Telomerase-Specific Virotheranostics for Human Head and Neck Cancer

Yuji Kurihara,1 Yuichi Watanabe,2,3 Hideki Onimatsu,2 Toru Kojima,4 Tatsuo Shirota,1 Masashi Hatori,1 Dong Liu,5 Satoru Kyo,6 Hiroyuki Mizuguchi,7 Yasuo Urata,2 Satoru Shintani,1 and Toshiyoshi Fujiwara3,4

Abstract

Purpose: Long-term outcomes of patients with squamous cell carcinoma of the head and neck (SCCHN) remain unsatisfactory despite advances in combination of treatment modalities. SCCHN is characterized by locoregional spread and it is clinically accessible, making it an attractive target for intratumoral biological therapies.

Experimental Design: OBP-301 is a type 5 adenovirus that contains the replication cassette in which the human telomerase reverse transcriptase promoter drives expression of the E1 genes. OBP-401 contained the replication cassette and the green fluorescent protein (GFP) gene. The antitumor effects of OBP-301 were evaluated in vitro by the sodium 30-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro)benzene sulfonic acid hydrate assay and in vivo in an orthotopic xenograft model. Virus spread into the lymphatics was also orthotopically assessed by using OBP-401.

Results: Intratumoral injection of OBP-301 resulted in the shrinkage of human SCCHN tumors orthotopically implanted into the tongues of BALB/c nu/nu mice and significantly recovered weight loss by enabling oral ingestion. The levels of GFP expression following ex vivo infection of OBP-401 may be of value as a positive predictive marker for the outcome of telomerase-specific virotherapy. Moreover, whole-body fluorescent imaging revealed that intratumorally injected OBP-401 could visualize the metastatic lymph nodes, indicating the ability of the virus to traffic to the regional lymphatic area and to selectively replicate in neoplastic lesions, resulting in GFP expression and cell death in metastatic lymph nodes.

Conclusions: These results illustrate the potential of telomerase-specific oncolytic viruses for a novel therapeutic and diagnostic approach, termed theranostics, for human SCCHN.

Cancer remains a leading cause of death worldwide despite improvements in diagnostic techniques and clinical management (1, 2). An estimated 500,000 patients worldwide are diagnosed with squamous cell carcinoma of the head and neck (SCCHN) annually. This aggressive epithelial malignancy is associated with a high mortality rate and severe morbidity among the long-term survivors (3). Current treatment strategies for advanced SCCHN include surgical resection, radiation, and cytotoxic chemotherapy. Although a combination of these modalities can improve survival, most patients eventually experience disease progression that leads to death; disease progression is often the result of intrinsic or acquired resistance to treatment (4, 5). A lack of specificity for tumor cells is the primary limitation of radiotherapy and chemotherapy. To improve the therapeutic index, there is a need for anticancer agents that selectively target only tumor cells and spare normal cells.

Replication-selective tumor-specific viruses present a novel approach for cancer treatment (6, 7). We reported previously that telomerase-specific replication-competent adenovirus (OBP-301, Telomelyn), in which the human telomerase reverse transcriptase (hTERT) promoter element drives the expression of E1A and E1B genes linked with an IRES, induced selective E1 expression, and efficiently killed human cancer cells but not normal cells (8–10). We also found that intratumoral injection of telomerase-specific replication-selective adenovirus expressing the green fluorescent protein (GFP) gene (OBP-401, TelomeScan) causes viral spread into the regional lymphatic area with subsequent selective replication in...
metastatic lymph nodes in nu/nu mice (11). Although up to 25% of patients with SCCHN develop distant metastasis to the lung, liver, or bone, lymph node metastases are more common in SCCHN patients (12); therefore, locoregional disease control with telomerase-specific oncolytic viruses may be a novel therapeutic strategy that is clinically applicable for the treatment of human SCCHN.

In the present study, we explore the therapeutic as well as diagnostic ability of telomerase-specific oncolytic viruses in vitro and in vivo. To this end, we adopted an orthotopic head and neck cancer xenograft model by inoculating human SCCHN cells into the tongues of nu/nu mice; this model resembles human SCCHN in a number of biological properties (13).

