Papillomaviruses in Anogenital Cancer as a Model to Understand the Role of Viruses in Human Cancers

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Abstract

Infections with specific types of human papillomaviruses (HPV) have emerged as necessary but not sufficient factors for the development, at least, of the majority of cervical, vulvar, penile, and perianal cancers. Evidence has accumulated for their causal role in the induction of anogenital premalignant lesions. Genetic events underlying the mechanism of anogenital carcinogenesis have become increasingly understood. A host cell-mediated intracellular control down-regulating specific HPV genes (E6, E7) in replicating normal cells appears to be interrupted in cancer cells, probably due to structural modifications of the respective host cell genes acquired in the course of HPV DNA persistence. Since genital HPV infections are ubiquitous, cofactors which modify controlling host cell genes are likely to determine the different geographic rates of cervical cancer incidence.

Prevalence of HPV Infections

It appears that HPV 16 is the most prevalent virus and thus far accounts for probably about 50% of all genital HPV infections (16). HPV infections are ubiquitous and have been found in all populations studied. From data in Germany at least 10–12% of sexually active adults are HPV positive (17). This is likely to represent a substantial underestimate since pregnant women are positive by about 30% (18) and repeated consecutive frame; URR, upstream regulatory region; CIF, cellular interfering factor.2 E-M. de Villiers, personal communication.

Historical Aspects

Papillomavirus research had a slow start; since the first demonstration of transmissibility of canine (1) and human (2) warts by cell-free extracts, decades elapsed before a role of one of these agents was established in the induction of malignant tumors in rabbits (3). It took an additional 40 years before the first experimental studies on a possible role of these viruses in cancer of the cervix were reported (4). In the 1970s advances became more rapid. The hypothesis was published that cancer of the cervix is caused by papillomavirus infection (5, 6), specific cytological changes in cervical smears were identified as papillomavirus specific (7), and the genetic heterogeneity of the HPV1 group was established (8–10). In 1979 specific HPV types were identified in a rare form of human carcinomas arising in patients with epidermodysplasia verruciformis at sunlight-exposed sites (11). One year later the first genital isolate (HPV 6) was characterized (12). This was soon followed by the description of additional types; HPV 11 (13), HPV 16 (14), HPV 18 (15), followed by approximately 15 additional types more recently (16). Today we know of about 60 distinct HPV types, 22 of which regularly or sporadically also infect the human genital tract.

Role of HPV in Precursor Lesions of Anogenital Cancer

Anogenital cancer develops within typical precursor lesions: cervical, penile, and vulvar intraepithelial neoplasias, the latter also designated in the past as Bowenoid papulosis (21). The first evidence for a role of a specific type of HPV (HPV 16) in the induction of these lesions resulted from the regular demonstration of HPV 16 DNA in 80% of biopsies from anogenital Bowen’s disease and Bowenoid papulosis (22), subsequently confirmed in numerous additional studies (23). Induction of similar proliferative changes in heterografted human tissues, successfully applied for the induction of condylomatous proliferations by HPV 11 infections (24), has not yet been reported for HPV 16 or other anogenital HPV isolates. It has been possible, however, to induce Bowenoid type changes by HPV 16 DNA transfection of human foreskin keratinocytes on a raft tissue culture system permitting differentiation of these cells in vitro (25). Moreover HPV 16 and HPV 18 DNA has been shown to effectively immortalize human foreskin and cervical keratinocytes in tissue culture (26–28). These cells change their growth characteristics, retain the HPV DNA in an integrated state, and express early functions of the viral genome. They become quickly aneuploid but fail to induce tumors on hetero-transplantation into nude mice. They clearly differ in this respect from cells derived from cervical carcinoma biopsies or cervical carcinoma cell lines.

The available data are in strong support of a causal role of HPV infections, particularly of HPV 16, at least for a large proportion of cervical, vulvar, and penile intraepithelial neoplasias. It is, however, presently not understood which molecular events lead to the development of these proliferations and what discriminates them from apparently symptom-free HPV carriers. Do the latter harbor as yet undetectable microlesions or do “surface” infections exist affecting only the superficial differentiating cells without touching the proliferating basal layer cells (21)? From in situ hybridization studies on HPV 16-positive cervical dysplasias, it became obvious that basal layer cells do contain HPV DNA (29). The regularity of the nuclear grain pattern in some of these samples even points to a monoclonal origin of these lesions (Fig. 1).

HPV in Anogenital Cancer: State and Expression of Viral DNA

An etiological role of specific HPV types for obligatory anogenital precancerous conditions instantly implies a role of
these viruses in the respective malignant tumors. Yet support for a direct role of certain HPV types in the induction of anogenital cancer remained circumstantial for a number of years. Only very recently have data accumulated which seem to permit a basic understanding of intracellular genetic events underlying the development of anogenital cancer. They point to a central role of an intracellular surveillance system in proliferating normal cells for persisting HPV DNA (20). Its breakdown, occurring in rare instances and probably mediated by a variety of mutagenic cofactors, appears to be a mandatory event for cancer development. Circumstantial evidence for a role of specific HPV types in anogenital cancer resulted from a number of observations.

