Highly Efficient Gene Delivery for Bladder Cancers by Intravesically Administered Replication-Competent Retroviral Vectors

Eiji Kikuchi, Silvia Menendez, Choichiro Ozu, Makoto Ohori, Carlos Cordon-Cardo, Christopher R. Logg, Noriyuki Kasahara, and Bernard H. Bochner

Abstract

Purpose: In an attempt to improve viral delivery of potentially therapeutic genes via an intravesical route, we have recently developed murine leukemia virus-based replication-competent retrovirus (RCR) vectors.

Experimental Design: We evaluated the transduction efficiency of intravesically administered RCR vectors to bladder tumor using orthotopic animal models to determine their potential as delivery vectors for bladder cancer.

Results: The RCR vector containing green fluorescent protein (GFP) marker gene achieved efficient in vitro transmission of the GFP transgene. Murine bladder tumor-2 mouse bladder tumors exposed to intravesically administered RCR vectors exhibited 0%, 9.2 ± 2.9%, and 30.0 ± 6.2% of GFP expression at 9, 18, and 27 days after exposure in the orthotopic model, respectively. Orthotopic KU-19-19 human bladder tumors exposed to intravesically administered RCR vectors exhibited 3%, 85 ± 1.0%, and 100% of GFP expression at 7, 21, and 35 days after exposure, respectively. GFP staining was observed only in the tumor cells in the bladder. No detectable PCR products of GFP gene could be observed in distant organs. Treatment with RCR vectors containing yeast cytosine deaminase (CD) gene plus 5-fluorocytosine (5-FC) dramatically inhibited the growth of preestablished murine bladder tumor-2 tumors. A single course of 5-FC treatment resulted in a 50% animal survival in mice exposed to RCR-CD compared with a 0% survival in all controls over a 70-day follow-up period.

Conclusions: Intravesically administered RCR vectors can efficiently deliver genes to orthotopic bladder tumor without viral spread in distant organs. RCR-CD/5-FC suicide gene therapy promises to be a novel and potentially therapeutic modality for bladder cancer.

Transitional cell carcinoma of the bladder is the second most common genitourinary malignancy and the second most common cause of genitourinary cancer-related death (1). Seventy percent to 80% of patients with transitional cell carcinoma present with noninvasive tumors that show a 70% local recurrence rate following standard treatment (2). Despite repeat transurethral resection and select administration of intravesical bacillus Calmette-Guerin immunotherapy or chemotherapy, up to 30% of recurrent tumors progress to a higher grade and/or stage (3). Radical cystectomy and urinary tract reconstruction provide optimal control of progressive superficial disease as well as muscle invasive tumors. Although advances in reconstructive techniques of the lower urinary tract have decreased the lifestyle changes associated with radical cystectomy, significant quality of life alterations occur after radical surgery. Gene therapy that provides improved control of high-risk superficial lesions would be an attractive potential strategy for the management of bladder cancer. The development of a viral-based gene therapy for bladder cancer has been the subject of several recent investigations (4, 5). The bladder represents an ideal target for gene therapy based on its relatively noninvasive access provided through catheterization, eliminating the need for systemic virus administration. Intravesical administration of viral vectors allows for direct local tumor contact, circumventing the difficulties associated with tumor targeting via systemic administration. Despite these apparent advantages, it has still been investigated to find an efficiency of gene transfer system to bladder tumor by intravesical administration of viral vectors (6). In an attempt to improve viral delivery of potentially therapeutic genes, we have recently developed murine leukemia virus (MuLV)-based replication-competent retrovirus (RCR) vectors (7, 8). Intratumoral injection of MuLV-based RCR vectors provided ~100% transduction efficiency in an intracranial malignant glioma model (9). Advantages to the MuLV-based vectors rest in their ability to only transduce actively dividing cells (10), potentially providing a relative selection for tumor cells.