Materials and Methods

Cell lines and cell culture. The human oral squamous carcinoma cell lines SAS-L, SCC-4, SCC-9, HSC-2, HSC-3, and HSC-4 were maintained in vitro as monolayers in DMEM supplemented with 10% heat-inactivated fetal bovine serum, 100 units/mL penicillin, and 100 mg/mL streptomycin (complete medium). The human non–small-cell lung cancer cell line H460 and the human esophageal cancer cell line TE8 were routinely propagated in monolayer culture in RPMI 1640 supplemented with 10% fetal bovine serum. The normal human lung diploid fibroblast cell line WI38 (JCRB0518) was obtained from the Health Science Research Resources Bank (Osaka, Japan) and grown in Eagle’s MEM with 10% fetal bovine serum. The normal human lung fibroblast NHLF (TaKaRa Biomedicals) and the normal human embryonic lung fibroblast MRC-5 (RIKEN BioResource Center) were cultured according to the vendors’ specifications.

Adenoviruses. The recombinant replication-selective, tumor-specific adenovirus vector OBP-301 (Telomelyn), in which the hTERT promoter element drives the expression of E1A and E1B genes linked with an IRES, was previously constructed and characterized (8–10). OBP-401 is a telomerase-specific replication-competent adenovirus variant with the replication cassette, and GFP gene under the control of the cytomegalovirus promoter was inserted into the E3 region for monitoring viral replication (11, 14). The viruses were purified by ultracentrifugation in cesium chloride step gradients, their titers were determined by a plaque-forming assay using 293 cells, and they were stored at −80°C.

Cell viability assay. An sodium 30-[1-phenylaminocarbonyl]-3,4-tetrazolum]-bis(4-methoxy-6-nitro)benzene sulfonic acid hydride (XTT) assay was done to assess the viability of tumor cells. Human SCCHN cells (1,000 per well) were seeded onto 96-well plates 18 to 20 h before viral infection. Cells were then infected with OBP-301 at a multiplicity of infection (MOI) of 1, 10, 50, and 100 plaque-forming units (pfu) per cell. Cell viability was determined at the indicated time points by using a Cell Proliferation Kit II (Roche Molecular Biochemicals) according to the protocol provided by the manufacturer.

Fluorescence microplate reader. Cells were infected with OBP-401 at the indicated MOI values in a 96-well black-bottomed culture plate and further incubated for the indicated time periods. GFP fluorescence was measured by using a fluorescence microplate reader (DS Pharma Biomedical) with excitation/emission at 485 nm/528 nm.

Animal experiments. SAS-L and HSC-3 human oral squamous cell carcinoma cells were harvested and suspended at a concentration of 5 × 10^6/mL in the medium. To generate an orthotopic head and neck cancer model, 6-wk-old female BALB/c nu/nu mice were anesthetized and injected directly with 20 μL of cell suspension at a density of 10^6 cells. The cells were injected into the right lateral border of the tongue with a 27-gauge needle. When the tumor grew to 2 to 3 mm in diameter (~5 to 7 days later), 20 μL of solution containing 1 × 10^6 pfu of OBP-301, OBP-401, or PBS were injected into the tumor. The perpendicular diameter of each tumor was measured every 3 d, and tumor volume was calculated by using 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 body weights of mice were monitored and recorded. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Okayama University.

In vivo fluorescence imaging. In vivo GFP fluorescence imaging was acquired by illuminating the animal with a Xenon 150-W lamp. The reemitted fluorescence was collected through a long-pass filter on a Hamamatsu C5810 3-chip color charge-coupled device camera (Hamamatsu Photonics Systems). High-resolution image acquisition was accomplished by using an EPSON PC. Images were processed for contrast and brightness with the use of Adobe Photoshop 4.0.1 software (Adobe). A fluorescence stereomicroscope (SZX7; Olympus) was also used to visualize GFP-positive tissues.

Statistical analysis. The statistical significance of the differences in the in vitro and in vivo antitumor effects of viruses was determined by using the Student’s t test (two-tailed). The antitumor effect viruses on orthotopically implanted tumors in nude mice were assessed by plotting survival curves according to the Kaplan-Meier method. P values <0.05 were considered statistically significant.

