Review

Molecular events in uterine cervical cancer

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Objective: To review the literature regarding the molecular events which occur in the development of uterine cervical cancer, with particular reference to human papillomavirus (HPV) infection.
Methodology: Bibliographic searches of Medline and the ISI citation databases using appropriate keywords, including the following: papillomavirus, cervix, pathology, cyclin, chromosome, heterozygosity, telomerase, smoking, hormones, HLA, immune response, HIV, HSV, EBV.
Conclusions: It has become clear that most cervical neoplasia, whether intraepithelial or invasive, is attributable in part to HPV infection. However, HPV infection alone is not sufficient, and, in a small proportion of cases, may not be necessary for malignant transformation. There is increasing evidence that HPV gene products interfere with cell cycle control leading to secondary accumulation of small and large scale genetic abnormalities. This may explain the association of viral persistence with lesion progression but, in many patients, secondary factors, such as smoking and immune response, are clearly important. However, the mechanisms involved in the interaction between HPV and host factors are poorly understood.
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Keywords: cervix; carcinoma; papillomavirus; aetiology; molecular biology

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Introduction

Human papillomaviruses (HPV) have been identified as the major aetiological factor in cervical carcinogenesis.[1] Epidemiological evidence indicates that the majority of cervical neoplasia is attributable to HPV infection but, although certain HPV genes are capable of immortalisation and can cooperate in the process of transformation, not all non-invasive lesions progress to the full malignant phenotype indicating that other cofactors are required.[2] This review considers the molecular biology of cervical neoplasia, particularly that of the HPVs, the interaction of HPVs with epithelial cells, and the influence of possible associated cofactors. The combination of such factors leads to a consortium of molecular events involved in the evolution of intraepithelial and invasive disease (see fig 1).

Human papillomaviruses and cervical neoplasia

VIRAL STRUCTURE

Papillomaviruses are small DNA viruses approximately 55 nm in diameter. Mature viral particles have an icosahedral outer capsid coat composed of two structural proteins. One of these (the L1 protein) comprises 80% of the total viral protein and has a relative molecular mass (M_r) of 53 000–59 000. The other (the L2 protein) is a minor component, and has a M_r of 70 000. Contained within the capsid is the viral genome which is a double stranded circular DNA approximately 7.9 kilobases (kb) in length.[3] The molecular organisation is similar for all 78 different types of HPV which have so far been isolated. Each genome can be divided into early (E) and late (L) regions, containing seven early and two late open reading frames (ORFs), and a non-coding region, referred to as the upstream regulatory region (URR). Expression of the early genes occurs at the onset of infection, and the products of these genes mediate specific functions controlling viral replication and, in the case of the oncogenic viruses, cellular transformation. The E1 gene is involved in viral replication and genome maintenance.[4] The E2 gene is a transcriptional regulator[5] and is also involved in viral DNA replication.[4] The E4 gene encodes several proteins which disrupt the cytoplasmic keratin network.[6] This produces the classic cytoplasmic halo effect known as koilocytosis. The E5 gene may play a role in cellular transformation by its interactions with cell membrane growth factor receptors.[7] The E6 and E7 genes, which lie immediately downstream of the URR, encode the major transforming proteins which are capable, under appropriate conditions, of inducing cell proliferation, immortalisation, and transformation.[1] Finally, the L1 and L2 ORFs encode for the viral protein coat and are activated towards the final stages of the viral cycle, and hence within superficial, terminally differentiated cells.[8] The L1 gene is frequently used for HPV typing[9 10] and encodes the common papillomavirus antigen which is targeted by antibodies used in the immunohistochemical detection of productive HPV infection.

