YM155, a Novel Small-Molecule Survivin Suppressant, Induces Regression of Established Human Hormone-Refractory Prostate Tumor Xenografts

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Abstract

Various accumulating evidence suggests that survivin, a member of the inhibitor of apoptosis (IAP) family, plays an important role in drug resistance and cancer cell survival in many types of cancer, including hormone-refractory prostate cancer (HRPC). Here, we characterized YM155, a novel small-molecule survivin suppressant, using a survivin gene promoter activity assay. YM155 suppressed expression of survivin and induced apoptosis in PC-3 and PPC-1 human HRPC cell lines at 10 nmol/L. In contrast, YM155 up to 100 nmol/L showed little effect on expression levels of other IAP- or Bcl-2–related proteins. In a s.c. xenografted PC-3 tumor model in mice, 3-day continuous infusions of YM155 at 3 to 10 mg/kg induced massive tumor regression accompanied by suppression of intratumoral survivin. YM155 also completely inhibited the growth of orthotopically xenografted PC-3 tumors. No significant decreases in body weight were observed in mice treated with YM155 during the experimental period. Pharmacokinetic analyses indicated that YM155 is highly distributed to tumors and at concentrations ~20-fold higher than those in plasma. Our findings represent the first attempt to show tumor regression and suppression of survivin in p53-deficient human HRPC cells by a single small molecular compound treatment. Further extensive investigation of YM155 in many types of cancer, including HRPC, seems to be worthwhile to develop this novel therapeutic approach. [Cancer Res 2007; 67(17):8014–21]

Introduction

Prostate cancer is the most frequently diagnosed cancer and the second leading cause of cancer-related death in the United States (1). The main treatment strategy for advanced prostate cancer involves androgen deprivation therapy, to which patients initially respond very well. However, this therapy eventually fails in most patients, who frequently develop hormone-refractory prostate cancer (HRPC) with an accompanying poor prognosis (2, 3). Although taxanes are among the more effective chemotherapeutic agents used in the clinical treatment of HRPC, many patients develop acquired resistance after exposure. Novel and more effective therapeutic options for HRPC treatment have therefore been sought.

Survivin is a member of the inhibitor of apoptosis (IAP) protein family and has been implicated in both cell survival and regulation of mitosis in cancer (4–6). Survivin is highly expressed in all primary tumor types (5) but is undetectable in most normal differentiated tissues, with the exception of placenta, testes, and rapidly dividing cells such as CD34+ bone marrow stem cells (7). Because a correlation exists between high expression of survivin in tumors and poor survival among patients with various cancers (8–10), survivin is considered a putative novel target in various cancer therapies.

Accumulating evidence suggests that survivin plays an important role in the survival strategies of HRPC. Survivin is not expressed in normal prostate secretary epithelial cells but is strongly expressed in prostate cancer cells (11). High expression of survivin has been associated with the established features of biologically aggressive prostate cancers, such as those with higher Gleason scores that metastasize to regional lymph nodes (12). Thus, foiling the survival strategy of cancer cells in HRPC by suppressing survivin may be a novel and effective therapeutic approach. To date, however, no therapeutic option has been developed that would counteract the physiologic functions of survivin specifically. Recent extensive research on survivin has revealed that a number of existing anticancer drugs show survivin-suppressive activity through several cell signaling pathways [i.e., doxorubicin (13), hedamycin (14), ErbB2 inhibitor lapatinib (15), Janus-activated kinase-2 inhibitor AG-490 (16), heat shock protein-90 antagonist 17-N-allylamino-17-demethoxygeldanamycin (17), histone deacetylase inhibitor LAQ824 (18), and cyclin-dependent kinase inhibitor flavopiridol (19)]. When exerting their antitumor activities, however, these drugs are also thought to act on other normal cell signaling pathways, which often results in severe systemic toxicity. Thus, additional research is necessary to identify a novel and specific antisurvivin therapeutic strategy with a clearer screening rationale.

Here, we characterized YM155, a novel small, imidazolium-based compound that specifically inhibits survivin, using a survivin promoter activity assay as an initial screening process. We then determined whether this survivin-suppressing molecule shows distinct antitumor activity in preclinical HRPC models.