Here, we have evaluated and characterized the transduction efficiency of intravesically administered RCR vectors to the
bladder using orthotopic murine models of bladder cancer to determine its potential as a delivery vector. Furthermore, using this delivery system, we have investigated cytosine deaminase (CD) plus 5-fluorocytosine (5-FC) suicide gene therapy as a potential cytotoxic treatment of bladder cancer. CD is one of the most widely studied enzymes for suicide gene therapy (11). This enzyme converts the prodrug 5-FC to its toxic metabolite, 5-fluorouracil (5-FU). CD is found in many bacteria and fungi but not in mammalian cells (12). Consequently, mammalian cells are resistant to the toxic effects of 5-FC.

Materials and Methods

Cell lines. Murine bladder tumor-2 (MBT-2), an N-[4-(5-nitro-2-furyl)-2-thiazolyl]-formamide–induced bladder cancer cell line, was originally isolated and characterized in a syngeneic C3H/HeJ mouse (obtained from Dr. Timothy Ratliff, University of Iowa, Iowa City, IA). The human bladder cancer cell line KU-19-19 was kindly provided by Dr. M. Murai (Keio University School of Medicine, Tokyo, Japan). These cell lines were cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum. 293T human kidney cells (American Type Culture Collection) were maintained in Eagle’s MEM in Eagle’s balanced salt solution with nonessential amino acids supplemented with 10% fetal bovine serum. These cell lines were incubated in medium with 5% CO2 humidified atmosphere.

Construction of RCR vectors and viral production. The MuLV proviral genomic sequence was modified by replacement of the U3 region in the 5′ long terminal repeat with the cytomegalovirus (CMV) promoter and the ecotropic envelope gene with the amphotropic envelope (env) from 4070A. An expression cassette consisting of GFP and CD preceded by an IRES was inserted precisely at the boundary between the env and 3′ untranslated region sequences of the modified MuLV genome.

Fig. 1. Schematic structure of a RCR vector proviral construct consisting GFP or yeast CD. The MuLV proviral genomic sequence was modified by replacing the U3 region in the 5′ long terminal repeat with the cytomegalovirus (CMV) promoter and the ecotropic envelope gene with the amphotropic envelope (env) from 4070A. An expression cassette consisting of GFP and CD preceded by an IRES was inserted precisely at the boundary between the env and 3′ untranslated region sequences of the modified MuLV genome.

medium was replaced with 1 mL of fresh complete medium, virus stock at a multiplicity of infection of 0.05 or PBS, and 4 μg/mL of hexadimethrine bromide (Sigma Chemical Co.) to assist the uptake of viral particles. Following 3-h exposure to the virus stock or PBS, the medium was discarded and replaced with 1 mL of fresh medium. Three, 7, and 21 days after infection, the cells were analyzed for GFP expression by fluorescence-activated cell sorting analysis. Cells were harvested with 0.25% trypsin in PBS, centrifuged, and washed in PBS. The number of GFP-positive live cells was expressed as a percentage of all cells in the sample. Data for GFP expression were acquired on a FACSCalibur machine equipped with CellQuest software (Becton Dickinson). All experiments were done in triplicate. For retroviral vector titers determination, serial dilutions of viral supernatant were added to cells in six-well plates. Three hours after infection, the cells were incubated in medium with 50 μmol/L 3′-azido-3′-deoxythymidine for 24 h and subjected to fluorescence-activated cell sorting analysis to determine its potential as a delivery vector. Furthermore, using this delivery system, we have investigated cytosine deaminase (CD) plus 5-fluorocytosine (5-FC) suicide gene therapy as a potential cytotoxic treatment of bladder cancer. CD is one of the most widely studied enzymes for suicide gene therapy (11). This enzyme converts the prodrug 5-FC to its toxic metabolite, 5-fluorouracil (5-FU). CD is found in many bacteria and fungi but not in mammalian cells (12). Consequently, mammalian cells are resistant to the toxic effects of 5-FC.

