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The Combination of Methionine Restriction and Docetaxel Synergistically Arrests Androgen-independent Prostate Cancer But Not Normal Cells

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Abstract. *Background/Aim: Androgen-independent prostate cancer (AIPC) is resistant to androgen-depletion therapy and is a recalcitrant disease. Docetaxel is the first-line treatment for AIPC, but has limited efficacy and severe side-effects. All cancers are methionine-addicted, which is termed the Hoffman effect. Recombinant methioninase (rMETase) targets methionine addiction. The purpose of the present study was to determine if the combination of docetaxel and rMETase is effective for AIPC. Materials and Methods: The half-maximal inhibitory concentrations (IC_{50}) of docetaxel and rMETase alone were determined for the human AIPC cell line PC-3 and Hs27 normal human fibroblasts in vitro. The synergistic efficacy for PC-3 and Hs27 using the combination of docetaxel and rMETase at their IC_{50}s for PC-3 was determined. Results: The IC_{50} of docetaxel for PC-3 and for Hs27 was 0.72 nM and 0.94 nM, respectively. The IC_{50} of rMETase for PC-3 and for Hs27 was 0.67 U/ml and 0.76 U/ml, respectively. The combination of docetaxel and rMETase was synergistic for PC-3 but not Hs27 cells. Conclusion: The combination of a relatively low concentration of docetaxel and rMETase was synergistic and effective for AIPC. The present results also suggest that the effective concentration of docetaxel can be reduced by using rMETase, which may reduce toxicity. The present results also suggest the future clinical potential of the combination of docetaxel and rMETase for AIPC.*

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Key Words: Androgen-independent prostate cancer, AIPC, PC-3, normal fibroblasts, Hs27, docetaxel, methionine addiction, Hoffman effect, methionine restriction, recombinant methioninase, rMETase, combination therapy, synergy.

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Prostate cancer is the second most common cancer in men (1). The prognosis is poor for androgen-independent prostate cancer (AIPC), which has a much higher frequency of local recurrence and distant metastasis than androgen-dependent prostate cancer (2, 3). Docetaxel is first-line chemotherapy for AIPC. Docetaxel has improved clinical outcomes for AIPC (3, 4). However, it has dose-limiting toxicity, and tumors treated with docetaxel can become resistant to the drug. The median overall survival of patients with metastatic AIPC with docetaxel-based chemotherapy is 18 months (5-7). Therefore, improved therapy for AIPC is urgently needed.

Methionine restriction targets the methionine addiction of cancer (8-36), which is termed the Hoffman effect (8-10, 23, 25, 26). AntiCancer Inc. has developed recombinant methioninase (rMETase) that targets methionine addiction of cancer (11). rMETase is synergistic with chemotherapy of numerous types (12).

In the present study, we determined if the combination of docetaxel and rMETase is effective for AIPC cells and not toxic for normal cells *in vitro*.

Materials and Methods

Cell culture. The human AIPC cell line PC-3 and normal human Hs27 fibroblasts were obtained from the American Type Culture

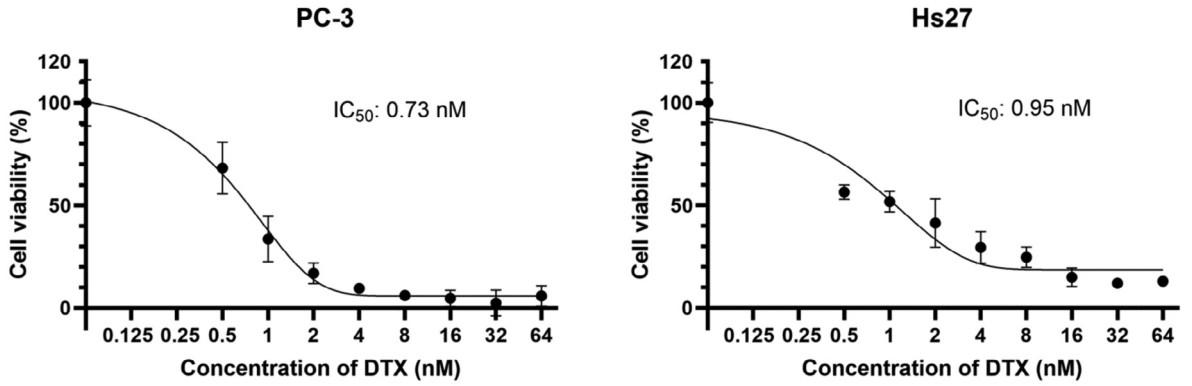

Figure 1. *Determination of the half-maximal inhibitory concentration (IC_{50}) of docetaxel (DTX) for PC-3 and Hs27 cells in vitro. Cell viability was measured using the WST-8 reagent. Data are shown as the mean±standard deviation. The concentration axis is log_2 scale.*

Collection (Manassas, VA, USA). The cells were cultured in Dulbecco's modified Eagle's Medium/Nutrient Mixture F-12 with GlutaMAX™ supplement (DMEM/F-12), 10% fetal bovine serum, and 100 IU/ml of penicillin/streptomycin.

