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MP-2, Isolated from *Dendropanax Morbifera* Leveille Induces Mitochondria-Mediated Apoptosis in HeLa Cells

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Objectives

In an effort to develop new anti-cancer bioactive agents, we isolated MP-2 compound from *Dendropanax Morbifera* Leveille (Araliaceae), which is well known in Korea traditional medicine for a variety of diseases. We evaluated inhibitory effects of MP-2 on human epithelial cancer cells. We used HeLa cells to gain further insight into the mechanisms of MP-2-induced anti-proliferative action and apoptosis. We examined the effects of MP-2 on sub-G1-phase DNA content, DNA fragmentation and appearance of apoptotic bodies. In a parallel experiment, we also assessed for apoptosis by staining with V-FITC and PI double staining. apoptosis-inducing factor (AIF) and endonuclease G (Endo G) were determined by Western blot analysis and Immunofluorescence confocal microscopy.

Materials and Methods

- Isolation of MP-2 from *Dendropanax Morbifera Leveille*
- Cell line : HeLa 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

Results

In the present study, we used HeLa cells to gain further insight into the mechanism of MP-2-induced anti-proliferative action and apoptosis in a time-dependent manner in human epithelial cancer cells.. we demonstrated that treatment with MP-2 increased sub-G1-phase DNA content, DNA fragmentation and

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appearance of apoptotic bodies in a time-dependent manner. MP-2 induced apoptosis appeared to be correlated with the modulation of death receptor, inhibitor of apoptosis (IAP) member, Bcl-2 family proteins and activation of caspases, which resulted in the cleavage of poly(ADP-ribose)polymerase (PARP). Furthermore, MP-2 induced apoptosis via mitochondria with the release of apoptosis-inducing factor and endonuclease G in a time dependent. In summary, our results indicate that treatment with MP-2 may be a safe strategy for treatment of resistant epithelial cancer.

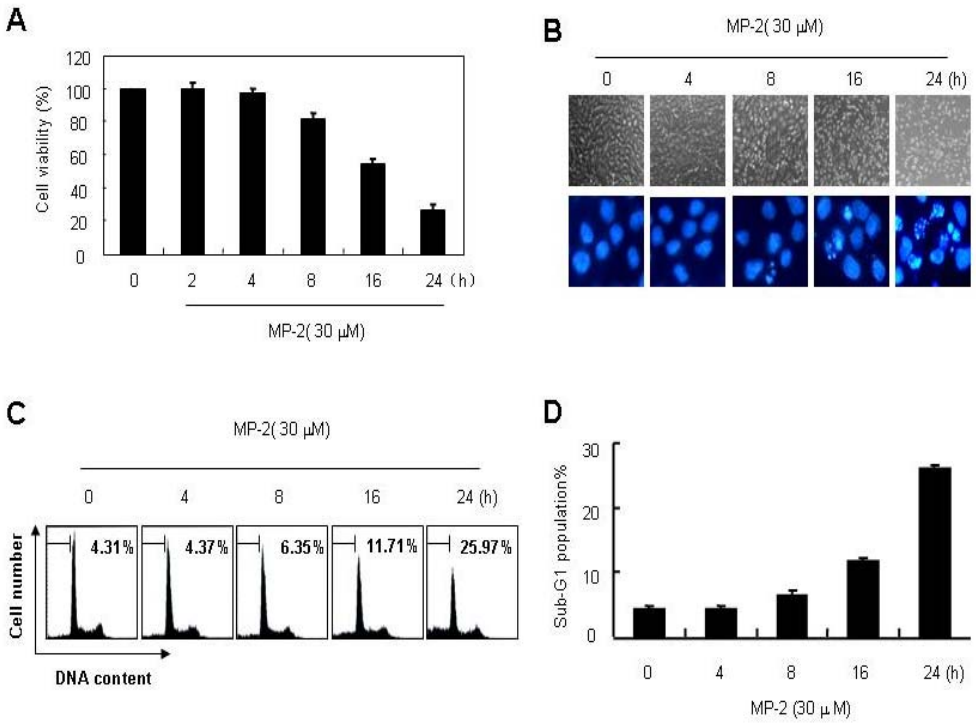

Fig. 1. Loss of cell viability and induction of apoptosis by MP-2 treatment in HeLa cells. (A) Cells were plated at 1x 10^5 cells per 60 mm plate, and the cells were pretreated with 30 μM MP-2 in a time dependent, prior to the determination of cell viability using the MTT assay. (B) The cellular (upper panels) and nuclear (lower panels) morphological changes in cells incubated with 30 μM MP-2 in a time dependent were examined under inverted and fluorescence microscopes, respectively. For DAPI staining (lower panels), the cells were fixed and stained with DAPI solution for 10 min at room temperature. (C) To quantify the degree of apoptosis induced by MP-2, cells grown under the same conditions as (A) were evaluated by flow cytometry for sub-G1 DNA content, which represents the cells undergoing apoptotic DNA degradation. Data represent the mean ± S.E. of three-independent experiments.