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REVIEW ARTICLE

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Multidisciplinary oncolytic virotherapy for gastrointestinal cancer

Toshiyoshi Fujiwara

Department of Gastroenterological Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

Correspondence
Toshiyoshi Fujiwara, Department of Gastroenterological Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Kita-ku, Okayama 700-8558, Japan.
Email: toshi_f@md.okayama-u.ac.jp

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Abstract

Replication-selective tumor-specific viruses represent a novel approach for treating neoplastic diseases. These vectors are designed to induce virus-mediated lysis of tumor cells after selective intracellular virus propagation. For targeting cancer cells, the use of tissue- or cell-specific promoters that are expressed in diverse tumor types but silent in normal cells is required. Human telomerase is highly active in more than 85% of primary cancers, regardless of tissue origin, and its activity is closely correlated with human telomerase reverse transcriptase (hTERT) expression. We constructed an attenuated adenovirus 5 vector (telomelysin, OBP-301) in which the hTERT promoter element drives expression of E1 genes. As only tumor cells that express the telomerase can activate this promoter, the hTERT proximal promoter allows for preferential expression of viral genes in tumor cells, leading to selective viral replication and oncolytic cell death. Upon US Food and Drug Administration approval, a phase 1 dose-escalation study of intratumoral injection of telomelysin for various solid tumors has been completed to confirm the safety, tolerability, and feasibility of the agent. Moreover, we found that adenoviral E1B 55-kDa protein in telomelysin inhibits the radiation-induced DNA repair machinery. Thus, tumor cells infected with telomelysin could be rendered sensitive to ionizing radiation. Recently, we assessed the safety and efficacy of intratumoral injection of telomelysin with radiotherapy in esophageal cancer patients not suited for standard treatments. This review highlights some very promising clinical advances in cancer therapeutic technologies using telomerase-specific oncolytic virotherapy.

KEYWORDS
adenovirus, clinical trial, esophageal cancer, radiotherapy, telomerase

1 | INTRODUCTION

Viruses are the simplest form of life, carrying genetic materials and the capacity to efficiently enter target host cells. Because of these properties, numerous types of viruses have been adapted as gene transfer vectors,[1–3] for which purpose adenoviruses have been well studied and characterized. Adenoviruses are large, double-stranded DNA viruses exhibiting tropism for many human tissues, such as bronchial epithelia, hepatocytes, and neurons. Moreover, adenoviruses can transduce nonreplicating cells and be grown to high titers *in vitro*, which allows for their potential clinical use. Replication-defective adenoviruses can be produced at high titers and have been successfully used to express genes in eukaryotic cells.[4,5] A variety of adenoviral gene therapy agents has been tested in various studies using *in vitro* and animal models. The tolerability, safety, and potential beneficial effects of these agents have been described for different target diseases[6,7] however, the three-dimensional spread of replication-deficient adenoviruses after intratumoral administration might be less than ideal.

Oncolytic viruses that can selectively replicate in tumor cells and lyse infected cells have been extensively investigated as novel anticancer agents.[8,9] These vectors are designed to induce virus-mediated lysis of tumor cells after selective intracellular propagation while remaining innocuous to normal tissues.[10] The optimal treatment of human cancers requires improvement of the therapeutic ratio to increase the cytotoxicity to tumor cells and decrease that against normal cells. This may not be an easy task, because the majority of normal cells surrounding tumors are sensitive to cytotoxic agents. Thus, to establish reliable therapeutic strategies for human cancers, it is important to seek genetic and epigenetic targets present only in cancer cells. One targeting strategy involve the use of tissue-specific promoters to restrict gene expression or virus replication to specific tissues. A large number of different tissue-specific promoters such as prostate-specific antigen (PSA),[11] Mucin 1,[12] osteocalcin,[13] L-plastin,[14] midkine,[15] and E2F-1[16] have been used for virotherapy applications. However, for targeting tumors derived from various tissues, tumor-specific, rather than tissue-specific, promoters would be more advantageous. For example, the promoter for human telomerase reverse transcriptase (hTERT) is highly active in most tumor cells but inactive in normal somatic cells.

