ORIGINAL ARTICLE

Intraperitoneal administration of telomerase-specific oncolytic adenovirus sensitizes ovarian cancer cells to cisplatin and affects survival in a xenograft model with peritoneal dissemination

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Despite tremendous development in chemotherapy for ovarian cancer over the past few decades, the prognosis of advanced cases with massive peritoneal dissemination is still unsatisfactory, and novel treatment modalities that can combine with chemotherapy are urgently needed. We recently developed virotherapy for solid tumors using telomerase-specific replication-selective adenoviruses (Telomelysin: OBP-301), in which the human telomerase reverse transcriptase (hTERT) gene promoter has been inserted to direct tumor-specific E1 gene expression. In this study, we investigated the anti-tumor effects of OBP-301, combined with cisplatin (CDDP), on ovarian cancer cells. In vitro treatment of SKOV3 cells with OBP-301 at a multiplicity of infection (MOI) of 0.01–100 induced significant cell death in a dose-dependent manner, with moderate cytotoxicity at an MOI of 1–10 and maximal cytotoxicity at an MOI of 100. In contrast, OBP-301 treatment of normal human cells showed no significant cell death at an MOI of 1–10 and exhibited modest cytotoxicity at an MOI of 100. The effects of low-dose CDDP at 0.5–1 μM, which induced only 20% cell death, were significantly augmented by combination with OBP-301 at an MOI of 1–10, finally achieving 40% cell death. Such enhancement of CDDP sensitivity was also observed in CDDP-resistant ovarian cancer cells. The combinatorial effects were further tested using a xenograft mouse model of SKOV3 with peritoneal dissemination. After intraperitoneal administration of OBP-301, we confirmed that injected OBP-301 fused with the green fluorescent protein (GFP) gene (OBP-401) was preferentially localized to peritoneal disseminations, as determined by fluorescence imaging. Treatment of mice with CDDP at low dose (0.5 mg kg^{-1}) had modest effects, showing a 10% decrease in disseminations, whereas combination with intraperitoneal administration of OBP-301 at an MOI of 10 led to enhanced effects, achieving an approximately 80% decrease in disseminations. Kaplan–Meier analysis showed improved overall survival of mice treated with CDDP plus OBP-301 compared with CDDP alone. These findings support the therapeutic potential of intraperitoneal administration of OBP-301 to sensitize ovarian cancer cells to CDDP.
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Introduction

Surgical tumor reduction and subsequent platinum-based chemotherapy has been considered as the standard therapy for advanced ovarian cancer over the past few decades.[1] Despite advances in the development of chemotherapeutic agents, the prognosis of advanced ovarian cancer with massive peritoneal disseminations is still unsatisfactory.[2–6] Hence, there is a great deal of interest in developing novel therapeutic agents, hopefully as chemosensitizers.

As such candidates, a variety of gene therapies have been tested by many laboratories researching on ovarian cancer. These studies have used specific transgenes, most of which induce apoptosis or cell cycle arrest, introduced into tumors through plasmid or viral vector systems using tumor-specific promoters.[7–9] Despite these efforts, the levels of transgene expression have been insufficient to eradicate tumors, mainly because of the unfavorable characteristics of adenoviral vectors, in which the E1 gene

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is deleted to inhibit the replicative capacity of the vector. These non-replicative vectors have limited distribution within the tumor mass after injection and are therefore not suitable for advanced ovarian cancers, as this tumor type frequently has multiple disseminated lesions throughout the whole peritoneal cavity. To increase viral spread to neighboring tumor cells or even distant lesions, the use of replication-competent adenoviruses has become a reality.

Many efforts have been made to realize cancer-specific adenoviral replication using a variety of gene promoters, including the *prostate-specific antigen, MUC1, osteocalcin, L-plastin, midkine* and *E2F-1* genes.[10–15] Unfortunately, these promoters have tissue-type specificity and exhibit transcriptional activity only in cells that express such tumor markers. Furthermore, the transcriptional activity is relatively low. We were prompted by these studies to use the human telomerase reverse transcriptase (*hTERT*) promoter and established an oncolytic adenovirus vector (Telomelysin, OBP-301), which contains the *hTERT* gene promoter upstream of the *E1* gene in adenovirus type 5 genome.[16] As hTERT expression is highly specific to cancer cells[17–19] and the *hTERT* promoter has stringent cancer specificity,[20] OBP-301 can express *E1* genes preferentially in cancer cells and thereby replicate there with much higher efficiency than in normal cells.[16]

This virus system does not require any specific transgenes to deliver, because vigorous viral replication itself induces cell death as a result of viral toxicity. In previous studies, *in vivo* direct injection of OBP-301 into primary tumor sites led to efficient eradication of the tumor without significant adverse effects in various organs.[16,21,22] Considering the superior infectivity of OBP-301 to not only primary tumors but also to the surrounding and even distant tumors, we sought to apply this virus to the treatment of ovarian cancers with multiple disseminations. As a major therapeutic protocol for ovarian cancer includes platinum-based chemotherapy, we have a special interest in whether OBP-301 has potential for additive or synergistic effects with cisplatin (CDDP). Here, using an *in vitro* and *in vivo* mouse model with peritoneal dissemination, we show the therapeutic efficacy of intraperitoneal administration of OBP-301 combined with cisplatin on ovarian cancer.

