Feasibility of Neoadjuvant Ad-REIC Gene Therapy in Patients with High-Risk Localized Prostate Cancer Undergoing Radical Prostatectomy

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Abstract

In a phase I/IIa study of *in situ* gene therapy using an adenovirus vector carrying the human *REIC/Dkk-3* gene (Ad-REIC), we assessed the inhibitory effects of cancer recurrence after radical prostatectomy (RP), in patients with high risk localized prostate cancer (PCa). After completing the therapeutic interventions with initially planned three escalating doses of 1.0×10^{10}, 1.0×10^{11}, and 1.0×10^{12} viral particles (VP) in 1.0–1.2 mL ($n = 3$, 3, and 6), an additional higher dose of 3.0×10^{12} VP in 3.6 mL ($n = 6$) was further studied. Patients with recurrence probability of 35% or more within 5 years after RP as calculated by Kattan's nomogram, were enrolled. They received two ultrasound-guided intratumoral injections at 2-week intervals, followed by RP 6 weeks after the second injection. Based on the findings of MRI and biopsy mapping, as a rule, one track injection to the most prominent cancer area was given to initial 12 patients and 3 track injections to multiple cancer areas in additional 6 patients. As compared to the former group, biochemical recurrence-free survival of the latter showed a significantly favorable outcome. Neoadjuvant Ad-REIC, mediating simultaneous induction of cancer selective apoptosis and augmentation of antitumor immunity, is a feasible approach in preventing cancer recurrence after RP. (199) Clin Trans Sci 2015; Volume 8: 837–840

Keywords: REIC/Dkk-3, gene therapy, neoadjuvant therapy, localized prostate cancer

Introduction

With the advent of prostate specific antigen (PSA) screening, PCa has been detected commonly when localized. Nevertheless, outcomes in patients with high risk localized prostate cancer (PCa) undergoing RP alone, have not improved significantly with time.[1] In order to improve long-term outcomes of these patients, the establishment of a new, efficient neoadjuvant therapy is much awaited. Recently, a variety of clinical trials including *in situ* gene therapy have been conducted as a form of neoadjuvant therapy, since this approach provides a paradigm for evaluating the activity and mechanism of action of new agents with histopathological analysis using tumor tissues before and after therapy.[2]

The expression of reduced expression in immortalized cells (REIC) /Dickkoph-3 (Dkk-3) gene is significantly reduced in a wide variety of cancer cells including prostate cancer [3–10] and its forced expression using Ad-REIC, induces cancer-selective apoptosis as a result of unfolded protein response, due to endoplasmic reticulum stress (ER stress).[6] ER stress mediates the enhanced IL-7 expression in co-infected normal fibroblasts, resulting in the activation of innate immunity involving NK cells.[11] In addition, secreted REIC protein with potent immunomodulatory function creates an optimal environment for activation of host immune cells, inducing cytotoxic T lymphocytes.[12,13]

We are developing an Ad-REIC gene therapy agent as a therapeutic cancer vaccine in the treatment of various intractable solid cancers. The First-In-Human clinical study, a phase I/IIa study of *in situ* Ad-REIC gene therapy for prostate cancer was initiated at Okayama University from January 2011. In this phase I/IIa study, two groups of patients were treated: group A consisting of patients with castration-resistant PCa (CRPC) with or without metastasis, and group B consisting of patients with high-risk, localized PCa scheduled to undergo radical prostatectomy (neoadjuvant study). In group A, direct and indirect systemic effects induced by *in situ* gene therapy were clearly illustrated in a case of chemotherapy resistant advanced CRPC with bulky lymph node metastases.[14]

