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[^{11}C] Methionine-PET Imaging as a Cancer Biomarker for Methionine Addiction and Sensitivity to Methionine-restriction-based Combination Chemotherapy

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Abstract. *Background/Aim: Methionine addiction is a fundamental and universal hallmark of cancer, termed the Hoffman effect. Methionine addiction of cancer is greater than glucose addiction, termed the Warburg effect, as shown by the comparison of PET imaging with [^{11}C]methionine and [^{18}F]fluorodeoxyglucose. The aim of the present study was to determine whether [^{11}C]methionine PET (MET-PET) images could be a biomarker of methionine addiction of cancer and potential response to methionine-restriction-based combination chemotherapy. Patients and Methods: In the present study a patient with invasive lobular carcinoma of the breast metastatic to axillary lymph nodes was imaged by both MET-PET and [^{18}F]fluorodeoxyglucose PET (FDG-PET) before and after combination treatment with methionine restriction, comprising a low-methionine diet and methioninase, along with first-line chemotherapy. Results: MET-PET gave a much stronger and precise image of the patient's metastatic axillary lymph nodes than FDG-PET. The patient had a complete response to methionine restriction-based chemotherapy as shown by MET-PET. Conclusion: MET-PET imaging is a biomarker of methionine-addicted cancer and potential response to methionine-restriction-based chemotherapy.*

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Methionine addiction is a fundamental and general hallmark of cancer, termed the Hoffman effect (1-6). Cancer cells synthesize larger-than-normal amounts of methionine from homocysteine but still need an external source of methionine to survive and proliferate. Hence, cancer cells are addicted to methionine. In contrast normal cells proliferate equally well with either methionine or its precursor homocysteine (2-6). Methionine addiction of cancer is due to excess use of methionine by cancer cells for transmethylation reactions (6-11). Methionine addiction is tightly linked to the malignancy of cancer (10, 12-15) and appears to be a universal hallmark of cancer (5, 16, 17).

Therefore, methionine addiction of cancer is a promising target of methionine-restriction therapy. The bacterial enzyme methioninase was initially developed to target methionine addiction of cancer in mouse models (18-21) and humans (22, 23), as an injectable therapy. However, monkey studies showed that anaphylaxis could occur with injectable methioninase (24). It was later discovered that methioninase was effective when administered orally (25-41) in mouse models of cancer and in the clinic (42-48). We originally showed that methionine restriction was synergistic with chemotherapy (49-51), which appears to be a general phenomenon (50-52).

Recently a patient with axillary lymph-node metastases of invasive lobular carcinoma (ILC) of the breast was treated with methioninase and a low-methionine diet in combination with first-line chemotherapy. Before treatment [^{11}C]methionine PET (MET-PET) and [^{18}F]deoxyglucose PET (FDG-PET) imaging

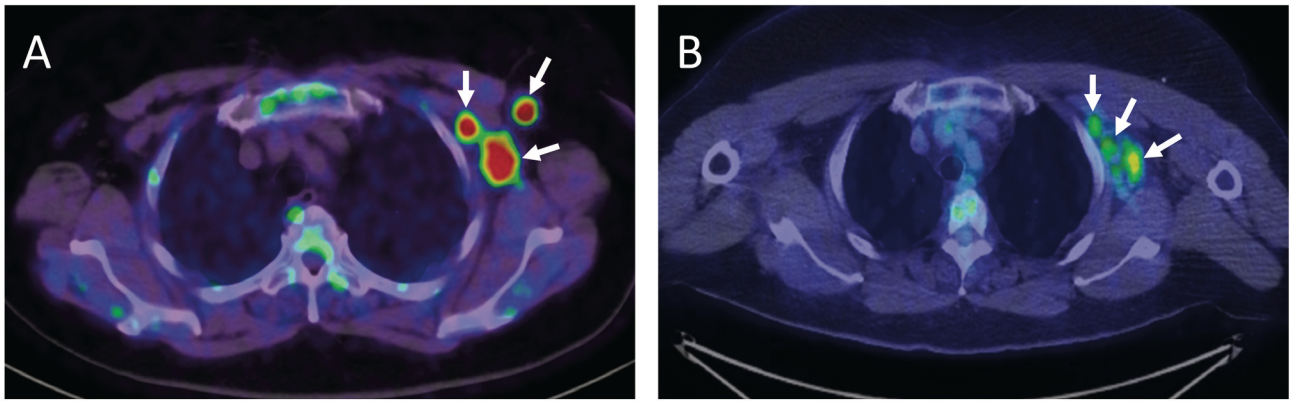

Figure 1. *Axillary lymph-node metastases. (A): [^{11}C]MET-PET imaging. (B): [^{18}F]FDG-PET imaging. Arrows indicate axially lymph-node metastases.*

demonstrated a very strong signal in the metastatic axillary lymph nodes and then demonstrated a complete response after 6 months of first-line chemotherapy, oral methioninase and a low-methionine diet. The MET-PET images were much stronger and delineated than the FDG-PET images. The present report suggests that MET-PET images can serve as a biomarker for methionine addiction and sensitivity to methionine-restriction combination chemotherapy.

