Preclinical Safety and Efficacy of in Situ REIC/Dkk-3 Gene Therapy for Prostate Cancer

Keichiro Kawauchi\textsuperscript{a}, Masami Watanabe\textsuperscript{a,b,c,*}, Haruki Kaku\textsuperscript{a,b,c}, Peng Huang\textsuperscript{b}, Kasumi Sasaki\textsuperscript{b}, Masakiyo Sakaguchii\textsuperscript{d}, Kazuhiko Ochiai\textsuperscript{c}, Nam-ho Huh\textsuperscript{d}, Yasutomo Nasu\textsuperscript{a,c}, and Hiromi Kumon\textsuperscript{a,b,c}

\textsuperscript{a}Department of Urology, \textsuperscript{b}Center for Gene and Cell Therapy, \textsuperscript{c}Innovation Center Okayama for Nanohio-Targeted Therapy, and \textsuperscript{d}Cell Biology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama 700-8558, Japan

The preclinical safety and therapeutic efficacy of adenoviral vectors that express the REIC/Dkk-3 tumor suppressor gene (Ad-REIC) was examined for use in prostate cancer gene therapy. The Ad-human (h) and mouse (m) REIC were previously demonstrated to induce strong anti-cancer effects \textit{in vitro} and \textit{in vivo}, and we herein report the results of two \textit{in vivo} studies. First, intra-tumor Ad-hREIC administration was examined for toxicity and therapeutic effects in a subcutaneous tumor model using the PC3 prostate cancer cell line. Second, intra-prostatic Ad-mREIC administration was tested for toxicity in normal mice. The whole-body and spleen weights, hematological and serum chemistry parameters, and histological evaluation of tissues from throughout the body were analyzed. Both experiments indicated that there was no significant difference in the examined parameters between the Ad-REIC-treated group and the control (PBS- or Ad-LacZ-treated) group. In the \textit{in vitro} analysis using PC3 cells, a significant apoptotic effect was observed after Ad-hREIC treatment. Confirming this observation, the robust anti-tumor efficacy of Ad-hREIC was demonstrated in the \textit{in vivo} subcutaneous prostate cancer model. Based on the results of these preclinical experiments, we consider the adenovirus-mediated REIC/Dkk-3 \textit{in situ} gene therapy to be safe and useful for the clinical treatment of prostate cancer.

\textbf{Key words:} REIC, Dickkopf-3, gene therapy, prostate cancer, preclinical study

Prostate cancer is a common and significant disease in the male population of the United States and many other developed countries [1]. Cancer progression accelerates death in cancer patients, and is often accompanied by a decrease in quality of life. The current therapeutic strategies, such as androgen deprivation therapy, radiation, and cytotoxic chemotherapy, often fail to stop the disease progression. Therefore, novel and effective therapies against prostate cancer are urgently needed.

Cancer progression is often accompanied by the down-regulation of apoptosis [2] and the increased invasive and motile activity of the cancer cells [3]. Cancer cells modulate the apoptotic and metastatic processes by producing both positive and negative effectors [4, 5]. REIC/Dkk-3, a member of the Dickkopf (Dkk) gene family known to interfere with Wnt signaling via Wnt receptors [6, 7], was previously reported to play a distinct role in the induction of apoptosis and inhibition of metastasis [8, 9].
ReIC/Dkk-3 induced robust anti-tumor effects in vivo and cancer-specific apoptosis in a variety of cancer types when expressed by adenovirus-mediated gene transfer [8–13]. Moreover, our recent study disclosed that the REIC/Dkk-3 protein also has a role in monocyte differentiation and tumor regression [14]. Intratumoral administration of the REIC/Dkk-3 protein significantly suppressed tumor growth, with CD11c⁺ and CD8⁺ (dendritic and killer T cell marker, respectively) cell accumulation, and enhanced the anti-cancer cytolytic activity of splenocytes. These data indicated a cytokine-like role of the REIC/Dkk-3 protein in monocyte differentiation that might be exploited therapeutically.

Gene therapy has been applied to treat human diseases in clinical trials and is considered to have therapeutic potential. Our group has previously demonstrated the feasibility of using adenoviral vectors for the treatment of prostate cancer in the clinic [15]. Based on the background information noted above, we are promoting the translation of in situ REIC/Dkk-3 gene therapy from the lab to the clinic for patients with prostate cancer. To perform the necessary preclinical study as part of the progression to clinical use of the strategy, we herein examined the toxicity and therapeutic effects of the adenoviral vectors encoding the novel tumor suppressor REIC/Dkk-3.