In the neoadjuvant study, patients treated with the initially planned three escalating dose levels (DLs) of 1.0×10^{10}, 1.0×10^{11}, and 1.0×10^{12} viral particles (VP) in 1.0–1.2 mL ($n = 3$, 3, and 6) showed remarkable safety profiles of Ad-REIC (primary endpoint) and dose-dependent immunopathological effects (secondary endpoint) without reaching the maximum tolerated dose. Then, an additional study with a higher dose level (DL-4) of 3.0×10^{12} VP in 3.6 mL ($n = 6$) was conducted to assess the safety and the inhibitory effects of cancer recurrence after RP. In this preliminary report, we discuss the potential of neoadjuvant Ad-REIC gene therapy to proceed the next comparative study. The precise clinical data on the entire phase I/IIa study of *in situ* Ad-REIC gene therapy including the data for advanced CRPC will be published elsewhere.

Patients and Methods

Kattan's nomogram score of ≥115 (calculated 5-year recurrence-free probability of ≤65%) [15,16] was used to select high risk localized PCa and eighteen patients were enrolled in the study. Initially, three escalating DLs of Ad-REIC (1.0×10^{10}, 1.0×10^{11}, and 1.0×10^{12} VP in 1.0–1.2 mL) were studied in 3, 3 and 6 cases, respectively. Based on MRI findings and biopsy mapping, one track injection to the most prominent cancer area was conducted. Secondarily, DL-4 of 3.0×10^{12} VP in 3.6 mL with 3 track injections (3 times injection of 1.0×10^{12} VP in 1.2 mL) to multiple cancer areas was given to 6 cases. The original Ad-REIC, a replication-deficient adenovirus vector, was constructed at Okayama University [3] and its cGMP product

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		Dose Level 1+2 ($n = 6$)	Dose Level 3 ($n = 6$)	Dose level 4 (N = 6)	p Value
Age		**62.5 (59–74)**	**68 (63–71)**	**66 (57–74)**	**0.533**
Clinical T stage	T2a	1	0	2	0.485
	T2b	0	0	0	
	T2c	2	2	2	
	T3a	3	4	2	
Biopsy Gleason score	7	0	2	1	0.392
	8	2	3	3	
	9	3	1	2	
	10	1	0	0	
PSA		**13.425 (5.02–26.62)**	**13.775 (9.82–16.18)**	**19.5 (10.87–33.60)**	**0.309**
Kattan's nomogram score		**140.5 (124–176)**	**140 (118–167)**	**137 (122–173)**	**0.894**
Pathlogical stage	pT2b	0	0	0	0.148
	pT2c	1	3	4	
	pT3a	0	1	1	
	pT3b	5	2	1	
	Margin+	4	4	4	1
	Node+	2	0	0	0.085

Table 1. Clinical and pathological characteristics of 18 patients enrolled in the study.

was supplied by Momotaro-Gene Inc., a start-up biotech company originating from Okayama University. All patients received two ultrasound-guided intraprostatic injections at 2-week intervals, followed by RP 6 weeks after the second injection.

Using standard sections of RP specimens with hematoxylin and eosin staining, final pathological diagnosis and antitumor effects mediated by Ad-REIC were determined. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining for the detection of apoptosis of cancer cells and immunohistochemical staining for the analysis of tumor infiltrating lymphocytes were conducted in selected cases. Peripheral blood lymphocyte subsets were analyzed by flow cytometry after Ad-REIC treatment in all patients. Serum PSA levels were analyzed before and after Ad-REIC injections. After RP, PSA was measured at months 1, 2, 3 and every 3 months thereafter or as clinically indicated. Biochemical recurrence was defined as an initial PSA value exceeding 0.2 ng/mL, followed by a subsequent confirmatory PSA value >0.2 ng/mL . If PSA levels did not decrease to less than 0.2 ng/mL after surgery, the date of RP was defined as the date of disease recurrence.

The present clinical protocols were approved by the Okayama University Institutional Review Board and the Japanese Government. Patients reviewed the informed consent document and received individual counseling with a thorough discussion as to alternative treatments, including nonparticipation.

