Integrated Systems and Technologies

An Optimized Telomerase-Specific Lentivirus for Optical Imaging of Tumors

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Abstract

Advances in medical imaging techniques, such as ultrasound, computed tomography, magnetic resonance imaging, and positron emission tomography, have made great progress in detecting tumors. However, these imaging techniques are unable to differentiate malignant tumors from benign ones. Recently developed optical imaging of tumors in small animals provides a useful method to distinguish malignant tumors from their surrounding normal tissues. Human telomerase reverse transcriptase (hTERT) is normally inactivated in most somatic cells, whereas it is commonly reactivated in many cancer cells. In this study, we constructed a lentiviral vector that expresses green fluorescent protein (GFP) driven by an optimized hTERT promoter to create a noninvasive tumor-specific imaging methodology. The activity of this optimized hTERT promoter was found to be equal to the activity of SV40 and cytomegalovirus promoters. In vitro experiments showed that GFP was only expressed in telomerase-positive tumor cells infected with this lentivirus, whereas there was no GFP expression in telomerase-negative tumor cells or normal somatic cells. We also found that subcutaneous telomerase-positive tumors could be visualized 24 hours after an intratumoral injection with this lentivirus by using a charge-coupled device (CCD) camera. In contrast, telomerase-negative tumors could not be imaged after an intratumoral injection even for 30 days. These results suggest that infection with lentivirus containing this optimized hTERT promoter might be a useful diagnostic tool for the real-time visualization of macroscopically invisible tumor tissues using a highly sensitive CCD imaging system. Cancer Res; 70(7); 2585–94. ©2010 AACR.

Introduction

Early detection of malignant tumors and their metastasis is a vital factor for patients’ prognosis. In the past several decades, the world has seen a dramatic improvement in tumor detection by medical imaging techniques. Ultrasound, computed tomography, and magnetic resonance imaging are among the most common and effective tools that are used to locate and image tumors. However, these tools are unable to differentiate malignant tumors from benign ones, and also have limits in their target sizes. Positron emission tomography (PET), when combined with proper target agent, is better to identify malignant tumors than the above methodologies. However, the choosing of PET target reporter and injection of matched marker can be a hurdle for its application. For this reason, tumor-specific imaging that allows for the definitive identification of malignant tissues and the differentiation of tumor tissues and their surrounding normal tissues is of considerable value in the diagnosis and treatment of human cancers.

Optical imaging technology uses fluorescent proteins or luciferase genes as internal reporters and uses a supersensitive high-resolution charge-coupled device (CCD) camera as the external signal capture device to observe the location, movement, and distribution of genetically tagged targets (1, 2). This imaging strategy provides precise real-time information for researchers. With a proper tumor-specific promoter, fluorescent proteins or luciferase can be directionally tagged in tumor cells, which in turn could be noninvasively detected at a single-cell level by various kinds of multispectrum supersensitive CCD cameras, making it possible for researchers to create a noninvasive real-time diagnosis and tracking model for early-stage tumors.

To guarantee that the reporter gene is only expressed in tumor cells, selection of a tumor-specific promoter is important. At present, many tumor-associated antigens and their promoters, including carcinoembryonic antigen, α-fetoprotein, and prostate-specific antigen, are restricted to a very few number of tumor types. One consequence is a limit in the scope of their application as a tumor-specific promoter. Therefore, it is necessary to identify a universal tumor-specific promoter, which would allow for reporter gene expression specifically in tumor cells.

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Telomerase was first discovered in 1985 by Greider and Blackburn (2). A few years later, telomerase was commonly recognized as a unique and most effective anticancer target and was widely studied in tumor diagnosis and treatment (3–5). As a complex, human telomerase contains a reverse transcriptase, also known as the human telomerase catalytic subunit (hTERT), as its rate-limiting component (6, 7). The expression of hTERT is limited to telomerase-positive cells, whereas the other members of the complex are constitutively expressed in all cells (8). It has been shown that human telomerase is expressed in most malignant tumors (9). The tumor-specific activity of hTERT provides an opportunity for using it in cancer diagnosis and treatment. Numerous studies have been undertaken to uncover the molecular mechanisms of hTERT regulation in malignant tumors, including the first cloning and functional study of the hTERT promoter (10–12). Deletion analysis studies have identified a 260-bp region in the hTERT promoter as the key component in the regulation of hTERT expression. Within this core promoter region, Wang and colleagues (13) identified a distinct type of transcription factor–binding sites (E-box). These sites are able to bind a basic helix-loop-helix zipper encoded by the Myc family. Several other studies have confirmed that c-Myc binds to E-boxes on the hTERT promoter and activates its downstream targets (12–16).

