Combined Effect Of Zoledronic Acid And Telomerase-specific Oncolytic Adenovirus For Human Osteosarcoma Cells.

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Introduction: Osteosarcoma is the most frequent type of primary malignant bone tumor and common between the ages of 10 to 25. Because of a high rate of systemic spread, preoperative and postoperative chemotherapy in combination with aggressive surgery is the standard treatment. Based on preoperative and postoperative chemotherapy, the prognosis of osteosarcoma patients has improved dramatically, but still it is reported that 30% of osteosarcoma patients still die from tumor or metastasis. Therefore, the development of a novel therapeutic strategy is required.

We previously reported that telomerase-specific, replication selective adenovirus (Telomelysin, OBP-301), efficiently killed human bone and soft tissue sarcoma cells but not normal human somatic cells. However, some human osteosarcoma cells were less sensitive to OBP-301 and bone destruction was not inhibited in orthotopic mouse model1.

Zoledronic acid (ZOL), which is a third-generation bisphosphonates, is widely used to prevent bone destruction for bone metastasis in clinical situation. It is also reported that ZOL has direct antitumor effects and synergistically augment the effects of other anticancer agents in osteosarcoma cells2. Therefore, we focused on ZOL and investigated a potential of ZOL to augment OBP-301 treatment.

In this study, we examined the combined effect of ZOL and OBP-301 in human osteosarcoma cell lines.

Methods: Cell lines
We used four human osteosarcoma cell lines (U2OS, SaOS-2, MNNG/HOS, 143B) and two normal human cells, which are osteoclast precursor cell (OCP) and osteoblast cell (NHOst). OCP was pre-treated with RANKL and M-CSF for 7 days to differentiate as manufacture’s protocol.

Recombinant adenoviruses
We previously developed tumor-specific replication-competent oncolytic adenovirus, OBP-301, in which the human telomerase reverse transcriptase (hTERT) gene promoter drives the expression of the E1A and E1B genes linked to an internal ribosome entry site (IRES). OBP-301 cannot replicate themselves in normal cells because of no telomerase activity in normal cell, however, OBP-301 can replicate themselves in tumor cells according to high telomerase activity of tumor cells and induce the cells to oncolysis.

Cell viability assay
We used XTT-assay to examine the antitumor effect of ZOL and OBP-301. The cells were infected by OBP-301 at multiplicity of infections (MOI) of 0, 1, 10, 50, 100, 200 plaque forming units (PFU)/cell and...
treated with ZOL at concentration of 0, 1, 5, 10 µM at the same time 24 hours after cell seeding. Cell viability was examined at day 3 and 5 after treatment. We also examined the combined effect of OBP-301 and ZOL at 5 day after treatment by calculating combination index with using CalcuSyn software (Biosoft).

Apoptosis analysis
To investigate the apoptotic cell death by ZOL and OBP-301, western blot and flow cytometry was performed.
Cells were seeded in a 100-mm dish at a density of 3×105 cells/dish for western blot and in a 6 well plate at a density of 5×104 cells for flow cytometry 24 hours before single or combination treatment of ZOL and OBP-301. 3 days after treatment, whole cell lysates were subjected to western blot analysis to investigate Poly (ADP-ribose) polymerase (PARP), cleaved PARP and β-actin. And also expression of active caspase-3 was analysed with flow cytometry.

Effect of ZOL on virus replication
To investigate the effect of ZOL on OBP-301 replication, the E1A copy number were measured at 2h, 24h, 48h after infection of OBP-301 in OBP-301 single treatment and combination treatment of OBP-301 and ZOL group. E1A copy number was determined using TaqMan real-time PCR systems (Applied Biosystems).

In vivo experiment
Luciferase was transfected to 143B cells and 2.0×106 of 143B luciferase cells were inoculated into left tibia of female athymic nude mice. One week after inoculation, OBP-301 was injected into the tumor and ZOL was injected intraperitoneally every week for three times. Tumor growth was investigated by IVIS imaging system every week and bone destruction was investigated by micro CT at day 28.

Results: XTT-assay showed that treatment with ZOL and OBP-301 decreased the cell viability of osteosarcoma cells in time- and dose-dependent manner respectively. ZOL decreased the cell viability of osteoclast cell, but not osteoblast cell and OBP-301 did not have any influence to normal cells. Moreover, combined treatment of ZOL and OBP-301 showed synergistic antitumor effect to all osteosarcoma cells.
Western blot analysis showed that single treatment of ZOL or OBP-301 showed a little increasing of cleaved PARP, but synergistic increasing of cleaved PARP was observed in combination treatment. And synergistic increasing of active caspase-3 expression was observed in combination treatment compared to single treatment.
There was no difference of E1A copy number between single treatment of OBP-301 and combination treatment of OBP-301 and ZOL.
In vivo experiment, tumor growth was inhibited by OBP-301 and ZOL, and significant inhibition was observed in combination treatment group. Bone destruction was observed in control and OBP-301 treatment group, but inhibited in ZOL and combination treatment group.

Discussion: We revealed that combination treatment of OBP-301 and ZOL had a synergistic antitumor effect to osteosarcoma cells and it is considered that the mechanism of synergistic antitumor effect is owing to apoptosis increase. And it is also proved that ZOL did not affect to OBP-301 replication. There is a close relation between osteosarcoma and bone destruction, tumor cells activates the osteoclast cells and induces bone destruction. In this study, OBP-301 could inhibit tumor growth but
could not inhibit associate bone destruction. But in combination with ZOL, tumor growth was inhibited synergistically and bone destruction had also inhibited. Combination treatment of OBP-301 and ZOL would be a good new strategy to osteosarcoma. **Significance:** A telomerase-specific oncolytic adenovirus and zoledronic acid combined therapy will be a novel treatment on osteosarcomas.