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REVIEW ARTICLE

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Association of human papillomavirus infection with carcinoma of the cervix uteri and its precursor lesions: theoretical and practical implications

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Abstract Human papillomaviruses (HPVs) are the major aetiological agents of cervical carcinoma. In this review, epidemiological and molecular data are combined to present a model for HPV-induced cervical carcinogenesis. The impact of current knowledge regarding diagnostic and therapeutic approaches is shown, i.e. the use of HPV tests in cervical cancer screening, in the management of atypical smears of uncertain diagnosis and in smears indicative of mild dysplasias, as well as in follow-up examinations during and after therapy. In addition, the value of the two most frequently used HPV detection systems, polymerase-chain reaction (PCR) and hybrid capture (HC) analysis, is discussed.

Keywords HPV · Cervical cancer · Cervical dysplasia

Introduction

More than 20 years after the detection of human papillomavirus (HPV) DNA in cervical neoplasia and the identification of the first genital HPV types [46, 71], there is no doubt about the important aetiological role of papillomaviruses in cervical carcinogenesis. The characterisation of new genital HPV types and the development and application of highly sensitive HPV detection systems have shown that nearly 100% of all cervical squamous cell carcinomas (SCCs) and more than 70% of cervical adenocarcinomas are associated with papillomavirus DNA [29, 67].

Of the more than 80 HPV types known today, 23 infect the genital mucosa. These types differ in their transforming potential and are therefore subdivided into risk groups. "Low-risk" HPV types (HPVs 6, 11, 42, 43 and 44) are found in exophytic condylomata acuminata, flat condylomata and weak dysplasias, but not in invasive carcinomas (a rare example is HPV 6/11 in verrucous carcinomas), whereas "high-risk" types (HPVs 16, 18, 31, 33, 35, 39, 45, 50, 51, 53, 55, 56, 58, 59, 64 and 68) are detectable in samples from carcinomas and dysplasias in different ratios [4, 33]. Some HPV types of the latter group, i.e. HPVs 31, 33, 35, 51 and 52, were named "intermediate-risk" types by some authors [33] because of their lower prevalence in carcinomas than in mild or severe dysplastic lesions. Others, including ourselves, avoid this term because of the unclear implications for patients harbouring one of these types. Among the genital papillomaviruses, HPV 16 is the most frequent type in SCCs. More than 50% of these tumours worldwide harbour HPV16 DNA [4, 67]. In contrast, more than 50% of cervical adenocarcinomas are associated with HPV18 [39, 53]. The prevalence of the other HPV types vary with geographic regions.

Large epidemiological and prospective studies have shown that the risk of developing cervical cancer is strongly associated with the presence and persistence of "high-risk" genital papillomavirus types [59]. Additional factors which were suspected as risk factors for cervical carcinomas (chemical and hormonal influences, other viral or bacterial infections) did not prove significant in multivariate analysis [45, 59], except smoking, for which controversial data were obtained and which might act as a cofactor in the progression to high-grade lesions [20].

The common viral aetiology of cervical SCCs and their development from well-characterised precursor lesions make them an interesting model system of human carcinogenesis. More and more, the fundamental effects of the viral oncoproteins E6 and E7, which are crucial for malignant transformation, are unravelled [47]. According to our current knowledge, the natural history of cervical HPV infection and its consequences can be described as follows.

Asymptomatic/submorphological infection

HPV infection of cervical mucosal cells after sexual transmission generally occurs via microabrasions, pre-

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dominantly at the transformation zone. Basal epithelial cells are the target cells of HPV infection, with α6β4 integrin probably acting as a viral receptor [14]. However, viral replication and maturation proceeds during migration towards the mucosal surface and is tightly linked to full epithelial differentiation. In cases of latent infection, the virus is only replicated along with the cellular DNA. If the viruses undergo the full infectious cycle with the opportunity for sexual transmission to other people, only minimal or no morphological changes are observed at this stage. These transient infections disappear within some months in most cases, probably as a result of host immune response. Asymptomatic infections can be detected by sensitive molecular methods in 5–30% of randomly taken cervical scrapes, the likelihood of infection being dependent on factors associated with sexual behaviour [6, 33, 58, 59]. Studies with US college girls have shown that more than 60% undergo a transient HPV infection as detected by polymerase-chain reaction (PCR) within 3 years after diagnosis of HPV infection. Only 10% of these women develop cervical dysplasia [59]. However, in contrast to the high rate of HPV infection in the general population, cytological changes associated with cervical dysplasia [low-grade squamous intraepithelial lesions (LSIL, CIN I) cervical intraepithelial neoplasia] are found in only 2–3% of the women. The risk of developing a CIN increases with the length of persistence of HPV infection [21].

