Role of the Human Papillomaviruses in Human Cancer

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Abstract

The papillomaviruses associated with human anogenital carcinomas encode two transforming proteins, E6 and E7. The oncoprotein products of these two genes complex with the tumor suppressor gene products p53 and pRB, respectively. The loss of the normal function of these tumor suppressor gene products, either as a consequence of their association with E6 and E7 or by mutation, appears to be a common event in human cervical carcinogenesis.

Compelling evidence now associates specific human papillomaviruses with certain human anogenital cancers, most notably cervical cancer. Numerous epidemiological studies have implicated a venereally transmitted agent in human cervical carcinoma (1, 2). Sexual transmission of an agent with prolonged latency of perhaps 20 to 25 years is suggested by these epidemiological studies. The suggestion of an infectious agent in the etiology of cervical cancer has led investigators to evaluate genital pathogenic organisms as potential candidate factors. Approximately 20 years ago, HSV type 2 was considered a potential etiological factor (3, 4). Molecular biological studies, however, failed to provide any convincing evidence for the presence of HSV nucleic acids in cervical cancers (5). A prospective epidemiological study carried out by Vonka et al. (6, 7) failed to support an involvement of HSV-2 infection as a principal etiological factor in cervical cancer or in preneoplastic lesions. At this point, however, it is not possible to totally exclude HSV as a potential cofactor perhaps working in concert with the HPVs.

The first concrete evidence suggesting a role of HPV infection with cervical cancer was the recognition that morphological abnormalities that constitute cervical dysplasia (also referred to as CIN) were the cytopathic effects of a papillomavirus infection (8–10). Further support for a papillomavirus etiology in cervical dysplasia came from the demonstration of papillomavirus capsid proteins in the nuclei of some cells in approximately 50% of cases of cervical dysplasia examined (11, 12). Initial experiments examining for the presence of HPV DNA sequences in cervical cancers were generally negative. In retrospect, however, these negative data can be explained by the marked plurality of HPV types which are now recognized (13) and the fact that the papillomavirus DNA probes used at that time were not appropriate. The recognition, however, that cervical dysplasia (CIN) was a papillomavirus infection of the cervix, which is accepted as a preneoplastic lesion which may in some cases proceed to cervical cancer, inspired a search of cervical cancers for the evidence of HPV DNA.

There are approximately 65 different types of HPVs which have now been described (13). Approximately 20 of these HPV's are associated with anogenital lesions, and these HPVs can be further classified as either “high risk” or “low risk” based on whether or not the genital tract lesions with which these HPVs are associated have a risk for malignant progression. The “low risk” viruses such as HPV-6 and HPV-11 are associated with venereal warts or condyloma acuminata and these rarely progress to malignancy. In contrast, the “high risk” viruses such as HPV-16 and 18 are associated with CIN, which are at risk for progression to malignancy. It was the laboratory of Harald zur Hausen that initially cloned HPV-16 and HPV-18 DNA's from cervical carcinoma tissues (14, 15) and, using these two DNA's as probes, were able to demonstrate that approximately 70% of the human cervical carcinomas contain DNA of these HPV types (16). HPV-33 was subsequently cloned and appears to be present in a subset of invasive cervical carcinomas (17), and other specific HPVs now are recognized as part of this “high risk” HPV group. In general, HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, and 56 are found in cases of moderate and severe dysplasia and invasive cervical carcinomas and therefore constitute the “high risk” types (18).

A recent study has demonstrated that approximately 84% of cervical carcinomas contained a “high risk” HPV DNA (19). Studies of cervical cancers and of cervical cancer cell lines that are HPV positive have shown that the DNA is usually found to be integrated (16) although there are some cases where the DNA is apparently extrachromosomal. In those cases where the DNA is integrated, the pattern of integration is clonal indicating that the association of the HPV precedes the clonal outgrowth of the tumor.

In contrast to the integrated state of the HPV DNA that is generally found in cervical cancers, the viral DNA is usually extrachromosomal in the premalignant CIN lesions (20). Integration appears to be a random event as far as the site of integration in the host chromosome is concerned since the viral genome can be found in different locations in different cancers and cell lines. In some cell lines, however, the integration has occurred in the vicinity of known oncogenes. For instance in the HeLa cell line (which is a HPV-18 positive cervical carcinoma cell line) the integration of the viral genome has occurred within approximately 50 kilobases of the c-myc locus on human chromosome 8 (21). Whether such an integration event provides a selective advantage to the progression of a preneoplastic lesion to a cancer is currently unknown. In those cancers or derived cell lines in which the number of integrated viral genomes is low enough to permit a detailed analysis, the integration pattern reveals a remarkable specificity with respect to where the double-stranded circular viral genome is opened and disrupted as a consequence of the integration event. Integration generally occurs in the E1/E2 region of the viral genome (22, 23) disrupting the E2 viral transcriptional regulatory circuit.

