Diagnostic and therapeutic application of telomerase-specific oncolytic adenoviral agents

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1. ABSTRACT

Replication-selective tumor-specific viruses present 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. Telomerase activation is considered to be a critical step in carcinogenesis and its activity correlates closely with human telomerase reverse transcriptase (hTERT) expression. Since only tumor cells that express telomerase activity would activate this promoter, the hTERT proximal promoter allows for preferential expression of viral genes in tumor cells, leading to selective viral replication. We constructed an attenuated adenovirus 5 vector (Telomelysin, OBP-301), in which the hTERT promoter element drives expression of E1A and E1B genes linked with an internal ribosome entry site (IRES). Telomelysin replicated efficiently and induced marked cell killing in a panel of human cancer cell lines, whereas replication as well as cytotoxicity was highly attenuated in normal human cells lacking telomerase activity. We further modified the E3 region of OBP-301 to contain green fluorescent protein (GFP) gene for monitoring viral replication (TelomeScan, OBP-401). When TelomeScan was intratumorally injected into human tumors orthotopically implanted into the rectum in mice, para-aortic lymph node metastasis could be visualized at laparotomy under a three-chip color cooled charge-coupled device camera. Our results indicate that TelomeScan causes viral spread into the regional lymphatic area and selectively replicates in neoplastic lesions, resulting in GFP expression in metastatic lymph nodes. This article reviews recent highlights in this rapidly evolving field: cancer therapeutic and cancer diagnostic approaches using the telomerase-specific oncolytic adenoviruses.

2. INTRODUCTION

The optimal treatment for human cancer requires an improvement of therapeutic ratio to increase cytotoxic efficacy on the tumor cells and decrease that on the normal cells. This may not be an easy task because most of normal cells surround tumors are sensitive to the cytotoxic treatment. Thus, to establish reliable therapeutic strategies for human cancer, it is important to seek the genetic and epigenetic targets present only in cancer cells. The emerging fields of functional genomics and functional proteomics provide an expanding repertoire of clinically applicable targeted therapeutics (1).

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) (2), the telomerase-associated protein (hTEP1) (3), and the catalytic subunit (hTERT, human telomerase reverse transcriptase) (4, 5). Both hTR and hTERT are required for the reconstitution of telomerase activity in vitro (6) and, therefore, represent the minimal catalytic core of telomerase in humans (7). However, while hTR is widely expressed in embryonic and somatic tissue, hTERT is tightly regulated and is not detectable in most somatic cells. The hTERT proximal promoter can be used as a molecular switch for the selective expression of target genes in tumor cells (9-11), since almost all advanced human cancer cells express telomerase and most normal cells do not (12, 13).

Replication-defective, E1-deleted adenoviral vectors facilitate the efficient delivery of a variety of transgenes to target tissues and have demonstrated clear therapeutic benefits and safety in a variety of clinical
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Figure 1. Scheme of the mechanisms of tumor destruction with viral agents. Left, limited tumor destruction with non-replicating gene-therapy vector. Right, intratumoral replication, spread and cell death induction by virotherapy agent within tumor mass as well as metastatic lesions.

studies (14); a significant obstacle, however, is the limited distribution of the vectors within the tumor mass even after direct intratumoral administration (Figure 1). To confer specificity of infection and increase viral spread to neighboring tumor cells, the notion of using replication-competent adenoviruses has become a reality (15). We hypothesized that an adenovirus containing the hTERT promoter-driven E1 genes could be used to target a variety of tumor cells that display telomerase activity.

3. TELOMERASE-SPECIFIC ONCOLYTIC VIRUS FOR CANCER THERAPEUTICS

The use of modified adenoviruses that replicate and complete their lytic cycle preferentially in cancer cells is a promising strategy for treatment of cancer. We have developed Telomelysin (OBP-301), in which the tumor-specific hTERT promoter regulates both the E1A and E1B genes (Figure 2) (16, 17). Telomelysin controls the viral replication stringently, thereby providing profound therapeutic effects in tumor cells as well as the attenuated toxicity in normal tissues. Indeed, Telomelysin induced selective E1A and E1B expression in cancer cells, which resulted in viral replication at 5-6 logs by 3 days after infection; Telomelysin replication, however, was attenuated up to 2 logs in cultured normal cells. These data indicate that selective replication of Telomelysin is both therapeutically beneficial and safe.

