

CERVICAL CANCER: DISEASE AND ITS PATHOLOGY**Kumud Bala^{1*}, Reny Das² and Manisha Lall³**^{1,2}Amity Institute of Biotechnology, Sector 125, Noida.³Magadh Mahila College, Magadh University, Patna.Article Received on
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Accepted on 19 Dec 2015***Correspondence for****Author****Kumud Bala**Amity Institute of
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125, Noida.**INTRODUCTION**

Cervical cancer is an avoidable cause of death among women in sub-Saharan Africa. The most common cancer affecting women after breast, colorectal and lung cancers; it is the most notable in the lower resource countries of sub Saharan Africa, Latin America and Caribbean with 528000 cases every year. With 266000 deaths reported in 2012, it is also the fourth most common cause of cancer death in women worldwide. The highest rate of cervical cancer was reported in Malawi, followed by Mozambique and Comoros and the lowest incidence was found in Northern America and Oceania. According to the most recent data produced by National Institute of Health, 8.1 cases

of cervical cancer per 100,000 women per year have been reported. The mortality rate was observed to be 2.4 deaths per 100,000 women per year. According to a report by World Health Organisation in 2009, cervical cancer had a global age standardised incidence rate of 16 per 100,000 in 2004 and 1-year prevalence of 381,033 and 5-year prevalence of 1.41 million in 2003. It also had 3,719,000 DALYs (disability adjusted life-years).

In 2008, cervical cancer was the third largest cause of cancer mortality in India and had an age-standardised incidence rate of 30.7 per 100,000 women. Cervical cancer also had 1-year prevalence of 101,583 and 5-year prevalence of 370,243 in 2007 and 10% out of 729,600 cancer deaths in India. 987,000 DALYs were also reported. The mortality rates are higher in low and middle income countries (LMICs) (WHO, 2009). Within a country, the cervical cancer deaths are higher in low socio-economic groups within countries (Kurkure and Yeole, 2006). It has been reported by WHO that around 80% of global cervical cancer cases are in low and middle income countries (Waggoner, 2003). In India, Chennai has the highest age-adjusted incidence and Thiruvananthapuram has the lowest (National Cancer Registry Programme and World Health Organisation).

A worldwide trend has been observed in developing countries that are trying to undergo rapid economic and societal changes and shift towards a lifestyle that is atypical of developed countries. Such countries have shown an increasing burden of cancers related to hormonal, dietary and reproductive risk factors. The most important risk factor for the cause and development of cervical cancer is Human papilloma virus (HPV) infection. Almost all the case of cervical cancer have been found with the DNA of HPV (Bosch and de Danjose, 2003).

HPV being a sexually transmitted infection makes cervical cancer a chronic disease with an infectious aetiology (Alliance for Cervical Cancer Prevention, Cancer Research UK). Not all the HPV infections develop into cancer because the infection is usually resolved. About 3-10% of women infected with HPV develop the infection persistently and are at a higher risk of developing cervical cancer (Monsonogo et al, 2004).

Though a number of strains of HPV infection which increase the risk of developing cervical cancer have been discovered but HPV 16 and 18 are accounted for 70% of cervical cancer cases. HPV 31, 33, 35,45,52 and 58 are five other strains which account for an additional 20% cases. HPV 16 predominates in squamous cell carcinoma whereas HPV 18 plays a crucial role in adenocarcinoma [WHO/ICO Information Centre on Human Papilloma Virus and Cervical Cancer; Bosch and de Sanjosé, 2003].

Cervical cancer has devastating effects on the socio and economic life of a women but it shouldn't be a death sentence. Low-tech and inexpensive screening tools can considerably reduce the risk of deaths by cervical cancer in the under-developed countries. Cervical cancer can be prevented and cured if detected at an early stage. The already available tools for screening and treatment, especially HPV vaccination along with well organised national programmes should be actively implemented.

