CpG Oligodeoxynucleotides Enhance the Activities of CD8+ Cytotoxic T-Lymphocytes Generated by Combined hMUC1 Vaccination and hNIS Radioiodine Gene Therapy

Yong Hyun Jeon · Yun Choi · Jaetae Lee ·
Chul Woo Kim · June-Key Chung

Received: 10 March 2010 / Revised: 14 May 2010 / Accepted: 26 May 2010 / Published online: 13 July 2010
© Korean Society of Nuclear Medicine 2010

Abstract

Purpose The authors evaluated whether the cytotoxicity of CD8+CTLs generated by combined hMUC1 vaccination and hNIS radioiodine gene therapy was enhanced in the presence of CpG in an established tumor model.

Methods CMNF cells (CT26 cells expressing hMUC1, hNIS and Firefly luciferase) were transplanted into BALB/c mice. Four and 10 days later, tumor-bearing mice were immunized intramuscularly with pcDNA3.1 or pcDNA-hMUC1 or pcDNA-hMUC1+CpG, and subsequently administered PBS or ^{131}I [five groups (seven mice/group): referred to as the pcDNA3.1+PBS, phMUC1+PBS, pcDNA3.1+ ^{131}I , phMUC1+ ^{131}I , and phMUC1+ ^{131}I +CpG groups]. The number of CD8+IFN γ + T cells of splenocytes as well as the number of CD8+IFN γ + T cells of splenocytes re-stimulated with CD11c+ cells was determined using FACS analysis. The activities of cytotoxic T cells (CTLs) from splenocytes were investigated.

Results Marked tumor growth inhibition was observed in the phMUC1+ ^{131}I and phMUC1+ ^{131}I +CpG groups, but not in the other three single therapy groups. Particularly the number of CD8+IFN γ + T cells of splenocytes was more increased in the phMUC1+ ^{131}I +CpG group than in the phMUC1+ ^{131}I group. The number of CD8+IFN γ + T cells of splenocytes stimulated with CD11c+ cells was the most enhanced in the phMUC1+ ^{131}I +CpG group among the five groups. Concurrently, the activities of hMUC1-associated CTLs obtained from splenocytes in the phMUC1+ ^{131}I +CpG group were significantly greater than in the other four groups (pcDNA+PBS, phMUC1+PBS, pcDNA+ ^{131}I , phMUC1+ ^{131}I , and phMUC1+ ^{131}I +CpG, 16 $\pm$ 2%, 20 $\pm$ 1%, 30 $\pm$ 2%, 60 $\pm$ 2%, and 87 $\pm$ 2%, respectively, $P<0.01$).

Conclusion Our data suggest that adjuvant CpG ODNs can increase the killing activities of CTLs generated by combined hMUC1 DNA vaccination and hNIS radioiodine gene therapy.

Yong Hyun Jeon and Yun Choi contributed equally to this study.

Y. H. Jeon · J.-K. Chung (✉)
Department of Nuclear Medicine,
Seoul National University College of Medicine,
28 Yongon-dong, Chongno-gu,
Seoul 110-744, Korea
e-mail: jkchung@plaza.snu.ac.kr

Y. Choi · C. W. Kim
Department of Pathology,
Seoul National University College of Medicine,
Seoul, Korea

Y. H. Jeon · Y. Choi · C. W. Kim · J.-K. Chung
Tumor Immunity Medical Research Center,
Seoul National University College of Medicine,
Seoul, Korea

Y. H. Jeon · J.-K. Chung
Laboratory of Molecular Imaging
and Therapy of Cancer Research Institute,
Seoul National University College of Medicine,
Seoul, Korea

Y. H. Jeon · J. Lee
Department of Nuclear Medicine, School of Medicine,
Kyungpook National University,
Daegu, Korea

C. W. Kim (✉)
Department of Pathology, Tumor Immunity Medical Research
Center and Cancer Research Institute, Seoul National University,
College of Medicine,
28 Yongon-Dong, Jongno-Gu,
Seoul 110-799, Korea
e-mail: cwkim@plaza.snu.ac.kr