Patients and Methods

Recombinant methioninase (rMETase) production and formulation. rMETase was produced in *Escherichia coli*, which was transformed with the *methioninase* gene obtained from *Pseudomonas putida*. The purification of rMETase was carried out using heat precipitation of non-methioninase proteins at 60˚C, subsequent polyethylene glycol precipitation of methioninase, followed by diethylaminoethyl-sepharose fast-flow chromatography. rMETase was prepared in a solution of normal saline with pyridoxal 5'-phosphate (PLP), at a concentration of 5 mg/ml (19, 53).

Methionine restriction and rMETase administration. The patient, with invasive lobular carcinoma of the breast, was administered a low-methionine dietary regimen, with daily intake of methionine below 2 mg per kg body weight (53). A total of 250 units of rMETase were orally administered four times daily, as a dietary supplement.

[^{11}C]MET-PET and [^{18}F]FDG-PET imaging. The patient adhered to a minimum fasting period of 6 hours before the MET-PET examination. The patient received intravenous administration of [^{11}C]methionine at a dosage of 370 MBq per kilogram of body weight, along with hydration using a solution of 0.9% sodium chloride. Physical activity was minimized, and a rest period of 10 min was implemented after the injection. At 10 min post-injection, the MET-PET scan was conducted utilizing a Vereos Digital PET/computed tomography (CT) apparatus (Philips, Amsterdam, The Netherlands). The PET scanner utilized 23,040 lutetium-yttrium oxyorthosilicate (LYSO) crystals arranged in 64 rings. Subsequently, the patient received intravenous administration of [^{18}F]deoxyglucose at a dosage of 4.4 MBq/kg body weight, followed by the FDG-PET using the same protocol as described above. To provide attenuation correction for the PET images, a low-amperage CT scan was obtained using the following parameters: 213 mA current, 120 kV for voltage, and a CT slice thickness of 5 mm. The CT dosage index was recorded as 10.7 milligrays (mGy). Following a non-enhanced CT scan, a comprehensive PET examination of the entire body was conducted in the caudocranial direction, spanning from the upper thighs to the vertex. Each bed position was imaged for a duration of 2 min. The reconstruction process was executed *via* the 3D reconstruction technique known as ordered subset expectation maximization. This method involved employing 30 subsets and conducting two iterations. A nuclear-medicine physician conducted an examination of the images.

Results

Case report. A 59-year-old Japanese female presented with invasive lobular carcinoma of the breast in 2018 and underwent a left mastectomy at the National Cancer Center, Tokyo. The patient subsequently took the estrogen-blocker anastrozole. Three years later, the patient presented with extensive axillary lymph-node metastasis as shown by MET-PET imaging, with very strong and sharply delineated signals in the left axillary lymph nodes (Figure 1A). The MET-PET image was much stronger and more precise than the FDG-PET image (Figure 1B). The patient was then treated with doxorubicin-cyclophosphamide (AC) chemotherapy for 3 months, along with a low-methionine diet (52) and oral recombinant methioninase [250 units, q4×day, administered orally (p.o)]. In the subsequent 3 months the patient received docetaxel under methionine restriction with the low-methionine diet and methioninase. At the end of the 6 months treatment period, MET-PET imaging showed a complete response (CR) (Figure 2A and B). One year after standard chemotherapy combined with methionine restriction with a low methionine diet, and oral

