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CANCER VACCINES WITH SPECIAL REFERENCE TO HUMAN PAPILLOMA VIRUS (HPV)

Shripad P. Joshi[1], *Mausumi Bharadwaj*[2], *Bhudev C. Das*[1*]

[1]Division of Molecular Oncology, [2]Division of Molecular Genetics & Biochemistry, Institute of Cytology and Preventive Oncology (ICMR), I-7, Sec-39, NOIDA - 201 301

Abstract

Despite various advances in diagnostics and therapeutics, cancer is the second most leading cause of death worldwide, accounting for almost 12.5% of deaths annually. Multiple factors like various mutagens, carcinogens, viral and bacterial infections individually or in combination are responsible for development of cancer. Infectious agents are responsible for about 15% of the cancers, such as cervical cancer, liver cancer, stomach cancer etc. The prophylaxis against these agents is an attractive strategy towards prevention of these cancers. This is evident from the successful Hepatitis B virus (HBV) vaccination to prevent hepatocellular cancer. Two prophylactic vaccines against HPV which have been shown to be highly immunogenic and successful in protecting from HPV infection are to be available for vaccinating females of age 9 to 26 years. Current research focuses on the development of cheaper second generation vaccines including DNA vaccines and therapeutic vaccines that can effectively curb the established cancers at their initial stages and are in the phase I/II clinical trials. Therefore prophylactic vaccine, early diagnosis and therapeutic vaccine certainly hold the promise of controlling the most dreaded disease of humankind.

Key Words : Cancer, Cervical Cancer, Human Papillomavirus, Vaccine, Cancer Prevention

Correspondence : **Bhudev C. Das**, Division of Molecular Oncology, Institute of Cytology and Preventive Oncology (ICMR), I-7, Sec-39, NOIDA - 201 301 **Email:** bcdas48@hotmail.com

Introduction

Cancer is the leading cause of death worldwide with approximately 10.9 million new cases diagnosed each year and about 6.7 million deaths. Current estimates bring the grim scenario that the number of new cancer cases is expected to grow by 50% over the next 20 years to reach 15 million by 2020 and more than 70% of them will be from developing countries. There are currently more than 24.6 million persons living with cancer and are in need of life saving drugs.

Cancer is a multistep and multifactorial disease arising due to uncontrolled clonal growth of cells. This is due to alteration in the structure and / or function of the genes controlling cellular growth and proliferation that may be because of exposure to various mutagens, carcinogens and viral/bacterial infections. The infectious agents are responsible for more than 15% of the cancers and some of these infections at different organ sites may also cause benign tumors. Some important examples include cancer of the uterine cervix in women due to persistent infection with oncogenic Human Papillomavirus (HPV) types, liver cancers caused by infection of Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV). Recently, stomach cancer has also been found to be linked to the infection with the bacterial pathogen, *Helicobacter pylori*.

As most of the cancers are detected in late stage, the only option is to conduct chemotherapy, radiotherapy, surgery etc which will extend the life span but certainly no cure is possible. Recent developments in immunology, genetics and molecular biology have fostered development of vaccines for infectious diseases, cancer, allergies and auto immune diseases. As 15% of the cancers are caused due to infectious agents, the prophylactic vaccine against these pathogens is expected to reduce the burden of the cancer cases.

This article reviews the development of different cancer vaccines with a special emphasis on cervical cancer vaccines and the different approaches being adopted for their development.

Cancer and Cancer Vaccine

The extensive research in understanding the role of infectious agents in cancer causation has lead to unraveling of oncogenic mechanisms and pathogenesis of these infectious agents. Fruitfully this has lead to development of number of prevention strategies against cancer including vaccines. The best example is the prophylactic recombinant vaccine against HBV which is the first vaccine that has been shown to prevent cancer. Recently, the US-FDA has approved marketing of the virus like particle (VLP) based HPV vaccine which will be second such vaccine that can prevent cervical cancer. Taken together the widespread implementation of both these vaccines will reduce the cancer burden to a great extent. There are

several other viruses and bacteria that are shown to be causative agents for cancer and the vaccines against them are at different stages of development. The infectious agents, cancer and the vaccine status is summarized in Table-1.