Materials and Methods

Cell cultures and reagents. Human cervical epithelial carcinoma cell line HeLa-S3, Chinese hamster ovary cell line CHO, human non–small-cell lung carcinoma cell line A549, and human HRPC cell lines PC-3, 22Rv1, and DU145 were obtained from American Type Culture Collection. Human HRPC cell lines PPC-1 and TSU-P1 were obtained from The University of Utah (Salt Lake City, UT) and Teikyo University (Tokyo, Japan), respectively.

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Cells were maintained in RPMI 1640 (Life Technologies, Inc.) or DMEM (Life Technologies), supplemented with 10% heat-inactivated fetal bovine serum in a humidified incubator with 5% CO₂ at 37°C. YM155 monobromide was synthesized at Astellas Pharma, Inc., used as YM155 in this study and is uniformly expressed as “YM155.” In the *in vitro* studies, dose levels are expressed in terms of YM155, the cationic moiety of the drug substance. Doxorubicin and methotrexate were purchased from Sigma-Aldrich. For the *in vitro* experiments, the reagents were dissolved in DMSO and diluted in saline to a final DMSO concentration of <1%. For the *in vivo* experiments, YM155 was dissolved and diluted in saline immediately before administration.

**Promoter-luciferase reporter assay.** A 2,767-bp sequence of human survivin gene promoter was isolated from human genomic DNA (Clontech) by PCR using Pyrobest polymerase (TaKaRa) and the following primers: 5′-GGCCAGTTC-3′ (forward) and 5′-GCAGCCCTGGTGACCAG-3′ (reverse); human G3PDH, 5′-CTGCCTGGCAGCCCTTT-3′ (forward) and 5′-GCAGCCCTGGTGACCAG-3′ (reverse). Real-time PCR was performed using the ABI Prism 7900 sequence detection system and the SYBR Green PCR Master Mix (Applied Biosystems) according to the manufacturer's instructions. Dissociation curve analyses were done to verify that there was neither unspecific amplification nor formation of primer dimers. Values were calculated based on standard curves generated for each gene. Normalization of samples was determined by dividing copies of survivin transcripts by copies of GAPDH. All sets of reactions were conducted in triplicate. The relative expression levels are expressed as a percentage of the indicated control.

**Western blot analysis.** Affinity-purified rabbit antisurvivin antibodies were prepared by immunization of rabbits using a recombinant survivin protein. Proteins were extracted from cells as previously described (20). Equal amounts of proteins (20 μg) were resolved by SDS-PAGE and transferred to a polyvinylidene difluoride membrane. After blocking with TBS-T buffer [20 mmol/L Tris-HCl (pH 7.5), 0.137 mol/L NaCl, and 0.05% Tween 20] containing 10% nonfat milk for 1 h at room temperature, each membrane was incubated for 1 h at room temperature with one of the following primary antibodies: affinity-purified rabbit antisurvivin (500-fold dilution), goat anti–cellular inhibitor of apoptosis protein 2 (c-IAP2; 1 μg/mL; R&D Systems), rabbit anti–X-linked inhibitor of apoptosis protein (XIAP; 1 μg/mL; R&D Systems), rabbit anti–Bcl-2 (1,000-fold dilution; Cell Signaling), rabbit anti–Bcl-Xj (1,000-fold dilution; Cell Signaling), rabbit anti–Bad (1,000-fold dilution; Cell Signaling), mouse monoclonal anti–α-tubulin (1 μg/mL; Santa Cruz Biotechnology), or rabbit anti–actin (500-fold dilution; Sigma-Aldrich), for 1 h at room temperature. After washing the membranes with TBS-T buffer, they were incubated with horseradish peroxidase–conjugated secondary antibody for 1 h at room temperature. Proteins of interest were detected by enhanced chemiluminescence using ECL plus (Amersham) followed by autoradiography.

**Trypan blue exclusion staining for determination of cell viability.** After culture for 48 h in the absence or presence of YM155, PC-3 and PPC-1 cells were collected by trypsinization and centrifugation (0.05% trypsin-EDTA; Life Technologies) and resuspended in DMEM. The cell suspension was diluted in equal volumes of trypan blue (0.4% working solution; Life Technologies). Viable (unstained) and dead (stained) cells were counted on a hemocytometer, and the ratio of viable cells to the total number of cells was expressed as viability percentage.