Keywords CpG oligodeoxynucleotides · hMUC1 vaccination · hNIS radioiodine gene therapy

Abbreviations

CMNF	CT26/hMUC1-hNIS-Fluc
<i>Fluc</i>	firefly luciferase
CMN therapy	combined hMUC1 vaccination and hNIS radioiodine gene therapy

Introduction

Because single modality cancer therapies are not usually effective against aggressive tumors, many researchers have focused on the merits of combinatorial therapies with new modalities of cancer therapy [1, 2]. For example, combinatorial therapies comprised of radiotherapy and a cancer vaccine have been investigated. Although radiation alone can destroy cancer cells directly through apoptosis or necrosis, not all cancer cells are killed, and some of these may become resistant to radiation. Alternatively, some cancer vaccines have been shown to induce anti-tumor immunity, and several trials have concluded that cancer vaccines alone inadequately inhibit tumor progression and recurrence because of low immunogenicities and adverse effects on regulatory T cells and other cellular entities.

Previously, we reported that combined hMUC1 vaccination and hNIS radioiodine gene therapy showed synergistic therapeutic effects in a murine tumor model and that these effects substantially augment the comparatively weak therapeutic effects of hMUC1 [3]. In addition, our group found that combined hMUC1 vaccination and hNIS radioiodine gene therapy can prevent tumor recurrence by synergistically enhancing hMUC1-associated immunity in an animal model [4].

It is important that cytotoxic T cell (CTL) activity against cancer be maintained during immunotherapy, since tumor-associated CTLs are essential effectors [2]. Of the various strategies used to maintain and enhance CTL activity, the adjuvant used of CpG oligonucleotides (ODNs) has attracted attention. In particular, CpG ODNs have been reported to have an immunostimulatory effect and to promote the cancer cell-killing activities of CTLs [5, 6]. Furthermore, CpG ODNs have been suggested to be a powerful enhancer of radiation-induced immune response [7–9].

In this study, we attempted to increase the killing activities of cytotoxic T lymphocytes (CTLs) generated by combined hMUC1 vaccination and hNIS radioiodine gene therapy by introducing CpG ODNs as an adjuvant.

Materials and methods

Animals

Specific pathogen-free 6-week-old female BALB/c mice were obtained from SLC Inc. (Japan). All animal experiments were performed after receiving approval from the Institutional Animal Care and Use Committee (IACUC) of the Clinical Research Institute at Seoul National University Hospital. In addition, the National Research Council (NRC) guidelines for the care and use of laboratory animals (revised 1996) were observed throughout.

Cell line

Previously, we established the CMNF cell line, which is a stable clone of CT26 cells (an adenocarcinoma colon cancer cell line) that expresses the hMUC1, hNIS and firefly luciferase genes (Fig. 1) (CT26/hMUC1-hNIS-*Fluc*), and examined gene expression in this cell line by FACS and by performing ^{125}I uptake and in vitro luciferase assays (data not shown).

DNA vaccine

The human pancreatic mucin 1 gene, hMUC1 (accession no. J05582), was cloned into the BamHI site of pcDNA3 vector (Invitrogen, Carlsbad, CA). Plasmid DNA was amplified in *E. coli* DH5 α and purified by large-scale plasmid preparation using endotoxin-free Giga Prep columns (QIAGEN, Chatsworth, CA). DNA was dissolved in endotoxin-free TE buffer for storage purposes.

