

Gene-based vaccine strategies against cancer

Review Article

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Summary

In recent years, the characterization of gene-based cancer vaccines has been an important step in the development of different treatment options for human carcinoma. These particular vaccines make use of proteins that are specifically produced at very high levels by tumor cells. These tumor-associated antigens (TAAs) are not only used in diagnostic situations, but also in the development of cancer vaccines. In this review we will focus on two well characterized TAAs, carcinoembryonic antigen (CEA) and prostate specific antigen (PSA). The two methods of *in vivo* delivery we will examine are recombinant vaccinia virus and nucleic acid immunization. The TAA gene can be cloned into vaccinia virus and the viral infection stimulates an adequate immune response in the host. In the case of nucleic acid immunization, DNA constructs encoding for TAAs are directly injected into the host and are taken up by its cells. The cells express the specific encoded antigen upon which the immune system acts.

The effects of CEA recombinant vaccinia virus (rV-CEA) have been characterized in rodents, macaques, and humans. It was shown that the vaccine induced both humoral and cellular immune responses in mice and monkey models. In a phase I clinical trial, a CEA-specific cytotoxic T-lymphocyte response was observed. The effects of a CEA DNA vaccine were investigated in both mice and dogs and both humoral and cellular immune responses were found as well. A recombinant vaccinia virus expressing PSA was tested in rhesus monkeys and induced a PSA-specific long term cellular immune response. Experiments were also performed injecting a PSA DNA construct into both mice and rhesus monkeys. PSA-specific humoral and cellular immune responses were observed in both cases. All these experimental approaches demonstrate the efficacy and advantages of gene-based cancer vaccine strategies and support further clinical investigations.

I. Introduction

Although advances in science have led to countless theories and methods designed to combat human carcinoma, the battle is far from being over. Surgical excision of tumors, drug therapies, and chemotherapy have been effective in certain cases but in other situations, particularly when the tumor has begun to metastasize, effective treatment is far more difficult and far less potent.

Thus, researchers are continually investigating novel and more effective treatment strategies for various forms of cancer. Research, in recent years, has turned toward the use of vaccines to treat cancer. To this end, several proteins produced by tumor cells became a target for vaccine development. These tumor-associated antigens are predominantly expressed in a tissue-specific manner and are expressed at greatly increased levels in affected cells. Besides being important

diagnostic aids, these antigens represent appropriate targets for the development of cancer vaccines (Sogn et al, 1993).

Tumor-associated antigens (TAA) are proteins produced by tumor cells which can be presented on the cell surface in the context of major histocompatibility complexes (Kelley and Cole, 1998). Recently, these antigens have been the focus of study as a viable option for immunotherapy of various types of cancer. In this review we will examine the progress in the investigation of the immunological effects of two such TAAs, carcinoembryonic antigen (CEA) and prostate specific antigen (PSA).

II. Background

The use of therapeutic cancer vaccines has several distinct advantages. The immune response can be directed against carcinomas with a high degree of specificity. They can also generate immunological memory, for continued protection. The immune response induced by the vaccine can be modified or enhanced with other forms of immunotherapy such as using cytokines and other cellular therapies (Jones and Mitchell, 1996). Gene-based cancer vaccine strategies have yielded promising results, and several different methods of *in vivo* delivery are currently being explored (Roth and Cristiano, 1997). Two such approaches are recombinant vaccinia virus and nucleic acid, or DNA immunization (**Table 1**).

Vaccinia virus is one of the most heavily investigated viral delivery vehicles; it is a type of pox virus which was used in the successful eradication of smallpox (Kantor et al, 1992a). It is extremely immunogenic and is capable of stimulating both humoral and cellular immune responses

(Kaufman et al, 1991). Among its many advantages is that it greatly enhances the immune response when coupled with a weak immunogen such as a TAA. Through recombinant DNA technology, TAA genes can be cloned into the vaccinia viral vector and this recombinant vaccinia virus can be used to stimulate an effective immune response. Another advantage is that it can infect professional antigen presenting cells (APCs), such as dendritic cells or macrophages, and express the antigen along with MHC class I and/or class II complexes (Tsang et al, 1995). Finally, the stability and efficiency of vaccinia allows it to successfully incorporate fairly large inserts, which is advantageous in the context of cloning the genes for different TAAs (Kaufman et al, 1991). Potential disadvantages are toxicity effects, immunogenicity to the virus, and risk of viral reversion. Moreover, recombinant vaccinia viruses cannot be used to target specific cells.