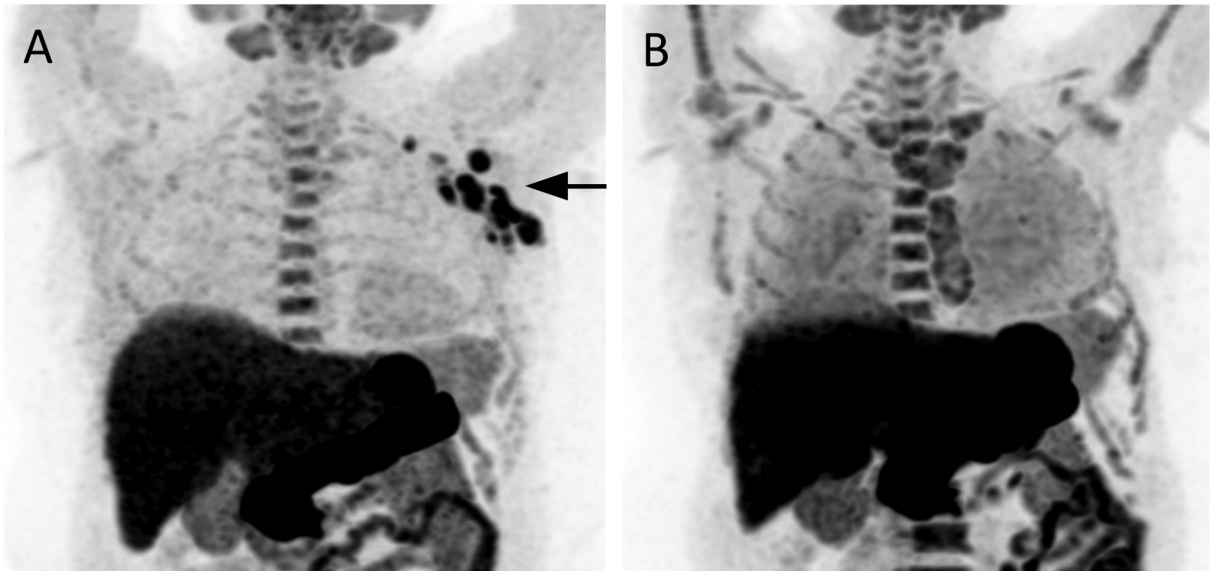

Figure 2. *[^{11}C]MET-PET imaging of axillary lymph-node metastases (arrow). Before (A) and after 1st-line chemotherapy combined with methionine restriction (B).*

rMETase, MET-PET showed that the CR was durable (Figure 3).

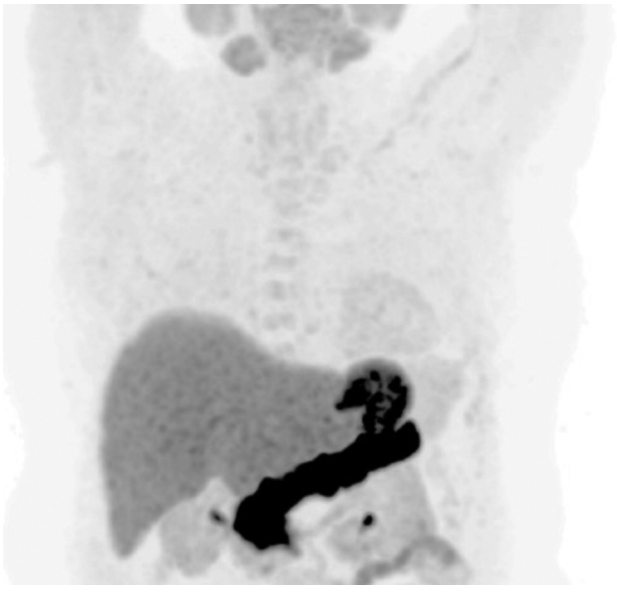

Figure 3. *[^{11}C]MET-PET imaging. One year after the start of methionine-restriction-based chemotherapy.*

Discussion

It has been known since 1969 that methionine restriction can inhibit cancer growth in rats (55). This result was confirmed in cell culture 14 years later (56, 57). We showed that cancer cells are methionine addicted in 1976 (2). Wang *et al*. observed that cancer stem cells are highly methionine addicted in 2019 (6). We first showed in 1986 that methionine restriction and chemotherapy are synergistic (49). This study was followed by numerous other studies with a wide variety of chemotherapy drugs demonstrating synergy with methionine restriction, including oral methioninase (52). The demonstration that methioninase could be administered orally showed the clinical potential of methionine restriction. Initial clinical case studies of oral methioninase treatment with or without chemotherapy had efficacy in prostate (42-44), pancreatic (45), rectal (46, 47) and ILC breast (48) cancer.

However, there has not been a biomarker of methionine addiction of cancer that could indicate that a cancer patient is a strong candidate for methionine-restriction-based combination chemotherapy. The present study demonstrates a strong and precise signal with [^{11}C]methionine PET imaging of the patient's axillary-lymph-node metastases (Figure 1A), which was much stronger and precise than [^{18}F]deoxyglucose PET imaging of the axillary-lymph-node metastasis (Figure 1B).

To move forward, more radiology clinics should offer [^{11}C]methionine-PET for whole body imaging (58) and awareness should be increased among oncologists of the general potential of combining chemotherapy with methionine restriction (52, 59).

Conflicts of Interest

The Authors declare no competing interests regarding this work.

Authors' Contributions

YK, and RMH wrote the article. QH provided methioninase. TS, CH, SM, KM and TT critically reviewed the article.

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