CLASSIFICATION OF HPVS

HPVs were originally classified according to their degree of solution phase homology.[11] With the advent of widespread polymerase chain reaction (PCR) amplification and sequencing, classification is now usually based on partial sequencing of 399 bp in the E6 region and/or 291 bp in the L1 region of the virus.[12] A new HPV type is assigned when there is less than 90% homology with other previously typed HPVs, a subtype being 90–98% homologous and a variant >98%.[12] A number of phylogenetic trees have been generated using this

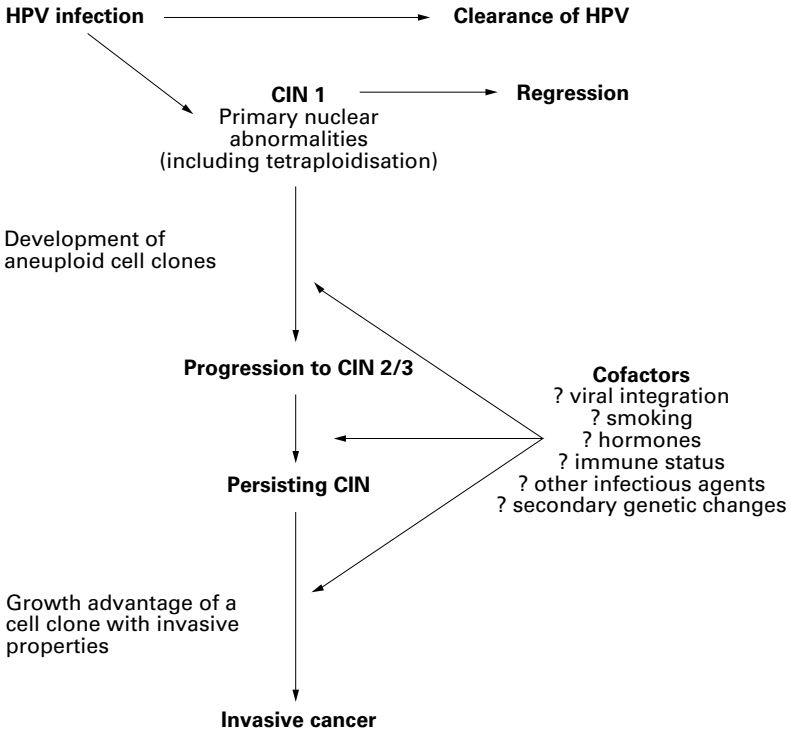

Figure 1 Schematic overview of the human papillomavirus (HPV) associated molecular events involved in cervical carcinogenesis (CIN = cervical intraepithelial neoplasia).

system but a more recent classification of HPVs based on clinicopathological associations as well as genetic structure gives a better view of both functional and genetic properties.[13] This system provides a more robust basis for the investigation of clinical associations and biological properties of these viruses. For example, recent studies have demonstrated not only that HPV 16 variants are widely prevalent in cervical cancers[14] but also that such variants differ in their ability to interact with host cell mechanisms.[15]

CLINICAL ASSOCIATIONS OF HPV GENOTYPES

Molecular cloning of viral nucleic acids, amplification by PCR and sequencing have demonstrated over 78 different HPV genotypes, at least 30 of which have been detected in the genital tract.[13 16] These HPVs have been divided into low, intermediate, and high risk types according to their segregation with the different grades of intraepithelial and invasive disease. Low risk HPV types (for example, HPV 6, 11, 40, 42, 43, 44) are usually associated with benign exophytic genital warts. HPV 6 and 11 are present in over 90% of condylomas, with about two thirds of these containing HPV 6 and one third HPV 11. They are also associated with low grade squamous intraepithelial lesions (SILs) (wart virus change and CIN 1) but are only rarely found in high grade SILs (CIN 2 and 3) and invasive carcinomas. By contrast, intermediate (particularly HPV 31, 33, 39, 52, and 58) and high risk (HPV 16, 18, 45, and 56) HPV types are associated with "flat" condylomas, all grades of SIL and invasive carcinoma.[17 18] The concept of high, intermediate, and low risk HPV is supported by many epidemiological case controlled studies which have collectively shown a consistent association between high and intermediate risk HPV and high grade cervical disease. In a worldwide study of more than 1000 specimens from patients with invasive cervical cancer HPV DNA was identified in 93% of tumours. HPV 16 was present in 50% of the specimens, HPV 18 in 14%, HPV 45 in 8%, and HPV 31 in 5%. In squamous cell tumours HPV 16 predominated (51%) but HPV 18 was the commonest type found in adenocarcinomas (56%) and adenosquamous tumours (39%).[19] Another study involving cervical specimens from 2627 women showed HPV present in 79.3% of women with definite cervical neoplasia (627 of 791) and in 23.7% of patients with borderline atypia. Low risk HPV (HPV 6, 11, 42, 43, and 44) was present in 20.2% (76 of 377) of low grade lesions but absent in all cancers. Intermediate risk HPV (HPV 31, 33, 35, 51, 52, and 58) was detected in 23.8% (62 of 261) of high grade lesions but in only 10.5% (16 of 153) cancers. HPV 16 was associated with 47.1% of both high grade SILs (123 of 261) and cancers (72 of 153) and HPV 18, 45, and 56 were found in 26.8% (41 of 153) of invasive carcinomas but only 6.5% (17 of 261) of high grade SIL.[17]

