INTRODUCTION:

Soft tissue sarcomas are rare neoplasms which arise mainly in extremity or retroperitoneal space. Despite progress in multimodality treatment, the prognosis for the patients with these tumors is still poor. In order to improve the prognosis of the patients with soft tissue sarcomas, novel anti-tumor drugs are needed.

Bisphosphonates (BPs) are developed primarily to treat bone diseases such as osteoporosis, which is caused by excessive bone resorption or metastatic bone involvement. The nitrogen-containing BPs blocks the mevalonate pathway by inhibiting the activation of small GTP-binding protein prenylation. Many investigators have reported that BPs have direct inhibitory effects on various malignant cells including multiple myeloma, leukemia, prostate cancer, and so on. However, as far as we know, there is no report on the effect of BPs against primary soft tissue tumors. Our group reported that third-generation BPs, zoledronic acid (ZOL) significantly inhibit the in vitro growth of murine osteosarcoma cell lines in a time- and dose-dependent manner by preventing prenylation of small GTPases and inducing apoptosis (51st ORS), and so, we studied whether BPs can be a novel anti-tumor therapeutic agents to soft tissue sarcomas.

MATERIALS AND METHODS:

We used a third-generation BP, ZOL which were obtained from Novartis Pharmaceuticals. We used human fibrosarcoma cell lines, HT1080.

(1) Effects of ZOL on cell proliferation: The cell proliferation was determined by the trypan blue dye exclusion method.

(2) Cell cycle analysis and induction of apoptosis: Untreated HT1080 cells or HT1080 cells treated with ZOL for 48 hours were analyzed for alterations in the cell cycle by staining with propidium iodide (PI) (Sigma Aldrich) and made histogram.

(3) Western blot analysis: Protein samples were extracted from \( \times 10^6 \) cells and then, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then electroblotted onto a nitrocellulose membrane (Amersham Biosciences, Tokyo, Japan). The membranes were saturated with 5% (wt/vol) nonfat dry milk in TBST [25 mM Tris HCl (pH 7.8), 140 mM NaCl, 0.1% (vol/vol) Tween 20] and then incubated overnight with goat polyclonal anti-Rap1A antibody (diluted 1:1,000) (Santa Cruz Biotechnologies, CA). The membranes were washed thoroughly with TBST and incubated for 1 hour with anti-goat IgG coupled to horseradish peroxidase (Santa Cruz Biotechnologies) for Rap1A. Detection was performed with enhanced chemiluminescence kits (Amersham Biosciences).

RESULTS:

(1) Inhibition of cell growth by ZOL: The growth of HT1080 cells was inhibited by ZOL in a time and dose dependent manner. The IC_{50} values of the HT1080 cells for ZOL at the time of 48 and 72 hours exposure was 1.26 \( \mu \)M and 0.91 \( \mu \)M respectively (Figure 1).

(2) Effects of ZOL on the cell cycle: In the histogram, after exposure for 48 hours, ZOL increased the HT1080 cells in the S phase between the G1/G0 and G2/M phases (Figure 2).

DISCUSSION:

The aim of this study was to identify a novel antitumor therapeutic agents to soft tissue sarcomas. Effective Ras-related signaling requires the attachment of Ras-related proteins to the plasma membrane. This is suggesting that blocking Ras binding to the plasma membrane may be a good anti-tumor therapeutic target.

The nitrogen-containing BPs blocks the mevalonate pathway by inhibiting the activation of small GTP-binding protein prenylation. In our study, the direct anti-tumor effects of ZOL are evaluated to human fibrosarcoma cells like osteosarcoma cells in vitro. ZOL blocked the prenylation of Rap1A proteins in a dose- and time-dependent manner and induced fibrosarcoma cells into cell arrest from S phase to G2/M boundary. Taken together, these findings indicate that ZOL has anti-tumor effects to fibrosarcoma in vitro, which may important implications for future therapy of soft tissue sarcomas.