2 | TELOMERASE ACTIVITY FOR TRANSCRIPTIONAL CANCER TARGETING

One of the hallmarks of cancer is the unregulated proliferation of specific cell populations, which eventually affects normal cellular function throughout the body, and this is almost universally correlated with telomerase reactivation. Telomerase is a ribonucleoprotein complex that mediates the addition of TTAGGG repeats to the telomeric ends of chromosomes. The enzyme consists of three components: an RNA subunit (known as hTR, hTER, or hTERC),[17] telomerase-associated protein (hTEP1),[18] and the catalytic subunit (hTERT).[19,20] Both hTR and hTERT are required for the reconstitution of telomerase activity *in vitro*[21] and thus represent the minimal catalytic core of telomerase in humans.[22] However, although hTR is widely expressed in embryonic and somatic tissues, hTERT expression is tightly regulated and not detectable in most somatic cells. The strong association between telomerase activity and malignant tissues suggests that telomerase is a plausible target for the treatment of cancer.[23] Thus, the hTERT proximal promoter can be used as a molecular switch for the selective expression of target genes in tumor cells, as almost all advanced human cancer cells express telomerase, whereas most normal cells do not.[24,25]

3 | GENETIC ENGINEERING OF AN HTERT PROMOTER-DRIVEN ONCOLYTIC ADENOVIRUS

The use of modified adenoviruses that replicate and complete their lytic cycles preferentially in cancer cells is a promising strategy for the treatment of cancer. One approach to achieve tumor specificity of viral replication is based on the transcriptional control of genes that are critical for virus replication, such as *E1A* and *E1B*. The catalytic subunit of telomerase, hTERT, is expressed in the majority of human cancers, and the hTERT promoter is preferentially activated in a variety of human cancer cells.[26] Therefore, the hTERT promoter may be a suitable regulator of adenoviral replication. It was previously reported that transcriptional control of E1A expression by the hTERT promoter restricts adenoviral replication to telomerase-positive tumor cells, resulting in efficient lysis of the tumor cells.[27–30]

We also examined the adenovirus *E1B* gene, which is expressed early in viral infection, and its gene product inhibits E1A-induced p53-dependent apoptosis, which in turn promotes the cytoplasmic accumulation of late viral messenger RNA (mRNA), leading to a shutdown of host cell protein synthesis. In most vectors that replicate under transcriptional control of the *E1A* gene, including hTERT-specific oncolytic adenoviruses, the *E1B* gene is driven by the endogenous adenovirus *E1B* promoter. However, it was previously reported that transcriptional control of both the *E1A* and *E1B* genes by a single tumor-specific promoter with the use of an internal ribosome entry site significantly improves the specificity and therapeutic index in particular human tumor cells.[31] Thus, we developed telomelysin (OBP-301), in which the tumor-specific hTERT promoter regulates expression of both the *E1A* and *E1B* genes (Figure 1). Telomelysin is expected to control viral replication more stringently, thereby providing greater therapeutic efficacy against tumor cells, as well as attenuated toxicity in normal tissues.[32]

4 | PRECLINICAL STUDIES OF THE HTERT PROMOTER-DRIVEN ONCOLYTIC ADENOVIRUS

In preclinical experiments, telomelysin-induced selective E1A and E1B expression in cancer cells, which resulted in virus replication at 5-6 logs by 3 days after infection, but telomelysin replication was attenuated up to 2 logs in cultured normal cells.[32] *In vitro* cytotoxicity assays demonstrated that telomelysin efficiently kills both epithelial and mesenchymal types of malignant tumor cells (including those of lung cancer, gastric cancer, esophageal cancer, colorectal cancer, head and neck cancer, hepatic cancer, cervical cancer, breast cancer, osteosarcoma, pancreas cancer, prostate cancer, melanoma, and mesothelioma) in a dose-dependent manner (Figure 2).[33] In addition, intratumoral injection of telomelysin in subcutaneous and orthotopic xenograft tumor models was efficacious in treatment of both primary tumors[32,34] and regional lymph node metastasis.[35,36] Indeed, when telomelysin was intratumorally injected into human colorectal tumors orthotopically implanted into the rectum in nude mice, telomelysin caused viral