Materials and methods

Cell culture
The human ovarian cancer cell line, SKOV3, and normal primary human fibroblasts (Takara, Tokyo, Japan) were cultured at 37 °C under 5% CO_2 in Dulbecco's modified Eagle's medium, supplemented with 10% heat-inactivated fetal calf serum (Sigma-Aldrich, St Louis, MO), $100 \, \mu g \, ml^{-1}$ streptomycin and $100 \, IU \, ml^{-1}$ penicillin. KF28 cells were derived from serous cystadenocarcinoma of the ovary, from which CDDP-resistant KFr13 cells were established.[23] Both cells were cultured in the above conditions.

Reagents and viruses
Cisplatin was provided by Bristol Pharmaceuticals KK (Tokyo, Japan). OBP-301 (Telomelysin) is a telomerase-specific replication-competent adenovirus, in which the *hTERT* gene promoter has been inserted upstream of the *E1* gene in adenovirus type 5 genome.[16] A replication-deficient variant of adenovirus type 5 (dl312) was used as the control for oncolytic activity.[16] To visualize viruses infected *in vitro* and *in vivo*, the green fluorescent protein (*GFP*) gene was inserted into the OBP-301 genome (OBP-401) under control of the cytomegalovirus (CMV) promoter, so that OBP-401 expresses GFP in infected cells and this expression can be detected by fluorescence imaging.[24,25] The construction and features of these viruses have been described in detail in our previous studies.[16,24,26,27] The viruses were purified by $CsCl_2$ linear gradient ultracentrifugation. The viral titers were determined by a plaque-forming assay using 293 cells.

Cell viability assay
The cytotoxicity of the viruses and/or CDDP was evaluated using WST-1 reagent (Roche Diagnostics, Tokyo, Japan). Briefly, 1×10^3 cells were seeded in 96-well plates 24 h before treatment. Cells were treated with virus alone at the indicated multiplicity of infections (MOIs) and concentrations, respectively, for 24, 48, 72 and 96 h. In combination assay, cells were infected with virus at various MOIs and exposed to CDDP at various concentrations for 48 h. Then, a total of $10 \, \mu l$ of WST-1 reagent was added to each well, and the cells were further incubated for 2 h at 37 °C. Absorbance was measured using a microscope reader at test and reference wavelengths of 450 and 655 nm, respectively, to evaluate the relative viability of the cells treated.

Animal experiments
Female 7–9-week-old BALB/c nu/nu mice (SLC, Hamamatsu, Japan) were housed under specific pathogen-free conditions. Experiments proceeded according to the institutional guidelines. To confirm that we could produce a mouse model with peritoneal dissemination, SKOV3 cells (1×10^7 cells) were injected into the peritoneal space of the mice in 500 μl phosphate-buffered saline (PBS) and successful formation of disseminated cancer foci in the peritoneal cavity was apparent ~ 2–3 weeks after injection. We then decided to test four groups of mice as follows: OBP-301 alone, CDDP alone, CDDP + OBP-301 and PBS only (the mock group). Disease-free BALB/c nu/nu mice were inoculated with 1×10 SKOV3 cells (day 0) and then $0.5 \, mg \, kg^{-1}$ CDDP in a total volume of 1 ml was injected into the peritoneal space once per day on days 1, 3 and 5. Then 10^8 pfu of OBP-301 was injected into the peritoneal space once per day on days 7, 9 and 11, followed by weekly injections of 10^8 pfu of OBP-301 until the killing. In the OBP-301 alone and CDDP alone groups, PBS was injected instead of CDDP or OBP-301, respectively. In the mock group only PBS was injected. Six mice in each group were treated according to this protocol and were killed 50 days after inoculation of SKOV3 cells to observe the peritoneal

lesions. The effects of the treatment were evaluated by counting the number of peritoneal disseminations and evaluating the overall survival (OS).

