Novel Survivin Inhibitor YM155 elicits Cytotoxicity in Glioblastoma Cell Lines with Normal or Deficiency DNA-Dependent Protein Kinase Activity

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Background: Pediatric glioblastoma is a malignant disease with an extremely poor clinical outcome. Patients usually suffer from resistance to radiation therapy, so targeted drug treatment may be a new possibility for glioblastoma therapy. Survivin is also overexpressed in glioblastoma. YM155, a novel small-molecule survivin inhibitor, has not been examined for its use in glioblastoma therapy.

Methods: The human glioblastoma cell line M059K, which expresses normal DNA-dependent protein kinase (DNA-PK) activity and is radiation-resistant, and M059J, which is deficient in DNA-PK activity and radiation-sensitive, were used in the study. Cell viability, DNA fragmentation, and the expression of survivin and securin following YM155 treatment were examined using MTT (methylthiazolyldiphenyl-tetrazolium) assay, ELISA assay, and Western blot analysis, respectively.

Results: YM155 caused a concentration-dependent cytotoxic effect, inhibiting the cell viability of both M059K and M059J cells by 70% after 48 hours of treatment with 50 nM YM155. The half-maximal inhibitory concentration (IC50) was around 30–35 nM for both cell lines. Apoptosis was determined to have occurred in both cell lines because immunoreactive signals from the DNA fragments in the cytoplasm were increased 24 hours after treatment with 30 nM YM155.
YM155. The expression of survivin and securin in the M059K cells was greater than that measured in the M059J cells. Treatment with 30 nM YM155, for both 24 and 48 hours, significantly suppressed the expression of survivin and securin in both cell lines. Conclusion: The novel survivin inhibitor YM155 elicits potent cytotoxicity in glioblastoma cells in vitro via DNA-PK-independent mechanisms. YM155 could be used as a new therapeutic agent for the treatment of human glioblastomas.

1. Introduction

Brain tumors are one of the most common solid neoplastic disorders that present in children. The death of a pediatric patient with a brain tumor, especially a high-grade glioma, usually results from cancer. Gloioblastoma demonstrates the worse therapeutic response among high-grade gliomas, with a very low 5-year survival rate despite aggressive chemotherapy and/or radiation therapy. Thus, new strategies for treating glioblastomas should be investigated.

Survivin, a member of the inhibitor of apoptosis protein family, is overexpressed in human glioblastoma tissues. Studies have shown that the 3-year survival rate of glioblastoma tissues with positive nuclear survivin expression is zero; thus, survivin may be a useful biomarker for predicting clinical prognosis in patients with glioblastoma. Treatments aimed at inhibiting survivin expression could be a new strategy for the treatment of glioblastomas. However, specific survivin suppressants have not been examined for use in glioblastoma treatments.

A small molecule, YM155 [1-(2-Methoxyethyl)-2-methyl-4,9-dioxo-3-(pyrazin-2-ylmethyl)-4,9-dihydro-1H-naphtho[2,3-d]imidazolium bromide], has been identified by high-throughput screening as an inhibitor of the activity of the survivin gene promoter. YM155, as a survivin suppressant, has been shown to inhibit the progression of some neoplasms in vitro and in vivo (e.g., prostate cancer and lung cancer). Until now, YM155 has not been studied for its use in glioblastoma therapy. Therefore, we conducted an in vitro study to evaluate the therapeutic effects of YM155 in two glioblastoma cell lines: one cell line that expresses normal DNA-dependent protein kinase (DNA-PK) activity, and another cell line that is deficient in DNA-PK activity. The former is resistant to radiation, whereas the latter is sensitive to radiation. This study’s aim was to demonstrate that YM155 exerts potent cytotoxicity through survivin inhibition and induces apoptosis in both of these glioblastoma cell lines.

2. Materials and Methods

2.1. Cell culture

Human glioblastoma cell lines M059J and M059K were obtained from Dr. Shu Jun Chiu (Department of Life Sciences, Tzu Chi University, Hualien, Taiwan). M059J and M059K exhibit deficient DNA-PK and normal DNA-PK activities, respectively. The cells were cultured in F-12/ Dulbecco’s modified Eagle medium in combination with 10% fetal bovine serum (FBS) and 1% penicillin plus streptomycin.

