acid that is particularly abundant in foods rich in animal protein.
- Dietary methionine restriction inhibits growth and causes death of a variety of cancers growing in culture and as tumors in animals. Yet, methionine restriction is relatively well tolerated by normal tissues.
- Preliminary clinical trials in patients with advanced cancer indicate that dietary methionine restriction offers promise as a novel treatment strategy.
- A synergistic effect between methionine restriction and other treatment modalities may improve cancer outcome or at least reduce the adverse side effects of treatments such as chemotherapy.

## INTRODUCTION

Recent clarification of the molecular pathogenesis of cancer has led to the identification of several novel treatment strategies for advanced cancer. One such strategy is dietary restriction of the essential amino acid methionine. This paper reviews the history of amino acid restriction as cancer treatment, selective antitumor activities of methionine restriction in cell culture and experimental animal studies, specialized functions and metabolism of this amino acid, and potential synergistic effects between methionine restriction and other cancer treatment modalities. We also describe promising preliminary results of a phase I clinical trial of dietary methionine restriction for patients with advanced cancer.

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## BACKGROUND

Smoking cessation, consumption of diets rich in fruits and vegetables, avoidance of excessive sun exposure, and other lifestyle measures can prevent the majority of cancers [1]. Unfortunately, implementation of these conceptually simple measures remains elusive. As a result, each year hundreds of thousands of Americans develop metastatic cancer. Chemotherapy cures only a few types of metastatic cancer, including certain hematological malignancies, germ cell tumors, and a small fraction of other tumors. Unfortunately, the vast majority of common metastatic cancers, including those originating in the breast, prostate, gastrointestinal tract, and lung, are lethal. We therefore desperately need novel treatment strategies for metastatic cancer. Dietary methionine restriction is one such strategy.

Dietary amino acid restriction for the treatment of cancer is not a new concept. Animal experiments published in the early 1900s focused on the potential antitumor activity of dietary protein or amino acid restriction [2]. These initial studies demonstrated no antitumor activity, leading Drummond in 1917 to conclude "it is not possible to bring about an inhibition of tumor growth by an employment of dietary restrictions" [2]. This pessimistic conclusion, however, was not justified in light of later, more sophisticated studies involving chemically defined diets. In 1959, Sugimura and colleagues [3] studied the antitumor activity in tumor-bearing animals of diets lacking one essential amino acid. They found that tumor growth in animals was considerably slowed by diets lacking methionine, isoleucine, or valine. Of the three diets, the methionine free diet was the least toxic, which strongly indicates that dietary methionine restriction has highly specific effects on tumors and host tissues and does not represent indiscriminate tumor "starvation."

Shortly after the study by Sugimura and colleagues [3], several investigations focused on phenylalanine and tyrosine restriction to treat melanoma [4–6]. Phenylalanine and tyrosine are required for the synthesis of melanin, an abundant pigment in melanomas and melanocytes. In 1966, Demopoulos [6] described the results of a phase I clinical trial of dietary phenylalanine and tyrosine restriction for treatment of patients with metastatic melanoma. Remarkably, three of the five patients treated responded to the therapy and experienced minimal toxicity. Results of additional pilot studies were equally promising [4,5]. Unfortunately, examination of the effect of phenylalanine and tyrosine restriction was not pursued aggressively in the clinic thereafter, presumably because it was found to be somewhat "cumbersome, complex, and unpalatable" [6]. Nonetheless, studies of phenylalanine and tyrosine restriction strongly support the concept of amino acid restriction for the treatment of cancer, and excellent mechanistic studies are still underway [7,8].

## TUMOR GROWTH INHIBITORY EFFECTS OF METHIONINE RESTRICTION

Beginning in the early 1970s, many studies focused on the potential antitumor activity of methionine restriction. Mammalian cells cannot synthesize methionine from any of the other standard amino acids but can remethylate homocysteine to methionine [9]. Normal cells can therefore grow in culture when methionine is replaced by homocysteine in the growth medium [10], and animals fed diets in which methionine has been replaced by homocysteine suffer no ill effects and grow normally [11,12]. In contrast, a wide variety of cancer cell lines are methionine dependent even in the presence of homocysteine.

