Hormozgan University of Medical Sciences

REVIEW ARTICLE

Health Institute

Hormozgan University of Medical Sciences

Recent Applications of DNA Vaccines in Cancer Therapy

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Accepted 21 July 2016; Published Online 1 August 2016

ABSTRACT

DNA vaccination has been developed as a noteworthy immunotherapeutic approach for the battle against many serious challenges to human and animal safety, including infectious illnesses, autoimmunity, allergy and cancer. Clinical trials prepare chances to examine whether DNA vaccines can fulfill their final aim of demonstrating efficiency in treating humans. Today, anticancer DNA vaccines are quickly moving from the bench to the bedside, and several therapeutic and prophylactic preparations have already been licensed by FDA for application in humans. In this review, we describe an insightful and unbiased summary of outcomes from a set of current clinical trials on DNA vaccines against diverse cancers.

Key words: DNA Vaccine, Clinical Trial, Cancer Therapy

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Introduction:

The previous studies showed that the direct injection of naked plasmid DNA containing plasmids encoding the vaccine antigen along with a potent eukaryotic promoter applied for protein expression[1].The novel approach of immunization was engineered in response to a set of emerging illnesses that remain without suitable prophylactic and therapeutic treatment. More than 50 years ago, pioneering experiments conducted by Atanasiu *et al.* and Orth *et al.* demonstrated that inoculation of mouse-derived tumor DNA elicited tumors and resulted in seroconversion in injected mice [2, 3]. The task of Wolff *et al.* indicated that DNA vaccines injected intramuscularly could produce long-term gene expression *in vivo* without the requirement for a particular delivery system [4]; this result generated much incitement for the scientific research community. Then, Tang *et al*. indicated that injection of a DNA plasmid encoding human growth hormone (hGH) into mouse skin elicited an antibody response against the produced protein [5]. Ultimate proof that a DNA encoded antigen can induce efficient vaccine protection came from the experiment that intramuscular injection of DNA plasmid encoding influenza nuclear protein produced CTL responses that then protected the mice from challenge with virulent influenza strain [6]. Whiles these reports confirmed the theoretical usage of DNA vaccines, applied considerations remained. For instance, DNA inoculation leads to antigen expression in the low level and most transfected somatic cells aren't professional antigen presenting cells (APC). At least three diverse mechanisms have been suggested to have key role in the immunogenicity of DNA vaccines: (1) the host-synthesized antigens are presented by somatic cells (keratinocytes or muscle cells) by their MHC class I molecules to CD8 T cells; (2) DNA vaccination lead to direct transfection of professional APC such as dendritic cells and (3) transfected somatic cells is phagocytosed by professional APCs and the recombinant antigen is present to T cells. Myocytes are not effective at presenting antigens by MHC class I molecules, therefore the latter two mechanisms can be more important to DNA plasmids [7, 8].

Such nucleic acid based vaccines can be delivered dermally, intramuscularly (i.m.), mucosally or subcutaneously. Applying the host cellular machinery, the DNA vaccine arrives the nucleus of transfected local cells (such as keratinocytes or myocytes), including resident antigen presenting cells (APCs). After protein expression in host cells, the foreign antigenic proteins will convert to peptide strings. The host-synthesized antigenic proteins can become the subject of immune supervision in the context of both major histocompatibility complex (MHC) class I and class II molecules of APCs in the immunized host. Antigen-loaded APCs migrate to the draining lymph nodes where they present antigenic peptide-MHC complexes in composition with signaling by co-stimulatory molecules to naive T cells. The interaction allows the essential secondary signals to trigger an immune response and to activate and develop T cells or, alternatively, to activate B cell and antibody generation cascades. So, both cellular and humoral immune responses will be induced [9]

DNA vaccination has been developed further into a promising approach for the battle against many serious challenges to human and animal safety, including infectious illnesses, cancer, allergy and autoimmunity. The advantages of this approach over conventional live virus or protein subunit vaccines include safety, the possibility to induce strong cellular immune responses, fast adaptation to antigenic variants, easy and inexpensive production, the feasibility of combinatory vaccines, and the potential to be applied in settings devoid of a cold chain [10, 11].