Results

Clinical and pathological characteristics of 18 patients in three groups (DL-1+2, 3, and 4) are demonstrated in *Table 1*. Although the number of patients in each dose level is small, there are no significant differences in patient characteristics, including Kattan's nomogram scores among three groups of neoadjuvant Ad-REIC treatment. Most patients were regarded as a very high risk for recurrence; 83% (15/18) had a Gleason score of ≥8 and 72% percent (13/18) had a Kattan's nomogram score of >130 (5-year recurrence free probability of <5%). All 4 dose levels including the additional dose level 4, were feasible with no adverse events except for fever. Grade 1 or 2 fever was a common symptomatic toxicity in high dose levels of 3 and 4, but was transient and treatable with antipyretics. Neither intraoperative nor postoperative complications related to neoadjuvant Ad-REIC were observed.

In terms of antitumor effects, no clear effects were detected in DL-1 but 2 out of 3 in DL-2 showed PSA decline and moderate cytopathic effects with tumor infiltrating lymphocytes (TIL). All 6 cases in dose level 3 showed PSA decline and clear cytopathic effects with TIL. As illustrated in *Figure 1A*, massive degeneration with cytolysis and pyknosis was detected in the targeted tumor areas of Case B-8. Using serial sections of the surgical specimen from Case B-8 (*Figures 1B–E*), pyknotic cells undergoing apoptosis were confirmed by TUNEL staining (*Figure 1C*) and remarkable, concurrent infiltrations of $CD8^+$ lymphocytes (*Figure 1D*) and dendritic cells (*Figure 1E*) were clearly demonstrated in the area of apoptotic cancer cells by immunohistochemical staining.

Biochemical recurrence-free survival (BRFS) of each dose group was compared using the Kaplan-Meier survival analysis. Although the follow-up duration of DL-4 was short (median 12.0, range 6.1 to 29.7 months), no recurrence was observed in patients treated with dose level 4. BRFS in DL-4 was significantly more favorable than in DL-1+2 plus DL-3 group patients (*Figure 2*). Peripheral blood lymphocyte subsets were analyzed by flow cytometry after Ad-REIC treatment. Changes in B cells, T cells, NK cells and $CD4^+$ lymphocytes showed no tendency to increase, while $CD8^+$ lymphocytes increased in response to Ad-REIC treatment (*Figure 3A*). The HLA-DR marker of activation was used to double label $CD3^+$, $CD4^+$ and $CD8^+$ lymphocytes as a relative measure of activated T cells. $HLA\text{-}DR^+CD8^+$ (activated

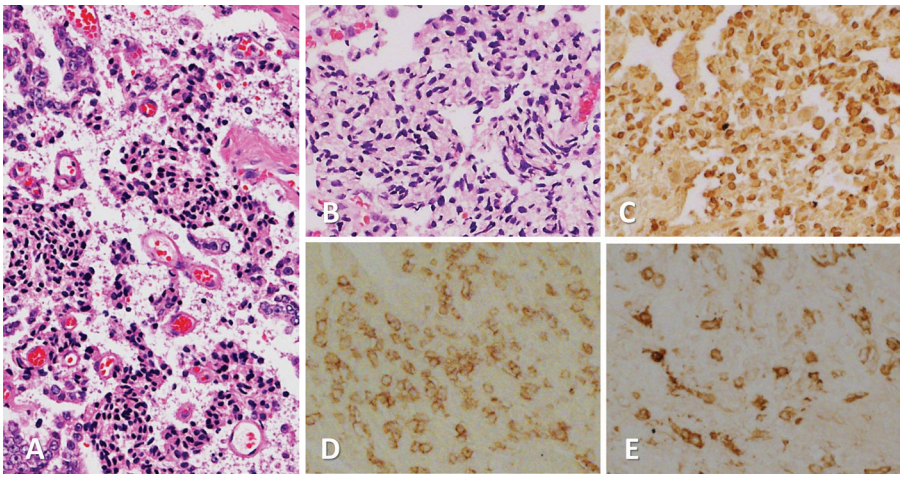

Figure 1. Surgical specimen from Case B-8 treated with DL-3 of 1×10^{12} VP of Ad-REIC. (A) Massive degeneration with cytolysis and pyknosis detected in the targeted tumor (H&E, x100). Serial sections stained differently (×100). (B) H&E, (C) TUNEL staining, illustrating pyknotic cells undergoing apoptosis. (D) Immunohistochemical staining, illustrating remarkable, concurrent infiltrations of CD8+ lymphocytes and (E) dendritic cells in the area of apoptotic cancer cells.