Scientists had explored the tumor-selective effect of hTERT or human telomerase RNA (hTR) promoter. An outstanding work done by Groot-Wassink and colleagues (17) studied and compared the tumor selectivity of hTERT and hTR promoter using an adenovirus containing PET reporter gene. They successfully proved that both promoters had tumor selectivity and were able to specifically detect tumors by PET scanning. Following this inspiring study, we used an optimized hTERT promoter and a green fluorescent protein (GFP) reporter gene in a lentivirus system and achieved long-time optical imaging of the tumor growth in vivo. In this study, we adopted a 295-bp hTERT promoter sequence as previously described (18). This optimized hTERT contains the core region of the hTERT promoter and three inserted E-box (CACGTG) domains, ensuring upregulation of the transgene in telomerase-positive cells. The activity of the promoter was tested in a standard promoter function analysis system, pGL3-Basic, with cytomegalovirus (CMV), SV40, and original hTERT promoter as controls. Then, the promoter was subcloned into a lentivirus expression plasmid to detect its hTERT specificity in tumor cells in vitro and in vivo. Briefly, the lentivirus containing this optimized hTERT promoter was used to infect telomerase-positive or telomerase-negative tumor cell lines, as well as normal cell lines in vitro, to confirm the tumor-selective transgene expression of the lentiviral vector. An in vivo study was carried out by injecting this hTERT promoter lentivirus into mice bearing telomerase-positive or telomerase-negative tumors. The GFP expression was imaged by a whole-body optical imaging system. With the above in vitro and in vivo studies, we hope to provide a novel model for early detection of primary tumors and their metastasis.

### Materials and Methods

**Cell lines and telomerase determination.** Eight cell lines were chosen for this study. Telomerase-positive cell lines, including 293FT human embryonic kidney cell line, HepG2 liver cancer cell line, SGC-7901 human gastric cancer cell line, SW480 colon cancer cell line, and A375 human melanoma cell line, were purchased from the Institute of Biochemistry and Cell Biology (Shanghai, China). Telomerase-negative cell lines, including U2OS and SaoS-2 human osteosarcoma cell lines, were purchased from the American Type Culture Collection. Human primary embryonic fibroblast (HF) cells were a gift from Dr. Liang (Burn Research Institute, Third Military Medical University, Chongqing, China). 293FT, HepG2, SGC-7901, and HF cells were cultured in DMEM containing 10% FCS. SW480 and A375 cells were cultured in RPMI 1640 containing 10% fetal bovine serum. U2OS and SaoS-2 cells were maintained in McCoy’s 5A medium (Life Technologies, Inc.; Invitrogen). All cell cultures contained 100 units/mL penicillin, 100 μg/mL streptomycin, and 2 mmol/L l-glutamine.

**Optimized hTERT promoter design and luciferase assay.** An optimized hTERT promoter sequence was adopted as previously described (18). The promoter contains the reported core region of the hTERT as described by Takakura and colleagues (10). To enhance the upregulating effect of Myc binding at E-box sites in telomerase-positive cells (15, 19, 20), three more Myc-binding E-box (CACGTG) motifs were designed downstream of the transcriptional start site of the hTERT promoter core sequence. The optimized 295-bp promoter was synthesized and subcloned in pUC57, with enzyme sites ClaI and BamHI at either end for subsequent plasmid construction.

The optimized 295-bp hTERT promoter was synthesized in pUC57, then subcloned in pGL3-Basic (Promega), and named pGL3-hTERTp. The activity of the optimized hTERT was tested by a standard luciferase assay as described in a previous study (21). In this assay, we used plasmids pGL3-CMVp that contained CMV promoter, pGL3-SV40p that contained SV40 promoter, and pGL3-hTERTp (original) that contained the full-length cDNA of hTERT promoter as positive controls. pGL3-Basic plasmid that contained an empty promoter served as negative control. The five plasmids were used to transfect five telomerase-positive cell lines (293FT, HepG2, SGC-7901, SW480, and A375 cells) and three telomerase-negative cell lines (U2OS, SaoS-2, and HF cells), respectively. Luciferase activity in cells was assayed using Promega’s luciferase assay kit according to the manufacturer’s instruction.