Table-1 : Infectious agents causing cancer and status of cancer vaccine development

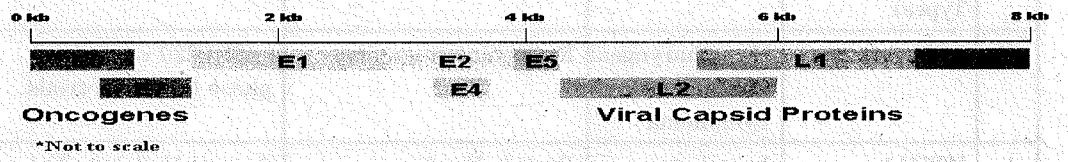
Sr. No.	Infectious Agent	Cancer	Vaccines Strategy	Current Status
1.	Hepatitis B Virus	Liver Cancer	Recombinant vaccine	HBsAg vaccine available
	Hepatitis C Virus	Liver Cancer	Recombinant vaccine/ Peptide vaccine	Phase II Clinical Trials Phase I clinical Trial
2.	Human Papillomavirus (High Risk Types)	Cervical Cancer	Recombinant vaccine	VLP based vaccine against types 16, 18 available
3.	EBV	Nasopharyngeal Carcinoma Lymphoma	Recombinant vaccine	gp350/220 vaccine in phase II clinical trials
4.	HTLV-1	Lymphoma	Peptide Vaccines	Animal Model studies.
5.	Helicobactor pylori	Stomach Cancer	Enzyme targeted/ Attenuated vaccine	Several Phase I clinical trials

Cervical Cancer and Human Papillomavirus

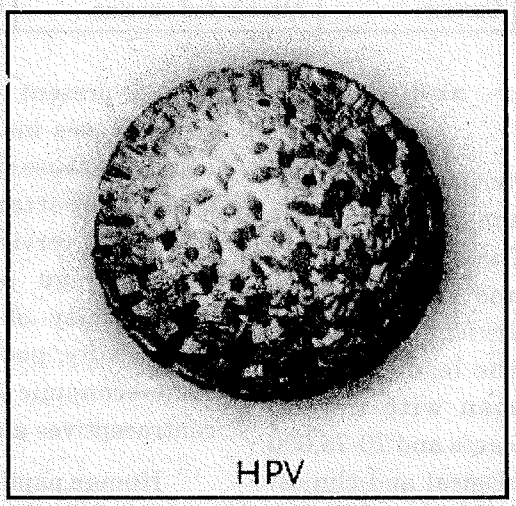
Cancer of the uterine cervix is the second-most common cancer among women worldwide. There are estimated 493,000 new cases and 274,000 deaths annually due to cervical cancer (1). Cervical cancer is the leading cancer among Indian women with annual incidence of 130,000 cases and 70-75,000 deaths (2). Epidemiological and clinical studies have confirmed that cervical cancer develops due to the persistent infection of High Risk HPV (HR-HPV) types. Recent studies show that HPV-DNA is present in 99.7% of the cervical cancer cases indicating that HPV is a necessary cause of cervical cancer (3). The other important risk factors for developing cervical cancer are early age of marriage or sexual exposure, multiplicity of sexual partners or promiscuity, poor genital hygiene, low socio-economic status, smoking, oral contraceptives and multiparity (3).

Human papillomaviruses (HPVs) are small nonenveloped DNA viruses having approximately 8.0 kb double stranded circular genome that encodes L1, L2 structural proteins and several other

early proteins (E1-E7) responsible for replication, transcription and transformation (See Fig1). Till date, more than 100 genotypes of HPV have been described, among them about 30 are associated with ano-genital infections. The two most important oncogenic HPV types termed as 'high risk' HPVs (HR-HPV) responsible for cervical cancer are HPV types 16 and 18 (4, 5). There are at least twelve more HPV types also designated as high risk types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 and 73). 'Low risk' HPVs such as HPV 6 and HPV 11 and others (40, 42, 43 and 44) are mainly associated with benign cervical lesions such as condylomata, accuminata and genital warts (6). More than 70-90% of the HPV infection shows natural clearance while about 10-30% cases show persistent infection with "high risk" types leading to malignant transformation and invasive cervical cancer. The virus has a long latent period and takes at least 10-15 years to develop cancer if persists after initial infection (6).



A. Human Papillomavirus Genome



B. Human Papillomavirus Particle

Fig. 1 : Schematic representation of (A) the linearised HPV genome and (B) the viral particle

HPV infects basal layer of epidermis in genital tract, anal, and perianal areas, oral cavity, esophagus and larynx where viral replication occurs. High risk HPV types responsible for cervical cancer infect the basal epithelial cells in the transformation zone between ectocervix and endocervix at the female genital tract. A persistent infection with HR-HPVs may lead to transformation due to loss of cell cycle control imparted by the viral oncoproteins E6 and E7 (7,8). This leads to different grades of cervical lesions that may eventually lead to invasiveness resulting in cervical cancer. Figure 2 and 3 shows the diagrammatic representation of the biology of HPV infection, its persistence, precancerous conditions and development of invasive carcinoma along with the factors contributing to the development of cervical cancer.