Results

In vitro cytopathic efficacy of OBP-301 on human SCCHN cell lines. We examined the cytopathic effect of OBP-301, which is an attenuated adenovirus in which the hTERT promoter element drives expression of E1A and E1B genes linked with an internal ribosome entry site (IRES; Fig. 1A), on various human SCCHN cell lines by the XTT cell viability assay. OBP-301 infection induced cell death in human SCCHN cells in a dose-dependent manner; the sensitivity, however, varied among different cell lines (Fig. 1B). The ID_{50} values calculated from the dose-response curves confirmed that SAS-L cells could be efficiently killed by OBP-301 at a multiplicity of infection (MOI) of <150 (ID_{50} = 148), whereas HSC-3 cells were less sensitive to OBP-301 (ID_{50} = 500; Fig. 1C).
To confirm the specificity of telomerase activity in human SCCHN cells, we next measured the expression of hTERT mRNA in a panel of human SCCHN cell lines and normal cell lines by using a real-time reverse transcription-PCR method. Although the levels of expression varied widely, all SCCHN cell lines expressed detectable levels of hTERT mRNA, whereas human fibroblast cells such as NHLF and WI38 were negative for hTERT expression (Supplementary Fig. S1A). We also examined the expression levels of coxsackievirus and adenovirus receptor on the cell surface of each type of cell by flow cytometric analysis. Apparent amounts of coxsackievirus and adenovirus receptor expression were detected on SAS-L and HSC-3 human SCCHN cells (Supplementary Fig. S1B).

To assess whether viral replication was restricted to tumor cells, we next examined the replication efficiency of OBP-301 by measuring the relative amounts of E1A DNA. SAS-L human SCCHN cells and MRC-5 human fibroblasts were harvested at indicated time points over 72 h after infection with OBP-301 and subjected to quantitative real-time PCR analysis. The ratios were normalized by dividing the value of cells obtained 2 h after viral infection. OBP-301 replicated 3 to 4 logs within 48 h after infection; the viral replication, however, was attenuated up to 2 logs in normal MRC-5 cells (Supplementary Fig. S2).

The response of tumor cells to DNA-damaging stimuli such as chemotherapeutic drugs and ionizing radiation is predetermined by the functional status of their p53 gene (15); however, the p53 status of human SCCHN cell lines (wild-type p53 [SAS-L], mutant p53 [SCC-4, HSC-2, HSC-3, HSC-4], and deleted p53 [SCC-9]) is not related to their sensitivity to OBP-301. Indeed, OBP-301 similarly killed parental SAS-L cells and cells stably transfected with the mutant p53 gene (Supplementary Fig. S3), suggesting that OBP-301 induces cell death in a p53-independent manner.

Selective replication of OBP-401 in human SCCHN cell lines in vitro. OBP-401 is a genetically engineered adenovirus that expresses GFP by inserting the GFP gene under the control of the cytomegalovirus (CMV) promoter into the E3 region of OBP-301 (Fig. 1A). To determine whether OBP-401 replication is associated with selective GFP expression in human SCCHN cells, cells were analyzed and recorded by using a time-lapse fluorescent microscope after OBP-401 infection. Representative images at the indicated time points are shown (Fig. 2A).
human SCCHN cells expressed bright GFP fluorescence as early as 12 h after OBP-401 infection at a MOI of 50. The fluorescence intensity gradually increased in a dose-dependent manner, followed by rapid cell death due to the cytopathic effect of OBP-401, as evidenced by floating, highly light-refractile cells under phase-contrast photomicrographs.

We also quantified GFP expression in human SCCHN cells following OBP-401 infection by using a fluorescence plate reader. Relative expression levels of GFP gradually increased in a dose-dependent manner (Fig. 2B). Moreover, we found an apparent inverse correlation between relative GFP expression at 72 h after OBP-401 infection and the ID_{50} values of OBP-301 in various human cancer cell lines, indicating that the outcome of OBP-301 treatment could be predicted by measuring GFP expression following OBP-401 infection.

