# REVIEW

# Papillomaviruses Causing Cancer: Evasion From Host-Cell Control in Early Events in Carcinogenesis

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**During the past 20 years, several types of human papillomaviruses (HPVs) have been identified that cause specific types of cancers. The etiology of cancer of the cervix has been linked to several types of HPV, with a high preponderance of HPV16. The role of these virus infections has been established 1) by the regular presence of HPV DNA in the respective tumor biopsy specimens, 2) by the demonstration of viral oncogene expression (E6 and E7) in tumor material, 3) by the identification of transforming properties of these genes, 4) by the requirement for E6 and E7 expression for maintaining the malignant phenotype of cervical carcinoma cell lines, 5) by the interaction of viral oncoproteins with growth-regulating host-cell proteins, and 6) by epidemiologic studies pointing to these HPV infections as the major risk factor for cervical cancer development. In addition to cancer of the cervix, a major proportion of anal, perianal, vulvar, and penile cancers appears to be linked to the same HPV infections. In addition, close to 20% of oropharyngeal cancers contain DNA from the same types of HPV. Recent evidence also points to a possible role of other HPV infections in squamous cell carcinomas of the skin. This review covers recent developments in understanding molecular mechanisms of HPV carcinogenesis, mainly discussing functions of viral oncoproteins and the regulation of viral oncogenes by host-cell factors. Modifications in host-cell genes, most likely engaged in the control of HPV gene expression in proliferating cells, emerge as important events in HPV-mediated carcinogenesis. [J Natl Cancer Inst 2000;92:690–8]**

## Introduction

Human papillomaviruses (HPVs) reveal a remarkable plurality of different genotypes. Until now, 85 HPV types have been identified and fully sequenced; more than 120 putative novel types have been partially characterized [*(1);* de Villiers EM: personal communication]. Thus far, all identified types appear to be strictly epitheliotropic: They infect epithelial cells either of the skin or of the anogenital and oropharyngeal mucosa. No evidence for HPV infections has yet been found in the gastric, ileojejunal, or colon mucosa.

The reasons for the enormous diversity of types as well as for the restriction of viral propagation to external skin or specific internal mucosal sites are presently unknown. The possibility exists, however, that both of these observations are interrelated: The replication of viral DNA and the subsequent particle formation in external differentiated epithelial cells of skin, anogenital, and oral mucosa may expose these infections less to immunologic interference. The absence of immunologic interactions could be the major reason for reduced evolutionary restraints, permitting the adaptation of new HPV variants to different types of differentiated epithelia *(2).* We currently do not know the basis for the apparent absence of HPV infections in internal organs and within the gastrointestinal epithelia, although the latter, in particular, must be frequently exposed to papillomavirus particles because of the consumption of contaminated food.

Structural properties of these viruses and viral gene functions have been reviewed repeatedly *(3,4)* and will not be discussed in detail here. It is, however, of interest to recall the different biologic activities of individual HPV types. Only a limited number of the approximately 40 types infecting the anogenital tract is found in anogenital cancer biopsy specimens. Specifically, these types possess cell-immortalizing and cell-transforming properties. In addition, close to 20% of oropharyngeal cancers contain DNA from the same types of HPV. The main representatives are HPV types 16, 18, 31, 33, 39, 45, 52, 58, and 69. They can be considered as high-risk types, while HPV types only rarely detected in malignant lesions, such as the two main types found in genital warts (condylomata acuminata), HPV6 and HPV11, are considered as low-risk types *(5).* In recent years, functional differences between oncoproteins of these virus subgroups have been intensively studied and provided some clues for the molecular mechanisms of HPV-induced cell immortalization and carcinogenesis. The availability of different types of infection within the same virus family represents one reason that HPV infections permit, at present, probably the most advanced insight into mechanisms of virus-induced carcinogenesis.