Historical perspective

Cervical cancer accounts for 12% of cancers found in women and is the second most frequently occurring gynaecological malignancy in the world. Cervical Cancer is caused by specific kinds of Human Papillomavirus. It was almost 30 years ago when the first association between Human Papillomavirus and cervical cancer was detected. In 1974 and 1976, scientists started to postulate the role of Human Papillomavirus in cervical cancer. In

1976, a report was published by Meisels and Fortin in which they analysed the appearance of Koilocytes in the smears obtained from cervix. The appearance of Koilocytes indicated the presence of HPV infection. They also differentiated between benign lesions that did not lead to cancer and non-viral precursors that lead to cervical cancer. In 1983 and 1984, HPV16 and HPV18 were the first types of HPV that were cloned after being directly isolated from cervical biopsies. Within four years, the expression of E6 and E7 which are specific viral genes was depicted in cervical biopsies and cervical cancer cell lines. The encoded viral oncogenes, a typical opening found in the virus molecule and the immortalization property shown by the viral DNA also explained the role of Human Papillomavirus in the aetiology of cervical cancer. In the subsequent 20 years, the scientists have analysed and understood the functions of viral oncogene to have a detailed knowledge of the history of HPV infection.

HUMAN PAPILOMAVIRUS AND DISEASE

Human Papillomavirus infects the cervical epithelial cells and cause a diverse range of lesions like common warts, neoplasia and cancer. On the basis of sequence analysis, over 100 different types of HPV have been identified. According to the evolutionary model, HPVs are categorised into distinct genera and the lesions caused by them also have different characteristics. There are two main groups of HPV named Alpha Papillomavirus and Beta Papillomavirus. Approximately 90% of HPVs which have been characterised fall into one of these two genera. Beta papillomavirus causes cutaneous infections and are mainly associated with the development of nonmelanoma skin cancer. Alpha Papillomavirus is the largest genera of HPVs. The genital mucosa consisting of the HPV types are consisted in this group. There are 30 different types of HPV that infect the cervical epithelium and cause lesions that progress to cancer. These HPVs are high risk HPVs. The most important HPV that is responsible for 50% of cervical cancer is HPV16.

Animal Model

The BALB/c nude mice were taken as the animal model to establish cervical cancer. This was executed by transplantation through abdominal subcutaneous tissue and the rate of tumor formation and the biological features of the tumor were investigated. Cervical cancer tissue were obtained from surgical specimens and were transplanted into subcutis of the mice. The generic characteristics, the rate of tumor growth, formation and transplantation were observed. On 85th day the mice were killed and the histological features of the tumor masses were analysed. Genomic DNA of all the organs were used as template and the DNA

expression of HPV was detected using Polymerase Chain Reaction. HE staining was done for tumor analysis to check the histological characteristic of the tumor. It was observed that the appearance of the tumors were similar before and after the transplantation. Also the tumor tissue had 100% positive rate of Human Papillomavirus DNA. The HPV DNA had same appearance before and after transplantation. The results of PCR showed the subtype of a high risk HPV. This suggested an HPV16 infection in the cervical cancer patient. The nude mice model of cervical cancer successfully established the biological features of human cervical cancer and provides a convenient tool for the research and study of the mechanism of human cervical cancer (Zhang Kaiju, Zhao Yanzhong et al. 2008).

Chemically induced cervical cancer

Diethylstilbestrol (DES), a synthetic form of estrogen was prescribed to pregnant women in the period of 1940 to 1970 to prevent complications during pregnancy like miscarriage and premature labor. In 1971, researchers at the Food and Drug Administration associated prenatal DES exposure to a type of vaginal and cervical cancer called cell adenocarcinoma (Herbst et.al 1971). The daughters of women who took DES during pregnancy were 40 times at a risk of cervical cancer than the unexposed women. DES daughters are at a high risk of developing abnormal cells in the vagina and the cervix. These are the precursors of cervical cancer like cervical intraepithelial neoplasia, dysplasia and squamous intraepithelial lesions and if left untreated can progress into cancer.

Many epidemiological studies have linked the use of oral contraceptives to cervical cancer. Scientists have shown that oral contraceptive is cofactor that elevates the risk of cervical cancer by four times in women who are tested positive for cervical HPV DNA (Victor Merino et al. 2002).

