Telomerase-Dependent Oncolytic Adenovirus Sensitizes Human Cancer Cells to Ionizing Radiation via Inhibition of DNA Repair Machinery

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Abstract

The inability to repair DNA double-strand breaks (DSB) leads to radiosensitization, such that ionizing radiation combined with molecular inhibition of cellular DSB processing may greatly affect treatment of human cancer. As a variety of viral products interact with the DNA repair machinery, oncolytic virotherapy may improve the therapeutic window of conventional radiotherapy. Here, we describe the mechanistic basis for synergy of irradiation and OBP-301 (Telomelysin), an attenuated type-5 adenovirus with oncolytic potency that contains the human telomerase reverse transcriptase promoter to regulate viral replication. OBP-301 infection led to E1B55kDa viral protein expression that degraded the complex formed by Mre11, Rad50, and NBS1, which senses DSBs. Subsequently, the phosphorylation of cellular ataxia-telangiectasia mutated protein was inhibited, disrupting the signaling pathway controlling DNA repair. Thus, tumor cells infected with OBP-301 could be rendered sensitive to ionizing radiation. Moreover, by using noninvasive whole-body imaging, we showed that intratumoral injection of OBP-301 followed by regional irradiation induces a substantial anti-tumor effect, resulting from tumor cell-specific radiosensitization, in an orthotopic human esophageal cancer xenograft model. These results illustrate the potential of combining oncolytic virotherapy and ionizing radiation as a promising strategy in the management of human cancer. Cancer Res; 70(22); 9339–48. ©2010 AACR.

Introduction

Current treatment strategies for advanced cancer include surgical resection, radiation, and cytotoxic chemotherapy. Preoperative or postoperative chemoradiation may improve local control and the survival of advanced cancer patients by minimizing the risk of dissemination during the surgical procedure, increasing the complete resection rate, and eradicating microscopic residual tumor cells that are not surgically removed. The lack of restricted selectivity for tumor cells is the primary limitation of radiotherapy, despite improved technologies such as stereotactic and hyperfractionated radiotherapy. Although radiotherapy is generally considered to be less invasive, the maximum doses and treatment fields are limited to avoid the influence on the surrounding normal tissues. Therefore, to improve the therapeutic index of radiotherapy while maintaining a tolerance of normal tissue toxicity, there is a need for agents that effectively lower the threshold for radiation-induced tumor cell death; the safety and efficacy of some candidates are already being explored in clinical trials (1–3).

Ionizing radiation primarily targets DNA molecules and induces double-strand breaks (DSB; ref. 4). Radiosensitization can result from a therapeutic increase in DNA DSBs or inhibition of their repair. Ataxia-telangiectasia mutated (ATM) protein is an important signal transducer of the DNA damage response, which contains DNA repair and cell cycle checkpoints, and activation of ATM by autophosphorylation occurs in response to exposed DNA DSBs (5). Cells mutated in the ATM gene have defects in cell cycle checkpoints and DNA repair and are hypersensitive to DSBs (6, 7); thus, agents that inhibit the ATM pathway can be useful radiosensitizers (8). The Mre11, Rad50, and NBS1 (MRN) complex is quickly stimulated by DSBs and directly activates ATM (9, 10). Defects in the MRN complex lead to genomic instability, telomere shortening, and hypersensitivity to DNA damage (11). We reported previously that telomerase-specific, replication-selective adenovirus (Telomelysin, OBP-301), in which the human telomerase reverse transcriptase (hTERT) promoter element drives the expression of E1 genes, induced selective

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E1 expression, and efficiently killed human cancer cells but not normal human somatic cells (12–15). Adenoviral E1B55kDa protein, a gene product in the adenoviral early region, inhibits the functions of p53 and the MRN complex by cooperating with adenoviral E4orf6 protein, leading to the proteolytic degradation of these proteins (10, 16–18). In the present study, we showed the synergistic efficacy of combined treatment with ionizing radiation and OBP-301 against human cancer cells, and we clarified the E1B55kDa-mediated mechanism used by OBP-301 to inhibit DNA repair.

Materials and Methods

Cell lines and cell cultures

The human non–small-cell lung cancer cell line A549 was propagated in DMEM containing Nutrient Mixture (Ham’s F-12) and supplemented with 10% FCS. The human esophageal squamous carcinoma cell line TE8 was cultured in RPMI 1640 supplemented with 10% FCS. The human esophageal adenocarcinoma cell line SEG1 was cultured in DMEM supplemented with 10% FCS. TE8 cells transfected with the firefly luciferase plasmid vector (TE8-Luc) were maintained in medium containing 0.2 mg/mL Geneticin (G418).

