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ORIGINAL ARTICLE

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

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glioblastoma;
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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

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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.[1] The death of a pediatric patient with a brain tumor, especially a high-grade glioma, usually results from cancer.[2,3] Glioblastoma demonstrates the worse therapeutic response among high-grade gliomas, with a very low 5-year survival rate despite aggressive chemotherapy and/or radiation therapy.[4–6] 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.[7] 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.[8] Treatments aimed at inhibiting survivin expression could be a new strategy for the treatment of glioblastomas.[9–13] 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.[14] YM155, as a survivin suppressant, has been shown to inhibit the progression of some neoplasms *in vitro* and/or *in vivo* (e.g., prostate cancer[14,15] and lung cancer).[16,17] 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.[18] 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.[19]

2.2. MTT cytotoxicity assay

For the cytotoxicity assay, 1.0×10^4 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.[20]

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 2.0×10^4 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.[20] 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 $\pm$ standard error of mean (SEM) and were analyzed using the Student *t* test. In all