Review Article

The Role of Human Papilloma Virus (HPV) in the aetiology of Cervical Cancer

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Introduction

Cervical cancer is one of the commonest cancers of the female anogenital tract and a leading cause of morbidity and mortality. The association of HPV and cervical cancer was first suggested by zur Hausen in 1976.[1] It is now believed that 94-100% of cervical cancers - as well as tumours of the penis, anus, vagina, and vulva - are associated with sexually transmitted genital infection by the human papilloma virus (HPV).[2,3] There are at least 118 fully described forms of the papillomavirus which structurally consists of double-stranded circular DNA surrounded by a viral capsid protein.[4] Here we review how the genes of specific HPV serotypes interact with host cell DNA and protein to produce cervical epithelial dysplasia. This can then progress to invasive cancer in conjunction with other cofactors like oral contraceptives, increasing parity, smoking and Chlamydia infection.

The HPV Genome - key players

The circular HPV DNA is 6800 to 8000 base pairs in length and codes for eight genes - E6, E7, E1, E2, E4, E5, L1 and L2. The first six are "early" viral genes which code for proteins produced during the early phase of infection in the basal cell layer. They result in enhanced proliferation of the infected cells and their lateral expansion.[5]

The E5 Protein has been shown to complex with epidermal-growth-factor receptor, platelet-derived-growth factor receptor and the colony-stimulating factor-1 receptor, which promotes growth.[6] E5 also appears to inhibit programmed cell death.[7] Nevertheless the fact that the viral E5 gene is often deleted during the process of viral DNA integration with the host cell genome suggests a dispensable role in oncogenesis.

E6 and E7 genes and their proteins appear to have a central role in HPV-induced cervical cancer. They are expressed in cervical cancers and are individually able to immortalise various human cell lines in vitro but when expressed together their efficiency is enhanced.[8]

The E6 Protein has significant effects by virtue of its interaction with, and degradation of, p53.[9] p53 is also known as the "guardian of the genome" and is crucial in protecting normal cells when exposed to stress (e.g. radiation, UV light or chemicals). In such cells it causes cell cycle arrest preventing a cell with damaged DNA from multiplying, and allowing the cellular repair systems to fix any damaged DNA. If repair is not feasible then p53 induces apoptosis (programmed cell death). Since all cancers arise on a background of DNA mutations, p53 has a key role in preventing carcinogenesis and unsurprisingly 50-60% of all cancers have p53 mutations.

Other effects of the E6 protein include degradation of the pro-apoptotic BAK protein which is involved in the intrinsic (mitochondrial) death pathway. BAK has a physiological role in the cellular response to stress, in that it can promote opening of the mitochondrial permeability pores releasing intra-mitochondrial cytochrome-c which induces apoptosis. E6 also activates telomerase and stabilises active Src-family kinases involved in enhanced cell survival, proliferation, and motility.

The E7 Protein binds to and degrades the Retinoblastoma (Rb) protein.[10] The RB gene, initially identified as the gene responsible for childhood eye tumours, was one of the first tumour suppressor genes to be discovered and led to Knudson's famous "two-hit" hypothesis of cancer development.[11] The Rb protein normally inhibits proliferation by binding to the E2F transcription factor - a key player controlling the G1/S phase checkpoint of the cell cycle. Loss of Rb by HPV E7 protein can therefore result in uncontrolled cell division. A normal cell would react to excessive E2F-mediated growth signals by p53-dependent apoptosis, however the presence of E6 protein counteracts this by p53 and BAK degradation which prevents apoptosis.[5] The end result of their combined action is host cell DNA which is prone to accumulate chance errors unchecked by physiological repair or programmed cell death.

HPV Type and Neoplastic Progression

Some HPV serotypes are high-risk (HR) for inducing squamous and adenosquamous cervical cancers, while others are only capable of producing genital warts or low-grade dysplastic lesions (table 1).[5] A DNA test for thirteen HR-serotypes (HPV-16,-18,-31,-33,-35,-39,-45, -51,-52,-56,-58,-59,-68) has been approved for use in the USA.[12] Particular HPV serotypes (e.g. HPV-31,-32,-52,-58) allow neoplastic progression to CIN3 only, whereas others (e.g HPV-16,-18 and -45) preferentially progress from CIN3 to cancer.[13]

Progression to high grade neoplasia occurs because HR-serotypes of HPV are unable to complete their life cycle in the transitional zone of the ectocervix-vaginal junction. Dysregulated expression of E6/E7 proteins leads to increased cell proliferation in the lower epithelial layers coupled with an inability to repair mutations in the host DNA. Integration and high expression of viral E7 genes within host DNA is a feature of progression from CIN3 to invasive cancer, as are loss of E2 and E4 genes which can exert a negative growth effect.[14]

Table 1. Commonly studied HPV serotypes according risk of malignancy.