Numerous cell culture studies using normal and malignant cell lines (e.g., leukemia, prostate) demonstrated that methionine restriction suppresses cancer cell growth, with little or no deleterious effect on normal cells [13–18]. Likewise, tumors are methionine dependent in vivo. Dietary methionine restriction causes regression of a variety of animal tumors and inhibits metastasis in animal models [12,17,19–22]. For example, altering the dietary arginine-methionine balance inhibited tumor growth without causing cachexia in rats with subcutaneously transplanted Morris hepatoma [21,22]. Also, substituting homocysteine for methionine reduced tumor growth and metastasis in a rat rhabdomyosarcoma cell line [12,17]. In addition, many fresh patient tumors in primary histoculture and human tumor xenografts in nude mice are methionine dependent [23,24].

Methioninase, an enzyme that specifically degrades methionine and homocysteine, inhibits growth of a variety of cancer cells in culture as well as solid tumors and leukemia in animals [13,25–33]. Animal studies also support an anti-tumor effect of methioninase [27,28,30]. In laboratory animals with Lewis lung carcinoma, synergistic antitumor activity was demonstrated between methioninase and the chemotherapy drug 5-fluorouracil [30]. In an orthotopic lung cancer model, recombinant methioninase plus methioninase gene therapy was effective in suppressing cancer [34]. The fact that methioninase inhibits tumor growth in preclinical models further supports the concept of dietary methionine restriction as cancer treatment. Recombinant methioninase was recently tested in a phase I clinical trial in Mexico. Patients in the trial experienced no significant toxicity, and plasma methionine levels fell dramatically as expected [35,36]. The antitumor activity of methioninase was not assessed in this trial. A polyethylene glycol conjugation of methioninase has been developed to reduce the potential antigenicity and lengthen the half-life of the recombinant enzyme [37,38].

## MOLECULAR MECHANISMS FOR THE TUMOR INHIBITORY EFFECT OF METHIONINE RESTRICTION

Determining the molecular mechanisms for the tumor specific growth inhibitory effects of methionine restriction will require an understanding of the specialized functions of methionine. Methionine is the major methyl donor for methylation of DNA, RNA, proteins, and other molecules. Overall rates of methylation are much higher in tumors than in normal tissues [39–41]. Cytosine methylation within CpG islands is one of the mechanisms by which gene expression is regulated [42]. Several growth inhibitory and pro-apoptotic genes are transcriptionally silenced in tumors as a result of focal DNA hypermethylation. DNA methylation also compacts and stabilizes chromatin structure and decreases its susceptibility to DNA-damaging agents. Loss of methylated cytosines reduces the stability of chromatin by decreasing binding sites for methyl-specific DNA-binding proteins [43]. In the absence of methyl-directed protein binding, affected DNA sequences are rendered more accessible to oxidant and/or enzyme-induced DNA strand breakage [44–47]. Animal studies have demonstrated that severe, prolonged methyl deficiency induced by dietary restriction of methionine, choline, homocysteine, and folate leads to global demethylation of normal liver DNA and resultant increased susceptibility to DNA strand breaks [44]. Inhibition of DNA methylation by methionine restriction may therefore make cancer cell DNA susceptible to damage.

Methionine is also required for synthesis of polyamines, which have far-ranging effects on nuclear structure and cell division [48], and for glutathione homeostasis. Glutathione ($\gamma$-glutamylcysteinylglycine) is a ubiquitous tripeptide that reduces oxidative stress in cells. Oxidative stress is primarily due to reactive oxygen species generated from mitochondrial respiration that are known to damage nuclear and mitochondrial DNA, as well as many other molecules [49,50]. Certain toxins and drugs, such as cancer chemotherapy drugs, also cause oxidative stress. Many tumors contain elevated levels of glutathione that confer resistance to a variety of chemotherapy drugs [51,52]. Methionine maintains intracellular glutathione levels by acting as a sulfur donor for synthesis of cysteine and by preventing efflux of glutathione from within cells [53,54]. Therefore, methionine restriction potentially could inhibit tumor growth by inducing oxidative DNA damage in cancer cells.