CTL) and HLA-DR$^+$CD3$^+$ (activated T) lymphocytes showed increases after Ad-REIC treatment with a tendency of dose-dependent manner (*Figures 3B and C*).

Discussion

Neoadjuvant therapy is widely accepted in the treatment of patients with localized or locally advanced high-risk breast cancer and other solid cancers.[17–19] In prostate cancer, however, a number of neoadjuvant trials with androgen deprivation therapy (ADT) and chemotherapy with or without ADT demonstrated that the beneficial effects on pathological outcomes, including pCR rate, did not translate to improved disease-free survival or overall survival.[20–25] Therefore, neoadjuvant therapy with ADT and chemotherapy is not currently recommended in patients with high-risk localized PCa undergoing RP. To establish a new standard of neoadjuvant therapy, a variety of clinical trials with various novel agents have been conducted. Different from ordinary systemic therapy, *in situ* immune gene therapy is expected to provide a new option for neoadjuvant therapy by generating indirect systemic effects.[26–28]

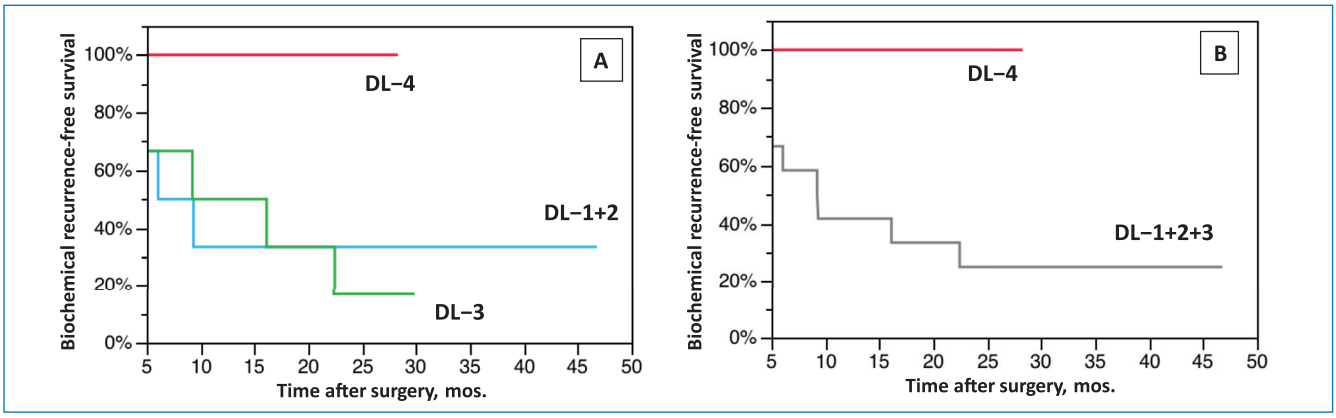

Figure 2. Kaplan–Meier curves representing biochemical recurrence free survival (BRFS) in patients with high risk prostate cancer treated with neoadjuvant Ad-REIC followed by radical prostatectomy. (A) BRFS curves of 3 dose level groups. The differences were not significant (log-rank test). (B) BRFS curves of DL-1,2,3 pooled group and DL-4 group. The difference was significant ($p < 0.05$, log-rank test).

