

# The Therapeutic Effect of Tumor-specific Oncolytic Adenovirus in Combination with Systemic Chemotherapy for Osteosarcoma

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**Background:** Osteosarcoma is the most common malignant primary bone tumor. One of the important clinical problems is that the patient prognosis has plateaued over the several decades, which are mostly attributed to the drug resistance and distant metastasis. Therefore, a novel treatment modality must be developed to overcome the current problems for osteosarcoma patients. We recently developed a telomerase-specific replication-competent oncolytic adenovirus, Telomelysin (OBP-301), in which the promoter element of the hTERT gene drives the expression of E1A and E1B genes. A Phase I clinical trial in the U.S. showed the safety of OBP-301 in advanced cancer patients, and a Phase I/II clinical trial for malignant tumors is currently undergoing. In this study, we evaluated the antitumor effect of OBP-301 on human osteosarcoma cells. In addition, we investigated the antitumor effect of OBP-301 in combination with chemotherapy against human osteosarcoma cells, anticipating for suppression of drug resistance for wide application of the limb-sparing surgery.

**Materials and Methods:** Osteosarcoma cells (OST, HOS, MNNG/HOS, SaOS-2 and U2OS) were seeded on 96-well plates at a density of  $1 \times 10^3$  cells/well and were infected with OBP-301 at the indicated MOIs. We also analyzed the antitumor effect of OBP-301 in combination with doxorubicin (DOX) and cisplatin (CDDP). Cell viability was determined using XTT assay. The combination index was calculated with the CalcuSyn software (BioSoft). In animal experiment, OST cells ( $5 \times 10^6$  cells per site) were inoculated into the tibia or the flank of female athymic nude mice. Palpable tumors developed within 14 to 21 days and were permitted to grow to approximately 5 to 6 mm in diameter. Then, a solution containing OBP-301, dl312, E1A-deleted adenovirus vector, or PBS was injected into the tumors. Tumor size was monitored by measuring tumor length and width by using calipers. We further investigated the *in vivo* combined effect of OBP-301 and chemotherapy. MNNG/HOS-luc cells ( $5 \times 10^6$  cells) were inoculated into the flanks of 5-week-old female nude mice. Three weeks after inoculation, OBP-301 ( $5 \times 10^7$  PFU/tumor) or control PBS was intratumorally injected once per week for 3 cycles. CDDP and DOX (4 and 2 mg/kg, respectively) or

control PBS was injected intraperitoneally 2 days after OBP-301 injection for 3 cycles. The luminescent intensity was measured by the IVIS imaging system.

**Results:** OBP-301 infection induced cell death in a time dependent manner in all osteosarcoma cell lines. Moreover, combination treatment with OBP-301 and chemotherapy showed synergistic or additive antitumor effect in all osteosarcoma cells. Western blot analysis showed that combination treatment increased the expression of cleaved-PARP. In animal experiment, we first identified a dose of OBP-301 that was suitable for induction of an antitumor effect in the subcutaneous OST bone sarcoma xenograft model. Then, OBP-301 and dl312 was injected into the tumor once a day for 3 days, with  $10^8$  PFUs per day. Tumor growth was significantly suppressed by OBP-301 injection compared with injection of dl312 or PBS. X-ray examination revealed that OBP-301-treated tumors resulted in less bone destruction than dl312- or PBS-treated tumors. Furthermore, the combination of chemotherapy with OBP-301 significantly suppressed tumor growth compared to monotherapy in MNNG/HOS xenograft tumor model, since the total flux of the combination-treated group was significantly smaller than that of OBP-301 or chemotherapeutic agents alone.

**Conclusions:** OBP-301 is a promising antitumor reagent for the treatment of osteosarcoma. Moreover, it synergistically enhanced the sensitivities to chemotherapeutic agents in osteosarcoma cells *in vitro* and *in vivo*. These results suggest that the combination therapy with chemotherapy and a telomerase-specific oncolytic adenovirus would provide a novel therapeutic strategy for osteosarcomas, anticipating for wide application of the limb-sparing surgery.