Cancer Cell International

CANCER CELL INTERNATIONAL

BioMed Central

Poster presentation

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Preclinical investigation of DNA immunization with a rearranged human papillomavirus type 16 (HPV16) E7 oncogene

P Öhlschläger[1], M Pes[1], W Osen[2], M Dürst[1], A Schneider[1], L Gissmann[2] and AM Kaufmann*[1]

Address: [1]Frauenklinik der FSU Jena, Bachstrasse 18, 07743 Jena, Germany and [2]Deutsches Krebsforschungszentrum, INF 242, 69120 Heidelberg, Germany

Email: AM Kaufmann* - andreas.kaufmann@med.uni-jena.de

* Corresponding author

from Association for Immunotherapy of Cancer: Cancer Immunotherapy – 2nd Annual Meeting
Mainz, Germany, 6–7 May 2004

Published: 1 July 2004

Received: 28 April 2004

Cancer Cell International 2004, **4**(Suppl 1):S53

This article is available from: http://www.cancerci.com/content/4/S1/S53

Infection with human papillomaviruses (HPV) is the major risk factor for the development of cervical cancer. The HPV E7 oncogene is constitutively expressed in infected cells hence it represents a promising target for immune therapy of HPV-related disease. For safety reasons the use of a transforming gene for DNA vaccination is not feasible. Therefore, we have generated a rearranged ('shuffled') HPV16 E7 artificial gene (HPV16 E7SH) specifically dissected at the sites associated with cell transformation. Sequence duplications were added in order to supply all original T cell epitopes. The potential risk of back to wild type recombination was reduced by the use of different codons in the duplicated sequences.

Our objective was to investigate whether the HPV16 E7SH gene is lacking detectable transforming properties and shows E7-specific tumor rejection in mice and immunogenicity in humans. The E7SH genes were generated by fusion-PCR using overlapping primers. For DNA immunization, the genes were cloned into vector pTHamp (kindly provided by T. Hanke, Oxford, UK) and injected intramuscularly (i.m.) into female C57BL/6 mice. CD8+ T cell responses were measured by ELISPOT and ^{51}Cr-release assays. Tumor regression experiments in immunized mice were performed after inoculation of HPV16 E7 wild type-expressing syngeneic C3 cells. Soft-agar transformation assays were performed to compare transforming capacity of wild type and artificial E7SH genes in murine cells. E7SH recombinant retroviral vectors were generated for infection of NIH3T3. Human monocyte-derived dendritic cells were transfected by nucleoporation and HPV16 E7SH expression was investigated by RT-PCR, Western blot, and specific T cell stimulation *in vitro*.

Intramuscular immunization with the pTHamp-E7SH expression plasmid induced E7-specific cytotoxic T cell responses as detected *ex vivo* by IFN-γ ELISPOT and after *in vitro* restimulation by ^{51}Cr-release assays. Two i.m. immunizations with pTHamp-E7SH were sufficient to mediate regression of established C3 tumors in C57BL/6 mice. The E7SH gene has lost its transforming properties as analyzed by *in vitro* soft-agar-transformation assays. After transfection of immature and mature human monocyte-derived dendritic cells recombinant HPV16 E7SH expression was detected by RT-PCR, Western blot analysis, and *in vitro* stimulation of autologous E7-specific T cells suggesting potential immunogenicity in humans. Our data indicate that the HPV16 E7SH gene efficiently induces cytotoxic T cells directed against the wild type E7 antigen as measured *in vitro* and *in vivo*. The construct will be tested in clinical phase I trials to proof safety and immunogenicity in patients.