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Therapeutic vaccines for high-risk HPV-associated diseases

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ABSTRACT

Cancer is the second leading cause of death worldwide, and it is estimated that Human papillomavirus (HPV) related cancers account for 5% of all human cancers. Current HPV vaccines are extremely effective at preventing infection and neoplastic disease; however, they are prophylactic and do not clear established infections. Therapeutic vaccines which trigger cell-mediated immune responses for the treatment of established infections and malignancies are therefore required. The E6 and E7 early genes are ideal targets for vaccine therapy due to their role in disruption of the cell cycle and their constitutive expression in premalignant and malignant tissues. Several strategies have been investigated for the development of therapeutic vaccines, including live-vector, nucleic acid, peptide, protein-based and cell-based vaccines as well as combinatorial approaches, with several vaccine candidates progressing to clinical trials. With the current understanding of the HPV life cycle, molecular mechanisms of infection, carcinogenesis, tumour biology, the tumour microenvironment and immune response mechanisms, an approved HPV therapeutic vaccine seems to be a goal not far from being achieved. In this article, the status of therapeutic HPV vaccines in clinical trials are reviewed, and the potential for plant-based vaccine production platforms described.

1. Introduction

Cancer is a global leading cause of death [1], and it is estimated that Human papillomavirus (HPV) related cancers account for 5% of all human cancers [2,3]. Cervical cancer is an important disease, more so than other cancers (breast, colorectal) as it affects women below the age of 45, resulting in more life years lost [4,5]. HPV is the most common cause of cervical cancer, the 4th most common cancer in women, which results in an estimated 528,000 cases and 266,000 deaths every year [6]. There are at least 170 HPV genotypes described, which are categorised into two groups: these are the low-risk types, including HPV-6/11/40/42/43/44/54/61 and -72 which cause genital warts, and high-risk (hr) types including HPV-16/18/31/35/39/45/51/52/56/58/66 and -68, which are responsible for 99.7% of cervical cancer cases [7–9]. HPV-16 and -18 are the most prevalent types associated with cervical cancer worldwide, causing more than 70% of cases. HPVs are also responsible for many penile, vulvar and anal carcinomas and contribute to over 40% of oropharyngeal cancers [10,11]. Persistent infection with hrHPVs results in the development of squamous intraepithelial lesions (SILs): in the cervix, these are also called cervical intraepithelial neoplasia, CIN; and in the vulva, vulval intraepithelial neoplasia (VIN). SILs can progress to malignant cancers [9].

1.1. HPV structure and pathogenesis

HPVs are small non-enveloped double-stranded DNA viruses with a genome size of approximately 8 kb [12]. The genome encodes for six early regulatory proteins - E1, E2, E4, E5, E6 and E7 - and the two late structural proteins L1 and L2. The early genes encode proteins responsible for viral DNA replication, transcription and oncogenic transformation, and the late genes encode the virus capsid proteins [13,14]. The capsid is 50–60 nm in diameter and is arranged in a T = 7 quasi-icosahedral formation consisting of 360 copies of L1 that assemble into 72 pentamers, with up to 72 copies of L2 integrated into each capsid [15,16].

HPV infects basal epithelial cells through anatomically accessible points such as microlesions in the skin, genital organs and oropharyngeal areas. Capsid proteins L1 and L2 attach to epithelial cell receptors and a long process of entry commences, resulting in cytoplasmic uncoating of the virus and entry of its genome into the nucleus of the

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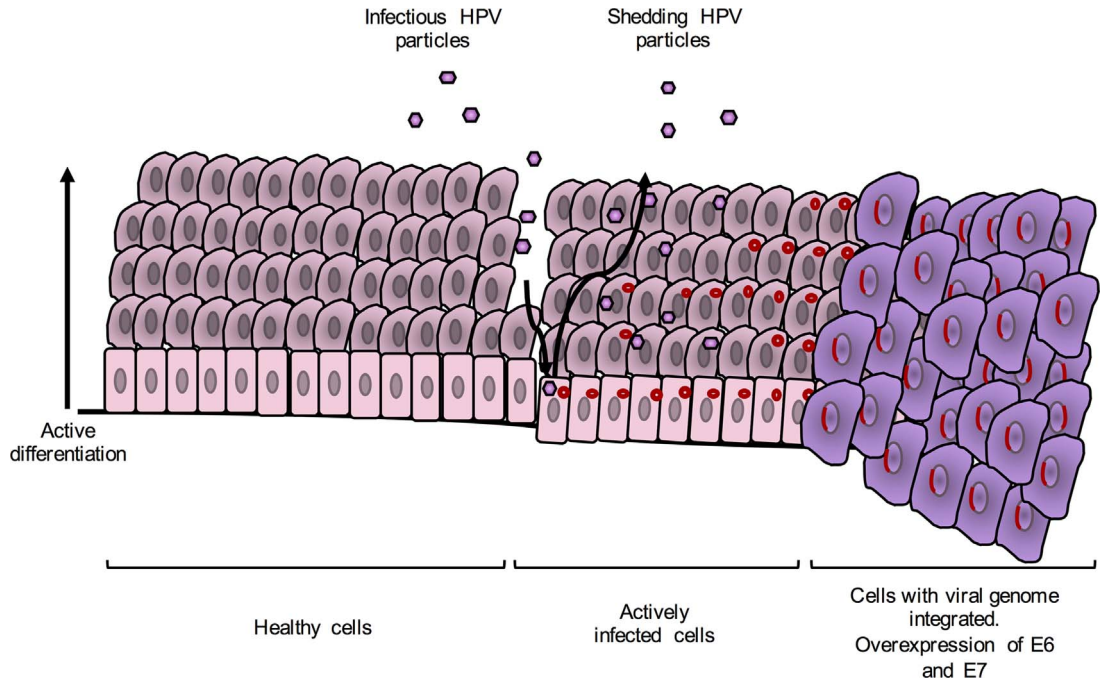