The DNA of HPV types 16, 18, and 33 and less frequently of other types can be found in about 90% of cervical, vulvar, and penile cancer biopsies, if such investigations are carried out under standardized experimental conditions. HPV 16 is present in about 50% of these biopsies, HPV 18 in close to 20%, and HPV 33 may be seen come in up to 10%. The remaining positive biopsies contain several additional HPV types, respectively (21).

Most carcinomas analyzed thus far reveal a tumor-specific pattern of viral DNA integration into host cell chromosomes. This indicates monoclonality of the respective tumor. Integration in malignant tumors seems to contrast the situation of premalignant lesions containing predominantly the vegetative episomal form of viral DNA (30).

The integrational pattern reveals a remarkable specificity for the site of disruption within the circular viral DNA. This regularly occurs within the 3' end of the E1 ORF, or the 5' end of the adjacent E2 ORF (31–33) obviously disrupts an intragenomic viral regulation exerted by E2 functions on other early gene expressions as originally demonstrated for bovine papillomaviruses (34–36).

The persisting viral DNA is regularly transcribed in HPV-positive primary tumors as well as in HPV DNA-containing cell lines derived from cancer of the cervix (31, 37). The transcriptional pattern is specific, the E6-E7 ORFs are regularly expressed, and transcripts frequently extend into the flanking host cell sequences (31, 38).

Although viral DNA persistence, a specific mode of integration, and a specific transcriptional pattern strongly argue in support of a role of HPV in the maintenance of the malignant phenotype, these data are unable to prove the point. In view of the ubiquity of these infections and their high prevalence in sexually active adults, moreover, epidemiological studies thus far failed to provide supporting correlations.

Functional Analysis of the HPV Gene Expression in Cervical Cancer Cells

An alternative approach has been attempted in analyzing the biological functions of viral genes, particularly those expressed in malignant tumors, in modulating their expression in malignant cell lines and in studying their role in nonmalignant hybrid cells obtained after fusion of cervical carcinoma cells with normal human keratinocytes or fibroblasts. As will be discussed below, the available data permit the statements: that E6-E7 genes code for transforming functions; that expression of the same genes is the major determinant for the malignant phenotype of those cervical carcinoma tissue culture cells studied up to now; that HPV DNA-positive nonmalignant human cells possess a mechanism selectively down-regulating HPV transcription after heterografting these cells into nude mice or after *in vitro* treatment with 5-azacytidine; that this mechanism is not detectable in cervical carcinoma cell lines; and that this cell-mediated regulation involves the URR of the E6-E7 ORFs.

As previously outlined, HPV 16 and 18 DNA immortalizes human cervical and foreskin keratinocytes (26–28). As shown earlier for bovine papillomaviruses (39), HPV 16 DNA also malignant transforms NIH 3T3 cells (40) and cooperates with ras genes in transforming primary rat kidney cells to a malignant phenotype (41). Recently, the causation of these changes by E6-E7 gene expression has been convincingly demonstrated (42, 43). The role of E6-E7 gene expression for the proliferation of cervical carcinoma cells *in vitro* and for the maintenance of the malignant phenotype has been analyzed by various approaches; von Knebel-Doebert et al. (44) used HPV 18 antisense DNA constructs, also carrying a selectable marker, under the control of the hormone-dependent mouse mammary tumor virus long terminal repeat. After transfection of HPV 18-positive C4-I cells, clones were isolated which revealed dexe-
methasone-dependent induction of the antisense message. It was shown under these conditions that E7 protein synthesis was diminished, and correspondingly cell growth was significantly reduced. Control C4-I cells in contrast were growth stimulated by dexamethasone treatment. Here the hormone led to increased transcription of the endogenous HPV 18 E6-E7 DNA resulting from the stimulation of a hormone-inducible element within the upstream regulatory region of HPV 18 DNA.1

Harris and Klein (45) demonstrated about two decades ago that fusion of malignant cells with normal cells yields hybrids which regularly lost the malignant phenotype. Stanbridge (46) analyzed this negative control of the malignant phenotype by fusing cells of the HPV 18-positive HeLa line, established almost 40 years ago from an adenocarcinoma of the cervix (47), with human fibroblasts or keratinocytes. The resulting nonmalignant hybrids were relatively stable. Only occasionally and after prolonged cultivation could malignant segregants be isolated. This was attributed to the loss of chromosome 11 originating from the normal fusion partner (48).