## Functions of Viral Oncoproteins and Their Interactions With Cellular Proteins

HPV genomes code for at least six different early and two late proteins. The structure of the genome and characteristic properties of individual viral proteins have been reviewed previously *(3).* High-risk HPVs code for at least three proteins with growth-stimulating and transforming properties (E5, E6, and E7).

### E5 Protein

The E5 protein is expressed in productive infections and may fulfill a function in the early expansion of an infected cell clone. The open reading frame coding for E5 is frequently deleted in cervical carcinoma cells *(6),* indicating the absence of an essential role of this gene in maintaining the malignant phenotype of cervical cancer cells. E5 represents a hydrophobic protein, pref-

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erentially found in the Golgi apparatus and in the plasma membrane *(7)*. It complexes with a variety of other transmembrane proteins, like epidermal growth factor receptor, platelet-derived growth factor β receptor, and colony-stimulating factor-1 receptor *(8)*, as studied particularly in cells infected by bovine papillomaviruses. E5 also binds to proteins of the gap–junction complex, such as the membrane-bound protein adenosine triphosphatase (ATPase) *(9,10)*. The HPV16 E5 protein possesses weak transforming activity and induces a protein kinase C (PKC)-mediated and, in addition, a receptor tyrosine kinase-mediated, PKC-independent, activation of membrane-associated protein kinases *(11,12)*. Its transient induction in mouse 3T3 cells or immortalized human keratinocytes results in suppression of the cyclin-dependent kinase inhibitor $p21^{CIP1}$ and in the induction of c-jun expression *(13)*. However, at present, the contribution of E5 to naturally occurring infections is still poorly understood.

## E6 Protein

More data are available relating to the function of the two other oncoproteins, E6 and E7. Their binding properties and functional characteristics are shown in Tables 1 and 2, respectively. Both proteins are consistently expressed in HPV-carrying anogenital malignant tumors *(6)* and are able to immortalize a wide variety of human cell types cooperatively *(14,15)*. Of interest, even the individual genes may immortalize human cells, such as mammary epithelial cells or different types of human keratinocytes [reviewed in *(16)*].

A number of interactions have been reported between high-risk E6 and host-cell proteins that provide some clues for the understanding of viral oncoprotein functions. The initial observation of E6 binding to the cellular protein p53 *(17)* mediated by the E6-associated protein (E6-AP) protein ligase *(18)* turned out to be particularly important. It emerges as one of the prime causes for chromosomal instability of high-risk HPV-containing cells *(19–21)*, with resulting mutational consequences for HPV-positive cells. The presence of E6 also enhances the integration of foreign DNA into the host-cell genome *(22)*. Elimination of p53 is, however, not a requirement for immortalization by E6 *(23)*.

Other consequences are presently emerging from the E6/E6-AP interaction: Potentially important is the recent observation of E6-AP-mediated ubiquitination and degradation of the src family tyrosine kinase Blk *(24)*. It is conceivable that the presence of E6 partially blocks this degradation and thereby stabilizes the respective kinase and stimulates mitotic activity *(24)*. This could explain, in part, growth-stimulatory functions of the E6 protein of high-risk HPVs. In addition, however, E6 reveals a remarkable pleiotropism in binding further host-cell proteins: It interacts with the calcium-binding protein ERC 55 *(25)*, with the focal adhesion protein paxillin *(26,27)*, with the human homologue of the *Drosophila* discs large tumor suppressor protein *(28)*, and with a novel putative GAP protein E6TP1 *(29)*. These interactions may lead to substantial functional consequences for E6-expressing cells, although they are, at present, not fully understood. This underlines the multifunctionality of the E6 protein. E6 also binds to the interferon regulatory factor 3 and inhibits the induction of interferon β messenger RNA (mRNA) following Sendai virus infection *(30)*. This inhibition is not mediated by ubiquitination or degradation.