The risk of progression from low grade to high grade SIL is greater in patients with persistent HPV infection,[20 21] and in those with high viral load[20] suggesting that prolonged exposure to viral effects is important in the pathogenesis of cervical disease. Whether this is related to viral factors, such as sequence variation, or is a reflection of host factors, such as immune status, remains to be determined.

Recently, a PCR based method for determining clonality has shown a strong correlation between HPV type and clonal status. Morphologically low grade SILs could be classed into two biologically distinct lesions. That associated with HPV types 16, 18, 31, 33, 35, 39, 45, 56, 58, or 65 was monoclonal indicating clonal expansion of infected keratinocytes, while that associated with other HPV types was polyclonal and probably represents non-neoplastic virally induced proliferation.[22] Whether the distinction between monoclonal and polyclonal HPV infection gives information additional to morphology and HPV type remains to be determined.

PATHOLOGY OF HPV INFECTION

HPVs are epitheliotropic by nature, infecting the cervical squamous epithelium possibly through small abrasions in the tissue. The ensuing virus life cycle is then closely linked to keratinocyte differentiation. In the proliferating basal epithelial cells, thought to be the site of initial infection, the viral genome is maintained as a low copy episome.[23] As the keratinocyte undergoes progressive differentiation, viral genome amplification and gene expression increase until late "L" gene expression and virion production occur in the terminally differentiated superficial cells. This form of infection leads to koilocytosis, nuclear enlargement, dyskeratosis, multinucleation, and in some cases low grade SIL. Such lesions may regress, persist, or progress.

A second form of infection (non-permissive transformable infection) occurs when viral

replication and vegetative viral production does not occur and may be found in both squamous and glandular tissue; infection of reserve cells which are committed to glandular differentiation and which do not allow permissive infection results in either aborted or non-permissive transformable infection. Viral DNA persists as either an extrachromosomal element or by integration into the host DNA as a single copy or multiple head to tail tandemly repeated copies at many chromosomal fragile sites.[24] The site of integration into the host genome does not appear to be consistent, although late replicating regions are targeted,[25] suggesting that structural and functional factors may be important. The viral breakpoint is more consistent as integration often causes disruption of the viral E2 gene in a manner that results in loss of function.[26] Moreover, disruption of either the E1 or E2 gene can lead to enhanced immortalisation capacity.[27] Viral integration also precludes late gene expression, even if the late genes are retained in the integrated viral genomes.[28] The mechanisms of viral integration are not well understood, although it has been demonstrated that expression of E6 or E7 of HPV 16 increases integration of foreign DNA compared with HPV 6 or 11[29]: this is consistent with the observation that viral integration is rare in lesions infected with low risk HPVs.