For tumor implantation, 8-week-old female C3H/HeJ mice or nu/nu mice were anesthetized with an i.p. injection consisting of ketamine (100 mg/kg; Fort Dodge Animal Health) and xylazine (20 mg/kg; Lloyd Laboratories). A 22-gauge catheter was inserted into
the bladder transurethrally, and the urethra was ligated with 3-0 silk suture tightly. Subsequently, $2 \times 10^6$ MBT-2 ($n = 24$) or $1 \times 10^7$ KU-19-19 ($n = 9$) cells in 100 µL of PBS were instilled into the bladder. Cells remained for 3 h in the bladder. Five days after tumor implantation, 100 µL of ACE-GFP vector ($3.2 \times 10^5$ transduction units/100 µL) were instilled into the bladder. The animals instilled with MBT-2 tumor cells were humanely sacrificed on days 9 ($n = 6$), 18 ($n = 5$), and 27 ($n = 9$) after viral instillation. The animals instilled with KU-19-19 tumor cells were sacrificed on day 7 ($n = 1$), 21 ($n = 2$), and 35 ($n = 3$) after viral instillation. Control tumor-bearing mice were treated with 100 µL of PBS vehicle only at day 5 ($n = 4$ in MBT-2 tumor-bearing mice and $n = 3$ in KU-19-19 tumor-bearing mice). Following cystectomy, bladders were placed in OCT compound (Sakura) and immediately frozen in liquid nitrogen. Other tissues, including samples from brain, lung, liver, kidney, heart, spleen, ovary, and uterus, were harvested. Frozen sections (5 µm) were cut on a cryostat and stained with H&E. Total tumor area in the largest histologic section of bladder was digitally determined by the medical image analysis program (Image-Pro Plus version 4.1, Media Cybernetics).

For normal bladder studies, either 100 µL of PBS vehicle or ACE-GFP vector was instilled into the bladder of C3H/HeJ mice (each, $n = 2$). Four and 8 weeks after vector instillation, bladder was harvested, along with tissues including brain, lung, liver, kidney, heart, stomach, spleen, ovary, and uterus.

**Immunohistochemical analysis.** For immunostaining, sections were fixed in 4% paraformaldehyde for 1 h. After endogenous peroxidase activity was blocked by use of 0.1% hydrogen peroxide for 15 min, sections were incubated for 30 min in a blocking solution containing 10% appropriate goat serum. Sections were incubated with 1:2,000 dilution of rabbit anti-GFP polyclonal antibody (Molecular Probes) for 2 h. Slides were incubated with biotinylated secondary antibody for 30 min and exposed to avidin-biotin-peroxidase complexes (Vector Laboratories, Inc.). Sections were treated with 0.06% 3,3′-diaminobenzidine (Sigma Chemical) used as final chromogen and counterstained with hematoxylin. The area stained with GFP antibody in the largest histologic section of bladder was also analyzed by the medical image analysis program (Image-Pro Plus version 4.1). The percentage of positive staining area with GFP (%GFP) was calculated from the area stained with GFP antibody divided by total tumor area in the largest section of bladder.

**PCR analysis in tumors and organs.** After the isolation of genomic DNA from all frozen tumor and organ sample, PCR assay was done to examine transduction and vector spread of RCR vector in MBT-2 orthotopic bladder tumor model and normal mice. Briefly, amplification was done in a reaction volume of 20 µL under the following conditions: 200 ng sample DNA, 0.2 µmol/L deoxynucleotide triphosphates, 0.5 µmol/L of each primer, 1× PCR buffer with magnesium, and 5 units Taq DNA polymerase (Roche Diagnostics).
GmbH). Products were amplified by 40 cycles of incubation at 94°C for 30 s, 53°C for 30 s, and 72°C for 1 min, respectively. Amplification cycles were then followed by 5-min extension at 72°C and hold at 4°C.

The primers used to amplify the GFP transgene were as follows: 5'-AAGGGCGAGGAGCTGTTC-3' (5' primer) and 5'-TACTTGTA-CAGCTCGTCCATGC-3' (3' primer).