The majority of human cancer cells acquire immortality and unregulated proliferation by expression of the hTERT (18) and, therefore theoretically, hTERT-specific Telomelysin can possess a broad-spectrum antineoplastic activity against a variety of human tumors. In vitro cytotoxicity assays demonstrated that Telomelysin could efficiently kill various types of human cancer cell lines including head and neck cancer, lung cancer, esophageal cancer, gastric cancer, colorectal cancer, breast cancer, pancreas cancer, hepatic cancer, prostate cancer, cervical cancer, melanoma, sarcoma, and mesothelioma in a dose-dependent manner (Figure 3). The dose of Telomelysin that causes about 50% reduction in cell viability in monolayer cultures (defined as ID$_{50}$) was less than 20 multiplicity of infections (MOIs) in almost all tumor cell lines examined in our study. These data clearly demonstrate that Telomelysin exhibits desirable features for use as an oncolytic therapeutic agent, as the proportion of cancers potentially treatable by Telomelysin is extremely high.

The in vivo antitumor effect of Telomelysin was also investigated by using athymic mice carrying
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Telomelysin (OBP-301)

TelomeScan (OBP-401)

Figure 2. Schematic DNA structures of telomerase-specific oncolytic viruses. Telomelysin (OBP-301), in which the hTERT promoter element drives the expression of E1A and E1B genes linked with an IRES. TelomeScan (OBP-401) is a telomerase-specific replication-competent adenovirus variant, in which GFP gene is inserted under CMV promoter into E3 region for monitoring viral replication.

Figure 3. Effect of Telomelysin on human cancer cells grown in culture. (a) Oncolytic efficacy induced by Telomelysin infection in vitro. Cell killing efficacy of human tumor (SW620 human colorectal cancer and human non-small cell lung cancer) and normal cells (normal human lung fibroblasts, NHLF) was evaluated by XTT assay. Statistical analysis was performed using Student's t-test for differences among groups. Statistical significance (*) was defined as $p < 0.01$. (b) Cytopathic effects associated with Telomelysin infection. Seven days after Telomelysin infection, cells were stained with Coomassie brilliant blue and documented as photographs.
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Figure 4. Effect of Telomelysin on human cancer cells grown in nu/nu mice. (a) Antitumor effects of intratumorally injected Telomelysin against established flank H1299 xenograft tumors in nu/nu mice. Eight mice were used for each group. The tumor growth was expressed by the tumor mean volume ± SD. Statistical significance (*) was defined as \( p < 0.05 \) (Student's \( t \)-test). (b) Macroscopic appearance of H1299 tumors on nu/nu mice 0, 14, and 28 days after treatment.

Intratumoral injection of Telomelysin into human tumor xenografts resulted in a significant inhibition of tumor growth and enhancement of survival (Figure 4) (16, 17). Macroscopically, massive ulceration was noted on the tumor surface after injection of high-dose Telomelysin, indicating that Telomelysin induced intratumoral necrosis of tumor cells due to direct lysis by virus replication in vivo. For effective treatment of distant metastatic tumors, intravenously infused chemotherapeutic drugs will need to distribute in sufficient quantities into the the tumor sites; oncolytic viruses, however, could still replicate in the tumor, cause oncolysis, and then release virus particles that could reach the distant metastatic lesions. Therefore, intratumoral administration that causes the release of newly formed virus from infected tumor cells might be theoretically suitable for oncolytic virus rather than systemic administration. Indeed, it was confirmed that, following intratumoral injection, Telomelysin replicated within tumors, spread into the bloodstream, and then replicated in distant tumor sites (16, 17). The biodistribution of Telomelysin as assessed by PCR amplification targeting for the viral E1A provides evidence that viral replication is highly specific for tumors despite its presence in the circulation. No significant elevation of liver enzymes was observed in mice intratumorally injected with Telomelysin. In addition, histopathological analysis of liver sections demonstrated absence of apoptotic hepatocytes and other histological signs of hepatocellular damage (17).

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 the hTERT transcriptional activity. These promising data led us to design a phase I clinical trial of Telomelysin as a monotherapy. The proposed protocol “A phase I dose-escalation study of intratumoral injection with telomerase-specific replication-competent oncolytic adenovirus, Telomelysin (OBP-301) for various solid tumors” sponsored by Oncolys BioPharma, Inc. is an open-label, phase 1, 3 cohort dose-escalation study. The Recombinant DNA Advisory Committee (RAC) at the National Institutes of Health (NIH) has already reviewed this protocol. The safety, tolerability, and feasibility of intratumoral injection of the agent will be assessed in patients with advanced cancer. The trial will be started upon approval of the US Food and Drug Administration (FDA).