DNA vaccination is a relatively new approach towards disease prophylaxis and/or treatment. DNA expression cassettes introduced *in vivo* can be taken up and expressed by host cells, leading to the production of specific foreign proteins. The presence of these foreign proteins can then elicit specific humoral and cellular immune responses against the foreign antigens (Wolff et al, 1990; Tang et al, 1992; Wang et al, 1993; Ulmer et al, 1993). This technique can be applied more widely than delivery through a recombinant vaccinia virus because there is no limitation on the size and type of nucleic acid used (Roth and Cristiano 1997). DNA vaccines are non-replicating, thereby minimizing the risk of any primary infections. It is also possible to alter or delete undesirable genes, such as those which may inhibit the immune response. More recently, the

	Advantages	Disadvantages
Vaccinia	<ul style="list-style-type: none"> -highly immunogenic -infects APCs -induces both humoral and cellular responses -large insert size 	<ul style="list-style-type: none"> -toxicity -risk of viral reversion -no targeting -induces vaccinia specific immune response
DNA	<ul style="list-style-type: none"> -possible to specifically target cells -no limit on size and type of nucleic acid -induces both humoral and cellular responses -non-replicating -able to genetically alter and enhance -use of molecular adjuvants to modulate response -repeated use without decrease in effect 	<ul style="list-style-type: none"> -low immunogenicity -difficult to incorporate into cells <i>in vivo</i>

Table 1. Comparison of recombinant vaccinia virus and nucleic acid immunization as *in vivo* delivery vehicles for gene-based cancer vaccine therapy.

use of molecular adjuvants such as cytokines and costimulatory molecules has proven to be effective in modulating and directing the desired immune responses

(Kim et al, 1998). Nucleic acid immunization is promising in the development of vaccinations for a wide array of pathogens, including cancer (Kim et al, In Press). Using DNA expression cassettes, DNA sequences that encode certain cancer proteins, such as those found in colon cancer or prostate cancer, are introduced into host cells. These cells then synthesize the antigenic cancer proteins which can then elicit an immune response against those proteins.

The first clinical studies for DNA vaccines tested the effects of the HIV-1 env/rev DNA vaccine in HIV-infected patients (MacGregor et al, 1998). Each patient in the trial received three injections each separated by ten weeks with increasing dosage (3 dosage groups of 5 subjects) of envelope vaccine. The clinical results reveal no significant clinical or laboratory adverse effects measured in all three dosage groups (30, 100, 300 µg). The immunized individuals developed increased antibody responses to envelope proteins and peptides after receiving the 100 µg dose of env/rev. Some increased cellular responses were also observed. These preliminary results demonstrate that the injection of even relatively low doses of a single immunogen DNA vaccine can augment both existing humoral and cellular immune responses in humans in a safe and tolerant manner.

III. Gene-based cancer vaccine strategies using CEA

Human CEA is a 180-kDa glycoprotein expressed in elevated levels in 90% of gastrointestinal malignancies, including colon, rectal, stomach, and pancreatic tumors, 70% of lung cancers, and 50% of breast cancers (Zaremba et al, 1997, Kelley and Cole, 1998). CEA is also found in human fetal digestive organ tissue, hence the name carcinoembryonic antigen (Foon et al, 1995). It has been discovered that CEA is expressed in normal adult colon epithelium as well, albeit at far lower levels (Conry et al, 1996a). Sequencing of CEA shows that it is associated with the human immunoglobulin gene superfamily and that it may be involved in the metastasizing of tumor cells (Foon et al, 1995).

A. CEA recombinant vaccinia virus vaccine

Recombinant vaccinia virus expressing the human CEA gene (rV-CEA) has been investigated as a potential therapy for colon and other gastrointestinal carcinomas. A number of groups have shown that immunization of these constructs into rodents induced both cellular and humoral responses. More importantly, immunization with rV-CEA led to antigen-specific inhibition of tumor growth in mice. Using an adoptive transfer experiment, Abrams, et. al. found that anti-tumor responses after rV-CEA

immunization were predominantly mediated by CEA-specific CD8⁺ T-cell response (Abrams et al, 1997). Splenocytes from rV-CEA immunized C57BL/6 mice were adoptively transferred to syngeneic immune deficient, tumor-bearing mice. They exhibited strong anti-tumor activity compared to splenocytes transferred from non-immunized mice. Adoptive transfer of CD4⁺, but not CD8⁺ T cells did not show anti-tumor activity. However, transfer of CD8⁺, but not CD4⁺ T cells still showed some anti-tumor response, although this response was less compared to when both CD8⁺ and CD4⁺ cell populations are present. CD4⁺ cells therefore may play an important helper or regulatory role in anti-tumor responses. Immunization of mice with rV-CEA induced anti-tumor activity that was mediated mainly by CD8⁺ cells, but both CD8⁺ and CD4⁺ cells were necessary to achieve optimal anti-tumor responses (Abrams et al, 1997).

