YM155, A Novel Small Molecule Survivin Suppressant, Reduces Tumor Progression of Human Musculoskeletal Malignancies

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Introduction: Musculoskeletal malignancies, particularly high-grade sarcomas such as malignant fibrous histiocytoma (MFH), which has recently been classified as undifferentiated pleomorphic sarcoma (UPS), and osteosarcoma, are clinically aggressive and demonstrate high metastatic behavior in various organs (1, 2). Advances in the treatment of musculoskeletal malignancies have led to multidisciplinary treatment, including surgery, chemotherapy and radiation therapy, resulting in great improvements in the quality of life of patients with the disease, however the chemotherapy and radiation therapy are not as effective as those for other malignancies. Therefore, new therapeutic strategies against high-grade sarcomas need to be established. Survivin is a member of the inhibitor of apoptosis (IAP) family, which usually expresses in the embryonic lung and fetal organs in the developmental stages, but is undetectable in normal adult tissues (3). Several studies reported that survivin is highly expressed in various human malignancies, and increased expression of survivin is an unfavorable prognostic marker correlating with decreased overall survival in cancer patients (4). YM155, a novel small molecule survivin suppressant, selectively suppresses survivin expression, resulting in activation of caspases and induction of apoptosis in several malignant tumors (5). We have previously reported that survivin was strongly expressed in human musculoskeletal malignancies and the antitumor effects of YM155 on a human MFH cell line. The aim of this study was to evaluate the effect of YM155 on apoptotic activity in human MFH and osteosarcoma cell lines in vitro and in vivo.

Methods: Cells: Three human MFH cell lines (Nara-H, Nara-F and TNMY1) and an osteosarcoma cell line (MG63), that express the high levels of survivin, were used in this study. Cells were routinely cultured in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin at 37°C in a humidified atmosphere containing 5% CO2.

In vitro studies: To evaluate the effect of YM155 on sarcoma cell growth, we performed WST-8 assays at 24, 48, 72 and 96 hours of YM155 treatment (at concentrations from 0 to 1000 nM), and relative cell viability was calculated. For evaluation of apoptosis, cell lysates were collected from cells at 48 and 72 hours of YM155 treatment, and expressions of apoptosis-related proteins, such as caspase-3, caspase-9 and PARP, were assessed by immunoblot analysis.

In vivo studies: Male BALB/c nude mice, aged 5 weeks, were obtained from CLEA Japan, Inc. (Tokyo, Japan), and each cell line was injected into dorsal, subcutaneous area of eighteen mice. Then, mice were randomly divided into three groups: YM155-4mg group (n=6), YM155-2mg group (n=6) and control group (n=6). Treatment commenced 2 weeks after cell implantation, and was performed five times a week for 2 weeks. Tumor volume and body weight in mice were monitored twice weekly until the end of treatment. At the end of experiment, mice were sacrificed and tumors were excised. Survivin expression
in tumor tissues was assessed by quantitative real time PCR, and the apoptotic activity was evaluated by DNA fragmentation assay and FACS analysis.

Statistical analysis: ANOVA with post hoc test was used to compare for continuous values. All tests were considered significant at p<0.05. The data were presented as the mean ± SE. For distributed data, the two-tailed t-test was used for a comparison among the groups.

**Results:** In *in vitro* studies, YM155 inhibited cell proliferation of all sarcoma cell lines in a dose- and a time-dependent manner at concentrations of 10 nM or more (Fig.1). Immunoblot analysis revealed that expressions of cleaved forms of caspase-3, caspase-9 and PARP were increased in YM155 treated cells, while the expressions were barely detected in control cells.

In *in vivo* studies revealed that tumor volume in YM155-4mg treated group of all cell lines was significantly reduced compared with that in control group after 14 days of the treatment, and no apparent body weight loss was observed during the experimental periods (Fig. 2, p<0.05). Especially, massive tumor regression was observed in the YM155-4mg group of Nara-H injected mice, and some tumors finally disappeared. Survivin expression in YM155 treated tumors was significantly decreased compared with control tumors (Fig.3). DNA fragmentation assay and FACS analysis revealed that apoptotic activity was significantly increased in YM155 treated tumors.

**Discussion:** Previous studies revealed that overexpression of survivin is associated with tumor growth, progression, and resistance to conventional anticancer agents in various human malignancies. YM155 was characterized a novel small molecule survivin suppressant using a survivin gene promoter activity assay. YM155 selectively suppresses survivin expression, resulting in activation of caspases and induction of apoptosis. YM155 has also been found to induce tumor regression and intratumoral survivin suppression in established human hormone refractory prostate cancer, non-Hodgkin lymphoma, and non-small cell lung cancer tumor xenografts. In the previous study, we reported that YM155 increased the expressions of apoptosis-related proteins, and the apoptotic activity in Nara-H cells in vitro, and then, YM155 showed a dose- and time-dependent antitumor effect on human MFH xenografts without any severe systemic toxicities in vivo. In this study, we demonstrated that YM155 increased the apoptotic activity in other human MFH cells and a human osteosarcoma cell line in vitro. Then, in vivo, YM155 showed an antitumor effect on a MFH and an osteosarcoma tumors without remarkable side effect. The findings in this study strongly suggest that YM155 may contribute to the suppression of tumor progression via promoting the mitochondrial apoptosis, and survivin may be considered as a potent therapeutic target for the novel treatment of human musculoskeletal malignancies.

**Significance:** YM155 selectively suppresses survivin expression, resulting in induction of apoptosis in human musculoskeletal malignancies, and survivin may be considered as a potent therapeutic target for the treatment of human musculoskeletal malignancies.
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