Fig. 1 Schematic representation of CMNF cells simultaneously expressing hMUC1, hNIS and Fluc. CMNF cells were derived from CT26 cells, a murine colon cancer cell line, and stably express three genes: hMUC1 gene for immunotherapy, hNIS gene for radioiodine gene therapy and firefly luciferase gene for monitoring tumor progression in living animal tumor models

In vitro clonogenic assay

The procedure used has been described previously [10]. Briefly, cells were grown in a 75-cm² flask and incubated for 7 h at 37°C in 5 ml of HBSS containing 37 MBq/10 ml (1 mCi/10 ml) of Na¹³¹I. The reaction was terminated by removing the radioisotope-containing medium and washing the cells twice with HBSS. Cells were then trypsinized, counted, and plated at densities of 250 or 1,000 cells per well in DMEM in six-well plates. They were then grown for 10 days, fixed with 3:1 methanol/acetic acid and stained with crystal violet, and macroscopic colony numbers were counted. Survival rates are presented as colony numbers expressed as percentages of colony numbers in plates treated with HBSS only.

Animal experiments

The five experimental groups are referred to as the pcDNA+PBS, phMUC1+PBS, pcDNA+¹³¹I, phMUC1+¹³¹I and phMUC1+¹³¹I+CpG ODNs groups. The experimental procedure detailed below was used to examine the relative efficacies of combined hMUC1 vaccination and hNIS radioiodine gene therapy in the absence and presence of CpG ODNs (Fig. 2).

CMNF cells (1×10^5) were transplanted subcutaneously into the right thighs of 35 BALB/c mice. Four and 10 days later, mice were immunized with pcDNA3.1 (50 µg/50 µl) or phMUC1 (50 µg/50 µl) or phMUC1 (50 µg/25 µl)+endotoxin-free CpG ODNs (30 µg/25 µl, 5'-TCC ATG ACG TTC CTG ACG TT-3'; BIONICS) into right hind leg quadriceps muscles, and 14 days later were administered PBS or 2 mCi of ¹³¹I i.p. This procedure

Fig. 2 Animal experimental procedure. CMNF cells (1×10^5) were transplanted s.c. into the right thighs of immunocompetent Balb/c mice. Tumor-bearing mice were immunized i.m. with the vectors pcDNA3-hMUC1+CpG, pcDNA3-hMUC1 or pcDNA3.1 at 4 and 10 days post-tumor challenge, and scintigraphic images were acquired at 14 days. In addition, after obtaining scintigraphic images, ¹³¹I was injected i.p. Bioluminescent imaging was performed, and tumor volume measurements were taken at the designated times

produced the five study groups (seven mice/group) mentioned above. Mice received a low-iodine diet with T4 supplementation in drinking water for 2 weeks post-tumor challenge to maximize radioiodine uptake in tumors and to reduce iodide uptake by thyroid glands. Tumor sizes were measured using a caliper at 7, 14, 21, 24, 28 and 32 days post-tumor challenge. For bioluminescence imaging acquisition, mice were repeatedly imaged at 3, 10, 14, 17, 21, 24 and 32 days post-challenge using an optical CCD camera, and for scintigraphic imaging acquisition, ^{99m}Tc-pertechnetate (0.5 mCi) was injected i.p. and mice were imaged using a γ-ray camera (ON-410) at 14 days post-challenge.

Intracellular cytokine staining and flow cytometric analysis

Mouse splenocytes (seven per group) were isolated from treated mice and stimulated in vitro for 72 h using 10 µg/ml of hMUC1 peptide (PDTRPAPGSTAPPAHGVTSAPDTRPAPGST) and IL-2 (50 U/ml). Stimulated splenocytes were treated with Golgistop (BD Pharmingen, NJ). Splenocytes were then washed once in FACS buffer and stained with FITC-conjugated monoclonal rat antimouse CD8 (BD Pharmingen, NJ). Cells were immunostained for cytokines using a Cytofix/cytoperm kit (BD Pharmingen, NJ) and with PE-conjugated anti-IFN-γ (BD Pharmingen, NJ). Flow cytometric analysis was performed using a Becton-Dickinson FACScan using CELLQuest software (Becton Dickinson Immunocytometry Systems, CA).

Re-stimulation of hMUC1-associated CD8+ T cells by enriched CD11c+ cells from immunized mice

Ten days after treating tumor-bearing mice (five mice/group) with ¹³¹I, splenocytes were harvested, and CD8+ T cells were separated using CD8 (Ly-2) microbeads (Miltenyi Biotec, Auburn, CA). The enriched CD8+ T cells so obtained were analyzed by FACS and restimulated in vitro for 72 h with 10 µg/ml of hMUC1 peptide (PDTRPAPGSTAPPAHGVTSAPDTRPAPGST) and IL-2 (50 U/ml).