**Measurement of caspase-3 activity.** The caspase-3 activity was measured with a CPP32/Caspase-3 Fluorometric Protease Assay Kit (MBL) according to the manufacturer's instructions. After incubation with YM155 for 48 h, PC-3 and PPC-1 cells were lysed in 100 μL of a cell lysis buffer (provided with the kit) and equal volumes (50 μL) of cell lysate were obtained (100 μg of protein). After addition of 2× reaction buffer, the mixture was added to a black 96-well plate. The DEVD-afc substrate (appended with the kit) was then added at 5 μL/well and the mixture incubated at 37°C for 30 min. Fluorescence emissions were quantified with a spectrofluorometer at an excitation wavelength of 390 nm and an emission wavelength of 460 nm.

**Table 1. In vitro cell growth inhibition by YM155 in various human cancer cell lines**

<table>
<thead>
<tr>
<th>Structure of YM155</th>
<th>Origin</th>
<th>Cell line</th>
<th>p53 status</th>
<th>GI₅₀ (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Diagram]</td>
<td></td>
<td>PC-3</td>
<td>Null</td>
<td>8.2</td>
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<tr>
<td></td>
<td></td>
<td>PPC-1</td>
<td>Mutant</td>
<td>2.3</td>
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<tr>
<td></td>
<td></td>
<td>DU145</td>
<td>Mutant</td>
<td>4.0</td>
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<tr>
<td></td>
<td></td>
<td>TSU-Pr1</td>
<td>Mutant</td>
<td>8.2</td>
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<tr>
<td></td>
<td></td>
<td>22Rv1</td>
<td>Mutant</td>
<td>11</td>
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<tr>
<td></td>
<td></td>
<td>SK-MEL-5</td>
<td>Wild-type</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A375</td>
<td>Wild-type</td>
<td>6.3</td>
</tr>
</tbody>
</table>

NOTE: The cells were treated with YM155 for 48 h. All the cell lines have wild-type, mutated, or null p53 and presented. The GI₅₀ value for each cell line was calculated by logistic analysis. The assay was done in triplicate, and the mean GI₅₀ value was obtained from the results of four independent assays.
In vitro cell growth inhibition assay. The antiproliferative activity of YM155 was measured by the method used at the National Cancer Institute (21). After treatment with YM155 for 48 h, the cell count was determined by sulforhodamine B assay. The GI50 value was calculated by logistic analysis, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by sulforhodamine B staining) in control cells during the drug incubation. The assay was done in triplicate, and the mean GI50 value was obtained from the results of four independent assays.

Time dependency of antitumor activity in vitro. A549 cells were treated with YM155, methotrexate, or doxorubicin. Each compound was removed after various lengths of exposure time by washing five times with fresh medium. At 72 h of incubation after the beginning of cell treatment, the effect of the selected compound on cell proliferation was determined with the Alamar Blue assay (Serotec; ref. 22), done in duplicate for each concentration (n = 1). The IC50 was calculated by logistic analysis. To understand the in vitro mode of action and pharmacodynamic properties of each compound, the log slope (exposure time) and the reciprocal of the log slope (IC50 of the antiproliferative effect on A549) were plotted on a logarithmic scale for YM155, doxorubicin (as area under the curve–dependent drug), and methotrexate (as time-dependent drug), and the slope of the IC50-exposure time curve was compared among the three agents (23, 24).

In vivo antitumor activities against PC-3 s.c. xenograft model. Five-week-old male nude mice (BALB/c nu/nu) were purchased from Charles River Japan, Inc. PC-3 cells (2 \times 10^5) were injected into the flanks of the mice and allowed to reach a tumor volume of >100 mm^3 in tumor volume (length \times width^2 \times 0.5). YM155 was s.c. administered as a 3-day continuous infusion per week for 2 weeks using an implanted micro-osmotic pump (Alzet model 1003D, Durect) or i.v. administered five times a week for 2 weeks. The percentage of tumor growth inhibition 14 days after initial YM155 administration was calculated for each group using the following formula: 

\[ MTV = \frac{100 \times (MTV \text{ of the treated group on day } 14) - (MTV \text{ of the treated group on day } 0)}{(MTV \text{ of the control group on day } 14) - (MTV \text{ of the control group on day } 0)}, \]

where MTV is mean tumor volume. For both the frozen tumors and plasma samples, survivin expression levels were analyzed by Western blotting and YM155 drug concentration by high-performance liquid chromatography/triple quadrupole mass spectrometry (LC/MS/MS) using validated methods.