## ACHIEVING SYNERGY BETWEEN METHIONINE RESTRICTION AND OTHER TREATMENTS

Dietary methionine restriction may act synergistically with other cancer treatments to increase their efficacy and/or reduce their toxic side effects. There are several potential strategies for achieving synergy between methionine restriction and other treatments. One such strategy is to combine dietary methionine restriction with methionine analogs. For instance, several studies have demonstrated that methionine restriction and the methionine analog ethionine (S-ethyl-L-homocysteine) have synergistic antitumor activity against a variety of tumors, including prostate cancer and sarcoma [18,55–58]. Other methionine analogs, including selenomethionine, as well as polyamine analogs, SIBA (an analog of S-adenosylhomocysteine), and trifluoromethylhomocysteine have also shown promise in animal studies in combination with methionine restriction [57–63]. Another approach to maximize the antitumor activity of methionine restriction is to target chemotherapy to tumors by restricting dietary methionine and then giving methionine conjugated to an anticancer drug, such as mitomycin C [64].

Dietary methionine restriction has also been combined with methioninase treatment to achieve maximal methionine depletion, as shown in human brain tumor xenografts in athymic mice [31]. Methioninase is being developed by a pharmaceutical company and hopefully will soon be available for clinical trials in the U.S.

Another potential strategy for optimizing the clinical effectiveness of methionine restriction will be to combine it with chemotherapy. Several preclinical studies have demonstrated synergy between methionine restriction and various cytotoxic chemotherapy drugs, such as 5-fluorouracil [30,65,66]. Methionine restriction is thought to enhance the antitumor activity of 5-fluorouracil by raising levels of 5,10-methylenetetrahydrofolate, which is the same mechanism by which leucovorin modulates 5-FU action. In Yoshida sarcoma bearing rats, methionine +/− cysteine restriction enhances the antitumor activity of 5-fluorouracil [65]. Likewise, a synergistic beneficial effect of methionine-depletion and 5-fluorouracil has been demonstrated in gastric cancer xenografts in nude mice [66]. Cisplatin, another commonly used chemotherapy drug, acts synergistically with methionine restriction by inhibiting methionine uptake in tumors, as demonstrated with animal breast cancer and colon cancer models [25,67]. Methionine restriction has also shown promise in animal studies in combination with vincristine [68], the alkylating agents ACNU [69] and CCNU [70], and the anthracycline doxorubicin [71,72]. The optimal sequence and schedule for combining methionine restriction with chemotherapy will need to be determined empirically. One possible approach will be treat patients with “cyclic” methionine restriction, in much the same way as cancer patients are treated with “cycles” of chemotherapy. Patients who are on methionine-restricted diets could be allowed to resume normal diets briefly at regular intervals. In this scenario, chemotherapy would most likely be given during the brief periods of methionine repletion. Preclinical studies involving human carcinoma and sarcoma cell lines lend support to this approach. In those studies, methionine restriction was combined with doxorubicin for 10 days, and cells were then treated with methionine repletion plus vincristine [10]. Clinical trials of various sequences

and schedules should become feasible in the near future following completion of the ongoing phase I clinical trial of dietary methionine restriction, described below.

Ultimately, optimization of dietary methionine restriction will depend upon clarification of the molecular mechanisms by which methionine restriction inhibits tumor growth. For instance, the fact that methionine restriction causes certain cancer cells to enter G2 cell cycle arrest [18] may be exploitable therapeutically. Cancer cells that are forced to leave G2 and reenter the cell cycle prematurely following exposure to chemotherapy die much more rapidly than those remaining in G2 [73–75]. "Abrogation" of G2 cell cycle arrest therefore accelerates cancer cell death. Perhaps drugs that abrogate G2 arrest can be used to increase the efficacy of dietary methionine restriction. Additional studies demonstrating that methionine restriction inhibits cyclin dependent kinases [76], modulates glutathione (GSH) levels [53,54,77–81], and possibly inhibits transmethylation of DNA and other molecules [39,40,70,82–86] may also lead to the development of strategies for optimizing the effectiveness and minimizing the toxicity of methionine restriction.

## CLINICAL FEASIBILITY AND POTENTIAL EFFICACY OF DIETARY METHIONINE FOR TREATMENT OF ADVANCED CANCERS

Based on the strength of many preclinical studies and one small clinical study involving preoperative methionine restriction for patients with localized gastric cancer [87], we initiated a phase I clinical trial of dietary methionine restriction for adults with advanced solid tumors at Baylor College of Medicine and the Houston VA Medical Center. Twelve patients have enrolled so far. Patients in the trial have been prescribed a medical food that provides 0.8 g methionine-free protein/kg/day, 35 kcal/kg/day, vitamins, minerals, and all standard amino acids other than methionine. Participants consume no methionine for the first two weeks. Thereafter, their methionine intake is restricted to 2 mg/kg/day, which is about 5–10% of normal intake. Participants receive no cancer treatments other than the dietary modification.