Thirteen days after treating tumor-bearing mice (four mice/group) with ¹³¹I, inguinal draining lymphoid cells were harvested, and CD11+ cells were enriched using CD11c (N418) Microbeads (Miltenyi Biotec, Auburn, CA). These enriched cells were then analyzed by FACS. Re-stimulated CD8+ T cells (treated with Golgistop for 6 h (BD Pharmingen, NJ)) were then harvested for co-culture with the enriched CD11c+ cells. The enriched CD11+ cells (1×10^5) were then co-cultured with re-stimulated 1×10^6 hMUC1-associated CD8+ T cells for 16 h. The re-stimulated CD8+ T cells were then stained with CD8 and IFN-γ Ab (BD Pharmingen, NJ) and analyzed by flow cytometry.

In vitro splenocyte cytotoxicity assays

The CytoTox 96 nonradioactive cytotoxicity assay (Promega, Madison, WI) was used to measure the cytotoxic activities of splenocytes in treated mice (eight mice/group), according to the manufacturer's protocol with minor modification. Briefly, splenocytes of treated immunocompetent BALB/c mice were incubated in the presence of 50 U/ml of human IL-2 and 10 µg/ml of hMUC1 peptide (PDTRPAPGSTAPPAHGVTSPDTRPAPGST). After 3 days, irradiated CT26 and CMNF target cells were plated at 1×10^4 cells/well on 96-well U-bottomed plates (Costar), and splenocytes (effectors) were then added to a final volume of 100 µl in a 1:12 splenocyte-to-target cell ratio. Plates were then incubated for 4 h in a humidified 5% CO₂ chamber at 37°C and centrifuged at 500g for 5 min.

Aliquots (50 µl) were transferred from all wells to fresh 96-well flat-bottom plates, and an equal volume of reconstituted substrate mix was added to each well. The plates were then incubated in the dark at room temperature for 30 min. Stop solution (50 µl) was then added, and absorbance values were measured at 492 nm. Cell death percentages at each effector-to-target cell ratio were calculated using: $[A(\text{experimental}) - A(\text{effector spontaneous}) - A(\text{target spontaneous})] \times 100 / [A(\text{target maximum}) - A(\text{target spontaneous})]$.

Statistical analysis

All data are expressed as means $\pm$ SDs and are representative of at least two separate experiments. Tumor protection-related experimental findings were analyzed using the Kruskal-Wallis

Fig. 3 In vivo visualization of the anti-tumor effects of combinational hMUC1 vaccination/¹³¹I plus CpG therapy. **(a)** In vivo monitoring of the minor anti-tumor effects of hMUC1 vaccination by scintigraphic imaging. Before administering a therapeutic dose of ¹³¹I to mice on day 14 post-challenge, scintigraphic images were acquired. T: thyroid, S: stomach, B: urinary bladder, CMNF: CT26/hMUC1-hNIS-Fluc. **(b)** The anti-tumor effects of hMUC1 vaccination+¹³¹I+CpG therapy were visualized by using the IVIS living imaging system at the designated times. **(c)** To quantify light intensities, regions of interest (ROI) were drawn over tumor regions,

and total photon effluxes were measured. *P<0.02 (pcDNA+PBS vs. phMUC1+PBS), **P<0.01 (pcDNA+¹³¹I vs. phMUC1+¹³¹I and pcDNA+¹³¹I vs. phMUC1+¹³¹I+CpG). **(d)** Tumor volumes [defined as length (mm) $\times$ width (mm)] were measured using a caliper. *P<0.02 (pcDNA+¹³¹I vs. phMUC1+¹³¹I and pcDNA+¹³¹I vs. phMUC1+¹³¹I+CpG), **P<0.02 (phMUC1+¹³¹I vs. phMUC1+PBS and phMUC1+¹³¹I+CpG vs. phMUC1+PBS). ***P<0.02 (pcDNA+PBS vs. phMUC1+PBS). **(e)** Numbers of tumor-free mice are expressed as percentages post-tumor challenge. Experiments were performed in triplicate, n=seven mice/group

test and Wilcoxon's rank sum test with Bonferroni's correction. Kaplan-Meier curves were used to estimate the tumor-free mice. P values of <0.05 were considered significant.