In vivo antitumor activities against PC-3 orthotopic xenograft model. For orthotopic implantation, a PC-3 cell suspension (1 \times 10^6/20 \mu L per mouse) was injected into the prostate dorsolateral lobe (right side) of 7-week-old male nude mice (BALB/c nu/nu). Two weeks after implantation, YM155 was administered as a 3-day continuous infusion per week for 3 weeks at 5 mg/kg/d using an implanted micro-osmotic pump (Alzet model 1003D, Durect). Body weight was measured periodically starting from the first day of YM155 administration. Three weeks later, the tumors adhering to the prostate and seminal vesicle were taken and weighed as tumor weight. The antitumor activity of YM155 was expressed as a percentage of tumor growth inhibition calculated using the following formula:

\[ \frac{100 \times (MTV \text{ of the treated group on day } 21) - (MTV \text{ on day } 21 \text{ of the treated group})}{(MTV \text{ on day } 21 \text{ of the control group})}. \]

Statistical analysis. Values are expressed as mean ± SE. Differences between groups were analyzed with Dunnett’s multiple range test. Relative mRNA expression levels for all YM155-treated cell groups were compared with the control group by Dunnett’s multiple range test using within-subject error. All data analyses were done with the SAS statistical software (SAS Institute), with P < 0.05 considered significant.

Results

YM155 inhibits survivin gene promoter activity and down-regulates survivin in vitro. To identify a novel small molecule that specifically inhibits survivin expression in human cancer cells, we conducted high-throughput screening of in-house chemical compound libraries using the transformants stably expressing survivin gene promoter–driven luciferase reporter or SV40 enhancer/promoter–driven luciferase reporter. We identified YM155 as a novel small-molecule survivin gene suppressant (Table 1). YM155 potently inhibited survivin promoter activity with an IC50 value of 0.54 nmol/L but did not significantly inhibit SV40 promoter activity at concentrations up to 30 μmol/L.

<table>
<thead>
<tr>
<th>YM155 (nmol/L)</th>
<th>Control</th>
<th>Survivin</th>
<th>Actin</th>
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<td>1</td>
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Figure 1. YM155 selectively suppresses survivin expression in human HRPC cells. A, PC-3 and PPC-1 cells were treated with YM155 (1, 10, 100, and 1,000 nmol/L) for 24 h (control: 0.1% DMSO). Cells were analyzed to detect survivin protein by Western blot analysis. B, PC-3 cells were treated with YM155 (100 nmol/L) for the indicated periods of time. Cells were analyzed to detect survivin protein by Western blot analysis and the mRNA by real-time PCR. Expression levels of survivin mRNA were normalized to that of G3PDH and expressed as relative expression levels (% of 0 h). Points, mean from four separate experiments; bars, SE. **P < 0.01, versus 0 h (Dunnett’s test using within-subject error). C, PC-3 cells were treated with YM155 at the indicated concentrations for 24 h (control, 0.1% DMSO), and the protein expression levels of survivin, c-IAP2, XIAP, Bcl-2, Bcl-xL, Bad, actin, and β-tubulin were compared.
We further confirmed the in vitro effect of YM155 on endogenous survivin expression in PC-3 and PPC-1 human HRPC cells with deficient p53 (Fig. 1A–C). YM155 administered from 10 to 1,000 nmol/L significantly suppressed survivin expression in a dose-dependent manner (Fig. 1A), as observed at 6 h after the drug addition (Fig. 1B). These results clearly show that at similar time points, YM155 suppresses survivin at the mRNA and protein levels, which suggests that the suppression of survivin by YM155 is through transcriptional inhibition of the survivin gene promoter.