The E2 open reading frame encodes a transcriptional regulatory protein, which is a DNA-binding protein. Products of the E2 open reading frame have been most extensively studied in the bovine papillomavirus and its products have been shown to have both positive and negative acting transcriptional regulatory functions (24). For HPV-16 and HPV-18, E2 appears to act principally as a repressor of the promoter from which the E6 and E7 genes are transcribed (25–27). The HPV genomes in cervical cancers and in derived cell lines are transcriptionally active, and the patterns of viral RNA expression are specific.


2 The abbreviations used are: HSV, herpes simplex virus; HPV, human papillomavirus; CIN, cervical intraepithelial neoplasia.
with regular expression of the E6 and E7 genes (28–30). Thus, it has been proposed that integration of the viral genome in cervical cancers, with the disruption of the E2 gene, provides a selective advantage leading to the uncontrolled proliferation of the cell due to the deregulated expression of the E6 and E7 genes.

Several studies have indicated that expression of E6 and E7 contributes to the proliferative growth phenotype of cervical carcinoma cells. Antisense experiments targeted to the E6 and E7 genes suggest a requirement for their expression for the proliferation of C4-1 cells, an HPV-18-positive cervical carcinoma cell line (31). Furthermore, the addition of the bovine papillomavirus BPV-1 E2 gene product to HeLa cells prevents their continued cell proliferation, and it has been proposed that this occurs through the transcriptional repression of the E6 and E7 genes (25).

A variety of studies have indicated that the E6 and E7 genes of the “high risk” papillomaviruses, such as HPV-16 and -18, are transforming genes. These genes contrast with the genes of the “low risk” HPVs, such as HPV-6 and 11, where the gene products are either nontransforming or very weakly transforming. The E6 and E7 genes of the “high risk” HPVs together are capable of readily immortalizing primary human keratinocytes (32, 33) whereas these same gene products from the “low risk” viruses are incapable of efficiently immortalizing primary human keratinocytes (34).

The E7 gene product of HPV-16 has been best studied. It is a small protein of only 98 amino acids. It migrates aberrantly with a slow mobility in polyacrylamide gels and has been shown to be a zinc-binding phosphoprotein, phosphorylated on serines by casein kinase II (35–37). E7 is expressed in benign cervical dysplasias, in cervical carcinomas, and in derived cell lines (30). E7 has adenovirus E1A-like transcriptional modulatory and transformation properties (38). Like adenovirus E1A, it is capable of transactivating the adenovirus E2 promoter (38). This transactivation property is perhaps most analogous to that of the 125 E1A product, rather than the 135 E1A product, in that E7 is not capable of transactivating the adenovirus E3 promoter or the adenovirus E4 promoter.2 E7 is a viral oncoprotein. It is sufficient for the transformation of established rodent cells such as NIH 3T3 cells (38–42). It is capable of cooperating with an activated ras oncogene to fully transform baby rat kidney cells or rat embryo fibroblasts (38, 43). Finally, as a viral oncoprotein it is able to cooperate with the E6 gene of the “high risk” HPVs for efficient immortalization of the primary human keratinocytes (32, 33) and human diploid fibroblasts (44). These similarities in function prompted our examination of the sequence of E7 and comparison with the amino acid sequence of adenovirus E1A. Striking similarities in the sequence exist between the amino-terminal 37 amino acids with 2 separated domains of adenovirus E1A (12).

An emerging theme among DNA tumor viruses (including the HPVs) is that the viral encoded oncoproteins interact specifically with critical cellular regulatory proteins. It is thought that the oncogenic effects of these viruses are at least in part a consequence of the specific interactions. The E7 proteins encoded by the oncogenic genital HPVs, adenovirus E1A, and the SV40 large T-antigen all localize to the nucleus and share certain characteristics. Each can extend the life span of primary cells, cooperate with other cytoplasmic oncoproteins such as ras to fully transform primary rat cells, induce DNA synthesis and growth-arrested cells, transform established rodent cells, and modulate transcription from certain promoters (reviewed in Ref. 45). The regions of amino acid sequence similarity between E7 and adenovirus E1A are also shared with SV40 large T-antigen (12, 46). These conserved regions have been shown to participate in the binding of a number of important cellular regulatory proteins, including the product of the retinoblastoma tumor suppressor gene, pRB (47–50). It seems likely that the comparable biological properties of these different oncoproteins may derive from their ability to target a similar set of regulatory proteins. The amino-terminal domain of the E7 proteins is highly conserved among the “high risk” HPVs and the “low risk” HPVs. The E7 proteins, of all of these genital tract HPVs, are capable of forming a complex with pRB, although there is a significant difference in the affinity of these complexes (50). The E7 proteins from the “low risk” HPVs types 6 and 11 associate with pRB with an approximately 10-fold lower affinity than do the E7 proteins of the “high risk” HPV types 16 and 18. Complex formation between the E7 proteins and pRB requires the integrity of the region similar to conserved region 2 of adenovirus E1A (50–52).