THE HUMAN PAPILLOMAVIRUS LIFE CYCLE

The Human Papillomavirus has a life cycle that is very different from other families of virus. Availability of epidermal or mucosal epithelial cells like basal layer cells of skin or mucosa which can proliferate are required for the HPV infection. The viral gene expression is greatly suppressed in these cells. There is a limited expression of early and specific viral genes like E5, E6 and E7. This largely enhances the proliferation and lateral expansion of the infected cells. Some of the progeny enters into the differentiating cells of superbasal layers and the expression of viral gene is initiated and the viral genes get activated. The circular viral DNA is replicated and formation of structural proteins such as capsid proteins takes place.

Assembly of complete viral particle happens at the upper layer of epidermis or mucosa. The viral particles are then released at the surface and infect other tissues.

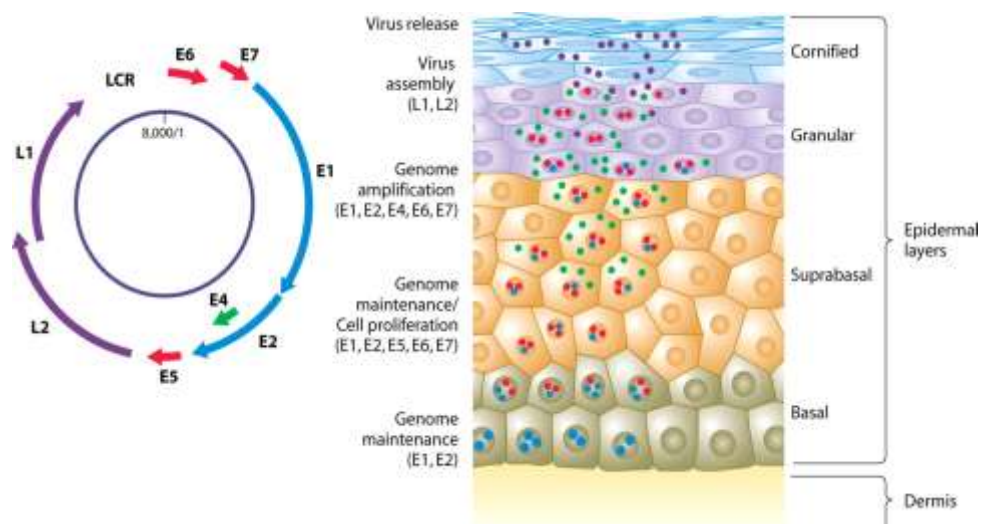


Figure1: Human Papillomavirus life cycle.

HPV PATHOGENESIS

The Human Papillomavirus genome consists of 6000 to 8000 base pairs. It is divided into eight open reading frames (ORFs) namely E1, E2, E4, E5, E6, E7, coding for 'early' functions and L1, L2 coding for 'late' functions. E5, E6, E7 are the three genes that possess proliferation-stimulating activity. E5 plays an important role in the early course of infection by stimulating cell growth. It forms a complex with the platelet derived growth receptor, epidermal growth factor and colony stimulating factor-1 receptor. Integration of episomal viral DNA into host cell DNA takes place and E5 coding sequence is deleted as HPV-infected lesions progress to cervical cancer. The ring molecule opens in the E2 ORF. The ORFs that are adjacent to E2 (E4, E5 and a part of L2) and a part of E2 itself gets deleted during integration. E6 and E7 play a more prominent role in malignant transformation. The E2 protein plays a prominent role in initiation of viral DNA replication and productive infection in basal cells. Is a DNA binding protein and recognizes a palindromic motif in the non-coding region of viral genome. HPV16 consists of four such motifs. Recruitment of E1 helicase into the viral genome requires E2 binding. This in turn binds to replication protein A and DNA polymerase alpha primase. Subsequently, the E2 protein is dissociated from the viral genome. The E1 protein is assembled into a double hexameric ring.