The effects of rV-CEA vaccination were further characterized in experimental trials with non-human primates. After injection, the rhesus macaques of the experimental group showed both humoral and cellular immune responses to CEA. The immunization also resulted in toxic effects such as mild fever, irritation of the skin near the injection point, and lymphadenopathy (Kantor et al, 1992b). The results of this experiment along with the results from various rodent experiments demonstrated potential utility and limitations of the rV-CEA vaccine.

Additional information in this regard has been provided in the clinical setting. Tsang, et al. in conjunction with the National Cancer Institute, recently conducted a phase I clinical trial testing the effects of rV-CEA in 26 patients with advanced metastatic carcinoma (Tsang et al, 1995). Peripheral blood lymphocytes (PBLs) were taken from patients both before and after vaccination and analyzed for their response to specific CEA peptides with human leukocyte antigen (HLA) class I-A2 motifs. It was observed that CEA-specific MHC class I restricted cytotoxic T-lymphocyte response could be elicited (Tsang et al, 1995). However, following the first vaccination, there was an anti-vaccinia immune response which suppressed the effects of subsequent vaccinations (Kelley and Cole, 1998).

B. CEA DNA vaccine

The immune response to nucleic acid vaccination using a CEA DNA construct was characterized in a murine model. The CEA insert was cloned into a vector containing the cytomegalovirus (CMV) early promoter/enhancer and injected intramuscularly. CEA spe-

	Humoral response	Cellular response
rV-PSA	+	+
PSA DNA	+	+

Table 2. Induction of PSA-specific immune responses in rhesus macaques.

cific humoral and cellular responses were detected in the immunized mice. These responses were comparable to the immune response generated by rV-CEA (Conry et al, 1994). The CEA DNA vaccine was also characterized in a canine model, where sera obtained from dogs injected intramuscularly with the construct demonstrated an increase in antibody levels (Smith et al, 1998). Cellular immune responses quantified using the lymphoblast transformation (LBT) assay also revealed proliferation of CEA-specific lymphocytes. Therefore a CEA nucleic acid vaccine was able to induce both arms of the immune responses (Smith et al, 1998). CEA DNA vaccines are currently being investigated in humans.

IV. Gene-based cancer vaccine strategies using PSA

Prostate cancer is the most common form of cancer and the second most common cause of cancer related death in American men (Boring et al, 1994). The appearance of prostate cancer is much more common in men over the age of fifty (Gilliland and Keys, 1995). Three of the most widely used treatments are surgical excision of the prostate and seminal vesicles, external beam irradiation, and androgen deprivation. However, conventional therapies lose their efficacy once the tumor has metastasized, which is the case in more than half of initial diagnoses (Wei et al, 1997, Ko et al, 1996).

PSA is a serine protease and a human glandular kallikrein gene product of 240 amino acids which is secreted by both normal and transformed epithelial cells of the prostate gland (Wang et al, 1982; Watt et al, 1986). Because cancer cells secrete much higher levels of the antigen, PSA level is a particularly reliable and effective diagnostic indicator of the presence of prostate cancer (Labrie et al, 1992). PSA is also found in normal prostate epithelial tissue and its expression is highly specific (Wei et al, 1997).

A. PSA recombinant vaccinia virus vaccine

Recombinant vaccinia virus vaccines expressing

human PSA (rV-PSA) were studied in rodent as well as in non-human primate models (Hodge et al, 1995). Hodge, et al. investigated the immunological effects of a recombinant vaccinia virus expressing human PSA (rV-PSA) in rhesus monkeys. Because of the high degree of similarity between the rhesus and human prostate gland and PSA (>90%), this animal model was well suited to accurately assess the effects of rV-PSA. Murine and other models did not share this homology. A control group receiving high-dose V-Wyeth, a group receiving low-dose rV-PSA and a group receiving high-dose rV-PSA were all given 3 injections at four week intervals. Before the initial injection, one monkey in each group was given a prostatectomy in order to mimic the situation of human patients who have undergone the same procedure. Following injection, the rhesus monkeys exhibited the expected low-grade fever and other symptoms of vaccinia infection. It was found that the monkeys receiving the high dose rV-PSA vaccination expressed long term cellular immune responses specific to PSA (**Table 2**). Also, there was no difference in the immune response of the monkeys who had their prostates removed (Hodge et al, 1995). Much like the experiments with rV-CEA, this experiment showed the effectiveness of rV-PSA in inducing an immune response in macaques.