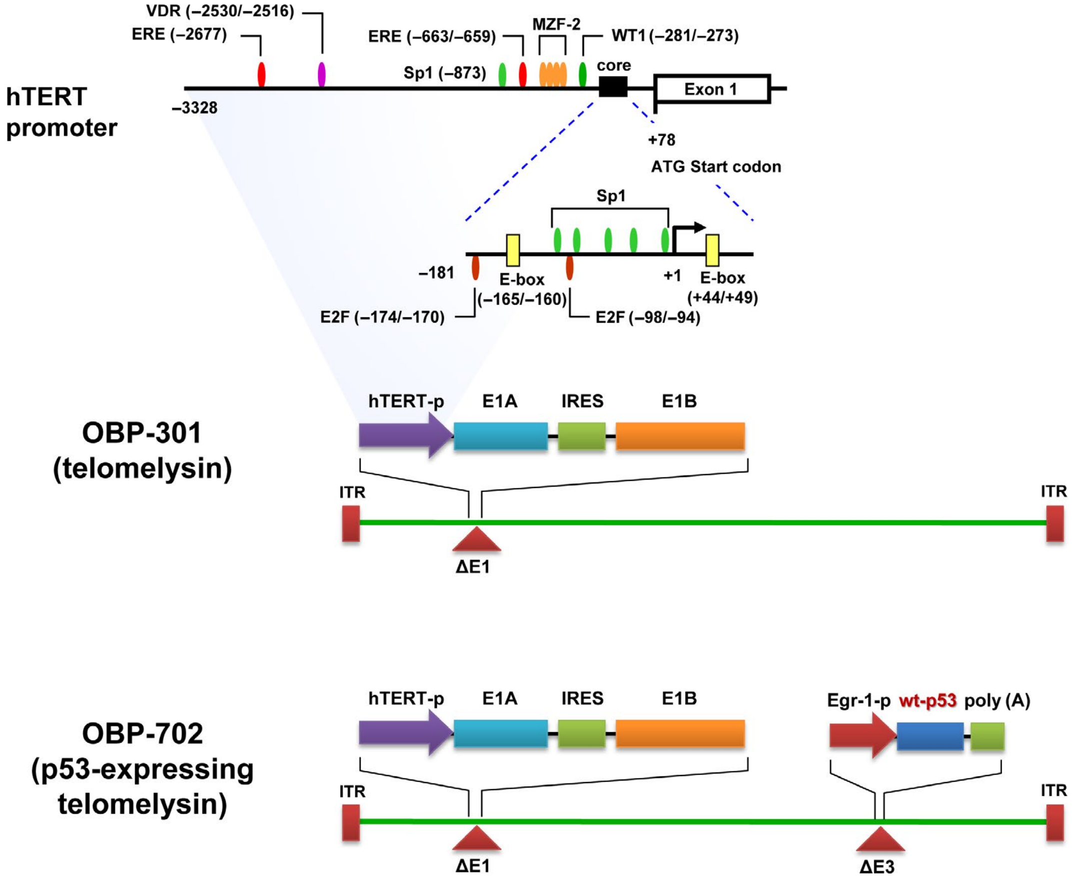

FIGURE 1 Structures of telomerase-specific oncolytic adenoviruses. In telomelysin (OBP-301), the human telomerase reverse transcriptase (hTERT) promoter element drives the expression of *E1A* and *E1B* linked with internal ribosome entry site. OBP-702 is a telomerase-specific replication-competent adenovirus variant, in which the Egr-1 promoter drives expression of the wild-type *p53* (wt-p53) gene inserted into the E3 region. Upper panel, scheme of the proximal hTERT promoter. Putative protein binding sites for various transcriptional factors are indicated. ITR, inverted terminal repeat

spread into the regional lymphatic area and selectively replicated in neoplastic lesions, resulting in tumor cell-specific death in metastatic lymph nodes.[35] Thus, telomelysin not only exhibits features that make it desirable for use as an oncolytic therapeutic agent, the proportion of cancers potentially treatable using telomelysin is extremely high.

To further enhance the antitumor effect of telomelysin-based oncolytic virotherapy, we evaluated the therapeutic potential of telomelysin in combination with conventional radiotherapy. Ionizing radiation primarily induces double-strand breaks (DSBs) in DNA molecules. Radiosensitization can result from a therapeutic increase in DNA DSBs or inhibition of their repair. The MRN complex, consisting of Mre11, Rad50, and NBS1, is quickly stimulated by DSBs and directly activates ataxia-telangiectasia mutated (ATM), an important signal transducer in the DNA damage repair response, which involves DNA repair and cell cycle checkpoints.[37] Therefore, defects in the MRN complex induce hypersensitivity to DNA damage.[38] We found that expression of the MRN complex in cancer cells decreased after telomelysin infection when E1B 55-kDa protein expression began, leading to inhibition of ATM phosphorylation by ionizing radiation and inhibition of DNA repair (Figure 3). Telomelysin infection apparently sustains elevated levels of γH2AX (a hallmark of DNA DSBs) for longer periods in irradiated tumor cells. These findings indicate that tumor cells infected with telomelysin could be rendered sensitive to ionizing radiation.[39]