The E2 plays an important role in the anchorage of viral episomes to mitotic chromosomes and correct segregation. In high risk HPV types, this association happens through spindle and

additional cellular proteins. E2 also acts as a transcription factor that regulates viral early promoters such as p97 in HPV16 and p99 in HPV31 and viral oncogenes such as E6 and E7 expression. E2 acts as a transcriptional activator at low levels and represses the expression of oncogene at high level by displacing SP1 transcriptional activator from a position near to the early promoter. E6 interacts with p53 and E7 interacts with RB33 (Retinoblastoma 33) and blocks the activity of the tumor suppressors. This interaction is followed by degradation of p53 and BAK34, a pro-apoptotic protein. This resists apoptosis and increases chromosomal instability. E6 oncoprotein³⁶ helps in growth stimulation by activating telomerase³⁵ and inhibiting degradation of SRC-family kinases. But cyclin dependent kinase inhibitor INK4A which is also known as p16 can counteract these functions. E7 interacts with and degrades RB. This leads to the release of the transcription factor E2F and leads to the up-regulation of INK4A^{33, 37}. This results in a high E2F activity and may lead to apoptosis in E7-expressing cells. E7 blocks the activity of the cyclin dependent kinase inhibitors WAF1 and KIP1 also stimulates the S-phase genes – cyclin E38 and cyclin A. E6 is also associated with BAK49 and BAX⁵⁰ which is crucial in the development of cervical cancer. This happens because it compromises the effectiveness of the response of cellular DNA damage and unchecks the accumulation of secondary mutations.

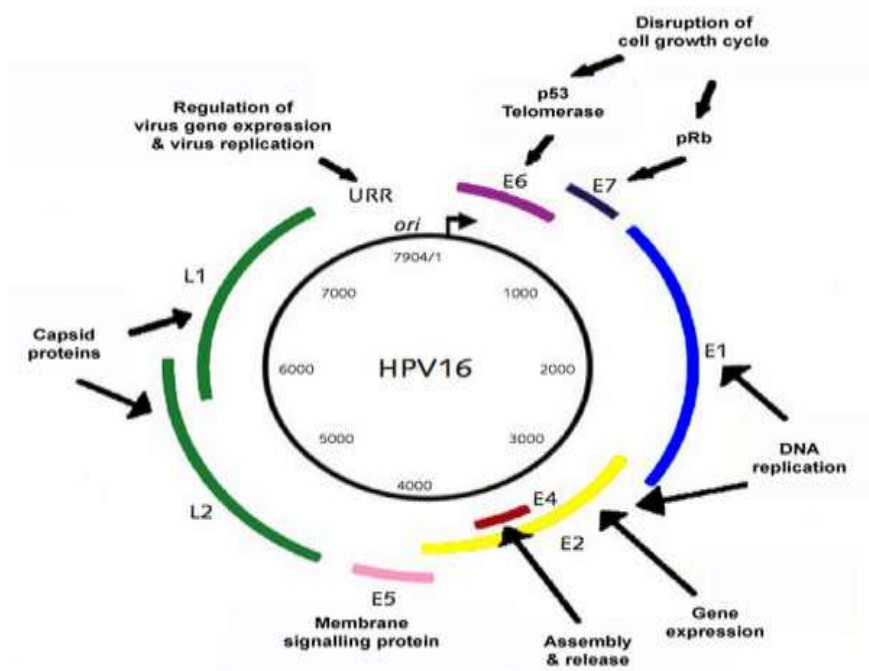


Figure2: Organisation of Human Papillomavirus circular DNA.

STIMULATION OF CELL-CYCLE PROGRESSION BY HIGH RISK HPV TYPES

HPV infection deregulates the cell cycle. Presence of high risk HPV leads to alteration of the regulatory proteins needed for cell proliferation. This allows HPV to activate entry into the S-phase in the upper epithelial cells. CDK4/CDK6 or cyclinD gets activated in the presence of growth factors. This leads to the phosphorylation of Rb and E2F transcription factor is released. In the cell, the levels of active Cdk or cyclinD is regulated by p16. A feedback mechanism is further provided through which the levels of Proliferating cell nuclear antigen (PCNA), MCM and cyclinE are regulated. Murine double minute (MDM) ubiquitin ligase regulates the level of p53 and maintains it below the one required for apoptosis. In the HPV infected cervical epithelium, cell cycle progression is independent of external growth factors and is regulated by E7. Normal feedback is also bypassed irrespective of rise in levels of p16. E7 helps in E2F mediated expression of cellular proteins required for entry into S-phase. It also binds and degrades pRB. When feedback mediated by p16 is absent, p14ARF level rises. This inhibits the function of MDM and increases p53 levels. E6 counters this and stimulates p53 degradation by associating with E6AP to prevent apoptosis.