Viral integration with associated disruption of the E2 gene and consequent removal of transcriptional repression is one mechanism of upregulation of E6/E7 expression. However, in one study, integration of HPV 16 was associated with increased stability of E6/E7 mRNA suggesting that alternative pathways may occur.[30] Moreover, episomal viral DNA is frequently present and amplified in cervical carcinomas,[31 32] and this amplification is dependent on retention of intact E1 and E2 genes in these lesions.[31] It has therefore been suggested that HPV amplification may provide an alternative mechanism for the upregulation of early gene expression in some tumours. These effects are clearly important in the process of squamous cell transformation as E6 and E7 expression is required for maintenance of the transformed phenotype.[33 34]

Several cellular transcriptional control factors are involved in the control of HPV early gene expression but the mechanisms involved are not well understood. It has been demonstrated that E6/E7 expression is dependent on expression of AP1, a cellular transcriptional control factor.[35] Interestingly, upregulation of the expression of c-jun (a component of AP-1) has been demonstrated in association with low risk HPV infection of condylomas so this pathway may be common to both high and low risk HPV types.[36]

EXPERIMENTAL HPV INDUCED TRANSFORMATION

HPV DNA, particularly the E6 and E7 genes, can immortalise primary cervical cells and human foreskin keratinocytes in culture[37 38] but transformation of normal cells generally requires cooperation between HPV and other oncogenic sequences, such as EJ-*ras*,[39] in keeping with other factors being involved in this process in vivo. Transfection of normal keratinocytes with HPV sequences can prevent cellular differentiation resulting in induction of morphological changes similar to CIN in raft culture.[40 41] Culture of naturally occurring lesions in a similar system leads to the production of morphological changes in vitro similar to those present in vivo.[42]

Fusion of HPV 18 containing HeLa cells with normal fibroblasts results in the repression of the malignant HeLa cell phenotype.[43] This system led to the localisation of a putative normal cellular factor, localised to chromosome 11, involved in the suppression of HPV E6/E7 expression. This was termed cellular interfering factor[44] but, to date, it has not been precisely identified. There is, however, evidence that a tumour suppressor gene important in cervical neoplasia may be located on chromosome 11 (see below).

Another approach to the study of HPV early genes involves the production of mice transgenic for the E6/E7 genes of high risk HPVs, particularly HPV 16. By introducing these genes under the control of the keratin 14 promoter, expression can be targeted to squamous epithelium and the effects of such expression analysed in vivo.[45 46] These mice develop hyperplastic and dysplastic squamous lesions but only progress to invasive malignancy when back crossed with certain strains, indicating that genetic background is important in the determination of susceptibility to invasive disease. This is in keeping with the effect of HLA genotype seen in human disease (see below).

HPV AND CELL CYCLE CONTROL

Recently, it has become increasingly clear that HPVs, like many other DNA viruses, achieve their replication by interference with normal cell cycle control mechanisms. Given that malignant transformation is also intimately related to these processes, it is likely that the oncogenic potential of papillomaviruses lies in their ability to alter cell cycle checkpoints, thereby leading to accumulation and transmission of genetic abnormalities. The initial observation that the E6 and E7 oncoproteins bind to the p53 and pRB proteins respectively, both of which are involved in the regulation of growth control, demonstrated that the HPV genes possessed functions analogous to those already known for SV40 and adenovirus.[47–49] Moreover, the E6 proteins of high risk HPVs, particularly HPV 16 and 18, bound more effectively to, and led to degradation of, the p53 protein via a ubiquitin mediated pathway,[50] indicating a functional difference between high and low risk HPVs. Introduction of mutations into HPV 11 and 16 E6 proteins by exchanging the p53 binding domains altered the ability of these proteins to bind to and degrade p53[51] and confirmed these differences. In addition, the E6 proteins of naturally occurring variants of HPV 16 differ in their abilities to bind to and degrade p53 protein,[15] indicating that these differences are determined by relatively small sequence variations.

p53 appears to protect the physical integrity of the genome by regulating the G1 cell cycle "checkpoint" preventing entry into S phase of cells containing DNA strand break damage, thus allowing DNA repair or apoptosis and avoiding replication of a damaged template.[52] DNA damage causes an increase in wild type p53 which activates expression of the WAF 1 gene whose protein product, p21 WAF, binds and inhibits cyclin/cyclin dependent kinase (CDK) activity, in particular CDK 2.[53] The inhibition of CDK 2 activity alters the phosphorylation state of the retinoblastoma gene product (pRB). Under normal cyclin/CDK control the RB protein is in the underphosphorylated form in G1 phase and becomes highly phosphorylated through the S and G2 phases. The underphosphorylated pRB readily complexes with the transcription factor E2F, but the phosphorylated form does not, allowing free E2F to activate transcription of a range of genes whose products are essential for cell cycle progression.[54] Therefore, temporary inhibition of the CDK 2 indirectly by p53 conserves the underphosphorylated pRB/E2F complex halting transcriptional activation and allowing DNA repair before entering the S phase. Cells expressing HPV 16/18 E6 and E7 lack this G1 cell cycle checkpoint. The resulting failure of the normal control system may ultimately contribute to the accumulation of genetic alterations required for tumour development and/or progression and lead to DNA instability which may facilitate integration of viral DNA into host chromosomes and further malignant progression.