B. PSA DNA Vaccine

The immune responses induced by a DNA vaccine encoding for human PSA has been investigated in a murine model. The vaccine construct was constructed by cloning a gene for PSA into expression vectors under control of a CMV promoter (**Figure 1**). The expression of 30 kD PSA protein was determined in vitro using immunoprecipitation following a transfection with the PSA construct (**Figure 1**). In vivo expression of PSA was determined by intramuscularly injecting BALB/C mice with the DNA vaccine and performing an immunohistochemistry analysis on their quadriceps muscles (**Figure 2**).

Following the injection of the PSA DNA construct (pCPSA), various assays were performed to measure both

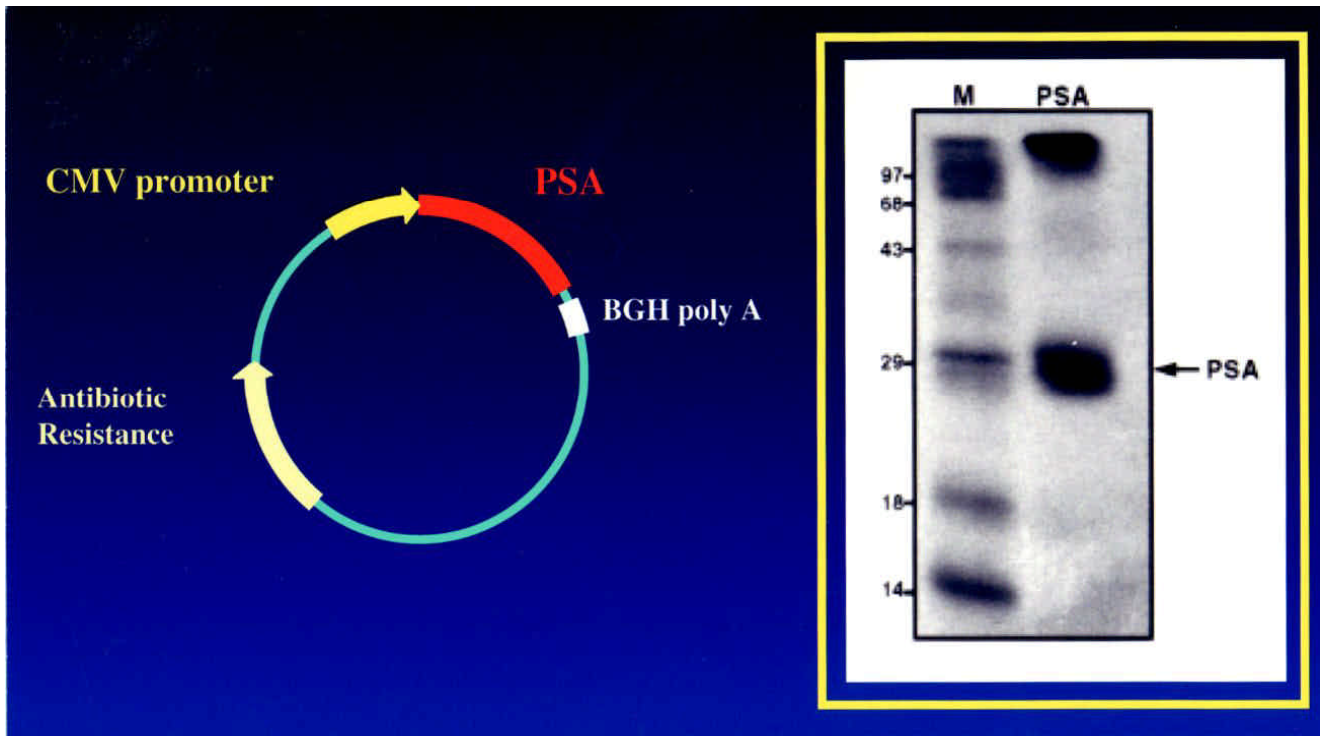
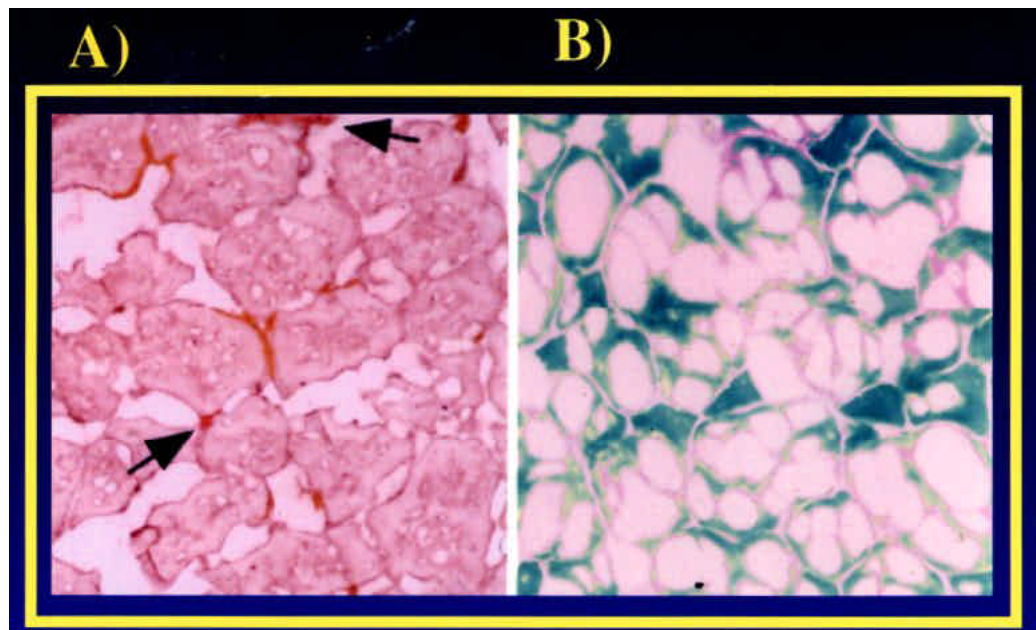


