Efficacy of Oral Recombinant Methioninase and Eribulin on a PDOX Model of Triple-negative Breast Cancer (TNBC) Liver Metastasis

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Abstract. *Background/Aim:* The aim of the present study was to identify effective drugs for a highly-aggressive liver-metastasis of triple-negative breast cancer (TNBC) in a patient-derived orthotopic xenograft (PDOX) mouse model. *Drugs tested* were oral recombinant methioninase (o-rMETase), low-dose eribulin and their combination. *Materials and Methods:* Patient-derived TNBC was implanted in the liver of nude mice by surgical hepatic implantation. Two weeks after transplantation, 32 mice were randomized (n=8 per group) into a phosphate-buffered saline vehicle-control group; o-rMETase-treatment group (100 units, o-rMETase, oral, daily for 2 weeks); eribulin-treatment group (0.05 mg/kg intraperitoneally once per week for 2 weeks); or combination-treatment group (100 units o-rMETase, oral, daily for 2 weeks + 0.05 mg/kg eribulin intraperitoneally once per week for 2 weeks). *Results:* After 2 weeks, the three treatment groups exhibited significantly-inhibited TNBC growth in the liver compared to the vehicle-control group ($p \leq 0.05$). *Conclusion:* o-rMETase and low-dose eribulin monotherapy and their combination were efficacious against the highly-aggressive TNBC PDOX growing in the liver. The TNBC PDOX model can be used to identify highly-effective drugs for therapy of TNBC with liver metastasis.

Triple-negative breast cancer (TNBC) is negative for estrogen receptor, progesterone receptor and human epidermal-growth-factor receptor 2 (1). TNBC is highly

recalcitrant to chemotherapy such as anthracyclines and taxanes and has very poor prognosis, as most patients die of metastatic disease (2, 3).

Eribulin is an anti-microtubule agent that prevents microtubule polymerization with anti-mitotic activity. Recently, eribulin was shown to be active clinically against metastatic breast cancer, increasing overall survival in the EMBRACE trial (4).

The patient-derived orthotopic xenograft (PDOX) mouse model, which mimics clinical cancer, was developed by our laboratory in 1991 (5). We established the first PDOX model of breast cancer, which showed metastases to the lung in 1993 (6). We previously developed PDOX models of TNBC (7-10) and showed the primary tumor to be sensitive to eribulin and oral recombinant methioninase (o-rMETase). We also developed a liver-metastasis PDOX model of TNBC (11). In the present study, we show that the liver-metastasis PDOX model is sensitive to low-dose eribulin and o-rMETase and their combination.

Materials and Methods

Mice. Female nu/nu nude female mice (AntiCancer Inc., San Diego, CA, USA), 4-6 weeks old, were used in this study. The breeding and maintenance of the nude mice are described in our previous reports (7, 8). All mouse studies were carried out under the National Institutes of Health Guide for the Care and Use of Animals (Assurance Number A3873-1).

Patient-derived TNBC and establishment of PDOX with liver metastasis. The TNBC tissue used was from a 74-year-old female patient diagnosed with invasive ductal-carcinoma in the right breast. Breast-conserving surgery was performed at the Department of Surgery, Samsung Medical Center, Seoul, Korea as previously described elsewhere (9, 10). Written informed-consent was obtained from the patient with Samsung Medical Center Institutional Review Board approval. The TNBC tumor had initially been established subcutaneously in nude mice, and PDOX models were subsequently established (9, 10). The liver-metastasis model was established by implanting a tumor fragment, harvested from subcutaneous growth,

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Key Words: Triple negative breast cancer, TNBC, patient-derived orthotopic xenograft, PDOX, liver metastasis, eribulin, oral recombinant methioninase, o-rMETase.

Figure 1. Comparison of inhibitory efficacy on the liver-metastasis triple-negative breast cancer (TNBC) patient-derived orthotopic xenograft. A: Final tumor weight in the phosphate-buffered-saline-vehicle (G1); oral-recombinant-methioninase (o-rMETase) monotherapy (G2); eribulin monotherapy (G3); and combination therapy (o-rMETase + eribulin; G4) groups. B: Representative images of TNBC growing in the liver at necropsy. Data are the mean $\pm$ SD of eight animals. Significantly different at * $p=0.05$ and ** $p<0.01$ compared to G1.

into the liver (11). The tumor fragments were implanted into the left liver-lobe by surgical hepatic-implantation to establish the liver-metastasis PDOX model. The wound was closed with a 7-0 nylon suture (11).

Treatment dose and schedule. Two weeks after implantation, the TNBC PDOX mouse models were randomized into four groups of eight mice each: Phosphate-buffered saline (PBS) vehicle-control group (0.2 ml PBS orally, daily for 2 weeks); o-rMETase-treatment

group (100 units of rMETase orally daily for 2 weeks); low-dose-eribulin-treatment group (0.05 mg/kg eribulin intraperitoneally once per week for 2 weeks); combination-treatment group (100 units r-METase orally daily for 2 weeks plus eribulin intraperitoneally at 0.05 mg/kg once per week for 2 weeks).

Body weights were measured twice a week, and a monitoring curve was plotted. The study was terminated 14 days after the initiation of treatment. All mice were sacrificed, the tumor was completely resected and weighed using an electronic scale.

Figure 2. Body-weight monitoring in the phosphate-buffered-saline-vehicle (G1); oral-recombinant-methioninase (o-rMETase) monotherapy (G2); eribulin monotherapy (G3); and combination therapy (o-rMETase + eribulin; G4) groups during the treatment period. There was no significant difference between the groups. Data are the mean $\pm$ SD of eight animals.

Statistical analyses. All statistical analyses were performed with R ver. 3.6.1 (R foundation, Vienna, Austria). All data were analyzed using one-way ANOVA followed by Dunnett's correction. Graphs show the mean tumor volume and number of mice, and error bars represent standard deviation. A probability value of $p \leq 0.05$ was defined as statistically significant.

Results

As shown in Figure 1, after 2 weeks of treatment, all the treatment groups exhibited significantly lower weight of the TNBC in the liver compared to the PBS-vehicle control ($p=0.05$ for o-rMETase monotherapy; $p=0.01$ for eribulin monotherapy and combination treatment groups).

Clinical observation of animals during the study showed no physical or behavioral signs that indicated adverse effects due to any treatment. A stable body weight in the treated groups, without significant loss compared to the vehicle control, indicated that both eribulin and o-rMETase had no obvious toxicity (Figure 2).

Discussion

Our previous studies have shown eribulin is highly effective against PDOX TNBC (7-10). The present study showed that low-dose eribulin is effective against a liver-metastasis TNBC PDOX.

We also previously showed that o-rMETase is active against the primary TNBC PDOX (9) with a tendency for efficacy

against liver metastasis of TNBC (11). The present study showed o-rMETase significantly inhibited growth of liver-metastasis TNBC. o-rMETase is of particular importance as it targets the methionine addiction of cancer, which is a fundamental and general hallmark of cancer discovered by us (12-20) and which is termed the Hoffman effect (21-23). Future clinical studies will combine low-dose chemotherapy with o-rMETase for TNBC to overcome the recalcitrance of this disease. o-rMETase has currently shown clinical efficacy against advanced prostate cancer (24-26). Previous clinical studies have shown injectable methioninase is safe in metastatic breast-cancer patients (27).

Authors' Contributions

HIL and RMH conceived the study. HIL, YS and JY performed the experiments. QH produced the methioninase. HIL and YS wrote the manuscript. RMH revised the manuscript.

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