Fig. 1. The life-cycle of a typical hr-HPV. Infection occurs at basal epithelial cells through anatomically accessible points such as microlesions. The genomes of HPVs stay as episomes in the host's cell nuclei. Cells proliferate and differentiate. The expression of structural proteins, L1 and L2, viral assembly and release only occur at late stages of the cell life cycle. Integration of the viral genome into the host's genome leads to overexpression of E6 and E7 which disrupt the cell life cycle regulation which promotes prolonged cell life leading to genomic instability and cancer. At this stage no viral structural proteins are expressed. Adapted from Moody and Laimins [10].

infected cell, where it is transcribed and then replicated. Early proteins are expressed first and regulate the host cell life cycle and genome replication. They also regulate the expression of late proteins in a cell differentiation-dependent manner: L1 and L2 are only expressed in mature squamous cells. Maturation of virions occurs after terminal differentiation of epithelial cells, and their release coincides with natural shedding of senescent cells at the end of the epithelial cell life cycle [9]. Most infections are cleared by the immune system [17,18]; however, some benign cervical lesions progress to cancer. Continuous infection results in low-grade CIN 1 lesions. Progression to high-grade CIN 2/3 lesions caused by hrHPVs leads to invasive cervical cancer (ICC), where the viral genome may integrate into the host genome [9] (Fig. 1). In a typical hrHPV carcinogenesis, the genome of the virus is integrated into the host's chromosomal DNA and the E2 sequence is disrupted during the linearisation of the genome. The E2 protein is the transcriptional repressor of E6 and E7; therefore, expression from these genes becomes constitutive once E2 is disrupted. The E6 protein subsequently promotes the degradation of the host apoptosis regulator protein p53, and activates telomerase which results in extended cell life. The E7 protein targets the tumour suppressor retinoblastoma protein (pRb) for degradation and leads to the transition of the cell life cycle to the S-phase and subsequent host cell genome replication. E6 and E7 disrupt the cell cycle regulation and promote prolonged host cell life, leading to genomic instability and eventually cancer [9].

1.2. Prophylactic vaccines and limitations

Currently, there are three commercially available HPV vaccines: these are Cervarix®, a bivalent HPV-16/18 vaccine; Gardasil®, a quadrivalent HPV-6/11/16/18 vaccine; and Gardasil®9, a nonavalent HPV-6/11/16/18/31/33/45/52/58 vaccine. All exploit the fact that HPV L1 protein can form virus-like particles (VLPs) when expressed alone in a variety of cell types, that are morphologically and antigenically highly similar to native virions [19]. These three vaccines effectively prevent HPV infections caused by the targeted types by eliciting the production of neutralising antibodies that bind to the viral particles and block their entrance into host cells [20–22]. However, these vaccines are not effective at eliminating pre-existing infections, since the target antigens, L1 capsid proteins, are not expressed in infected basal epithelial cells [23–25]. Therefore, the large number of individuals already infected with HPV do not benefit from the current vaccines. Developing countries carry the greatest burden of HPV infections and malignancies due to their lack of resources to implement efficient vaccination and screening programmes [8]. Thus, many women only detect infections when they have already progressed past CIN 1 or when cancer has already developed. Additionally, the high cost of these vaccines puts them out of the reach of low-income populations. Although HPV immunization programmes have been implemented in 76 countries and territories worldwide, only 1% of women in low and low-middle income countries are covered by these programmes [26]. Furthermore, the incidence of human immunodeficiency virus (HIV) has been shown to influence HPV acquisition, the prevalence of multiple HPV types, persistence of infection and alters the carcinogenicity of hrHPV types [27–32]. A meta-analysis looking at the carcinogenicity of HPV in HIV positive women worldwide, showed HPV-16, -18 and particularly HPV-45 in African women accounted for a greater proportion of HPV infection in ICC compared to normal cytology, and other high-risk types accounted for important proportions of other low and high-grade lesions [33]. Therefore, therapeutic vaccines that broadly target oncogenic HPV types, and that are inexpensive, are urgently required.

1.3. Therapeutic vaccines

Cell-mediated rather than humoral immune responses are important for the clearance of established infections. It has been observed that spontaneous clearance and slow progression of HPV infections are associated with a strong cell-mediated immune response involving mainly T-helper type 1 cells and cytotoxic T-cells derived from CD4+ and CD8+ T-cells, respectively [34]. The HPV E6 and E7 oncoproteins are essential for the onset and maintenance of malignancy; thus, they are unlikely to escape immune responses by mutation. They are also expressed constitutively and at high levels, and therefore represent near-