様式 7

論　文　内　容　要　旨

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学位論文題目	The study on Endoplasmic Reticulum-Mitochondria relationship in Tocopheryl ester induced Apoptosis （トコフェロールエステル体によって誘導されるアポトーシスにおける小胞体とミトコンドリアの関係に関する研究）		

The esters of α-tocopherol have drawn the attraction of researchers for their versatile biological functions. Recently, α-tocopheryl succinate (TS), a redox-silent succinyl ester of natural α-tocopherol, has emerged as a novel anticancer agent. Several reports have shown that TS inhibits mitochondrial respiratory complex II and generates superoxide and cause apoptosis. Another research suggests that endoplasmic reticulum (ER) stress was involved in TS-induced apoptosis. Furthermore, the terminal dicarboxylic moiety of tocopheryl esters contributes to apoptosis induction. However, the ER-mitochondria relationship and the influence of the dicarboxylic moiety of tocopheryl esters in this relationship are unclear. In this study, I found that TS increased intracellular superoxide and Ca^{2+} in cultured cells, suggesting induction of ER stress. In addition, TS downregulated glucose-regulated protein 78 (GRP78), which maintains ER homeostasis and promotes cell survival, further supporting ER stress induction. Moreover, TS caused mitochondrial depolarization. However, inositol 1,4,5-trisphosphate (IP3) receptor antagonist 2-aminoethyl diphenylborinate (2-APB) which is the inhibitor of Ca^{2+} efflux, decreased TS-induced intracellular $Ca^{2+,}$ restored mitochondrial activity and cell viability in TS-treated cells, establishing the ER-mitochondria relationship in apoptosis induction by TS. Further investigation on the ER-mitochondria contact site revealed that TS-treated cells exhibited increased ER-mitochondria contact compared to the control cells. These results confirm a potential relationship between ER and mitochondria in apoptosis induction. Furthermore, to investigate the role of the terminal dicarboxylic moiety of the TS on this ER-mitochondria relationship, I compared the apoptogenic ability of TS, which has four carbon atoms in the terminal dicarboxylic moiety, to that of a newly synthesized, tocopheryl glutarate (Tglu), which has five. Cytotoxicity assays in vitro confirmed that TS stimulated apoptosis, while Tglu was non-cytotoxic. In investigating biological mechanisms leading to these opposing effects, I found that Tglu treatment upregulated GRP78 but did not elevate intracellular superoxide and $Ca^{2+,}$ and thus no mitochondrial depolarization was observed like TS treatment. Taken together, these results suggest a model in which TS-mediated superoxide production and GRP78 inhibition induce ER stress, which elevates intracellular Ca^{2+} and, at the same time, increases ER-mitochondria contact and mitochondrial depolarization, leading to apoptosis. Because Tglu does not affect superoxide generation and increases GRP78 expression, it inhibits ER stress and is thereby non-cytotoxic. Moreover, increasing the carbon number in the terminal dicarboxylic moiety by only one carbon results in the opposite regulation of the ER-mitochondria relationship.