An interesting observation has been initially reported by Reznikoff et al. *(31)* revealing the regular absence of the $p16^{INK4}$ protein in uroepithelial cells immortalized by HPV16 E6. This has also been found in cervical keratinocytes immortalized by infection with amphotropic retroviruses expressing the E6 gene

**Table 1.** Binding of cellular proteins by the high-risk human papillomavirus oncoproteins E6 and E7

| Viral oncoprotein | Cellular-binding protein | Investigator(s), y (reference No.) |
|---|---|---|
| E6 | p53 | Werness et al., 1990 *(17)* |
| | E6-associated protein | Scheffner et al., 1993 *(18)* |
| | ERC55 | Chen et al., 1995 *(25)* |
| | hDLG | Kiyono et al., 1997 *(28)* |
| | Paxillin | Tong and Howley, 1997 *(26)*<br>Vande Pol et al., 1998 *(27)* |
| | Interferon regulatory factor 3 | Ronco et al., 1998 *(30)* |
| | Bak | Thomas and Banks, 1999 *(35)* |
| | E6TP1 | Gao et al., 1999 *(29)* |
| E7 | Retinoblastoma protein (Rb) | Dyson et al., 1989 *(39)* |
| | Rb-related pocket proteins | Dyson et al., 1992 *(40)* |
| | E2F/cyclin A complex | Arroyo et al., 1993 *(51)* |
| | Histone H1 kinase | Davies et al., 1993 *(46)* |
| | TATA box-binding protein | Massimi et al., 1996 *(68)* |
| | Cyclin E | McIntyre et al., 1996 *(50)* |
| | Subunit 4 (S4) adenosine triphosphatase | Berezutskaya and Bagche, 1997 *(49)* |
| | c-jun | Nead et al., 1998 *(54)* |
| | hTid-1 | Schilling et al., 1998 *(48)* |
| | Mi2 (histone deacetylase complex) | Brehm et al., 1999 *(45)* |
| | M2-pyruvate kinase | Zwerschke et al., 1999 *(47)* |
| | p48 | Barnard and McMillan, 1999 *(64)* |

**Table 2.** Identified functions of the high-risk human papillomavirus oncoproteins E6 and E7

| Viral oncoprotein | Identified function | Investigator(s), y (reference No.) |
|---|---|---|
| E6 | • Cell immortalization | Band et al., 1990 *(122)* |
| | • Binding of E6-associated protein results in degradation of specific host cell proteins (p53) | Werness et al., 1990 *(17)*, Scheffner et al., 1993 *(18)* |
| | • Antiapoptotic effect | Werness et al., 1990 *(17)*, Thomas and Banks, 1998 *(34)* |
| | • Chromosomal destabilization | White et al., 1994 *(19)* |
| | • Enhancement of foreign DNA integration and mutagenicity | Kessis et al., 1996 *(22)*, Havre et al., 1995 *(21)* |
| | • Activation of telomerase | Klingelhutz et al., 1996 *(36)* |
| | • Blockade of interferon functions (?) | Ronco et al., 1998 *(30)* |
| E7 | • Cell immortalization | Münger and Phelps, 1993 *(121)* |
| | • Activation of cyclins E and A | Arroyo et al., 1993 *(51)*, Zerfass et al., 1995 |
| | • Inactivation of retinoblastoma protein-related pocket proteins | Dyson et al., 1989, 1992 *(39,40)* |
| | • Induction of apoptosis | Puthenveettil et al., 1996 *(59)* |
| | • Inhibition of cyclin-dependent kinase inhibitors | Jones et al., 1997 *(57)*, Funk et al., 1997 *(56)* |
| | • Enhancement of foreign DNA integration and mutagenicity | Kessis et al., 1996 *(22)*, Reznikoff et al., 1996 *(31)* |
| | • Degradation of tyrosine kinase Blk (?) | Oda et al., 1999 *(24)* |

(Whitaker N, zur Hausen H: unpublished data). These data suggest a counteracting function of $p16^{INK4}$ to E6. In high-risk HPV infections, E6/E7 protein-synthesizing cells even reveal an increase in $p16^{INK4}$ production *(32)*, pointing to an E7 property overriding the inhibitory function of $p16^{INK4}$. In this case, cyclin D–cdk4 complexes are disrupted and replaced by cyclin D–$p16^{INK4}$ complexes *(33)*.