Much of the control of the cell cycle is mediated by the cyclin proteins which regulate the activity of CDKs. Cyclin control immediately before and during S phase is mediated by cyclins E and A and the overexpression of these cyclins in association with HPV infection suggests that the virus can "take over" certain host cell cycle control mechanisms to facilitate viral, rather than cellular, DNA replication.[55] The cell is then held in a prolonged replication phase until degradation of the increased cyclin is completed. Cyclin D is responsible for cells passing through G1 phase and complexes and inactivates Rb protein in a similar fashion to HPV E7. Intuitively, therefore, E7 could circumvent cellular requirements for cyclin D expression: this appears to be the case in cervical neoplasia.[56]

All of the cell cycle events are intimately related to viral early gene expression and upregulation of E6 and E7, by whatever mechanism, is likely to lead to such cells having a growth advantage over their neighbours. All HPV infected cells, regardless of HPV type, undergo proliferation whether uncontrolled or self limited. However, HPV 16/18 infected cells lack G1 checkpoints[57] favouring viral integration and inducing genetic instability. This mechanism remains intact with HPV 6/11 in which the virus remains in the episomal form, in part due to the differences in interaction between p53 and the E6 proteins of HPV 16/18 and HPV 6/11.[51] The inability to integrate and an intact G1 checkpoint, with overexpression of cyclin/CDKs, could account for HPV 6/11 infected lesions proliferating while remaining benign.

In addition to affecting early cell cycle control, HPV 16/18 infection is associated with the presence of abnormal mitotic figures suggesting disruption of mitotic events.[58] Before M phase, cyclin B/p34 cdc 2 complex together forming mitosis promoting factor (MPF).[59] In the normal cell cycle, p34 cdc 2 (CDK 1) is at a constant level throughout, its activity being controlled by the altering levels of the complexing cyclin B and by phosphorylation. Dephosphorylation of MPF causes an increase in associated histone 1 kinase activity and initiation of mitosis.[60] Cyclin A also complexes with p34 cdc 2 and is required for the onset of mitosis.[61] Exit from mitosis is attributed to cyclin B degradation.[62] Increased expression and altered activity of these proteins by HPV[60] could contribute to mitotic defects and chromosomal aberrations. Indeed, overexpression of the HPV 16 E6 and E7 genes in the presence of mitotic spindle inhibitors has been shown to induce genome wide DNA rereplication without intervening mitosis.[63] Similarly, overexpression of the E2 gene alone leads to S phase arrest and reduplication of keratinocyte DNA content.[64]

Thus, HPV genes are capable of interfering with both G1/S and G2/M cell cycle checkpoints. Abrogation of the former may lead to accumulation of small scale genetic abnormalities while induction of abnormalities of mitotic control is more likely to lead to more gross genetic changes involving whole chromosomes (chromosomal instability). The differences between HPV types may well be reflected in their differing abilities to block these checkpoints in vivo.

Although the binding of high risk HPV E7 proteins to pRb may be involved in immortalisation and transformation owing to disturbances in cell cycle control, HPV 16 E7 has been shown recently to be capable of binding directly to members of the AP1 family of transcription factors.[65] As (i) the AP1 transcription factors play an important regulatory role in the differentiation of keratinocytes and (ii) the HPV life cycle is closely tied to cell differentiation, it was hypothesised that binding of Jun proteins by E7 may result in inhibition of the cell differentiation required for efficient virus replication. The E7 protein may therefore play an additional role in transformation which is independent of its ability to bind to pRb.