We also investigated the effects of YM155 on protein expression of other IAP family proteins, Bcl-2 family proteins, and cellular skeletal proteins in PC-3 cells (Fig. 1C). YM155 strongly suppressed survivin expression at 10 and 100 nmol/L concentrations. In contrast, YM155 showed no significant effect on protein expression of c-IAP2, XIAP, Bcl-2, Bcl-XL, Bad, α-actin, and β-tubulin at concentrations up to 100 nmol/L. These findings confirm that YM155 specifically abrogated survivin expression without affecting other antiapoptotic proteins in cancer cells.

YM155 induces cancer cell apoptosis in vitro. To determine induction of cell death by YM155 in human cancer cells, PC-3 and PPC-1 cells were treated with YM155 for 48 h, after which cell viability was assessed with the trypan blue exclusion method and the triggering of apoptosis with the caspase-3 activity assay. In both cell lines, YM155, at concentrations from 10 to 1,000 nmol/L, significantly decreased the viability of the cells in a dose-dependent manner (Fig. 2A and B). When exposed to YM155, PC-3 and PPC-1 showed a concomitant increase in caspase-3 activity (Fig. 2C and D). These results suggest that YM155 induces apoptosis in human HRPC cells.

YM155 inhibits the growth of human cancer cell lines with mutated or truncated p53 in vitro. The antiproliferative activities of YM155 were tested against a panel of five human HRPC cell lines with mutated or truncated p53 and two human malignant melanoma cell lines with wild-type p53. YM155 potently inhibited the growth of all the cell lines tested, with GI50 values ranging from 2.3 nmol/L for PPC-1 to 11 nmol/L for 22Rv1 (Table 1), suggesting that YM155 potently inhibited the growth of human cancer cell lines regardless of p53 status.

YM155 shows time-dependent antitumor activity in vivo and induces regression of human s.c. xenografted prostate tumors. We evaluated the in vivo antitumor activity of YM155 with continuous infusion and bolus injection in PC-3 s.c. xenografted mice (Fig. 4). Three-day continuous infusions (weekly for 2 weeks) of YM155 at 3 and 10 mg/kg completely inhibited the tumor growth and induced massive tumor regression (Fig. 4A). For mice receiving continuous YM155 infusions, no sign of tumor regrowth or systemic toxicity, such as body weight loss or blood cell count decrease, was observed during the experimental period (data not shown). Mice receiving daily i.v. bolus injections of YM155 at ≥6 mg/kg did not survive, whereas those receiving YM155 by i.v. bolus injection at 2 mg/kg showed a maximum tumor growth inhibition of only 64% compared with the control (Fig. 4B). The half life of plasma YM155 concentrations after the i.v. bolus injection of YM155 was 1.06 h (data not shown); therefore, sufficient exposure time of YM155 cannot be achieved by i.v. bolus injection. These results suggest that continuous infusion of YM155 is highly efficient in human HRPC models compared with YM155 bolus injections, with massive tumor regression, and that YM155 exhibits time-dependent antitumor activity in vivo.

YM155 concentrations in plasma and tumor were analyzed during and after 3-day continuous infusion of YM155 at 3 mg/kg to mice bearing PC-3 xenografts (Fig. 4C). In s.c. PC-3 xenografts, the
observed mean drug concentration at steady state ($C_{\text{ss,obs}}$) ranged from 18.4 to 23.7 ng/mL in plasma and from 351 to 397 ng/g in tumors. Compared with plasma, a >20-fold YM155 concentration can be achieved in tumors. These results show that YM155 is highly distributed to tumor tissue.

YM155 suppresses survivin in vivo. To confirm whether YM155 alters survivin expression in tumors during drug treatment, we evaluated the effect of YM155 on intratumoral survivin expression (Fig. 4D). Mice with large established s.c. xenografted PC-3 tumors received a 3-day continuous infusion of YM155 at 10 mg/kg. Saline control animals showed rapid tumor growth from day 0 (366 mm$^3$) to day 7 (1,123 mm$^3$), with no change in intratumoral survivin and actin protein levels. In contrast, animals treated with YM155 showed tumor regression from day 0 (292 mm$^3$) to day 7 (162 mm$^3$), and a clear decrease in intratumoral survivin levels on days 3 and 7 was observed. No apparent change in actin protein levels was observed during the experimental period. These results show that the rapid tumor regression induced by YM155 also suppresses intratumoral survivin protein levels, and that YM155 shows potent antitumor activity in human HRPC models by suppressing intratumoral survivin levels.

