Sulforaphane Induces Cell Cycle Arrest and Apoptosis in Murine Osteosarcoma Cells \textit{in vitro} and Inhibits Tumor Growth \textit{in vivo}

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\textbf{Introduction:} Sulforaphane (SFN), a naturally occurring member of the isothiocyanate family, is produced from cruciferous vegetables, such as broccoli. SFN is an effective agent in the chemoprevention of chemically induced breast, colon, and stomach cancers in rats. In a chemotherapeutic study, SFN drastically inhibited the growth of xenografts of human prostate cancer by oral administration and breast cancer by intravenous injection. SFN suppresses the growth of cancer cells \textit{in vitro} by inhibiting cell cycle progression and/or causing apoptosis in T-cell leukemia, colon, breast, and prostate cancer cells. In addition, it was reported that SFN induces p21 and induces G1- and G2/M-phase cell cycle arrest in human colon cancer cells. Previously, we reported that SFN up-regulates DR5 expression and the combined treatment with SFN and TRAIL induced apoptosis in human osteosarcoma cells. In this study, we confirmed that SFN causes cell growth arrest \textit{in vitro}. Furthermore, we investigated the anti-tumor activity of SFN against osteosarcoma cells \textit{in vivo}.

\textbf{Materials and Methods:} We used SFN (LKT), and murine osteosarcoma cell line, LM8.

(1) Cell growth study
LM8 cells were inoculated at a density of 10,000 cells in 12-well plate. Next day, SFN was added at various concentrations. The number of viable cells was counted by a trypan blue dye exclusion test.

(2) Analysis of cell cycle progression
The nuclei were stained with propidium iodide (PI). DNA content was measured using a FACSCalibur flow cytometer and Cell Quest software (Becton Dickinson).

(3) Western blot analysis
We used rabbit polyclonal anti-p21 antibody (1:500; Santa Cruz). Enhanced chemiluminescence (Amersham Bioscience) was used for detection.

(4) \textit{In vivo} Effect of SFN on LM8 cells
The LM8Luc, stably expressing luciferase (Luc) LM8 cells, were generated. LM8Luc cells were mixed in PBS, and a suspension containing 10,000,000 LM8Luc cells was administered to the right flank of mice via s.c. injection. Mice were randomized into three groups of 5 mice / group. Twenty four hours later, intraperitoneal injections of SFN (1,5 or 10 mg / week) were performed. Control mice received an equal volume of the vehicle. We analyzed primary tumor lesion and lung metastasis by using the in vivo imaging system (Xenogen). Statistically significant differences in tumor volume between control and treated mice were assessed by Student’s t-test.

\textbf{Results:} (1) SFN inhibited the growth of LM8 cells in a dose-dependent manner (Figure 1).

(2) SFN arrested LM8 cells at the G2/M phase in cell cycle progression (Figure 2A). SFN up-regulated p21 expression in LM8 cells in a dose-dependent manner (Figure 2B).

(3) SFN (5 and 10 mg / week) treatment caused a significant inhibition of LM8 xenograft growth (Figure 3A). As shown in Figure 3B, the growth of the lung metastasis was significantly inhibited by SFN (10 mg / week). No remarkable signs of toxicity were observed following SFN administration.

\textbf{Discussion:} Our study revealed that SFN induces cell growth inhibition via cell cycle arrest specifically at the G2/M phase in murine osteosarcoma LM8 cells in culture studies. Furthermore, based on the encouraging \textit{in vitro} antitumor efficacy of SFN against osteosarcoma, we found that intraperitoneal administration of SFN (5 and 10 mg / week) significantly retarded the growth of LM8 xenografts to 30% less than controls in a preclinical animal model without causing any toxicity. In osteosarcoma cells, our findings in this study provide \textit{in vivo} evidence for the efficacy of SFN against the advanced growth of tumors. SFN is a food factor that is contained in vegetables. Hu et al. reported that plasma concentrations reached 20 μM after oral administration of SFN in rats. After the administration of purified SFN, the murine plasma concentrations in this \textit{in vivo} study might reach a concentration used in our \textit{in vitro} study. In the clinic, we will use purified SFN as an anti-tumor agent for osteosarcoma cells. In conclusion, our results showed that SFN inhibits cell growth and induces cell cycle arrest in murine osteosarcoma cells. Furthermore, findings in xenograft studies translate the anti-tumor effects in a preclinical osteosarcoma model. These results raise a possibility that treatment with SFN is promising for the chemotherapy of osteosarcoma.