In vitro *and* In vivo *fluorescence imaging*

SKOV3 and normal human fibroblasts were infected with OBP-401 at an MOI of 10 and GFP expression was assessed and photographed ($\times 200$) by an Eclipse TS-100 fluorescent microscope (Nikon, Tokyo, Japan) 24 h after infection. *In vivo* GFP fluorescence imaging was acquired by illuminating the animal with a Xenon 150-W lamp after an intraperitoneal injection of 10^8 pfu of OBP-401. The re-emitted fluorescence was collected through a long pass filter on a Hamamatsu C5810 three-chip color cooled charged-coupled device (CCD) camera (Hamamatsu Photonics Systems, Hamamatsu, Japan). High-resolution image acquisition was accomplished using an EPSON (Tokyo, Japan) personal computer. Images were processed for contrast and brightness with the use of Adobe Photoshop 4.0.1J software.

Statistical analysis

To assess the statistical significance of differences of the number of disseminated lesions, a standard two-tailed Student's *t*-test was performed using Microsoft Excel 2004. Life tables were computed using the Kaplan–Meier method, and the Log-rank test was used to assess statistical significance. A *P*-value of <0.05 was considered to indicate statistical significance.

Results

In vitro *effect of OBP-301 on human ovarian cancer cell line and normal cells*

To examine the *in vitro* effect of OBP-301 on an ovarian cancer cell line, we infected the human ovarian cancer cell line SKOV3 and normal human fibroblast with OBP-301 and a replication-deficient variant (dl312) at various MOIs. Cell viability was assessed by the WST-1 assay 4 days after infection. Approximately 30–70% of SKOV3 cells were killed by OBP-301 at MOIs of 10–100, respectively (Figure 1a), whereas dl312 failed to kill any cells even at an MOI of 100 (Figure 1b), showing that the replicative capacity of the virus is crucial for the cytotoxic effect on ovarian cancer cells. In contrast, no cytotoxic effects were observed in normal fibroblasts 4 days after infection of OBP-301, even at an MOI of 100 (Figure 1c), clearly indicating the cancer-specific cytotoxicity of OBP-301. Next, we examined the combinatorial effect of OBP-301 with CDDP, which is a key drug for advanced ovarian cancer. OBP-301 was infected into SKOV3 cells at various concentrations, with or without 0–4 μM CDDP. Cell variability was measured 72 h after infection. In the absence of CDDP, ~40% of SKOV3 cells were killed by OBP-301 at an MOI of 100, whereas 20% were killed at an MOI of 1–10 (Figure 2). The treatment of SKOV3 with 0–4 μM CDDP alone (mock) induced about 20% cell death at 0.5–1 μM and 40% death at 2 μM. When CDDP at 0.5–1 μM was combined with OBP-301 (1–100 MOI),
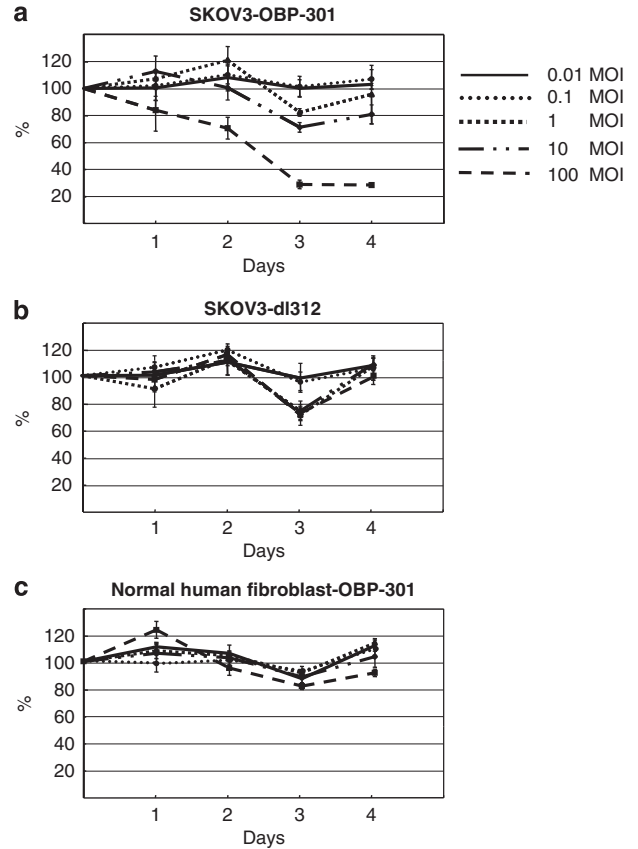