Low-grade squamous intraepithelial lesions

Low-grade squamous intraepithelial lesions (LSIL, CIN I) are characterised by increased proliferation of basal epithelial cells and clonal expansion, leading to the typical morphological changes with partial dedifferentiation and mild atypia. Koilocytic changes can occur, but are not obligatory. Mild dysplasias are associated with many different HPV types ("high-risk" and "low-risk"), with more than one HPV type found in about 30% of the cervical scrapes from these lesions [59] (K. Milde-Langosch, unpublished data). According to our observations, 60% of dysplasias harbour high-risk HPV types, pure low-risk infections are rare (less than 10%) and in about 30% no HPV DNA can be detected by consensus primer PCR [41]. The lesions are monoclonal or polyclonal [48], and the viral DNA is found in its episomal, circular form. Expression of the viral oncogenes E6/E7 is very weak or undetectable in these lesions, being suppressed by various viral and cellular factors [64]. The majority of mild dysplasias undergoes spontaneous regression in 3 years, only 15–25% showing progression to high-grade lesions (CIN III, HSIL; 59) within 2–4 years. The risk of progression is dependent on the HPV type, with HPV16 infections showing the highest rate of progression [19, 45]. Other risk factors, such as smoking, hormonal influences or immunological factors, might influence the outcome of mild dysplastic lesions [20, 21].

High-grade squamous intraepithelial lesions

High-grade squamous intraepithelial lesions (HSILs, CIN III) probably develop within mild dysplasias (LSILs) by clonal expansion of atypical cells with increasingly undifferentiated phenotype and high proliferative potential. According to another view, infection by some high-risk HPV types can directly lead to high-grade lesions, but the relevance of this mechanism is not proven unequivocally [27]. HSILs are monoclonal lesions that harbour high-risk HPV types in more than 90% of the cases. The main difference from LSILs is the high expression of the HPV oncogenes E6 and E7 [13]. This can be the result of HPV integration into the host genome leading to disruption of the viral E2 gene encoding a negative transcriptional regulator. However, changes in cellular transcriptional regulators, for example alterations of the AP-1 complex composition [62] or a reduced expression of the chemokine MCP-1 [28, 51, 56], might lead to upregulation of E6/E7 expression. In the host cells, normal cellular control mechanisms that prevent unregulated cell growth and division are disrupted by these oncoproteins in various ways:

- E6 binds to the p53 protein, leading to rapid degradation of this tumour suppressor. P53 is known as the "guardian of the genome", and loss of its function results in subsequent accumulation of genetic lesions [9, 47, 72].
- E7 binds to the retinoblastoma protein Rb, which displays a central function in normal cell cycle control. By binding to E7, the suppressive effect of Rb is abrogated, leading to accelerated cell cycle progression and proliferation [17].

Besides these important functions, viral oncogenes were shown to influence the host cell in additional ways:

- E6 proteins can enhance telomerase activity, leading to immortalisation of the infected cells [30, 54]. Bovine papillomavirus (BPV)-E6 interacts with Paxillin and contributes to disruption of the actin cytoskeleton [63].
- E7 trans-activates the expression of cyclin A and cyclin E, which represent important positive regulators of cell cycle progression [60, 65, 68]. In addition, it binds to the cdk inhibitor proteins p21/WAF1 and p27/Kip1, blocking their binding to cyclins and, in the case of p21, PCNA and disrupting normal cell cycle control [70]. Similar to Rb, the Rb-related protein p107 is also bound by E7, leading to release of E2F transcription factors from p107 in the G1 phase [69]. Furthermore, E7 binds to Mi2β, a member of the histone deacetylase complex which influences the histone–DNA interaction and the access of transcription factors [3], probably leading to deregulation of genes that govern the cell cycle.

As a result of these functions, E6/E7 expression confers a strong growth advantage to the host cells, leading to further proliferation and dedifferentiation. In vitro studies with cultured keratinocytes induced to over-

express the oncogenes E6 and E7 confirmed these functions. Morphological changes similar to precancerous alterations were produced and, under certain conditions, these cells formed malignant tumours after injection into mice [2]. The E6 and E7 proteins of different HPV types exert these effects with varying efficiencies: HPV 16 and 18 proteins are highly potent in these oncogenic functions, whereas HPV 6 and HPV 11 E6/E7 proteins are incapable of binding or inactivating to p53 or Rb [9, 17].