In vivo antitumor effect of intratumoral injection of OBP-301 in an orthotopic nude mouse model of human SCCHN. To assess the effect of OBP-301 on SCCHN in vivo, we used an orthotopic animal model for SCCHN in which SAS-L cells were implanted into the tongues of BALB/c nu/nu mice. Histopathologic examination of the excised primary tumors showed a tumor formation composed of implanted SAS-L cells with a solid architecture (Fig. 3A). Mice bearing palpable SAS-L tumors with a diameter of 3 to 5 mm received three courses of intratumoral injections of 10^8 pfu of OBP-301 or PBS (mock treatment) every 3 days beginning on the 7th day (regimen 1) or 10th day (regimen 2) after the initial tumor inoculation (Fig. 3B). Representative images from each group showed that tumors treated with OBP-301 starting on day 7 after tumor inoculation were consistently smaller than those of mock-treated mice 28 days after the first viral injection (Fig. 3C).
Tumor growth at the primary site and body weight were continuously monitored. Intratumoral injection of OBP-301 in both regimens induced a gradual reduction in tumor volumes compared with mock-treated mice. Mice with tumor shrinkage significantly recovered body weight starting on day 10 (regimen 1) or day 15 (regimen 2) after the last virus injection ($P < 0.05$), although there was a decrease in body weight in the control group (Fig. 3D). This antitumor effect could be observed in mice orthotopically implanted with HSC-3 cells; the appearance of the effect, however, was ~4 to 5 days slower than that of SAS-L tumor-bearing mice (Supplementary Fig. S4).

**Locoregional spread of virus following virotherapy in an orthotopic human SCCHN model.** SCCHN patients with metastases to regional lymph nodes have a poorer prognosis than patients without nodal metastases (16). To verify whether adenoviruses could traffic to regional lymph nodes through the lymphatics, we injected $1 \times 10^8$ pfu of OBP-401 into SAS-L tumors implanted into the tongues of mice. Five days after virus injection, primary tongue tumors as well as lymph node metastases could be detected as light-emitting spots with GFP fluorescence under the optical charge-coupled device imaging (Fig. 5A). We also found that OBP-401 could infect and replicate in SAS-L cells trafficking in lymphatic vessels (Fig. 5B). These results suggest that although adenoviruses could effectively drain to regional lymph nodes, OBP-401 replicated only in metastatic lymph nodes, which was confirmed by a histopathologic analysis. Metastatic SCCHN cells were mostly observed in the lymph nodes with fluorescence emission, whereas most of GFP-negative lymph nodes contained no tumor cells (Fig. 5C).

The optical imaging detected 13 lymph nodes labeled in spots with GFP fluorescence in 14 metastatic nodes (sensitivity of 92.9%). Among 21 metastasis-free lymph nodes, 3 nodes were GFP positive (specificity of 85.7%). In another orthotopic model implanted with HSC-3 human SCCHN cells, we could also detect GFP signals in one or two metastatic lymph nodes but not in other nonmetastatic nodes and salivary glands (Fig. 5; Supplementary Fig. S5).

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Fig. 3. Antitumor effects of OBP-301 in vivo in an orthotopic SCCHN model. A, tumor sections were obtained 35 d after tumor cell implantation. Paraffin-embedded sections of SAS-L tongue tumors were stained with H&E. Scale bar, 100 μm. Top, ×40 magnification; bottom, detail of the boxed region of the top panel; magnification, ×400. B, orthotopic animal experiment regimens. The tongues of BALB/c nu/nu mice were inoculated with $1 \times 10^5$ SAS-L human SCCHN cells. Orthotopic tumor-bearing mice received three courses of intratumoral injection of $1 \times 10^8$ pfu of viruses every 3 d starting on day 7 (regimen 1) or day 10 (regimen 2) after tumor cell inoculation. Eight mice were used in each group. C, macroscopic appearance of SAS-L tongue tumors on BALB/c nu/nu mice 5 d (top) or 35 d (bottom) after tumor cell inoculation. Representative tumors treated with PBS or OBP-301 are shown. Note the eradicated tumors in mice that received OBP-301 injection. Green arrowhead, SAS-L tumors. D, orthotopic tumor-bearing mice received three courses of intratumoral injection of $1 \times 10^8$ pfu of viruses every 3 d starting on day 7 (regimen 1; top) or day 10 (regimen 2; bottom) after tumor cell inoculation. The tumor volume (left) and the body weight (right) were monitored and plotted. Point, mean; bars, SD. Statistical significance was defined as $P < 0.05$. 

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Prolonged survival following OBP-301 virotherapy in an orthotopic human SCCHN model. Finally, we assessed the effect of intratumoral injection of OBP-301 on survival time of SAS-L–bearing mice. Mice treated with OBP-301 beginning either on the 7th day (regimen 1) or the 10th day (regimen 2) after tumor implantation survived significantly longer (mean = 27.4 or 33.7 days) than mice without treatment (mean = 14.7 or 24.3 days; regimen 1, \( P = 0.017 \); regimen 2, \( P = 0.016 \); Fig. 6). The prolonged survival might reflect an antitumor effect of oncolytic adenoviruses spreading into the locoregional area, including regional lymph nodes.