The disadvantages of DNA vaccines are based chiefly on health and safety matters. Most of the safety matters concerning the system are based on the activation of oncogenes or the inactivation of tumor suppressor genes and chromosomal instability through the stimulation of

chromosomal breaks or rearrangements as a result of genomic incorporation of DNA vaccine, as well as inducing anti-DNA antibodies. Another disadvantage of DNA vaccines is poor immunogenicity. Whereas DNA vaccines have obtained prosperous licensure for veterinary usages, their low immunogenicity in humans in comparison to protein-based vaccines has delayed their development. So, sufficient adjuvants will be critical to overcome this barrier. Other drawback of DNA vaccines is generation of antibiotic resistance. Production process involves choice of bacterial cells by using antibiotic resistance, which is conferred by a gene on plasmid backbone. There is a danger that antibiotic resistance is transferred to patients immunized with DNA vaccine via the unintended transfer of bacteria [12-14]

Despite the disadvantages, significant development has been made in the context of DNA immunization and a set of experiments have shown good results, confirming that DNA vaccines are able to stimulate impressive immune responses using genes from a diversity of infectious microorganisms. However, each DNA vaccine has to be well evaluated considering its usage, the nature of the pathogenic agent being vaccinated against, the nature of the antigen and the kind of immune response required for protection [15-18].

Tumor antigens

Since viral infections, such as hepatitis B virus (HBV) or human papilloma viruses (HPV), can cause cancer, it is conceivable to target viral proteins and by hindering infection, to reduce the outbreak of the associated cancer. Once infection has happened, it may still be feasible to protect against the expansion or progression of cancer, such as for HPV infection. A set of groups are now targeting the E6 and E7 proteins of HPV, as oncoproteins, have a key role in the transformation of infected cells into cancer cells. One such example applying DNA vaccines to target E6/E7 proteins in patients with high-grade cervical lesions owing to HPV, led to CD8+ T cell immune responses [19].

Efforts to break tolerance to purely endogenous tumor antigens demonstrated in large levels on tumor cell surfaces such as prostatic acid phosphatase (PAP), carcinoembryonic antigen (CEA) or alpha-fetoprotein (AFP) have been more difficult to carry out. For treatment of prostate cancer, the most prosperous example has been Sipuleucel-T licensed in 2010 in the USA [20].

OnceptTM, as a veterinary cancer product, is a DNA vaccine encoding the human enzyme tyrosinase, and has been licensed for the melanoma therapy, in dogs. The human tyrosinase is different from the canine version, inducing tolerance breakage. In humans, after electroporation delivery of a similar kind of heterogenous tyrosinase vaccine, 40% of the patients showed enhanced CD8+ at the greatest dose [20]. A clinical trial of DNA vaccine encoding a modified CEA caused some immune responses but unknown tumor decrease [21].

DNA vaccines may have advantages for the extension of idiotype-specific vaccines for B cell lymphomas because these types of vaccines could easily be produce directed against the patient's idiotype (Id) [22, 23]. Additionally, it has been indicated that a DNA vaccine could stimulate cross-reactive anti-idiotype antibody responses directed against human B cell lymphomas [24].

So far, the safety and efficiency of naked DNA plasmids have been examined in a relatively limited number of clinical trials. Constructs expressing for autogenic tumor-associated antigens (TAAs) or allogeneic elements that would perform cross-immunizing functions have been examined in B-cell lymphoma, colorectal carcinoma, head and neck cancer (HNC), prostate cancer, HPV-16+ cervical intraepithelial neoplasia (CIN) and melanoma. The data of these experiments offer that the intramuscular, intranodal and intratumoral immunization of cancer patients with naked DNA vaccines is effective and can induce TAA-specific immunity that represent bona fide therapeutic efficacies [23,25-34]

