Abstract of PhD. Thesis

Gene Immunotherapy of Cancer: DNA Vaccines against HPV 16

Mgr. Ingrid Poláková

Cervical carcinoma (CC) represents the second most frequent cancer in women, mostly associated with human papillomavirus (HPV) infection. Nowadays, two prophylactic vaccines, protecting against HPV 16 and HPV 18, are licensed. Nevertheless, development of therapeutic vaccines is desirable to eliminate current HPV infections and to treat progressing tumours. Suitable targets for vaccination are viral E6 and E7 oncoproteins. Since its discovery, DNA vaccination has become an effective strategy for development of vaccines against cancer including CC. Unfortunately, the immunogenicity of DNA vaccines in large animals and particularly in humans is low. Therefore, several ongoing studies are focused on strategies enhancing the efficacy and safety of DNA vaccines.

In this work, the immunogenicity of DNA vaccines against HPV 16 delivered by a gene gun was evaluated after the fusion of the E7 and E6 genes with GUS. The increased steady-state level of the E7GGG.GUS deletion mutants and the GUS.E7GGG fusion protein enhanced the production of E7-specific antibodies after immunisation with these vaccines but did not improve the CTL response. Joining of the signal sequence with GUS.E7GGG led to ER-localisation of the SS.GUS.E7GGG fusion protein, enhancement of the cell-mediated immune responses and slower tumour growth in immunised mice. Enhanced immunogenicity was showed after immunisation with the E6 gene fused to the 3'- terminus of the GUS (GUS.E6).The abolishment of the splice site in the E6 gene resulted in complete elimination of the expression of the truncated E6 transcripts. This modification moderately reduced the immunogenicity of the non-fused (E6cc) or fused (GUS.E6cc) genes. The oncogenicity of the E6 protein was reduced by two point mutations and the modified E6GT protein was unable to induce p53 degradation. These substitutions in the E6 protein did not substantially influence the immunogenicity of the vaccines.

Moreover, the thesis demonstrates one of the possible ways of tumour escape and a comparison of two different administration methods of vaccines, the delivery by the tattoo device and s.c. needle injection using peptide vaccines. The N53S substitution in the RAHYNIVTF immunodominant epitope (aa 49-57) of the E7 protein was responsible for the immunoresistance of TC-1 clones derived from tumours of immunised mice. The administration of the E7- and E6-derived peptide vaccines by tattooing induced higher cellular and humoral immune responses than the s.c. injection.