Materials and Methods

Experimental animals. We used adult male mice (C57BL/6: 30–39g, BALB/C nu/nu: 24–31g) to evaluate the toxicity and therapeutic efficacy of the treatment strategy of using adenoviral vectors for the treatment of prostate cancer. The mice were purchased from Japan SLC, Inc. (Hamamatsu, Japan) and maintained in a specific pathogen-free environment with free access to food and water at the laboratory animal center of Okayama University. They were allowed to adapt to their environment for more than one week before beginning the experiments. The animals were housed and handled in accordance with the Okayama University Animal Research Committee Guidelines.

Adenovirus vector carrying REIC/Dkk-3 (Ad-REIC). The full-length cDNA of mouse or human REIC/Dkk-3 was integrated into the cosmid vector, pAXCAwt, and then transferred into an adenoviral vector by the COS-TPC method (Takara Bio, Shiga, Japan) [8]. An adenoviral vector carrying the LacZ gene (Ad-LacZ) was used as a control. The adenoviral vectors are a replication-defective adenovirus of serotype 5 that contain the indicated gene under the control of the CMV early enhancer/chicken β actin (CAG) promoter. For the Ad-human REIC, in vivo transduction ability after intratumoral vector injection was confirmed by detecting the REIC protein expression [14].

Treatment protocol. The experimental design and doses of virus administered are shown in Fig. 1. The first experiment was done using the prostate tumor mouse model. To generate this model, PC3 human prostate cancer cells (2.5 × 10⁶ in 50 μl phosphate buffered saline (PBS)) were subcutaneously injected into the right thigh of BALB/C nu/nu mice. The tumors were permitted to grow to approximately 5mm in diameter. The mice were then randomly

![Table 1](image-url)

Fig. 1 The experimental design of the preclinical study is shown. Toxicological analysis of mice administered Ad-hREIC and Ad-mREIC was performed in both experiment 1 and 2 using the indicated mouse models. A study of the efficacy of intratumoral administration of Ad-hREIC in a subcutaneous PC3 prostate cancer model was performed in experiment 1.
assigned into the treatment group with (1) intratumoral injection of Ad-LacZ, (2) intratumoral injection of Ad-human REIC (Ad-hREIC) at the dose of \(5 \times 10^{10}\) viral particles/tumor in 50\(\mu\)l buffer, or (3) intratumoral injection of 50\(\mu\)l PBS. The selection of the dose was based on previous clinical studies of prostate cancer gene therapy in which adenovirus-mediated gene delivery was performed using a dose between \(10^{10}\) and \(10^{11}\) viral particles/prostate [16–18]. The injections were targeted to the center and periphery of each mass to deliver the agent diffusely. The size of the tumor was measured with vernier calipers until 25 days after each treatment. Tumor volume was calculated using the following formula: \(1/2 \times (\text{the shortest diameter})^2 \times (\text{the longest diameter})\). In the second experiment, the same number of Ad-mouse REIC (Ad-mREIC) viral particles in 50\(\mu\)l buffer was inoculated into the bilateral lobes of the dorsolateral prostate in normal C57BL/6 mice without any tumors.

The body weight of each mouse was monitored using a sensitive balance during the entire study period. All the animals were observed daily for physical appearances, activity levels, and mortality patterns. The first day of vector administration was taken as Day 0, while the day of sacrifice was designated as Day 32 and Day 21 in the first and second experiments, respectively. In both experiments, the spleen weight was measured at the time of sacrifice. For the histological analysis of the potential side effects of the Ad-REIC treatment, the dorsolateral prostate (the injection site) and selected organs (brain, lungs, heart, liver, stomach, spleen, kidneys, bladder, and rectum) were dissected at the sacrifice of animals in the second experiment. The tissues were then fixed in formalin and embedded in paraffin sections. The sections (5\(\mu\)m) were stained with hematoxylin and eosin and were examined for histopathological and cytotoxic changes.

