Enhanced oncolysis by a tropism-modified telomerase-specific replication selective adeno viral agent OBP-702 for osteosarcomas

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INTRODUCTION

The current combination treatment, chemotherapy and surgery, has significantly improved the cure rate and the survival rate of primary bone osteosarcoma. The 5-year survival rate has increased in the last 30 years from 10% to 70%. Even in patients with poor prognosis, such as those with metastases at diagnosis, the 5-year survival rate has reached 20–30% due to chemotherapy and the surgical removal of metastases and primary tumor. However, the most effective drugs for neoadjuvant or adjuvant chemotherapy are still the same for 20 years: Doxorubicin, Cisplatin, Methotrexate, Ifosfamide. The treatment outcome of osteosarcoma was little growth in recent years. Therefore, the development of a novel strategy is required.

Replication-competent oncolytic viruses are being developed for human cancer therapy. In our study, Telomelysin (OBP-301) was effective for bone and soft tissue sarcomas. OBP-301 has hTERT promoter that regulates viral replication and efficiently kills human cancer cells. However, some cell lines were not showed antitumor effect well. Human p53 protein works as a tumor suppressor, and we developed a novel telomerase-specific p53-armed oncolytic virus (OBP-702) to grow in efficiency against tolerant cell lines.

The objective of this study was to examine the in vitro antitumor effect of OBP-702 against osteosarcoma cell lines.

METHODS

We used 5 osteosarcoma cell lines (OST, HOS, U2OS, SaOS2, MNNG/HOS). ADVEXIN is a replication-defective adenovirus serotype 5 (Ad5) vector with a p53 cDNA expression cassette that replaces the E1 region of the virus. OBP-702 was telomerase-specific replication-competent adenovirus. It expresses the wild-type p53 by inserting the human p53 cDNA under the control of Egr-1 promoter at the deleted E3 region of OBP-301.

We used XTT [sodium 3’-[1-(phenylaminocarbonyl)-3, 4-tetrazolium]-bis(4-methoxy-6-nitro)benzene sulfonic acid hydrate] assay to examine the antitumor effect of OBP-702. Cells were seeded 24 hours before viral infection. All cell lines were infected with OBP-702 at multiplicity of infections (MOI) of 0, 0.1, 1, 10, 50, 100 plaque forming units (PFU)/cell. OBP-301 resistance cell lines, SaOS2 and MNNG/HOS were infected with OBP-301, OBP-702, or ADVEXIN at multiplicity of infections (MOI) of 0, 0.1, 1, 10, 50, 100 plaque forming units (PFU)/cell. Cell viability was determined at 1, 2, 3, and 5 days after virus infection using Cell Proliferation Kit II according to the protocol provided by the manufacturer. Using the cell viability data at 5 days after virus infection, we determined the ID50 value of each cell line. And we checked p53 expression of cell lines with Western blotting.

RESULTS

OBP-702 expressed great effect of cellular disorder for all sarcoma cell lines. According to calculated ID50 values, all sarcoma cell lines were more sensitive to OBP-702 than OBP-301. OBP-702 began to kill all sarcomas at day 2, and ID50 value was below the level of 8 MOI (Fig. 1). These results suggest that it had better effect than OBP-301 and ADVEXIN against SaOS2 and MNNG/HOS. In SaOS2, ID50 value was OBP-702: 5.5, OBP-301: 56.2, ADVEXIN: 104.7. In MNNG/HOS, ID50 value was OBP-702: 6.7, OBP-301: 25.8, ADVEXIN: 85.7. OBP-702 was effective even at low-dose, and was showed antitumor effect earlier (Fig. 2). In Western blotting, we could detect p53 protein in all cell lines except SaOS2.

DISCUSSION

OBP-301 has been reported to show a strong antitumor activity on a variety of human cancers. OBP-301 is sufficient to treat CAR-positive human bone and soft tissue sarcomas, but it could not kill CAR-negative cell lines at all. On the other hand, activity of OBP-301 is closely correlated with human telomerase reverse transcriptase (hTERT) expression. It means OBP-301 could hardly show efficacy for cells with low hTERT expression. OBP-301 was not effective against SaOS2 and MNNG/HOS. One reason is that SaOS2 has low hTERT, and MNNG/HOS has low CAR expression. Therefore, we made theses 2 cell lines infected by OBP-301, OBP-702 or ADVEXIN, and compared the result. OBP-702 expressed better effect of cellular disorder than OBP-301 and ADVEXIN. The difference of result between OBP-301 and OBP-702 was attributed to p53. We could not detect p53 protein in SaOS2, and detect p53 mutation protein in MNNG/HOS. Therefore, even if fewer virus of OBP-702, it can express good result against low hTERT or low CAR expression cell. In our study, OBP-702 infection resulted in a strong p53 protein expression within 24 hours after infection in various human cancer cell lines. Our data indicates that OBP-702 has possibilities to be more effective for osteosarcomas than OBP-301 in vivo.

In conclusion, this preclinical study clearly shows that OBP-702 has remarkable in vitro antitumor effects against human osteosarcoma cell lines. It could kill osteosarcoma cell lines earlier with low-dose than OBP-301. These findings suggest that OBP-702 provides a new platform for treating patients with low hTERT and low CAR expression human osteosarcomas.