Oncolytic adenovirus-mediated p53 gene transfer induces antitumor effect in human bone and soft tissue sarcoma cells

INTRODUCTION:
Bone and soft tissue sarcomas are annually diagnosed as the third most common cancer in children in the United States. Despite major advances in the treatment of bone and soft tissue sarcomas, such as neoadjuvant and adjuvant multigent chemotherapy and aggressive surgery, about one fourth of the patients show a poor response to conventional therapy, resulting in subsequent recurrence and leading to a poor prognosis. Therefore, the development of a novel therapeutic strategy is required to cure patients with bone and soft tissue sarcomas.

Replication-competent oncolytic adenovirus is emerging as a promising novel anticancer reagent. We previously developed an oncolytic adenovirus, OBP-301, in which human telomerase reverse transcriptase gene promoter drives viral E1 gene for replication. OBP-301 induces tumor-specific oncolytic cell death in a telomerase-dependent manner (1). Recently, we reported that OBP-301 shows cytopathic activity in human bone and soft tissue sarcoma cells (2). However, some human sarcoma cells were less sensitive to cytopathic activity of OBP-301. To enhance the cytopathic activity of OBP-301, we further developed a novel telomerase-specific oncolytic adenovirus, OBP-702, which expresses tumor suppressor p53 gene. In this study, we investigated the antitumor effect of OBP-702 in OBP-301-sensitive and OBP-301-resistant human bone and soft tissue sarcoma cells.

METHODS:

Cell lines
The human osteosarcoma cells (U2OS, OST, HOS, SaOS-2, MNNG/HOS) and human synovial sarcoma cell (SYO-1) were used.

Recombinant adenoviruses

The recombinant telomerase-specific replication-competent adenovirus OBP-301 was previously constructed (1). For OBP-301 induction of exogenous p53 gene expression, a human wild-type p53 gene expression cassette was inserted into the E3 region of OBP-301. Ad-p53 is a replication-defective adenovirus serotype 5 vector with a p53 gene expression cassette at the E1 region.

Cell viability assay

We used XTT [sodium 3-[1-(phenylaminocarbonyl)-3, 4-tetrazolium]-bis(4-methoxy-6-nitro)benzene sulfonic acid hydrate] assay to examine the antitumor effects of OBP-702 and OBP-301. The 50% inhibiting dose (ID50) value of OBP-301 and OBP-702 for each cell was calculated using cell viability data obtained on day 5 after virus infection.

Western blot analysis

Cells were seeded in a 100-mm dish at a density of 1 × 10^6 cells/dish 24 hours before infection and were infected with OBP-702 or Ad-p53 at the indicated multiplicity of infection (MOI) for 3 days. Whole cell lysates were subjected to western blot analysis for p53, p21 and β-actin.

RESULTS:
To compare the in vitro antitumor activity of OBP-702 and OBP-301, we used the four OBP-301-sensitive sarcoma cells (U2OS, OST, HOS, SYO-1) and the two OBP-301-resistant human sarcoma cells (SaOs-2, MNNG/HOS) that were recently reported (2). The cell viability of each cell was assessed over 5 days after infection using the XTT assay. OBP-702 suppressed the viability of OBP-301-sensitive and OBP-301-resistant cells more efficiently than OBP-301. Calculation of the ID50 values indicated that all cell lines were more sensitive to OBP-702 than to OBP-301 (Fig. 1). These results indicate that OBP-702 is more cytopathic for human sarcoma cells than OBP-301.

We next investigated the expression level of p53 and p53-downstream target p21 proteins in OBP-301-resistant SaOs-2 cells infected with OBP-702 or Ad-p53. OBP-702 infection induced higher p53 expression than that induced by Ad-p53 at the indicated MOIs. However, p21 upregulation was observed in SaOs-2 cells infected with Ad-p53, but not OBP-702. These results suggest that OBP-702 induces profound p53 upregulation without p21 activation.

DISCUSSION:
We previously reported that OBP-301 has a strong antitumor activity on a variety of human epithelial and non-epithelial malignant cells (1-3). However, some human sarcoma cells were resistant to OBP-301 (2). In this study, a novel oncolytic adenovirus OBP-702 shows more strong antitumor effect than OBP-301 (Fig. 1) through induction of profound p53 expression (Fig. 2). Although OBP-301 itself shows the cytopathic activity, p53 overexpression would enhance the OBP-301-mediated antitumor effect.

OBP-702 infection induced more profound p53 expression than Ad-p53. However, p53-downstream target p21 proteins were not activated by OBP-702 (Fig. 2). Overexpression of p53 is well known to induce cell cycle arrest and apoptosis. Especially, p53-downstream target p21 protein has been shown to induce cell cycle arrest, but suppress apoptosis (4). These results suggest that OBP-702 induces more profound apoptosis than Ad-p53 thorough p53 upregulation without p21 activation.

In conclusion, we have clearly demonstrated that the p53-expressing oncolytic adenovirus OBP-702 has a much stronger antitumor effect than OBP-301. Oncolytic adenovirus-mediated p53 gene transduction would induce profound p53 expression without p21 activation, resulting in the enhancement of antitumor effect.

REFERENCES:

SIGNIFICANCE:
A telomerase-specific oncolytic adenovirus is a promising antitumor reagent for the treatment of bone and soft tissue sarcomas.