Intratumoral versus Intravenous Gene Therapy Using a Transcriptionally Targeted Viral Vector in an Orthotopic Hepatocellular Carcinoma Rat Model

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ABSTRACT

Purpose: To evaluate the feasibility of intratumoral delivery of adenoviral vector carrying a bidirectional two-step transcriptional amplification (TSTA) system to amplify transcriptional strength of cancer-specific Survivin promoter in a hepatocellular carcinoma model.

Materials and Methods: MCA-RH7777 cells were implanted in rat liver, and tumor formation was confirmed with [^{18}F]fluorodeoxyglucose (18F-FDG) positron emission tomography (PET). The adenoviral vector studied had Survivin promoter driving a therapeutic gene (tumor necrosis factor-α–related apoptosis-inducing ligand [TRAIL]) and a reporter gene (firefly luciferase [FL]; Ad-pSurvivin-TSTA-TRAIL-FL). Tumor-bearing rats were administered Ad-pSurvivin-TSTA-TRAIL-FL intravenously (n = 7) or intratumorally (n = 8). For control groups, adenovirus FL under cytomegalovirus (CMV) promoter (Ad-pCMV-FL) was administered intravenously (n = 3) or intratumorally (n = 3). One day after delivery, bioluminescence imaging was performed to evaluate transduction. At 4 and 7 days after delivery, 18F-FDG-PET was performed to evaluate therapeutic efficacy.

Results: With intravenous delivery, Ad-pSurvivin-TSTA-TRAIL-FL showed no measurable liver tumor FL signal on day 1 after delivery, but showed better therapeutic efficacy than Ad-pCMV-FL on day 7 (PET tumor/liver ratio, 3.5 $\pm$ 0.58 vs 6.0 $\pm$ 0.71; P = .02). With intratumoral delivery, Ad-pSurvivin-TSTA-TRAIL-FL showed positive FL signal from all tumors and better therapeutic efficacy than Ad-pCMV-FL on day 7 (2.4 $\pm$ 0.50 vs 5.4 $\pm$ 0.78; P = .01). In addition, intratumoral delivery of Ad-pSurvivin-TSTA-TRAIL-FL demonstrated significant decrease in tumoral viability compared with intravenous delivery (2.4 $\pm$ 0.50 vs 3.5 $\pm$ 0.58; P = .03).

Conclusions: Intratumoral delivery of a transcriptionally targeted therapeutic vector for amplifying tumor-specific effect demonstrated better transduction efficiency and therapeutic efficacy for liver cancer than systemic delivery, and may lead to improved therapeutic outcome for future clinical practice.

ABBREVIATIONS

Ad-pCMV-FL = adenovirus encoding a cytomegalovirus promoter and firefly luciferase, Ad-pSurvivin-TSTA-TRAIL-FL = adenoviral vector carrying two-step transcriptional amplification of Survivin promoter driving tumor necrosis factor-α–related apoptosis-inducing ligand and firefly luciferase, BLI = bioluminescence imaging, CMV = cytomegalovirus, 18F-FDG = [^{18}F]fluorodeoxyglucose, FL = firefly luciferase, HCC = hepatocellular carcinoma, PBS = phosphate-buffered saline, PET = positron emission tomography, ROI = region of interest, TRAIL = tumor necrosis factor-α–related apoptosis-inducing ligand, TSTA = two-step transcriptional amplification

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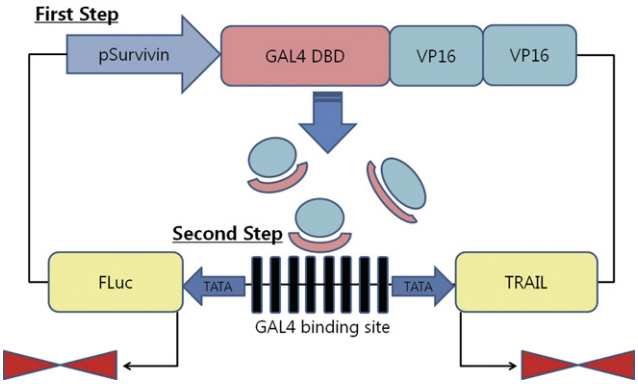

Figure 1. Schematic illustration of the Survivin-targeted bidirectional TSTA system. In the first step, pSurvivin is used to drive the expression of the transactivator fusion protein Gal4-VP16, composed of a Gal4-DNA–binding domain (*DBD*) fused in frame to two copies of the VP16-transactivation domain. The use of the GAL4-VP16 fusion helps in amplifying the expression of tumor-specific promoter to induce high level of the target protein ultimately. In the second step, the transactivator fusion protein Gal4-VP16 binds to Gal4-binding sites situated in between two thymine-adenine-thymine-adenine promoters, each of which simultaneously drives a downstream gene (TRAIL or FLuc). Therefore, this system is helpful in indirectly imaging the expression of TRAIL (ie, therapeutic gene) by monitoring the level of expression of the transcriptionally coupled FLuc (ie, reporter gene). (Available in color online at *www.jvir.org*.)

