A Novel Molecular Therapy Using Bioengineered Adenovirus for Human Gastrointestinal Cancer

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Replication-selective tumor-specific viruses constitute a novel approach for treatment of neoplastic disease. These vectors are designed to induce virus-mediated lysis of tumor cells after selective viral propagation within the tumor. Human telomerase is highly active in more than 85% of primary cancers, regardless of their tissue origins, and its activity correlates closely 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. Since only tumor cells that express telomerase activity would activate this promoter, the hTERT proximal promoter would allow for preferential expression of viral genes in tumor cells, leading to selective viral replication and oncolytic cell death. Lymphatic invasion is a major route for cancer cell dissemination, and adequate treatment of locoregional lymph nodes is required for curative treatment in patients with gastrointestinal tumors. We demonstrated that intratumoral injection of Telomelysin mediates effective in vivo purging of metastatic tumor cells from regional lymph nodes. Moreover, using noninvasive whole-body imaging, we found that intratumoral injection of Telomelysin followed by regional irradiation induces a substantial antitumor effect, resulting from tumor cell-specific radiosensitization, in an orthotopic human esophageal cancer xenograft model. These results illustrate the potential of oncolytic virotherapy as a promising strategy in the management of human gastrointestinal cancer.

Key words: telomerase, adenovirus, metastasis, lymph node, colorectal cancer

Viruses are the simplest form of life carrying genetic materials and are capable of entering host cells efficiently. Because of these properties, many 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 with tropism for many human tissues such as bronchial epithelia, hepatocytes, and neurons. Furthermore, they are capable of transducing nonreplicating cells and can be grown to high titers in vitro, a feature beneficial for clinical use. High titers of replication-defective adenoviruses can be produced and have been successfully used in eukaryotic gene expression [1, 4, 5]. Numerous studies using in vitro and animal models have tested a wide variety of adenoviral gene therapy agents and reported potential beneficial effects for different target diseases, including their tolerability and safety [6–9].

Oncolytic viruses that can selectively replicate in tumor cells and lyse infected cells have been extensively investigated as novel anticancer agents [3, 10]
11]. These vectors are designed to induce virus-mediated lysis of tumor cells after selective viral propagation within the tumor cell while remaining innocuous to normal tissues [12]. Onyx-015 is one such adenovirus with the E1B 55-kDa gene deleted, engineered to selectively replicate in and lyse p53-deficient cancer cells; clinical trials of intratumoral injection of Onyx-015 [13] alone or in combination with cisplatin/5-fluorouracil have been conducted in patients with recurrent head and neck cancer [14, 15]. However, a subsequent study clarified that the capacity of Onyx-015 to replicate independently of the cell cycle does not correlate with the status of p53 [16] but is determined by the late viral RNA export [17].

The optimal treatment of human cancer requires improvement of the therapeutic ratio, maximizing the cytotoxic efficacy of an agent on tumor cells while minimizing its effect on 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 cancer, it is important to seek genetic or epigenetic targets present only in cancer cells. One targeting strategy has involved the use of tissue-specific promoters to restrict gene expression or viral replication in specific tissues. A large number of different tissue-specific promoters have been used for virotherapy applications; however, tumor-specific rather than tissue-specific promoters would be more advantageous targets. For example, the promoter of human telomerase reverse transcriptase (hTERT) is highly active in most tumor cells but inactive in normal somatic cell types.

This review highlights some very promising advances in cancer therapeutic technologies using the hTERT promoter against human gastrointestinal cancer.

**Telomerase Activity for Transcriptional Cancer Targeting**

One of the hallmarks of cancer is unregulated proliferation of a certain cell population, which eventually affects normal cellular function in the human body. This process almost universally correlates with the activation of telomerase. Tumor cells can maintain telomere length predominantly due to telomerase, and its activity is detected in about 85% of malignant tumors [18], whereas telomerase is absent in most normal somatic tissues [19], with a few exceptions including peripheral blood leukocytes and certain stem cell populations [20, 21]. The strong association between telomerase activity and malignant tissue makes telomerase a plausible target for the diagnosis and treatment of cancer [22].