**Lentiviral vector construction.** Lentivirus expression vector pLenti6/V5-D-TOPO, as well as its packaging plasmids pMDLg/pRRE, psV-Rev, and envelope plasmid pMD2.G, was purchased from Invitrogen. All endonuclease enzymes and markers used in this study were purchased from TaKaRa. GFP was amplified from pGFP-C1 and ligated into pLenti6/V5-D-TOPO using a pLenti6/V5 directional TOPO cloning kit from Invitrogen. This plasmid was named pLenti-CMVp-GFP. To replace the CMV promoter,
the optimized hTERT promoter was digested from pUC57 vector with Clal and BamHI and then ligated into the linearized pLenti-CMVp-GFP fragment that was digested using Clal and BamHI. This plasmid was named pLenti-hTERTp-GFP.

Lenti-CMVp-GFP and Lenti-hTERTp-GFP lentiviral vectors were produced by cotransfection of 293FT with the four plasmids as previously described (22). Briefly, 293FT cells were transduced with 20 μg of transferring vector construct pLenti-CMVp-GFP or pLenti-hTERTp-GFP, 10 μg of third-generation packaging construct pRSE-Rev, 15 μg of pMDLg/pRRE, and 7.5 μg of envelope plasmid pMD2.G by calcium phosphate. Seventy-two hours after transfection, cell supernatants were collected and filtered through 0.22-μm pore size filters. The vectors were concentrated 100-fold by ultracentrifugation before freezing and storing at −70°C.

Real-time PCR for lentiviral titer. Lentiviral titer was detected by real-time PCR. HeLa cells (5 × 10⁴) were infected with a serial dilution of prepared vectors in a 24-well plate in the presence of 8 μg/mL polybrene (Sigma). Seventy-two hours later, genomic DNA from infected HeLa cells was extracted and genic lentiviral RNA, proviral DNA copies, and transgene mRNA expression were analyzed by real-time PCR. Probes were labeled at the 5′ end with the reporter dye FAM (emission wavelength, 518 nm), whereas the 3′ end was labeled with the quencher dye TAMRA (emission wavelength, 582 nm). The 3′ end of the probe was additionally phosphorylated to prevent extension during PCR. For the detection of the WPRE sequence, the following primers and probes were used: forward primer (1277F), 5′-CCGGTTCAGCAACTGTGTCAAGGTT-3′; reverse primer (1361R), 5′-CCAGCCATGTACGTTGCTATCCAGGC-3′; probe (1314P), 5′-FAM-TTCAGGACCGAAACCCCCACTGT-TAMRA-3′. For detection of β-actin, the sequences of primers and probe were as follows: forward primer, 5′-GGCAGAAGATGACCCAGCTC-3′; reverse primer, 5′-CCAGTGTTAGCGCAGAAGG-3′; probe, 5′-FAM-CCAGGCTGTACGTCATTCCAGC-TAMRA-3′. The concentration of lentivirus was calculated using the following equation: transducing units (TU)/mL of lentiviral vectors = copies (WPRE/β-actin) × cell number (5 × 10⁴) × dilution times × 1,000 μL/mL.

Determination of the optimal multiplicity of infection. To determine the optimal multiplicity of infection (MOI) of the lentivector, we transduced the 293FT cell line with the two lentiviral vectors (Lenti-CMVp-GFP and Lenti-hTERTp-GFP) at a MOI of 1 and 2, visualized GFP, and photographed the cells at 0, 2, 12, 24, 48, and 72 h.

In vitro visualization of tumor cell lines. Telomerase-positive cell lines 293FT, HepG2, SGC-7901, SW480, and A375 and telomerase-negative cell lines U2OS, SaoS-2, and HepG2, SGC-7901, SW480 and telomerase-negative tumor cell lines U2OS and SaoS-2 were used for lentivirus-mediated noninvasive whole-body tumor imaging. Telomerase-positive tumor cell lines HepG2, SGC-7901, and SW480 and telomerase-negative tumor cell lines U2OS and SaoS-2 were used for lentivirus-mediated noninvasive whole-body tumor imaging. Three mice of each group were s.c. injected with 1 × 10⁶ cells of each tumor cell line at both sides of gluteal areas. Ten days after the tumor implantation when tumors were palpable, 8 × 10⁶ TUs of Lenti-hTERTp-GFP or Lenti-CMVp-GFP lentiviral vector were intratumorally injected into the left and right tumors, respectively. Mice were imaged under a KODAK In-Vivo Multispectral Imaging System FX (Carestream Health) 24 h after injection. In this study, we chose an exposure time of 10 s, a standard excitation spectrum of 470 nm (which permits an emission spectrum of 535 nm), and a field of view of 150 mm. For each group, both white light and fluorescent images were obtained and analyzed. Thirty days after vector injection, mice were imaged again by the same procedures described above.

