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Oral-recombinant Methioninase Converts an Osteosarcoma from Docetaxel-resistant to -Sensitive in a Clinically-relevant Patient-derived Orthotopic-xenograft (PDOX) Mouse Model

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Abstract. *Background/Aim: Osteosarcoma is the most frequent malignant bone tumor. Failure of first-line therapy results in poor prognosis of osteosarcoma. In the present report, we examined the efficacy of the combination of oral recombinant methioninase (o-rMETase) and docetaxel (DOC) on an osteosarcoma patient-derived orthotopic xenograft (PDOX) mouse model. Materials and Methods: Osteosarcoma-PDOX models were established by tumor insertion within the tibia of nude mice. The osteosarcoma PDOX models were randomized into four groups (4-5 mice per group): control; o-rMETae alone; DOC alone; o- rMETase combined with DOC. The treatment period was 3 weeks. Results: The combination of o-rMETase and DOC showed significant efficacy compared to the control group (p=0.03). In contrast, there was no significant efficacy of o-rMETase alone or DOC alone (p=0.65, 0.60, respectively). Conclusion: o-rMETase converted an osteosarcoma PDOX from DOC-resistant to -sensitive. This combination therapy may be effective against recalcitrant osteosarcoma and other recalcitrant cancers.*

Osteosarcoma is classified as a rare cancer, but it is the most frequent malignant bone tumor. Failure of first-line therapy results in very poor prognosis of osteosarcoma patients (1-4).

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In order to individualize and improve therapy for recalcitrant osteosarcoma, our laboratory has established a patient-derived orthotopic xenograft (PDOX) mouse model of osteosarcoma to identify potential effective treatment strategies (5-20).

Methionine addiction (21) is a fundamental and general hallmark of cancer, resulting in the requirement of very high levels of methionine compared to normal cells (21-26). Methionine addiction of cancer is termed the Hoffman effect (27-29), which is analogous to the glucose addiction of cancer cells, termed the Warburg effect. The methionine-degrading enzyme, recombinant methioninase (rMETase), effectively targets methionine addiction to inhibit or arrest cancer cells in late-S/G_2 phases of the cell cycle (30-36).

Docetaxel (DOC) arrests cells in the M-phase of the cell cycle (37), complementing the effect of rMETase (38). The efficacy of the combination of DOC and gemcitabine (GEM) in osteosarcoma, especially in relapsed or refractory cases, has been reported (37). DOC has also shown synergy with AG-270, an inhibitor of methionine adenosyl-transferase 2α (MAT2A), which is involved in methionine addiction (39).

In 2018, our laboratory discovered that rMETase could be effectively administrated orally (o-rMETase) (32), which greatly facilitated treatment of recalcitrant cancer in both PDOX models and patients (19, 20, 32-34, 40-57).

In the present study, we examined whether the combination of o-rMETase and DOC is effective in an osteosarcoma-PDOX mouse model.

Materials and Methods

Mice. Athymic nu/nu nude mice in the present study, (AntiCancer, Inc., San Diego, CA, USA), 4-6week old, were used as previously described (5-20), with Institutional Animal Care and Use Committee (IACUC) approval, following the principles and procedures provided in the National Institutes of Health (NIH) Guide for the Care and Use of Animals, under Assurance Number A3873-1 (5-20).

Patient-derived tumor. An osteosarcoma biopsy specimen from a 14-year-old boy with pelvic osteosarcoma was previously surgically obtained from the UCLA Medical Center after patient and parent informed written consent and Institutional Review Board approval (IRB#10-001857) and established in nude mice as previously reported (20).

Tibia-insertion osteosarcoma PDOX model. A 1-mm diameter medullary cavity was made in the proximal tibia and 1 mm^3 tumor fragments, previously grown subcutaneously in nude mice, were implanted into the medullary cavity, as previously described (58).

Recombinant methioninase (rMETase) production. The protocol for the production of rMETase has been previously reported (59).

Treatment study design. The osteosarcoma-PDOX mouse models were randomized into four groups of four or five mice per group as follows: G1, control PBS (0.2 ml/day, oral, twice a day); G2, o-rMETase (50 units/mouse, oral, twice a day); G3, DOC [20 mg/kg, intraperitoneal (*i.p.*) injection, once a week]; G4, combination of o-rMETase (50 units, oral, twice a day) and DOC (20 mg/kg, *i.p.* injection, once a week). The treatment was initiated once tumor size reached a volume of 40 mm^3. Tumor measurement and tumor-volume calculation were performed as previously described (5-20). The treatment period was 3 weeks for each group, and all mice were sacrificed after treatment as previously described (5-20) (Figure 1). Data are presented as mean±standard deviation.