5 | CLINICAL APPLICATION OF THE HTERT PROMOTER-DRIVEN ONCOLYTIC ADENOVIRUS AS MONOTHERAPY

Many clinical trials of adenoviruses testing their oncolytic properties are well underway (Table 1).[40–44] To explore the clinical application of telomelysin, Oncolys BioPharma Inc. was established in March 2004 as an Okayama University-launched bio-venture company. Based on preclinical studies, an open-label, phase 1 dose-escalation study of intratumoral injection of telomelysin was initiated in the USA to validate the safety, tolerability, and feasibility of telomelysin as monotherapy in patients with advanced solid tumors.[45] The trial commenced following approval by the US Food and Drug Administration in October 2006. The doses of telomelysin were escalated in 1-log increments from low to high virus particles (vp). Sixteen patients with a variety of solid tumors, such as melanoma, head and neck cancer, breast cancer, lung cancer, and sarcomas,

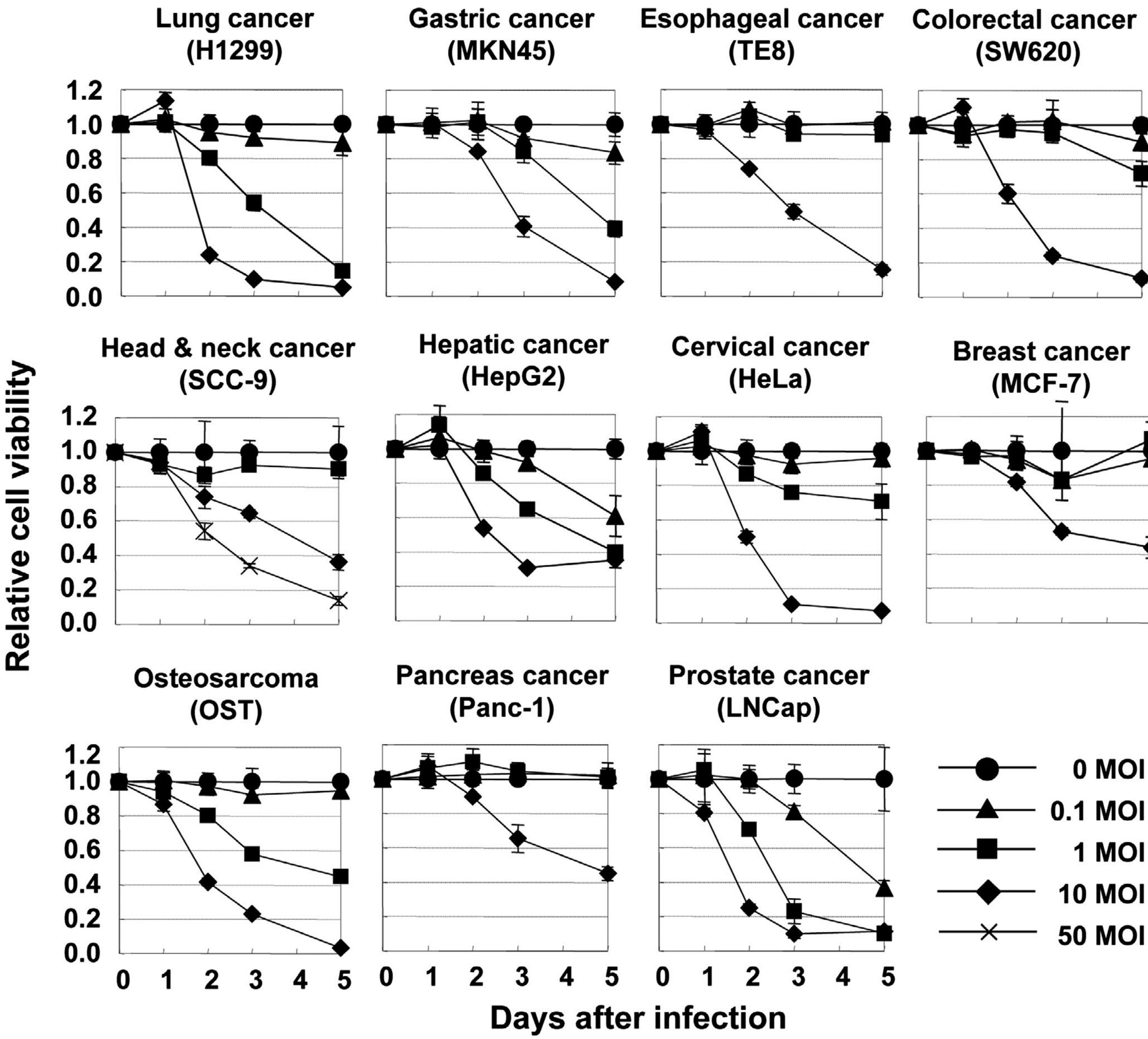

FIGURE 2 Oncolytic effects of telomelysin *in vitro* on a variety of human cancer cell lines.[33] Cells were infected with telomelysin at indicated multiplicity of infections (MOI) values, and surviving cells were quantitated over 5 days by XTT assay. Data are mean ±SD

were treated with a single-dose intratumoral injection of telomelysin and then monitored over 1 month.