High risk malignant and benign mucosal lesions	HPV-16, HPV-18, HPV-26, HPV-30, HPV-31, HPV-33, HPV-35, HPV-39, HPV-45, HPV-51, HPV-52, HPV-53, HPV-56, HPV-58, HPV-59, HPV-66, HPV-67, HPV-68, HPV-69, HPV-70, HPV-82
Low risk mucosal and cutaneous lesions (male and female genital warts, condyloma acuminata of cervix, laryngeal papillomas)	HPV-2, HPV-6, HPV-7, HPV-11, HPV-13, HPV-27, HPV-40, HPV-43, HPV-44, HPV-57, HPV-74

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The HPV life cycle and associated genetic events

The HPV life cycle consists of initial infection, uncoating, genome maintenance, genome amplification, and packaging to form new viral particles. Most of the work in this area and its associated genetic events has focused on HPV type 16 (HPV16) which is a major cause of cervical cancer.

Initial infection is thought to require viral access to cells in the basal layer of the epithelium, via breaks, abrasions or other micro-traumas in the stratified epithelium. Hair follicles seem to have abundant amounts of viral DNA[15] leading to suggestions that epithelial stem cells may be an important target for the virus.[16]

The virus attaches to the basal epithelial cells via specific cell surface receptors[17] leading to internalisation of the virus followed by uncoating of the viral particles and release of the viral genome. The viral E1 and E2 proteins have a role in maintaining the viral genome as an independent DNA element which can replicate extra-chromosomally or can be maintained by integration into the host genome (episome) (table 2).

Normal basal epithelial cells undergo cell division but then exit the cell cycle as they reach the suprabasal layers and undergo a process of terminal differentiation. In HPV infected cells the viral E6 and E7 proteins are expressed which prevent the suprabasal cells from exiting the cell cycle and retard the differentiation process.[14] Excessive amounts of p21/p27 can bind E7 and reduce the proliferative effect. It has therefore been suggested that E7 functions to promote S-phase entry in a subset of suprabasal cells with intrinsically low levels of p21/p27 or alternatively high levels of E7 expression.[14]

Amplification of viral genome occurs in the mid to upper epithelial layers and requires the activity of E1, E1, E4 and E5 proteins. The exact details are still to be elucidated but key events include up-regulation of a promoter present within the E7 open reading frame and increased E1/E2 expression. The freshly replicated genome can act as a template for further gene expression leading top increased amounts of E1/E2 and other replication proteins. The minor (L2) and major (L1) capsid proteins are expressed in the upper layers of the epithelium and by packaging the viral DNA allow assembly of the complete icosohedral shaped virus (Modis Y, 2002).[18]

Table 2. Human papilloma virus early and late genes with postulated functions.

E1	Maintains viral genome as episome after uncoating of viral coat in basal cell layer
	Facilitates correct segregation of genomes during cell division
	Failure to express full length E1 protein leads to integration of viral genome into host cell chromosome (Frattini, 1996)
	Acts as viral DNA helicase during viral genome amplification (Doorbar, 2005)
E2	Maintains viral genome as episome after uncoating of viral coat in basal cell layer
	Facilitates correct segregation of genomes during cell division
	Regulatory role in viral genome amplification (binds to regulatory region on viral genome and forms initiation complex with E1 protein)
E4	Levels rise in the mid-basal epithelial layers suggesting role in viral genome amplification
E5	Complexes with epidermal-growth-factor receptor, platelet-derived-growth factor receptor and the colony-stimulating factor-1 receptor promoting growth
E6	Binds to and degrades p53
	Inhibits Bak and Bax
	Activates telomerase
	Stabilises (i.e. inhibits degradation of) Src-family kinases
E7	Binds to and degrades Rb protein promoting E2F release and S-phase entry
	Activates histone deacetylases, AP-1 transcription complex, cyclin A and E
	Inhibits p21/cip1, p27/kip1 and INK4A
L1	Major capsid (coat) protein expressed in upper epithelial layers allowing assembly of full virus; each virus contains approx. 360 copies
L2	Minor capsid (coat) protein expressed in upper epithelial layers allowing assembly of full virus; each virus contains approx. 12 copies; in its absence virus-like particles are formed instead