Adenovirus

The recombinant, replication-selective, tumor-specific adenovirus vector OBP-301 (Telomelysin), in which the hTERT promoter element drives the expression of E1A and E1B genes linked with an internal ribosome entry site, was previously characterized (12–15). The wild-type adenovirus type 5 (Ad-wt) and the E1B55kDa-defective adenovirus mutant dl1520 (Onyx-015) were also used (19).

Cell viability assay

A549, TE8, and SEG1 cells were infected with OBP-301 at the indicated multiplicities of infection (MOI) and then irradiated at the indicated dosages by using an MBR-1520R device (Hitachi Medical Co.). Cell viability was determined 5 days after irradiation with a Cell Proliferation Kit II (Roche Molecular Biochemicals), according to the manufacturer’s protocol. Synergy between radiation and OBP-301 was calculated with the CalcuSyn software (BioSoft), and the protocol was approved by the Ethics Review Committee for Animal Experimentation of Okayama University.

In vivo subcutaneous human tumor model

A549, TE8, and SEG1 cells (2 × 10^6 per mouse) were injected s.c. into the flanks of 5- to 6-week-old female BALB/c nu/nu mice. When tumors reached ∼3 to 5 mm in diameter, the mice were irradiated at a dosage of 3 Gy/tumor every 2 days (for A549) or 2 Gy/tumor every week (for TE8 and SEG1) for three cycles starting at day 0. When irradiated, mice were placed prone in custom-made holders that contain lead collimators to shield the upper half of the mice. Immediately after radiation, OBP-301 at a dose of 1 × 10^8 plaque-forming units (PFU)/tumor or PBS was injected into the tumor. In experiments with larger tumors, subcutaneous TE8 tumors with a diameter of 8 to 10 mm were treated with radiation at 2 Gy/tumor and intratumoral injection of OBP-301 at 1 × 10^8 PFU/tumor three times per week (every 2 days) for three cycles (nine times in total). The perpendicular diameter of each tumor was measured every 3 to 4 days, and tumor volume was calculated with the following formula: tumor volume (mm^3) = a × b^2 × 0.5, where a is the longest diameter, b is the shortest diameter, and 0.5 is a constant to calculate the volume of an ellipsoid. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Okayama University.

Orthotopic human esophageal cancer model

TE8-Luc cells (2 × 10^6 per mouse) suspended in Matrigel were inoculated into the abdominal esophagus of 6-week-old female BALB/c nu/nu mice during laparotomy. Three weeks later, mice were irradiated with 2 Gy/tumor in holders that contain lead collimators to shield the head, neck, and chest of the mice. Immediately after radiation, mice were intratumorally injected with OBP-301 at 1 × 10^8 PFU/tumor during laparotomy, every 2 days for three cycles. To monitor tumor progression, the substrate luciferin was injected i.p. at a dose

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of 150 mg/kg body weight. Images were collected in the supine position every few minutes from 10 to 30 minutes after luciferin injection with the IVIS Imaging System (Xenogen), and photons emitted from the abdominal esophagus region were quantified by using Living Image Software (Xenogen).

Statistical analysis
All data were expressed as mean ± SD. Differences between groups were examined for statistical significance with the Student’s t test. P values <0.05 were considered statistically significant.

Results
Radiosensitizing effect of OBP-301 in vitro
To examine the potential interaction between OBP-301 and ionizing radiation in vitro, we first evaluated their combined effect in human lung (A549) and esophageal (TE8 and SEG1) cancer cell lines. The cells received a single dose of ionizing irradiation 24 hours after either mock or OBP-301 infection, and the cell viability was assessed by 2,3-bis[2-methoxy-4-nitro-5-sulphophenyl]-2H-tetrazolium-5-carboxanilide inner salt (XTT) assay 5 days after irradiation. The addition of OBP-301 increased the cytotoxicity of ionizing radiation in a dose-dependent manner. The combination index showed potent, statistically significant synergy between OBP-301 and radiation in all three cell lines (Fig. 1A). In contrast, synergy was not observed in normal human lung fibroblasts (NHLF) because the viral replication of OBP-301 was attenuated in telomerase-negative normal cells (Supplementary Fig. S2A, B).