Figure 1 *In vitro* cytotoxic effects of OBP-301 on cancer and normal cells. Human ovarian cancer cell line, SKOV3 cells (**a**), and normal human fibroblasts (**c**) were infected with OBP-301 at various multiplicity of infections (MOIs), and the cell viability was assessed daily by WST-1 assay until 4 days after infection. SKOV3 cells were also infected with replication-deficient adenoviral vectors (dl312) lacking the *E1A* gene (**b**) to test the specific effects of OBP-301. Bars indicate s.e.
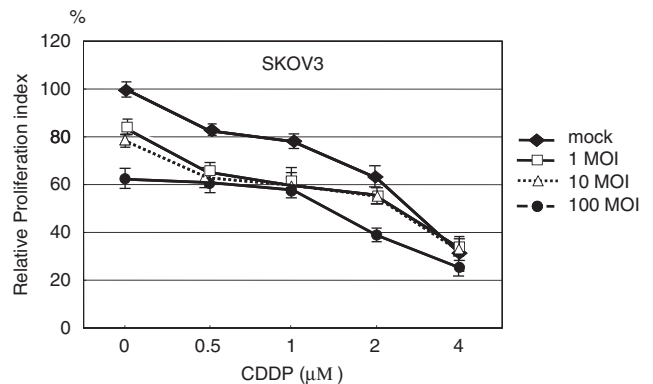

Figure 2 Combinatorial effects of OBP-301 and cisplatin on SKOV3 cells. SKOV3 cells were infected with OBP-301 at various multiplicity of infections (MOIs) and exposed to cisplatin at the indicated concentrations for 72 h. Then, the cell viability was measured by WST-1 assay. Bars indicate s.e.

the ratio of killed cells increased by 20%, resulting in a total of 40% cell death. When CDDP at 2 μM was combined with OBP-301 (100 MOI), the ratio of killed

cells increased again by another 20%, resulting in 60% cell death. The treatment with CDDP alone at 4 μM resulted in ~70% cell death, and the additive effects of OBP-301 were limited, with <10% increase in cell death even at 100 MOI. These findings suggest that OBP-301 has potent cytotoxic activity against ovarian cancer cells *in vitro* at an MOI of 1–100, and significantly enhances the effects of low-dose CDDP, which otherwise showed lesser cytotoxicity.

We further examined whether OBP-301 has cytotoxic activity or sensitizing effect on CDDP-resistant ovarian cancer cells as well. For this purpose, we used CDDP-resistant ovarian cancer cell line, KFr13 cells, established from KF28 cells derived from human serous cystadeno-carcinoma of the ovary.[23] The IC50 of CDDP was compared among KF28, KFr13 and SKOV3 cells and is shown in Figure 3a. KFr13 cells exhibited much higher IC50 than KF28 or SKOV3 cells, as expected. We then treated KFr13 cells with CDDP at various concentrations in the presence or absence of OBP-301 and examined the combinatorial effect. As shown in Figure 3b, the treatment of CDDP alone at 1–4 μM induced no significant death of this cell line. When CDDP at 1–4 μM was combined with OBP-301 at 1 MOI, the ratio of killed cells increased up to 10–40%, according to the concentration of CDDP. The treatment with CDDP alone at 8 μM resulted in ~70% cell death, and the ratio of killed cells increased by 20% by the combination.
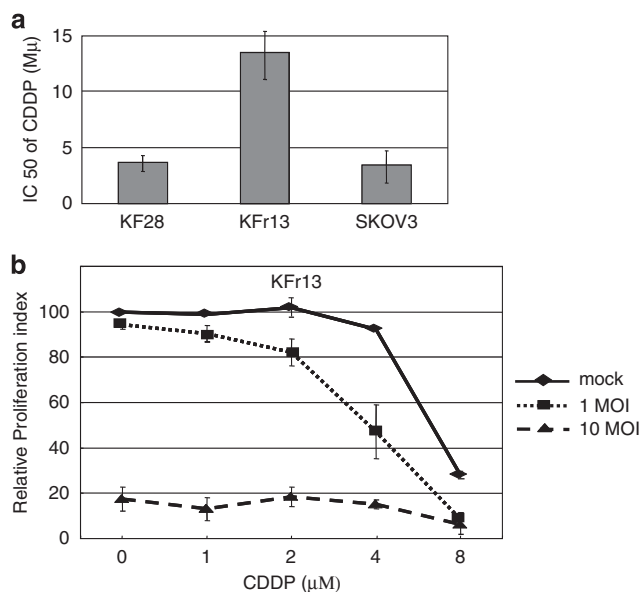

Figure 3 Combinatorial effects of OBP-301 and cisplatin on CDDP-resistant KFr13 cells. (**a**) CDDP-sensitive KF28 and SKOV3 cells and CDDP-resistant KFr13 cells were treated with CDDP at various concentrations for 5 days. Then, the cell viability was measured by WST-1 assay, and IC50 of CDDP was calculated in each cell line. Bars indicate s.e. (**b**) CDDP-resistant KFr13 cells were infected with OBP-301 at various multiplicity of infections (MOIs) and exposed to cisplatin at the indicated concentrations for 72 h. Then, the cell viability was measured by WST-1 assay. Bars indicate s.e.