Hematoxylin and eosin (H&E) staining. Procedures for H&E staining were performed according to standard protocols.

Statistical analyses. All statistical analyses were performed with JMP ver. 15.0.0 (SAS Institute, Cary, NC, USA). Welch's *t*-test was applied as the parametric test to compare the means between two related groups. Tukey-Kramer HSD was performed for the parametric test of comparison between groups. Bar graphs show the mean, and error bars indicate standard deviation of the mean. A *p*-value ≤0.05 was defined as statistically significant.

Results

Treatment efficacy on the osteosarcoma PDOX. There were no significant differences in tumor volume of the osteosarcoma-PDOX between the control and those treated with o-rMETase alone, or DOC alone, at the end of the treatment period (p=0.65, 0.60, respectively). In contrast, the combination of o-rMETase and DOC showed significant efficacy to reduce tumor volume compared to the control group (p=0.03) (Figure 2). There were no animal deaths in any group. Mouse weight showed no significant differences between the four groups (Figure 3).

Histology of osteosarcoma-PDOX. The osteosarcoma-PDOX tissue of the control group comprised high-density spindle-shaped cancer cells (Figure 4A). Treatment with o-rMETase-alone or DOC alone had no effect on the histologic phenotype of the osteosarcoma PDOX, which was similar to the control. Treatment with the combination of DOC and o-rMETase reduced cancer-cell density in the osteosarcoma PDOX (Figure 4B-D).

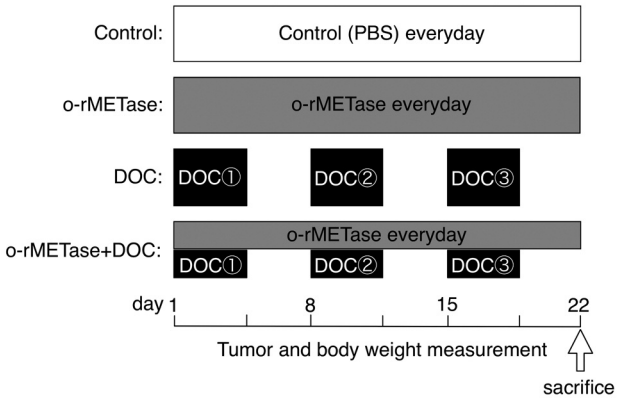

Figure 1. *Treatment scheme*.

Discussion

The present study showed that o-rMETase converted an osteosarcoma PDOX from DOC-resistant to -sensitive. The combination of DOC and GEM has shown efficacy as second-line therapy for soft-tissue sarcoma, following failure of first-line treatment with doxorubicin (DOX) and ifosfamide (IFO) (60). In the present study, DOC alone had no efficacy on the osteosarcoma PDOX, but it was highly effective in combination with o-rMETase. The present study was performed in a clinically-relevant osteosarcoma PDOX model, compared to an un-physiological subcutaneous-tumor model of sarcoma (61).

The present results are consistent with a previous study reporting that the combination of AG-270, a methionine adenosyltransferase 2α (MAT2A) inhibitor, with DOC showed efficacy on non-small-cell lung carcinoma (NSCLC) and esophageal squamous cell carcinoma (SCC) in patient-derived xenograft (PDX) models, where neither DOC alone nor AG-270 alone showed significant efficiency (39).

AG-270 targets methionine addiction, as does o-rMETase, and the efficacy of the combination of AG-270 and DOC suggested that the combination of o-rMETase and DOC would be effective. Indeed, our present results confirmed this hypothesis. o-rMETase and DOC are complementary as o-rMETase selectively arrests cancer cells in late-S/G_2-phases of the cell cycle (35, 36), DOC arrests cells in the M phases (37). The combination of methionine restriction and an anti-mitotitc has been previously shown to be selectively effective on cancer cells on a co-culture of cancer and normal cells, as cancer cells which escaped from the late-S/G_2 arrest by methionine restriction were arrested by the antimitotic in M phase (38).

The present results suggest that the combination of o-rMETase and DOC should be effective against recalcitrant osteosarcoma and other recalcitrant cancers. o-rMETase and combination chemotherapy for blockade of the methionine-methylation axis (40, 41) is also a promising strategy as o-rMETase has shown clinical efficacy (33).

