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Review

Methionine Restriction: Ready for Prime Time in the Cancer Clinic?

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Abstract. *Attempts to selectively starve cancers in the clinic have been made at least since the time of Warburg beginning 100 years ago. Calorie-restriction or low-carbohydrate diets have had limited success with cancer patients. Methionine restriction is another strategy to selectively starve cancer cells, since cancers are addicted to methionine, unlike normal cells. Methionine addiction of cancer is termed the Hoffman effect. Numerous preclinical studies over the past half century have shown methionine restriction to be highly effective against all major cancer types and synergistic with chemotherapy. Low-methionine medical diets can be effective in lowering methionine and have shown some clinical promise, but they are not palatable and thereby not sustainable. However, selectively choosing among plant-based foods allows a variety of low-methionine diets that are sustainable. Our laboratory has developed a methioninase that can be administered orally as a supplement and has resulted in anecdotal positive results in patients with advanced cancer, including hormone-independent prostate cancer, and other recalcitrant cancers. The question is whether methionine restriction through a low-methionine diet, or even greater methionine restriction with methioninase in combination with a low-methionine diet, is ready for prime time in the clinic, especially in combination with other synergistic therapy. The question will hopefully be answered in the near future, especially for advanced cancer patients who have failed all standard therapy.*

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Otto Warburg observed in the late 1920s that cancer cells used glycolysis to metabolize glucose, an anaerobic process, even in the presence of oxygen. Warburg's hypothesis was that cancer cells had defective mitochondria and could not carry out oxidative phosphorylation (1). This is called the Warburg effect. Warburg's idea was that cancer cells could be starved to death by depriving them of energy. The study of the Warburg effect has been revived in the 21st century (2) and it is hypothesized that the Warburg effect is a property of rapidly proliferating cells – both cancer and non-malignant, whereby the intermediate metabolites of glucose are converted into biomass as well as energy.

However, depriving tumors of energy involves starving the whole body of energy. Hints of a better way to starve cancer cells come from different sources. The first hint came from Sugimura *et al*. (3) who observed that removal of the amino-acid methionine from the diet of rats with tumors slowed tumor growth more than when other amino-acids were removed from the diet. Another important hint came from positron emission tomography (PET) imaging in the clinic where PET images of tumors obtained with [^{11}C] methionine were compared to PET images obtained with [^{18}F] deoxyglucose, whereby the images with [^{11}C] methionine had a stronger signal and were more specific than images obtained with the [^{18}F] deoxyglucose analog of glucose (4, 5). These results suggest that differences between cancer cells and normal cells in their requirement for methionine may be larger than their differences in their requirement for glucose. Cancers of all types have been shown to be methionine-addicted (6, 7).

Another important hint came from the discovery how cancers are addicted to methionine: cancer cells can biosynthesize methionine from its immediate precursor homocysteine at normal or higher rates, but still require large amounts of methionine for excess transmethylation reactions, in contrast to normal cells of all types (8-12). It appears that methionine addiction is a universal hallmark of cancer and is is termed the Hoffman effect. Important early studies showed that methionine-restricted (MR) cancer cells selectively arrest in late S/G_2 of the cell cycle (13). This unusual cell-cycle arrest was exploited to demonstrate that cell-cycle-specific cancer chemotherapy was very effective when combined with MR (14-16).

Recently Locasale et al found that normal people on a low-methionine diet re-programmed their methionine metabolism (17).

The enzyme methionine γ-lyase (methioninase) was first used for cancer treatment in a mouse model in 1973 (18). Soda *et al*. (19) and Tan et al (20) subsequently developed a methioninase from *Pseudomonas putida* and later cloned it in *E. coli*, thereby establishing recombinant methioninase (rMETase). Methioninase was tested in cancer patients for its ability to deplete circulating methionine after intravenous injection (*i.v.*). Methionine was rapidly depleted to undetectable levels in the patients with no adverse effects (21, 22). rMETase was then tested by *i.v.* administration in macaque monkeys and found to cause anaphylacsis unless the rMETase was conjugated to polyethylene glycol (PEG) (23, 24). This made methioninase difficult to develop as a clinical therapeutic.

However in 2017, we showed that methioninase was highly effective when administered orally (o-rMETase) in patient-derived orthotopic xenograft (PDOX) models of various cancers. For example, o-rMETase could overcome gemcitabine resistance in a PDOX model of pancreatic cancer (25). o-rMETase overcame chemotherapy-resistance in sarcoma (26-28) and triple-negative breast cancer (29, 30). Early clinical studies have shown that o-rMETase decreases or stabilizes prostate specific antigen (PSA) levels in patients with advanced-prostate cancer with no adverse effects (31-33). Currently, approximately 40 patients with advanced cancer are taking o-rMETase as a supplement.

Future Directions

Currently o-rMETase is given to cancer patients as a supplement. o-rMETase can also be developed as a drug in the future. In the near future, o-rMETase, as a supplement, will be given to patients to enhance chemotherapy, especially combined with agents that inhibit DNA synthesis or anti-mitotic agents, including 5-fluoruracil (5-FU), cisplatinum, gemcitabine, and paclitaxel, which have been shown to be effective in combination with o-rMETase in PDOX models (34-36). The patients taking o-rMETase are urged to also have a low-methionine diet (37). It is hoped in the near future, that methionine restriction, with both a low-methionine diet and o-rMETase, will be ready for prime time in the clinic, especially in combination with synergistic therapy, for advanced cancer patients who have failed standard therapy. The combination of rMETase, a methionine-restricted diet and synergistic chemotherapy has been shown to be very effective in a mouse-model of brain cancer (38). Early clinical trials of nutritional MR, with and without chemotherapy showed promising results in some advanced cancer patients. Epner was among the first to administer cancer patients a methionine-free diet and found that it may have slowed or arrested the cancer in advanced patients (39, 40). Thivat *et al*. (41) reported that a methionine-free diet combined with cystemustine had some efficacy in melanoma and glioma patients in a phase I clinical trial (41). Durando et al (42) reported that a methionine-free diet and FOLFOX chemotherapy had efficacy in some patients with metastatic colon cancer. Goseki et al (43) reported methionine-free total parenteral nutrition (TPN) with chemotherapy was significantly more effective than methionine-containing TPN with chemotherapy, in advanced gastro-intestinal- cancer patients. The addition of o-rMETase to regimens with methionine-free nutrition (44) and chemotherapy holds clinical promise.

Conflicts of Interest

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

Authors' Contributions

JY and RMH: writing, review, and/or revision of the article. QH, MS, and DT gave technical support and conceptual advice.

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