The enzyme telomerase is a ribonucleoprotein complex responsible for the addition of TTAGGG repeats to the telomeric ends of chromosomes, and contains three components: the RNA subunit (known as hTR, hTER, or hTERC) [23], the telomerase-associated protein (hTEP1) [24], and the catalytic subunit (hTERT) [25, 26]. Both hTR and hTERT are required for the reconstitution of telomerase activity in vitro [27] and, therefore, represent the minimal catalytic core of telomerase in humans [28]. Both hTR and hTERT transcripts are easily detectable in cancer cells but are either absent or at low levels in normal cells [29]; however, the hTR promoter is always stronger than hTERT with presumably more background [30]. Thus, the hTERT promoter region can be substantially used as a fine-tuning molecular switch that works exclusively in tumor cells (Fig. 1).

**hTERT Promoter-driven Telomerase-specific Oncolytic Adenovirus**

The use of modified adenoviruses that replicate and complete their lytic cycle preferentially in cancer cells is a promising strategy for the treatment of cancer. One approach to achieving tumor specificity of viral replication is based on the transcriptional control of genes that are critical for virus replication such as EIA or E4. As described above, telomerase, especially its catalytic subunit hTERT, is expressed in the majority of human cancers, and the hTERT promoter is preferentially activated in human cancer cells [18]. Thus, the broadly applicable hTERT promoter might be a suitable regulator of adenoviral replication. Indeed, it has been reported previously that the transcriptional control of EIA expression via the hTERT promoter could restrict adenoviral replication to telomerase-positive tumor cells and efficiently lyse tumor cells [31–36]. Furthermore, Kuppuswamy et al. have recently developed a novel oncolytic adenovirus
(VRX-011), in which the replication of the vector targets cancer cells by replacing an adenovirus E4 promoter with the hTERT promoter [37]. VRX-011 could also overexpress the adenovirus death protein (ADP) (also known as E3-11.6K), which is required for efficient cell lysis and release of virions from cells at late stages of infection.

The adenovirus E1B gene 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 mRNA, leading to a shut-down of host cell protein synthesis. In most vectors that replicate under the transcriptional control of the E1A gene including hTERT-specific oncolytic adenoviruses, the E1B gene is driven by the endogenous adenovirus E1B promoter. However, Li et al. [38] have demonstrated that transcriptional control of both E1A and E1B genes by the α-fetoprotein (AFP) promoter with the use of IRES significantly improved the specificity and the therapeutic index in hepatocellular carcinoma cells. Based on the above information, we developed Telomelyn (OBP-301), in which the tumor-specific hTERT promoter regulates both the E1A and E1B genes (Fig. 2). Telomelyn is expected to control viral replication more stringently, thereby providing better therapeutic effects in tumor cells as well as attenuated toxicity in normal tissues [39].

**In Vitro and In Vivo Antitumor Efficacy of Telomelyn in Human Gastrointestinal Cancer**

As the majority of human cancer cells acquire immortality and unregulated proliferation by expression of hTERT [18], hTERT-specific Telomelyn could theoretically possess a broad-spectrum antineoplastic activity against a variety of human tumors [39, 40]. Telomelyn induced selective E1A and E1B expression in cancer cells, which resulted in viral replication at 5-6 logs by 3 days after infection; on the other hand, Telomelyn replication was attenuated up to 2 logs in cultured normal cells [39, 40].

**In vitro** cytotoxicity assays demonstrated that Telomelyn could efficiently kill various types of human gastrointestinal cancer cell lines including esophageal cancer, gastric cancer, and colorectal cancer in a dose-dependent manner [41]. These data clearly demonstrate that Telomelyn exhibits desirable features for use as an oncolytic therapeutic agent, as the proportion of cancers potentially treatable by Telomelyn is extremely high.

The **in vivo** antitumor effect of Telomelyn was also investigated using athymic mice carrying xenografts, because most murine tumor cells are known to express
low levels of the coxsackievirus and adenovirus receptor (CAR). Intratumoral injection of Telomelyn into human colorectal tumor xenografts resulted in a significant inhibition of tumor growth and enhancement of survival [39, 40]. Macroscopically, massive ulceration was noted on the tumor surface after injection of high-dose Telomelyn, indicating that Telomelyn induced intratumoral necrosis due to direct lysis of tumor cells by virus replication in vivo [42].