Our group carried out experiments to analyze HPV 18 DNA transcription in these nonmalignant HeLa-fibroblast or HeLa-keratinocyte hybrids as well as in their malignant segregants. Although the in vitro rate of HPV transcription did not differ significantly in nonmalignant hybrids and their malignant segregants from that of the parental HeLa line (49), heterografting of those cells into diffusion chambers implanted on a collagen matrix into nude mice (50) changed the pattern. By using in situ hybridizations,4 significant reduction in HPV transcription occurred within 6 days selectively within the nonmalignant hybrids. In contrast, human vimentin gene expression continued under the same conditions at approximately the same level in the nonmalignant hybrids and their malignant segregants demonstrating survival of the implanted cells. These data indicate that HPV transcription is regulated differently in nonmalignant hybrids kept under in vitro or in vivo conditions. They imply the selective down-regulation of HPV transcription in vivo, possibly triggered by a humoral factor (20).

Corresponding observations were made under in vitro conditions if the same cells had been exposed to 5-azacytidine treatment (49). This demethylating compound leads to a selective rapid switch-off of HPV 18 transcription in nonmalignant hybrids and of HPV 16 transcripts in in vitro immortalized human keratinocytes. Cells from different cervical carcinoma lines and malignant segregants remain unaffected. The down-regulation occurs at the level of transcriptional initiation and is accompanied by a rapid growth inhibition. The effect is reversible by cycloheximide treatment, pointing to a protein factor involved in its induction. The latter should be contributed by activated cellular genes originating from the normal fusion partner.

Model of Virus-Host Cell Interaction in Normal and Malignant Cells

These observations imply in molecular terms an as yet unspecified effect of host cell proteins inducible in normal cells, affecting the upstream regulatory region (URR) of integrated HPV DNA.

The interpretation of the combined data is shown in Fig. 2. According to this model, the basic features of which had been outlined before (20, 51), cellular genes down-regulate HPV transcription in proliferating cells. In view of the present lack of knowledge on their mode of interaction they are designated as cellular interfering factors (CIFs). CIF genes appear to be activated after transplantation of human cells into animal hosts by a putative humoral mechanism. Similarly, treatment of tissue culture cells by 5-azacytidine may lead to their induction. They seem to be functionally inactive in cervical carcinoma cells where they should be structurally modified. In vitro immortalization is interpreted as the consequence of uncontrolled viral genetic activity resulting from a reversible inactivation of CIF genes.

This concept provides a convenient explanation for epidemiological features of cervical cancer development and may possibly also contribute to the understanding of other virus-linked cancers in humans (20). The rarity of at least three events occurring within the same cell (uptake of viral DNA, inactivation of the first copy of a CIF gene and subsequently of its allelic counterpart) readily explains the relatively low number of infected individuals developing anogenital cancer and the long latency period between primary infection and tumor appearance. Position effects due to viral DNA integration may play an additional role. Monoclonality of the arising tumor can be predicted. The concept also stresses the important role of mutagenic cofactors which should substantially elevate the risk for modifications within the CIF genes (52). It seems likely that geographic differences in anogenital cancer incidence reflect different exposures to such factors rather than variations in the (ubiquitous) HPV infections. On the other hand, hormonal factors (progesterone) may contribute to an increased HPV production (18), to enhanced genetic activity of persisting viral DNA (53), and to a higher number of genome-carrying cells (54). This may also elevate the risk for cancer development at a later stage. A low increase in the relative risk for cancer of the cervix has been reported for long-term oral contraceptive users (reviewed in Ref. 55).

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1 U. Bernhard, personal communication.
2 E. Schwarz, F. Bosch, and H. zur Hausen, unpublished data.
Conclusions

Based on the described experimental data it is hard to escape the conclusion that HPV infection is a causative factor for anogenital cancer, although classical criteria (Koch's postulates) appear to be barely applicable (56).

At present, infections with specific HPV types probably permit the best experimental documentation of a viral etiological involvement in a widespread human cancer. In addition, they reveal new insights into intracellular surveillance mechanisms against potentially deleterious effects of persisting viral genomes. These considerations should not distract us from the fact that major questions still remain unresolved. They concern epidemiological aspects, the mode of transmission and genome persistence, the humoral and cell-mediated immune response to HPV infections, the intracellular interactions of HPV-coded transforming proteins, the pathogenesis of premalignant lesions, the characterization of cellular genes down-regulating transforming proteins, the pathogenesis of premalignant lesions, the characterization of cellular genes down-regulating HPV transcription, the identification of cofactors interacting with HPV-infected cells in anogenital carcinogenesis, possible modes of prevention and therapy of HPV infections, and many others.

HPV-related research obviously requires increasing attention. Part of it needs to be focused on the possible involvement of specific HPV types in nongenital human cancers. Carcinomas developing in the ororespiratory and digestive tract as well as in the bladder may be of particular interest. The small number of positive biopsies reported thus far, specifically from ororespiratory sites and the skin, containing defined HPV genotypes (see review, Ref. 16) should stimulate intensified research in this remarkable group of human pathologies.

References


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