2.2. MTT cytotoxicity assay

For the cytotoxicity assay, 1.0 × 10⁴ glioblastoma cells per well were seeded onto 96-well plates. After overnight incubation, the cells adhered to the plate. YM155 (Selleck, Houston, TX, USA) at a concentration of 0–50 nM was dissolved in dimethyl sulfoxide (DMSO; J.T. Baker, Phillipsburg, NJ, USA) and incubated for 48 hours. The MTT (methylthiazolyldiphenyl-tetrazolium bromide; Sigma, St. Louis, MO, USA) assay was then performed, using the protocol described in a previous study.

2.3. DNA fragmentation assay

The Cell Death Detection ELISA PLUS (Roche, Mannheim, Germany) assay kit was used to differentiate apoptotic and necrotic glioblastoma cells after treatment with YM155. Twenty-four hours after drug treatment, both supernatants of the cultured medium and the cytoplasmic fraction from 2.0 × 10⁶ cells were collected in 24-well dishes. The optical density (OD) value at 405 nm, which represents the extent of DNA fragmentation, was measured.

2.4. Western blot analysis

After YM155 treatment, the expression levels of survivin and securin in the glioblastoma cells were detected using Western blot analysis. The procedures we followed were described in our previous report. Primary antibodies, including anti-survivin (#2808; Cell Signaling Technology, Inc., Danvers, MA, USA) and anti-securin (ab3305; Abcam, Cambridge, MA, USA), were used. The expression of α-tubulin (anti-α-tubulin, sc-8305; Santa Cruz, CA, USA) was used as the internal standard. The intensity of the immunoreactive proteins was calculated using NIH software ImageJ V.1.40. The intensity ratio, which was calculated by dividing the intensity of survivin or securin to that of α-tubulin, was used to compare the effects of YM155 treatment.

2.5. Statistical analysis

Data are presented as the mean ± standard error of mean (SEM) and were analyzed using the Student t test. In all
cases, \( p < 0.05 \) was considered statistically significant and is labeled using an asterisk (*) in the figures. Double and triple asterisks (**) and (***) in the figures indicated \( p < 0.01 \) and \( p < 0.005 \), respectively.

### 3. Results

#### 3.1. Cytotoxic effects of YM155 on glioblastoma cells

After 48 hours of treatment, YM155 elicited potent cytotoxicity in a concentration-dependent manner in both DNA-PK-deficient M059J (Figure 1A) and DNA-PK-normal M059K (Figure 1B) human glioblastoma cells. The half-maximal inhibitory concentration (IC50) was about 30–35 nM (Table 1). YM155 exerted significant cytotoxicity on both cell lines when the concentrations were \( > 10 \) nM. The viabilities of the M059J and M059K cells were not

**Table 1** Cell viability of glioblastoma cells 48 hours after YM155 treatment.

<table>
<thead>
<tr>
<th>Concentration of YM155 (nM)</th>
<th>M059J (n = 3)</th>
<th>M059K (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0*</td>
<td>99.7 ± 2.8</td>
<td>99.8 ± 2.2</td>
</tr>
<tr>
<td>5</td>
<td>93.4 ± 1.7</td>
<td>95.8 ± 0.9</td>
</tr>
<tr>
<td>10</td>
<td>88.0 ± 2.6</td>
<td>80.1 ± 1.7</td>
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<tr>
<td>20</td>
<td>69.4 ± 3.6</td>
<td>60.9 ± 1.9</td>
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<tr>
<td>30</td>
<td>60.1 ± 5.0</td>
<td>49.6 ± 2.3</td>
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<tr>
<td>40</td>
<td>45.7 ± 3.0</td>
<td>42.9 ± 2.4</td>
</tr>
<tr>
<td>50</td>
<td>31.9 ± 1.9</td>
<td>28.7 ± 1.0</td>
</tr>
</tbody>
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* Only DMSO was administered.
significantly different at each concentration of YM155 (5–50 nM; Table 1).

3.2. YM155 induces apoptosis in glioblastoma cells

To determine the characteristics of the cytotoxicity that is mediated by YM155 in glioblastoma cells, a DNA fragmentation ELISA assay was carried out. Low basal levels of DNA fragments bound to anti-histone plus anti-DNA antibodies were detected in the culture medium of the M059J and M059K cells 24 hours after treatment with 30 nM YM155. A significantly large amount of cytoplasmic DNA fragments was found after treating the M059J and M059K cells with 30 nM YM155 in comparison with the control.

