Available online at www.sciencedirect.com
SCIENCE DIRECT
Vaccine 24 (2006) 2880–2893
Vaccine
www.elsevier.com/locate/vaccine

An improved rearranged Human Papillomavirus Type 16 E7 DNA vaccine candidate (HPV-16 E7SH) induces an E7 wildtype-specific T cell response

Peter Öhlschläger[a,b,*], Michaela Pes[a], Wolfram Osen[c], Matthias Dürst[a], Achim Schneider[b], Lutz Gissmann[c], Andreas M. Kaufmann[b]

[a] *Frauenklinik der FSU Jena, Abteilung Gynäkologische Molekularbiologie, D-07743 Jena, Germany*
[b] *Charité Berlin, Campus Benjamin Franklin, D-12200 Berlin, Germany*
[c] *Deutsches Krebsforschungszentrum, D-69120 Heidelberg, Germany*

Received 26 July 2005; received in revised form 19 December 2005; accepted 22 December 2005
Available online 19 January 2006

Abstract

A new and very promising approach in vaccine development is the application of naked DNA. In comparison to conventional vaccines it offers several advantages, especially if there is a need for the development of low cost vaccines. Infection with high-risk human papillomaviruses (hr-HPVs) is the major risk factor for the development of cervical cancer (cc), the third most common cancer in women worldwide. The HPV E7 oncogene is constitutively expressed in HPV-infected cells and represents an excellent target for immune therapy of HPV-related disease. Therefore, we chose the HPV-16 E7 as model antigen in the development of a therapeutic DNA vaccine candidate. For safety reasons the use of a transforming gene like the HPV-16 E7 for DNA vaccination is not feasible in humans. In consequence we have generated an artificial ("shuffled") HPV-16 E7-gene (HPV-16 E7SH), containing all putative cytotoxic T-lymphocyte (CTLs) epitopes and exhibiting high safety features. Here, we show the induction of a strong E7-wildtype (E7WT) directed cellular and humoral immune response including tumor protection and regression after in vivo immunization in the murine system. Moreover, the vaccine candidate demonstrated immunogenicity in humans, demonstrated by priming of antigen-specific T cells in vitro. Importantly, the artificial HPV-gene has completely lost its transforming properties as measured in soft agar transformation assays. These results may be of importance for the development of vaccines based on oncogenes or oncoproteins.

Keywords: Gene shuffling; Cervical cancer; Immunotherapy; Tumor regression; In vitro immunization; T cells

1. Introduction

A naked DNA vaccine provides remarkable advantages in comparison to protein- or peptide-based vaccines making it of potential interest also for the use in Third World countries [1]. Its production costs are relatively low and predictable (M. Schleef, PlasmidFactory, Bielefeld, Germany, personal communication). Furthermore, DNA is stable, does not require refrigeration for storage and can easily be modified. With respect to prime-boost regimen naked DNA lacks unwanted immune reactions against other components of the vaccine as is observed in case of vector based-vaccines [2].

Abbreviations: APCs, antigen presenting cells; B-LCLs, B-lymphoblastic cell line; cc, cervical cancer; CIN, cervical intraepithelial neoplasia; CpG, unmethylated cytidine phosphate guanosine motifs; CS, calf serum; CT, cardiotoxin; CTLs, cytotoxic T lymphocytes; C-X-X-C, Zn binding motifs; DCs, dendritic cells; E7SH, rearranged ("shuffled"=SH) gene of the E7WT; E7WT, E7-wildtype gene/antigen of Human Papillomavirus Type 16; HLA, human leukocyte antigen; hr-HPVs, high-risk human papillomaviruses; i.m., intramuscularly; nt, nucleotides; PBMC, peripheral blood mononuclear cell; pRB, retinoblastoma protein; s.c., subcutaneously; Th, T helper cells; VP22, herpes simplex virus VP22

* Corresponding author at: Frauenklinik der Friedrich-Schiller-Universität Jena, Abteilung Gynäkologische Molekularbiologie, Bachstrasse 18, 07740 Jena, Germany. Tel.: +49 3641 934274; fax: +49 3641 934216; mobile: +49 160 5502270.
E-mail address: peter_oehlschlaeger@yahoo.de (P. Öhlschläger).

0264-410X/$ – see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2005.12.061

In this study, we have selected cc as a model for the development of a safe therapeutic DNA vaccine candidate. Worldwide approximately 370,000 cases of cc are being diagnosed each year and almost 200,000 deaths are attributed to this disease [3]. In Third World countries cc is one of the major cause of cancer-related deaths. About 80% of women dying from this disease originate from low-budget countries where screening programs for early detection and the medical infrastructure for treatment are not available. In contrast in the more developed world the mortality was reduced (by 70% in the US) during the last 50 years as a consequence of cytological screening programs [4]. Treatment of cc patients by surgery, radiotherapy or chemotherapy results in a significant loss of quality of life. Even when optimal treatment is available about 40% of all cc patients die of this disease [5]. Therefore, the development of an effective and save therapeutic vaccine is needed.