Surprisingly, this cell line was very sensitive to OBP-301 because the treatment with OBP-301 alone at 10 MOI resulted in >80% cell death, irrespectively of the CDDP treatment. We also tested the effects of OBP-301 on another CDDP-resistant ovarian cancer cell, CaOV3.[28] OBP-301 had similar cytotoxic effect on this cell line (data not shown). These findings indicate that OBP-301 has cytotoxic and sensitizing effect on CDDP-resistant ovarian cancer cells.

Visualization of infected viruses using the GFP gene in vitro *and* in vivo

To confirm the specific effects of OBP-301 on ovarian cancer cells, we decided to confirm the localization of infected viruses, hopefully by visualizing the viruses. Thus, the *GFP* gene driven by the *CMV* promoter was inserted into OBP-301 so that the viruses could express GFP by efficient replication in infected cells (Figure 4a). This chimeric virus was named OBP-401.[24,25] We first infected OBP-401 into SKOV3 and normal human fibroblast at an MOI of 10 *in vitro*. Apparent expression of GFP was observed in SKOV3 cells (Figure 4b, right panel); however, only faint GFP signals were detected in normal human fibroblasts (Figure 4b, left panel), clearly indicating the cancer-specific replication of OBP-401 *in vitro*. Next, to assess the localization of intraperitone-ally injected virus, we established an *in vivo* mouse model of ovarian cancer cells, in which 1×10^7 SKOV3 cells were inoculated into the peritoneal cavity of nude mice, leading to the formation of peritoneal dissemina-tions. OBP-401 was then injected into the peritoneal cavity 4 weeks after the initial inoculation with the cancer cells. The mice were killed 72 h after OBP-401 injection and GFP expression was observed. GFP expression was found to be localized mainly at the surface of dissemi-nated cancer lesions (Figure 4c, left panel). We even detected GFP expression in small lesions that were only detectable with a microscope, not by macroscopic observation (Figure 4c, right panel). These findings suggest that our viruses injected into the peritoneal cavity exhibit preferential and specific distribution in disseminated cancer foci.

In vivo *effect of intraperitoneal administration of OBP-301 in combination with CDDP on peritoneal dissemination of ovarian cancer cells*

As the use of platinum-based chemotherapeutic agents has commonly been accepted as the standard therapy for advanced ovarian cancer, we examined the *in vivo* effects of OBP-301 alone, CDDP alone or OBP-301 combined with CDDP using a mouse model with peritoneal dissemination (Figure 5a, see Materials and methods). The numbers of peritoneal disseminated lesions were then counted in each treatment group (Figure 5b). Administration of CDDP alone did not significantly reduce the number of disseminated lesions compared with the mock-treated mice, whereas treatment with OBP-301 alone led to a significant reduction in disseminated lesions ($P < 0.05$). The combination of CDDP with OBP-301 resulted in a significant decrease in
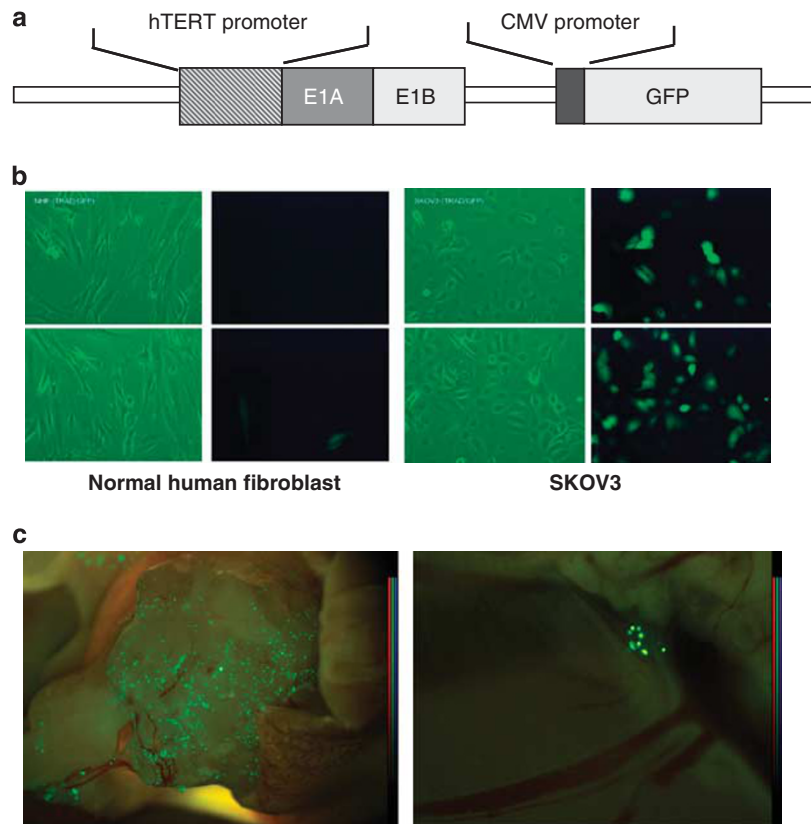
Figure 4 Visualization of infected OBP adenoviruses by green fluorescent protein (GFP) expression. To visualize OBP-301 *in vitro* and *in vivo*, the *GFP* gene driven by the cytomegalovirus (CMV) promoter was inserted into the genome of OBP-301, named OBP-401 (**a**). SKOV3 and normal human fibroblasts were infected with OBP-401 at an multiplicity of infection (MOI) of 10 and GFP expression was assessed using a fluorescence microscope (**b**). SKOV3 cells (1×10^7 cells) were inoculated into the peritoneal cavity of BALB/c nu/nu mice and OBP-401 was injected into the peritoneal cavity 4 weeks later. The mice were killed and GFP expression was assessed 72 h after injection of OBP-401 (**c**).