**Lymph Node Metastasis in Human Gastrointestinal Cancer**

Lymph node status provides important information for both the diagnosis and treatment of human gastrointestinal cancer. Lymphatic invasion is a major route for cancer cell dissemination, and lymph node metastasis represents aggressive tumor behavior and is associated with high rates of regional recurrence, which portends a poor outcome and may produce marked morbidity [43-45]. Therefore, adequate resection of the locoregional lymph nodes is required for curative treatment in patients with gastrointestinal malignancies such as esophageal, gastric, and colorectal cancers [46, 47]. Extended lymphadenectomy, however, may greatly impair quality of life, especially for patients with early-stage epithelial neoplasms in the gastrointestinal tract [48]. Their primary tumors can be removed by new endoluminal therapeutic techniques such as endoscopic submucosal dissection; however, patients with submucosal invasion, lymphovascular infiltration of cancer cells, or undifferentiated histology often become candidates for surgical organ resection with lymphadenectomy, because there is a risk of regional lymph node metastasis, although the frequency is relatively low [49]. For example, resection of upper gastrointestinal organs such as gastrectomy and esophagectomy may result in body weight loss and microgastria. A less invasive way to selectively treat lymph node metastasis would benefit these patients by allowing them to avoid prophylactic surgery.

**In Vivo Lymphatic Spread of Virus on Regional Lymph Nodes**

The therapeutic potential of viral agents against primary tumors as well as their systemic biodistribu-
tion targeting distant metastases has been intensively investigated [3, 10, 50]. Few studies, however, have examined the ability of the virus to progress to the regional draining lymph nodes. Recently, Burton et al. showed that replication-deficient adenovirus could be successfully transported to the regional lymph nodes and non-invasively detect metastasis by expressing the prostate-specific reporter gene in an orthotopic prostate xenograft [51].

To verify that oncolytic adenoviruses progress through the lymphatic vessels to the regional lymph nodes, we used an orthotopic mouse model of human rectal cancer with spontaneous lymph node metastasis. We have demonstrated that intratumoral injection of the telomerase-specific replication-selective GFP-expressing adenovirus TelomeScan (OBP-401) (Fig. 2) could efficiently visualize metastatic lymph nodes with GFP fluorescence signals in human cancer xenograft models [52, 53]. These studies suggest the possible application of the adenovirus vectors as a lymphotropic agent for the treatment of lymph node metastasis.

**In Vitro Purging of Human Colorectal Cancer Cells by Telomelyn**

**In vitro** purging experiments demonstrated that Telomelyn infection could selectively eliminate human tumor cells in the presence of human or mouse lymphocytes [54]. We used TelomeScan to visualize viable human tumor cells after purging with Telomelyn, as we have previously shown the high sensitivity and specificity of this molecular imaging method [52, 53]. It has been reported that the fiber-modified adenovirus serotype 5 (Ad5) and an adenovirus vector based on another serotype such as 35 efficiently transduce exogenous genes into hematopoietic cells, including stem cells; the unmodified Ad5, however, could rarely infect these cells because of the lack of CAR expression [55]. Indeed, Ad5-based Telomelyn had no apparent effects on the viability of lymphocytes in vitro. These results suggest that normal lymphocytes in the regional lymph nodes could be strictly protected from Telomelyn-induced oncolysis because lymphocytes are not subject to Telomelyn infection and viral replication is also unlikely to occur in normal cells due to their low telomerase activity [20].