Among different TAAs, promising results have also been presented with mammaglobin-A (MAM-A) DNA immunization. MAM-A is present in more than 80% of primary and advanced breast tumors and expression is not phase dependent, exhibiting it as suitable target for

immunization [35, 36]. Narayanan *et al*. showed for the first time *in vivo* that immunization with MAM-A cDNA caused a CD8+ immune response against MAM-A positive cancer cells, and that passive transfer of CD8+ CTLs could regress such cancer *in vivo* [37]. In this instance, there was no electroporation to increase cellular uptake, and without application of delivery methods, cellular uptake can be significantly decreased. Nonetheless, the preclinical results indicated the efficiency of the vaccine, and the MAM-A cDNA was examined in a Phase I clinical trial. Seven patients with phase IV metastatic breast tumor and HLA-A2+ status were given 3 doses of the MAM-A DNA vaccine 4 weeks apart. Although most studies detail the CD8+ and antibody response, the clinical trial concentrated on the CD4+ population, recognized by the expression of the inducible costimulator molecule (ICOS) on currently activated T-cells. Data demonstrated a high development in CD4+ ICOS T-cells coupled with a decrease in regulatory T-cells, which is indicative of antitumor immune responses, although more investigations are required to examine the clinical outcome [38].

MAM-A is a protein with 93 amino acids that displays various features of an appropriate antigen for breast cancer vaccine therapy. First, the protein is significantly expressed in breast cancer cells but is absent or expressed at very low degrees in normal cells [39]. Second, MAM-A is significantly immunogenic. *In vitro*, MAM-A expressing cells can be applied to produce MAM-A-specific $CD4^+$ and $CD8^+$ T cells that enable to specific identification and eradication of MAM-A expressing breast cancer cells. It is necessary to mention MAM-A-specific $CD8^+$ T cells have been identified in patients with breast cancer but are not observed in patients without disease [38, 40].

The potential of applying xenogeneic versions of antigens in DNA plasmids to bypass central immune tolerance has been tested, frequently in melanoma. Interestingly, a current study demonstrated the application of xenogeneic p53 in colon cancer. In many cancer cells, p53 is mutated and over expressed.P53 indicates another self-antigen in which only low affinity $CD8^+$ T cells against p53 may be produced causing weak antigen-specific immunity [41]. A report demonstrates that a xenogeneic version of the p53 gene can stimulate robust p53-specific immunity. Notably, intramuscular immunization of DNA vaccine encoding the human p53 gene followed by electroporation induces a potent CD8+ T cell response against mouse p53 in mice model. Additionally, these effects are demonstrated to elicit both therapeutic and prophylactic antitumor effects against mouse colon cancer MC38 expressing mouse p53. The xenogeneic version of p53 is likely identified by the immune system as foreign owing to its source from another species. It is momentous to note that the special approach apply DNA sequences encoding genes homologous between 2 species. So, the expressed antigen has to be analogous to be identified as the same host antigen, yet distinct to bypass tolerance against the self-antigen. More experiments are required to examine the efficiency of DNA vaccine encoding the xenogeneic p53 on other p53-expressingcancer cells [42]. In next section, we attempt to provide an informative and unbiased overview of current clinical trials of DNA vaccines in cancer therapy.

Human Clinical Trials

Clinical trials prepare chances to examine whether DNA vaccines can fulfill their final aim of demonstrating efficiency in treating humans. The broadly accepted safety profile of DNA vaccines has resulted in relaxed requirements for FDA approval and the repeated combination of first and second phase clinical trials. Since the safety of DNA plasmids is almost confirmed, the basic concern in clinical trials has become demonstrating efficiency [43]. In this section, we describe a short summary of outcomes from a set of current clinical trials on DNA vaccines against different cancers.

Melanoma

Malignant melanoma expresses a set of TAAs that can be applied as targets for DNA immunization. Intranodal injection of DNA vaccines encoding Melan-A (MART-1) and tyrosinase have been indicated to induce both humoral and cellular immune responses in stage IV melanoma patients [30].A phase I trial has been managed by using DNA plasmid encoding xenogeneic mouse gp100 or human gp100.

Human or mouse gp100 plasmid DNA were administered with 3 doses (100, 500, or 1500 µg) i.m. every 21 days, and then with the gp100 of the other species 3 times. Only low toxicity was determined at the immunization site in 12 out of 19 patients. Additionally, CD_8^+ T cells binding gp100 HLA-A2 restricted tetramers were induced in 5 patients while one patient showed an enhancement in IFN γ+ CD8 + T cells. However, no discernable diversity in progression-free survival was detected between patients with or without immune responses [20].