Gene therapy for cancer can target a variety of pathways to achieve apoptosis, cell lysis, antitumor immunity, or angiogenesis inhibition by transferring genetic materials into targeted cells (1). It requires a series of complex processes for its efficacy, which is affected not only by the selection of the appropriate therapeutic genes, but also by several factors such as vector, delivery method, cell transduction, and transgene expression. Many efforts have been made during the past several years to develop strategies for cancer gene therapy aiming at specific targeting to tumors to avoid unwanted, immunogenic, or toxic transgene expression in normal tissues while achieving high levels of transgene expression in cancer cells (1). Transcriptionally targeted cancer gene therapy uses cancer-specific promoters that induce therapeutic gene expression exclusively in cancer cells rather than in normal cells (2). However, the cancer-specific promoters usually demonstrate low levels of transcription and require amplification strategies to reach adequate levels for therapeutic efficacy. We previously developed an adenoviral vector carrying the cancer-specific promoter pSurvivin and a bidirectional dual gene-expression system composed of the therapeutic apoptosis-inducing protein tumor necrosis factor-α–related apoptosis-inducing ligand (TRAIL) gene and the reporter firefly luciferase (FL) gene, which a has two-step transcriptional amplification (TSTA) system with GAL4-VP16 fusion protein to amplify the promoter activity (Ad-pSurvivin-TSTA-TRAIL-FL) (3) (**Fig 1**). The GAL4-VP16 fusion protein amplifies expression of genes under the control of multiple GAL4-binding sites and a thymine-adenine-thymine-adenine minimal promoter (3,4). The ideal gene therapy requires not only efficient targeting but also monitoring the location and extent of therapeutic gene expression, which can be provided by the coexpression of a reporter gene. Our system has been designed so that TRAIL (ie, therapeutic gene) expression can be inferred from the signal generated by FL (ie, reporter gene) expression with bioluminescence imaging (BLI; **Fig 1**). The expressions of TRAIL and FL in our system were reported to highly correlate in a previous study (3).

Another strategy for gene targeting and amplification is a localized gene delivery. When therapeutic genes for cancer are delivered by intravenous injection, undesirable interactions between the administered genes and blood components or nontarget cells would be expected (5), and induce more loss of the DNA complex into the systemic circulation. In addition, although the intravenously administered genes targeting liver cancer enter the liver efficiently, it has been shown that most of them are completely trapped by Kupffer cells, thereby making it impossible to direct genes to tumor cells (6). Therefore, it would be desirable to administer the genes in a way that avoids the barriers that can be expected with systemic delivery. Local delivery can increase the direct uptake of the DNA complex into the target tissue compared with systemic delivery, because the local delivery of genes helps in bypassing multiple anatomic and physiologic barriers to a target, leading to higher target concentration with lower systemic exposure (7). Various methods available to accomplish this strategy include direct intratumoral injection or transarterial injection through the vessels that directly feed the tumor. We hypothesize that combining the transcriptional targeting strategy with local delivery techniques may lead to even more specific and amplified transgene expression in cancer gene therapy than using either strategy alone. In this study, we used molecular imaging to compare the transgene expression and therapeutic efficacy of intratumoral versus intravenous delivery of Ad-pSurvivin-TSTA-TRAIL-FL.

MATERIALS AND METHODS

Tumor Model

Cell preparation. McA-RH7777, a syngeneic rat hepatocellular carcinoma (HCC) cell line, was obtained from American Type Culture Collection (Manassas, Virginia) and cultured in Dulbecco modified Eagle medium with 10% fetal bovine serum and 1% penicillin/streptomycin. All cells were maintained in a humidified incubator with 5% CO_2 in atmosphere in air at 37°C. A total of 10^6 cells suspended in 100 μL of phosphate-buffered saline (PBS) solution were prepared for injection into each animal.

Animal preparation. Animal experiments (**Fig 2**) were approved by our institutional animal care and use committee. Twenty-three Buffalo rats weighing 300–350 g (Charles River Laboratory, Wilmington, Massachusetts) were used for the orthotopic tumor model (8), and kept separately

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