（学位第３号様式）

学 位 論 文 要 旨

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題　目	Molecular mechanisms of anti-cancer activity of Wasabi 6-MSITC in human colorectal cancer cells (ワサビ機能性成分のヒト大腸がん細胞における抗がん活性の分子機構に関する研究)

Recently, cancer is the first leading cause of death, and the increase of its incidence and mortality as colorectal cancer has been observed in Japan. Lifestyles are associated with cancer risk, and consumption of fruit and vegetables on daily basis has provided preventive effects against colorectal cancer. Apoptosis, programmed cell death, plays an important role in the removal of seriously damaged cells or tumor cells. The tumor suppressor gene, *p53* plays a central role in apoptosis induction. Many chemotherapeutic drugs revealed their anti-cancer effects by activating P53, however, the *p53* gene is one of the most frequently mutated genes in many cancers.

6-(Methylsulfinyl)hexyl isothiocyanate (6-MSITC), a major bioactive compound in Wasabi, is a very popular pungent spice in Japan. Wasabi 6-MSITC has been reported to have cancer chemopreventive activities against colorectal carcinogenesis in rat model, however, the underlying mechanism is unclear. In this study, I investigated the anticancer activity and molecular mechanisms, using two types of human colorectal cancer cells (HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$).

First, 6-MSITC caused cell viability inhibition, cell cycle arrest in G_2/M phase and apoptotic cell death in both types of cells. The increase levels of P21, death receptor 5 (DR5) and pro-apoptotic BCL-2-associated X protein (BAX), and the decrease levels of anti-apoptotic B-cell lymphoma 2 (BCL-2) and B-cell lymphoma-extra large (BCL-XL) and inhibitor of apoptosis protein (IAP) family were observed in both types of cells treated with 6-MSITC. These data indicated that 6-MSITC inhibited cell viability of human colorectal cancer cells through cell cycle arrest in G_2/M phase and apoptosis induction by *p53*-independent molecular events.

Moreover, investigation of molecular mechanisms revealed that the activation of extracellular signal-regulated kinase 1/2 (ERK1/2), rather than *p53*, is recruited for 6-MSITC-induced apoptosis. 6-MSITC stimulated ERK1/2 phosphorylation, and then activated ERK1/2 signaling including ELK1 phosphorylation, and upregulation of C/EBP homologous protein (CHOP) and death receptor 5 (DR5). This mechanism was confirmed as the MAP/ERK kinase 1/2 (MEK1/2) inhibitor U0126 blocked all of these molecular events induced by 6-MSITC, and enhanced the cell viability in both types of cells. These results indicated that 6-MSITC induced apoptosis in colorectal cancer cells via *p53*-independent, ERK1/2-mediated ELK1/CHOP/DR5 pathway

Further, 6-MSITC enhanced the ratio of pro-apoptotic BAX/anti-apoptotic myeloid cell leukemia 1 (MCL-1), and sequentially caused mitochondrial membrane potential ($\Delta\Psi_m$) loss, cytochrome *c* release, and caspase-3 activation in both types of cells. 6-MSITC-activated ERK1/2 has been involved in extrinsic apoptotic cell death via ELK1/CHOP/DR5, however, the MEK1/2 inhibitor U0126 had no effect on mitochondria dysfunction caused by 6-MSITC. Taken together, 6-MSITC induced apoptosis of human colorectal cancer cells in *p53*-independent mitochondrial dysfunction pathway differently from ERK1/2-mediated ELK1/CHOP/DR5 pathway.

In conclusion, these findings will help in understanding the chemoprevention mechanisms of Wasabi 6-MSITC on colon carcinogenesis previously reported in animal model, and offer on opportunity that Wasabi 6-MSITC might be a potential compound for colorectal cancer chemoprevention even with *p53* mutation.