Dietary methionine restriction has reduced plasma methionine levels from 22 ($\pm$6) $\mu$M to 9 ($\pm$2) $\mu$M within eight weeks without affecting levels of other amino acids or albumin. The observed reduction in plasma methionine levels is therapeutically relevant, since similar reductions inhibit growth of human cancer cells in culture. The fact that serum albumin levels have remained stable indicates that the experimental diet does not indiscriminately block protein synthesis. Side effects include weight loss of approximately one pound per week and mild fatigue, both of which are reversible.

Although the trial was designed primarily to assess safety, it has yielded preliminary evidence of antitumor activity. Patients have remained in the trial for 2–39 weeks. One patient with hormone independent prostate cancer experienced a >25% reduction in serum prostate-specific antigen (PSA) after 12 weeks on the trial, and another patient with progressive renal cell carcinoma experienced a radiographic objective response. These results, although very preliminary, strongly suggest that dietary methionine restriction has antitumor activity.

## CONCLUSION

Despite many promising preclinical and clinical studies in recent years, dietary methionine restriction and other dietary approaches to cancer treatment have not yet gained wide clinical application. Most clinicians and investigators are probably unfamiliar with nutritional approaches to cancer. Many others may consider amino acid restriction as an "old idea," since it has been examined for several decades. However, many good ideas remain latent for decades if not centuries before they prove valuable in the clinic. For example, who would have anticipated that arsenic, which has been used as both a medicine and toxin for centuries, would prove to be a highly effective, modern treatment for acute leukemia [88]? With the proper development, dietary methionine restriction, either alone or in combination with other treatments, may also prove to have a major impact on patients with cancer.

## REFERENCES

1. American Institute for Cancer Research. "Food, Nutrition and the Prevention of Cancer: A Global Perspective." World Cancer Research Fund, American Institute for Cancer Research, 1997.
2. Drummond JC: A comparative study of tumor and normal tissue growth. Biochem J 11:825, 1917.
3. 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 81:448–455, 1959.
4. Lorincz AB, Kuttner RE, Brandt MB: Tumor response to phenylalanine-tyrosine-limited diets. J Am Diet Assoc 54:198–205, 1969.
5. Lawson DH, Stockton LH, Bleier JC, Acosta PB, Heymsfield SB, Nixon, DW: The effect of a phenylalanine and tyrosine restricted diet on elemental balance studies and plasma aminograms of patients with disseminated malignant melanoma. Am J Clin Nutr 41:73–84, 1985.
6. Demopoulos HB: Effects of reducing the phenylalanine-tyrosine intake of patients with advanced malignant melanoma. Cancer 19:657–664, 1966.
7. Pelayo BA, Fu YM, Meadows GG: Inhibition of B16BL6 melanoma invasion by tyrosine and phenylalanine deprivation is associated with decreased secretion of plasminogen activators and increased plasminogen activator inhibitors. Clin Exp Metastasis 17:841–848, 1999.
8. Fu YM, Yu ZX, Pelayo BA, Ferrans VJ, Meadows GG: Focal

