Suberoylanilide hydroxamic acid (SAHA) inhibits growth of chondrosarcoma cells via induction of apoptosis and autophagic cell death

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Introduction: Since chondrosarcoma has high resistance to conventional chemotherapy and radiotherapy, surgical resection is only effective treatment for chondrosarcoma. Therefore, a novel approach to therapeutic strategy is needed to improve prognosis of the patients with chondrosarcoma.

Histone deacetylase inhibitors (HDACIs) are novel and promising anti-tumor agents. Previous studies demonstrated that HDACIs can activate transcription of specific genes through the accumulation of histone acetylation and subsequently lead to a variety of phenotypic changes, including cell cycle arrest, differentiation and apoptosis(1).

In this study, we investigated the anti-tumor effects of a HDACI, suberoylanilide hydroxamic acid (SAHA), on chondrosarcoma cell lines.

Materials and Methods: Cells and Reagents: Chondrosarcoma cell lines SW1353, Rat chondrosarcoma (RCS) and OUMS-27 were cultured in Dulbecco modified Eagles medium. SAHA was dissolved in 100% ethanol at a concentration of 10 mM for in vitro and also dissolved in DMSO at a concentration of 100 mg/ml for in vivo and stored at –80 degree until use.

Cell Viability Assay: The cell viability assay was carried out using a CellTiter-Glo Luminescent Cell Viability Assay Kit (Promega).

Western Blot: Lysates from the cells were subjected to western blot analysis using various antibodies.

Flow Cytometry: Cells treated with SAHA were harvested and fixed with 70% ethanol for 30 min at 4 degree, and resuspended in PBS with 10 mg/ml RNase A. The samples were centrifuged and resuspended with 95 µl PBS and 5 µl propidium iodide (50µg/ml), and mixed up to 1ml with 0.1% BSA. Alterations in the cell-cycle distribution were analyzed using flow cytometer (Becton Dickinson). The cell proportions were analyzed using Cell Quest (Becton Dickinson). For each sample, 2500 events were scored.

Electron microscopy: The cells (1x107) were fixed with 1x fixing buffer, and 60 nm-80 nm thin sections were stained with uranyl acetate for 10 min and lead acetate for 15 min. Then the sections were examined under a JEM2000EX (JEOL, Tokyo) electron microscope.

Results: We first investigated whether SAHA could reduce the viability of chondrosarcoma cell lines. When the cells were treated with various concentrations of SAHA for 48 hr, the number of viable cells was decreased in a dose-dependent manner. We next examined the acetylation status of histone H3 (Ac-H3) in the cells. After treatment with SAHA for 24 hr, the expression of Ac-H3 and the proteolytic cleavage of poly (ADP-ribose) polymerase (PARP) which is the marker of apoptosis was increased in SW1353 but not in RCS and OUMS-27 (Figure 1). FACS analysis revealed that SAHA caused G1/S arrest in RCS and G2/M arrest in OUMS-27.

Recently, a new type of programmed cell death has been reported, that is autophagic cell death defined as massive macro degradation of cytoplasmic organelles. Since an autophagosome is found in cytoplasm at the first stage of the autophagic cell death, we examined the expression of autophagosome specific protein known as microtubule-associated protein 1 light chain 3 (LC3). The result revealed that the expression of LC3, especially type 2 isoform of LC3 (LC3-2), was increased in a dose dependent manner in RCS and OUMS-27 (Figure 2). We further examined the ultrastructural morphology of RCS and OUMS-27 after 20µM SAHA treatment for 24h. A number of huge vacuoles and double/multiple-membrane vesicles were appeared in the cytoplasm. These morphological features clearly reflected the specific autophagic characteristics.

Discussion: In the present study, we demonstrated that SAHA exhibited the anti-tumor effects on chondrosarcoma cell lines. We also examined the biochemical mechanisms of programmed cell death induced by SAHA, and found that this agent could exert two different types of programmed cell death in chondrosarcoma cells. Several types of programmed cell death have been reported; Type I cell death corresponded to apoptotic cell death, and type II cell death to autophagic cell death characterized by the appearance of double- or multiple-membrane cytoplasmic vesicles known as autophagosomes. Unlike apoptosis, caspases are not activated in autophagic cell death, thus the nucleus stay intact until the end of cell life. It has been shown that the changes in the intracellular localization of LC3 protein are indicative of autophagic activity, and that two forms of LC3, LC3-1 and -2, are produced post-translationally in various cells. LC3-1 was cytosolic, whereas LC3-2 was membrane bound. The amount of LC3-2 was correlated with the extent of autophagosome(3). In the present study, we detected the autophagosome specific protein LC3 by western blot analysis in RCS and OUMS-27. The results showed that LC3-2 expression was dose dependently increased by the treatment with SAHA. Therefore we concluded that these massive degradation of essential cellular structures might lead to autophagic cell death. These results suggest that SAHA induces apoptosis and/or autophagic cell death in chondrosarcoma cells and that SAHA is a promising reagent for molecular target therapy for chondrosarcomas.

References: 1. Finzer P et al; Oncogene 2001
2. Clarke PG et al; Embryol 1990

Figure 1: Acetylation status of histone H3 and proteolytic cleavage of poly (ADP-ribose) polymerase by SAHA in chondrosarcoma cells.

Figure 2: Detection of Autophagosome specific protein, LC3 type 2 in RCS and OUMS-27 treated with SAHA.