Cofactors in the evolution of cervical neoplasia

The epidemiological evidence that many HPV infections regress, and that progression of intraepithelial lesions is associated with viral persistence, taken in conjunction with the in vitro data that HPV genes are capable of immortalisation of normal cells, but not their transformation (see above), indicate that secondary changes are important in HPV associated cervical carcinogenesis. These changes may occur as a direct consequence of HPV infection or indirectly through the action of

cofactors, either innate or acquired. This is consistent with current multistage models of carcinogenesis, in which several genetic events are required to effect transformation.

GENETIC CHANGES

Genetic damage with consequent loss of tumour suppressor genes, or activation of cellular oncogenes, can lead to cellular immortalisation and transformation. Such genetic changes may occur as a result of cell cycle checkpoint abnormalities induced by HPV gene expression, or may be related to environmental factors such as the formation of smoking related DNA adducts. However, loss or mutation of conventional oncogenes and tumour suppressor genes is uncommon in cervical neoplasia.[2 66]

Conventional cytogenetic studies have demonstrated non-random chromosome abnormalities involving chromosomes 1, 3, 5, 11, and 17 in cervical carcinoma[67] but relatively few studies have examined the correlation between HPV and chromosome abnormalities in cervical cells and tissues. In a cell culture model involving transfection of high risk HPV DNA, immortalisation was associated with clonal allele loss on chromosomes 3p, 11q, 18q, and 10p as assessed by microsatellite loss of heterozygosity (LOH).[68] Similarly, comparative genomic hybridisation demonstrated abnormalities of chromosomes 3, 4, 10, and 11 in keratinocytes stably transfected with HPV 16.[69] Numerical abnormalities of chromosomes 11, 17, and X were identified in 88% of invasive squamous carcinomas of the cervix by interphase cytogenetics but there was no relation of these abnormalities to the type or morphological distribution of the HPV sequences present.[70]

Analysis of LOH in naturally occurring tumours has demonstrated changes in almost all chromosome arms,[71] but the most frequent abnormalities are present in similar chromosomes to those involved in the in vitro studies.[72–80] Several studies have shown LOH on the short arm of chromosome 3 in cervical carcinoma, particularly in the 3p13–21.1, 3p21, and 3p21–22 regions.[72–75] Using comparative genomic hybridisation, gain of chromosome 3q was identified in severe dysplasia/CIS and was overrepresented in 90% of carcinomas, suggesting that gain of this region was important in progression from non-invasive lesions to invasive carcinoma.[81] In other studies, frequent LOH has been identified on chromosome 17p,[73 76 77] chromosome 11q,[78 79] chromosome 4,[80] and chromosomes 6p and 18q.[75] Microsatellite instability, which is frequently associated with some tumour types—for example, colorectal carcinoma, does not appear to be common in cervical carcinoma, being identified in only 5.6% of tumours in one study.[82]

There is increasing evidence that cellular immortalisation requires restoration of chromosome telomere length by activation of telomerase.[83] Recently, it has been demonstrated that expression of the E6 protein of HPV 16 can induce telomerase activity but that this induction is not sufficient for keratinocyte immortalisation.[84] The observation that clonal allele loss on chromosomes 3p, 10p, 11q, and 18q was accompanied by telomerase activation in keratinocytes immortalised by HPV transfection[68] is in keeping with these findings. It is therefore not surprising that telomerase activity was identified in all of 10 cervical carcinomas reported in a recent study.[85]

Although there is an emerging pattern of genetic changes in neoplastic cervical lesions, little is know of the mechanisms of induction of these abnormalities, or of the identity of the genes involved. The relation between productive viral infection, viral gene expression, viral integration, keratinocyte differentiation, and DNA abnormalities is also poorly understood.