Two additional functions of E6 obviously influence its role as oncoprotein: Its antiapoptotic effect could be initially deduced from the E6-mediated degradation of p53 *(17)*. E6 also mediates the degradation of another proapoptotic protein, Bak, a member of the Bcl-2 family *(34,35)*. Furthermore, E6 activates host-cell telomerase *(36)*. This might be associated with a loss of alleles in chromosomes 3p and 10p and thus represents a late event in the steps toward immortalization *(37)*.

It appears, at present, that the two most prominent functions of E6 can be summarized as follows: because of its mutagenic and antiapoptotic effect, E6 (in high-risk HPV-genome-carrying cells) appears to act as an important factor in tumor progression, acting through an endogenous pathway. Because of its binding to p53, it overcomes the $G_1$/S checkpoint control in DNA-damaged cells. In addition, via different pathways, expression of the E6 oncoprotein may lead to immortalization of specific human cells. Immortalization by E6 results in a large number of modifications in the respective host cells, among them the activation of host-cell telomerase.

### E7 Protein

Similar to the functions of E6, E7 oncoprotein functions are also pleiotropic. Its carboxyl-terminal zinc-binding domain can be functionally replaced by the homologous sequences of the E6 protein *(38)*, indicating the relationship between the two proteins. A key observation for an important function of E7 was the demonstration of its binding to pRb *(39)* and retinoblastoma protein (Rb)-related pocket proteins *(40)*. This binding results in phosphorylation of these proteins, in their enhanced degradation by ubiquination *(41)*, and in the release of transcription factors of the E2F family, activating transcription of genes regulating cell proliferation *(42,43)*. Under certain conditions, this binding is apparently not an essential component for immortalizing properties of E7 *(44)*. Mutations in the zinc finger domain of E7, which is dispensable for Rb binding, abolish its transformation functions *(45)*. This region binds a protein of the nucleosome remodeling deacetylase histone deacetylase complex, Mi2β, pointing to a possible engagement of E7 in targeting deacetylation pathways *(43)*. The binding of E7 to the histone H1 kinase at the $G_2$/M phase of the cell cycle is apparently also not related to Rb binding *(46)*. Kinase binding-deficient mutants were described as transformation defective. Another interesting interaction of E7 has recently been reported to be the glycolytic enzyme M2 pyruvate kinase, modulating the activity of this enzyme *(47)*. The functional importance of additional E7–host-cell protein interactions, like hTid-1, a homologue of the *Drosophila* tumor suppressor protein Tid56 *(48)* and the subunit 4 (signal anchor) ATPase *(49)*, remains to be determined.

E7 proteins of high-risk HPVs are found in cyclin E *(50)* and in cyclin A *(51)* complexes. These complexes exhibit kinase activity. The activation of cyclin E, followed by the activation of cyclin A, is mediated by E7 sequences required for transformation *(52)*. An interaction of the E7 gene product has also been noted with the AP-1 family of transcription factors that are transactivated by E7 *(53)*. The E7-induced S-phase entry is not accompanied by cyclin D activation, probably because of the formation of cyclin D/$p16^{INK4}$ complexes in high-risk HPV-infected cells *(33)*. The binding of E7 to c-jun *(54)* and to the TATA box-binding protein *(55)* points to further interactions with transcription factors.

An important function of E7 proteins is the inactivation of the cyclin-dependent kinase inhibitors $p21^{CIP-1}$ *(56,57)* and $p27^{KIP-1}$ *(58)*. This interaction uncouples cdk activity from cdk inhibitors and should be a major factor in growth stimulation of HPV-infected cells.

In contrast to the E6 protein, the E7 protein sensitizes p53-reactive cells to apoptosis *(59–61)*. In cells with p53 mutations, E7 expression exerts an antiapoptotic effect *(62)*. It is interesting to note, in this respect, that the growth arrest in damaged keratinocytes caused by p53 induction is bypassed by HPV16 E7 *(63)*. Similar to E6, the E7 protein inhibits interferon signaling pathways by binding to the interferon regulatory protein p48 *(64)*.

