Honokiol Induces Cell Apoptosis in Human Chondrosarcoma Cells through Mitochondrial Dysfunction and Endoplasmic Reticulum Stress

Chondrosarcoma is a malignant primary bone tumor that responds poorly to both chemotherapy and radiation therapy. Honokiol, a major bioactive compound extracted from Chinese traditional herb Magnolia officinalis, has been reported to exhibit a potent cytotoxicity by inducing cell apoptosis in cancer cells. Although the effects of honokiol-induced tumor apoptosis have been studied in many cancers, the role of honokiol in the process of cell apoptosis in chondrosarcoma remains largely unknown. To the best of our knowledge, this study is the first attempt to determine the apoptosis activity of honokiol in human chondrosarcoma cell lines. Our data provide evidence that honokiol reduced cells survival and tumor growth in human chondrosarcoma cells in vitro and in vivo.

METHODS:
Cell culture: The human chondrosarcoma cell line (JJ012) was kindly provided from the laboratory of Dr. Sean P Scully (University of Miami School of Medicine, Miami, FL, USA). The human chondrosarcoma cell line (SW1353) was obtained from the American Type Culture Collection. The cells were cultured in DMEM/α-MEM supplemented with 10% Fetal Bovine Serum and maintained at 37°C in a humidified atmosphere of 5% CO₂. Primary cultures of human chondrocytes were isolated from articular cartilage as previously described. The cells were grown in plastic cell culture dishes in 95% air-5% CO₂ with DMEM which was supplemented with 20 mM HEPES and 10% heat-inactivated FBS, 2 mM glutamine, penicillin (100 U/ml) and streptomycin (100 μg/ml).

Detection of Ca²⁺ concentrations: Approximately 2 × 10⁴ cells/well of JJ012 cells in 12-well plates were incubated with honokiol for 4, 8, 12 and 24 hr to detect changes in Ca²⁺ levels. Cells were harvested and washed twice, and re-suspended in Indo 1/AM (3 μg/ml) at 37°C for 30 min and analyzed by flow cytometry.

MTT assay; Quantification of apoptosis by flow cytometry; siRNA transfection; Reporter assay; Quantitative real time PCR; Western blot analysis; Calpain Activity Assays; In vivo tumor xenograft study

RESULTS
To investigate the potential cell death of honokiol in human chondrosarcoma cells, we first examined the effect of honokiol on cell survival in human chondrosarcoma cells (JJ012). Treatment of JJ012 cells with honokiol-induced cell death in a concentration-dependent manner by using MTT assay (Fig. 1A&B). The IC50 value of honokiol was 10 μM for JJ012 cells (Fig. 1C). Next we investigated the anti-cancer effect of honokiol in other chondrosarcoma cell lines. Fig. 1C also shows that honokiol-induced cell death in other chondrosarcoma cell line (SW1353). However, honokiol did not affect the cells viability of normal chondrocytes (primary chondrocytes) (Fig. 1C). To investigate the anti-cancer effect of honokiol in human osteosarcoma cells, MG-63 and U2OS osteosarcoma cell lines were used. Honokiol also increased cell death in osteosarcoma cells (Fig. 1C).

We next investigated whether honokiol induces cell death through an apoptotic mechanism. Annexin-V-PI double-labeling was used for the detection of PS externalization, a hallmark of early phase of apoptosis. As compared to vehicle-treated cells, a high proportion of annexin V+ labeling was detected in cells treated with honokiol (Fig. 2A&B). Next we investigated the effect of honokiol-induced apoptosis by using TUNEL assay. Compared with vehicle-treated JJ012 cells, those treated with honokiol showed significant cell apoptosis (Fig. 2C). To determine whether honokiol induces apoptosis by triggering the mitochondrial apoptotic pathway, we measured the change in the expression of Bcl-2 family proteins. Treatment of JJ012 cells with honokiol induced Bax and Bak protein levels (Fig. 2D). In addition, honokiol decreased the expression of Bcl-2, which led to an increase in the proapoptotic/antiapoptotic Bcl-2 ratio (Fig. 2D).

DISCUSSION:
Natural product drugs have been suggested to play a dominant role in pharmaceutical care. Natural products are one of the important sources of potential cancer chemotherapeutic and chemopreventive agents. Honokiol has been widely used in the traditional Chinese and Japanese medicine for several thousand years, mainly, for the treatment of anti-thrombocytic, anti-bacterial, anti-inflammatory, and anxiolytic effects. Previous reports have demonstrated that honokiol is also possessing potent anti-neoplastic and anti-angiogenic properties. However, the precise molecular mechanism of exhibited anti-tumor activity by honokiol is not well understood. Thus, the results of this study provide evidences for the anti-tumor activity of honokiol in chondrosarcoma cells, and more importantly, the molecular basis for its effect. The present study has demonstrated that honokiol causes apoptosis in chondrosarcoma cells in vitro and in vivo. Honokiol-induced apoptosis in chondrosarcoma cells through the mitochondria dysfunction leading to activate caspase-9 and involves a caspase-3-mediated mechanism. Honokiol also induced cell death is mediated by increasing ER stress, GPR78 activation, Ca²⁺ release, which subsequently triggers calpain activity, resulting in apoptosis.

This study is the first to attempt to determine the apoptosis activity of honokiol in human chondrosarcoma cell lines. Our data provide evidence that honokiol reduced cells survival and tumor growth in human chondrosarcoma cells in vitro and in vivo.