学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

Regulation of apoptosis related factors in intrinsic signaling pathway by myricetin *in vitro*

（ミリセチンによる内因性シグナル伝達経路のアポトーシス関連因子の試験管内での調節）

Polyphenols, which are abundant in our daily food, exhibited its antioxidant activities have been reported by many researches previously such as cardio-protective, anti-inflammatory and anti-diabetes. Recenly, another effect of polyphenols has been paid more attention. Prooxidant activity has been applied in some targeted treatment especially anticancer. Myricetin, widely exists in various vegetables or fruits especially berries, exhibited its potent antioxidant effect for protecting our human bodies from aging or diseases. Although many studies have shown the prooxidant activity of myricetin such as anticancer, the mechanism of anticancer is still unclear. In the present study, two cell lines were utilized for clarifying the anticancer effect of myricetin, and deloping anticancer mechanism through a consistent apoptotic signaling pathway.

PC12 cells were treated with myricetin in two concentration levels comprising 0.1 and 1 μM under serum-free condition. MCF-7 cells were treated with three concentration levels including 10, 20 and 40 μM. Morphological changes were observed using trypan blue assay. DNA fragmentation was determined by DNA ladder assay after treatment of myricetin in PC12 cells under serum deprivation condition. IC50 by MTT assay and GSH levels were determined for analyzing cytotoxicity and oxidative stress after treatment of myricetin in MCF-7 cells. The expression of cytochrome c, p53, Bax, Bcl-2, caspase-3 and 9 were determined by western blot analysis in the experiment of PC12 cell. In the experiment of MCF-7 cells, the expression of p53, NF-kB, Bax, Bcl-2, Bcl-xL, Apaf-1 and caspase-3 were determined by western blot analysis.

For the treatment of PC12 cells under serum-free condition after 72 h, western blot results showed that cytosolic cytochrome c which was released from mitochondria.

Subsequently, tumor suppressor gene p53, pro-apoptotic and anti-apoptotic Bcl-2 family proteins Bax and Bcl-2 were expressed. The caspase cascade reaction was through caspase 3 and 9 expression.

For the treatment of MCF-7 cells, IC50 was 44, 29.5 and 21.3 μM after 24, 48 and 72 h. A decreasing GSH levels were determined. P53 activation, which showed an increasing tend after myricetin dose increased, had a negative relation to NF-kB activation, and induced the transcriptional activation of Bax versus Bcl-2 dimer. It was found the adaptor receptor Apaf-1 and caspase-3 was also activated when apoptosis occurred by myricetin treatment. It could directly reflect the release of cytochrome c because Apaf-1 binds with released cytosol cytochrome c, forms a complex with oligomerizated caspase 9 in the presence of ATP to activate caspase 3 and sequentially cleave cellular death substrates.

P53 activation evokes a series of complicated and closed link to execute apoptosis by the release of cytochrome c. In fact, the release of cytochrome c, which is induced by the transcriptional activation of Bcl-2 family members like pro-apoptotic Bax and anti-apoptotic Bcl-2, triggers the downstream caspase cascade reaction and apoptogenic protein expression. Generally, the released cytosolic cytochrome c which binds with the adaptor molecular Apaf-1, forms a complex with oligomerizated caspase 9 in the presence of ATP to activate caspase 3 and sequentially cleave cellular death substrates. Herein, a consistent mechanism of apoptotic signaling pathway was demonstrated.

Generally, myricetin exhibited a potent inhibitory effect on the growth of PC 12 cells and MCF 7 cells through a consistent apoptotic signaling pathways. Herein, the activation of p53 plays a critical role for triggering a series of intracellular morphology and molecular changes including cell shrinkage, blebbling, nuclear fragmentation, chromatin condensation, expression of apoptogenic protein and disruption on the balance of intracellular redox environment. Actually, myricetin exhibited its potent anti-oxidant property on protecting from ageing. However, more potential pro-oxidant activity function should be paid attention as the data shown in the present study. A potent proof was provided in present study and it is expected that myricetin shows synergistic effect with anticancer drug or therapies.