adhesion kinase-dependent apoptosis of melanoma induced by tyrosine and phenylalanine deficiency. Cancer Res 59:758–765, 1999.
9. Hoffman RM, Erbe RW: High in vivo rates of methionine biosynthesis in transformed human and malignant rat cells auxotrophic for methionine. Proc Natl Acad Sci USA 73:1523–1527, 1976.
10. 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 76:629–639, 1986.
11. Du Vigneaud V, Chandler JP, Moyer AW, Keppel DM: The effect of choline on the ability of homocysteine to replace methionine in the diet. J Biol Chem 131:57–76, 1939.
12. Breillout F, Hadida F, Echinard-Garin P, Lascaux V, Poupon MF: Decreased rat rhabdomyosarcoma pulmonary metastases in response to a low methionine diet. Anticancer Res 7:861–867, 1987.
13. Kreis W: Tumor therapy by deprivation of L-methionine: rationale and results. Cancer Treat Rep 63:1069–1072, 1979.
14. Tisdale M, Eridani S: Methionine requirement of normal and leukaemic haemopoietic cells in short term cultures. Leuk Res 5:385–394, 1981.
15. 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: 429–434, 1983.
16. 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 119:29–34, 1984.
17. Breillout F, Antoine E, Poupon MF: Methionine dependency of malignant tumors: a possible approach for therapy. J Natl Cancer Inst 82:1628–1632, 1990.
18. Poirson-Bichat F, Gonfalone G, Bras-Goncalves RA, Dutrillaux B, Poupon, MF: Growth of methionine-dependent human prostate cancer (PC-3) is inhibited by methionine combined with methionine starvation. Br J Cancer 75:1605–1612, 1997.
19. Guo H, Lishko VK, Herrera H, Groce A, Kubota T, Hoffman RM: Therapeutic tumor-specific cell cycle block induced by methionine starvation *in vivo.* Cancer Research 53:5676–5679, 1993.
20. Goseki N, Onodera T, Koike M, Kosaki G: Inhibitory effect of L-methionine-deprived amino acid imbalance using total parenteral nutrition on growth of ascites hepatoma in rats. Tohoku J Exp Med 151:191–200, 1987.
21. Millis RM, Diya CA, Reynolds ME: Growth inhibition of subcutaneously transplanted hepatomas by alterations of the dietary arginine-methionine balance. Nutr Cancer 25:317–327, 1996.
22. Millis RM, Diya CA, Reynolds ME, Dehkordi O, Bond VJ: Growth inhibition of subcutaneously transplanted hepatomas without cachexia by alteration of the dietary arginine-methionine balance. Nutr Cancer 31:49–55, 1998.
23. Guo HY, Herrera H, Groce A, Hoffman RM: Expression of the biochemical defect of methionine dependence in fresh patient tumors in primary histoculture. Cancer Res 53:2479–2483, 1993.
24. Hoshiya Y, Guo H, Kubota T, Inada T, Asanuma F, Yamada Y, Koh J, Kitajima M, Hoffman RM: Human tumors are methionine dependent in vivo. Anticancer Res 15:717–718, 1995.
25. Tan Y, Sun X, Xu M, Tan X, Sasson A, Rashidi B, Han Q, Wang X, An Z, Sun FX, Hoffman RM: Efficacy of recombinant methioninase in combination with cisplatin on human colon tumors in nude mice. Clin Cancer Res 5:2157–2163, 1999.
26. Yoshioka T, Wada T, Uchida N, Maki H, Yoshida H, Ide N, Kasai H, Hojo K, Shono K, Maekawa R, Yagi S, Hoffman RM, Sugita K: Anticancer efficacy in vivo and in vitro, synergy with 5-fluorouracil, and safety of recombinant methioninase. Cancer Res 58: 2583–2587, 1998.
27. Tan Y, Xu M, Guo H, Sun X, Kubota T, Hoffman RM: Anticancer efficacy of methioninase in vivo. Anticancer Res 16:3931–3936, 1996.
28. Kreis W, Hession C: Biological effects of enzymatic deprivation of L-methionine in cell culture and an experimental tumor. Cancer Res 33:1866–1869, 1973.
29. Tisdale MJ, Jack GW, Eridani S: Differential sensitivity of normal and leukaemic haemopoietic cells to methionine deprivation by L-methioninase. Leuk Res 7:269–277, 1983.
30. Yoshioka T, Wada T, Uchida N, Maki H, Yoshida H, Ide N, Kasai H, Hojo K, Shono K, Maekawa R, Yagi S, Hoffman RM, Sugita K: Anticancer efficacy in vivo and in vitro, synergy with 5-fluorouracil, and safety of recombinant methioninase. Cancer Res 58: 2583–2587, 1998.
31. Kokkinakis DM, Schold SCJ, Hori H, Nobori T: Effect of long-term depletion of plasma methionine on the growth and survival of human brain tumor xenografts in athymic mice. Nutr Cancer 29:195–204, 1997.
32. Kreis W, Baker A, Ryan V, Bertasso A: Effect of nutritional and enzymatic methionine deprivation upon human normal and malignant cells in tissue culture. Cancer Res 40:634–641, 1980.
33. Miki K, Al-Refaie W, Xu M, Jiang P, Tan Y, Bouvet M, Zhao M, Gupta A, Chishima T, Shimada H, Makuuchi M, Moossa AR, Hoffman RM: Methioninase gene therapy of human cancer cells is synergistic with recombinant methioninase treatment. Cancer Res 60:2696–2702, 2000.
34. 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 7:332–338, 2000.
35. Tan Y, Zavala JS, Xu M, Zavala JJ, Hoffman RM: Serum methionine depletion without side effects by methioninase in metastatic breast cancer patients. Anticancer Res 16:3937–3942, 1996.
36. Tan Y, Zavala JS, Han Q, Xu M, Sun X, Tan X, Magana R, Geller J, Hoffman RM: Recombinant methioninase infusion reduces the biochemical endpoint of serum methionine with minimal toxicity in high-stage cancer patients. Anticancer Res 17:3857–3860, 1997.
37. Tan Y, Sun X, Xu M, An Z, Tan X, Tan X, Han Q, Miljkovic DA, Yang M, Hoffman RM: Polyethylene glycol conjugation of recombinant methioninase for cancer therapy. Protein Expr Purif 12:45–52, 1998.
38. Tan Y, Sun X, Xu M, An Z, Tan X, Han Q, Miljkovic DA, Yang M, Hoffman RM: Polyethylene glycol conjugation of recombinant methioninase for cancer therapy. Protein Expr Purif 12:45–52, 1998.
39. Stern PH, Hoffman RM: Elevated overall rates of transmethylation in cell lines from diverse human tumors. In Vitro 20:663–670, 1984.
40. Tisdale MJ: Effect of methionine deprivation on methylation and synthesis of macromolecules. Br J Cancer 42:121–128, 1980.
41. Judde JG, Ellis M, Frost P: Biochemical analysis of the role of