Blood parameters. Blood was drawn from the inferior vena cava of each animal under deep anesthesia with sodium pentobarbital. The blood samples were collected in a plastic tube with ethylenediaminetetra-acetic acid (EDTA). The measurements of white blood cells (WBC), hemoglobin, hematocrit, and platelets were carried out with the EDTA-treated anti-coagulated blood using a hematological instrument (MEK-6358, Celltac-alpha, Nihon Kohden, Tokyo, Japan) within hours of collection. To analyze the biochemical parameters, we centrifuged the anti-coagulated blood at 3,000 rpm for 10 min, and blood plasma was obtained and stored at \(-80^\circ\)C. Albumin concentrations were determined by the Bromo Cresol Green (BCG) assay, using a commercially available kit (Wako Pure Chemical Industries Osaka, Japan) and following the manufacturer’s instructions. The plasma levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) were determined by the JSCC transferable method by employing the standard ready-to-use kits (Wako Pure Chemical Industries). Total bilirubin was evaluated by the azobilirubin method, using a commercial kit (Daiichi Pure Chemicals Co. Tokyo, Japan).

Western blotting analysis. The PC3 cells were washed twice with PBS and then lysed with lysis buffer (50 mM HEPES, pH 7.4, 250 mM NaCl, 1 mM EDTA, 1% NP-40, 1 mM DTT, 1 mM PMSF, 5\(\mu\)g/ml leupeptin, 5\(\mu\)g/ml aprotinin, 2 mM NaVO₄, 1 mM NaF, 10 mM β-GP), and the proteins were extracted. After centrifugation, the supernatants were adjusted to achieve equal protein concentrations in each sample and then diluted with the same volume of 2 \(\times\) SDS sample buffer and heated for 5 min at 95°C. Samples (10\(\mu\)g of protein) were separated on 7.5% SDS-PAGE gels and electroblotted onto a polyvinylidene fluoride (PVDF) membrane. The blots were blocked for 1 h with 10% nonfat milk powder, 6% Glycine, and 0.1% Tween-20 in Tris buffered saline (TBS) at room temperature. The proteins were then identified with the use of the primary antibody, a rabbit anti-human REIC/Dkk-3 polyclonal antibody raised in our laboratory [8] (1:1,000 dilution). After extensive washing with 0.1% Tween-20 in TBS (T-TBS), the blots were exposed to the appropriate horseradish peroxidase-conjugated secondary antibody. After another round of extensive washing with T-TBS, membranes were developed using the enhanced chemiluminescence detection method (ECL kit, Amersham Pharmacia Biotech, Chandler, AZ, USA).

Apoptosis assay. To examine in vitro apoptosis induction after the treatments, we seeded cells in flat-bottom 6-well plates and incubated them for 24 h. The cells were then treated with Ad-LacZ and Ad-REIC at the indicated multiplicity of infection (MOI) in serum-free medium (500\(\mu\)l) for 2 h, and the medium was exchanged for fresh complete medium...
(2 ml). After an additional 48 h of incubation, Hoechst 33342 stock solution was added into the medium at a 2 μg/ml concentration, and the cells were incubated in the dark for 10 min. Hoechst 33342 is an intercalating dye that allows the determination of variations in the total chromatin quantity and the degree of chromatin condensation [11]. Using fluorescence microscopy, we identified apoptotic cells by the presence of highly condensed or fragmented nuclei. Apoptotic cells were counted at 5 different fields under microscopic observation. One hundred cells were judged under each field.

**Statistical analysis.** The data are expressed as the means ± standard error (S.E.). An unpaired Student’s t-test was performed for the statistical analysis between the 2 groups. The differences were considered statistically significant at p < 0.05.

**Results**

In the first experiment, we examined the in vivo toxicity and efficacy of the Ad-hREIC vector using the subcutaneous human prostate tumor mouse xenograft model. The experiment was designed as a preclinical study to support the future clinical use of Ad-hREIC, and we therefore evaluated its toxicological profile and anti-tumor effects. We also conducted a second experiment in non-tumor-bearing mice to examine the safety of Ad-mREIC, to ensure that specifically overexpressing REIC/Dkk3 via intra-prostatic injection does not result in toxicity.

None of the mice died during the first experiment in any of the treatment groups (n = 5). In the second experiment, 1 or 2 mice died in each group during the experiment [PBS (n = 15): one mouse died on day 18; Ad-LacZ (n = 16): 1 mouse died on day 7 and day 18; Ad-mREIC (n = 16): 1 mouse died on day 18 and one on day 19]. At necropsy, there was no obvious cause of death such as extensive inflammation, abnormal bladder distension, trauma of various organs, or abundant ascites.