Immunohistochemical staining. The expression of hTERT in the above cell lines and GFP in nude mice tumors was detected by immunohistochemistry. Immunohistochemistry was performed as described in a previous study (23). Briefly, after endogenous peroxidase activity was blocked by treatment with a 0.3% H₂O₂-methanol solution for 30 min at a temperature of 25°C, tumors were incubated with a GFP or hTERT primary antibody (the mouse anti-GFP primary antibody was purchased from ZhongShan Golden Bridge Biotechnology Co. and the rabbit anti-hTERT primary antibody was purchased from Santa Cruz Biotechnology) overnight at 4°C, respectively. After thoroughly washing sections with PBS containing 0.1% Triton X-100, the sections were incubated with a secondary antibody for 30 min at a temperature of 25°C. Finally, slides were incubated for 15 min with an avidin-biotin enzyme reagent. The staining was developed by immersing the slides into a 3,3′-diaminobenzidine/H₂O₂ solution. PBS was used as a negative control in place of the primary antibody.

Western blot. Western blot was performed to detect hTERT protein in the above cell lines following the procedure described in our previous study (24). One bottle of each cell line was lysed with the M-PER extraction reagent (Pierce Co.) and quantified by the bicinchoninic acid assay method. The proteins of these cells were separated by 10% SDS-PAGE and then electrophoretically transferred onto a polyvinylidene difluoride membrane. Skim milk (5%) in TBS–TWEEN 20 containing 50 mmol/L Tris-base, 50 mmol/L NaCl, and 0.1% (v/v) Tween 20 was used to block nonspecific binding of the membrane at room temperature. The membrane was
hybridized with 1:1,000 dilution of the polyclonal antibodies against hTERT (Santa Cruz Biotechnology) overnight at 4°C. The membranes were washed and incubated with an alkaline phosphatase-conjugated goat anti-rabbit IgG antibody for 1 h at room temperature. Immunoreactive bands were detected using an enhanced chemiluminescent Western blot analysis system (Amersham Biosciences).

Results

**Immunohistochemical staining and Western blot for hTERT determination.** Both immunohistochemical staining and Western blot analysis confirmed that hTERT proteins were expressed in 293FT, HepG2, SGC-7901, SW480, and A375 cells, whereas there were no hTERT proteins detected in U2OS, SaoS-2, and HF cells (Fig. 1). This result indicated that the first group of cells is telomerase positive and the second group is telomerase negative.

**Activity of hTERT promoter determined by luciferase assay.** The activity of promoters was determined by luciferase assay. As shown in Fig. 2A, in the telomerase-negative cell lines, luciferase activity in pGL3-hTERTp–transfected cells was significantly lower than pGL3-CMVp–transfected and pGL3-SV40p–transfected cells. There was no significant difference in luciferase activity among pGL3-hTERTp–transfected, pGL3-Basic–transfected (blank), and pGL3-hTERTp–transfected (original) cells, whereas in the five telomerase-positive cell lines, the luciferase activity in pGL3-hTERTp–transfected cells was equal to pGL3-CMVp–transfected and pGL3-SV40p–transfected cells. Moreover, we also found that luciferase activity in pGL3-hTERTp–transfected cells was slightly higher than that in pGL3-hTERTp–transfected (original) cells (Fig. 2B). These results indicate that the optimized hTERT promoter has stronger activity in telomerase-positive cell lines compared with the original hTERT promoter. Moreover, our results also show that this optimized hTERT promoter is telomerase selective.