transmethylation in the methionine dependence of tumor cells. Cancer Res 49:4859–4865, 1989.
42. Baylin SB, Herman JG, Graff JR, Vertino PM, Issa JP: Alterations in DNA methylation: a fundamental aspect of neoplasia. Adv Cancer Res 72:141–96, 1998.
43. Nan X, Campoy FJ, Bird A: MeCP2 is a transcriptional repressor with abundant binding sites in genomic chromatin. Cell 88:471–481, 1997.
44. Pogribny IP, Basnakian AG, Miller BJ, Lopatina NG, Poirier LA, James SJ: Breaks in genomic DNA and within the p53 gene are associated with hypomethylation in livers of folate/methyl-deficient rats. Cancer Res 55:1894–1901, 1995.
45. Davey C, Pennings S, Allan J: CpG methylation remodels chromatin structure in vitro. J Mol Biol 267:276–288, 1997.
46. Razin A: CpG methylation, chromatin structure and gene silencing-a three-way connection. EMBO J 17:4905–4908, 1998.
47. Wolffe A: Chromatin Structure. In: "Chromatin: Structure and Function." San Diego: Academic Press, pp 7–172, 1998.
48. "Polyamines in Cancer: Basic Mechanisms and Clinical Approaches." Austin, Texas: R.G. Landes Company, 1996.
49. Beckman KB, Ames BN: Oxidative decay of DNA. J Biol Chem 272:19633–19636, 1997.
50. Davies KJ: Oxidative stress: the paradox of aerobic life. Biochem Soc Symp 61:1–31, 1995.
51. Chen X, Carystinos GD, Batist G: Potential for selective modulation of glutathione in cancer chemotherapy. Chem Biol Interact 111–112:263–275, 1998.
52. Calvert P, Yao KS, Hamilton TC, O'Dwyer PJ: Clinical studies of reversal of drug resistance based on glutathione. Chem Biol Interact 111–112:213–224, 1998.
53. Fernandez-Checa JC, Maddatu T, Ookhtens M, Kaplowitz N: Inhibition of GSH efflux from rat liver by methionine: effects of GSH synthesis in cells and perfused organ. Am J Physiol 258: G967–G973, 1990.
54. Aw TY, Ookhtens M, Kaplowitz N: Inhibition of glutathione efflux from isolated rat hepatocytes by methionine. J Biol Chem 259:9355–9358, 1984.
55. Guo H, Tan Y, Kubota T, Moossa AR, Hoffman RM: Methionine depletion modulates the antitumor and antimetastatic efficacy of methionine. Anticancer Res 16:2719–2723, 1996.
56. Poirson-Bichat F, Goncalves RA, Miccoli L, Dutrillaux B, Poupon MF: Methionine depletion enhances the antitumoral efficacy of cytotoxic agents in drug-resistant human tumor xenografts. Clin Cancer Res 6:643–653, 2000.
57. Sasamura T, Matsuda A, Kokuba Y: Effects of D-methionine-containing solution on tumor cell growth in vitro. Arzneimittelforschung 49:541–543, 1999.
58. Sasamura T, Matsuda A, Kokuba Y: Nutritional effects of a D-methionine-containing solution on AH109A hepatoma-bearing rats. Biosci Biotechnol Biochem 62:2418–2420, 1998.
59. Porter CW, Sufrin JR, Keith DD: Growth inhibition by methionine analog inhibitors of S-adenosylmethionine biosynthesis in the absence of polyamine depletion. Biochem Biophys Res Commun 122:350–357, 1984.
60. Porter CW, Sufrin JR: Interference with polyamine biosynthesis and/or function by analogs of polyamines or methionine as a potential anticancer chemotherapeutic strategy. Anticancer Res 6:525–542, 1986.