Figure 3  Expression of survivin after YM155 treatment. Figures A, B and C, D refer to M059J (n = 3) and M059K (n = 3) cells, respectively. (A, C) Representative Western blots of survivin expression that were examined 24 and 48 hours after treatment with 30 nM YM155. Expression of α-tubulin was used as the internal standard. (B, D) Quantitative comparisons of survivin expression. ** indicates p < 0.01; *** indicates p < 0.005.

Figure 4  Expression of securin after YM155 treatment. Figures A, B and C, D refer to M059J (n = 3) and M059K (n = 3) cells, respectively. (A, C) Representative Western blots of securin expression that were examined 24 and 48 hours after treatment with 30 nM YM155. Expression of α-tubulin was used as the internal standard. (B, D) Quantitative comparisons of securin expression. * indicates p < 0.05; ** indicates p < 0.01.
also present with decreased DNA-PK activities.27 This resistance in cancer cells.28,29 Survivin is undetectable in radiation-sensitive M059J cells in this study. This is consistent with previous studies. Furthermore, Chen et al described the interaction between nuclear survivin and DNA-PKcs following exposure to irradiation, demonstrating that survivin knockdown by RNAi impairs the repair of double-stranded breaks via nonhomologous end-joining (NHEJ) and V(D)J recombination.23 Cells that are deficient in DNA-PK may be a good cell model for investigating new treatment strategies against radiation-resistant glioblastoma.

Our results show that the novel survivin inhibitor YM155 elicits cytotoxicity in the glioblastoma cell lines M059K and M059J, indicating that DNA-PK is not involved in survivin inhibition-induced apoptosis of glioblastoma cells. Capalbo et al described the interaction between nuclear survivin and DNA-PKcs following exposure to irradiation, demonstrating that survivin knockdown by RNAi impairs the repair of double-stranded DNA breaks.27 Survivin-depleted cells also present with decreased DNA-PK activities.27 This evidence explains why survivin expression in radiation-resistant M059K cells was greater than that measured in radiation-sensitive M059J cells in this study. This is consistent with the observation that survivin mediates radiation-resistance in cancer cells.28,29 Survivin is undetectable in normally differentiated human adult tissues.30 Thus, the targeting of survivin inhibition may not influence the functioning of normal cells. Knockdown of survivin by shRNA delays the growth of glioblastoma xenografts in nude mice.31 However, the clinical applications of genetic knockdowns may not be feasible in the near future. A few phase I/II clinical trials on the use of YM155 in cancer therapies have already been reported, and these treatments were well tolerated by patients with minimal adverse effects such as fatigue, nausea, and pyrexia.32–34 Thus, YM155 may offer a new hope for glioblastoma patients, including those with radiation resistance.

Human survivin is identical to the product of PTTG (pituitary tumor-derived transforming gene), and the overexpression of securin has been observed in several human cancers other than pituitary tumors.35–37 The securin that is expressed in the nuclei was originally found to be an inhibitor of premature sister chromatid separation and a potential activator of transcription, but recently it has also been found to mediate tumor invasiveness and recurrence.38 Until now, there have been no studies that have investigated the expression of securin in human glioblastoma cells and tissues, and our results provide the first results. In our previous report, concomitantly decreased expression of survivin and securin in bladder cancer cells was noted after TrkB antibody treatment.39 Chao and Liu have also demonstrated that the transfection of survivin siRNA significantly induces apoptosis and decreases the levels of survivin and securin proteins in A549 cells.40 Thus, the decreased expression of securin and elevated apoptosis following YM155 treatment observed in our study is consistent with previous studies. Furthermore, Chen et al reported that securin mediates radiosensitivity.41 Thus, the higher expression level of securin that was observed in M059K cells in comparison with M059J cells may at least partly explain why M059K cells exhibit radiation resistance.

In conclusion, survivin inhibition by YM155 elicits potent cytotoxicity in human glioblastoma cells via DNA-PK-independent mechanisms. YM155 could be used as a new therapeutic drug for treating glioblastomas, including those that are resistant to radiation.

Acknowledgments

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References


43. Muller C, Calsou P, Frit P, Cayrol C, Carter T, Salles B. UV sensitivity and impaired nucleotide excision repair in DNA-