All patients received telomelysin without dose-limiting toxicity. Common grade 1 and 2 toxicities included injection site reactions (pain, induration) and systemic reactions (fever, chills). Pharmacokinetic and biodistribution data for telomelysin were of particular interest. Circulating viral DNA was transiently (<6 h after injection) detected in plasma from 13 of 16 patients within 24 h after injection. This dose-dependent initial peak in circulating virus titer was followed by a rapid decline; however, three patients demonstrated evidence of prolonged viral replication through detection of viral DNA in plasma on days 7 and 14, suggesting the replication of telomelysin in primary tumors. In one of these patients, the injected malignant lesion and loco-regional uninjected satellite nodules disappeared, fulfilling the definition of a complete response (CR) at day 28. Eleven patients satisfied RECIST criteria for stable disease response in the injected lesion at day 28, whereas three patients had progressive disease, and two patients were unevaluable. Six patients exhibited reductions in tumor size of 6.6%-33%, and one of these patients exhibited a 33% reduction in the size of the injected lesion at day 28 and 56.7% reduction at day 56. These clinical data suggest that telomelysin is well-tolerated and warrants further clinical studies of its use in treating solid cancers.

6 | MULTIDISCIPLINARY APPROACH USING THE HTERT PROMOTER-DRIVEN ONCOLYTIC ADENOVIRUS

The advantage of combination therapy with telomelysin plus conventional radiotherapy is that the areas in which each treatment demonstrates its