61. Breillout F, Poupon MF, Blanchard P, Lascaux V, Echinard-Garin P, Robert-Gero M: Association of SIBA treatment and a Met-depleted diet inhibits in vitro growth and in vivo metastatic spread of experimental tumor cell lines. Clin Exp Metastasis 6:3–16, 1988.
62. Poirson-Bichat F, Lopez R, Bras GR, Miccoli L, Bourgeois Y, Demerseman P, Poisson M, Dutrillaux B, Poupon MF: Methionine deprivation and methionine analogs inhibit cell proliferation and growth of human xenografted gliomas. Life Sci 60:919–931, 1997.
63. Rodriguez-Vicente J, Vicente-Ortega V, Canteras-Jordana M: The effects of different antineoplastic agents and of pretreatment by modulators on three melanoma lines. Cancer 82:495–502, 1998.
64. Yoshida S, Yamana H, Tanaka T, Ishibashi N, Toh U, Ishii H, Shirouzu Y, Shirouzu K: Effect of combination therapy with a methionine-mitomycin C conjugate and a methionine-deficient diet on tumor growth. In Vivo 12:351–355, 1998.
65. Goseki N, Endo M, Onodera T, Kosaki G: Anti-tumor effect of L-methionine-deprived total parenteral nutrition with 5-fluorouracil administration on Yoshida sarcoma-bearing rats. Ann Surg 214:83–88, 1991.
66. Hoshiya Y, Kubota T, Inada T, Kitajima M, Hoffman RM: Methionine-depletion modulates the efficacy of 5-fluorouracil in human gastric cancer in nude mice. Anticancer Res 17:4371–4375, 1997.
67. Hoshiya Y, Kubota T, Matsuzaki SW, Kitajima M, Hoffman RM: Methionine starvation modulates the efficacy of cisplatin on human breast cancer in nude mice. Anticancer Res 16:3515–3517, 1996.
68. Goseki N, Nagahama T, Maruyama M, Endo M: Enhanced anticancer effect of vincristine with methionine infusion after methionine-depleting total parenteral nutrition in tumor-bearing rats. Jpn J Cancer Res 87:194–199, 1996.
69. Goseki N, Endo M: Thiol depletion and chemosensitization on nimustine hydrochloride by methionine-depleting total parenteral nutrition. Tohoku J Exp Med 161:227–239, 1990.
70. Kokkinakis DM, Von WM, Vuong TH, Brent TP, Schold SCJ: Regulation of O6-methylguanine-DNA methyltransferase by methionine in human tumour cells. Br J Cancer 75:779–788, 1997.
71. Goseki N, Yamazaki S, Endo M, Onodera T, Kosaki G, Hibino Y, Kuwahata T: Antitumor effect of methionine-depleting total parenteral nutrition with doxorubicin administration on Yoshida sarcoma-bearing rats. Cancer 69:1865–1872, 1992.
72. Nagahama T, Goseki N, Endo M: Doxorubicin and vincristine with methionine depletion contributed to survival in the Yoshida sarcoma bearing rats. Anticancer Res 18:25–31, 1998.
73. Chan TA, Hermeking H, Lengauer C, Kinzler KW, Vogelstein B: 14-3-3Sigma is required to prevent mitotic catastrophe after DNA damage. Nature 401:616–620, 1999.
74. Suganuma M, Kawabe T, Hori H, Funabiki T, Okamoto T: Sensitization of cancer cells to DNA damage-induced cell death by specific cell cycle G2 checkpoint abrogation. Cancer Res 59:5887–5891, 1999.
75. Guo HY, Hoffman RM, Herrera H: Unchecked DNA synthesis and blocked cell division induced by methionine deprivation in a human prostate cancer cell line. In Vitro Cell Dev Biol Anim 29A:359–361, 1993.
76. Lu S, Epner DE: Molecular mechanisms of cell cycle block by