Recombinant methioninase production. The *methioninase* gene from *Pseudomonas putida* was previously cloned in *Escherichia coli*. rMETase (Anticancer Inc., San Diego, CA, USA) was produced by fermenting recombinant *E. coli*. rMETase was purified with a high-yield technique that included a 60˚C thermal step, polyethyleneglycol precipitation, and diethylaminoethyl-sepharose fast-flow ion-exchange column chromatography (11).

Docetaxel and rMETase sensitivity assay. The sensitivity of PC-3 and Hs27 cells to rMETase and docetaxel was determined using the WST-8 reagent (Dojindo Laboratory, Kumamoto, Japan). The cells were cultured in 96-well plates at 1.0×10^3 cells/well in DMEM/F-12 overnight at 37˚C with 5% CO_2. Cells were then treated with rMETase at different doses ranging from 0.125 U/ml to 16 U/ml or with docetaxel ranging from 0.5 nM to 64 nM for the 72 h at 37˚C with 5% CO_2. Following rMETase or docetaxel treatment, the WST-8 (10 μl) reagent was added to each well, and the cells were incubated for 1 hour. The absorbance was then measured using a microplate reader (Sunrise; Tecan, Männedorf, Switzerland) at 450 nm. Drug sensitivity was analyzed with Microsoft Excel for Windows 2016 ver. 2309 (Microsoft, Redmond, WA, USA), ImageJ ver. 1.53t (National Institutes of Health, Bethesda, MD, USA) and GraphPad Prism 10.0.3 (GraphPad Software, Inc., San Diego, CA, USA) to create drug-sensitivity curves and calculate half-maximal inhibitory concentration (IC_{50}) values. Experiments were repeated twice, in triplicate.

Efficacy of the combination of rMETase and docetaxel. The viability of PC-3 and Hs27 cells after treatment with the combination of docetaxel and rMETase, using the IC_{50} concentrations for PC-3 cells, was determined with the WST-8 reagent. Following combination treatment, the absorbances were measured, and cell viability was calculated. Experiments were repeated twice, in triplicate.

Statistical analysis. Tukey's multiple comparison test was used to compare data between groups. Data are presented as mean±standard deviation. Statistical analyses were performed with GraphPad Prism 10.0.3. Values of $p\leq0.05$ were considered significant.

Results

Determination of the IC_{50} of rMETase and docetaxel. The IC_{50} of docetaxel alone was 0.73 nM for PC-3 and 0.95 nM for Hs27 cells (Figure 1). The IC_{50} of rMETase alone was 0.67 U/ml for PC-3 and 0.76 U/ml for Hs27 cells (Figure 2).

Determination of synergy of the combination of rMETase and docetaxel. With the combination of rMETase and docetaxel, the viability of PC-3 cells was significantly reduced compared with docetaxel or rMETase alone ($p=0.0081$). In contrast, the viability did not differ significantly between Hs27 cells treated with docetaxel or rMETase alone and Hs27 cells treated with the combination (Figure 3).

Discussion

In the present study, we observed greater efficacy with the combination of docetaxel and rMETase than with either agent alone for PC-3 AIPC cells but not for Hs27 normal fibroblasts.

Docetaxel inhibits microtubule depolymerization and arrests cells in the G_2/M phase of the cell cycle, leading to apoptosis (13). Synergy with the combination of docetaxel and rMETase may be due to cancer-cell-selective arrest in late-S/G_2 phase by rMETase (14, 15). The synergy of combination therapy for PC-3 cells might occur because rMETase leads to cell death in the S/G_2 phase, and some cells that escape rMETase treatment are then killed by docetaxel in the G_2/M phase. The combination of rMETase and docetaxel was shown to have *in vivo* efficacy in an osteosarcoma patient-derived orthotopic xenograft (PDOX) mouse model (16). The present and previous results indicate that the cancer-specific synergy of rMETase combined with chemotherapy is a general phenomenon (12, 16, 30, 37-45).