In both the first and second experiments, there was no statistically significant difference in the survival between the Ad-REIC-treated group and the control groups by Mantel-Cox log-rank analysis (data not shown). The physical appearances and activities were observed carefully, and there were no definite differences among the groups in either experiment. Inflammation-related findings such as abscesses were not detected at the time of sacrifice in any of the animals. The time courses of body weight loss in both experiments are shown in Fig. 2A. The average body weight at the start point was as follows: PBS 27.0 g, Ad-LacZ 26.1 g, Ad-hREIC 29.3 g in experiment 1 and PBS 34.2 g, Ad-LacZ 34.3 g, Ad-mREIC 33.7 g in experiment 2. Although the average body weight of Ad-mREIC-treated mice tended to decrease more rapidly in experiment 2, no significant difference was observed in the body weight at any time point between the Ad-mREIC- and Ad-LacZ-treated groups. The body weight change between pre-treatment and the time of sacrifice was calculated for each treatment group, and no significant changes were observed in any of the groups during either experiment (Fig. 2B).

To assess the systemic toxicology, we collected blood and performed hematological (WBC, hemoglobin, hematocrit, and platelets) and plasma chemistry (albumin, ALT, AST, ALP, total bilirubin) evaluations (Table 1). There were no statistically significant differences between the Ad-LacZ and Ad-REIC groups in any of the parameters for either experiment. Because the LDH levels were affected by hemolysis of the collected blood samples, the differences in LDH activity could not be attributed to the toxicity of Ad-REIC treatment (data not shown).

To confirm the protein expression and apoptotic effect induced by Ad-REIC, we treated PC3 prostate cancer cells with Ad-hREIC. A Western blotting analysis demonstrated significant expression of the REIC/Dkk-3 protein in PC3 cells on day 2 after the treatment (Fig. 3A). In an apoptosis assay using the Hoechst 33342 stain, apoptotic cells were frequently observed in Ad-hREIC-treated PC3 cells (Fig. 3B, upper panel). The mean rate of apoptosis at 10, 20, and 100 MOI was 13.7%, 30.1%, and 67.5%, respectively, and significant apoptotic induction was observed for each Ad-hREIC concentration in comparison to the control Ad-LacZ treatment (Fig. 3B, lower panel).

In the first in vivo experiment, we further demonstrated the anti-tumor effects of Ad-hREIC using a subcutaneous PC3 tumor model (Fig. 4). In the PBS- and Ad-LacZ-treated groups, the tumor size progressively increased during the observation period. In contrast, the tumor growth in the Ad-REIC-treated group was significantly suppressed. In fact, the tumor had macroscopically disappeared at the time of sacri-
fice in one of the mice in the Ad-hREIC-treated group. A statistically significant reduction of the tumor volume was observed on days 21 and 25 in the Ad-REIC-treated group compared to the Ad-LacZ group.

To further examine the systemic toxicology of the Ad-mREIC treatment, we performed histological analyses using sections of the various organs (brain, lungs, heart, liver, stomach, spleen, kidneys, bladder, and rectum) in the second experiment. There were no histopathological abnormalities in the observed sections in any of the treatment groups (data not shown). We also investigated the local effects of Ad-mREIC injection by a histopathological analysis of the dorsolateral prostate, the site where the vector was injected. In the Ad-LacZ and Ad-mREIC treatment groups, infiltration of mononuclear cells typical of lymphocytes was observed in the interstitial area (Fig. 5A). In the Ad-LacZ-injected prostate, some degenerated prostatic glands containing fewer epithelial cells than normal were observed, indicating cytotoxic changes after Ad-LacZ treatment. Epithelial cell proliferation was also observed in the prostatic glands of the Ad-LacZ group, indicating some degree of related inflammation. We could not detect any histological effects in the prostatic sections of the PBS-treated group.

Because the REIC/Dkk-3 gene product is reported to possess immunological activity [14], we also assessed the spleen weight to determine whether the treatment induces the side effect of splenomegaly. The spleen weight of each mouse was measured at the time of sacrifice in both in vivo experiments, and there