the number of disseminated lesions compared with CDDP or OBP-301 alone ($P < 0.001$, $P < 0.01$, respectively). A representative picture of the peritoneal cavities of two mice is shown in Figure 4c. Massive progression of the peritoneal disseminations can be seen in the mock-treated mouse, whereas markedly fewer and smaller disseminated tumors are evident in the CDDP/ OBP-301-treated mouse.

To clarify the survival effect of intraperitoneal administration of OBP-301, three groups of mice treated with PBS alone (mock), CDDP alone or CDDP combined with OBP-301 were further monitored for mortality until 120 days after inoculation of cancer cells (Figure 6). All mock-treated mice died around days 50–60, whereas CDDP alone extended survival, with a 50% OS rate at day 60 and 17% at day 120. Co-treatment with OBP-301 further extended survival, achieving 100% OS at day 80 and 83% at day 120. Thus, the prognosis was significantly improved by the combination of CDDP with OBP-301. Taken together, these findings suggest that intraperitoneal administration of OBP-301 sensitizes ovarian cancer cells to CDDP and may be a useful treatment option for advanced ovarian cancer with peritoneal dissemination.

Discussion

In this study, we showed the therapeutic potential of intraperitoneal administration of OBP-301 in combination with CDDP for advanced ovarian cancers with peritoneal dissemination. A series of our previous studies have shown the anti-tumor effects of OBP-301 in various types of cancers, including lung and colorectal cancers both *in vitro* and *in vivo*,[16,21,22,24–27] and a clinical trial of OBP-301 therapy as monotherapy is currently underway based on these preclinical studies.[29] However, these preclinical and clinical studies have been carried out mainly by direct injection of OBP-301 into primary tumor sites. This study is the first to test the effect of intraperitoneal administration of OBP-301.

