SULFORAPANE ENHANCES TRAIL-INDUCED APOPTOSIS THROUGH THE INDUCTION OF DR5 EXPRESSION IN HUMAN OSTEOSARCOMA CELLS

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INTRODUCTION:

Tumor necrosis factor (TNF) -related apoptosis-inducing ligand (TRAIL) selectively induces apoptosis in cancer cells in vitro and in vivo with little or no toxicity toward normal cells. Therefore, TRAIL is one of the most promising new candidates for antitumor therapeutics.

Death receptor 5 (DR5) and death receptor 4 (DR4) are members of the TNF-receptor family, and receptors for TRAIL. TRAIL transmits apoptotic signaling through cleavage and activation of caspases and Bid following interaction with DR5 and DR4. In addition, DR5 is a downstream gene of the p53 tumor-suppressor gene, and up-regulated by conventional anticancer drugs such as doxorubicin and etoposide. Moreover, down-regulation of DR5 promotes colon tumor xenograft growth in mice and confers resistance to chemotherapeutic agents. Therefore, DR5 is an attractive molecular target for effective cancer therapy.

Sulforaphane (SFN), a naturally occurring member of the isothiocyanate family, is produced from cruciferous vegetables. SFN has attracted particular attention due to its potent anticancer effects. Recent studies have indicated that SFN can suppress proliferation of cancer cells in vitro and in vivo by inhibiting cell cycle progression and/or causing apoptosis.

In this study, we tested an effective usage of the combined treatment of SFN with TRAIL in human osteosarcoma cells.

MATERIALS AND METHODS:

We used human osteosarcoma cell lines, Saos2. To examine the effect of SFN treatment on normal cells, we used normal human peripheral blood mononuclear cells (PBMC).

(1) Reagents
Sulforaphane and soluble recombinant human TRAIL/APO2L were purchased from LKT (St. Paul, MN) and PeproTech (London, UK) respectively. Recombinant human DR5 (TRAIL-R2)/Fc chimera, and the caspase inhibitors zVAD-fmk were purchased from R&D Systems (Minneapolis, MN).

(2) Detection of Apoptosis
DNA fragmentation was quantified as the percentage of cells with hypodiploid DNA (Sub-G1). The nuclei were stained with propidium iodide (SIGMA, St Louis, MO). The DNA content was measured using a FACSCalibur flow cytometer and Cell Quest software (Becton Dickinson, Franklin Lakes, NJ).

(3) Western Blot Analysis
We used rabbit polyclonal anti-DR5 antibody (1:250; Cayman Chemical, Ann Arbor, MI). Enhanced chemiluminescence (Amersham Chemical, Piscataway, NY) was used for detection.

RESULTS:

SFN enhances TRAIL-induced apoptosis synergistically in Saos2, human osteosarcoma cells.

As a single agent, both of SFN and TRAIL very weakly induced apoptosis in Saos2 cells. However, SFN strongly enhanced TRAIL-induced apoptosis (Figure 1). In addition, the SFN-mediated enhancement of TRAIL-induced apoptosis was markedly blocked by the DR5/Fc chimera, which has a dominant negative effect by competing with receptors for TRAIL, or the pancaspase inhibitor zVAD-fmk (Figure 1).

Figure 1
Saos2 cells were treated with SFN (30µM) and/or TRAIL (50ng/ml) for 24h with or without DR5/Fc chimera (1µg/ml) and zVAD-fmk (20µM).

DISCUSSION:

To improve the prognosis of osteosarcoma, new strategies are necessary. In the search for new strategies and antitumor agents, we found that SFN is a potent enhancer of TRAIL-induced apoptosis in osteosarcoma cells.

Furthermore, SFN up-regulates DR5 expression and sensitize TRAIL-induced apoptosis in a p53-independent manner because functionally-inactivated mutations of p53 gene exist in Saos2 cells. Several studies have shown the relationship of the inactivation of p53 with resistance to conventional antitumor agents. Therefore, the combined treatment with SFN and TRAIL may be effective for osteosarcoma with resistance to conventional agents caused by inactivated p53. On the other hand, SFN did not enhance TRAIL-induced apoptosis in normal human PBMC, suggesting that this regimen is expected to be safe in clinic.

In conclusion, we show for the first time that SFN synergistically enhances TRAIL-induced apoptosis through the induction of DR5 expression in human osteosarcoma cells but not in primary nonmalignant human cells. These results raise a possibility that the combination of SFN and TRAIL might be a promising molecular-targeting chemotherapy for malignant osteosarcoma.