Figure 1. Construction and in vitro expression of PSA DNA vaccine. The complete coding sequence of PSA was cloned into pCDNA3 vector. Expression of PSA was assayed by immunoprecipitation with α -PSA antibodies. The immunoprecipitated sample was analyzed by SDS-PAGE (12%).

Figure 2. Immunohistochemical assay for expression of PSA on muscle cells. Frozen muscle sections were prepared from DNA injected animals and stained with α -PSA antibody. Positive antigen expression is illustrated by PSA-specific staining and representative examples of in vivo expression are highlighted with black arrows. **A)** A slide from a leg immunized with PSA vaccine and stained with α -PSA antibody. **B)** A slide from control plasmid immunized leg stained with α -PSA antibody.



the humoral and cellular immune responses of the mice (Kim et al, In Press). PSA-specific immune responses induced *in vivo* by immunization were characterized by enzyme-linked immunosorbent assay (ELISA), T helper proliferation cytotoxic T lymphocyte (CTL), and flow cytometry assays. Strong and persistent antibody responses were observed against PSA for at least 180 days following immunization. In addition, a significant T helper cell proliferation was observed against PSA protein. Immunization with pCPSA also induced MHC Class I CD8⁺ T cell-restricted cytotoxic T lymphocyte response against tumor cell targets expressing PSA. The induction of PSA-specific humoral and cellular immune responses following injection with pCPSA was also observed in rhesus macaques (Table 2).

V. Conclusion

Research involving different gene-based vaccines demonstrate that they can induce effective immune responses in a variety of animal models, including rodents and macaques as well as in humans. This effect was found in both methods of *in vivo* delivery, though differences remain between the two. Although recombinant vaccinia virus may produce more potent immune responses than DNA, it has many side effects such as eliciting an immune response against the virus itself. This immune response reduces the effectiveness of subsequent inoculations. DNA, while less immunogenic, can be used repeatedly with less adverse side effects. Furthermore, co-administration of molecular adjuvants with DNA vaccine constructs enhance the level of antigen-specific immune responses (Kim et al, 1997a,b; Conry et al, 1996b; Kim and Weiner, 1997; Chow et al, 1997; Sin et al, 1998).

Additional studies are warranted to optimize these strategies. Areas of future study could focus on controlling the immune responses induced by these therapies and further explore their effects on humans. It would be advantageous to modulate and refine the effects of these vaccines in order to gain optimal response. There are a number of ongoing clinical studies that will help ascertain how to best use gene-based therapies.

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