SMOKING

Epidemiological studies have shown an important correlation between cigarette smoking and the development of cervical cancer. A twofold increased risk of CIN and invasive disease has been demonstrated among smokers[86]; passive smoking has also been implicated[87] and cessation of cigarette smoking is associated with a reduction in the size of CIN lesions by approximately 20%.[88]

The actual mechanism by which smoking leads to an increased risk of cervical cancer is not fully understood but Langerhans' cell number is reduced in the cervical epithelium of smokers,[89 90] suggesting that abnormalities of local immune surveillance may be important. Alternatively, the constituents of smoke and their derivatives may interact directly with HPV. Nicotine and cotinine are frequently found at high levels in cervical mucus[91] and can induce proliferation of HPV transformed cervical cells.[92] Polycyclic aromatic hydrocarbons such as benz[a]pyrene present in cigarette smoke have been shown to inhibit cell proliferation in both "normal" and HPV 16 immortalised cervical cell lines, but with inhibition occurring at a 20-fold lower concentration in the normal cells.[93] Moreover, as these compounds can form adduction products with DNA, they are potentially genotoxic. This effect may be important in vivo as DNA adduction products were found to be present in normal cervical epithelium at a higher level in smokers than in non-smokers.[94]

OTHER INFECTIOUS AGENTS

It has been suggested that more than one infectious agent may act in a synergistic manner in the development of cervical cancer but there is little evidence for the interaction of HPV and infectious agents other than herpes simplex virus (HSV)[95] and HIV. Experimental evidence has also shown that cells transformed by HPV 16/18 are capable of inducing tumours in mice only after transfection with HSV 2.[96] Moreover, women testing seropositive for HSV 2 alone had a risk ratio of 1.2 for cervical cancer, but those with HPV 16/18 in addition had a risk ratio of 8.8; in those with HPV 16/18 alone the risk ratio was 4.3.[97]

In HIV positive patients who are not immunocompetent there is an increased incidence of

HPV infection with associated epithelial cell abnormalities.[98] In addition, the HIV *tat* 1 protein is capable of transactivating HPV 16 transcription.[99] Thus, HIV infection may affect HPV associated neoplasia by both potentiation of the effects of HPV and by leading to immune deficits and defective viral clearing.

Epstein–Barr virus, although demonstrated in invasive squamous carcinoma cells and associated lymphocytes, has not at present been shown to interact with HPV directly.[100]

HORMONES

The URR of HPV 16 contains a glucocorticoid regulatory element, which permits E2 independent early gene transcription.[101] Therefore, steroid hormones may enhance viral transcription, as shown with oestrogens and progestagens in cell lines.[102 103] A recent study has demonstrated that glucocorticoids downregulate HLA class I expression in HPV containing cervical carcinoma cell lines, but only if the viral genome is integrated into the cellular genome.[104] These studies demonstrate that hormonal factors may not only be important in the control of viral gene transcription but that they may also influence immune competence.

IMMUNE STATUS

Certain HLA genotypes are found more frequently in patients with cervical neoplasia than in the normal population,[105–108] suggesting that susceptibility to cervical neoplasia may in part be determined by inherent genetic factors governing the immune response to HPV. Phenotypically, downregulation of class I HLA antigens, which are important in the recognition of viral antigens, is frequent in both preinvasive and invasive disease.[109 110] Conversely, normal cervical epithelial cells do not express class II HLA antigens but such antigens are found on the surface of epithelial cells in cervical neoplasia.[111] This upregulation is related to the morphological grade in preinvasive lesions and appears independent of the presence of HPV sequences.[112] The keratinocyte phenotype in these lesions therefore resembles that of antigen presenting cells and suggests that they may be involved directly in immune surveillance.

In renal transplant patients there is an increased prevalence of wart virus associated changes, CIN, and invasive squamous carcinomas.[113] HIV positive patients also have an increased incidence of these changes which may be associated with both reduced systemic immune competence, as assessed by CD 4 cell count[114] or with a local defect in immunity with reduced Langerhans cell number.[115] This is compounded by local shifts in immune surveillance related to alterations in Langerhans cell activity associated with HPV mediated epithelial cell injury.

In view of the association of viral persistence with lesion progression, variation in the ability of different class II alleles to effect clearance of HPV could explain the association of some HLA class II genotypes with cervical neoplasia. Similarly, acquired immune defects may exert their effects through defective viral clearing and hence viral persistence.