By the integration of HPV DNA into host chromosomes, the infection becomes irreversible. Indeed, spontaneous regression of high-grade lesions is rare, and probably 33–50% will eventually progress to invasive cancer if left untreated, although this process can require several years or even decades [59].

Invasive cancer

Cervical cancer cells are generally characterised by high E6/E7 expression, the disruption of cellular control mechanisms and accumulation of additional genetic defects, i.e. aneuploidy. HPV 16 DNA is integrated in approximately two-thirds of the SCCs [10], thus disrupting not only viral but also cellular genes at different chromosomal loci. In cases with episomal HPV 16 DNA, mutations of YY1 silencer elements within the regulatory region are frequently found. YY1 elements are binding sites for the transcription factor YY1, which is involved in the regulation of a large number of genes. As YY1 binding to HPV 16 and HPV 18 promoters leads to a reduction of E6/E7 expression, mutations in YY1 elements that abolish YY1 binding can greatly enhance E6/E7 oncogene expression [36]. HPV18 DNA, which is found in more than half of cervical adenocarcinomas, is always integrated [49].

Reduced expression of the cytokines interferon (IFN)γ, interleukin (IL)-10 and IL-12 leading to decreased local cellular immunity might facilitate progression [11]. In addition, allelic losses or gene amplifications at several chromosomal sites were found in various studies [31], suggesting functional loss of tumour suppressor genes or activation of proto-oncogenes located at these sites. In a subset of carcinomas, activation of the cellular oncogenes c-myc, erbB2, etc. was described [42]. Changed expression of cell cycle regulators, i.e. increased levels of the cyclin-dependent kinase cdk4 and decreased expression of the CDK inhibitor p27, are often found and might contribute to tumour progression. In addition, over-expression of the gene encoding the CDK-inhibitor p16 has been demonstrated in cervical cancer and HSILs infected with high-risk HPV types [57].

Practical aspects

How can our knowledge of the infection with HPV and its oncogenic potential influence diagnostic or therapeutic approaches? Prevention of papillomavirus infection itself would probably reduce cervical cancer to a minimum, but with a prevalence of HPV infection of greater than 50% in young, sexually active women [32], all attempts to eradicate HPV would certainly fail. As a viral infection, HPV-associated lesions are not accessible to directed therapy. Especially in high-grade lesions and carcinomas, where HPV DNA is mostly integrated, control of HPV infection is not promising and surgical measures are inevitable irrespective of the HPV status. Cellular immune response in CIN lesions is generally low, and immunotherapy protocols using stimulated lymphocytes are successful in experimental model systems and might be promising in the future [25]. Strong efforts are currently being made for development of prophylactic vaccines against high-risk HPV types [34]. If they are successful, the need for HPV testing in clinical practice will probably be eliminated.

Today, HPV tests might be useful in earlier stages of disease or as a screening method. The following indications are suggested.

HPV detection in cervical cancer screening

After the introduction of diagnostic cytology (Pap tests) as a screening test, the incidence of invasive cervical cancer has drastically dropped by 50–70% in women who attended screening programmes. A further reduction of cervical cancer deaths would be possible if all precursors, especially high-grade cervical intraepithelial lesions, were detected and subjected to therapy. The sensitivity of the Pap test was determined as approximately 70% [6, 15, 66]. Various studies have shown a high intra- and inter-observer variability in the interpretation of Pap tests leading to an unacceptable number of false-negative results [6, 66]. This problem is currently being addressed in two approaches: (1) optimisation of Pap tests by automatic devices, improving diagnostic performance and facilitating the detection of abnormal cells and (2) introduction of HPV tests into screening programmes for better identification of patients at risk, thus permitting longer screening intervals [51, 61].

Persistent infection with high-risk HPV types is a strong risk factor for the development of high-grade CIN and cervical cancer. However, there is a high rate of transient and asymptomatic HPV infections among young women [59]. Given the low incidence of cervical cancer, it may not be useful to apply HPV detection for cervical cancer screening in this age group. If each positive test in these women was followed by repeated cytological examination and colposcopy, it would lead to a huge number of superfluous diagnostic measures, high costs and mostly unnecessary frightening of the patients. Yet, HPV prevalence reaches its maximum (20–25%) in women 20–25 years old and decreases strongly with age (>30 years, 4.4%) [59]. Sexual behaviour and a changed immune response might be the reasons for these observations. In addition, persistence of HPV infection is more often observed in patients greater than 30 years old [18],

and the incidence of high-grade lesions increases with age to a maximum around 30 years. Consequently, the positive predictive value of high-risk HPV test results for the presence of HSIL or cervical cancer increases significantly after the age of 35 years, where they indicate underlying lesions in 70% of the cases. Therefore, combined routine cytology and HPV tests would be reasonable for cervical cancer screening in women older than 35 years. Whether this approach is realised depends on the readiness of the health systems to carry the additional costs for a more effective cervical cancer screening.

