Abstract

Major part of the thesis is focused on the development and increase of immunogenicity of experimental DNA vaccines against the human papillomavirus type 16 E7 oncoprotein and against the centrosomal proteins Aurora kinase A (Aurka) and Hmnr of which expression is increased in solid tumors but also in hematological malignancies. We modified DNA vaccines with signal sequences of the lysosomal LAMP-1 protein and with the helper p30 epitope derived from tetanus toxoid or the PADRE epitope designed in silico. After intradermal immunization of mice by a gene gun, we determined immune reactions in vitro with ELISPOT and ELISA and observed an antitumor effect of DNA vaccines in vivo. The PADRE epitope stimulated the specific Th1 immune response and increased antitumor effect of DNA vaccines more efficiently in comparison with the p30 epitope. However, we did not prove the antitumor effect of the DNA vaccines against the Aurka a Hmnr antigens. To achieve the antitumor effect of DNA vaccine against the Aurka antigen, we combined DNA immunization with antibodies against CD25 and PD-1. While the effect of anti-CD25 was dependent on a number of doses and type of depleted cells, a significant factor of anti-PD-1 efficacy was its administration delay from the first DNA immunization. Our results suggest that the inhibition of immunosuppressive mechanisms in tumors might be more important for the effect of combined immunotherapy than the level of specific immune response induced against a specific antigen. To consider a significance of centrosomal proteins as targets for immunotherapy of chronic myeloid leukaemia (CML), we determined the expression of the AURKA, HMMR, PLK1 and ESPL1 genes in mononuclear cells isolated from the peripheral blood of CML patients by RT-qPCR and antibody production against the centrosomal proteins in patients’ sera by ELISA. Compared to healthy donors, CML patients at diagnosis had significantly increased expression of all four tested genes. During treatment, the expression of the centrosomal genes decreased to the basal level in all patients. The majority of the patients reached the basal level during three months. The remaining patients (17%) with delayed decrease, had worse overall survival. In the CML patients, we also proved statistically significant increase of antibodies against the PLK1 and ESPL1. The patients with higher total production of antibodies against the four tested centrosomal antigens reached better major molecular response and survival without failure. These results confirm, that immune response against the centrosomal proteins might contribute to antitumor therapy.

Key words:
DNA vaccine, DNA immunization, helper epitope, centrosomal proteins, CD25 depletion