学位論文要旨

EFFECTS OF PHENOLIC AND STILBENOID COMPOUNDS ON THE PROLIFERATION OF HEPATOMA CELLS AND THEIR MODES OF ACTION
食品中のポリフェノール及びスチルベノイド化合物の肝癌細胞増殖に対する作用とその機構解析

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Cancer is a leading cause of death worldwide. According to the World Health Organization, in 2012, there were 14.1 million new cancer cases and 32.6 million people living with cancer. Epidemiological studies have consistently shown that regular consumption of fruit and vegetables is strongly associated with reduced risk of developing chronic diseases, such as cancer and cardiovascular diseases. Phenolic compounds derived from the olive tree, tyrosol and hydroxytyrosol, have been under investigation for many years. Among those phenolic compounds, tyrosol and hydroxytyrosol is the most studied phenolic compounds derived from olive oils. There have been some reports about the inhibitory effects of tyrosol and hydroxytyrosol on cancer cells such as human promyelotic leukemia, breast, prostate, and colon cancer. In the present study, the effect of tyrosol and hydroxytyrosol on the proliferation of hepatoma AH109A cells, as well as on mesentery derived mesothelial cells (M-cells), were evaluated using our *in vitro* assay system.

The treatment with tyrosol and hydroxytyrosol for 24 hours resulted in a dose-dependent inhibition on the proliferation hepatoma AH109A cells. Our data showed that tyrosol significantly inhibit the proliferation of AH109A cells up to 200 μM, and no effect on the proliferation of the M-cells up to 100 μM. Hydroxytyrosol significantly inhibit the proliferation of AH109A cells at 100 μM and continue the inhibition up to 200 μM. On normal cells, hydroxytyrosol had no effect on the proliferation of M-cells up to 50 μM, but significantly inhibit the

proliferation of the cells at higher concentration. We found that the anti-proliferative activity of hydroxytyrosol against AH109A hepatoma cells is stronger compared to tyrosol.

Pterostilbene is a naturally occurring stilbenoid compound isolated from several natural plant sources such as grapes and blueberries. Previous study demonstrated the effect of pterostilbene on several types of cancer such as breast, skin, gastric, and bladder cancer. However, little is known about the effect of pterostilbene on the proliferation of hepatoma cells. This study was undertaken to characterize its ability to suppress the proliferation of hepatoma AH109A cells and the possible mechanism(s) involved.

Pterostilbene dose-dependently inhibit the proliferation of AH109A cells. Pterostilbene significantly inhibit the proliferation of AH109A at 25 μM by 20% and continued the inhibition up to 200 μM by more than 90% as compared to correspondence control. Pterostilbene exerted little or no effect on the proliferation of rat L6 myoblasts and rat skin fibroblasts. *Ex vivo* experiment conducted by intubating 40 mg of pterostilbene/100 g body weight on male Donryu rat showed that pterostilbene-loaded rat sera could significantly inhibit the proliferation of AH109A cells. This data suggest that pterostilbene was absorbed through gastrointestinal tract and retain its anti-proliferative activity. Pterostilbene arrested the cell cycle of AH109A cells at G0/G1 phase and reduced the protein expression of cyclin-dependent kinase 4 (CDK4) and cyclin-dependent kinase 6 (CDK6) dose-dependently, without any induction on apoptosis. We also found that pterostilbene could significantly increase the intracellular peroxide level of AH109A cells in a dose- and time-dependent manner. The down-regulation of CDK4 and CDK6 protein expressions and the modification of intracellular peroxide level of the cells may be at least one of the mechanisms of anti-proliferative activity of pterostilbene against hepatoma AH109A cells.

In conclusion, our data indicated that hydroxytyrosol, tyrosol, and pterostilbene exerted anti-proliferative effect against AH109A hepatoma cells without any effect on normal cells at low concentration. Hydroxytyrosol have a higher anti-proliferative activity against AH109A cells compared to tyrosol. Pterostilbene suppressed the proliferation through cell cycle arrest at G0/G1 phase, modification of CDK4 and CDK6 protein expression, and the production of intracellular peroxide in AH109A cells. Further studies are needed to elucidate the precise mechanisms on the effect of above-mentioned components and are now in progress in our laboratory.