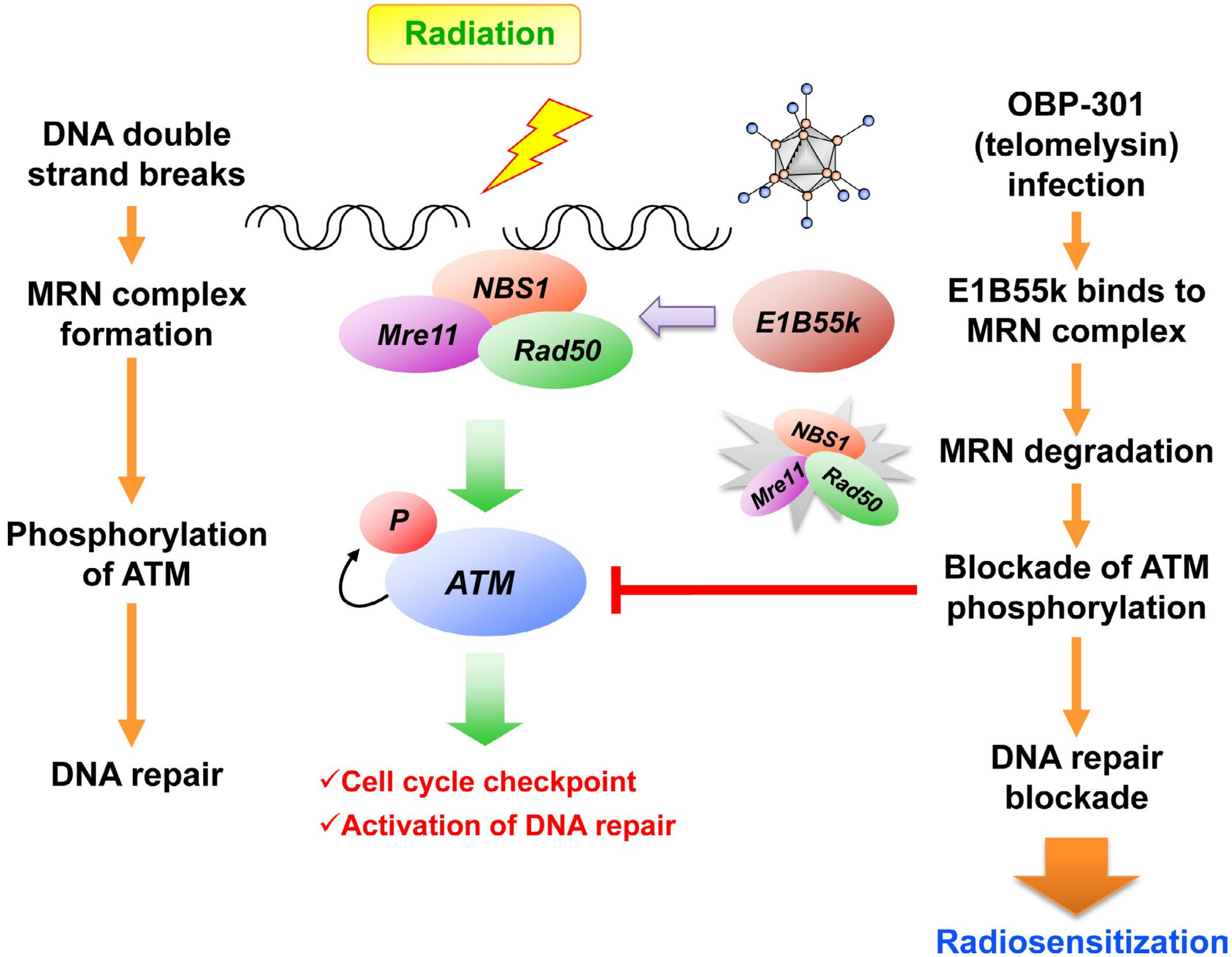

FIGURE 3 Molecular mechanism of telomelysin-induced radiosensitization. The MRN complex plays a role as an upstream sensor in response to DNA double-strand breaks. Degradation of the MRN complex by E1B 55-kDa protein prevents ataxia-telangiectasia mutated (ATM) autophosphorylation and signaling, leading to inhibition of the ATM-dependent G_2/M checkpoint

therapeutic effect overlap. We previously demonstrated that intratumorally injected telomelysin expressing the *GFP* gene effectively traffics to the regional lymph nodes, as viral replication produced green fluorescent protein-associated fluorescence signals in metastatic lymph nodes in orthotopic human colorectal and oral cancer xenograft models.[46,47] Therefore, we hypothesize that intratumoral administration of telomelysin would radiosensitize both primary tumors and regional lymph nodes.

An open-label, phase 1, dose-escalation study was conducted in esophageal cancer patients to further determine the feasibility, efficacy, and pharmacokinetics of telomelysin in combination with radiotherapy (UMIN 000010158; Figure 4). Thirteen patients with histologically confirmed esophageal cancer who were deemed unfit to receive standard therapies such as surgery and chemotherapy were enrolled into this study. Virus administration was performed by intratumoral injection of the primary or metastatic tumor via a flexible endoscope. Study treatment consisted of intratumoral needle injections of telomelysin on days 1, 18, and 32 of treatment. Radiation therapy was administered concurrently over 6 weeks, beginning on day 4, to a total of up to 60 Gy. Virus distribution and shedding into the body fluids, including the saliva, sputum, urine, and plasma, were monitored using a quantitative DNA-polymerase chain reaction assay.

Of 13 patients, seven, three, and three patients were included in cohorts treated with $10,^{10},^{11}$ and 10^{12} vp of telomelysin, respectively. The patient group comprised 10 men and three women, with a median age of 79.7 years (range, 53-92 years). Common grades 1 and 2 toxicities included fever, esophagitis, pneumonitis, anorexia, constipation, and gastroesophageal reflux. All patients developed a transient, self-limited lymphopenia. Distribution studies revealed the presence of viral DNA in five of the six patients who received 10^{11} or 10^{12} vp of telomelysin, but this was rarely detected in the gargle, saliva, and urine. Eight patients had a local CR; all of these patients exhibited no viable malignant cells in biopsy specimens. Three patients had a partial response. The objective response rate was 91.7%. The clinical CR rate was 83.3% in stage I and 60.0% in stages II and III. Histopathologic examination of post-treatment specimens showed massive infiltration of $CD8^+$ cells in three partial response tumors. These findings indicate that treatment involving multiple

TABLE 1 Oncolytic adenoviruses in clinical testing

Oncolytic adenovirus	Serotype	Mechanism of action	Indication(s)	Clinical phase	Ref.
CG0070	Adenovirus type 5	Human E2F-1 promoter drives *E1A* gene Armed by the addition of the *GM-CSF* gene	Bladder	Phases I/II	40
DNX-2401/AdΔ24-RGD (tasadenoturev)	Adenovirus type 5	E1A-Δ24 mutation in pRb-binding site Integrin-binding RGD motif	Brain	Phases I & II	41
ColoAd1 (enadenotucirev)	Adenovirus type 3/11p	Group B Ad11p/Ad3 chimeric adenovirus	Solid tumors	Phases I & I/II	42
ONCOS-102	Adenovirus type 5/3	E1A-Δ24 mutation in pRb-binding site Pseudotyped with Ad3 knob Armed by the addition of the *GM-CSF* gene	Solid tumors	Phases I & I/II	43
VCN-01	Adenovirus type 5	E1a mutated in the pRB-binding site Retargeting RGDK modification of the fiber Expression of hyaluronidase	Pancreatic cancer	Phase I	44
OBP-301 (telomelysin)	Adenovirus type 5	Human telomerase reverse transcriptase promoter drives *E1* genes	Esophageal cancer HCC, melanoma	Phases I/II & II	45