Discussion

The present study illustrates the potential application of replication-selective oncolytic adenoviruses as an anticancer agent in human SCCHN patients. We found that intratumoral administration of telomerase-specific oncolytic adenovirus induced tumor volume reduction as well as the recovery of weight loss by enabling oral ingestion in an orthotopic xenograft model, in which human SCCHN cells were implanted into the tongues of BALB/c nu/nu mice. Oncolytic virotherapy also prolonged the survival of SCCHN tumor-bearing mice, presumably due to the locoregional antitumor effect against primary tumors and lymph node metastases with viruses spreading into the lymphatics.

Telomerase-specific oncolytic adenovirus OBP-301 exhibits a broad cytopathic effect against human cancer cell lines of different tissue origins (8–10). In a panel of human SCCHN cell lines, OBP-301 also showed apparent antitumor effects in vitro in a dose-dependent manner (Fig. 1B), although the sensitivity varied greatly between cell lines despite hTERT and coxsackievirus and adenovirus receptor expression (Supplementary Fig. S1). We have previously found that the process of oncolysis is morphologically distinct from apoptosis and necrosis (17). The cell death machinery triggered by OBP-301 infection is still under the investigation, although autophagy is partially involved in this effect (17, 18). OBP-301 has been developed based on the ability of the hTERT promoter to control replication of the virus in the tumors, leading to selective killing of tumor cells and minimal undesired effects on normal cells; the ID\(_{50}\) values of OBP-301 in various human cancer cell lines, however, were not related to the levels of hTERT mRNA expression (8, 10). Indeed, HSC-3 and HSC-4 human SCCHN cells expressing high levels of hTERT mRNA were less sensitive to OBP-301 than SCC-4 and SCC-9 cells with low levels of hTERT expression. Thus, neither hTERT expression nor coxsackievirus and adenovirus receptor expression could be useful for predicting the outcome of OBP-301 treatment.

Biomarkers have been extensively studied and often used to predict the potential therapeutic benefit of new agents, including molecular-targeted therapies (19). There is a widely recognized need for biomarkers that could improve the...
clinician’s ability to select suitable drugs for appropriate patients. We found that the levels of GFP expression following OBP-401 infection were highly associated with ID_{50} values of OBP-301 in individual cell lines in vitro (Fig. 2C). This correlation may be an expected result, because OBP-301 and OBP-401 have the same genomic backbone except for the GFP expression cassette. Although it is necessary to establish the assay procedures for GFP-based fluorescence measurement in more detail, we propose the diagnostic application of OBP-401 to predict tumor responses to OBP-301. For example, when the biopsy tissue samples of the tumor are exposed to OBP-401 for a certain amount of time ex vivo, the levels of GFP expression may be of value as a positive predictive marker for the outcome of OBP-301 virotherapy. Further prospective clinical studies are required to confirm the direct correlation between the GFP expression in biopsy samples following ex vivo OBP-401 infection and the clinical responses to OBP-301 in patients with SCCHN.

An orthotopic nude mouse model to investigate the cellular and molecular mechanisms of metastasis in human neoplasia was first described by Fidler et al. (20, 21) and Killion et al. (22). The orthotopic implantation of tumor cells restores the correct tumor-host interactions, which do not occur when tumors are implanted in ectopic subcutaneous sites (20). To further explore the in vivo antitumor effects of telomerase-specific virotherapy for SCCHN, we used an orthotopic nude mouse model of human tongue squamous cell carcinoma. In our preliminary experiments, we inoculated tumor cells into the tongue of BALB/c nu/nu mice and confirmed the formation of tumors with a diameter of 3 to 5 mm after 5 days and the development of metastases in neck lymph nodes after 35 days. We also identified the presence of disseminated tumor cells in the regional lymph nodes at least 10 days after tumor cell implantation by using GFP-expressing SAS-L human SCCHN cells (data not shown). Intratumoral injection of OBP-301 done 7 or 10 days after tumor inoculation significantly shrunk the tongue SAS-L tumor volumes, which in turn increased the body weight of mice by enabling oral ingestion (Fig. 3D). Moreover, HSC-3 cells were relatively resistant to OBP-301 in vitro; intratumoral injection of OBP-301 was, however, effective for recovering the body weight in mice bearing HSC-3 tongue tumors after a long-term observation (Supplementary Fig. S4). These results suggest that although the appearance of the effect may be slower, the in vivo antitumor activity could be