We measured the amount of apoptosis in A549 and TE8 cells that were irradiated after OBP-301 infection. The cells were infected with OBP-301 at a MOI of 1.0, irradiated with a dose of 10 Gy 24 hours after infection, and analyzed for apoptosis. OBP-301 caused a significant increase in active caspase-3–positive cells in response to ionizing irradiation (Fig. 1B). Western blot analysis showed that ionizing radiation promoted the cleavage of PARP, a caspase-3 substrate and a biochemical marker of apoptosis, with prior OBP-301 infection (Fig. 1C). Moreover, OBP-301 infection before irradiation significantly increased the number of A549 cells with apoptotic nuclear morphology (Fig. 1D and E). Thus, OBP-301 combined with ionizing radiation synergistically increased the amount of apoptosis.

Degradation of the MRN complex by adeno viral E1B55kDa protein
To elucidate the molecular mechanism responsible for the synergy between OBP-301 and ionizing radiation, we examined the physical interaction between viral proteins and the MRN complex, which drives the DNA repair pathway as a sensor of DNA DSBs, through a series of confocal microscopy experiments. Immunofluorescence staining of A549 cells infected with OBP-301 at a MOI of 10 showed colocalization of the signals representing Mre11, NBS1, and Rad50, suggesting that these proteins exist as a complex. Moreover, there was marked overlap in the nuclear signals corresponding to adeno viral E1B55kDa and NBS1, indicating a direct interaction between the E1B55kDa protein and the MRN complex (Fig. 2A). Western blot analysis showed that the levels of Mre11, NBS1, and Rad50 protein gradually decreased after OBP-301 infection at an MOI of 10, as the E1B55kDa expression increased (Fig. 2B). The expression of the MRN complex remained unchanged in NHLF because E1B55kDa expression was absent after OBP-301 infection (Supplementary Fig. S2C).

To confirm the effect of adeno viral E1B55kDa on the MRN degradation, we compared the subcellular localization and degradation of Mre11 protein after infection with wild-type adenovirus (Ad–wt), an adenovirus mutant that lacked E1B55kDa (dl1520, Onyx-015), or OBP-301 in A549 cells. The Mre11 protein accumulated in the nucleus in a scattered pattern 24 hours after OBP-301 or Ad–wt infection and then relocated to the perinuclear area and was degraded; however, the scattered nuclear signals of Mre11 remained largely undegraded 72 hours after dl1520 infection (Fig. 2C). We measured the proportions of the cells with each staining pattern (scattered, perinuclear, or degraded) to compare the subcellular dynamics of the Mre11 protein. OBP-301 and Ad–wt infection caused a rapid decrease in Mre11-positive cells compared with dl1520 infection (Fig. 2D and E), suggesting that the E1B55kDa protein is essential for the degradation of the MRN complex.

Inhibition of radiation-induced DNA damage responses by OBP-301
The MRN complex functions as a DSB sensor that activates the ATM-dependent signaling pathway, which coordinates cell cycle arrest with DNA repair. To further investigate the relationship between E1B55kDa and ATM activation, we examined the effect of OBP-301 infection on the radiation-induced phosphorylation of ATM (pATM) in A549 cells. Immunofluorescence analysis revealed spot signals of pATM throughout the nuclei 30 minutes after ionizing radiation; however, OBP-301 or Ad–wt infection (10 MOI) 24 hours before irradiation blocked the formation of pATM foci (Fig. 3A). Pretreatment with OBP-301 significantly reduced the number of pATM-positive cells compared with pretreatment with dl1520 lacking E1B55kDa (Fig. 3B). Western blot analysis also showed that ionizing radiation induced the phosphorylation of ATM, whereas expression of E1B55kDa by OBP-301 infection led to the degradation of the MRN complex, which was accompanied by a greatly reduced level of pATM (Fig. 3C). Following dl1520 infection, ATM phosphorylation was seen in the absence of E1B55kDa expression and MRN degradation.

We also investigated whether OBP-301 infection could abrogate the DNA repair process by using γH2AX, which is a sensitive indicator of DSBs. Ionizing radiation induced γH2AX expression as early as 30 minutes after treatment in both mock- and OBP-301–infected A549 cells. The levels of γH2AX protein gradually decreased as the DNA DSBs were repaired in mock-infected cells, but remained elevated in cells infected with OBP-301 24 hours before irradiation (Fig. 3D). Densitometric quantification revealed that the relative density of γH2AX/β-actin at 3 hours after irradiation decreased by 64% without prior OBP-301 infection, but decreased by only...
19% with OBP-301 infection compared with the levels at 30 minutes postirradiation (Fig. 3E). These results indicate that OBP-301 infection interrupts the cellular DNA repair mechanism induced by ionizing radiation.