**Lentiviral vector construction and GFP visualization.** Enzyme digestion was done to confirm the structure of the pLenti-hTERTp-GFP and pLenti-CMVp-GFP vectors. For pLenti-hTERTp-GFP, a 301-bp fragment of the hTERT promoter is between ClaI and BamHI sites, and a 1,152-bp

![Figure 1. Expression of hTERT protein in various cell lines. A, immunohistochemical analysis of hTERT protein in 293FT (a), HepG2 (b), SGC-7901 (c), SW480 (d), A375 (e), U2OS (f), and SaoS-2 (g) tumor cell lines and HF (h) normal somatic cells. HTER protein was detected in 293FT, HepG2, SGC-7901, SW480, and A375 cells (brown stain), whereas none was detected in U2OS, SaoS-2, and HF cells. Magnification, × 200. B, Western blot for hTERT protein in various tumor cell lines and normal somatic cells. Lane 1, 293FT; lane 2, HepG2; lane 3, SGC-7901; lane 4, SW480; lane 5, A375; lane 6, U2OS; lane 7, SaoS-2; lane 8, HF. hTERT protein was detected in 293FT, HepG2, SGC-7901, SW480, and A375 cells but not in U2OS, SaoS-2, and HF.](image-url)
The titer of lentivirus was determined by real-time reverse transcription-PCR. The result showed that the titers of vectors Lenti-CMVp-GFP and Lenti-hTERTp-GFP lentivirus were imaged using a confocal laser scanner. A random field of each well was chosen and pictured (Fig. 4). The results showed that approximately 80% to 95% cells of all cell lines infected with Lenti-CMVp-GFP lentivirus expressed significant fluorescent signals. In contrast, in Lenti-hTERTp-GFP lentivirus–infected cells, GFP expression could be detected only in telomerase-positive cell lines (293FT, HepG2, SGC-7901, SW480, and A375 cells) but not in telomerase-negative cell lines (U2OS, Saso-2, and HP cells). These results suggest that GFP is selectively expressed in telomerase-positive tumor cells when infected with the lentivirus containing optimized hTERT promoter.

**In vitro selective visualization of tumor cell lines.** Cells that had been infected with Lenti-CMVp-GFP and Lenti-hTERTp-GFP lentivirus were digested with a confocal laser scanner. The wild type of each cell was chosen and pic-tured (Fig. 5). The results showed that approximately 80% to 95% cells of all cell lines infected with Lenti-CMVp-GFP lentivirus expressed significant fluorescent signals. In contrast, in Lenti-hTERTp-GFP lentivirus–infected cells, GFP expression could be detected only in telomerase-positive cell lines (293FT, HepG2, SGC-7901, SW480, and A375 cells) but not in telomerase-negative cell lines (U2OS, Saso-2, and HP cells). These results suggest that GFP is selectively expressed in telomerase-positive tumor cells when infected with the lentivirus containing optimized hTERT promoter.

**In vivo selective visualization of subcutaneous tumors.** To access the selectivity of the lentivirus containing optimized hTERT promoter, we detected the GFP signals in telomerase-positive and telomerase-negative tumors after intratumoral injection with Lenti-CMVp-GFP and Lenti-hTERTp-GFP lentiviral vectors by a CCD camera. Both white light and fluorescence images of the same position were collected at 24 hours and 30 days after vector injection. As shown (Fig. 5), GFP signals in tumor masses could be detected noninvasively at 24 hours after lentivirus injection. For mice that were injected with the Lenti-CMVp-GFP lentivirus, fluorescence was detected in both telomerase-positive and telomerase-negative tumors with considerable intensity. In contrast, for mice that were injected with the Lenti-hTERTp-GFP lentivirus, fluorescence was only exhibited in telomerase-positive tumors. All telomerase-negative tumors that were infected with the Lenti-hTERTp-GFP vector had no detectable fluorescence (Fig. 5A). The same results were obtained at day 30, which showed a larger area and much higher intensity of GFP expression (Fig. 5B). These results suggest that transfection with lentivirus containing this optimized hTERT promoter might be a useful diagnostic tool for the real-time visualization of macroscopically invisible tumor tissues using a highly sensitive CCD imaging system.

**GFP detection by immunohistochemical staining.** H&E staining of all tumor samples that were infected with a lentiviral vector showed typical malignant morphology, with increased nuclear content and nuclear arrangement (Fig. 6A). Immunohistochemical staining showed that GFP was strongly expressed in all cells that were infected with Lenti-CMVp-GFP, with no significant differences between telomerase-positive and telomerase-negative cell lines. However, cells that were infected with Lenti-hTERTp-GFP exhibited different GFP staining. Only telomerase-positive cells showed strong GFP expression, whereas telomerase-negative cells showed no detectable GFP expression (Fig. 6B).

