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Oleifoliosides B-mediated autophagy and apoptosis involved Caspase Independent Pathways in A549 cells

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실험목적 (Objectives)

The purpose of this study to investigate human cancer cell death mechanism, we isolated a compound from *Dendropanax Morbifera Leveille*(oleifoliosides B), and evaluated oleifoliosides B for their inhibitory effects of A549 cells. We further insight into the mechanism of oleifoliosides B-induced autophagy and apoptosis. we also assessed that the relationship between BafilomycinA1, a inhibitor of autophagy and oleifoliosides B -mediated apoptotic cell death. Furthermore, we also investigate that oleifoliosides B inhibited survival signaling pathways such as Akt and Nrf2 in A549 cells.

재료 및 방법 (Materials and Methods)

- Compound extract from *Dendropanax Morbifera* Leveille(oleifoliosides B)
- Cell line : A549 cells
- Cell viability and growth assay
- DNA flow cytometry assay.
- Determination of caspases activity
- Protein extraction and Western blot analysis
- Immunofluorescence confocal microscopy
- Nuclear staining

실험결과 (Results)

In the present study, we isolated a bioactive agent, oleifoliosides B, and demonstrated that this compound induced apoptosis in cancer cells in vitro. Here, we showed that oleifoliosides B induced autophagy at early time as evidenced the conversion of microtubule associated protein 1 light chain 3 (LC3-I) to LC3-II in A549 human non-small cell lung carcinoma cells. Pharmacological inhibition of autophagy with bafilomycin A1 decreased apoptotic cell death, suggesting that the autophagy caused by oleifoliosides B played a not protective role and promoted apoptotic cell death. Additional studies revealed that oleifoliosides B inhibited survival signaling pathways such as Akt and Nrf2 in A549 cells. Treatment with oleifoliosides B also reduced the expression of anti-apoptotic surviv in and cellular FLICE-inhibitory protein (c-FLIP). Taken together, these results indicated that the

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autophagy induced by oleifoliosides B promote apoptosis, implying that oleifoliosides B has significant potential as a chemotherapeutic enhancer.

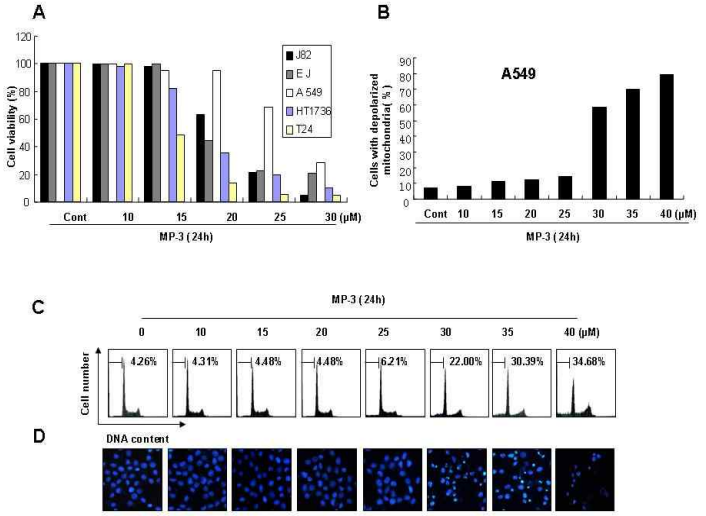

Fig. 1. The effects of oleifoliosides B on the viability of several cells. Cells (5 × 105 cells/ml) were incubated with the indicated concentrations of oleifoliosides B. (A) Cell viability was determined by MTT assay. (B) Loss of MMPs were determined by flow cytometry (C) The subG1 DNA contents by flow cytometry analysis. (D) Cellula morphology of cells incubated for indicated time was examined under light microscopy (x400).

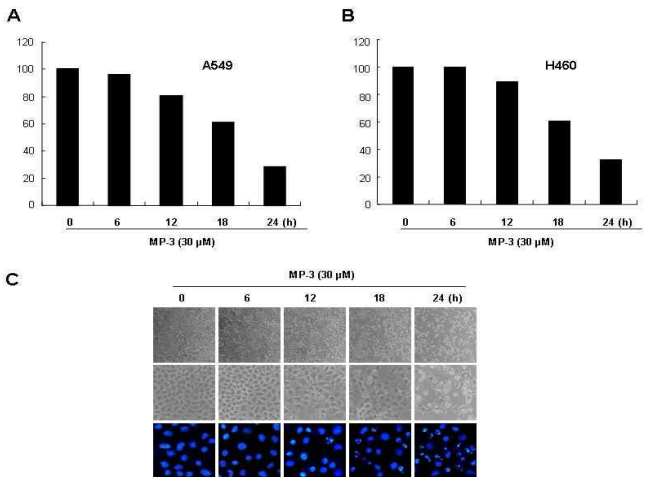

Fig. 2. The effects of oleifoliosides B on the viability of A549 and H460 cells. Cells (5 × 10^5cells/ml) were incubated with the indicated time of oleifoliosides B. (A and B) Cell viability was determined by MTT assay. (C) The cellula morphology of cells was examined under light microscopy (upper panels C, x200) or were stained with DAPI solution for 10 min, and then photographed with a fluorescence microscope using a blue filter (lower panels C, 400x).