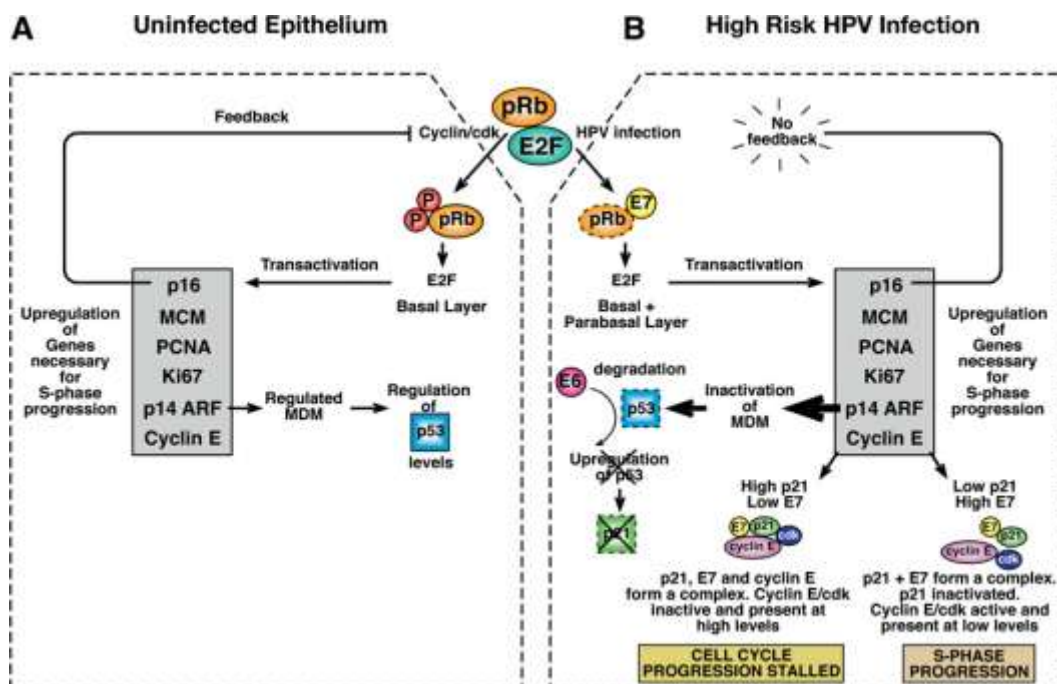


Figure3: Cell cycle progression by high risk Human Papillomavirus type.

GENOME AMPLIFICATION

To produce infectious virions, all the papillomaviruses should amplify and package their genomes. The onset of these late events is dependent on the changes that occur in the cellular environment when the infected cells have crossed the epithelial layer. The up regulation of E7 ORF, a differentiation dependent promoter, is critical for these events. P670 and p742 are

the differentiation dependent promoter present in HPV16 and HPV31 respectively. Changes that happen in cellular signalling lead to the activation of these promoters. This further elevates the level of viral proteins (E1, E2, E4, E5) essential for replication.

Cells that support genome amplification consist of E7. E2 and E1 also play a crucial role in genome amplification. E1 remains highly conserved in HPVs and has a weak affinity for AACNAT, a consensus motif that is repeated six times in viral origin. E1 expression is very low during natural infection and E2 is required to efficiently target E1 to its binding sites. E2 is linked to E1 with the help of its N-terminus and forms a dimer with DNA through its C terminus. A localized distortion happens in the viral DNA due to the E1-E2 complex formed at the origin of replication which leads to the recruitment of additional molecules of E1 and E2 is eventually displaced.

E1 associates with Hsp40 and Hsp70 that are cellular heat shock proteins and E1 dihexamers are formed. E1 and E2 also regulate p97 in HPV16 and p99 in HPV31 which are early promoters and E2 can down regulate E6 and E7 expression. E2 has different affinity for its binding sites and owing to this ability it can activate or repress the expression of viral genes. As E2 is increased, the basal transcription factors necessary for the activation of promoter like Sp1 and TATA box binding protein, get displaced. E2 stimulates genome amplification and down regulates expression of E6 or E7. It also deprives the virus of the replicative environment required for DNA synthesis. In the HPV 16 induced cervical lesion, the cells that support genome amplification scarce and differ greatly in their location in the lesion. Whereas in HPV1 induced verrucas, the cells supporting viral genome amplification are consistently prevalent in the upper basal layer.