Results

Treatment of tumor xenografts in animals

Minor tumor growth inhibitory effects were observed by scintigraphic imaging in the three hMUC1 vaccination groups, but not in the two pcDNA vaccination groups (Fig. 3a). A slight tumor growth inhibition effect was observed in the phMUC1+PBS group, but not in the pcDNA3.1+PB group

S group at 32 days post-challenge (Fig. 3d, $P < 0.02$, pcDNA+PBS vs. phMUC1+PBS). Rapid tumor growth inhibition was observed in ^{131}I -treated animals (pcDNA+ ^{131}I , phMUC1+ ^{131}I and phMUC1+ ^{131}I +CpG ODNs) (Fig. 3b-d), according to bioluminescence imaging and caliper measurements. Importantly, tumor-free mice were only observed in two groups, i.e., the phMUC1+ ^{131}I group and the phMUC1+ ^{131}I +CpG ODNs group, in both of which six of seven mice were tumor free (Fig. 3b and e).

hMUC1-associated CD8+IFN- γ + T cells

The frequency of CD8+IFN- γ + T cells in the phMUC1+ ^{131}I +CpG ODNs group was markedly higher than in the other

Fig. 4 Levels of hMUC1-associated CD8+IFN- γ + T cell immune response in immunocompetent mice treated with phMUC1+ ^{131}I +CpG. (a) Representative flow cytometry data. (b) Columns indicate numbers of IFN- γ -secreting hMUC1-associated CD8+ T cells per 1×10^5 cells.

Splenocytes were stained with PE-labeled IFN- γ and FITC-labeled CD8 Ab and analyzed by flow cytometry. * $P < 0.02$, ** $P < 0.01$. Experiments were performed in triplicate, and bars represent means $\pm$ SD, $n = 8$ mice/group

four groups (pcDNA+PBS, phMUC1+PBS, pcDNA +¹³¹I, phMUC1+¹³¹I, and phMUC1+¹³¹I+CpG ODNs groups, 55±1, 92±2, 679±3, 978±5, and 1,278±3, respectively; *P<0.02, **P<0.01; Fig. 4a and b).

Number of CD8+IFN-γ+ T cells stimulated with CD11c+ cells

As shown in Fig. 5, enriched CD11c+ cells from the phMUC1+¹³¹I+CpG ODNs group generated the highest number of CD8+IFN-γ+ T cells compared to the other four therapy groups (pcDNA+PBS, phMUC1+PBS, pcDNA+¹³¹I, phMUC1+¹³¹I group and phMUC1+¹³¹I+CpG ODNs, 7±3,

31±9, 87±2, 109±3 and 139±15, respectively, *P<0.02, **P<0.01).

Cytotoxic T cell (CTLs) activity against hMUC1-expressing cancer cells

As shown in Fig. 6, the killing effects of CTLs cells obtained from splenocytes against hMUC1-expressing cancers was greatest in the phMUC1+¹³¹I+CpG ODNs group (pcDNA+PBS, phMUC1+PBS, pcDNA+¹³¹I, phMUC1+¹³¹I and phMUC1+¹³¹I+CpG ODNs, 16±2%, 20±1%, 30±2%, 60±2% and 87±2%, respectively; *P<0.02, **P<0.01). Furthermore, the cytotoxic activity of