**Synergistic antitumor activity of OBP-301 plus radiation in human tumor xenografts**

We next assessed the therapeutic efficacy of OBP-301 in combination with ionizing radiation against A549, TE8, and SEG1 cells in vivo. To determine the treatment schedule, we examined whether radiation could modify adenoviral infectivity and replication in human cancer cells. OBP-301 infection following ionizing radiation showed synergistic antitumor effects in vitro (Supplementary Fig. S3) due to an increased expression density of coxsackievirus and adenovirus receptor, which resulted in enhanced adenoviral uptake in human cancer cells (Supplementary Figs. S4 to S6). We also confirmed that ionizing radiation does not interfere with OBP-301 replication (Supplementary Fig. S7).

Based on these preliminary results, we chose a therapy regimen with three cycles of regional radiation followed immediately by intratumoral administration of OBP-301. Mice bearing A549, TE8, and SEG1 subcutaneous tumors that were 3 to 5 mm in diameter received 3 Gy (for A549) or 2 Gy (for TE8 and SEG1) local irradiation followed by the intratumoral injection of either $1 \times 10^8$ PFU of OBP-301 or PBS every 2 days (for A549) or 7 days (for TE8 and SEG1) for three cycles. Intratumoral administration of OBP-301 or radiation
alone resulted in significant tumor growth suppression compared with mock-treated tumors. The combination of OBP-301 plus radiation produced a more profound and significant inhibition of tumor growth compared with either modality alone in all three types of tumors, despite the difference in treatment schedules (Fig. 4A).

Histopathologic analysis of A549 tumors excised 10 days after the completion of three cycles of either regional radiation or OBP-301 infection revealed the degeneration of tissues and reduced tumor cell density compared with untreated tumors. However, treatment with OBP-301 injection plus radiation yielded massive tissue destruction and further reduction in tumor cell density. Moreover, the cytolytic changes induced by the combination therapy led to the development of hyalinized acellular stroma (Fig. 4B). Terminal deoxyribonucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining showed that combining ionizing radiation with OBP-301 markedly increased the amount of apoptotic cells in A549 tumors excised 3 days after the completion of the treatment (Fig. 4C). OBP-301 plus irradiation apparently increased PARP cleavage in A549 tumors compared with ionizing radiation alone (Fig. 4D).

**Eradication of established human tumor xenografts by OBP-301 plus radiation**

To mimic the clinical characteristics of advanced cancer patients, we established TE8 xenografts with a 10-fold larger tumor burden. Mice bearing large TE8 subcutaneous tumors received nine cycles of 2 Gy irradiation followed by intratumoral injection of $1 \times 10^8$ PFU of OBP-301 three times per week for 3 weeks. Tumors treated with either OBP-301 or ionizing radiation alone exhibited a transient shrinkage,
but invariably started to regrow 14 days after the beginning of treatment, whereas OBP-301 plus radiation completely eradicated the established larger TE8 tumors on day 28 in 9 of 10 mice (Fig. 5A).

Tumors treated with OBP-301 or radiation alone were consistently smaller than tumors of the control cohort of mice (Fig. 5B; Supplementary Fig. S8A). Massive ulceration was noted on the tumor surface after injection of OBP-301, whereas no tumor burden was detected when ionizing radiation was combined with OBP-301 injection. Moreover, histologic analysis revealed the apparent destruction of tumor tissues after OBP-301 injection or ionizing radiation.

However, no residual tumor cells were observed in tumors treated with OBP-301 plus radiation; instead, massive cellular infiltrates were noted (Supplementary Fig. S8B). Mice with tumor eradication significantly recovered their body weight, although there was a gradual decrease in the body weight of the control group (Supplementary Fig. S9).

**Evaluation of in vivo antitumor effects on orthotopic human esophageal cancer model**

Finally, we assessed the therapeutic efficacy of intratumoral injection of OBP-301 and local irradiation in an orthotopic human esophageal cancer xenograft model by...
using noninvasive whole-body imaging. When TE8 human esophageal cancer cells stably transfected with the luciferase gene (TE8-Luc) were s.c. implanted into nude mice, a correlation was observed between tumor growth (volume) and the luciferase emission level (luminescent intensity; Supplementary Fig. S10). Our preliminary experiments revealed that when TE8-Luc cells were inoculated into the wall of the abdominal esophagus of athymic nu/nu mice, esophageal tumors appeared within 3 weeks after tumor injection (Fig. 5C).