Another clinical trial was managed to compare the immunological responses of intramuscular injection and particle mediated epidermal delivery (PMED) of the xenogeneic gp100 DNA plasmid. The results showed that the safety profile of PMED was determined to be comparable to that of the intramuscular immunization route. Additionally, PMED considered stimulate higher IFN+ CD8 + T cell generation while requiring a notably lower dose of DNA. Although 30% of the immunized patients showed immune responses, no remarkable clinical results were observed [44].

Myeloma

In recent study, McCann *et al*., applied DNA fusion gene vaccines encoding patient-specific single chain variable fragment, or idiotype (Id), fused to fragment C (FrC) of tetanus toxin. Patients were immunized i.m. with 1 mg DNA on six times. 14 patients were registered on study and completed immunizations. Idiotypic DNA plasmids were well tolerated with vaccine-associated side events restricted to low-grade constitutional symptoms. A boost of pre-existing anti-FrC antibody was determined by ELISA in 8/14 patients, whereas anti-Id antibody was produced in 1/13 patients. Altogether, 29% of patients made an immune response to FrC and Id, with 43% patients responding to FrC alone. Over the one year study period, serum paraprotein was undetectable, reduced or remained constant for 71 % of patients, whilst ongoing CR/PR was retained for 79 % of patients. The median time to progression was reported 38.0 months for 13/14 patients. Altogether, survival chance was 64 % after a median follow-up of 85.6 months [45].

Colorectal cancer

Usage of a DNA vaccine against colorectal cancer has also been examined in a phase I clinical trial. The DNA vaccine expresses a modified form of the human CEA gene linked to a promiscuous T helper epitope of the tetanus toxin, and has been indicated to be antigenic in mice. Before the first immunization, patients were treated with cyclophosphamide intravenously. CEA66 DNA vaccines were administered either IM (8mg) orintradermally (2mg) on week 0, 2, and 6, along with subcutaneous immunization of GM-CSF (150µg). Only minor side effects, such as fatigue, chest tightness, myalgia, and arthralgia were observed at the immunization site. During the follow-up course, one patient had a relapse, and interestingly, another patient was recognized with urinary bladder cancer, which was unrelated to the DNA immunization treatment [21].

Cervical cancer

DNA vaccines theoretically should produce the most potent immune responses against cervical cancer owing to its etiological element being HPV infection. HPV E6 and E7 are foreign antigenic proteins and are only expressed in transformed tumor cells, making them ideal objectives. Diverse DNA plasmids encoding the viral oncoproteins HPV E6 and E7 have indicated to produce robust humoral and cellular immune responses in mice. Different fusion DNA vaccines encoding HPV E7 and other manipulative factors have been designed. A phase I trial was managed in patients with grade 2/3 cervical intraepithelial neoplasia (CIN). The DNA plasmid encodes a modified form of HPV E7 unable of binding retinoblastoma protein and linked to heat shock protein 70 (HSP70). The patients received 3 intramuscular immunizations (0.5, 1, or3mg) on days 0, 28, and 56. Histologic results based on resection were examined at week 15, and histologic relapses were seen in 33% of the patients in the highest-dose cohort [46].

Prostate Cancer

In a phase I clinical trial, a DNA plasmid encoding PSA was evaluated in patients with castration-resistant prostate cancer [33]. To assess the biologically active dose of the DNA vaccine, patients were immunized with 1 of 3 doses, 100,

300, or 900 mg, 5 times at one month periods along with the cytokines interleukin-2 and GM-CSF as vaccine adjuvants. The DNA vaccine was found to be safe with no side effects. No PSA-specific immune responses, as determined by IFNγ generation, were indicated in patients before vaccination or in patients who immunized with the lowest doses of the DNA vaccine, whereas 2 of the 3 patients immunized with 900 mg of the vaccine showed PSA-specific IFNγ generation and anti-PSA antibodies [33, 34]. More investigation demonstrated that 5 of 6 patients analyzed represented an enhancement in PSA–specific immune responses after immunization, with the highest responses seen in patients who immunized with the highest dose of the DNA vaccine [47]. A reduction in PSA slope was seen in 2 patients representing PSA-specific IFN γ release.