Interestingly, the virus is non-lytic and remains within the epithelial cell until the latter reaches the epithelial surface. This viral retention within epithelial cells till it reaches the uppers layers has a role in immune evasion by the virus. In the vast majority of HPV infections however there is an immune response. Most lesions elicit lymphocytic response within 8-12 weeks and lesion regression occurs by 16 weeks.[19] Latent papillomavirus DNA may remain in the basal layers despite lesion regression and can be activated at a later date. Unsurprisingly those who develop immune system deficiencies (e.g. post-transplant, HIV, etc.) are prone to recurrent and persistent HPV infections.

Only a minority of infected individuals develop persistent lesions with progression to cervical intraepithelial neoplasia (CIN) type 1. This can be followed by CIN2, CIN3 and cervical cancer.[3] High-risk HPV serotypes are more likely to produce persistent and progressive lesions.

Immune evasion by HPV

Although 30-60% of sexually active individuals are infected with HPV, only 1% of infected individuals develop persistent HPV infection.[20] In these individuals HPV is able to avoid initiating an immune response by limiting availability of viral proteins to the immune surveillance mechanisms using several mechanisms.

Transfer RNA (tRNA) for specific amino acids bind to mRNA codons and allow the nascent protein to be synthesized. Redundancy of the genetic code allows more than one tRNA for a given amino acid and each species has a preferred codon usage for a particular amino acid. Papillomaviruses "late" genes utilise codons which are rarely used by mammalian cells[21] and this inhibits production of viral capsid proteins in the basal layers of the cervical epithelium. There may be still other transcriptional and translational control mechanisms reducing expression of viral proteins in the immunogenic lower epithelial layers.

Furthermore most E7 protein is sequestered within the nucleus thus moving one more step away from the view of antigen presenting cells.[21]

A cell infected by a virus will usually respond by producing interferons (IFN) producing an antiviral and antitumour effect. HPV16 E7 protein has been shown to block IFN-α activity[20] as well as inhibiting the IFN-β promoter.[22]

Normally dendritic cells - part of the adaptive immune system - "sense" foreign proteins present within the tissues. These are then sampled, processed and presented to T-cells in regional lymph nodes resulting in activated CD8 cytotoxic and CD4 helper T-cells which migrate to the tissues and attack the foreign cells or viruses. The process requires dendritic cells to become "mature" by inflammatory signals such as TNF or wounding of the tissues. This explains why exposure of mice to E7 along with a concomitant bacterial endotoxin produces an immune response to E7, whereas in the absence of endotoxin tolerance to E7 develops instead.[23] The exposure of antigen presenting cells to HPV E7, in a non-inflammatory epithelial environment may explain the peripheral immune tolerance which is present with persistent infection.

HPV-dependent cofactors for the development of cervical cancer

The knowledge that not all women with HPV infection develop cervical cancer, has led to interest in assessing role of other factors - like oral contraceptive use, parity, smoking and other sexually transmitted diseases (STDs) - which increase predisposition for progression to cancer. Previous studies have been marred by designs which fail to account for possible confounding between variables. For example oral contraceptive use, early age of first intercourse and parity have been shown to be associated with developing cervical cancer but these can be surrogates for sexual promiscuity which is associated with HPV infection. Fortunately recent well designed pooled analyses by the International Agency for Research on Cancer (IARC) in Lyon, France have shed light on some of these issues.

Role of Oral Contraceptives: Data pooled from eight case-control studies were analysed to compare oral contraceptive (OC) use in HPV-positive women with invasive cervical cancer (ICC) to HPV-positive women without ICC.[24] The researchers were able to control the effects of HPV infection since both groups of women had HPV DNA present in exfoliated cervical cells. OC use for less than 5 years did not increase risk for squamous ICC. However, women with squamous ICC were almost 3 times more likely to have used OCs for 5-9 years, and 4 times more likely to have used OCs for 10 years or longer, than those who did not have ICC.

Role of Parity: Serum concentrations of oestrogen and progesterone increase as pregnancy progresses and peaks during the third trimester and may be associated with the development of an atypical transformation zone and squamous metaplasia at the endo/ecto-cervical junction.