High-risk HPV E7 expression enhances the integration of foreign DNA into host-cell DNA *(22)*, results in increased mutagenesis *(19,31)*, and enhances the mutagenicity of chemical carcinogens *(62)*. The mechanism underlying this function is presently poorly understood.

Similar to E6 expression, E7 expression disrupts the $G_1$/S transition, possibly by the altered regulation of cyclin E *(66)*. Phosphorylation of E7 by casein kinase II, analogous to phosphorylation of the adenovirus E1a complex, enhances some of these functions *(67,68)*. High-risk HPV E7, in contrast to E6, does not activate cellular telomerase *(69)*, although E7-immortalized cells partially restore their telomere length.

Thus, E7 is a potent inhibitor of some cyclin-dependent kinase inhibitors, it inactivates Rb-related pocket proteins, it enhances mutagenic events in E7-expressing cells, despite its property to sensitize cells to apoptosis, it is able to immortalize various types of human cells. It will be interesting to analyze E7-immortalized cell clones for modifications in the p53 gene.

Although E6 and E7 proteins may immortalize various types of human cells independently, their cooperative interaction leads to substantially enhanced immortalization efficiency. The partial inactivation of p53-mediated checkpoint control by E6, its antiapoptotic effect, the bypass of $p16^{INK4}$-controlled inhibition of E6 functions by E7, and the functional inactivation of the cyclin-dependent kinase inhibitors $p21^{CIP-1}$ and $p27^{KIP-1}$ by E7 are probably the key events in this cooperation.

## E6 and E7 Genes are Necessary But Not Sufficient for Cell Immortalization and the Malignant Phenotype

As pointed out previously, immortalization of human cells of various origin can be achieved with either E6 or E7 oncogenes of high-risk HPVs but more efficiently by the joint function of both of them. Several sets of data exist pointing to the requirement for viral oncogene expression to maintain either the immortalized or the malignant phenotype of the respective cells [reviewed in *(70)*]. However, a substantial body of evidence supports the concept that neither the individual genes nor their cooperation is sufficient to convert normal cells into an immor-

talized or malignant state. This can be deduced from a number of experimental studies. Somatic cell hybridizations, initially performed with simian virus 40-immortalized cells, revealed that different immortalized clones were able to complement each other toward senescence, in spite of ongoing SV40 T-antigen synthesis *(71–73)*. Similar data have been subsequently reported for HPV-immortalized human cells *(74)*. These studies *(75,76)* emphasize that the expression of viral oncoproteins is not sufficient for the immortalized state, although their conditional expression points to their necessary role in HPV-mediated cell immortalization.

Several studies underline the importance of HPV oncoprotein expression in malignant cervical carcinoma cells: Reversible repression of E6/E7 expression in the HPV18-positive cervical carcinoma cell line SW756 by dexamethasone blocks their malignant phenotype *(74)*. Reintroduction of the two oncogenes under the control of a dexamethasone-inducible promoter restores malignant growth. Similarly, as initially shown by von Knebel Doeberitz et al. *(78)*, viral oncogene antisense constructs selectively inhibited growth of cervical carcinoma cells harboring the respective virus *(79–81)*. Similarly, specific ribozymes or antisense oligonucleotides inhibit growth of HPV-containing cervical cancer cells *(82–84)*.

However, somatic cell hybridization studies *(84)* also reveal that the expression of HPV oncoproteins is not sufficient for the maintenance of the malignant phenotype in cervical cancer cells. Hybrid clones derived from the fusion of different cervical carcinoma cell lines or immortalized by HPV16 and converted to malignant growth by additional x-irradiation either complemented each other to senescence or to nontumorigenic immortalized growth or retained their malignant characteristics. This set of data points to the existence of a separate signaling cascade blocking the progression of immortalized cells toward malignant conversion. This signaling pathway is obviously regulated by several cellular genes and may become interrupted during the progression to malignant growth in different individual genes. Thus, after somatic cell hybridization of different clones from malignant lines, complementation may occur within this signaling cascade, resulting in an immortalized, but not a malignant, phenotype of the respective clones. Complementation toward senescence of two different malignant cells after somatic fusion should involve complementation within two different signaling cascades.