Mice bearing macroscopic esophageal tumors were treated with local irradiation at 2 Gy followed by intratumoral injection during laparotomy of 1 × 10⁸ PFU of OBP-301 every 2 days for three cycles. The luminescent intensity of tumors treated with OBP-301 plus radiation was significantly lower than that of mock-treated, irradiated, or OBP-301–injected tumors (Fig. 5D and E). These results suggest that the biochemical interaction of OBP-301 with irradiation can be translated into a potential clinically applicable cancer treatment.

**Discussion**

A novel biological property of OBP-301 as a molecular radiosensitizer was verified, referring to a critical role of adeno viral E1B55kDa in inhibiting the DNA damage responses triggered by ionizing radiation. Radiation-induced cell death is dependent on DNA damage and, therefore, inhibition of

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**Figure 4.** Antitumor effects of OBP-301 and ionizing radiation against s.c. established xenograft tumors. A, cells (2 × 10⁶ per mouse) were injected s.c. into the right flanks of mice. When the tumors reached 3 to 5 mm in diameter, mice were exposed to 3 Gy (for A549) or 2 Gy (for TE8 and SEG1) of ionizing radiation and intratumorally administered OBP-301 (1 × 10⁸ PFU tumor) for three cycles every 2 d (for A549) or every week (for TE8 and SEG1). Six (for A549) or eight (for TE8 and SEG1) mice were used for each group. Tumor growth is expressed as the mean tumor volume ± SD. Arrows indicate each treatment. *, P < 0.01. B, mice bearing A549 xenografts were treated as described above. Tumor sections were obtained 10 d after the final administration of OBP-301. Paraffin sections of tumors were stained with hematoxylin and eosin. Scale bar, 100 μm. Magnification, ×200 (top), ×400 (bottom). C and D, in vivo induction of apoptotic cell death by OBP-301 and ionizing radiation. C, paraffin-embedded sections of A549 subcutaneous tumors excised 3 d after treatment as described above were subjected to TUNEL staining. D, Western blot analysis for PARP and β-actin was done with proteins extracted from A549 subcutaneous tumors 3 d after treatments.
DNA repair can enhance the sensitivity of human tumor cells to ionizing radiation. The ATM activation by the MRN complex is essential for sensing and signaling from DNA DSBs and plays an important role in DNA repair and checkpoints, indicating that this pathway may be a good target for enhancing the antitumor effects of DNA-damaging agents. Indeed, ATM inhibitors, such as KU55933 and CGK733, and an MRN complex inhibitor, mirin, could sensitize cancer cells to therapeutic agents that cause DNA DSBs (21–23). Hsp90 interacts with the MRN complex, and an Hsp90 inhibitor enhances the sensitivity of tumor cells to radiation (24). Molecular disruption of MRN function by a dominant-negative mutant Rad50 gene transfer sensitizes tumor cells to cisplatin (25). Although these approaches are effective in interrupting cellular DNA repair mechanisms, they lack tumor selectivity and may damage normal tissues when combined with DNA-damaging therapies. Our data show that OBP-301 could synergize with ionizing radiation only in tumor cells but not in normal cells due to its telomerase dependency, suggesting that the regional administration of OBP-301 enables targeted radiosensitization.

The adenovirus E1B gene encodes a 19-kDa polypeptide (E1B19kDa) and a 55-kDa protein (E1B55kDa). The E1B55kDa protein induces a cellular environment conducive for viral protein synthesis via a complex with the E4orf6 protein (26). This E1B55kDa/E4orf6 complex degrades the MRN complex, blocks the downstream ATM signaling, and leads to a defective G2-M checkpoint in response to DSBs (10). Although the impact of E1B55kDa-mediated disruption of the MRN-ATM pathway on the DNA damage responses triggered by ionizing radiation has not yet been studied, we showed that OBP-301-mediated E1B55kDa expression induced the degradation of all components of the MRN-ATM pathway.
complex, which in turn prevented ATM autophosphorylation following ionizing radiation. OBP-301 expresses the E1B gene under the control of the hTERT promoter through an internal ribosome entry site sequence, whereas dl1520 (Onyx-015, CI-1042), which has been used in many clinical trials, was genetically modified by disruption of the coding sequence of the E1B55kDa protein (19). Therefore, ionizing radiation–induced ATM activation was blocked more efficiently by OBP-301 than by dl1520, which lacks E1B55kDa, although dl1520 slightly inhibited ATM phosphorylation, presumably due to E4orf6 protein expression (27).