Fig. 2  A, The mouse body weight is shown on the indicated days after each treatment. Five to 9 mice were analyzed for each group; B, The body weight changes between pre-treatment and the time of sacrifice are shown for each treatment group for experiments 1 and 2. Five to 9 mice were analyzed for each group. No significant (n.s.) difference was observed in any group in terms of body weight change.
Table 1  The hematological (WBC, hemoglobin, hematocrit and platelets) and plasma chemistry (albumin, ALT, AST, ALP, total bilirubin) evaluations were performed with the blood samples collected at the time of sacrifice. Shown are the means and standard errors of values for 4 to 5 animals per group.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>WBC (10^³ cells/µl)</th>
<th>Hemoglobin (g/dl)</th>
<th>Hematocrit (%)</th>
<th>Platelets (10⁴/µl)</th>
<th>Alb (g/dl)</th>
<th>ALT (IU/l)</th>
<th>AST (IU/l)</th>
<th>ALP (IU/l)</th>
<th>Total Bilirubin (mg/dl)</th>
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<tbody>
<tr>
<td>PBS</td>
<td>53.0 ± 11.1</td>
<td>11.6 ± 1.5</td>
<td>43.5 ± 4.7</td>
<td>66.7 ± 21.6</td>
<td>2.2 ± 0.11</td>
<td>36.0 ± 1.9</td>
<td>100.2 ± 9.9</td>
<td>107.6 ± 4.2</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>Ad-LacZ</td>
<td>71.0 ± 5.8</td>
<td>10.2 ± 0.5</td>
<td>38.7 ± 1.8</td>
<td>98.3 ± 9.0</td>
<td>2.1 ± 0.10</td>
<td>36.6 ± 1.4</td>
<td>102.8 ± 13.1</td>
<td>76.8 ± 7.4</td>
<td>0.03 ± 0.00</td>
</tr>
<tr>
<td>Ad-hREIC</td>
<td>53.0 ± 5.1</td>
<td>10.2 ± 0.3</td>
<td>37.7 ± 1.0</td>
<td>87.6 ± 10.9</td>
<td>2.0 ± 0.08</td>
<td>42.4 ± 7.3</td>
<td>85.6 ± 12.5</td>
<td>94.2 ± 8.8</td>
<td>0.02 ± 0.01</td>
</tr>
</tbody>
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WBC, white blood cells; Alb, Albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase.

Fig. 3  A, REIC/Dkk-3 expression after Ad-LacZ and Ad-hREIC treatment at 100 MOI was examined in PC3 prostate cancer cells by Western blotting analysis. The total protein was extracted on day 2 after the treatment, and 10 µg of protein was loaded per lane; B, The induction of apoptosis after in vitro Ad-hREIC treatment was examined in PC3 cells by Hoechst 33342 staining. The upper panel indicates the appearance of apoptotic cells after Ad-hREIC treatment. The lower panel shows the apoptotic rate of PC3 cells after the indicated treatments. A total of 5 different fields were examined under a microscope to determine the apoptotic rate. A significant difference was observed (*p < 0.05) between the Ad-hREIC and the control Ad-LacZ treatment.
To examine the local effects of Ad-mREIC treatment, we performed histological analysis of the vector injection site during the second experiment. The dorsolateral prostates (the injection site) were dissected at the time of sacrifice and fixed in formalin. The sections, stained with hematoxylin and eosin, were analyzed for histopathological and cytotoxic changes. The asterisk shows a degenerated prostatic gland that contains fewer epithelial cells than normal. The daggers indicate the affected prostatic glands with epithelial cell proliferation. The selected area (black square) is magnified in the inset panel, and the arrowhead indicates a mononuclear cell typical of lymphocytes;

To assess the effects on the spleen after Ad-REIC treatment, we measured the spleen weight of each mouse at the time of sacrifice in both experiments. Four to 5 mice were analyzed for each group. No significant (n.s.) difference was observed in the spleens of any of the mice in any of the treatment groups.
were no statistically significant differences in spleen weight following treatment in either treatment group (Fig. 5B).

**Discussion**

We previously cloned the REIC (Reduced Expression in Immortalized Cells) gene and reported that its expression is down-regulated in a variety of cancer cell lines and tumors [8, 10–12, 19–21]. The REIC gene is identical to the Dickkopf-3 (Dkk-3) gene [20] and REIC/Dkk-3 seems to be one of the most innovative tumor suppressor genes used for cancer gene therapy [8, 9, 12, 21]. We have extensively utilized an adenoviral vector agent, Ad-hREIC, containing the human REIC/Dkk-3 gene under the control of the CAG promoter in preclinical cancer models in mice. The adenoviral vector carrying human REIC/Dkk-3 selectively induced apoptosis in prostate cancer and malignant mesothelioma cells through the activation of c-Jun-NH2 kinase (JNK) and c-Jun [8, 12]. The adenovirus-mediated expression of the REIC/Dkk-3 gene efficiently induces endoplasmic reticulum (ER) stress-mediated apoptosis in a cancer cell-specific manner [12, 13]. The current study also demonstrated that the Ad-hREIC treatment induced significant apoptosis in a prostate cancer cell line. We further demonstrated the robust therapeutic utility of Ad-hREIC in a subcutaneous prostate cancer model. Thus, the adenoviral agent appears to be effective against human prostate tumors in terms of the cancer-specific targeting and a more radical response to expression of the gene.

