Epitope composition of recombinant fragment of orthopoxvirus p35 protein

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Y. Khlusevich[I], A. Matveev[I], L. Emelyanova[I], O. Golota[I], N. Tikunova[I]

[I]Institute of Chemical Biology and Fundamental Medicine SB RAS, Novosibirsk, Russia

Oncolytic virotherapy - a revolutionary tool for cancer treatment. One of these viruses is the vaccinia virus. But the use of oncolytic viruses has a number of disadvantages associated with the appearance of neutralizing antibodies even after a single use. Therefore, approaches are needed to reduce the immune response in the body a patient with oncolytic virotherapy without reducing the oncolytic properties of viruses. Possible way the solution to this problem will be to reduce immunogenicity by disrupting the structure of epitopes recognized virus neutralizing antibodies. Earlier we obtained full-sized human antibodies capable of neutralizing various orthopoxviruses, and one of the virus-neutralizing epitopes of p35 protein was localized using synthetic biology and phage display. It shown that the localized virus-neutralizing epitope of the p35 protein is discontinuous, and amino acid residues in the 15–19 aa and 232–237 aa regions located on the ^{13}VIDRLPSETFPNVHEHINDQKF^{34} and ^{231}DNAAKYVEH^{239} loops, respectively, are involved in binding. To understand which critical amino acid residues are involved in the interaction with virus neutralizing antibodies, a fusion protein containing both regions of localized epitope (1-34 aa and 228-239 aa), united by a flexible peptide linker, was constructed. Based on this combined protein, a panel of 5 mutant proteins containing substitutions in both regions was obtained. Analysis of the specificity of the interaction of the obtained mutant variants of the main immunogenic protein of vaccinia virus p35 with existing neutralizing full-length human antibodies fh1A and fh8E, recognized the defined neutralizing epitope, showed that the changes introduced lead to the absence of antibody binding. The sera of donors immunized with vaccinia virus also did not detect deletion mutant variants of the p35 protein of orthopoxvirus in Western blot analysis.
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