4. TELOMERASE-SPECIFIC ONCOLYTIC VIRUS FOR CANCER DIAGNOSTICS

Metastatic spread of tumor cells plays a major role in the morbidity and mortality of human cancer. Although there are few life-prolonging treatments for the majority of patients with distant sites of metastasis, early detection of occult metastasis and early therapeutic interventions may decrease the rate of metastatic spread and extend survival. Lymphatic invasion is one of the major routes for cancer metastasis, and adequate resection of locoregional lymph nodes is required for curative treatment in patients with advanced malignancies. The risk of lymph node metastasis can be partially predicted by clinical data such as tumor stage, serum tumor marker level, and medical images; there are, however, no noninvasive approaches to accurately predict the presence of lymph node metastasis, in particular, microscopic metastasis. Although molecular analysis based on detection of genetic markers of cancer cells is clinically relevant in some patients, the procurement of sufficient tissue to confirm the diagnosis can be associated with significant morbidity and cost depending on the size and location of the lesion.

To distinguish normal from neoplastic tumor tissues, selective labeling of tumor cells is required. We modified Telomelysin to contain the GFP gene driven by the cytomegalovirus (CMV) promoter in the E3 deleted region to label efficiently and uniformly target tumor cells with green fluorescence. The resultant adenovirus was
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Figure 5. Selective visualization of lymph node metastasis in the orthotopic xenograft models of human colorectal cancer cells. (a) Macroscopic appearance of HT29 rectal tumor 4 weeks after tumor inoculation (b) External images of orthotopic HT29 tumor-bearing nu/nu mice injected with TelomeScan. (c) Gross appearance of the abdominal cavity. Five days after intratumoral injection of TelomeScan, HT29 tumor-bearing nu/nu mice were assessed for lymph node metastasis at laparotomy. (d) Internal imaging with the optical CCD camera visualized metastatic lymph nodes with GFP fluorescence.

There have been very impressive advances in our understanding of the molecular aspects of human cancer and in the development of technologies for genetic modification of viral genomes. Nevertheless, there are many remaining hurdles, ethical and technical that must be solved before virotherapy including virus-mediated gene therapy ever reach routine clinical application. The safety considerations in the virus manufacture and clinical protocols are among the most important issues to be studied. Another important issue is to find ways to selectively deliver viruses into a high percentage of malignant cells in an existing tumor mass. The use of tissue or cell-type specific promoters could perhaps achieve specificity of virus-mediated antitumor effect. The hTERT promoter-based transcriptional targeting in adenoviral constructs is a powerful tool for cancer diagnosis and therapy. In particular, the hTERT-specific oncolytic adenovirus achieves a more strict targeting potential due to the amplified effect by viral replication, and is a promising therapeutic alternative to replication-deficient gene therapy vectors. Several independent studies that used different regions of hTERT promoter and different sites of adenoviral genome responsible for viral replication, have shown that the hTERT promoter allows adenoviral replication as a molecular switch and induces selective cytopathic effect in a variety of human tumor cells (16, 22-24). Among these viral constructs, to the best of our knowledge, Telomelysin seems to be the first hTERT-dependent oncolytic adenovirus that will be used in a clinical trial based on preclinical pharmacological and toxicological studies.

The field of virotherapy is progressing considerably and is rapidly gaining medical and scientific acceptance. Although many technical and conceptual problems await to be solved, ongoing and future clinical studies will no doubt continue to provide important clues that may allow substantial progress in human cancer therapy.

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7. REFERENCES


5. CONCLUSIONS AND PERSPECTIVES

There have been very impressive advances in our understanding of the molecular aspects of human cancer and in the development of technologies for genetic modification of viral genomes. Nevertheless, there are many remaining hurdles, ethical and technical that must be solved before virotherapy including virus-mediated gene
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Abbreviations: hTERT: human telomerase reverse transcriptase; IRES: internal ribosome entry site; GFP: green fluorescent protein; hTEP1: telomerase-associated protein; MOIs: multiplicity of infections; CMV: cytomegalovirus

Key Words: telomerase, hTERT, adenovirus, GFP; Imaging, Review

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