Combined PCR-based HPV tests and classical cytology are also used in the Dutch population-based screening system. For women with cytologically normal smears and a negative high-risk HPV test result, screening intervals were prolonged to 5 years in contrast to 1 year in most countries, and even 8-year intervals were proposed [37, 55], leading to a significant cost reduction. Only patients with high-risk HPV positive tests or suspicious Pap smears are referred to the gynaecologist in this model. Whether these long screening intervals are sufficient for an efficient cervical cancer prevention largely depends on the quality of the cervical smear and the sensitivity of the applied HPV test.

HPV test in cervical smears of uncertain diagnosis

A substantial proportion of all cervical smears does not provide clear morphological criteria of CIN lesions, but are nevertheless not “normal”. According to the Bethesda system, these cases are termed “atypical squamous cells of undetermined significance” (ASCUS, Pap3, Pap2w) or “atypical glandular cells of undetermined significance” (AGUS) [43]. These categories summarise benign reactive changes, morphological changes associated with non-HPV infections and inflammations and a subset of LSILs. The frequency of this diagnosis is highly dependent on the individual cytologist, and there is considerable uncertainty about the further management of these patients.

Follow-up studies have shown that a significant proportion of cervical cancers had previously repeated ASCUS smears. Therefore, some authors suggest that all ASCUS results should be followed by repeated cytological examination at 6-month intervals for 2 years without HPV testing [26]. But this approach does not solve the problem of unclear diagnosis, especially if it is done by the same cytologist. Obviously, there is a need for a second criterion which might help to identify those smears from patients with low-grade or even high-grade lesions (CIN I–III) and the patients at risk of developing a cervical dysplasia, and reduce the rate of false-negative results in cancer screening.

The HPV detection rate in ASCUS smears is 40–50%, with one-third harbouring high-risk HPV types. In these patients, the risk of developing a CIN lesion is 50- to 400-fold that associated with negative or low-risk HPV results. Therefore, tests for high-risk HPV DNA are suggested in women with ASCUS and AGUS cytology [8, 16, 39]. Positive results should lead to thorough colposcopic examination, biopsy and treatment, whereas patients with negative results do not carry a significant cancer risk.

HPV tests in LSILs (mild dysplasia, Pap3d)

Approximately 40% of Pap3d smears are either HPV negative or positive for low-risk HPV types [41]. Schiffman et al. [58, 59] proposed that high-risk HPV-positive ASCUS and LSIL patients should be regarded as one group harbouring a potential of progression to high-grade lesions. In contrast, high-risk HPV-negative smears should be regarded as benign histologic/cytologic changes. However, a diagnostic classification solely relying on the accuracy of the HPV test might have the same drawbacks as a purely cytological approach, i.e. false-negative results because of technical problems or difficulties in interpretation.

In patients with histologically defined mild dysplasias, HPV tests can give valuable information for estimation of the individual risk of progression. Patients with HPV-negative or low-risk positive dysplasias carry only a minimal risk, and therefore follow-up intervals can be longer than with high-risk HPV-infected lesions. But even the latter might regress spontaneously and can be managed by careful cytological and colposcopic control every 3 months unless progression is observed.

HPV test in follow-up during or after therapy

HSILs are precancerous lesions with a high risk of progression to invasive cancer and must be removed by conisation, loop excision or laser vaporisation, the choice of method being dependent on clinical aspects (size of the lesion, extension into the endocervix, children planning, age of the patient, etc.). The target of these therapies is not the HPV infection, but the histologically defined lesion. Nevertheless, HPV tests can be included in follow-up examinations if the HPV status before therapy is known. Our own experiences with follow-up after loop excision in 51 patients have shown that in HPV-positive dysplasias, the recurrence rate was only 3% if HPV tests during follow-up were negative. In contrast, 33% of the patients who showed positive HPV results after therapy developed recurrent disease within the next 2 years (unpublished results). In another study with 48 patients who received conisation because of CIN III lesions, 96% of the patients with recurrences, but none of the patients who remained disease-free, had persistent HPV infection [5]. Thus, a positive HPV test after therapy might be an early indicator of recurrence and small follow-up intervals should be chosen in these cases.

