УДК 604

PRODUCTION OF RECOMBINANT HPV ANTIGEN FOR VACCINE

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According to recent data, around 620,000 new cases of cancer in women and 70,000 in men worldwide have been associated with human papillomavirus (HPV). Cervical cancer in 2022 ranked fourth among cancer-related causes of death, accounting for approximately 660,000 new cases and about 350,000 deaths among women globally. Cervical cancer accounts for over 90% of HPV-related cancers in women [1].

One of the most effective methods for HPV prevention is vaccination, which helps to prevent primary infection with high-risk HPV types and the subsequent development of oncological complications. Modern HPV vaccines are produced using recombinant technologies that enable the formation of virus-like particles (VLPs) based on the L1 capsid protein, which lack the viral genetic material.

The aim of this work is to analyze the principles of creating recombinant HPV antigen producers for vaccine development.

Based on literature data, an analysis of human papillomavirus antigen production was conducted. HPV is a large group of over 200 related viruses that are non-enveloped, double-stranded, circular DNA viruses belonging to the family Papillomaviridae. HPV specifically infects cutaneous or mucosal squamous epithelium [2]. Viral particles consist of a single double-stranded DNA molecule associated with cellular histones and enclosed in a protein capsid. The capsid contains two structural proteins – the late L1 (55 kDa; about 80% of the total viral protein) and L2 (70 kDa) [3].

Since the viral surface is composed mainly of the L1 protein, whereas L2 is minimally exposed, the L1 protein is used as an antigen for recombinant vaccine design. Currently available licensed HPV vaccines use either baculovirus expression systems (Cervarix, GlaxoSmithKline) or yeast expression systems (Saccharomyces cerevisiae for Gardasil, Merck & Co.; Pichia pastoris for Walrinvax, Walvax Biotechnology).

The baculovirus/insect cell expression system is widely used for the production of VLPs and represents a promising platform for generating viral antigens and vaccine products. The Spodoptera frugiperda (Sf9) cell line, characterized by higher cell density, faster growth rate, and greater tolerance to osmotic and pH stress, is probably the most widely used insect cell line for recombinant protein production [4]. Therefore, as an HPV-L1 producer, the Sf9 cell line infected with Autographa californica multiple nucleopolyhedrovirus (AcMNPV) containing the L1 gene under the control of the polyhedrin promoter can be selected.

For the genetic modification of the producer, the gene encoding the HPV L1 protein is isolated, codon-optimized for Sf9 cells, and then cloned into the pFastBacHTa (pFB) plasmid for use in the baculovirus expression system. pFastBacHTa is a donor plasmid that contains specific elements — the Tn7L/Tn7R transposon sequences, which ensure site-specific integration of the inserted gene into the bacmid (within the attTn7 locus of the bacmid DNA). In addition, the plasmid contains a strong baculoviral promoter — the polyhedrin (polh) promoter, an N-

terminal 6×His tag with high affinity for nickel (to facilitate protein purification using affinity chromatography), and a TEV protease cleavage site for subsequent removal of the 6×His tag [4].

After cloning the target gene, the pFB-HPV L1 plasmid is used to transform Escherichia coli DH10Bac cells. The DH10Bac cells carry a bacmid containing the mini-attTn7 target site [4].

The bacmid is a large vector that harbors a modified genome of the Autographa californica multiple nucleopolyhedrovirus (AcMNPV), with the lacZ gene and the attTn7 docking site inserted into the lacZ coding region. The bacmid behaves like a plasmid in bacteria (can be cultivated in E. coli, DNA can be isolated), but when introduced into insect cells it functions as a viral genome and gives rise to the formation of a recombinant baculovirus. The bacmid contains special sites (mini-attTn7), where expression cassettes from the donor plasmid pFB-HPV L1 can be integrated using Tn7 transposition. A helper plasmid expresses the Tn7 transposase, which mediates the transposition of the donor plasmid's expression cassette – flanked by the Tn7R and Tn7L sequences on the pFB-HPV L1 plasmid, carrying the target gene and the gentamicin resistance marker – into the attTn7 docking site of the bacmid. Colonies containing recombinant bacmids are identified using gentamicin selection and blue-white screening (non-recombinant colonies appear blue due to lacZ expression, while recombinant colonies appear white because the lacZ gene is disrupted by transposon insertion) [5]. The efficiency of transposition is confirmed by polymerase chain reaction (PCR) using L1-specific primers [4].

Purified bacmid DNA is then used for the transfection of insect cells in order to generate viable baculovirus that can be used for recombinant protein production [5].

The transfection process lasts 4–6 hours at 27 °C. Baculovirus proliferation is monitored over 72 hours by analyzing cytopathic effects. Successful transfection is confirmed by observing characteristic cytopathic changes in infected cells [4].

Afterwards, the culture is collected by centrifugation, and the cell pellet containing most of the target protein in the form of VLPs is obtained. The cells are lysed, the lysate is clarified, and the supernatant is purified. Since the genetic construct includes a histidine tag with high affinity for nickel at the N-terminus of the HPV L1 protein, one of the purification methods used is affinity chromatography [4].

Thus, the method of producing the human papillomavirus antigen in insect cell expression systems has been analyzed. The baculovirus-based Sf9 insect cell expression system provides a high level of protein synthesis and correct assembly of virus-like particles (VLPs) that are structurally similar to native HPV but lack the infectious genome. The obtained results and described technological approaches can be used for the development of highly efficient recombinant VLP vaccines against HPV using insect cell expression platforms.

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