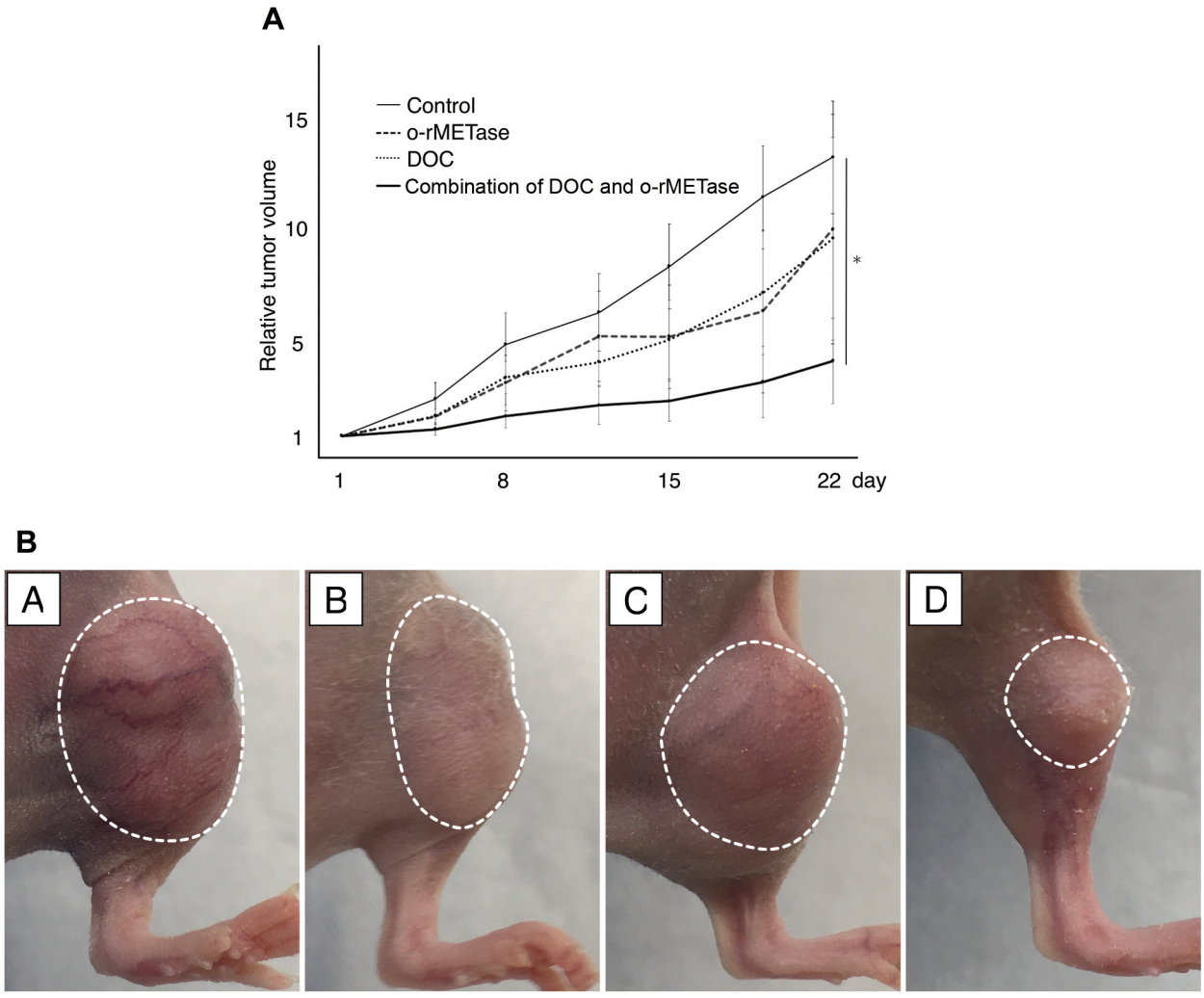

Figure 2. *A: Efficacy of drugs on the osteosarcoma-PDOX. Line graphs show relative tumor volume at each time point. Relative tumor volume is defined as the tumor volume at time (t) divided by the tumor volume at the onset of treatment. n=4-5 mice/group. *p<0.05. Error bars: ±SD. B: Representative photographs of osteosarcoma-PDOX mouse models from each treatment group at the end of treatment. A: Untreated control; B: o-rMETase alone; C: DOC; D: o-rMETase and DOC.*

Conflicts of Interest

The Authors have no conflicts of interest to declare in relation to this study.

Authors' Contributions

YA, YT and RMH were involved in study conception and design. YA and NFW were involved in acquisition of data. YA, YT, NFW, JY, KH and RMH analyzed and interpreted data. YA, YT and RMH wrote this manuscript. All Authors reviewed and approved the manuscript.