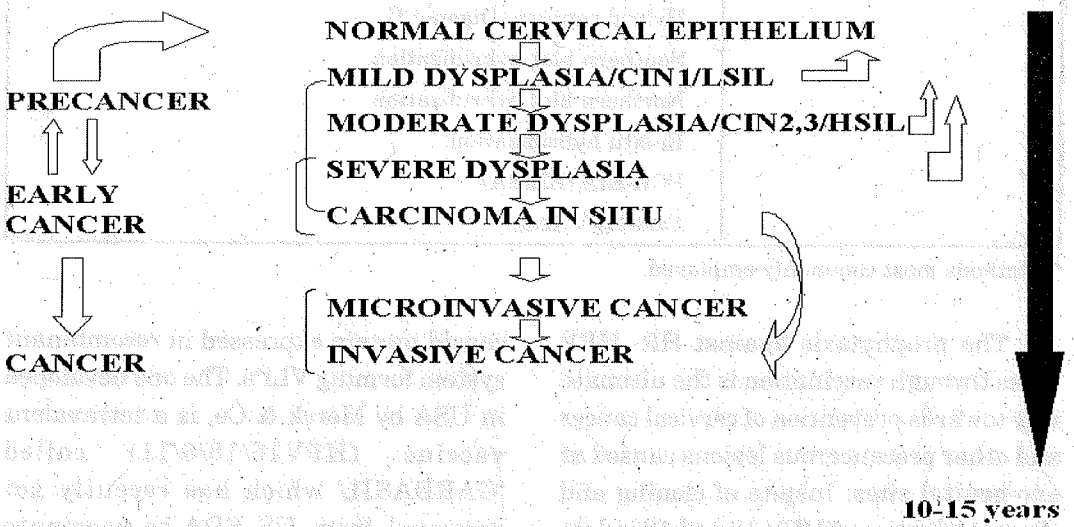


Fig. 2 : Diagrammatic presentation of different stages during progression of precancerous lesions to invasive cancer

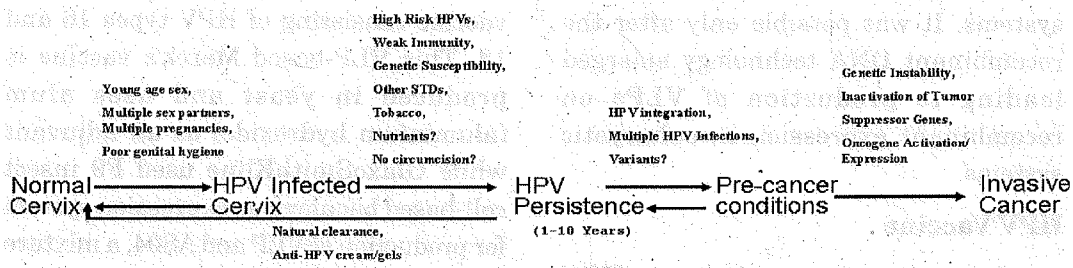


Fig. 3 : Biology of HPV infection and development of cerical cancer

Early diagnosis of the cervical lesions and their treatment helps in preventing development of invasive cancer. There are a number of cellular and molecular methods for diagnosis of HPV infection and cervical lesions at an early stage (See Table 2). Among them the most effective and widely employed method is the Pap smear test that is routinely followed in most of the developed countries. The availability of cytological Pap test has lowered the incidence of invasive cervical cancer by 70-75% in developed countries.

Table 2 : Cellular and molecular diagnostic methods for detection of lesions and HPV infection

Cytological Diagnosis	Papanicolaou-stained smear method (Pap Test)
HPV Diagnosis*	Polymerase Chain Reaction (PCR).
	Hybrid capture (Digene) II
	Southern blot hybridization.
	Northern blot hybridization.
	In-situ hybridization.
	PCR-EIA (ELISA).
	Fast-HPV Test.

* Methods most commonly employed.

The prophylaxis against HR- HPV types through vaccination is the ultimate way towards prevention of cervical cancer and other precancerous lesions caused at ano-genital sites. Inspite of cloning and characterization of HPV 16 and 18 in late seventies (9, 10), the development of HPV vaccine was delayed because of difficulty to propagate virus in tissue culture systems. It was possible only after the recombinant DNA technology emerged leading to production of VLPs on recombinant expression in eukaryotic systems.

