YM155 Reverses Cabazitaxel Resistance in Castration-resistant Prostate Cancer by Reducing Survivin Expression

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Abstract. Background/Aim: The purpose of the present study was to clarify whether treatment with YM155, a novel small-molecule inhibitor of survivin, reversed cabazitaxel resistance in castration-resistant prostate cancer (CRPC).

Materials and Methods: Cabazitaxel resistance was induced in the castration-resistant prostate cancer cell line, 22Rv1-CR. In vitro and in vivo models were used to test the efficacy of YM155 and cabazitaxel. Results: Survivin gene expression was significantly higher in 22Rv1-CR than its parent cells (22Rv1). In 22Rv1-CR cells, YM155 significantly reduced expression of the survivin gene in a concentration-dependent manner. YM155 alone was poorly effective; however, it significantly enhanced the anticancer effects of cabazitaxel on 22Rv1-CR in vitro and in vivo. Conclusion: Inhibition of survivin by YM155 overcomes cabazitaxel resistance in CRPC cells.

Cabazitaxel was more efficacious in terms of producing a significant difference in patients with castration-resistant prostate cancer (CRPC) compared with the control group in the TROPIC trial (1). Hence, cabazitaxel is now employed as the standard treatment for most patients with CRPC. However, treatment outcomes are not always completely successful. Thus, the development of a new therapeutic regimen is required.

Survivin is a member of a family of eight different proteins that function as inhibitors of apoptosis (2). In 1997, Altieri et al. reported, for the first time, that survivin controlled apoptosis in cancer cells (3). It was also reported that survivin is overexpressed in several kinds of cancer and is involved in the survival of cancer cells and cell division. Conversely, cancer cell apoptosis is induced when the function of survivin is restrained. Additionally, because survivin expression level correlates with malignant disease prognosis and drug resistance, it is considered a potential target of future cancer therapeutics.

A novel small-molecule inhibitor of survivin, YM155, was identified by cell-based high-throughput screening (4). YM155 suppresses the transactivation of survivin by directly binding to its promoter (5). Subsequently, Cheng et al. reported that YM155-mediated inhibition of survivin expression occurs, at least in part, through inhibition of survivin transcription by disrupting Sp1 interaction with the -149 to -71 region in the survivin core promoter (6).

YM155 exhibits potent antitumor activity in vitro, and induces tumor regression in established non-small cell lung cancer, non-Hodgkin lymphoma, melanoma, and hormone-refractory prostate cancer xenografts (4, 7-9). The anticancer efficacy of YM155 as monotherapy or in combination with docetaxel (10) or platinum compounds, such as cisplatin and carboplatin (11) has been shown in Xenograft models (4, 12).

The purpose of this study was to determine the efficacy of YM155 and whether YM155 treatment could reverse acquired cabazitaxel resistance in the prostate cancer cell line 22Rv1-CR both in vitro and in vivo.

Materials and Methods

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**Induction of cabazitaxel resistance in prostate cancer 22Rv1 cells.**

22Rv1 cells were incubated for few months in the presence of low-concentrations of cabazitaxel. The concentration of cabazitaxel was increased gradually until reaching a final concentration of 5 mmol/L. Cells that grew in 5 mmol/L cabazitaxel were designated as 22Rv1-CR.

**Quantitative real-time polymerase chain reaction.** Total RNA was extracted from cells or a portion of the isolated tumor tissue after treatment and reverse-transcribed to generate cDNA. Transcript levels were quantified using an ICycler IQ™ system according to the manufacturer’s instructions (Bio-Rad, Hercules, CA, USA). Amplification was carried out in 10 μl Premix Ex Taq™ using 2 μl cDNA and the survivin primer (No. Hs04194392_s1; Applied Biosystems, Foster City, CA, USA). The polymerase chain reaction was performed for 1 cycle of 10 min at 95 ºC, followed by 40 cycles of 15 s at 95 ºC and 60 s at 60 ºC. As the internal control, 18S rRNA (No. Hs99999901_s1; Applied Biosystems) transcript levels were used. Gene expression is shown as fold changes vs. controls.

**Cell proliferation assay.** The number of living cells was measured using CellTiter 96® Aqueous One cell proliferation assay according to the manufacturer’s instructions (Promega). 22Rv1-CR cells (1x10⁶) were incubated with various concentrations of cabazitaxel and/or YM155 in culture medium with 10% charcoal-stripped fetal bovine serum and antibiotics for various periods of time at 37 ºC in a 5% CO₂ atmosphere.