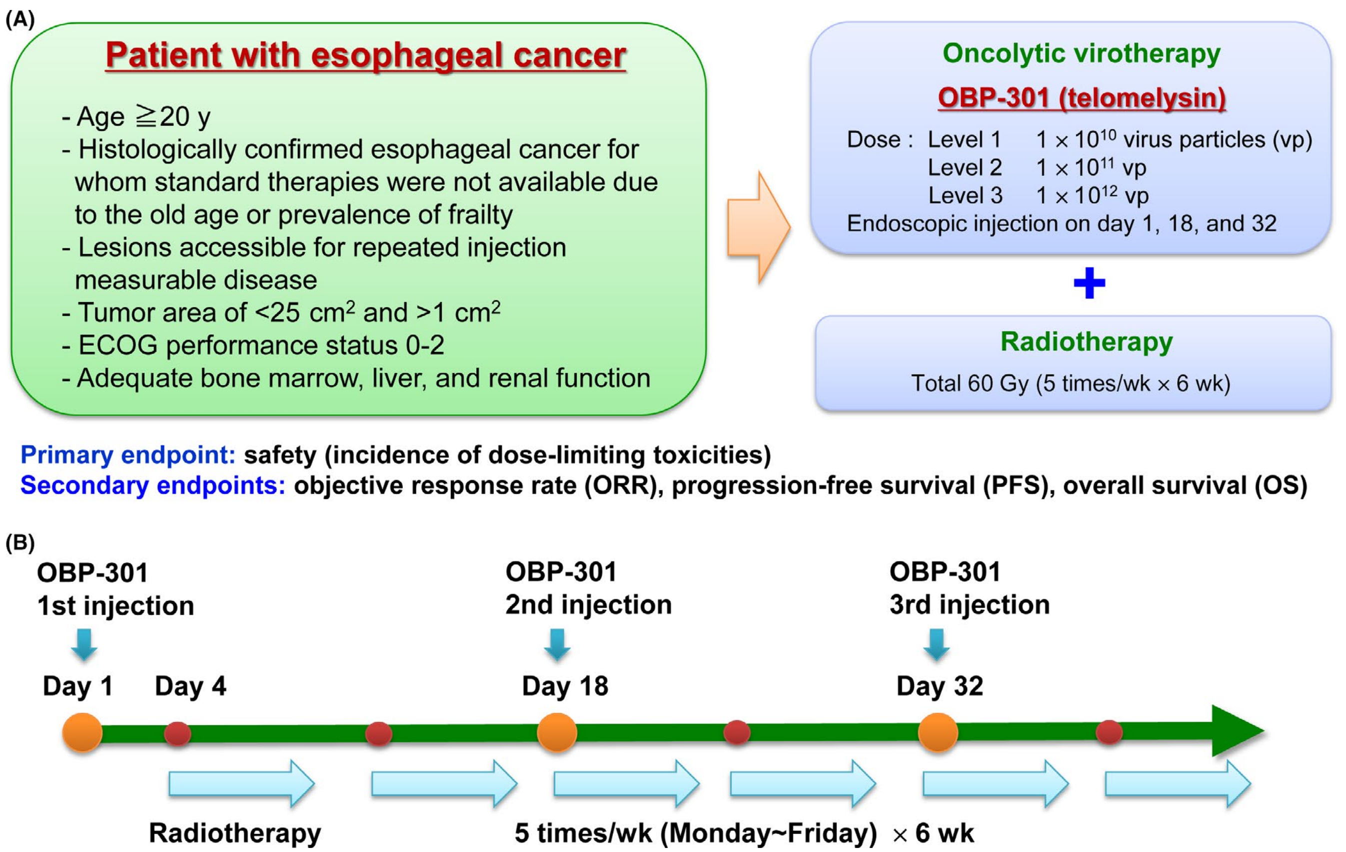

FIGURE 4 Schematic outline of strategies for treating esophageal cancer. A. Synopsis of the study design and objectives. B. Schematic illustration of the treatment schedule. Patients with esophageal cancer who are not eligible for standard treatments receive intratumoral needle injections of telomelysin on days 1, 18, and 32. Radiation therapy is administered concurrently over 6 weeks, beginning on day 4, up to a total of 60 Gy

courses of endoscopic telomelysin injection in combination with radiotherapy is feasible and provides clinical benefits in patients with esophageal cancer, particularly those who are unfit for standard treatments.