**Discussion**

The cloning of the core region of the hTERT promoter in 1999 facilitated the development of targeted cancer gene

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**Figure 2.** Determination of promoter activity by luciferase assay. A, activity of each promoter in five telomerase-positive cell lines and three telomerase-negative cell lines were transfected with pGL3-hTERTp, pGL3-CMVp (original), pGL3-CMVp, pGL3-SV40p, and pGL3-Basic (blank) vectors. Seventy-two hours after transfection, the cells were lysed and dual luciferase assays were conducted. Promoter activity was indicated by relative luciferase activity. A, activity of each promoter in five telomerase-positive cell lines. B, activity of each promoter in five telomerase-negative cell lines.
therapy approaches based on the hTERT promoter (10). Recent studies have provided mechanistic insight into how the hTERT promoter can be stimulated or suppressed by the activation or inactivation of oncogenes and tumor suppressor genes, respectively (12, 13, 19). The hTERT promoter contains two E-boxes (CACGTG) that are binding sites for the Myc/Max/Mad network of transcription factors. The oncoprotein c-Myc heterodimerizes with the Max protein to activate hTERT transcription (25). In contrast, Mad1 and Max protein heterodimers result in the repression of hTERT expression (19). These findings helped researchers to optimize the hTERT promoter as a highly tumor-specific promoter. In fact, gene diagnosis and therapy based on the hTERT promoter has been reported by several laboratories (26–29). Padmanabhan and colleagues (30) designed two truncated promoters of hTERT, which were 0.5 kb and 0.2 kb in length, respectively. The authors found that these two truncated promoters have the same activity as the original hTERT promoter. However, these truncated promoters, as well as the original hTERT promoter, expressed 55% to 68% of their activity from the CMV promoter. These results showed that the activity of the unmodified hTERT promoter is much weaker than commonly used viral promoters, such as the CMV and SV40 promoters. In the present study, to maximize the activity of the promoter in tumor tissues, and minimize its activity in normal somatic cells, a previously reported hTERT promoter sequence (18) was adopted to achieve transgene expression in a tumor-specific manner. To increase the activity of the promoter in telomerase-positive tumor cells and decrease its activity in normal somatic cells, the core sequence of the hTERT promoter was maintained in this optimized promoter, whereas the silencing sequence was knocked out. In addition, three E-box (CACGTG) motifs were inserted downstream of the hTERT promoter. This

Figure 3. Construction of pLenti-hTERTp-GFP and pLenti-CMVp-GFP lentiviral vectors and determination of MOI. A, construction of pLenti-hTERTp-GFP lentiviral vector. Lane 1, plasmid of pLenti-hTERTp-GFP was digested with ClaI and BamHI, and a 301-bp fragment containing the hTERT promoter was formed; lane 2, plasmid of pLenti-hTERTp-GFP was digested with BamHI and ApaI, and a 1,152-bp fragment of GFP was formed. B, construction of pLenti-CMVp-GFP lentiviral vector. Lane 1, plasmid of pLenti-CMVp-GFP was digested with ClaI and BamHI, and a 605-bp fragment containing the CMV promoter was formed; lane 2, plasmid of pLenti-CMVp-GFP was digested with BamHI and Xhol, and a 1,152-bp fragment of GFP was formed. C, determination of MOI. 293FT cells were infected with 1 and 2 MOI of the above lentiviruses, respectively. GFP expression was detected 12 h after infection and reached a peak at 72 h.
allows for the binding of Mad1 and Myc transcription factor family members to inhibit and enhance the activity of the promoter in normal and tumor tissues, respectively. The luciferase assay showed that the activity of this promoter was highly hTERT specific and as strong as the CMV and SV40 promoters. In telomerase-positive cells, the activity of the optimized promoter was as strong as both the CMV and SV40 promoters, and slightly higher than the activity of the original hTERT promoter. In contrast, the optimized hTERT promoter had similar levels of activity as the negative control in telomerase-negative cell lines. These results suggest that this optimized hTERT promoter has much more activity in telomerase-positive cancer cell lines than in telomerase-negative cell lines.