**Xenograft models.** To evaluate the effect of cabazitaxel and/or YM155 on cabazitaxel-resistant prostate tumor growth in vivo, we used a nude mouse tumor xenograft model. Mice (5 weeks of age) were transplanted subcutaneously into the right flank with 3.0×10⁶ 22Rv1-CR cells mixed with 100 μl Matrigel and 100 μl phosphate-buffered saline. Palpable tumors developed at the injection sites. The mean tumor volume was 517 mm³ using the following equation: m₃ × m₂ × 0.5236, where m₁ represents the short axis and m² represents the long axis. Following this, mice were stratified into different groups, so that mean tumor volumes in each group were comparable. Mice were daily treated with cabazitaxel (5 or 10 mg/kg) and/or YM155 (3 mg/kg) via intraperitoneal injections. Tumor volume measurements began on day 8 and continued weekly until the end of the study. After 28 days, primary tumors were carefully removed, photographed, and analyzed for survivin mRNA expression and immunohistochemical staining. The study was approved by Gunma University Animal Care and Experimentation Committee (approval no. 18-043).

**Tumor immunohistochemical staining.** An immunohistochemical study was performed using the labeled streptavidin-biotin method with a rabbit polyclonal antibody to survivin at a 1:8000 dilution. Secondary biotinylated anti-rabbit IgG (Vector Laboratories, Burlingame, CA, USA) was used at a 1:8000 dilution. A blinded pathological examination of all specimens was performed by a single physician to confirm immune stainability.

**Statistical analyses.** Data are expressed as means±SD. For a single comparison of two groups, Student’s t-test was used. Differences between values in the cabazitaxel and/or YM155 experiment were evaluated by an analysis of variance using Tukey’s post-hoc test. An analysis of variance was also used to compare tumor sizes in mice following different treatment protocols. In all analyses, p<0.05 was considered statistically significant.

**Results**

Survivin levels were increased in the cabazitaxel-resistant prostate cancer cell line. Cabazitaxel suppressed 22Rv1 cell growth in a concentration-dependent manner (Figure 1A). The survivin mRNA level was significantly higher in 22Rv1-CR cells than the parental cell line, 22Rv1 (Figure 1B).

YM155 significantly downregulated survivin gene expression and enhanced the antiproliferative effects of cabazitaxel treatment in cabazitaxel-resistant prostate cancer cells in vitro. We investigated whether YM155 was effective at inhibiting survivin expression in 22Rv1-CR cells in vitro. YM155 significantly downregulated survivin mRNA expression in a concentration-dependent manner (Figure 1C). YM155 alone was poorly effective; however, in combination with cabazitaxel, it significantly suppressed cell proliferation (Figure 1D). These results show that YM155 enhances the toxic effect of cabazitaxel in cabazitaxel-resistant prostate cancer in vitro.

YM155 enhances the therapeutic effect of cabazitaxel treatment in cabazitaxel-resistant prostate cancer in vivo. Our in vitro data indicated that YM155 significantly reversed cabazitaxel resistance in prostate cancer cells. To validate our in vitro results in vivo, we carried out a YM155 and cabazitaxel combination treatment study in a nude mouse xenograft model.

Cabazitaxel treatment did not reduce tumor growth in animals bearing 22Rv1-CR tumors. YM155 treatment of 22Rv1-CR tumors was also ineffective in reducing the tumor burden. However, YM155 in combination with cabazitaxel was effective in inhibiting tumor growth of 22Rv1-CR (CBZ 5 mg + YM155: p<0.05 on day 15; CBZ 10 mg + YM155: p<0.01 on days 8 and 15) (Figure 2A, B).

YM155 alone tended to decrease survivin gene expression of 22Rv1-CR tumor cells (p=0.09), while in combination with cabazitaxel there was a significant decrease (p<0.05) (Figure 2C). Microscopically, the intensity of survivin staining of viable cells was strong in the control group. Although we were unable to precisely quantitate the intensity, survivin staining of viable cells was weak in certain mice in the YM155-treated and cabazitaxel + YM155 -treated groups (Figure 2D).