7 | NEXT-GENERATION MULTIFUNCTIONAL HTERT PROMOTER-DRIVEN ONCOLYTIC ADENOVIRUS

Oncolytic viruses armed to express several types of therapeutic transgenes have been reported.[48,49] Among candidate therapeutic transgenes, the tumor suppressor *p53* gene is particularly potent and exhibits a variety of effects, including induction of cell cycle arrest, apoptosis, senescence, and DNA repair.[50] Indeed, a p53-expressing replication-deficient adenovirus (Ad-p53, Advexin) was shown to induce antitumor effects both *in vitro* and *in vivo*,[51–53] as well as in various clinical studies.[54,55] Therefore, to develop a next-generation hTERT promoter-driven oncolytic adenovirus, we modified telomelysin (OBP-301) to express the wild-type *p53* tumor suppressor gene (OBP-702; Figure 1) and compared the antitumor activity of OBP-702 with that of telomelysin.[56,57]

The antitumor effects of OBP-702 and telomelysin were compared using telomelysin-sensitive and -resistant human cancer cells. OBP-702 suppressed the viability of both telomelysin-sensitive and -resistant cancer cells more efficiently than telomelysin. OBP-702 exhibited greater induction of apoptosis of cancer cells compared to telomelysin and replication-deficient Ad-p53. Adenovirus E1A-mediated miR-93/106b upregulation induced p21 suppression followed by MDM2 downregulation, leading to p53-mediated apoptosis in OBP-702-infected cells. p53 overexpression enhanced adenovirus-mediated autophagy via activation of the damage-regulated autophagy modulator (DRAM). Moreover, OBP-702 significantly suppressed tumor growth in subcutaneous and orthotopic tumor xenograft models compared to monotherapy with telomelysin or Ad-p53. These findings suggest that OBP-702-mediated p53 transactivation is a promising antitumor strategy for inducing both apoptotic and autophagic cell death pathways via regulation of microRNA and DRAM in human cancers.

To produce high-titer virus in quantities required for clinical trials, large-scale manufacturing of OBP-702 is ongoing under Good Manufacturing Practice conditions.

8 | CONCLUSIONS AND PERSPECTIVES

Significant advances have been made in our understanding of the molecular aspects of human gastrointestinal cancers and the development of technologies for viral genome modification. Transcriptional targeting is a powerful tool that enables tumor selectivity in cancer therapy, and the hTERT promoter-driven oncolytic adenovirus we developed exhibits more specific targeting potential due to the amplifying effects of viral replication.

A promising future application for telomelysin includes combination therapy with conventional approaches such as radiotherapy, chemotherapy, surgery, and immunotherapy. Indeed, in April 2019, the Japanese Ministry of Health, Labour and Welfare designated telomelysin in combination with radiotherapy an innovative pharmaceutical product under the SAKIGAKE Designation System. Designated products are eligible for prioritized consultation services, with a reduction in the premarket review period to as short as 6 months, half the standard review period of 12 months. Moreover, Oncolys BioPharma Inc. granted an exclusive license to Chugai Pharmaceutical Co., Ltd., one of Japan's leading research-based pharmaceutical companies, concerning the development, manufacture, and marketing of telomelysin in Japan and Taiwan. It is expected these events will accelerate the clinical development of telomelysin.

Clinical trials of telomelysin in combination with immunotherapy or chemoradiotherapy are also underway. An open-label, phase 1 study (EPOC1505) has been conducted to evaluate the safety and efficacy of telomelysin with anti-programmed death 1 (PD-1) antibody, pembrolizumab, in patients with advanced solid tumors. A phase 2 study of telomelysin in combination with pembrolizumab for esophagogastric adenocarcinoma was initiated in May 2019 at Weill Cornell Medical College in the USA. Moreover, NRG Oncology, a non-profit research organization in the USA, is planning a phase 1 study of telomelysin combined with definitive chemoradiation consisting of carboplatin/paclitaxel for treating locally advanced esophageal cancer in patients who are medically inoperable.

The field of targeted oncolytic virotherapy has progressed considerably, such that it is rapidly gaining medical and scientific acceptance. Although some conceptual and regulatory problems remain to be solved, ongoing and future clinical studies are expected to provide important data that will lead to further substantial progress in human gastrointestinal cancer therapies.

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Author Contribution: TF is solely responsible for the literature review and drafting of the manuscript.

ORCID

Toshiyoshi Fujiwara https://orcid.org/0000-0002-5377-6051

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