YM155 completely inhibits the growth of human orthotopic xenograft prostate tumors. We further evaluated the in vivo antitumor activity of YM155 in PC-3 orthotopic xenografts, a more clinically relevant model of HRPC (Fig. 5). YM155 administered at 1 and 5 mg/kg showed 47% and 80% inhibition of tumor growth, respectively, compared with controls (Fig. 5A). For this evaluation, because the excised prostate and seminal vesicles were both included in the tumor weight, YM155 treatment almost completely inhibited tumor growth (Fig. 5C). After orthotopic implantation of PC-3, control mice showed a marked decrease in body weight and a deterioration of general health as the tumors progressed (Fig. 5B).
Mice treated with YM155, however, showed improved general health and body weight gain. These results strongly suggest that YM155 may potentially show clinical benefits in patients with HRPC.

Discussion

The present study had four major findings. First, we have shown that YM155, a novel imidazolium-based survivin suppressant, selectively suppressed the expression of survivin and induced apoptosis in p53-deficient human HRPC cells in vitro. Second, YM155 suppressed intratumoral survivin and showed potent antitumor activities, including tumor regressions in s.c. and orthotopically xenografted human HRPC models. Third, the antitumor activity of YM155 was time dependent, and continuous infusion led to maximal antitumor activity without the induction of severe systemic toxicities in vivo. Finally, pharmacokinetic analyses indicated that YM155 shows higher distribution to tumor tissues than plasma. These findings hold promise for the clinical use of YM155 as a novel chemotherapeutic option in the treatment of HRPC.

To identify YM155 from the chemical compound libraries, we conducted 2.3-kb survivin promoter (−2,761 to +6) luciferase assay using p53-deficient cancer cells. p53 is known as a negative regulator of survivin, and the survivin promoter contains several Sp1 canonical, Sp1-like, and p53 binding elements, suggesting the participation of the Sp1 transcription factor and/or p53 in its gene regulation (25). Hoffman et al. (26) reported that doxorubicin-induced cancer cell death is, at least in part, involved in the transcriptional inhibition of survivin, whereas Estève et al. (25) revealed that doxorubicin suppresses survivin by Sp1 recruitment of p53 on its promoter. In contrast, Yonesaka et al. (13) found that doxorubicin does not suppress survivin in human cancer cells with mutated or deleted p53. In this study, we found that YM155 suppresses expression of survivin and potently inhibits the growth of human cancer cell lines regardless of p53 status. We therefore speculate that YM155 has distinctive mechanisms of action over doxorubicin in its inhibition of survivin promoter activity in a p53-independent manner. In prostate cancer, p53 gene mutation is associated with advanced stage, loss of differentiation, and the transition from androgen-dependent to androgen-independent growth (27, 28). Thus, any new therapeutic option for the treatment of HRPC should show distinctive antitumor activity against p53-deficient cells, and YM155 is expected to show potent antitumor activity based on survivin suppression against human advanced HRPC. Extensive analyses using survivin promoters of different length are now in progress to identify an important region that involves survivin inhibition induced by YM155 on its promoter.

For the first time, we have shown that a small molecular compound suppresses intratumoral survivin and shows potent antitumor activities, including tumor regressions in both s.c. and orthotopically xenografted human HRPC models. In addition, YM155, in combination with established chemotherapeutic agents, also exhibited enhanced antitumor activities in several tumor xenografted models (1). Although the functional inhibition of survivin by its small interfering RNA or antisense molecules can induce mitotic defects and apoptosis in various human cancer cells including HRPC, clear tumor regression is unlikely to occur by its functional inhibition alone (13, 29–31). Zhang et al. (32) reported that antiandrogen therapy of LNCaP human hormone-responsive prostate cancer cells resulted in survivin down-regulation as well as

Figure 5. Inhibition of orthotopically xenografted PC-3 tumor growth in mice receiving YM155 treatment. YM155 was administered to PC-3 orthotopically xenografted mice as a 3-d continuous infusion per week for 2 wks at 1 and 5 mg/kg. Statistical analysis for each treated group was done for the values on day 21. *, P < 0.05; **, P < 0.01, versus control. N.S., not significant (P > 0.05).