E4 and E5 are other viral proteins that also contribute in viral genome amplification. A transmembrane protein, HPV E5, that is predominantly found in endoplasmic reticulum associates with the vacuolar proton ATPase to delay the acidification of endosome. Due to this, the recycling of the receptors of the growth factor is affected and the EGF mediated receptor signalling is also increased. This also maintains an environment in the upper epithelial cells conducive for replication. Association of certain types of HPV with CDK2 or cyclinB prevents their nuclear accumulation. This is essential for the progression into mitosis. E4 viral proteins are encoded by HPV11, HPV16, HPV18, HPV1 and it arrests the cell cycle in G2.

HIGH AND LOW RISK HPV INFECTIONS

High risk HPV types are those that are found in anogenital and cervical cancer. Whereas low risk HPV types are those that are present in non-malignant lesions and genital warts. Human cells could be immortalized only by high risk HPV types genes- E6 and E7. Alpha 7 and Alpha 9 are the main groups that consist of high risk HPV types. The most prominent in them are HPV16 and HPV18. These viruses exist in women with low grade squamous intraepithelial neoplasia (LSIL), high grade squamous intraepithelial neoplasia (HSIL), no cytological abnormalities and cancer. HPV16 DNA is present in 26% of low grade squamous intraepithelial neoplasia (LSIL) and in 63% of cervical squamous cell carcinomas (SCC).

The second most prevalent HPV type responsible for cervical cancer is HPV18 and causes 40% of cervical adenocarcinoma cases. HPV infection is most commonly transmitted through sexual contact. It initially results in inconspicuous squamous intraepithelial lesions most of which are get cleared in 6-12 months by immunological suppression. Some of the lesions progress to carcinoma of the cervix. This happens when the secondary point mutations get accumulated in the genome and gradually lead to cancer.

In LSIL, also known as grade 1 CIN caused by low and high risk HPV types, the viral coat proteins are found on the epithelial cells. The proliferative phase is very extensive in HSIL lesions. There is a change in pattern of gene expression during the cervical cancer progression. The events in CIN1 have an order similar to that found in productive lesions. However, the order remains same in CIN2 and CIN3 but the onset of the late events gets retarded. The infectious virions are produced only at the surface of the epithelial cells. HPV sequences get integrated into the host genome. This further deregulates E7 expression and loss of E1 and E2. For cervical cancer, the essential stages of the HPV life cycle are unsupported which leads to the loss of the viral episomes.

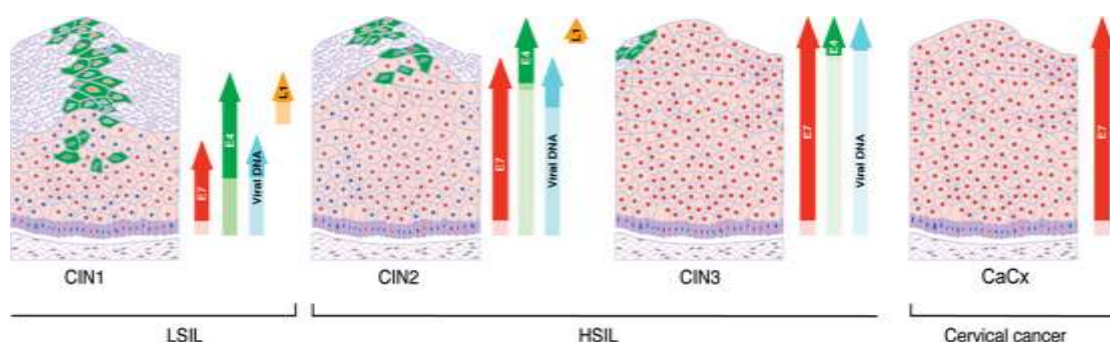


Figure4: Changes in viral expression patterns during progression of cervical cancer.