**In Vivo Antitumor Effect of Telomelyn on Lymph Node Metastasis**

Mice bearing orthotopic human colorectal tumors received 3 rounds of intratumoral injection of Telomelyn at 2-day intervals beginning 2 weeks after the tumor inoculation. Histopathological examination of the excised total lymph nodes showed that Telomelyn treatment considerably reduced the metastatic rates. We also used a simple real-time Alu PCR assay to quantify the few metastatic human tumor cells against a background of large numbers of mouse host cells [54]. This human-specific amplification method enabled us to detect human tumor cells in a linear range of $10^3$–$10^6$ cells/sample and to monitor the time-dependent exponential growth of spontaneous lymph node metastasis from orthotopic colorectal tumor xenografts. In accordance with the histologically confirmed results, the Alu PCR assay indicated that intratumoral injection of Telomelyn into the primary tumors significantly inhibited lymph node metastasis with high levels of viral replication. We also used TelomeScan and a three-dimensional optical detection system (IVIS 200) (Xenogen, Alameda, CA, USA) to compare the extent of metastasis after Telomelyn and control treatments. After 2 weeks of orthotopic implantation of human colorectal tumor cells, Telomelyn was administered intratumorally for 5 cycles. We then used the IVIS imaging system to explore the abdominal cavity at laparotomy following a single injection of TelomeScan into tumors. The number of GFP-positive lymph nodes and the GFP signal levels of individual lymph nodes were much higher in mock-treated control mice than in Telomelyn-treated mice. Indeed, the sum of GFP fluorescence intensity in the abdominal cavity was significantly lower in mice treated with Telomelyn, confirming the *in vivo* biological purging effect of Telomelyn. The fact that two independent and highly sensitive approaches showed comparable results suggests the potent *in vivo* purging effect of oncolytic virotherapy on regional lymph nodes.

For effective treatment of metastatic tumors, intravenously infused chemotherapeutic drugs must be distributed in sufficient concentrations into the tumor sites; by contrast, oncolytic viruses can replicate in the tumor, cause oncolysis, and then release virus particles that could reach distant metastatic lesions.
Moreover, intratumoral injection can avoid the hepatotoxicity that may be induced by systemic adenoviral administration. Therefore, low-dose intratumoral administration of oncolytic virus that causes the replication and release of newly formed viruses from tumor cells may be safer than traditional full-dose chemotherapy, and certainly, intratumoral administration of the virus allows for a safer dose than would its systemic administration.

Preoperative Intratumoral Administration of Telomelysin against Lymph Node Metastasis

Currently, surgery and radiation are the most effective and clinically reliable local management strategies for human malignancies including lymphatic metastases. Ionizing radiation targeting the lower half of the mouse body including primary tumors and the para-aortic lymphatic area did significantly inhibit lymph node metastasis, but systemic toxicity indicated by symptoms such as body weight loss was remarkable in irradiated mice compared to mice treated with Telomelysin. In fact, total body irradiation at a dose of 10 Gy has been reported to be lethal in mice because of acute radiation syndromes involving the hematopoietic system and gastrointestinal tract [56]. We demonstrated that regional injection of Telomelysin might be simpler and safer than radiotherapy as a treatment for metastatic lymph nodes [54].

We also assessed the effect of surgical resection of primary rectal tumors on lymph node metastasis. Unexpectedly, metastatic tumor cells in the lymph nodes considerably increased after surgical removal of primary rectal tumors, presumably due to the spread of tumor cells into the lymphatic circulation during the surgical procedure. Another possible explanation of this phenomenon includes a decrease in angiogenic inhibitors such as angiotatin and endostatin secreted from the primary tumor mass [57]. In contrast, intratumoral injection of Telomelysin prior to surgical resection significantly inhibited lymph node metastasis. Telomelysin causes viral spread into the regional lymphatic area and selectively replicates in neoplastic lesions, resulting in eradication of lymph node metastasis. Tumor cells infected with Telomelysin in the primary tumors are also unable to metastasize to the regional lymph nodes. Therefore, although the surgical procedure itself has the potential to promote regional metastasis, preoperative treatment with Telomelysin may prevent this undesirable event.

Ionizing Radiation and DNA Repair Machinery

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 cytotoxic effects on the surrounding normal tissues. Therefore, to improve the therapeutic index of radiotherapy, there is a need for agents that effectively lower the threshold for radiation-induced tumor cell death while not compromising the tolerance of normal cells. The safety and efficacy of some candidates are already being explored in clinical trials [58–60].