Our *in vitro* data showed that OBP-301 alone has marked anti-tumor effects on SKOV3 cells at an MOI of 100, whereas modest to mild effects were observed at an MOI of 10 or lower (Figure 1). However, such low-dose OBP-301 did have a sensitizing effect on SKOV3 cells to CDDP *in vitro* (Figure 2), rendering the cytotoxicity of low-dose CDDP (0.5 μM) equivalent to that of high-dose CDDP (2 μM). We also confirmed that similar sensitizing effect was observed in CDDP-resistant cells (Figure 3).
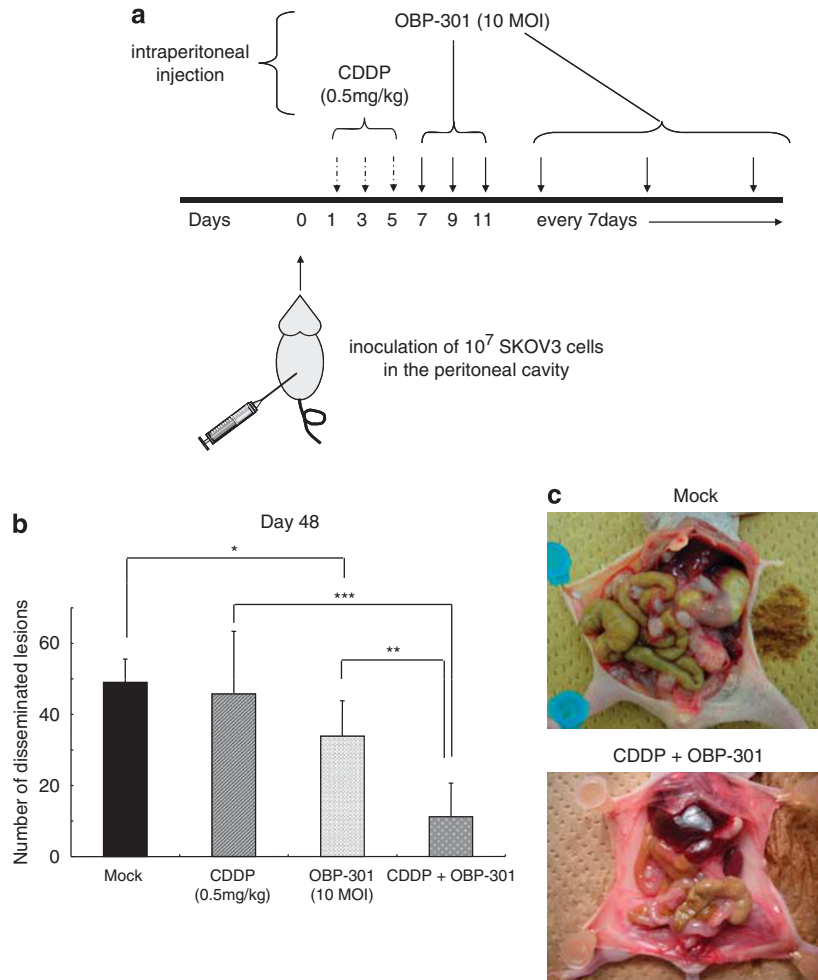
Figure 5 *In vivo* effect of intraperitoneal administration of CDDP and/or OB-301 on peritoneal disseminations of SKOV3. (a) Protocol of intraperitoneal administration of CDDP and/or OB-301. After the inoculation of 1×10^7 SKOV3 cells (day 0), 0.5 mg kg^{-1} CDDP was injected into the peritoneal cavity once a day on days 1, 3 and 5. Then, 10^8 pfu of OBP-301 was injected into the peritoneal cavity once a day on days 7, 9 and 11 followed by weekly injection of 10^8 pfu of OBP-301 until the killing. Arrows indicate the day of treatment. (b) The numbers of peritoneal disseminated lesions were counted in each treatment group 50 days after inoculation of SKOV3 cells. Bars, s.d. (c) Representative picture of the control mice (upper panel) and mice treated with CDDP + OBP-301 (lower panel). Bars, s.d.; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
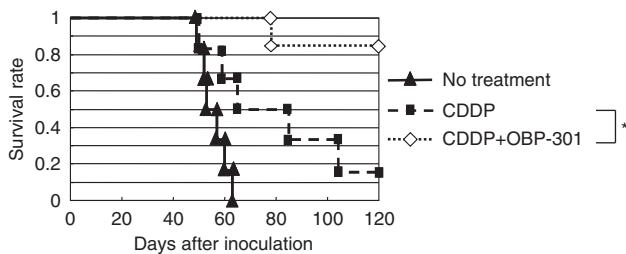

Figure 6 Survival of mice treated with intraperitoneal administration of CDDP and/ or OB-301. Kaplan–Meier plots of overall survival of the no treatment, CDDP alone and CDDP + OBP-301 groups. Each group consisted of six mice. *$P < 0.05$.

Of particular interest is that CDDP-resistant KFr13 cells seemed to have higher sensitivity to OBP-301 than CDDP-sensitive SKOV3 cells. These finding suggest the therapeutic potential of OBP-301, irrespectively of the CDDP sensitivity of target cells. The beneficial combined

effect of OBP-301 with CDDP was further supported by the *in vivo* analysis. Although the number of disseminated lesions was not significantly altered by low-dose CDDP (0.5 mg kg^{-1}) alone, the number significantly decreased by the addition of OBP-301 to CDDP ($P < 0.01$) (Figure 5).

The molecular mechanism through which OBP-301 sensitizes cells to CDDP was not analyzed in this study, but it is likely that OBP-301 and CDDP produce their cytotoxic activities through different mechanisms. We also confirmed that the replicative efficacy of OBP-301 was not affected by CDDP treatment *in vitro* and that OBP-301 infection had no effect on cell cycle distribution (data not shown). Therefore, the anti-tumor mechanisms of the two agents seem to be independent of each other. We also confirmed that the nuclear morphology of cells infected with OBP-301 did not exhibit findings of apoptosis characterized by chromosome condensation, nuclear shrinkage and fragmentation (data not shown). Furthermore, western blot analysis showed no change in a