Fig. 5 Levels of hMUC1-associated CD8+IFN-γ+ T cells stimulated by hMUC1 antigen-loaded DCs in the draining lymph nodes of mice treated with phMUC1+¹³¹I+CpG. (a) Representative flow cytometry data. (b) Columns indicate numbers of IFN-γ secreting hMUC1-

associated CD8+ T cells per 5×10⁴ cells. Re-stimulated cells were stained with PE-labeled IFN-γ and FITC-labeled CD8 Ab, and analyzed by flow cytometry. *P<0.02, **P<0.01. Experiments were performed in triplicate, and bars represent means ± SD

Fig. 6 Cytotoxic effects of T cells against hMUC1 expressing cancer cells generated by phMUC1+¹³¹I+CpG treatment. Splenocytes obtained from treated mice were prepared and re-stimulated with IL-2 and hMUC1 peptides for 3 days. Irradiated CMNF cells (target cells) were then incubated with splenocytes (effectors) at an E:T ratio of 12:1 for 4 h in 96-well plates. * $P<0.02$, ** $P<0.01$. Experiments were performed in triplicate, and bars represent means $\pm$ SD, $n=8$ mice/group

hMUC1-associated CTLs in the phMUC1+¹³¹I+CpG ODNs group was significantly greater than that of those in the phMUC1+¹³¹I group ($P<0.02$).

Discussion

Immunotherapies based on cancer DNA vaccines generate cancer-specific immunity by inducing Th1 or Th2 immune response [2, 11]. However, tumor cells can escape immune systems augmented by immunotherapies by not expressing MHC class molecules or antigens. Thus, other therapeutic strategies are required to overcome this therapeutic limitation of cancer DNA vaccines.

Radiation in combination with cancer vaccines has been found to kill tumor cells effectively [12, 13]. Radiation is known to kill cancer cells via its direct cytotoxic effect and via the induction of radiation-induced immune response [1, 14, 15]. Similarly, in a previous study, we found that combined hMUC1 vaccination and hNIS radioiodine gene therapy had a good therapeutic effect in a murine cancer model [3]. In particular, in a previous study, we found that combined hNIS radionuclide gene therapy and hMUC1 DNA vaccination completely inhibited tumor growth, increased hMUC1-associated CTL immunity and afforded long-term tumor protection in a tumor rechallenge model [4].

Despite the synergistic effects of combination therapy in animal tumor models, the maintenance of acquired adoptive host immunity against cancer and the enhancement of therapeutic effects can probably be best achieved by modulating immune response against cancer. Several immunotherapy

adjuncts using GM-CSF, IL-2, IL-12, IFN- α , IFN- γ and CpG ODNs have been suggested in this context. Recombinant GM-CSF or plasmid-encoded GM-CSF is known to increase the number of immature DCs at the vaccine site [16], enhance DC1 maturation and migration, and enhance the immunogenicity of tumor cell or DNA vaccines [17]. It was reported that IL-2 could act as an important T cell growth factor, and IL-12 generates interferon γ -expressing cytotoxic T cells [18]. CpG ODN is believed to be one of the most effective adjuvants to enhance anti-tumor immunity. Different antigen-presenting cell (APC) subsets express unique profiles of toll-like receptors (TLRs) that play important roles in activation of the APCs [19], and these influence the activation, maturation and features of the resulting immune response. It has been shown that the strong immunostimulatory activity of CpG ODNs is due to the presence of unmethylated CpG dinucleotides in the CpG molecule [20–22]. These bacterial DNA sequences are bound by toll-like receptor 9 (TLR9), which is expressed by dendritic cells, macrophages, natural killer (NK) cells and others. Also, CpG ODN is known to directly generate the activation of B-lymphocytes [23] and induce lymphocytes to secrete various cytokines such as IL-6, 12 and IFN- γ [24]. Of the various CpG types found to be promising in terms of activating immune response, we chose to use a CpG with the sequence TCC ATG ACG TTC CTG ACG TT. Previously, Chaput et al. reported that this bacterial DNA sequence generates Ag-specific immune response against melanoma when treated with human exosomal MHC class I molecules [25]. In addition, Ye et al. found that CpG ODNs induced antigen-specific CD8+ T cell immune response in a murine cervical cancer model. Thus, we decided to use this bacterial sequence (CpG) to enhance hMUC1-associated immune response induced by combined hMUC1 vaccination and hNIS radioiodine gene therapy [13].

