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Long-term Stable Disease in a Rectal-cancer Patient Treated by Methionine Restriction With Oral Recombinant Methioninase and a Low-methionine Diet

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Abstract. *Background/Aim: Rectal cancer is a recalcitrant disease with limited treatment options. Pre-clinical studies have shown the efficacy of methionine restriction with a low-methionine diet and oral recombinant methioninase (o-rMETase) for colorectal cancer. There are also clinical studies on methionine restriction with o-rMETase for other recalcitrant cancer types. The goal of the present study was to determine the efficacy of a low-methionine diet and o-rMETase on a rectal cancer patient. Patient and Methods: A 55-year-old man diagnosed with recurrent locally-advanced rectal-cancer was treated with o-rMETase and a low-methionine diet, during which time, he did not receive standard chemotherapy. Disease stability was monitored by carcinoembryonic antigen (CEA) levels, sigmoidoscopy, and computed tomography (CT). Results: The patient was diagnosed with stage II rectal cancer (adenocarcinoma) in 2018. After neoadjuvant chemo-radiotherapy, the patient received total mesorectal excision (TME) in 2018. Local recurrence was found by sigmoidoscopy one year later. The patient was given chemotherapy, the recurrent lesion shrunk, and was then removed endoscopically in December 2019, with positive margins. The tumor did not become apparent for about a year after that. An endoscopic examination performed in December 2020, revealed a local recurrence. Since that time, the patient had an elevated CEA. The patient went on o-rMETase and a low-methionine diet from January 2021. Since then, the patient's CEA level has remained stable for the next year and a half. He received sigmoidoscopy and CT regularly, and the tumor size has not changed. Conclusion: This patient's clinical course indicates that o-rMETase and a low-methionine diet may be effective for rectal cancer, for long-term disease stabilization. Further case studies and clinical trials are needed to determine the generality of the present result.*

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Key Words: Rectal cancer, patient, stable disease, methionine addiction, Hoffman effect, methionine restriction, oral recombinant methioninase, low-methionine diet.

Colorectal cancer is the fourth-most common cancer and the second leading cause of cancer death in the United States (1). Rectal cancer accounts for about one-third of these cases. Regarding locally-advanced disease (T3-4, N0), neoadjuvant chemoradiotherapy and adjuvant chemotherapy are the standard-of-care for rectal cancer. Although the 3-year disease-free survival rate has increased from 62.9 % to 75.9 % for locally-advanced disease, it is still not sufficient (2-4). It is also important for patients with rectal cancer to preserve anal function. Most patients with rectal cancer, especially those whose tumor is located close to the anal verge, have undergone total mesorectal excision with a colostomy.

Methionine addiction is a fundamental and general hallmark of cancer (5-7) and is termed the Hoffman effect (8). Since cancer cells require a greater amount of methionine than normal cells, which is due to the excess transmethylation reactions in cancer cells, they cannot survive under the condition of methionine restriction (9-11). We have developed recombinant methioninase (rMETase) (12), which catabolizes methionine, and have shown the efficacy of rMETase for colorectal cancers using patient-derived orthotopic xenograft (PDOX) models and cell lines (13-16). Durando *et al*. reported the clinical efficacy of a methionine-restricted diet in combination with standard first-line chemotherapy (fluorouracil and oxaliplatinum) for

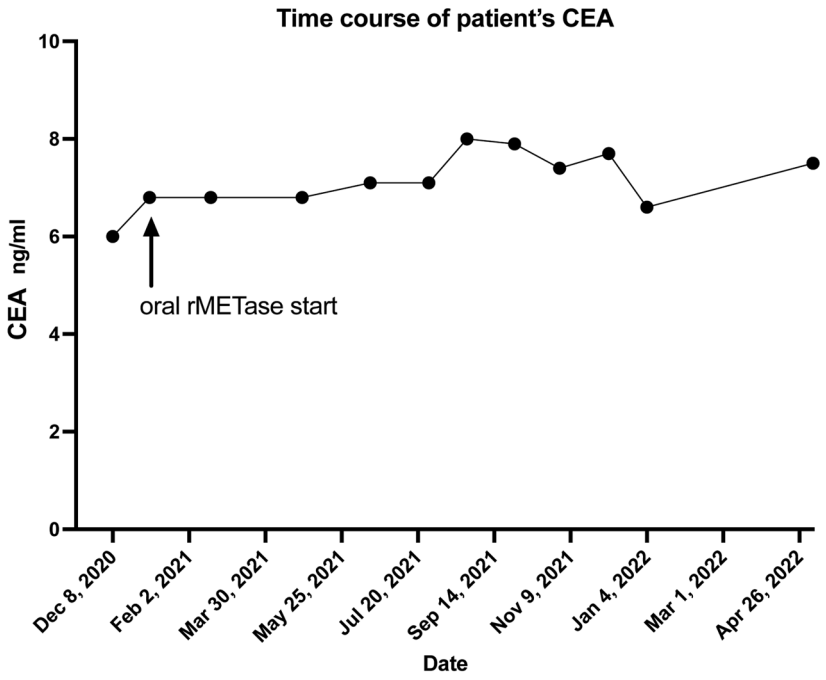

Figure 1. *Time course of patient's carcinoembryonic antigen (CEA) levels (ng/ml).*

patients with colorectal cancer (17). In this Phase I trial, 3 of 4 evaluable patients had a partial response, and one had stable disease for 29.4 months (17).