HOST CELL CONTROL IN HUMAN PAPILLOMAVIRUS INFECTION

The immune system is very crucial in order to control the HPV infections. The SIL also persists for a prolonged period of time in an immunosuppressed women. T-helper cells also regress the lesions and a cellular immune response and humoral response is generated during the regression against the HPV antigens. The HLA receptors, the proteasome transportation system and the cellular recognition system help the high grade SIL to escape the immunological system. Inhibition of viral oncoproteins and transcriptional factors also induce immunological surveillance to protect the HPV infected cells from malignant transformation. E6 DNA transfected cell lines have the CDKN2A gene inactivated either by mutation, deletion or methylation. CDKN2A encodes INK4A which inhibits the cell cycle. This happens because of the inactivation of cyclin D1- CDK6 and cyclin D1- CDK4 complexes by INK4A and it also prevents cyclin E expression. This block is overcome by E7 expression. E7 induces cyclin E and cyclin A by interacting with RB38. There is another pathway known as the cellular interference factor (CIF) concept which blocks the transcription of the viral DNA. This pathway is triggered when macrophages and TNF (Tumor Necrosis Factor) cause paracrine stimulation of the epithelial cells of cervix. Various effects are observed in the HPV immortalized cells like modification of AP1, a transcription factor. AP1 endogenously synthesises the antiviral interferon.

PREVENTIVE MEASURES FOR CERVICAL CANCER

Health awareness and education

Gynaecological patients were protected from vaginal, cervical and vulvar and squamous intraepithelial lesions of infectious origin by enforcing some specific standards of hygiene. This was mainly to prevent the iatrogenic transmissions. Condoms provide partial protection against HPV infections that are transmitted through sexual contact because the virus infected cells are present only at the external genital areas. WHO stresses on social awareness through control programmes to inform women and their families about the signs and symptoms of cervical cancer and the importance of regular screening for early detection. It has also encouraged women to avoid first intercourse at an early age, reduce tobacco consumption and avoid multiple sexual partners.

Vaccines

Many researches has been carried out to develop vaccines against the HPV infections. Though an immunological surveillance can clear the infection yet most of the viral antigens fail to induce an effective humoral or cellular response. Vaccines made with viral structural

proteins have shown to elicit an immune response in both animal system and humans. Most of the vaccines are being made from 'virus like particles' (VLPs) that are indeed derived from L1 or both L1 and L2 that are structural proteins. Two vaccines have been invented till now to protect against HPV16 and HPV18 infection. Prospects of polyvalent vaccines are also promising.

CANCER THERAPY

Chimeric vaccines are being developed as an approach to immunotherapy for cervical cancer. A deformed form of VLPs that are shown to have both immunopreventive and immunotherapeutic effect can traet early cervical lesions. These deformed VLPs are made by combining the antigenic epitopes from E7 oncoprotein and L1 protein. Many cytokines can repress transcription of HPV by transforming various growth factors such as Tumor necrosis factor and Tumor growth factor. HPV gene activity can also be repressed when retinoic acid receptors are activated by retinoic acid. Imiquimid is an immunomodulatory drug that stimulates cytokines to prevent HPV infection. A recent study also revealed that the HPV transcription can be partially and selectively checked in malignant and immortalised cells by pyrrolidine dithiocarbonate and 2-deoxyglucose. Trichostatin A and sodium butyrate can arrest HPV positive cells without changing the HPV oncogenes' expression.

FUTURE DIRECTIONS

The recent researches have shown promising developments in preventing high risk HPV type infections. Many vaccine formulas are being revealed to have therapeutic potential against the infection. But we need to find out a vaccine that gives optimal results. A vaccine that is cost effective needs to be developed for global application. It is also necessary to reach women in the most cervical cancer prone areas of the world because only then can the lives of more than 100,000 be saved every year.

CONCLUSION

Many factors contribute to the papillomavirus infection like host factor, site of infection and HPV type. The HPV types have been studied extensively since 1970s and their clinical manifestations have been an active area of research. Cervical cancer accounts for 12% of the global cancer burden and should therefore be prevented. A number of therapeutic and prophylactic vaccines are being developed to prevent malignant conversion of the early leasions. An advance approach should also be followed for screening procedures that are

based on host cell and HPV interactions. This will contribute to the prevention of human cervical cancer and also save the lives of millions of women around the world every year.

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