The same procedures were applied to amplify a 525-bp fragment of mouse β-casein DNA as an internal control using the following primers: 5'-GATGTGCTCCAGGCTAAAGTT-3' (5' primer) and 5'-AGAAACGGAAATGTTGTGGAGT-3' (3' primer).

The reaction products were loaded on 1% agarose gels and visualized by ethidium bromide staining. Positive controls for these experiments were viral-producing 293T cells. Bladder tumor treated with PBS only was also examined.

Treatment in vivo. On day 0, $2 \times 10^6$ MBT-2 ($n = 25$) cells in 100 µL PBS were instilled into the bladder for 3 h as describe above. Five days after tumor implant, 100 µL of ACE-GFP vectors (3.2 × 10^5 transduction units/100 µL) or ACE-CD vectors were instilled into the bladder for 3 h. Twelve days later, these animals were subsequently received daily i.p. injections of either 500 mg/kg of 5-FC ($n = 8$, for a group receiving ACE-CD vector; $n = 7$, for ACE-GFP vector group) or PBS ($n = 10$, for a group receiving ACE-CD vector) for 15 consecutive days. These animals were humanely sacrificed on day 32. Tissue sampling and the analysis of total tumor area in the largest histologic section of bladder were done as mentioned above.

The second set of experiments was done for the survival benefit with ACE-CD/5-FC treatment in the orthotopic model. Treatment groups included ACE-CD/5-FC, ACE-CD/PBS, and ACE-GFP/5-FC ($n = 14$ in each group). Survival rate was calculated by the Kaplan-Meier method and differences between the treatment group and controls were determined with the log-rank test.

Statistical analysis. Results were expressed as the mean ± SE. Differences between groups were examined with the Student’s t test with $P < 0.05$ considered significant. These analyses were done with the STATA version 7.0 statistical software package (Stata Corp.).

Results

RCR vectors mediate efficient in vitro expression and transmission of the GFP transgene in bladder cancer cells. We assessed the in vitro transduction efficiency of our MolV-based RCR vectors by monitoring expression of the GFP transgene over time in both MBT-2 (Fig. 2A) and KU-19-19 (Fig. 2B) cell lines following exposure to the RCR vector. After a 3-h viral exposure, the transduction levels of MBT-2 cells were 37.2 ± 30.4% at day 3. At days 7 and 21, GFP expression was observed in 38.0 ± 30.0% and 45.7 ± 14.3% of cultured MBT-2 cells, respectively. For KU-19-19 human bladder cancer...
5-FC treatment compared with the control groups. Kaplan-Meier curves showed the significant benefit for the ACE-CD/5-FC therapy in MBT-2 orthotopic bladder tumor growth. Preestablished MBT-2 tumors were treated with intravesical administration of ACE-CD or ACE-GFP vectors on day 5. On day 17, these animals were subsequently received daily i.p. injections of either 500 mg/kg of 5-FC or PBS for 15 consecutive days. Tumor area was estimated on day 32. ACE-CD/5-FC resulted in a significant reduction of total tumor area compared with other treatment regimen (P < 0.05, for both). In vivo survival experiment. Kaplan-Meier curve showed the significant benefit for the ACE-CD/5-FC treatment compared with the control groups.

Fig. 6. In vivo therapeutic experiments. A, effect of ACE-CD/5-FC therapy in MBT-2 orthotopic bladder tumor growth. Preestablished MBT-2 tumors were treated with intravesical administration of ACE-CD or ACE-GFP vectors on day 5. On day 17, these animals were subsequently received daily i.p. injections of either 500 mg/kg of 5-FC or PBS for 15 consecutive days. Tumor area was estimated on day 32. ACE-CD/5-FC resulted in a significant reduction of total tumor area compared with other treatment regimen (P < 0.05, for both). B, in vivo survival experiment. Kaplan-Meier curve showed the significant benefit for the ACE-CD/5-FC treatment compared with the control groups.