A, on autopsy on day 21, orthotopically implanted tumors, including adherent seminal vesicle at the implantation side, were weighed and plotted. B, body weight in each treatment group was measured periodically and plotted. Columns and points, mean (n = 9); bars, SE. C, tumors were photographed in all groups.
tumor regression. However, in the androgen-refractory stage, to mediate resistance to antiandrogen therapy, survivin is upregulated by an insulin-like growth factor I via the AKT pathway (32). Further, the PC-3 is known to overexpress other antiapoptotic family proteins or Bcl-2 family proteins (33), and hence we anticipate that survivin and these antiapoptotic molecules will act redundantly to protect HRPC cells from cell death stimuli. In this study, YM155 induced apoptosis in PC-3 without affecting the expression levels of c-IAP-2, XIAP. Bad, Bcl-2, or Bcl-xL. Furthermore, no appearance of mitotic defects, specific cell cycle arrest in G2-M phase, or alteration of the phosphorylation form of AKT was observed in PC-3 during the apoptosis induction induced by YM155.1 These findings lead us to conjecture that YM155 shows potent antiapoptosis activity based on suppression of survivin via novel mechanisms of action. Details of the mechanism of YM155 suppression of survivin and massive tumor regression in human HRPC cells remain to be elucidated in further extensive studies on factors that are altered by YM155.

Antitumor activities of YM155 in vivo were strengthened by continuous infusion, and no apparent systemic toxicity was observed during the experimental periods. Time-dependent anticancer drugs often show enhanced efficacy followed also by enhanced toxicity when dosing on a continuous basis. This would, in part, be explained by the lack of target molecule specificity of the drug between cancer and normal cells. However, for YM155, survivin is one of the major cancer transcriptomes and a cancer-specific target (34); therefore, these characteristics highlight one of the clear benefits of YM155 over other anticancer drugs that show enhanced efficacy with low toxicity during continuous dosing.

Our pharmokinetic study revealed that YM155 was distributed to tumors at concentrations ~20-fold higher than those in plasma, and with minimal differences in tumor distribution between tumor implantation locations. A lower concentration in plasma reflects lower exposure to normal tissues whereas a higher concentration in tumors reflects higher exposure of the drug to the tumor tissues.

This difference in tissue distribution between plasma and tumors is likely explained by the structure of YM155. Several kinds of organic cation transporters are known to be overexpressed in various human cancers (35, 36), and given that YM155 is a small-molecule compound with an organic cation moiety, the possibility exists that it is incorporated into cancer cells via these transporters. Further comprehensive investigation of these cancer-functional drug influx transporters is crucial to explaining the higher tumor tissue distribution of YM155.

In conclusion, YM155 is a new first-in-class molecular entity that suppresses survivin and shows potent antitumor activities in human HRPC cells in vitro and in vivo. The mechanism by which YM155 exhibits survivin suppression remains to be elucidated; nevertheless, our findings represent the first attempt to show tumor regression and suppression of survivin in p53-deficient human prostate cancer cells by a single small molecular compound treatment. YM155 is expected to show antitumor activity against various kinds of human cancers, including human HRPC, via the novel mechanism of inducing apoptosis with potent survivin suppression. Further extensive investigation of YM155 in various cancer types to develop this novel therapeutic approach seems to be worthwhile. A phase II, open-label study of YM155 with 7-day continuous i.v. infusion in HRPC patients is currently under way.

Acknowledgments


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Correction: YM155, a Novel Small-Molecule Survivin Suppressant, Induces Regression of Established Human Hormone-Refractory Prostate Tumor Xenografts

In this article (Cancer Res 2007;67:8014–21), which appeared in the September 1, 2007 issue of Cancer Research (1), Shinji Hatakeyama should have been listed as the eighth author. The amended author listing is provided below. The authors regret this error.

Takahito Nakahara, Aya Kita, Kentaro Yamanaka, Masamichi Mori, Nobuaki Amino, Masahiro Takeuchi, Fumiko Tominaga, Shinji Hatakeyama, Isao Kinoyama, Akira Matsuhisa, Masafumi Kudou, and Masao Sasamata.

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