family of cysteine proteases known as caspases on infection with OBP-301 (data not shown). The *in vitro* dose-response curves of CDDP combined with OBP-301 show that the interaction between the two agents is additive, not synergistic, suggesting that OBP-301 targets cells that have escaped death by CDDP, leading to an additive effect. Interestingly, intraperitoneal administration of OBP-301 in combination with CDDP induced a more pronounced growth inhibitory effect, that is, a synergistic effect, *in vivo* than *in vitro* (Figure 5), suggesting particular interactions between OBP-301 and CDDP *in vivo*. Similar synergistic effects of OBP-301 *in vivo*, but not *in vitro*, have been reported with other cytotoxic agents.[21] One possible explanation for the augmented cytotoxicity observed *in vivo* might be that the blood supply to tumor lesions is disturbed by the actions of OBP-301, in which intraperitoneal administration of OBP-301 may attack endothelial cells of microvessels in disseminated lesions and thereby enhance the cytotoxic effects of CDDP. Although microvessels are mouse origin, *hTERT* promoter might function in such cells because it has very similar context of the sequences with the mouse *TERT* promoter[30] and it can be activated in proliferation-competent normal cells, such as endothelial cells in tumor microvessels.[31–33] Alternatively, CDDP treatment affects the immunological circumstances of the host, disturbing the functions of macrophages and other immune cells, which may provide some advantage to OBP-301 in its infectivity and replicative capacity in cancer tissues. Finally, we tested the change in expression of various genes involved in drug resistance, such as *MDR1*, *MRP1*, *LRP* and *GST*, 24 h after the treatment with OBP-301 at 100 MOI. However, reverse transcriptase–PCR assays failed to find any specific factors whose expression was significantly regulated by the treatment (data not shown). Further analyses are needed to identify the molecular mechanisms of the synergistic effects by both agents *in vivo*.

Although this study did not investigate the adverse effects on mice, our previous studies showed no significant toxicity of OBP-301 on normal human cells.[16,21,22,24,25] This is likely because of the use of the *hTERT* promoter for tumor-specific expression of the *E1* gene. Although the *hTERT* promoter is reported to be silent in normal cells, it may provide some transcriptional activity in some cell types with telomerase activity, such as stem or germ-line cells.[31–33] It is therefore possible that these telomerase-positive normal cells might be targeted by OBP-301. Nevertheless, severe adverse effects due to OBP-301 have not been reported. One possible explanation of this favorable phenomenon is that cells with stem-like characteristics are usually non-epithelial in origin and are therefore not likely to be targeted by adenoviral vectors.[34] Alternatively, the levels of telomerase activity in cells with stem-like characteristics are not as high as in cancer cells unless the cells are exposed to ectopic mitogenic stimuli.[32,33] Therefore, the cytotoxicity by OBP-301 might be limited or permissive in such cells.

Platinum-based chemotherapy has widely been accepted as the standard regimen for both neoadjuvant and adjuvant settings for advanced ovarian cancer.[1] However, ∼30–40% of patients have resistance to platinum-based chemotherapy, and even sensitive patients frequently acquire resistance after repeated cycles of CDDP-based chemotherapy.[2–6] In this study, we found that OBP-301 was useful for CDDP-resistant ovarian cancer cells, indicating the therapeutic potential of this agent, irrespectively of the CDDP sensitivity of target cells. Furthermore, we found that use of OBP-301 alone had cytotoxic effects *in vitro* at an MOI of 100, but exhibited more pronounced effects *in vivo* at an MOI of 10, decreasing the number of disseminations (Figure 5). Although we did not examine the survival effect of OBP-301 alone, patients who relapse with acquired CDDP resistance may be able to use OBP-301 alone.

The most notable finding of this study is that addition of intraperitoneal injection of OBP-301 improved the survival of mice. Our treatment modality is characterized by continuous weekly administration of OBP-301 after CDDP treatment. As one of the major purposes of this strategy is to reduce the total dose of CDDP delivered, thereby reducing its adverse effects, we did not test weekly combined administration of OBP-301 and CDDP. Our data indicate that continuous weekly OBP-301 administration after CDDP treatment has some survival effect and is a practical regimen for minimizing the adverse effects of CDDP.

We are currently investigating the combinatorial actions of OBP-301 with taxans, other key drugs for ovarian cancers. In fact, the combination therapy of taxans and CDDP/carboplatin is now a novel standard of chemotherapy for ovarian cancer. Therefore, it is necessary to investigate the best modality by which to combine OBP-301 with such standard regimens. Furthermore, it is of great interest to test the possibility of combination therapy of OBP-301 with the recently established molecular target therapies, such as signal transduction inhibitors, to provide relief to patients with ovarian cancers who have acquired resistance to standard therapy.

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