In another clinical trial, prostate-specific membrane antigen (PSMA) was evaluated as DNA vaccine–after the 3 months vaccination period. No PAP-specific antibody responses were determined. No remarkable side effects were detected, and an enhancement in PSA-DT (PSA doubling time) from 6.5 months pretreatment to 8.5 months on-treatment was seen. Altogether, the DNA vaccine encoding PAP was safe, induced an antigen-specific T-cell response, and may be related to an enhanced PSA doubling time offers that a multi-institutional phase II trial designed to examine clinical efficiency is warranted [48]. In a longitudinal immune examination (1year post treatment), 75% of patients who undergo at least a doubling of the PSA-DT in the year follow-up, had observable long-term PAP-specific, IFNγ-producing T-cell immune responses [49].

Breast cancer

Her2/neu (HER2) is a transmembrane receptor overexpressed in breast cancer cells, and is applied as a target antigen for DNA vaccination in clinical trials. A DNA vaccine encoding signaling-deficient form of HER2 was used along with low doses of GM-CSF and IL-2 in patients with metastatic HER2-expressing breast cancer. The DNA vaccine was well tolerated with no clinical side effects or autoimmunity observed. Although no improved T cell responses were determined immediately after three cycles of immunization, but a high increase of MHC class II restricted T-cell responses to Her2 was determined for several years after vaccination. Additionally, the DNA vaccine could produce long-term antibody responses, and 33% of patients survived for more than 4 years after the immunizations [50]. Another DNA vaccine encoding chimeric rat/human HER2 was examined in 16 HER2 and 28 HER2patients with pancreatic and breast cancers, respectively. The chimeric DNA vaccine could stimulate T cell responses and highly prevent HER2+ tumor growth, with the capability to circumvent suppressor effects of regulatory T cells, TGF-β, and IL10. The data of the study suggested the proof of conception that plasmid encoding chimeric rat/human HER2 can be applied as efficient vaccines for any patients with HER2-overexpressing cancers with the benefit of being not restricted to specific MHC [51].

Among different TAAs, encouraging data have been reported with MAM-A DNA immunization. Nevertheless, the preclinical information showed the efficiency of the DNA vaccine, and the MAM-A cDNA was examined in a Phase I clinical trial. Seven patients with stage IV metastatic breast tumor and HLA-A2+ status were immunized with three doses of the MAM-A DNA plasmide4 weeks apart. Although most studies explain the CD8+ and antibody response, the clinical trial concentrated on the CD4 + population, known by the proliferation of the inducible costimulator molecule (ICOS) on currently activated T-cells. Data demonstrated a high development in CD4+ ICOS T-cells coupled with a decrease in regulatory T-cells, which is suggestive of antitumor immunity, although more analysis are required to examine the clinical outcome [38].

Conclusion:

Preclinical and clinical studies obtained during the last years represents that DNA vaccines have the potential to stimulate tumor-specific immunity that may result in a therapeutic benefit. DNA vaccines suggest great feasibilities in that they can be manipulated (1) so to express not only the TAA(s) of chosen but also immunostimulatory molecules, such as xenogenous proteins and cytokines that act as adjuvants; and (2) so that the intracellular routing of the TAA(s) of chosen is

pre-detected, causing the preferential induction of cellular or humoral immune responses.

Although different approaches to increase the immunogenicity and potency of DNA plasmids have already been developed and investigated, future DNA plasmids should aim to more increase antitumor immunity by circumventing immune tolerance, stimulating long-term memory and cleaving the immunosuppressive networks in the tumor microenvironment. Additionally, DNA plasmids can be applied in conjunction with other cancer therapies to more control and eradicate tumors. Nonetheless, in the future an increasing amount of DNA vaccines will enter more advanced steps of human investigations, aimed to determine their efficiency as real clinical products

Conflict of interest:

The authors declare that they have no competing interest.

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