Ionizing radiation primarily targets DNA molecules and induces double-strand breaks (DSBs) [61]. 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 [62]. Cells with ATM gene mutations have defects in cell-cycle checkpoints and DNA repair and are hypersensitive to DSBs [63, 64]; thus, agents that inhibit the ATM pathway can be useful radiosensitizers [65]. The MRN complex, consisting of Mre11, Rad50 and NBS1, is quickly stimulated by DSBs and directly activates ATM [66, 67] (Fig. 3). Defects in the MRN complex lead to genomic instability, telomere shortening and hypersensitivity to DNA damage [68].
Telomelysin Sensitizes Human Cancer Cells to Ionizing Radiation

Virus infection and replication produce exogenous viral proteins, many of which manipulate the host cellular machinery to allow viral persistence in the life-cycle. The adenovirus EIB gene encodes a 19-kDa polypeptide (E1B19kDa) and a 55-kDa protein (E1B55kDa). These gene products are expressed early in viral infection and promote the cytoplasmic accumulation of late viral mRNA, leading to a shutoff of host cell protein synthesis. The E1B55kDa protein induces a cellular environment conducive to viral protein synthesis via a complex with the E4orf6 protein [69]. This E1B55kDa/E4orf6 complex degrades the MRN complex, blocks downstream ATM signaling, and leads to a defective G2/M checkpoint in response to DSBs [67]. 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 demonstrated that Telomelysin-mediated E1B55kDa expression induced the degradation of all components of the MRN complex, which in turn prevented ATM autophosphorylation and signaling, leading to the inhibition of the ATM-dependent G2/M checkpoint.

Our in vitro studies suggest that Telomelysin infection and ionizing radiation may mutually sensitize human tumor cells, potentially leading to an effective combination treatment. Telomelysin 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 Telomelysin may yield optimal results. We confirmed the synergistic antitumor effect of three cycles of treatment with Telomelysin plus regional radiation and the in vivo induction of apoptotic cell death on subcutaneous human 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 [72]. Thus, we also demonstrated the significant synergy of combined treatments in an orthotopic mouse model of human esophageal cancer by using a noninvasive whole-body imaging system.

Clinical Application of Telomelysin

Preclinical models suggested that Telomelysin could selectively kill a variety of human cancer cells in vitro and in vivo via intracellular viral replication regulated by hTERT transcriptional activity. Pharmacological and toxicological studies in mice and cotton rats demonstrated that none of the animals treated with Telomelysin showed signs of viral distress (e.g., ruffled fur, weight loss, lethargy, or agitation) or histopathological changes in any organs at autopsy. These promising data led us to design a phase I clinical trial of Telomelysin as a monotherapy.

The protocol “A phase I study of intratumoral injection with telomerase-specific replication-competent oncolytic adenovirus, Telomelysin (OBP-301) for various solid tumors,” sponsored by Oncolys BioPharma, Inc. (Minato-ku, Tokyo, Japan), is an open-label, phase I, 3-cohort dose-escalation study [73, 74]. The trial commenced following approval of the US Food and Drug Administration (FDA) in October, 2006. The study to assess the safety, tolerability, and feasibility of intratumoral injection of the agent in patients with advanced solid cancer has been completed. The doses of Telomelysin were escalated from low to high virus particles (VP) in one-log increments. Sixteen patients with a variety of solid tumors such as melanoma, head and neck cancer, breast cancer, lung cancer, and sarcomas were treated with a single-dose intratumoral injection of Telomelysin and then monitored over one 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). The data obtained on the pharmacokinetics and biodistribution of Telomelysin may be of interest. Clinical trials of intratumoral and intravenous administration of CG7870, a replication-selective oncolytic adenovirus genetically engineered to replicate preferentially in prostate tissue, demonstrated a second peak of the virus genome in the plasma [75, 76], suggesting active viral replication and shedding into the bloodstream. In fact, circulating viral DNA was transiently (<6 h after injection) detected in plasma in 13 of 16 patients within 24 h after injection. This dose-dependent initial peak in circulating virus was followed by a rapid decline; however, three patients demonstrated evidence of prolonged viral replication through detection of plasma viral DNA at days 7 and 14, suggesting Telomelysin replication in primary tumors. One of these 3 had disappearance of the injected malignant lesion and loco-regional un.injected satellite nodules, fulfilling a definition of complete response at day 28. Seven patients fulfilled the RECIST definition for stable disease by day 56 after treatment, while 6 patients showed 6.6 to 43% tumor size reduction. Thus, Telomelysin appears well-tolerated and warrants further clinical studies for solid cancer.