HPV Vaccine

Recently, two prophylactic HPV vaccines have been developed using L1 capsid protein expressed in recombinant system forming VLPs. The one developed in USA by Merck & Co, is a tetravalent vaccine (HPV16/18/6/11) called 'GARDASIL' which has recently got approval from US FDA to vaccinate females of age 9 to 26 years (11). Another vaccine, 'CERVARIX' developed by GlaxoSmithKline in Belgium is a bivalent vaccine consisting of HPV types 16 and 18. This VLP-based Merck's vaccine is produced in yeast and uses alum (aluminium hydroxide) as an adjuvant while GlaxoSmithKline used F9 insect cell-based baculovirus expression system for production of VLP and AS04, a mixture of alum and monophosphoryl lipid A as adjuvant which provides more stability to

the vaccine. Both the vaccines have successfully undergone phase III clinical trials and are found to be well-tolerated, highly immunogenic and showed protection against persistent HPV infection for a period of 5 years (12,13).

Although prophylactic vaccination appears to be successful in young adolescents, it would take decades to perceive the ultimate benefits in reduction of cervical cancer cases. As the vaccine is not effective against already established HPV infection and there are estimated 5 million women worldwide already infected with HPV, development of therapeutic vaccine is an important aspect of current research. There are several therapeutic HPV vaccines in phase I and phase II clinical trials world over. Most of them target the HPV early proteins E6 and E7 or peptides derived from them largely because these are the transforming viral proteins that are expressed in cervical tumors. Frazer et al. 2004 showed in Phase I study of HPV 16-specific immunotherapy with E6E7 fusion protein and ISCOMATRIX ™ adjuvant in women with cervical intraepithelial neoplasia that this immunotherapy was well-tolerated and subjects developed HPV 16 E6E7 specific immunity (14). Some other approaches for development of therapeutic HPV vaccine uses autologous Dendritic Cell (DC) pulsed with full-length HPV 16 or 18 oncoproteins to induce HPV specific antitumor immune response (15).

DNA Vaccine

One of the second-generation vaccines that have great potential to address various limitations of recombinant protein-based vaccines is "Genetic vaccines" or "DNA vaccines". In last few years, DNA vaccines have been developed against different viral as well as bacterial and parasitic infections in animal models showing lasting immunity and protection. Clinical trials of DNA vaccines have been performed or are under way for various diseases, including cancer, influenza, hepatitis B, HIV, and malaria. Recently, first DNA vaccine against West Nile virus got approval from Department of Agriculture, United States (USDA) for commercial use in horse.

Since genetic vaccine is a plasmid-based vaccine, it is cost-effective to produce in large quantities. Robustness and stability of DNA vaccine even in higher temperature and single dose administration provide an edge over the other vaccines, particularly for distribution in the developing countries. Rocha-Zavaleta et al (2002) showed that parenteral and oral immunization with a plasmid DNA expressing HPV 16 L1 can induce systemic and mucosal antibody production together with cytotoxic T lymphocyte responses in animal models (16). The research towards development of chimeric DNA vaccines will be another milestone in vaccine research as it can serve as both prophylactic and therapeutic vaccine.