Fig. 5. Virus spread of OBP-401 via lymphatics to regional lymph nodes on HSC-3 tumor-bearing mice. A, selective visualization of lymph node metastasis in orthotopic xenografts of HSC-3 human SCCHN cells. Mice received intratumoral injection of OBP-401 at the concentration of 1 × 10^8 pfu after 24 d of tumor inoculation and were assessed for lymph node metastasis 5 d later under fluorescence stereomicroscope. B, HSC-3 primary tumor, salivary glands, and lymph nodes were excised 5 d after OBP-401 injection and then assessed for GFP fluorescence. 1 to 4, lymph nodes; 5 and 6, salivary glands. C, other HSC-3 tumor-bearing mice. Excised primary tumors, salivary glands, and lymph nodes were assessed for GFP fluorescence.
expected even in resistant SCCHN tumors. Because the body weight loss due to a feeding problem in this orthotopic SCCHN model resembles the disease progression in SCCHN patients, the finding that OBP-301 increased the body weight of mice suggests that OBP-301 virotherapy could potentially improve the quality of life in advanced SCCHN patients.

Amplified viruses can infect adjacent tumor cells as well as reach metastatic lymph nodes via the lymphatic circulation. We have previously shown that the telomerase-specific OBP-401—expressing GFP could be delivered into human tumor cells in regional lymph nodes and replicate with selective GFP fluorescence after injection into the primary tumor in an orthotopic rectal tumor model (11). In the orthotopic SCCHN model, OBP-401 spread into the neck lymph nodes after injection into the primary tongue tumor and selectively replicated in metastatic nodules (Figs. 4 and 5; Supplementary Fig. S5). The sensitivity and specificity of this imaging strategy for SAS-L tumors are 92.9% and 85.7%, respectively, which are sufficiently reliable to support the concept of this approach. These results suggest that surgeons may be able to excise primary tumors as well as metastatic lymph nodes precisely with appropriate margins by using this novel surgical navigation system with OBP-401. Moreover, the therapeutic profiles of OBP-401 and OBP-301 are considered similar, and a histopathologic analysis showed the destruction of micrometastases by virus in metastatic lymph nodes. This regional antitumor effect of oncolytic viruses could have a significant effect on the prolongation of the survival of mice bearing orthotopic tumors (Fig. 6).

Targeted therapies such as the anti–epidermal growth factor receptor monoclonal antibody cetuximab and other small-molecule epidermal growth factor receptor–tyrosine kinase inhibitors have been developed for SCCHN. Although a phase III trial showed a survival benefit with cetuximab and standard platinum-based therapy in SCCHN patients (23), some patients are exquisitely sensitive to these drugs and can develop particular and severe toxicities (24). A phase I study is currently under way in the United States to determine the feasibility and to characterize the pharmacokinetics of OBP-301 in patients with histologically proven nonresectable solid tumors (25). An interim analysis of the first 12 patients, including four SCCHN patients treated with escalating doses of OBP-301, indicates that OBP-301 virotherapy is well tolerated without any severe adverse events, suggesting that OBP-301 may be much more potent than other targeted therapies for human SCCHN in terms of specificity, efficacy, and toxicity.

In conclusion, our data clearly indicate that telomerase-specific oncolytic adenoviruses have a significant therapeutic potential against human SCCHN in vitro and in vivo. Moreover, these viruses can be used in an ex vivo diagnostic assay to predict the therapeutic potential of the virus in SCCHN patients. The combination of a diagnostic assay with a therapeutic entity is termed theranostics (26). Telomerase-specific oncolytic viruses can be used to treat the patients and to identify the patients who will likely benefit from virotherapy (Supplementary Fig. S6). In addition, telomerase-specific in situ imaging strategy has a potential of being widely available in humans as a navigation system in the surgical treatment of SCCHN. Thus, our oncolytic virus–based approach might be a novel “virotheranostics” for SCCHN. Phase II studies of telomerase-specific virotheranostics in advanced SCCHN patients are warranted.

Disclosure of Potential Conflicts of Interest

H. Onimatsu and Y. Urata are employed by Oncolyx BioPharma, Inc. T. Fujwara is a consultant to Oncolyx Biopharma, Inc.

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References

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