**Discussion**

Kaneko et al. reported that downregulating survivin by RNA interference induced apoptosis, while survivin overexpression rendered cells resistant to lovastatin-induced growth inhibition in a human colon cancer cell line (13). O’Connor et al. reported that survivin reduced sensitivity to taxanes (14). Yoon et al. reported that YM155 potentiates chemosensitivity to gemcitabine in pancreatic cancer cells by suppressing the
induction of survivin (15). In renal cancer, we reported that survivin inhibition by YM155 reversed rapamycin resistance (16). In that study, YM155 significantly decreased survivin gene and protein expression levels in a rapamycin-resistant clear cell carcinoma cell line (Caki-1-RapR) as well as cell proliferation, in a concentration-dependent manner. Furthermore, treatment with YM155 significantly restored rapamycin resistance in these cancer cells. In a nude mouse tumor xenograft model, YM155 significantly inhibited the growth of Caki-1-RapR tumor cells. Furthermore, YM155 significantly enhanced the antitumor effects of rapamycin in these cells. Additionally, we found that, in the statin-resistant renal cell clear cell carcinoma cell line, Caki-1-StaR, survivin knockdown by siRNA or YM155 significantly reversed simvastatin resistance in vitro (17). These findings prompted us to confirm the effects of survivin inhibition in a CRPC model.

In the present study, we investigated whether YM155 treatment could reverse resistance in a prostate carcinoma cell line with acquired cabazitaxel resistance (22Rv1-CR cells) in vitro and in vivo. A combination of YM155 and cabazitaxel inhibited 22Rv1-CR tumor growth; however, YM155 alone was poorly effective. A study conducted by Kita et al. in the prostate cancer cell line, PC-3, found that a concentration of YM155 greater than 10 nM decreased survivin mRNA and protein, and induced apoptosis (18). However, Nakahara et al. reported that the efficacy of YM155 in certain drug-resistant cell lines was poor (8). In the present study, YM155 alone did not inhibit the proliferation of 22Rv1-CR cells. Therefore, it is likely that the effect of cabazitaxel was augmented by YM155-mediated suppression of survivin in 22Rv1-CR cells.

In conclusion, we found that YM155 significantly enhances the therapeutic efficacy of cabazitaxel treatment on 22Rv1-CR cells. We chose 22Rv1 cells because they are hormone therapy resistant cells and a good model for CRPC. Although other prostate cancer cell lines might exhibit a different response, it is quite likely that YM155 could prove to be a novel treatment to reverse cabazitaxel resistance in prostate cancer.

Figure 1. A: 22Rv1 cells were treated for 72 h with cabazitaxel and cell proliferation was assessed by the MTS assay. Values are expressed as the mean±SD (n=5). **p<0.01 versus the control. B: Survivin mRNA expression in 22Rv1-CR cells and its parental cell line 22Rv1 was examined by RT-PCR. Values are expressed as the mean±SD (n=5). **p<0.01 versus the control. C: 22Rv1-CR cells were treated for 72 h with different concentrations of YM155 (nM), and survivin expression was examined by RT-PCR. Values are expressed as the mean±SD (n=5). **p<0.01 versus the control. D: 22Rv1-CR cells were treated for 72 h with YM155 [Y (nM)] alone or in combination with cabazitaxel [C (nM)] and cell proliferation was assessed by the MTS assay. Values are expressed as the mean±SD (n=5). **p<0.01 versus the control.
Figure 2. Animals bearing 22Rv1-CR tumors were daily treated with YM155 (3 mg/kg) or cabazitaxel (5 mg/kg) alone or cabazitaxel (10 mg/kg) alone or in combination or phosphate-buffered saline alone (control). A: Photographs of the 22Rv1-CR tumors from untreated (control), cabazitaxel (5 mg/kg)-, cabazitaxel (10 mg/kg), YM155-, cabazitaxel (5 mg/kg) and YM155-, or cabazitaxel (10 mg/kg) and YM155- treated groups of mice. B: Relative tumor volumes for 22Rv1-CR tumors in mice treated with cabazitaxel, YM155, or cabazitaxel and YM155. Values are expressed as the mean±SD (n=6). *p<0.05, and **p<0.01 versus the control. C: Survivin gene expression in 22Rv1-CR tumors from mice treated with YM155, or cabazitaxel and YM155. Values are expressed as the mean±SD (n=6). #p=0.09, and *p<0.05 versus the control. D: Immunohistochemical staining of survivin in 22Rv1-CR tumors.
Conflicts of Interest

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

Authors’ Contributions


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