In the present study, we found that CpG ODNs enhance the cytotoxic effects of CTLs generated by combined hMUC1 vaccination and hNIS radioiodine gene therapy. In a current mice cancer treatment model, marked tumor growth inhibition and complete tumor resolution were observed in the two combinatorial study groups (phMUC1+¹³¹I and phMUC1+¹³¹I+CpG ODNs), but not in the three single therapy groups (Fig. 3b, d). Furthermore, the immunostimulatory effects of CpG ODNs on combination therapy was evident by investigation of the number of CD8+IFN- γ + T cells, the number of CD8+IFN- γ + T cells stimulated with CD11c+ cells and the immune responses of hMUC1-associated cytotoxic T cells (CTLs).

In summary, in our study CpG ODNs were found to have a positive immunological effect on combined hMUC1 vaccination and hNIS radioiodine gene therapy. Specifically, CpG ODNs was found to increase the number of hMUC1-associated CD8+IFN- γ + T cells, the number of CD8+IFN- γ + T cells stimulated with CD11c+ cells, and the killing activities of

cytotoxic T lymphocytes induced by combined hMUC1 vaccination and hNIS radioiodine gene therapy against a hMUC1-expressing cancer. CpG ODNs can be used as an attractive immunomodulatory adjuvant that enhances CTL-based immunity generated by combinatorial hMUC1 DNA vaccine/hNIS radionuclide gene therapy.

Acknowledgments This work was supported in part by the Cancer Research Center, the Korean Science & Engineering Foundation (KOSEF) through the Tumor Immunity Medical Research Center at Seoul National University College of Medicine, Korea. Y.H. Jeon and Y. Choi were supported by the BK21 Project for Medicine, Dentistry and Pharmacy (2009).