CYTOKINES AND GROWTH FACTORS

In HPV immortalised cell lines E6 and E7 gene transcription can be inhibited by leukoregulin and interferon gamma, but not by interferon alfa, with associated reduction in cell proliferation.[116] HPV 16 and 18 E6 and E7 production is also inhibited by transforming growth factor β (TGF-β) in HPV transformed keratinocytes.[117] Conversely, HPV 16 E5 can interact with epidermal growth factor receptor (EGFR) in the process of cell transformation.[118] E-cadherin transfection leads to restoration of membrane E-cadherin/catenin complex together with downregulation of EGFR and reversion of the transformed phenotype in HPV transfected cells.[119] These studies suggest that interaction of HPV with cell surface molecules may be important in the determination of cellular phenotype.

HPV negative cervical tumours

Sensitive, broad spectrum HPV detection methods have demonstrated HPV DNA sequences in the vast majority of invasive cervical carcinomas. Whether truly HPV negative carcinomas exist is currently a matter for debate as there are several reasons why tumours may falsely appear HPV negative.[120] However, the epidemiological finding that patients with HPV negative CIN have a different spectrum of risk factors suggests that at least intraepithelial disease may arise in the absence of HPV infection.[121] HPV negative cervical cell lines and tumours often contain p53 mutations which could theoretically substitute for the presence of the HPV E6 protein.[122 123] Although there is a substantial inverse correlation between p53 mutation and HPV infection, it has become clear that they are not mutually exclusive in cervical carcinomas. Thus, p53 gene mutations have been identified in HPV positive tumours[124 125] and, similarly, p53 mutations are not always present in apparently HPV negative tumours.[126] This does not however entirely exclude the hypothesis that p53 mutation substitutes for E6 gene expression in HPV negative tumours as (i) p53 mutation may be acquired as a late event in HPV positive tumours[127]; and (ii) apparently HPV negative cervical tumours may be infected with either undetected or unknown HPV types.[120]

Cyclin D is responsible for cells passing through G1 phase and has been implicated in carcinomas when overexpressed.[128] PRAD 1 or *bcl* 1 oncogene, which is on chromosome 11q13 and is activated by translocation, is identical to cyclin D. A recent study has shown abnormalities of PRAD 1 (both amplification and rearrangement of DNA and overexpression of mRNA) in seven of 13 cervical and vulval squamous carcinoma derived cell lines.[129] The E7 protein has cyclin D-like activity and therefore overexpression of cyclin D could mimic, at least in part, the effects of this protein.

Mechanistically, therefore, other genetic abnormalities can substitute for HPV gene expression. This is consistent with the hypothesis that transformation of cervical epithelial cells, and hence carcinoma of the cervix, can occur by HPV independent pathways.

Glandular cervical tumours

The association between glandular neoplasia of the cervix and HPV infection is less strong than that of squamous neoplasia.[130 131] There are methodological difficulties in the study of glandular lesions as, without microdissection techniques, it is not possible to exclude the possibility that any HPV sequences identified in extracted nucleic acids were present in the accompanying squamous epithelium rather than in the glandular lesion.[132] Nevertheless, HPV sequences have been localised directly within glandular epithelial cells by both DNA and RNA in situ hybridisation in several studies.[133–137] Moreover, HPV 18 is found significantly more frequently in glandular than in squamous lesions of the cervix,[131 135 138] indicating the involvement of a different spectrum of HPV types. Small cell carcinoma of the cervix is also associated with HPV 18 infection, suggesting that this HPV type may be involved particularly in non-squamous tumours.[139]

Conclusions

It has become clear that most cervical neoplasia, whether intraepithelial or invasive, is attributable in part to HPV infection. However, HPV infection alone is not sufficient, and may, in a small proportion of cases, not be necessary for full malignant transformation. There is increasing evidence that HPV gene products interfere with cell cycle control leading to secondary accumulation of small scale and large scale genetic abnormalities. This may explain the association of viral persistence with lesion progression but, in many patients, secondary factors, such as smoking and immune response, are clearly important. However, the mechanisms involved in the interaction between HPV and host factors are poorly understood.

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