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자궁경부암 세포주 HeLa에 대한 강화약쑥에서 분리한 Eupafoin의 Apoptosis 유발에 관한 연구

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Eupafolin isolated from *Artemisia princeps* induced apoptosis via caspase dependent pathway in human cervical carcinoma HeLa cells

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Objectives

Many compounds that are used in cancer chemotherapy are derived from plant sources, such as vinblastin, paclitaxel, camptothecin, and etoposide. Natural compounds are still major sources of new drug development, and we are interested in the role of natural products in chemotherapy. Eupafolin is found in *Artemisia princeps* (known locally as "Sajabalssuk") which has been used traditional oriental medicine for the treatment of inflammation, cancer and other disorders. It also possesses several known biological properties such as antioxidant effect, antibacterial activity, antiproliferative activity and cytotoxicity for various cancer cells. In the present study, we investigated the effect of eupafolin on the apoptotic activity and the molecular mechanism of the action in human cervical carcinoma HeLa cells.

Materials and Methods

○ Materials

The eupafolin used for this study was isolated from the whole herb of *Artemisia princeps*, which was obtained on GangHwa Island, Korea. This compound used for this study was checked by HPLC and were >98% pure and dissolved in DMSO. Human cervical carcinoma HeLa cells were purchased from the Korean cell line bank and cultured in RPMI 1640.

○ Methods

The in vitro growth inhibition effect of eupafolin on HeLa cells was determined by MTT assay. To analyze an affect of eupafolin on the apoptosis, DNA fragmentation assay, DNA contents profiles, DAPI staining and Annexin V-PI staing were determined. Western blot of apoptosis-related proteins was determined and measurement of mitochondria membrane potential($\Delta\Psi_m$) was assessed using $DiOC_6(3)$ by flow cytometry.

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Results

We examined the effect of eupafolin on the growth of HeLa cells using a MTT assay. Dose dependent inhibition of cell growth was observed in HeLa cells with an IC_{50} of approximately 26 μM following teatment with eupafolin for 48 h. Treatment of HeLa cells with eupafolin resulted in DNA fragmentation, DNA ladder formation, and an increase in the sub-G1 phase. To further confirm and evaluate the induction of apoptosis, we stained cells with Annexin V and PI. As with the percentages of the sub-G1 group cells determined by flow cytometry, the proportion of annexin V-staining cells in the eupafolin-treated cells more strongly increased in a dose -dependent manner, which supports the idea that eupafolin-induced HeLa cells death occurs via apoptosis.

In addition, stimulation of HeLa cell with eupafolin induced a series of intracellular events that the activations of caspase-8, caspase-9, caspase-3 and PARP cleavage. And eupafolin activated the intrinsic pathway of apoptosis, which involved disrupting mitochondria membrane potential, release of cytochrom *c*, decreased Bcl-2 expression. Pre-treatment with a broad caspase inhibitor, caspases-8, -9 or -3 inhibitor (이름을 적어줌, v-zad-fmk등) neutralized the pro-apoptotic activity of eupafolin in HeLa cells. Taken together, these results suggest that eupafolin may play chemotherapeutic role for cervical cancer via the potent apoptotic activity through caspase dependent signaling pathway.

＊ 시험성적

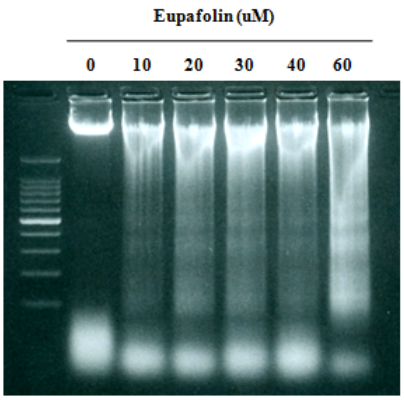

Fig. 1. Effect of Eupafolin on DNA fragmentation in HeLa cells

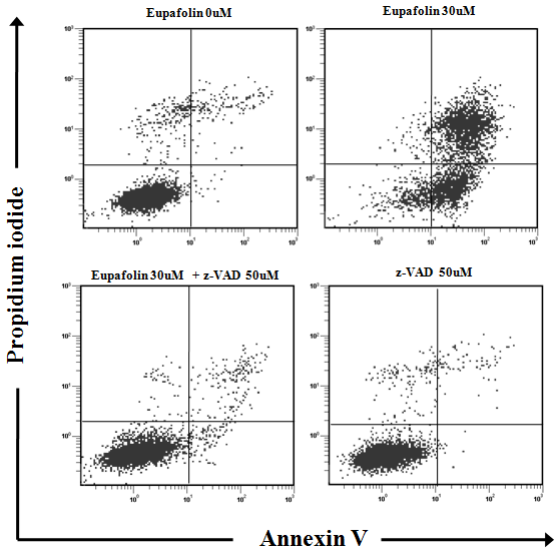

Fig. 2. Effects of broad caspase inhibitor on eupafolin-induces apoptosis