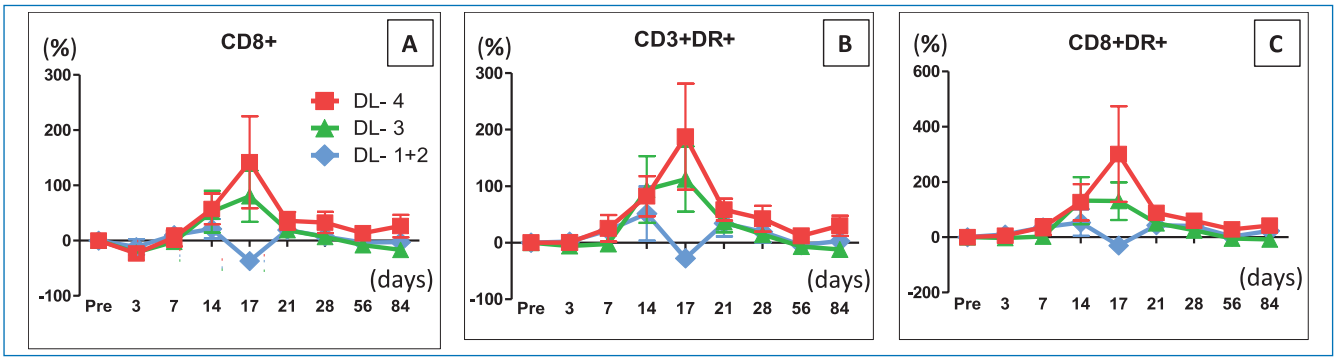

Figure 3. Flow cytometry analysis of circulating peripheral blood lymphocyte at indicated time points after the first injection (Day-0) of Ad-REIC. Data for each changing rate are presented as mean ± SE. (A) Changing rates of CD8$^+$ cells in three dose groups. (B) Changing rates of CD3$^+$ DR$^+$ cells in three dose groups. (C) Changing rates of CD8$^+$ DR$^+$ cells in three dose groups. Changing rates of CD8$^+$, CD3$^+$ DR$^+$,CD8$^+$ DR$^+$ cells increased after the second Ad-REIC injection (Day-13) in DL-3 and DL-4, but the differences among three dose groups were not significant (two-way ANOVA test).

REIC/Dkk-3 gene was isolated and cloned as an immortalization-related gene at Okayama University in 2000,[29] and has rapidly emerged as a key player in most human cancers.[30] Recently, we have reviewed previous fundamental studies and summarized the anticancer mechanisms of *in situ* Ad-REIC as a therapeutic cancer vaccine.[13] These anticancer mechanisms of Ad-REIC mediating direct and indirect systemic effects by augmented antitumor immunity have been confirmed in treating a patient with metastatic CRPC following chemotherapy.[14] In the present neoadjuvant study, the key principal of Ad-REIC anticancer mechanisms characterized by massive cancer selective apoptosis with concurrent infiltrations of $CD8^+$ lymphocytes and dendritic cells was clearly illustrated in histological sections of surgical specimens treated with Ad-REIC (see *Figure 1*). In addition, peripheral blood $CD8^+$ T cells were increased with a tendency of dose-dependent upregulation of HLA-DR expression (see *Figure 2*). Therefore, it is possible that cancer vaccine effects by neoadjuvant Ad-REIC could translate into improved postoperative outcomes in patients with high-risk localized PCa. BRFS in dose level 4, with three track injections using an optimum Ad-REIC dose of 1.0×10^{12} VP /1.2 mL, was significantly more favorable than other dose levels.

Conclusion

Although historically, PCa was not regarded as an immunogenic cancer, recent clinical results including the efficacy of sipuleucel-T for metastatic CRPC have led to a renewed interest in immunotherapy for PCa. Consequently, Ad-REIC gene therapy is regarded as a promising option for immunotherapy in the treatment of patients with high-risk localized PCa in neoadjuvant setting. Prospective randomized comparative study may warrant exploration. (1,568)

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