methionine restriction in human prostate cancer cells. Nutr Cancer, in press, 2001.
77. Meredith MJ, Cusick CL, Soltaninassab S, Sekhar KS, Lu S, Freeman ML: Expression of Bcl-2 increases intracellular glutathione by inhibiting methionine-dependent GSH efflux. Biochem Biophys Res Commun 248:458–463, 1998.
78. Wang ST, Chen HW, Sheen LY, Lii CK: Methionine and cysteine affect glutathione level, glutathione-related enzyme activities and the expression of glutathione S-transferase isozymes in rat hepatocytes. J Nutr 127:2135–2141, 1997.
79. Djurhuus R, Svardal AM, Mansoor MA, Ueland PM: Modulation of glutathione content and the effect on methionine auxotrophy and cellular distribution of homocysteine and cysteine in mouse cell lines. Carcinogenesis 12:241–247, 1991.
80. Aw TY, Ookhtens M, Kaplowitz N: Mechanism of inhibition of glutathione efflux by methionine from isolated rat hepatocytes. Am J Physiol 251:G354–G361, 1986.
81. Story MD, Meyn RE: Modulation of apoptosis and enhancement of chemosensitivity by decreasing cellular thiols in a mouse B-cell lymphoma cell line that overexpresses bcl-2. Cancer Chemother Pharmacol 44:362–366, 1999.
82. Chiang PK, Gordon RK, Tal J, Zeng GC, Doctor BP, Pardhasaradhi K, McCann PP: S-Adenosylmethionine and methylation. FASEB J 10:471–480, 1996.
83. Hoffman RM: Altered methionine metabolism and transmethylation in cancer. Anticancer Res 5:1–30, 1985.
84. Hoffman RM: Altered methionine metabolism, DNA methylation and oncogene expression in carcinogenesis. A review and synthesis. Biochim Biophys Acta 738:49–87, 1984.
85. Fiskerstrand T, Christensen B, Tysnes OB, Ueland PM, Refsum H: Development and reversion of methionine dependence in a human glioma cell line: relation to homocysteine remethylation and cobalamin status. Cancer Res 54:4899–4906, 1994.
86. Tisdale MJ: Changes in tRNA methyltransferase activity and cellular S-adenosylmethionine content following methionine deprivation. Biochim Biophys Acta 609:296–305, 1980.
87. Goseki N, Yamazaki S, Shimojyu K, Kando F, Maruyama M, Endo M, Koike M, Takahashi H: Synergistic effect of methionine-depleting total parenteral nutrition with 5-fluorouracil on human gastric cancer: a randomized, prospective clinical trial. Jpn J Cancer Res 86:484–489, 1995.
88. Niu C, Yan H, Yu T, Sun HP, Liu JX, Li XS, Wu W, Zhang FQ, Chen Y, Zhou L, Li JM, Zeng XY, Yang RR, Yuan MM, Ren MY, Gu FY, Cao Q, Gu BW, Su XY, Chen GQ, Xiong SM, Zhang T, Waxman S, Wang ZY, Chen SJ: Studies on treatment of acute promyelocytic leukemia with arsenic trioxide: remission induction, follow-up, and molecular monitoring in 11 newly diagnosed and 47 relapsed acute promyelocytic leukemia patients. Blood 94: 3315–3324, 1999.

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