References

- Demaria S, Bhardwaj N, McBride WH, Formenti SC (2005) Combining radiotherapy and immunotherapy: a revived partnership. *Int J Radiat Oncol Biol Phys* 63:655–66
- Gurunathan S, Klinman DM, Seder RA (2000) DNA vaccines: immunology, application, and optimization*. *Annu Rev Immunol* 18:927–74
- Jeon YH, Choi Y, Kim HJ, Kim CW, Jeong JM, Lee DS (2007) Human sodium iodide symporter gene adjunctive radiotherapy to enhance the preventive effect of hMUC1 DNA vaccine. *Int J Cancer* 121:1593–9
- Jeon YH, Choi Y, Yoon SO, Kim CW, Chung JK (2008) Synergistic tumoricidal effect of combined hMUC1 vaccination and hNIS radioiodine gene therapy. *Mol Cancer Ther* 7:2252–60
- Wooldridge JE, Ballas Z, Krieg AM, Weiner GJ (1997) Immunostimulatory oligodeoxynucleotides containing CpG motifs enhance the efficacy of monoclonal antibody therapy of lymphoma. *Blood* 89:2994–8
- Brazolot Millan CL, Weeratna R, Krieg AM, Siegrist CA, Davis HL (1998) CpG DNA can induce strong Th1 humoral and cell-mediated immune responses against hepatitis B surface antigen in young mice. *Proc Natl Acad Sci USA* 95:15553–8
- Mason KA, Ariga H, Neal R, Valdecana D, Hunter N, Krieg AM et al (2005) Targeting toll-like receptor 9 with CpG oligodeoxynucleotides enhances tumor response to fractionated radiotherapy. *Clin Cancer Res* 11:361–9
- Mason KA, Neal R, Hunter N, Ariga H, Ang K, Milas L (2006) CpG oligodeoxynucleotides are potent enhancers of radio- and chemoresponses of murine tumors. *Radiother Oncol* 80:192–8
- Cerkovnik P, Jezersek Novakovic B, Stegel V, Novakovic S (2009) Class C CpG oligodeoxynucleotides as a single agent and in combination with radiotherapy efficiently delayed growth of subcutaneous B16F1 tumors. *Innate Immun* 15:313–21
- Mandell RB, Mandell LZ, Link CJ Jr (1999) Radioisotope concentrator gene therapy using the sodium/iodide symporter gene. *Cancer Res* 59:661–8
- Donnelly J, Berry K, Ulmer JB (2003) Technical and regulatory hurdles for DNA vaccines. *Int J Parasitol* 33:457–67
- Gulley JL, Arlen PM, Bastian A, Morin S, Marte J, Beetham P et al (2005) Combining a recombinant cancer vaccine with standard definitive radiotherapy in patients with localized prostate cancer. *Clin Cancer Res* 11:3353–62
- Ye GW, Park JB, Park YJ, Choi YS, Sin JI (2007) Increased sensitivity of irradiated murine cervical cancer tumors to E7 subunit vaccine-driven CTL-mediated killing induces synergistic anti-tumor activity. *Mol Ther* 15:1564–70
- Chakraborty M, Abrams SI, Camphausen K, Liu K, Scott T, Coleman CN et al (2003) Irradiation of tumor cells up-regulates Fas and enhances CTL lytic activity and CTL adoptive immunotherapy. *J Immunol* 170:6338–47
- Klein B, Loven D, Lurie H, Rakowsky E, Nyska A, Levin I et al (1994) The effect of irradiation on expression of HLA class I antigens in human brain tumors in culture. *J Neurosurg* 80:1074–7
- Weber J, Sondak VK, Scotland R, Phillip R, Wang F, Rubio V et al (2003) Granulocyte-macrophage-colony-stimulating factor added to a multi-peptide vaccine for resected Stage II melanoma. *Cancer* 97:186–200
- Dranoff G, Jaffee E, Lazenby A, Golumbek P, Levitsky H, Brose K et al (1993) Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. *Proc Natl Acad Sci USA* 90:3539–43
- Berinstein NL (2007) Enhancing cancer vaccines with immunomodulators. *Vaccine* 25(Suppl 2):B72–88
- Iwasaki A, Medzhitov R (2004) Toll-like receptor control of the adaptive immune responses. *Nat Immunol* 5:987–95
- Gallichan WS, Woolstencroft RN, Guarasci T, McCluskie MJ, Davis HL, Rosenthal KL (2001) Intranasal immunization with CpG oligodeoxynucleotides as an adjuvant dramatically increases IgA and protection against herpes simplex virus-2 in the genital tract. *J Immunol* 166:3451–7
- Weiner GJ, Liu HM, Wooldridge JE, Dahle CE, Krieg AM (1997) Immunostimulatory oligodeoxynucleotides containing the CpG motif are effective as immune adjuvants in tumor antigen immunization. *Proc Natl Acad Sci USA* 94:10833–7
- Davis HL, Weeratna R, Waldschmidt TJ, Tygrett L, Schorr J, Krieg AM (1998) CpG DNA is a potent enhancer of specific immunity in mice immunized with recombinant hepatitis B surface antigen. *J Immunol* 160:870–6
- Krieg AM, Yi AK, Matson S, Waldschmidt TJ, Bishop GA, Teasdale R et al (1995) CpG motifs in bacterial DNA trigger direct B-cell activation. *Nature* 374:546–9
- Klinman DM, Yi AK, Beaucage SL, Conover J, Krieg AM (1996) CpG motifs present in bacteria DNA rapidly induce lymphocytes to secrete interleukin 6, interleukin 12, and interferon gamma. *Proc Natl Acad Sci USA* 93:2879–83
- Chaput N, Scharz NE, Andre F, Taieb J, Novault S, Bonnaventure P et al (2004) Exosomes as potent cell-free peptide-based vaccine. II. Exosomes in CpG adjuvants efficiently prime naive Tc1 lymphocytes leading to tumor rejection. *J Immunol* 172:2137–46