In line with the requirement for specific host-cell modifications in addition to the expression of viral oncoproteins are observations of specific chromosomal aberrations in HPV-immortalized or in cervical carcinoma cells *(86,87)*. A gene locus relatively frequently modified in cervical cancer is located in the chromosomal region 3p14.2 that harbors the fragile histidine triad (FHIT) gene *(88,89)*. Also in line with this interpretation, a large-scale, population-based study *(90)* from Sweden pointed to genetic links to the development of cervical cancer. An initially intriguing observation on a role of p53 polymorphism for the risk of cervical cancer *(90)* has not been confirmed in a number of other studies [reviewed in *(92)*].

As outlined previously, expression of high-risk HPV oncoproteins may, in part, induce these genetic modifications in host-cell DNA. Chemical and physical mutagens should also interact cooperatively in the development of these changes. In addition, integration of viral DNA could further contribute to specific alterations within the host-cell DNA.

## Intracellular and Intercellular Regulation of Viral Oncogene Function

### Intracellular Regulation Prevents Immortalization of Cells Infected With High-Risk HPV

Some lines of experimental studies point to the existence of an intracellular control of viral oncoprotein function, preventing immortalization of genetically unmodified high-risk HPV-infected human cells: One important aspect is the demonstration of complementation for senescence of different clones of HPV-immortalized cells after somatic cell hybridization *(74)*. The continued transcription of HPV E6/E7 mRNA in cells undergoing senescence corresponds to a similar situation observed in somatic cell hybrids obtained from SV40-immortalized cells *(72,73)*, where senescent cells still continue to express SV40 large T antigen.

The molecular mechanism of this functional impairment of viral oncoproteins is presently still not understood. It may involve modifications of viral oncoproteins, for instance, by phosphorylation or dephosphorylation. Phosphorylation of the E7 protein of HPV16 by casein kinase II has been described before *(67)*. On the other hand, it may as well involve inhibition due to direct interactions with other host-cell proteins, like cyclin-dependent kinase inhibitors. At least for E6-immortalized human cells, this interpretation gains substantial support from the observed consistent inactivation of $p16^{INK4}$ in the proliferating cells [*(31);* Whitaker N, zur Hausen H: unpublished data]. Although functional inactivation of cyclin-dependent kinase inhibitors $p21^{CIP-1}$ and $p27^{KIP-1}$ by the E7 protein has been reported *(57–59)*, an excess synthesis of these kinase inhibitors may have the opposite effect and block E7 functions.

The identification of the signaling cascade(s) involved would clarify one important step in elevating the risk of a normal cell toward malignant progression. The complementation studies pointing to the existence of at least four complementation groups suggest the participation of several host-cell genes in this control mechanism.

### Intercellular Regulation Prevents Malignant Conversion of HPV-Immortalized Cells

Early considerations, assuming an essential role of viral oncoproteins in carcinogenesis, postulated the existence of an intracellular control of viral oncogene transcription or viral oncoprotein expression in nonmalignant cells harboring the respective tumor virus genome *(93,94)*. The initial assumption of a cellular-interfering factor had to be changed subsequently into the cellular-interfering factor concept, postulating the existence of at least two, one intracellular and another intercellular signaling cascade, whose interruption in high-risk HPV-positive cells results in immortalization and malignant conversion.

Evidence for the existence of an intercellular regulation of HPV transcription originated from two experimental approaches: the repression of viral oncogene transcription in immortalized cells by specific cytokines and concordant observations after heterografting HPV-immortalized cells into immunocompromised mice.