The therapeutic effects of Ad-REIC were previously observed in a variety of in vivo tumor models generated with prostate, breast, and mesothelioma cancer cells [8, 9, 11, 12], but the toxicity of the Ad-REIC treatment remained unknown. Thus, we currently focused on the safety of local injection of Ad-REIC for the treatment of prostate cancer. Non-human primates have been used in some studies of adenoviral vector toxicity [22, 23], and mice have many advantages for preclinical evaluations prior to these primate studies [24]. To evaluate the toxicity of the Ad-REIC vector, we first analyzed the lethality and survival in 2 experiments. There was no toxicity when tumor-bearing mice were administered the human-REIC vector, although some lethality was observed in the mice during the second experiment. However, since the survival was not statistically different between the groups (1 or 2 mice died in both the control and treatment groups), it is likely that the Ad-REIC treatment itself did not specifically induce the deaths. The most conceivable reason for the lethality is that the mice in the second experiment died due to post-operative complications of the open surgery for the prostatic injections. The other possibility is that adenovirus toxicity may be the cause of the deaths. Although definite histological damage was not observed in the examined mice, we cannot exclude this possibility. To confirm the reproducibility of the deaths and to exclude the adenovirus-related death, we did similar additional experiments using C57BL/6 mice in which there was no death during the experimental schedule until day 28. Thus, it seems that the death in experiment 2 was not reproducible and not adenovirus-related. Based on these results, another possibility is that the mice deaths may have been procedure-related.

We next investigated the systemic toxicity of the Ad-REIC treatment. The liver has been widely recognized as the primary target organ of adenoviral vectors after an intravenous adenoviral administration [25]. Although the Ad-REIC was locally administered by intratumoral or intra-prostate injection in our experiments, the possibility could not be excluded that the viral particles might disseminate into the general circulation and affect the liver functions. However, in the current studies using Ad-hREIC and Ad-mREIC, we did not detect any differences in the liver enzymes or other biochemical parameters in any of the 3 treatment groups. None of the mice showed histopathological liver abnormalities such as cytotoxicity or inflammation in the PBS-, Ad-LacZ-, or Ad-mREIC-treated groups during the second experiment (data not shown), indicating the consistency of the results of biochemical evaluations. As for other distant organs, no histological abnormalities were observed (data not shown) in the screening, although there were some histological changes at the injection site, the dorsolateral prostate. In addition, there was no effect of Ad-REIC treatment on hematological parameters or spleen weight. It therefore seems that Ad-REIC and the gene product, secreted REIC protein, do not induce serious systemic toxicity or non-specific inflammation under the indicated conditions.
The second step of our work was to analyze the local side effects associated with an intra-prostatic delivery of the Ad-REIC vector by direct injection. To investigate the probable local toxicity due to the adenovirus or the gene products, including the secreted REIC protein, we examined the histopathological changes of the dorsolateral prostate after the Ad-mREIC injection. We could not detect any histological damage to the prostate gland after PBS or Ad-REIC treatment. In the Ad-LacZ group, there was some damage to the prostate gland, which may have been due to the toxicological profile of the adenoviral vectors [26]. The lack of local toxicity in the Ad-mREIC-injected prostate glands was consistent with the previous reports showing that Ad-hREIC induces cytotoxic or apoptotic effects only in cancer cells and not in normal cells [8, 11]. As for the lymphocyte infiltration in the prostatic interstitial space, the phenomenon was observed in both the Ad-LacZ and Ad-mREIC treatment groups. It is likely that the adenoviral vector may have caused the inflammatory reaction. In addition, a recent study reported that the REIC protein induced a chemokine-like action that caused the accumulation of immune cells when directly injected into subcutaneous tumors in C57BL/6 mice [14]. Thus, the other possibility is that the gene product, the REIC protein, might have been involved in lymphocyte infiltration in the Ad-mREIC-treated group. Further studies will clarify the mechanisms of the Ad-REIC-induced immunomodulatory effects in locally injected normal and tumor tissues.

Our results show that the adenoviral vector encoding the REIC/Dkk-3 gene and the CAG promoter has no significant toxicity and is therapeutically effective when directly injected into the tumors of mice with experimental prostate cancer or into the prostates of normal mice at the indicated doses. We believe that the current study provides valuable information for future clinical trials of Ad-REIC-mediated gene therapy against human prostate cancer.

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References