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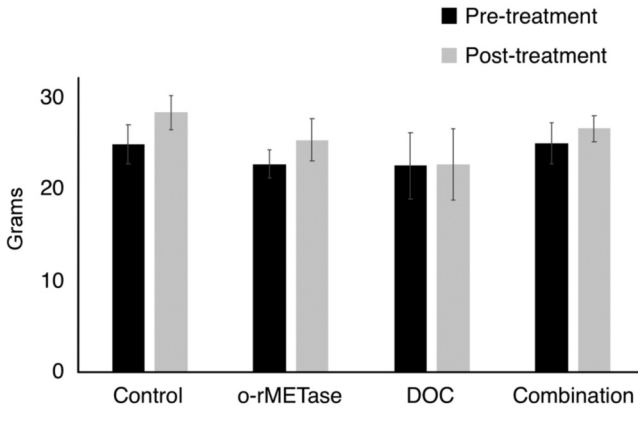

Figure 3. *Mouse body weight at pre- and post-treatment.*

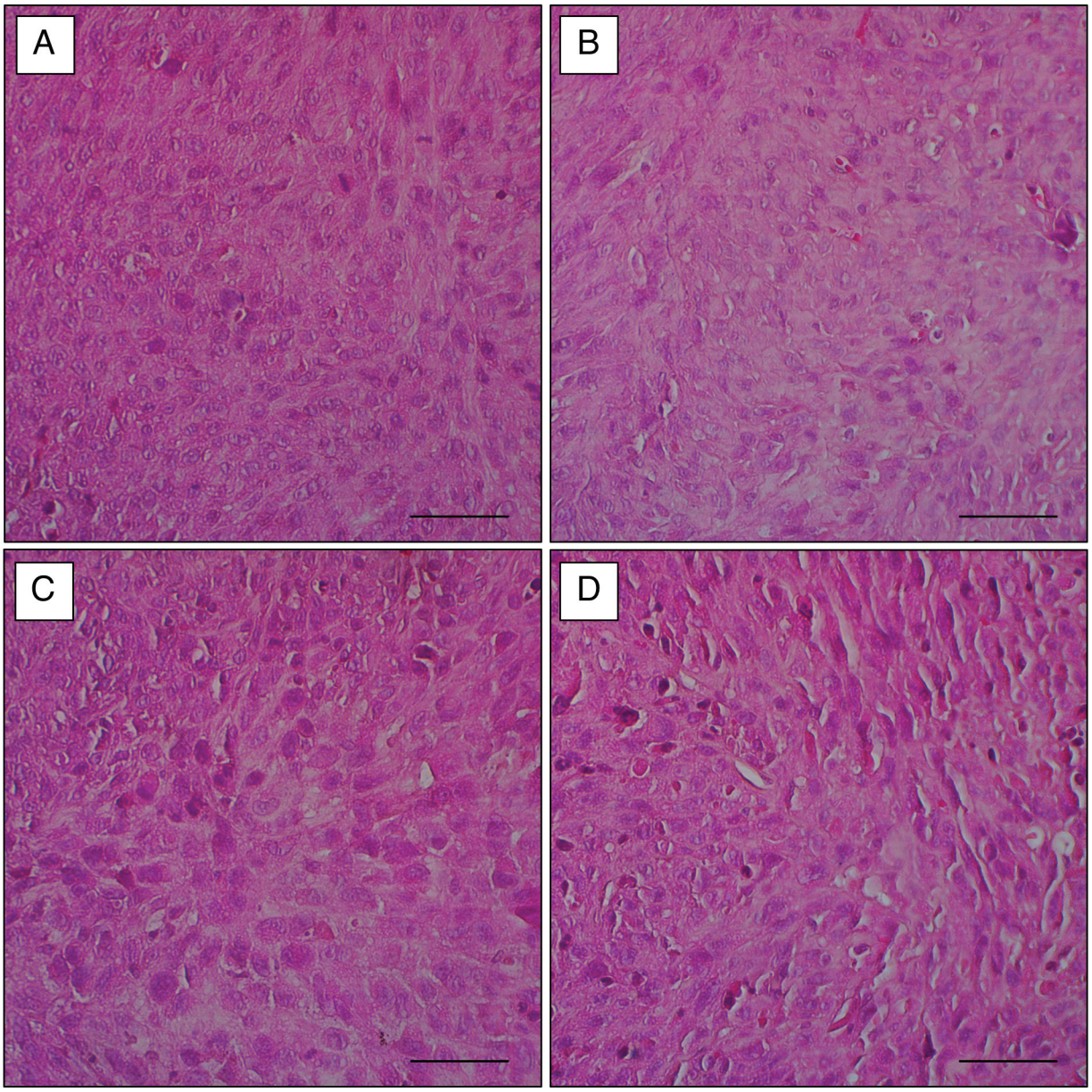

Figure 4. *Representative photomicrographs of H & E-stained tissue sections of the untreated and treated osteosarcoma-PDOX. (A) Control administered oral PBS. (B) o-rMETase. (C) DOC. (D) Combination of o-rMETase and DOC. Magnification: 200×. Scale bar: 50 μm.*

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