cells, 82.5 ± 8.2% of KU-19-19 cells were positive by day 3, which increased to 95.3 ± 1.4% at day 7, and 97.9 ± 0.3% by day 21. ACE-GFP vectors efficiently transduce orthotopic MBT-2 and KU-19-19 bladder tumors. To determine the ability of ACE-GFP to achieve efficient transgene delivery in bladder tumors in vivo, we injected the RCR vectors into preestablished MBT-2 and KU-19-19 orthotopic bladder tumors in C3H/HeJ mice and nude mice, respectively. Immunohistochemistry was used to evaluate GFP expression within the tumor for both quantification of transduction and location of expressed transgene. All tumors removed from control mice treated with PBS alone showed no GFP expression at any time point. In MBT-2 orthotopic tumors harvested 9 days after the instillation of ACE-GFP vectors, no transduction was observed in all six mice examined (Fig. 3A). By day 18, intratumoral GFP expression (mean ± SE) was 9.2 ± 2.9% (n = 5; Fig. 3B and E). Two bladder tumors exhibited a moderate level of transduction (14.0% and 17.3%). The region of the tumor showing GFP immunoreactivity was confined to the superficial layers of the tumor mass. By day 27, the area of GFP-positive tumor significantly increased to a mean %GFP ± SE of 30.0 ± 6.2% (n = 9; Fig. 3C and E). Four bladders harvested at this time showed significant levels of infection with 37.1%, 42.1%, 53.0%, and 59.3% of the entire tumor mass. Within the bladders, whereas tumor cells were strongly GFP positive, infiltrating immune cells and peritumoral muscle and fibroblasts were not (Fig. 3D). Additionally, endothelial cells that were part of the tumor-associated vasculature also showed GFP expression, supporting the endothelium as a secondary source of viral production (data not shown). An even more efficient transduction of tumor cells was observed using the KU-19-19 orthotopic human bladder model (Fig. 4). KU-19-19 tumors harvested after 7 days following exposure to a single instillation of ACE-GFP vector showed that only 3% of tumor cells were expressing GFP (Fig. 4A). However, the percentage of GFP-positive cells in ACE-GFP–infected tumors dramatically increased to 85 ± 1.0% on 21 days (Fig. 4B) and ~100% of tumor cells at day 35 (Fig. 4C).

To evaluate the extent of transduction of normal bladder tissues following intravesical administration of the RCR vectors, 3.2 × 10^5 transduction units of ACE-GFP vectors in 100 µl were instilled into the bladder of C3H/HeJ mice transurethrally and GFP expression was assessed by immunohistochemistry. No GFP positivity was detected in the bladder of normal mice instilled with PBS alone or with ACE-GFP vectors (data not shown). A PCR assay was applied to confirm the absence of ACE-GFP sequences in genomic DNA from non–tumor-bearing bladders treated with intravesically administered RCR vectors as well as to examine the biodistribution of RCR vectors in distant tissues and organs, including brain, lung, liver, kidney, heart, stomach, spleen, ovary, and uterus. No viral ACE-GFP sequences could be detected in the bladder or any systemic organs 28 (Fig. 5A) or 56 days following the instillation of our RCR vectors (data not shown).

Intravesically administered ACE-GFP vectors to MBT-2 tumor-bearing mice do not spread in distant organs. Orthotopic MBT-2 bladder tumor model could provide high tumor incidence and consistency of tumor growth in the bladder of immuno-competent C3H/HeJ mice (13). To evaluate viral spread in distant organs and its therapeutic effects in the presence of a normal immune system, biodistribution and therapeutic studies were done in the immunocompetent MBT-2 orthotopic model. Distant tissues, including bladder tumor, brain, lung, liver, kidney, heart, spleen, ovary, and uterus, were harvested from MBT-2 tumor-bearing mice 27 days after vector instillation in which GFP expression was ~60% of the total orthotopic MBT-2 tumor area. Using PCR techniques described above, ACE-GFP was only detected in the bladder tumor tissues exposed to RCR vectors. No viral sequence-related PCR products could be detected in any of the systemic organs tested (Fig. 5B).