Based on the above findings, a lentiviral vector containing hTERT promoter-driven GFP was constructed to detect its hTERT specificity in tumor cells in vitro and in vivo. Studies about the hTERT promoter in the diagnosis and treatment of cancers have been previously reported (29). However, these studies were generally based on adenoviral vectors. Compared with adenoviral vectors, lentiviral vectors can integrate target genes into the host genome to achieve long-term stable expression (31). Moreover, the infection efficiency of lentivirus is very high (32). The present study indicated that the GFP signal could be detected at 1 MOI for both hTERTp and CMVp lentiviruses 12 hours after transfection and could last for 40 days, which provides a valid property for in vivo tumor transfection and imaging. Additionally, lentiviruses have been reported to be able to infect quiescent cells such as cancer stem cells (33). As we know, hTERT is also expressed in cancer stem cells (34, 35). If a suicide gene was cloned downstream of this lentiviral
vector, then we might selectively kill these cancer stem cells, a possibility with tumor treatment implications. Of course, gene therapy based on the hTERT promoter lentivirus is carried on in our laboratory.

In the following experiment, the optimized hTERT promoter was applied to an in vitro and in vivo tumor-specific imaging model. Compared with Lenti-CMVp-GFP, Lenti-hTERTp-GFP showed no significant difference in infection efficiency in vitro and in vivo. More importantly, Lenti-hTERTp-GFP transfection resulted in GFP expression only in telomerase-positive tumor cells. Both telomerase-negative and normal somatic cells were GFP negative, suggesting that telomerase was the target of the Lenti-hTERTp-GFP virus. Because >85% of malignant tumors express telomerase (36), these results provide a solid basis for real-time diagnosis of tumors and their metastasis based on hTERT promoter through the use of optical imaging technology.

Other reporter such as luciferase is also commonly used for molecular detection by intravital or optical imaging. In contrast to luciferase systems, fluorescent protein detection by optical imaging has several important advantages. First, the imaging process for luciferase must be carried out in an almost light-free environment. Moreover, animals must be injected with the luciferin substrate, which has to reach every tumor cell to be useful (37). In contrast, there is no need for total darkness to capture real images by fluorescent proteins, which can also be captured by using a fairly simple apparatus. Second, fluorescent proteins have a much stronger signal. Ray and colleagues (38) reported that the red fluorescence protein signal was ~1,000 times stronger than that of the luciferase signal. Although the molecular imaging strategy using hTERT promoter-driven GFP is considered promising, some limitations of the system exist. The relatively short

Figure 5. Selective visualization of subcutaneous tumors in vivo. A, whole-body imaging 24 h after intratumoral injection of the lentivirus. B, whole-body imaging 30 d after intratumoral injection of the lentivirus. The mice from each group were sedated and imaged under the in vivo imaging system. Lenti-CMVp-GFP and Lenti-hTERTp-GFP lentiviruses were injected into the right and left tumor of each mouse, respectively. Both fluorescent (left) and white light (right) images were collected at the same position. For Lenti-CMVp-GFP lentivirus, GFP was expressed in both telomerase-negative and telomerase-positive tumors. For Lenti-hTERTp-GFP lentivirus, GFP was expressed only in telomerase-positive tumors. Thirty days after lentivirus injection, GFP expression of each tumor was consistent with the first imaging, except for a larger boundary and increased intensity.
wavelength of excitation is the major shortcoming of GFP. If the tumor mass is located in a deep layer, the excitation light for GFP may not be detected, therefore leading to a false-negative result (39). To overcome this obstacle, a handheld flashlight to excite GFP fluorescence has been developed, which could detect signals from fluorescent proteins during surgery (40). For the application of this technology on clinical patients, supersensitive optical device that is able to capture signals from much deeper tissues is being explored and studied (41). In the short future, this technology can be used as a supplementary method for during-surgery imaging of tumor-adjacent lymphatic metastasis (42) when vector-mediated reporter gene was locally delivered into tumor area.

In conclusion, the data presented here indicate that transfection of a telomerase-specific lentivirus based on this optimized hTERT promoter may be a useful diagnostic strategy for real-time visualization of macroscopically invisible tumor tissues through highly sensitive CCD imaging system. Although the sensitivity and specificity of the method must be assessed by additional experiments, this technology provides a foundation for future clinical applications.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

Correction: An Optimized Telomerase-Specific Lentivirus for Optical Imaging of Tumors

In this article (Cancer Res 2010;70:2585–94), which was published in the April 1, 2010 issue of Cancer Research (1), incorrect versions of Figs. 3 and 4 were published. The correct versions of these figures are provided below.

Figure 3.
Reference


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