DESING OF GENETIC CONSTRUCTION FOR DNA VACCINE AGAINST PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME

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Porcine reproductive and respiratory syndrome (PRRS) is an infectious disease, causing enormous economic damage to swine industry worldwide. First PRRS was registered in the United States in 1987 and later cases of the disease were found in Europe. The infectious agent causing this syndrome refers to a single-stranded RNA virus of the *Arteriviridae* family. The size of the viral genome is 15 kb. It contains ten open reading frames that encode 14 non-structural proteins and eight structural. PRRS virus is divided into two types based on genetic differences: the European (type 1) and North American (type 2). The clinical manifestations of the syndrome include respiratory disease, abortions and high mortality of young animals.

Live attenuated and inactivated vaccines are used for reducing the spread of porcine reproductive and respiratory syndrome virus. But the effectiveness of these types of vaccines, especially against some strains of PRRS virus, remains low. In this connection, development of new drugs such as DNA vaccines is very important. DNA vaccines has advantages over other types of vaccines, in particular, it induces humoral and cellular immune responses and activation of interferon system, which contributes to a long-lasting immunity. In the classical variant DNA vaccines consist of a plasmid or viral vectors which contain the genes of infectious agents.

For creation of a DNA vaccine against PRRSV was necessary to find the viral targets for neutralization by the immune system. For this purpose the viral proteins have the greatest potential immunogenicity was chosen on the basis of literature data. These proteins include viral envelope glycoprotein GP5 and the membrane protein M. M protein is the most conservative between all structural proteins of PRRSV. Glycoprotein GP5 is responsible for attachment of the virus to target cells. Conservatism and surface location of these proteins indicates on possibility of their effective recognition by cells of the immune system. But it is known that these structural proteins insufficiently for antibodies formation. It’s necessary to improve the presentation of antigens encoded by DNA vaccine with the help of MHC (major histocompatibility complex) class II molecules. This can be achieved by efficient translocation antigens to lysosomes. MHC class II molecules are transported into the lysosomal compartments only in complex with the invariant chain (Ii). Ii protein contains the endosomal localization signal on its N-terminal end. It allows to direct newly synthesized MHC class II from Golgi to lysosomal compartments. In this connection, it is assumed that the addition of this sequence to antigens would enhance their presentation to appropriate immunocompetent cells and thus ensure the formation of antibodies.

We designed genetic constructs which contains the genes encoding GP5 and M proteins of PRRSV based on the plasmid pVAX1 vector (Invitrogen). Firstly

design of G-block was developed. G-block contains the M gene on 5'-end of which a Kozak sequence surrounding the start codon was attached. A Kozak sequence is important for the initiation of translation in eukaryotic cells. Gene of GP5 protein followed by M gene and on the 3'-end stop codon was added. Also, before the M protein, 90 bp nucleotide sequence was added. It is a signal sequence of invariant chain which is responsible for MHC class II molecules transporting.

This obtained sequence was amplified using appropriate primers (primer design was performed using Primer 3 and The unafold programs) and cloned in the pVAX1 vector. The recombinant constructs were transformed into competent *E. coli XL-1 Blue* cells.

Presence of the desired area in plasmids isolated from transformed cells, confirmed by restriction analysis. DNA fragments of about 1300 base pairs, what corresponds to the size of the insertion encoding the genes of PRRSV structural proteins were identified in the electrophoregram. Vector's structure allows cloned genes to be expressed efficiently in eukariotic cells.

At the end of this work *E. coli XL-1 Blue* strain containing a plasmid vector with genes of porcine reproductive and respiratory syndrome virus was obtained. In the course of further work it is planned to estimate the expression efficiency of the target genes containing in DNA vaccine constructs in mammalian cell culture.