The transforming properties of the E6 protein were first revealed in studies using primary human cells, most notably primary human squamous epithelial cells (32, 33). Efficient immortalization of primary human keratinocytes for HPV-16 and -18 requires both E6 and E7 (32, 33, 53); the low frequency of primary human keratinocytes by E7 alone has been noted in the literature (54) and presumably other cellular genetic events are required for this event to occur. The ability of the E6 and E7 proteins together to efficiently immortalize primary human keratinocytes is a property of the “high risk” human papillomaviruses but not of the “low risk” viruses (34, 55). It was this transformation property of the E6 proteins of the “high risk” viruses that prompted our studies to investigate whether E6 targeted p53, similar to the SV40 large T-antigen and the Elb protein encoded by adenovirus 5. An association of the E6 proteins of the “high risk” HPV types, but not of the “low risk” HPV types with p53, was demonstrated using E6 and human p53 made in vitro using programmed rabbit reticulocyte lysates (56). In SV40-transformed cells and in adenovirus 5-transformed cells, the level of p53 cellular protein is increased because of an increased half-life in the protein (57, 58). It is clear, however, that although the oncoproteins of these different DNA tumor viruses all appear to target p53, the consequence of the protein-protein interaction is likely to be quite different. In contrast to the increased half-life of p53 seen in SV40 and Ad 5-transformed cells, HPV containing cervical carcinomas and HPV-transformed cells generally contain very low levels of p53.4 For example, in the HeLa cell line, which is an HPV-18-positive human cervical carcinoma cell line, p53 has been reported to be undetectable despite the presence of translatable mRNA (59). We therefore carried out a series of studies designed to examine the consequence of the interaction of the E6 oncoprotein of the “high risk” HPVs with p53 and were able to demonstrate that the E6 oncoprotein encoded by the oncopogenic HPVs that bind p53 stimulate the degradation of p53 in rabbit reticulocyte lysates (60). The E6-promoted degradation of p53 is ATP dependent and involves the ubiquitin protease system (60). The selective degradation of cellular proteins such as p53 with cellular regulatory functions by E6 is a novel mechanism of action for dominant-acting oncoproteins.

The demonstration of the transforming properties of the E6 and E7 oncoproteins of the “high risk” HPVs supports a role

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4 M. Scheffner and P. M. Howley, unpublished observation.
for these viruses in carcinogenic progression. The E6 and E7 genes are expressed in HPV-associated cancers. The papillomaviruses clearly exert some of their proliferative and oncogenic effects through the interactions of these oncoproteins with key cellular proteins having regulatory properties. In a benign HPV infection, the targeting of these tumor suppressor gene products by E6 and E7 must provide a growth advantage to the virus, possibly by expanding the pool of cells to make viral particles by inducing their proliferation, or by providing an appropriate intracellular milieu for efficient vegetative viral DNA replication. In a cancer associated with these HPVs, the expression of these viral genes becomes deregulated and their continued expression leads to the uncontrolled cellular proliferation. As with the other small DNA tumor viruses, it seems likely that expression of the viral oncoproteins of the HPVs may lead to aneuploidy and contribute to the other cellular events necessary for a full cancer. Other cytogenetic studies have implicated genetic changes in the short arm of human chromosome 3p as being associated with carcinogenic progression in HPV-positive cervical cancers (61). We have recently turned our attention to those cervical carcinomas which do not contain HPV. As noted above, approximately 15% of human cervical cancers are negative for HPV DNA sequences (19). In addition, two cervical carcinoma cell lines are negative for HPV DNA, the C33 cell line and the HT3 cell line (62). If indeed the p53 and the retinoblastoma tumor suppressor genes are important targets for carcinogenic progression by the human papillomaviruses in cervical cancer, then one might hypothesize that these genes would be mutated in the HPV-negative cancers. We have examined a total of seven cervical carcinoma cell lines, five of which contain human papillomaviruses and the two HPV-negative cell lines to test this hypothesis. In each of the HPV-negative lines, we have recently demonstrated that indeed p53 and pRB are mutated.5

Acknowledgments

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References


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