One hallmark of DNA DSBs is the phosphorylation of H2AX at Ser139, a specialized histone H2A variant (referred to as γH2AX; ref. 28); the reduction in γH2AX levels in irradiated cells correlates with the repair of DSBs (29). OBP-301 infection apparently sustained the elevated levels of γH2AX longer in irradiated tumor cells, indicating that tumor cells infected with OBP-301 could be rendered sensitive to ionizing radiation. We previously found that the process of oncolysis is morphologically distinct from apoptosis and necrosis, although autophagy is partially involved in this effect (30). The observation that OBP-301 infection significantly enhanced the induction of apoptosis when combined with ionizing radiation suggests that the radiosensitizing activity of OBP-301 is independent of virus-mediated oncolysis. Indeed, dephosphorylation of H2AX is associated with efficient DNA repair, whereas a pronounced increase in H2AX phosphorylation correlates with apoptosis (31, 32). Moreover, synergistically enhanced apoptosis by OBP-310 and ionizing radiation is likely to be p53 independent because p53 was ubiquitinated and degraded by E1B55kDa and E4orf6 proteins (16, 18).

Our in vitro studies suggest that OBP-301 infection and ionizing radiation may mutually sensitize human tumor cells, potentially leading to an effective combination treatment. OBP-301 infection requires a period of replication to induce the cytopathic effect and to sensitize cells to radiation, whereas ionizing radiation immediately causes DNA DSBs. Therefore, in a true clinical setting, multiple cycles of the external-beam radiotherapy followed by intratumoral injection of OBP-301 may yield optimal results. We confirmed the synergistic antitumor effect of three cycles of treatment with OBP-301 plus regional radiation and the in vivo induction of apoptotic cell death on subcutaneous human tumor xenografts. The orthotopic implantation of tumor cells, however, restores the correct tumor-host interactions, which do not occur when tumors are implanted in ectopic subcutaneous sites (33). Thus, we also showed the significant synergy of combined treatments in an orthotopic mouse model of human esophageal cancer by using a noninvasive whole-body imaging system.

There are some possible advantages of combining virotherapy with radiotherapy in vivo. First, OBP-301 may inhibit the vascular supply by killing endothelial cells because endothelial cell proliferation is increased in irradiated tumors (34), presumably with high telomerase activity. Alternatively, local irradiation itself may attack the vascular endothelial cells in the tumor site, which in turn can block the escape of locally injected OBP-301 into the blood circulation. Indeed, ionizing radiation inhibits endothelial cell proliferation, tube formation, migration, and clonogenic survival (35). Furthermore, in an immunocompetent environment, as we previously reported (36), OBP-301 stimulates host immune cells to produce endogenous antiangiogenic factors such as interferon-γ. Second, virotherapy and radiotherapy may target tumor cells in different parts of tumors with distinct mechanisms. For example, tumor hypoxia has been considered a potential therapeutic problem because it renders tumor cells more resistant to ionizing radiation (37) and, therefore, some cells in certain parts of tumors may survive and proliferate under hypoxic conditions. In contrast, because hypoxia induces the transcriptional activity of hTERT gene promoter through hypoxia-inducible factor 1α (38), OBP-301 can be expected to replicate and efficiently kill tumor cells even under hypoxic conditions. Thus, hTERT-specific oncolytic virotherapy can be effective in eliminating tumor cells that survive after local radiotherapy.

Another advantage of this combination therapy is that the area where each treatment shows the therapeutic effect is overlapping. The treatment field of radiotherapy includes primary tumors and regional lymph nodes. We previously showed that intratumorally injected OBP-301 expressing the GFP gene is effectively transported into the lymphatic circulation; viral replication produced GFP fluorescence signals in the metastatic lymph nodes in orthotopic human colorectal and oral cancer xenograft models (39, 40). Therefore, we anticipate that intratumoral OBP-301 administration will radiosensitize both primary tumors and regional lymph nodes.

In summary, our data show the molecular basis of radiosensitization induced by telomerase-specific virotherapy, in which the adenoviral E1B55kDa protein inhibits the radiation-induced DNA repair machinery through the interruption of the MRN function. OBP-301 infection and ionizing radiation mutually modulate their respective biological effects and thereby potentiate each other, profoundly enhancing in vivo antitumor activity in an orthotopic mouse model.

Disclosure of Potential Conflicts of Interest

Y. Urata is an employee of Oncolytic BioPharma, Inc., the manufacturer of OBP-301 (Telomelysin). The other authors disclosed no potential conflicts of interest.

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