In the present case report, a 55-year-old male with rectal cancer who received rMETase orally and was on a strict methionine-restriction diet, currently has stable disease for 1.5 years after local recurrence.

Patients and Methods

rMETase production and formulation. rMETase was produced by fermentation of recombinant E. coli transformed with the methioninase gene from *Pseudomonas putida*. Methioninase was purified using a heat step at 60 degrees, polyethylene glycol precipitation, and column chromatography with diethylaminoethyl (DEAE)-Sepharose FF, with high yield (18, 19).

Methionine restriction and rMETase administration. The patient underwent methionine restriction with a low-methionine diet according to the Nutritional Oncology Research Institute (NORI) protocol (20), which recommends less than 2 mg/kg methionine intake per day. rMETase was taken twice a day at a dose of 250 units as a supplement.

Case Report

A 55-year-old male was diagnosed with rectal cancer (adenocarcinoma) in 2018. There were no lymph-node or distant metastasis on computed tomography (CT), and magnetic resonance imaging (MRI) showed that the depth of the tumor invaded the perirectal tissues. The clinical stage was Stage IIA, and the treatment strategy included surgery after neoadjuvant chemoradiotherapy. The patient started capecitabine (orally twice daily at 850 mg/m^2 throughout radiotherapy) and radiotherapy (50.4 Gy) in March 2018 and received total mesorectal excision (TME) on June 05, 2018. Pathological findings showed that the final stage was Stage II (T3N0M0). After surgery, the patient decided not to receive adjuvant chemotherapy. However, local recurrence was found on sigmoidoscopy in June, 2019. The patient received chemotherapy and the tumor shrunk. The patient had the residual tumor removed endoscopically without a clean margin in December 2019. The tumor did not become apparent for about a year after that. An endoscopic examination performed in December, 2020, revealed recurrence. Until then, blood tests showed elevated carcinoembryonic antigen (CEA) levels. The patient did not want to receive chemotherapy because the tumor was localized and small. Accordingly, the patient started methionine restriction with o-rMETase and a low-methionine diet in January 2021. Since then, the patient's CEA levels have remained stable for the next year and a half (Figure 1). The patient received sigmoidoscopy and CT regularly, the tumor size has not changed, and no new lesions have been observed.

Discussion

This is the first clinical report that methionine restriction, combining rMETase and a low-methionine diet, prevents the progression of rectal cancer. In the present case, the patient's CEA levels were stable for about one year and a half with methionine restriction with o-rMETase and a low-methionine diet.

Methionine addiction is a fundamental and general hallmark of cancer cells (5-7) (21-23). We previously showed that even though cancer cells can synthesize methionine from homocysteine to a similar or greater extent than normal cells, they require a large amount of exogenous methionine due to excess transmethylation reactions (9-11, 24). Gao *et al*. reported that methionine restriction enhanced the therapeutic response in RAS-driven colorectal cancer and soft-tissue sarcoma in patient-derived xenograft models (PDX) (25), confirming results we found more than 25 years earlier (26-28). Gao *et al*. also showed that methionine restriction disrupts the flux of one-carbon metabolism and effects vulnerabilities involving redox and nucleotide metabolism in PDX models as well as human studies. These results indicated that methionine restriction itself may be effective in colorectal cancer patients.

Additionally, Zhang *et al*. reported that increasing levels of S-adenosylmethionine (SAM), which is the universal methyl donor, upregulated the expression of cell adhesion genes and promoted tumor cell migration and metastasis in colon cancer cell lines (HCT116, SW480) (29). This result indicates that methionine restriction may inhibit metastasis by depleting SAM.

Previously we have shown that o-rMETase has potential clinical efficacy in prostate and pancreatic cancer patients (30-33).

In the present rectal cancer case, methionine restriction with o-rMETase and a low-methionine diet showed significant efficacy for locally-recurrent rectal cancer, suggesting that methionine restriction has clinical potential for rectal cancer.

Positron emission tomography (PET) with [^{11}C] methionine (34) has shown that the Hoffman effect of methionine addiction of cancer is stronger than the Warburg effect of glucose addiction, suggesting that methionine restriction is an important target for rectal and other cancers. Although PD-1 blockade has been used to obtain complete responses in rectal cancer (35), the durability of the responses is not yet clear and potential side effects can occur from immunotherapy. Methionine restriction is a possible safe and effective alternative and targets a fundamental hallmark of cancer (36) and is synergistic with cytotoxic chemotherapy (37).

Conflicts of Interest

The Authors declare no competing interests regarding this work.

Authors' Contributions

YK, TT, and RMH wrote the paper. QH produced methioninase. YA, NM, and KO reviewed the manuscript.

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