Perspectives

There have been very impressive advances in our understanding of the molecular aspects of human gastrointestinal cancer and in the development of technologies for genetic modification of viral genomes. Transcriptional targeting is a powerful tool for tumor selectivity in cancer therapy, and the hTERT-specific oncolytic adenovirus achieves a more strict targeting potential due to the amplified effect by viral replication. Several independent studies using different regions of the hTERT promoter and different sites of the adenoviral genome responsible for viral replication have shown that the hTERT promoter allows adenoviral replication as a molecular switch and induces selective cytopathic effects in a variety of human tumor cells [31–33, 39–41]. Among these viral constructs, to the best of our knowledge, Telomelysin
seems to be the first hTERT-dependent oncolytic adenovirus that has been used in a clinical trial based on preclinical pharmacological and toxicological studies. Thus, this telomerase-specific targeted oncolytic adenovirus holds promise for the treatment of human cancer.

It has been shown that Telomelyn delivered to the primary tumor site could spread into the regional draining lymphatic vessels, selectively replicate in neoplastic foci, and then reduce the number of tumor cells in metastatic lymph nodes in an orthotopic human colorectal cancer xenograft model [54]. This virus-mediated molecular surgery for lymph node metastasis mimics the clinical scenario of lymphadenectomy; the technique, however, seems to be safer and less invasive. Moreover, we demonstrated that preoperative delivery of Telomelyn into primary tumors prevented the exacerbation of lymph node metastasis by surgical procedures. Telomelyn may offer advantages over other oncolytic viruses targeting lymphatic metastasis, as its safety profile as well as biodistribution pattern after intratumoral delivery have already been confirmed in a phase I clinical trial for various types of solid tumors [73, 74]. Our study provides evidence for the in vivo purging effect of Telomelyn in regional lymph nodes that is sufficiently reliable to support this approach.

A possible future direction for Telomelyn includes combination therapy with conventional therapies such as chemotherapy, radiotherapy, surgery, immunotherapy, and new modalities such as antiangiogenic therapy. Since the results of a phase I clinical trial demonstrated that even partial elimination of the tumor induced by intratumoral injection of Telomelyn could be clinically beneficial, combination approaches may lead to the development of more advanced biological therapy for human cancer. The combination of systemic chemotherapy and local injection of Telomelyn has been previously shown to be effective [77–79]. As a replication-deficient adenovirus could replicate in cancer cells and enhance the anticancer effect when co-transfected with Telomelyn, which can produce the E1 protein, we demonstrated the synergistic effects of Telomelyn combined with an E1-deleted replication-deficient adenoviral vector expressing the human wild-type p53 tumor suppressor gene (Ad5CMV-p53, Advexin) [80, 81]. As described above, Telomelyn is also synergistic with ionizing radiation against human esophageal cancer cells, and we clarified the E1B55kDa-mediated mechanism used by Telomelyn to inhibit DNA repair. Peri- or post-operative administration of Telomelyn may also be valuable as an adjuvant therapy in areas of microscopic residual disease at tumor margins to prevent recurrence or regrowth of tumors.

The field of targeted oncolytic virotherapy is progressing considerably and is rapidly gaining medical and scientific acceptance. Although many technical and conceptual problems await solutions, ongoing and future clinical studies will no doubt continue to provide important clues that may allow substantial progress in human gastrointestinal cancer therapy. Thus, phase II studies of telomerase-specific virotherapy in human cancer patients are warranted.

References

10. Liu TC, Galanis E and Kirn D: Clinical trial results with oncolytic


42. Fujiwara T, Urata Y and Tanaka N: Telomerase-specific oncolytic virotherapy for human cancer with the hTERT promoter. Curr