Therapeutic effects of ACE-CD vectors plus 5-FC in MBT-2 orthotopic bladder tumor model. We next examined the therapeutic efficacy of RCR vector–mediated suicide gene therapy using the combination of intravesically administered ACE-CD vector and systemic 5-FC using orthotopic MBT-2 bladder cancer model. Orthotopically preestablished MBT-2 tumors were exposed to a single intravesical instillation of ACE-CD or ACE-GFP vector followed by 5-FC or PBS i.p. for 15 consecutive days. Treatment groups included ACE-CD vector followed by 5-FC or PBS i.p. for 15 consecutive days. Treatment groups included ACE-CD vector followed by 5-FC or PBS i.p.
vectors/5-FC, ACE-CD vectors/PBS, and ACE-GFP vectors/5-FC. Data were reported as mean total tumor area within each treatment group. Treatment with ACE-CD vectors/5-FC (0.327 ± 0.080 cm²) significantly retarded tumor growth compared with ACE-CD vectors/PBS (0.796 ± 0.141 cm²) or ACE-GFP/5-FC (0.939 ± 0.103 cm²; P < 0.05, for both; Fig. 6A). Furthermore, ACE-CD/5-FC treatment resulted in a 50% animal survival over a 70-day follow-up period compared with a 0% animal survival for all control groups (P < 0.01, for both; Fig. 6B).

Discussion

The fundamental goals of all gene therapy strategies are to obtain effective gene delivery to the desired target cell population and to establish adequate transgene expression. Targeting systemically administered virus to a tumor cell population has remained a major obstacle toward the introduction of clinically efficacious viral-based gene therapy treatment strategies. Localized bladder cancer represents a potentially ideal tumor model for gene therapy strategies based on the relatively noninvasive direct access to the bladder. Elimination of the need for systemic viral delivery holds great appeal as it may avoid targeting issues that have proven difficult with most gene therapy strategies. Various viral vectors have been tested to determine their efficacy following intravesical administration in orthotopic bladder tumor models. Several authors have reported on intravesically administered replication-defective adenoviral vectors (14–19). Standard adenoviral vectors carrying reporter gene constructs resulted in infection of both normal urothelial cells and bladder tumor cells; however, transgene expression was low and restricted to the superficial layers of cells in contact with the luminal surface. Limited transduction was noted without the use of compounds capable of destroying or altering the superficial glycosaminoglycan layer of the bladder (20). Furthermore, the viral particles are found to be cytotoxic and immunogenic, raising the concern that repeated adenoviral exposures may be further limited in its infectivity due to potential immune rejection by the host. Additionally, heterogeneity of coxsackie adenovirus receptor expression in human bladder cancer cells represented an additional obstacle for efficient adenovirus uptake (21). Vaccinia virus (22), canarypox virus (23), and herpes virus (24) were also reported to have the capability of transfecting bladder tumors in situ.

To address the need for more efficient gene delivery systems, we have constructed a MuLV-based RCR vector containing IRES-GFP cassette between the env gene and the 3′ untranslated region (7). By intratumoral injection of the RCR vectors, the level of transduction efficiency has greatly improved compared with standard defective retrovirus vectors in mammary (8) and brain tumor models (9). We have previously shown the high level of bladder tumor transduction using our RCR vector in a s.c. bladder tumor model (25). In the present study, we have investigated the utility of intravesically administered MuLV-based RCR vectors as useful gene transfer vectors to orthotopic bladder tumors. Our in vitro studies showed that, at day 21 after a 3-h exposure to RCR vectors, ~50% of MBT-2 tumor cells and 100% of KU-19-19 tumor cells stably expressed our transgene. The RCR vector replication in vitro was considered more robust in the human KU-19-19 bladder cancer cells compared with murine MBT-2 cells. This may be due to differences in cell surface receptor availability or differences in host cell restrictions on virus replication involving innate defense mechanisms that affect virus entry or particle production levels. It has replicated very well in other bladder cancer cell lines of human origin (e.g., T24) but not of mouse origin (e.g., MB49; data not shown).

