Telomerase-Specific Replication-Selective Virotherapy for Bone and Soft Tissue Sarcomas

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INTRODUCTION:
Bone and soft tissue sarcomas represent a small percentage of cancers worldwide; however, they are the third most children cancer in United States. Despite major advances in the treatment of advanced bone and soft tissue sarcomas such as the introduction of novel chemotherapy regimens, therapy fails in about one fourth of the patients. Therefore, to treat the patients with advanced primary bone and soft tissue sarcomas, a multidisciplinary approach is required.

Tumor-specific replication-selective oncolytic virotherapy is a promising antitumor therapy for induction of cell death in tumor, but not of normal cells. We previously developed an oncolytic adenovirus, OBP-301, that kills human epithelial malignant cells in a telomerase-dependent manner. Furthermore, a Phase I clinical trial of OBP-301, which was conducted in the United States on patients with advanced solid tumors, indicated that OBP-301 is well tolerated by patients. Recent evidence suggests that non-epithelial malignant cells, which have low telomerase activity, maintain telomere length through alternative lengthening of telomeres (ALT). However, it remains unclear if OBP-301 is cytopathic for non-epithelial malignant cells. However, some populations of tumor cells lack CAR expression, suggesting a requirement for the development of a novel antitumor therapy against CAR-negative tumor cells. We recently developed fiber-modified OBP-405, which can bind to integrin molecules (αvβ3 and αvβ5) and efficiently kill cossack adenovirus receptor (CAR)-negative tumor cells.

In the present study, we first investigated the in vitro cytopathic efficacy of OBP-301 against 14 human bone and soft tissue sarcoma cells. Next, the relationship between the cytopathic activity of OBP-301, CAR expression, and telomerase activity in human sarcoma cells was assessed. The in vivo antitumor effect of OBP-301 was also confirmed using orthotopic animal models. Finally, the anti-tumor effect of OBP-405 against OBP-301-resistant sarcoma cells was evaluated in vitro and in vivo.

METHODS:
We used XTT assay to examine the antitumor effect of OBP-301 on human osteosarcoma cell lines (OST, U2OS, HOS, HuO9). MNN/HOS, SaOS-2, NOS-2, NOS-10), human chondrosarcoma cell line UOMS-27, human dedifferentiated chondrosarcoma cell line NDCS-1, human clear cell sarcoma cell line CCS, human synovial sarcoma cell line SYO-1, human malignant fibrous histiocytoma cell line NMFH and human malignant peripheral nerve sheath tumor cell line NMS-2. All cell lines were infected with OBP-301. Cell viability was determined at 1, 2, 3, and 5 days after virus infection.

Flow cytometry was used to analyze the expression of adenovirus primary receptor, the CAR. Real-time PCR was used to analyze the expression of hTERT mRNA. CAR-positive and hTERT mRNA-expressing human sarcoma cell lines were infected with OBP-301 at an MOI of 100 PPU/cell. Total RNA was extracted from the cells 2 days after virus infection. After synthesis of cDNA from total RNA, the levels of hTERT and GAPDH mRNA expression were determined using quantitative real-time PCR.

OBP-301 antitumor effects were assessed using orthotopic tumor xenograft models. OBP-405 was used to confirm an antitumor effect on OBP-301-resistant sarcomas.

RESULTS:
OBP-301 infection induced cell death in dose-dependent and time-dependent manner in all sarcoma cell lines except for the UOMS-27 and NMFH-1 cell lines. MNN/HOS and SaOS-2 cells were relatively less sensitive than the other 10 sarcoma cell lines.

The 12 OBP-301-sensitive sarcoma cell lines expressed CAR, whereas the OBP-301-resistant UOMS-27 and NMFH-1 cells did not express CAR. All cell lines expressed detectable levels of hTERT mRNA except SaOS2 human osteosarcoma.

We next investigated the relationship between CAR and hTERT mRNA expressions and the cytopathic activity of OBP-301 among the 11 CAR-positive sarcoma cell lines with hTERT gene expression. CAR expression levels significantly correlated with the cytopathic activity of OBP-301 against 8 of the bone sarcoma cell lines. CAR expression in three of the soft tissue sarcoma cell lines also correlated with the cytopathic effect of OBP-301. In contrast, there was no significant correlation between hTERT mRNA expression and the cytopathic activity of OBP-301. Sensitivity to OBP-301 was dependent on CAR expression, not on telomerase activity.

To determine if OBP-301 activates hTERT mRNA expression in both ALT-type and non-ALT-type human sarcoma cell lines, we infected 11 CAR-positive human sarcoma cells with OBP-301. Ten out of the 11 CAR-positive human sarcoma cell lines showed an increase in the expression level of hTERT mRNA after OBP-301 infection that ranged from 1.1 to 50.0 fold increase. These results suggest that OBP-301 is cytopathic for both ALT-type and non-ALT-type human sarcoma cells through activation of the hTERT gene promoter.

Intratumoral injection of OBP-301 significantly suppressed the growth of Oste and SYO-1 tumors. Furthermore, fiber-modified OBP-405 showed antitumor effects on OBP-301-resistant OUMS-27 and NMFH-1 cells.

DISCUSSION:
The cytopathic activity of OBP-301 significantly correlated with CAR expression, but not with telomerase activity, of human sarcoma cells. These results suggest that the cytopathic activity of OBP-301 depends primarily on infection efficiency rather than virus replication. Primary epithelial and non-epithelial malignant tumors frequently express CAR.

ALT-type sarcoma cells that express a low level of hTERT mRNA showed a sensitivity to OBP-301 that was similar to that of non-ALT-type sarcoma cells. We further demonstrated that OBP-301 infection upregulates hTERT gene expression and subsequently activates virus replication and cytopathic activity in ALT-type sarcoma cells. These results suggest that the hTERT gene promoter is a useful tool for enhancement of the oncolytic adenoviruses not only because it induces tumor-specific virus replication, but also because it enhances virus replication after infection. These results suggest that, if hTERT gene expression cannot be detected in tumor cells, then ALT-type sarcoma cells should be treated with high doses of OBP-301, or with fiber-modified OBP-405, to enhance OBP-301 infection efficiency (Fig.1).

In conclusion, we have clearly shown that OBP-301 has strong in vitro and in vivo antitumor effects against human bone and soft tissue sarcoma cells. Telomerase-specific replication-selective oncolytic virotherapy would provide a new platform for the treatment of patients with bone and soft tissue sarcomas.