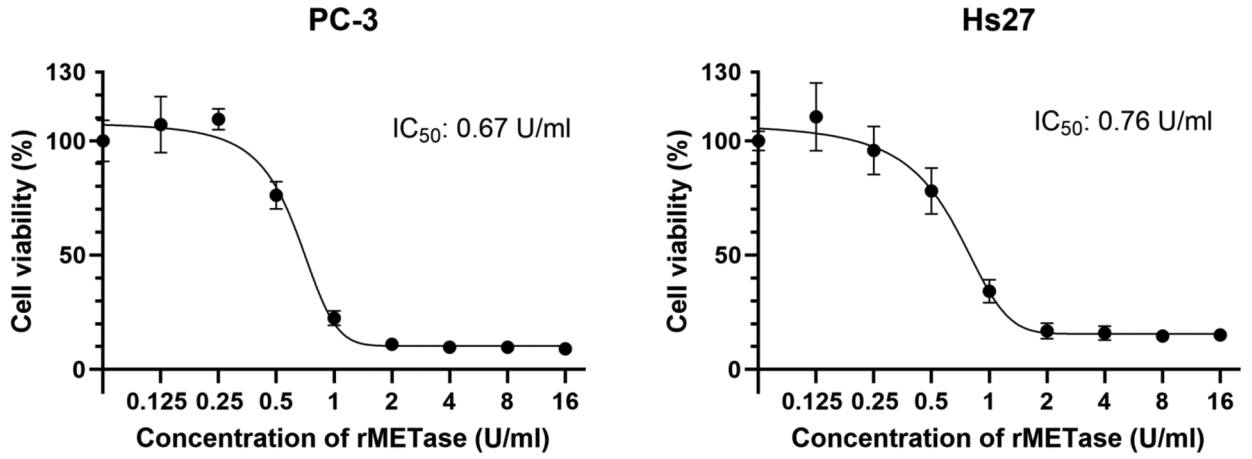

Figure 2. *Determination of the half-maximal inhibitory concentration (IC_{50}) of recombinant methioninase (rMETase) for PC-3 and Hs27 cells in vitro. Cell viability was measured using the WST-8 reagent. Data are shown as mean±standard deviation. The concentration axis is log_2 scale.*

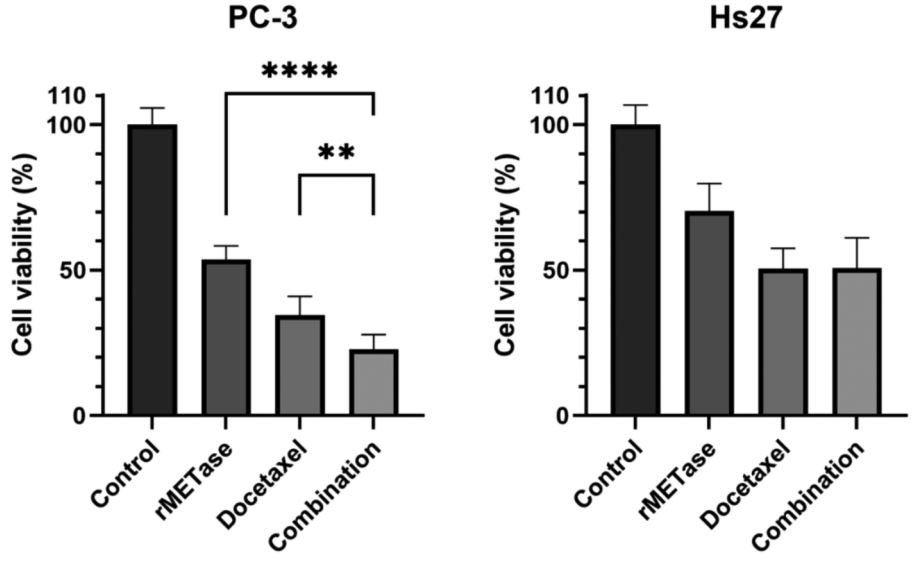

Figure 3. *Cell viability of PC-3 and Hs27 cells treated with the combination of docetaxel and recombinant methioninase (rMETase), using the half-maximal inhibitory concentrations (IC_{50}) for PC-3 cells. Data are shown as mean±standard deviation. Significantly different at: **p=0.0081 and ****p<0.0001.*

rMETase is effective because it targets the fundamental basis of cancer, methionine addiction (8-10, 14-15, 17-36), termed the Hoffman effect (8-10, 23, 25, 26).

Conclusion

The combination of docetaxel and rMETase was synergistic and effective for PC-3 AIPC cells but not for Hs27 normal fibroblasts. The effective concentration of docetaxel can be reduced by using rMETase, which may reduce toxicity, suggesting the clinical potential of the combination of docetaxel and rMETase for AIPC, as rMETase is showing clinical promise (46-58).

Conflicts of Interest

There are no conflicts of interest, according to the Authors.

Authors' Contributions

KM and RM performed experiments. KM, RM, and RMH wrote this article. QH provided methioninase. SM, MS, MB, YT, and KN reviewed this article.

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