The Lyon group used the same dataset to analyse the role of parity in women who had pre-existing HPV infection.[25] A direct association between the number of full term pregnancies and squamous ICC was found. The odds ratio for seven full-term pregnancies or more was 3.8 compared with nulliparous women, and 2.3 compared with women who had one or two full-term pregnancies. Interestingly a similar association was not found between cervical adenocarcinoma and parity suggesting an alternative aetiology.

Chlamydia infection is similar to HPV in that it is a

STD and can produce chronic cervical infection. The obligate intracellular localization of Chlamydia along with associated chronic inflammation, reactive oxygen release and epithelial damage could potentially promote neoplastic transformation. Akin to HPV it has evolved strategies to allow its replication within host cells. For example Chlamydia inhibits TNF-α, etoposide and Fas-antibody induced apoptosis of host cells by preventing release of cytochrome-c.[26]

Analysis of pooled IARC data from seven countries was published last year controlling for confounders.[27] HPV DNA positive cases with squamous ICC were 1.8 times more likely to have Chlamydia infection than HPV DNA positive controls. Again no such association was found in relation to cervical adenocarcinoma which suggests an alternative mode of carcinogenesis.

Cigarette smoking was first postulated to be a factor in cervical cancer by Winkelstein in 1977[28] and nicotine products have been detected in exfoliated cervical epithelial cells and in cervical mucus of smokers.[29] IARC pooled-analysis suggests that smoking increases the risk of squamous ICC in HPV DNA positive women.[30]

Why should smoking have this effect in cervical cancer? Cigarette smoke contains many carcinogens including N-nitrosamines, like NNK. NNK produces host DNA adducts resulting in point mutations (G≥A transitions) in DNA, particularly in the p53 and Ras genes altering their function in favour of carcinogenesis.

Polycyclic aromatic hydrocarbons (PAHs) are another group of chemicals - benzo(a)pyrene is one member - which are present in tobacco. PAHs are known to induce mutagenic G-T transversions in DNA. Of interest, a study comparing effects of benzo(a)pyrene in normal and HPV-16 immortalized cervical epithelial cells found a two and half fold greater level of adduct formation in the HPV-affected cells.[31]

In the same study, both the normal and HPV-affected cells were growth inhibited but the HPV-affected cells were less growth inhibited. Extrapolating the relative growth inhibition of normal cells to the clinical scenario makes one consider the possibility of a progressive "shift" of the epithelium (of smokers with HPV infection) towards having more HPV transformed cells.

Tobacco smoke carcinogens are processed within cells by enzyme systems-especially cytochrome-P450 (CyP450) - to form bioactive metabolites which in turn produce cellular damage. Individuals vary in the extent to which these enzymes are induced upon exposure to toxins. In fact there are toxin-related, CyP450 isoform-related and cell-specific variations in enzyme induction.[32] Although work characterising CyP450 variation in cells cervical exposed to HPV-16 E6/7 genes has been performed more could be done.

Other possible effects of tobacco smoking may include reduced immune function and altered metabolism of female hormones resulting in increased carcinogenesis.

Herpes Simplex Virus Type 2 (HSV2) the only study comparing HPV and HSV2 found that HSV2 had a role in increasing the risk of cervical cancer only in HPV-DNA negative subjects, suggesting that separate pathways may be involved in HSV2 oncogenesis.[33]

Further Cofactor Thoughts are the influences of co-factors additive or multiplicative/synergistic? A Swedish study comparing 834 women with and without squamous ICC, and the effects of HPV infection, Chlamydia infection and smoking found that each had an independent effect (Odds Ratios, OR, were 5.4, 3.4 and 1.8 respectively).[34] However among those positive for all three factors the OR was 2.5 - compared to an expected OR=33 if there were synergistic effects. While these findings of co-factor antagonism need corroboration they suggest that despite individually promoting cervical cancer a more complex interrelationship may be present.

Thoughts for the future

A major breakthrough has been the discovery that injected HPV capsid proteins can induce an immune response in healthy individuals. Prophylactic vaccinations containing HPV-16 (and/or -18) L1 protein are in clinical trials at present with the aim of preventing HPV infection in women.[35] Therapeutic vaccines for women already infected by high-risk HPV are also in the pipeline. Thus the potential for greatly reducing mortality from cervical cancer is now closer than ever before.

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