To investigate the efficiency of viral transduction via an intravesical route of administration, we tested our RCR vectors in orthotopic bladder tumor models of both immunocompetent and immunodeficient mice. In the MBT-2 tumor model, low transduction efficiencies of <15% were observed by day 18 following a single intravesical instillation of the RCR vector, which increased up to 20% to 60% by day 27. Our transgene expression was stably observed beyond day 40 after viral exposure (data not shown). Further enhancement of viral transduction of MBT-2 orthotopic tumors may have been obtained with repeat administrations of vector or the use of higher-titer vector preparations. In contrast, intravesically administered RCR vectors to preestablished KU-19-19 human tumors in immunodeficient mice noted a highly efficient transduction by day 21 (85%), which increased to ~100% of all tumor cells by day 35.

Because MuLV-based RCR vectors can transduce only cells that are actively dividing, their transduction would be expected to be relatively selective to tumor cells (10). We observed that intravesically administered MuLV-based vectors transduced actively dividing bladder tumor cells in the orthotopic model, but not quiescent normal cells, including epithelial and stromal cells. As expected from the results in vitro, the level of virus replication in vivo was more robust in the orthotopic KU-19-19 human bladder cancer model compared with MBT-2. Of interest, within the orthotopic MBT-2 tumor nest, endothelial cells of the tumor-associated vasculature did exhibit some degree of transgene expression. Additional virus spread in the MBT-2 model in vivo may have been mediated more efficiently by viral replication in proliferating tumor neovasculature and secondary horizontal spread to adjacent bladder cancer cells.

To address the concern of hematogenous spread of intravesically administered RCR vectors in our model, we assessed the viral spread in both normal mice and tumor-bearing animals. No GFP protein expression was observed in normal mice 4 or 8 weeks following exposure to intravesically administered vectors. Furthermore, no detectable levels of RCR vector sequences were observed by PCR analysis in any of systemic organs and upper urinary tract tissues tested in both normal and tumor-bearing mice. The normal, intact urothelium of the bladder (due in part to the integrity of the glycosaminoglycan layer; ref. 26) and/or immune system seemed to prevent systemic viral dissemination. In fact, previously, investigators have reported that removal of glycosaminoglycan layer by ethanol (27) or polyamides (20) has facilitated replication-defective adenovirus vector attachment and subsequent transduction. This limited viral uptake by normal bladder tissues provides a potential advantage for our RCR-mediated system, limiting systemic viral distribution from the bladder.

MuLV is capable of oncogenic transformation in primates under certain conditions. Investigators have observed the development of T-cell lymphomas in 3 of 10 rhesus monkeys.
In conclusion, we have shown that the RCR vectors can efficiently transduce bladder cancer cells and stably propagate albeit with some variation in the level of efficiency. Single intravesical administration of the vectors provides high transduction level only within bladder tumor cells and long-term transgene expression in orthotopic bladder tumor models. Furthermore, using this attractive gene delivery system, we have also shown that intravesical instillation of RCR vectors encoding the yeast CD gene followed by systemic 5-FC administration results in significant suppression of preestablished bladder tumor growth and improvement of mouse survival. These data suggest that the use of intravesically administered MuLV-based RCR vector might be an attractive and useful method for achieving efficient gene transduction and RCR vectors encoding CD gene plus 5-FC suicide gene therapy might be a novel and potential therapeutic modality for bladder cancer.

Acknowledgments

We thank Maria E. Dudas and Minglan Lu (Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, NY) for their expert technical assistance.

References

10. Miller DG, Adam MA, Miller AD. Gene transfer by retrovirus vectors occurs only in cells that are actively replicating at the time of infection. Mol Cell Biol 1990;10:4239–42.

Intravesical Gene Delivery for Bladder Cancer by RCR

Intravesical Gene Delivery for Bladder Cancer by RCR
Highly Efficient Gene Delivery for Bladder Cancers by Intravesically Administered Replication-Competent Retroviral Vectors

Eiji Kikuchi, Silvia Menendez, Choichiro Ozu, et al.