A necessary event for the development of premalignancies like cervical intraepithelial neoplasia (CIN) and cc is infection by hr-HPVs [6]. So far over 120 HPV types are identified [7], 18 of which were found to be associated with cc [8]. HPV-16 is responsible for about 50% of the cases [9]. Due to the fact, that the oncoprotein E7 of the hr-HPVs is exclusively and consistently expressed by HPV-infected tumor cells [10] it represents a specific target for an immune therapy directed against cc and its premalignant dysplasia. The E7, however, is an oncoprotein with transforming activity by interfering with the cell cycle control. The E7 alters cell growth regulation by inactivating the pRB (retinoblastoma) tumor suppressor protein [11,12] and contains two metal-binding motifs (C-X-X-C) [13,14].

For safety reasons a functional oncogene cannot be applied to humans. Therefore, efforts were made to inactivate the oncogenic properties of the HPV-16 E7. Some investigators have introduced point mutations into the sites of the E7-oncogene that are associated with transforming potential [15,16], whereas others have used HLA- (human leukocyte antigen) restricted singular epitopes [17,18].

These approaches, however, may lead to an unwanted loss of naturally occurring epitopes that is potentially associated with a decrease in vaccine efficacy. Our aim was to supply all potential naturally occurring T cell epitopes, covering the broad range of MHC restriction. In consequence, prior knowledge of the patient's HLA-haplotype is not required. This is especially important in the outbred human population. In addition, a more potent immune response may be induced, involving all occurring HLA-restriction elements in the vaccine. We had generated in a "proof-of-principle" study an artificial HPV-16 E7 gene (HPV-16 E7SH) of the first generation [19]. In this study we showed that an oncoprotein with a rearranged primary sequence still induces E7WT-specific CTLs in mice but is devoid of transforming properties. We had taken advantage of the earlier finding in our laboratory that fusion with the VP22 gene of Herpes Simplex Virus Type 1 strongly enhances the CTL response in mice [20]. In order to translate the therapeutic DNA vaccine candidate into a clinical trial we decided to further improve its safety features. For this reason we did not fuse any heterologous genes. We enhanced immunogenicity by placing a Kozak-sequence [21] in front of the gene [22] and chose the plasmid-vector pTH [23] applicable to humans [26]. More importantly we redesigned the E7 itself. The sequence was taken apart at exactly at the positions that are critical for transforming properties of the protein (pRB-binding site, C-X-X-C motifs) and reassembled in a "shuffled" order as "core" gene. This sequence was codon optimized to humans (almost identical to mice). The original junctions destroyed by the dissection were added as an "appendix" with non-codon optimized sequence to minimize recombination events reconstituting the wildtype sequences (see also Fig. 1).

Tumor protection and regression experiments will give a first impression on immunogenicity and effectivity of tumor vaccines. They do not fully reflect, however, the responses induced in humans. "In vitro immunization" of human lymphocytes by antigen-loaded dendritic cells (DCs) may be used as a model [24]. Loading of DCs by DNA transfection is a suitable technique [25] and specific T cell priming verifies the potential immunogenicity of the DNA vaccine candidate.

Here, we show that the HPV-16 E7SH DNA vaccine candidate of the second generation induces specific immunity in vivo in mice and after in vitro immunization of human lymphocytes and, therefore, holds promise for a safe therapeutic HPV-vaccine.

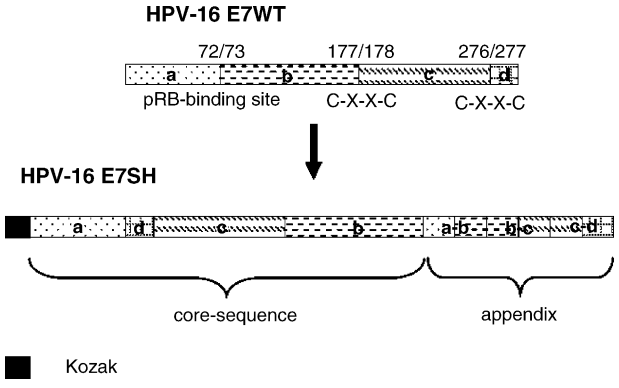

Fig. 1. Map of the artificial HPV-16 E7SH gene. The HPV-16 E7 wildtype gene (E7WT, above) was dissected at the positions corresponding to the pRB binding site (nt 72/37) and between the two C-X-X-C motifs (nt 177/178 and nt 276/277). The resulting four fragments a, b, c and d were rearranged ("shuffled") forming the core element with the sequence a, d, c, b. To avoid loss of putative CTL epitopes at the junctions a-b, b-c and c-d, these sequences (3×27 nt $= 3 \times 9$ amino acids) were added as an appendix forming the complete HPV-16 E7SH gene. To minimize the potential risk of "back-to-wildtype recombination" the codons of the core element were optimized for expression in humans according to the Kazusa codon usage database (www.kazusa.or.jp/codon/). A Kozak sequence was added in front of the gene to enhance translation.