Capsaicin is a major pungent ingredient found in hot pepper, has long been reported to control of obesity and anti-carcinogenic activities. Capsaicin induced apoptosis in a various cancer cells, however the precise molecular mechanisms have been poorly understood. In present study, the effect of capsaicin in cell viability the U87MG human glioma cells and its molecular mechanisms of cell death were investigated. Capsaicin induced reduction of cell viability in dose- and time-dependent manners. Apoptosis was determined based on the increase of positive TUNEL stained cells. The mechanisms of apoptosis were related with mitochondrial pathway (Bcl-2/Bax), activation of MAPK pathway. These data suggest that capsaicin could be a novel chemotherapeutic agent of human malignant gliomas.

Keywords Glioblastoma, U87MG cell, Capsaicin, Apoptosis, MAPKs

Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) is the active pungent ingredient found in the hot pepper of the genus Capsicum, has long been used commonly and frequently as drugs, food additives in the world1,2. In the previous researches, studies on the capsaicin have aimed on the control of obesity and anti-carcinogenic or chemo-preventive activities3,4. It has been recently reported that capsaicin could inhibit the growth of tumor cells and induce the apoptosis in the various cancer cells such as human leukemia HL-60 cells5, gastric adenocarcinoma cell line (AGS cells)6 and human breast cancer MCF-7 cells7. According to these results, capsaicin extracted from natural plants has been expected a part of the most promising anti-cancer agents. However, the molecular mechanisms of capsaicin-induced apoptosis have not been clearly understood. And there have been only a few reports on the effect of capsaicin in the human malignant glioma cells. Human glioma, glioblastom multiform (GBM), is one of the most aggressive and invasive malignant tumors8-10. In spite of optimal standard treatment, the prognosis of GBM still remains very poor with median survival of one year.

In this present study, we investigated the effects of capsaicin on cell viability and identify the molecular mechanisms of cell death in the human glioblastoma cells.

Inhibitory effects of capsaicin on human glioblastoma U87MG cell

In order to determine the effects of capsaicin on cell viability in the human glioblastoma, U87MG cells were treated with different concentrations of capsaicin (0, 50, 100, 200, 400, 600 and 800 μM) and time periods (0, 3, 6, 12, 18, 24 and 48 hr). The cell viability was measured by MTT assay. As shown in Figure 1A and 1B, cell viability was decreased in a dose- and time-dependent manner. These results suggest that capsaicin inhibited proliferation of human glioblastoma cells in a dose- and time-dependent through reduction of cell viability. Moreover, we examined the morphological change of U87MG cells for different concentrations (200 and 400 μM) of capsaicin in a dose-dependent manner. We found that cells were changed to spindle shape after treatment with capsaicin. The degrees of morphological change were proportional to the con-
capsaicin (Figure 1C).

**Capsaicin-induced apoptotic cell death in U87MG cells**

To demonstrate the effect of capsaicin on apoptosis in the human glioblastoma, U87MG cells exposed to capsaicin were experimented using western blot analysis and TUNEL assay. As shown in Figure 2A and 2B, expression levels of pro-caspase3 and Bcl-2 were decreased in the cells treated with capsaicin. Whereas, expression levels of Bax was increased. To confirm the apoptotic cells following treatment with capsaicin, we examined the expression of fluorescence of TUNEL assay by confocal microscope. It has been reported that TUNEL positive cells were models of apoptosis \(^{11-13} \). Cells treated with capsaicin revealed marked increase in fluorescence (green) (Figure 2C). On the contrary, little fluorescence was detected in control cells without capsaicin. These results indicate that capsaicin-induced apoptosis in the U87MG cells.

**Role of MAPK (mitogen-activated protein kinase) signaling in capsaicin-induced apoptosis**

There has been known that MAPKs are involved in the cell death of various cancer cells \(^{14-16} \). Therefore, we examined the association of MAPKs activity in the U87MG cells under exposure to capsaicin. To demonstrate whether MAPKs signaling are involved in the capsaicin-induced apoptosis, activity of MAPKs subfamilies were evaluated by using specific antibodies of phosphorylation of ERK and p-38. However, activation of JNK was not detectable (data not shown). Capsaicin induced transient activation of ERK and p-38 after 30 min (Figure 3A). Next, we examined the effect of MAPKs inhibitors, SB203580 (a p38-kinase inhibitor), SP 600125 (a c-Jun N-terminal kinase inhi-