HPV tests in metastases of unknown primary tumour

Several studies have shown that in cervical SCCs, the HPV infection is also detectable in metastases and recur-

rences [22, 40]. In certain cases, HPV detection can therefore resolve the problem of an unknown or occult primary tumour. By RNA/RNA in situ hybridisation using HPV oncogene-specific probes, even small metastatic cell groups can be identified [52], making this method potentially useful for the detection of minimal residual disease.

HPV detection systems

When material from cervical lesions is sent to pathology, many clinicians expect not only a cytological or histological diagnosis, but also information about HPV infection and type. For clinical applications, HPV tests are only useful and valuable if the HPV detection system is highly sensitive and specific, and if false-positive and false-negative results can be largely excluded. Only two test systems fulfil this need at present: the PCR and the hybrid capture (HC) analysis. Other HPV detection methods are restricted to certain research applications (RNA in situ hybridisation), too complicated for routine purposes (Southern-blot hybridisation) or not sufficient in sensitivity and specificity (dot-blot hybridisation, filter in situ hybridisation, DNA in situ hybridisation). Yet, for research applications, in situ hybridisation using HPV-specific DNA or RNA probes on paraffin sections can be a valuable tool to study the exact localisation of the HPV infection or the expression of HPV oncogenes [52, 53].

In order to detect a broad spectrum of genital papillomavirus types, all laboratories performing HPV PCR use one of two general primer pairs: primers GP5+/GP6+, which span a region of 140–150 bp from the L1 open reading frame [12], or the degenerate primer pair MY09/MY11 [35], which gives rise to a PCR product of approximately 450 bp also from the L1 region. GP5+/GP6+ amplification products can be used for HPV typing using an enzyme immunoassay with different HPV type-specific oligonucleotides in order to detect 20 individual HPV types [23, 24]. MY09/MY11 amplicons can be further analysed by restriction fragment length polymorphism (RFLP) and hybridisation analysis [38] or by hybridisation with type-specific oligonucleotide probes [1]. Alternatively, HPV detection by general primer PCR can be followed by HPV typing with type-specific primer pairs. Although the MY09/MY11 system is slightly less sensitive than the GP5+/GP6+ system, it is currently used more frequently for HPV screening. Both systems work well and with similar efficiency in cervical scrapes [44]. In formalin-fixed archival material, primers that yield small amplification products work better because of the fragmentation of the DNA as a result of fixation in these samples. For this purpose, Kleter et al. [29] developed a PCR test with a target product size of only 65 bp, with only 25 bp between the primers. Although extremely sensitive, this system is not yet widely applied. The problem is that HPV will be detected in a wide range of normal cytological smears and therefore the results are not easy to interpret. The disadvantage of all PCR-based HPV tests is the high risk of false-positive results because of the possible amplification of contaminants. Therefore, these tests should only be performed in carefully controlled laboratories with experience in molecular biology.

The HC system (Abbot) was developed for routine HPV detection in cervical scrapes. It is the only Food and Drug Administration (FDA)-approved HPV test and should be used in combination with special collection kits containing sterile cotton swabs or cytobrushes and tubes with a medium suitable for storage and transport of cells and small biopsies. Instead of HPV typing, the system only allows discrimination of low-risk and high-risk HPV types by the use of parallel tests for these risk groups. The current HC test (HCII) is a liquid hybridisation system which uses RNA probe cocktails and microtiter plates which are coated with antibodies directed against DNA-RNA hybrids. After binding of the hybrids and removal of unbound nucleic acids, detection is performed with a second, alkaline phosphatase-coupled antibody and a substrate which emits chemiluminescence. The latter is quantified in a luminometer. The HC II system detects 18 anogenital HPV types with a sensitivity that is only slightly below that of the commonly used general-primer PCR techniques [7]. The test can easily be performed by laboratory staff who are not experienced in molecular biology, and the resultant diagnosis of the HPV risk group gives enough information to the clinician in most cases. The major drawbacks of the HC test are the impossibility of exact HPV typing and the high costs of the system.

Conclusions

Molecular and epidemiological data have largely increased our knowledge of the natural history of HPV infection. The recognition that papillomaviruses are the primary aetiological agent of virtually all cervical carcinomas has important implications for preventive medicine. HPV diagnosis with highly sensitive methods might improve screening and provide important information for diagnosis and evaluation of prognosis and improve the management of patients with cervical neoplasia.

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