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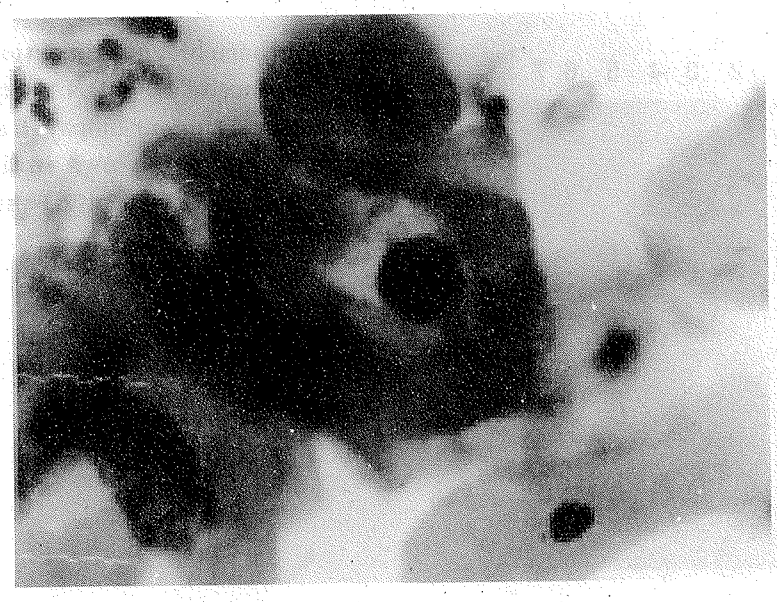
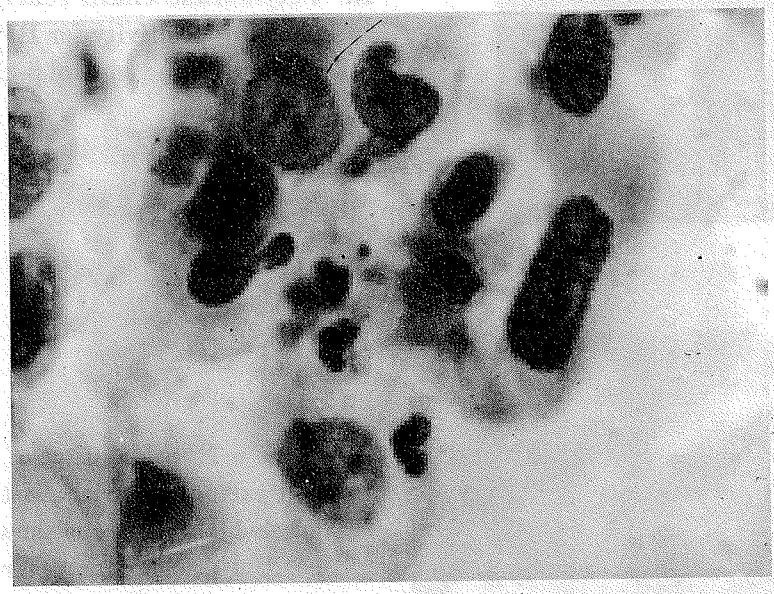
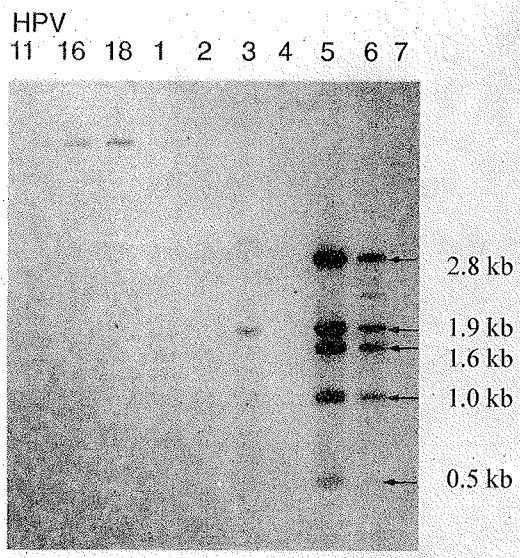


A. Kaliocytes showing infection of HPV at the LSIL stage of lesion

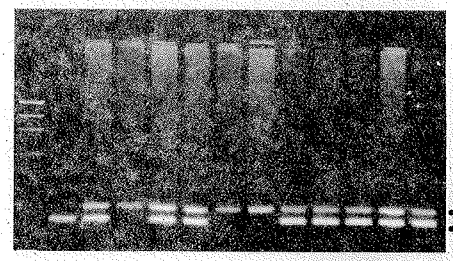


B. HSIL stage of cervical precancer lesion with or without HPV infection as revealed by Pap test during cytological screening

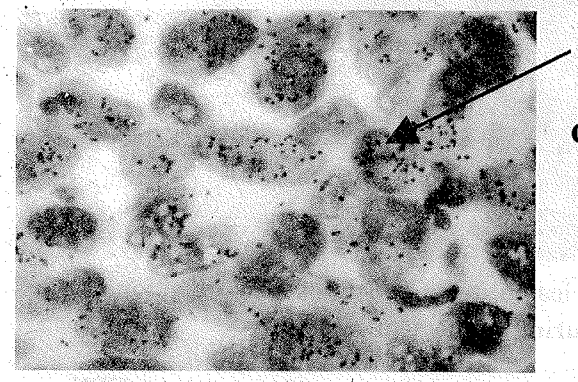
Fig. 4 : Different stages of precancerous cervical lesions with or without HPV infection as revealed by Pap test during cytological screening.



A. Southern blot hybridization showing presence of HPV DNA sequences in cervical tumour biopsy in samples 1, 3, 5 and 6. Arrows indicate PstI digested HPV-specific band pattern.



B. Polymerase chain reaction (PCR) showing positivity for HPV 16 DNA sequences and control β-globin gene.



C. In Situ Hybridization of squamous cell carcinoma with ^{3}H-thymidine-labeled HPV16 probe showing highly positive nuclei with silver gains.

Fig. 5 : Detection of HPV DNA in the cancerous tissues by different molecular approaches.