Treatment of HPV-immortalized and malignant cells with retinoic acid revealed a differential suppression of HPV transcription in the former and also similarly a differential regulation of the retinoic acid receptor β gene *(95)*. However, some sup-

pression also occurs in malignant cells. In combination with interferon α, squamous cell carcinomas of the cervix seem to respond in systemic treatment to retinoic acid *(96)*.

Some cytokines, however, reveal a more selective effect. Whereas two cytokines, interleukins 6 and 17, even promote tumorigenicity of cervical cancer cells in nude mice *(97,98)*, others suppress selectively HPV transcription of immortalized cells. Transforming growth factor-β (TGF-β) effectively suppresses HPV early gene transcription and cell growth in immortalized cells *(99,100)*, whereas, in malignant cells, viral transcriptional activity remains unaltered. Resistance to growth inhibition by TGF-β appears to be a late event in cervical carcinoma development *(101)*. Interleukin 1, leukoregulin, and particularly tumor necrosis factor-α (TNF-α) represent additional cytokines, selectively suppressing HPV transcription in HPV-immortalized cells *(102–104)*.

Interferons have obviously some effect on papillomavirus-immortalized but also on malignant cells. The available data are partially controversial, although interferons γ and β, in particular, appear to be effective *(102,105)*. The interferon α effect seems to be more cell line specific *(106)* and affects less the transcription than the level of HPV E7 protein expression *(107)*. An interesting observation is the recent report of a direct interaction of E7 with p48, the DNA-binding component of the interferon-stimulated gene factor 3, blocking the interferon transcription process *(64)*. This finding, in addition to the E6 binding of interferon regulatory factor 3 and its resulting functional inhibition *(30)*, may point to an important interference mechanism of HPV infections in modifying their intracellular and intercellular regulation.

The first direct evidence for a selective suppression of HPV transcription in immortalized cells under *in vivo* conditions resulted from *in situ* hybridization experiments after heterografting such cells into nude mice *(108,109)*. Within 3 days, E6/E7 gene transcription was drastically reduced when compared with *in vitro* cultivation of the same cells. In contrast, malignant cervical cancer cells were not negatively affected in their transcriptional activity by the same treatment. These observations underlying the clinical studies reveal a barely detectable rate of HPV E6/E7 gene expression in the proliferating layers of most biopsy specimens of low-grade cervical intraepithelial neoplasias, whereas abundant transcripts were noted in high-grade lesions *(109,110)*. Subsequent studies *(111)* indicated that the suppression of HPV transcription could be achieved under tissue culture conditions by the addition of murine or human macrophages and is most likely mediated by the excretion of TNF-α.

The analysis of intracellular changes mediated by TNF-α treatment selectively in HPV-immortalized but not in malignant cells points to an important role of modifications in the AP-1 transcription complex *(112)*. AP-1 expression during cellular differentiation determines E6/E7 expression in stratified epithelial cells *(113)*. Whereas, in immortalized cells, the AP-1 complex in the HPV promoter mainly consists of c-jun/c-jun homodimers, TNF-α treatment results in a shift toward c-jun/fra I heterodimers *(112)*. This shift is accompanied by the suppression of HPV transcription. In malignant cells, the AP-1 composition of the AP-1 complex in the HPV promoter frequently consists of c-jun/c-fos heterodimers. Overexpression of c-fos in immortalized cells results in preferential c-jun/c-fos heterodimers and in malignant conversion of the respective clones *(112)*.

These data stress the important role of jun/fos heterodimerization in determining independence from cellular regulatory functions and in converting immortalized cells to a malignant phenotype. This is emphasized by some previous observations showing induction of tumorigenicity in primary human keratinocytes by cotransfecting HPV18 DNA and v-fos *(114)*, the increased transcription of E6/E7 in H-ras-mediated malignant conversion of HPV-immortalized cells *(115)*, and by the requirement of c-fos for malignant progression of skin tumors *(116)*. The importance of AP-1 activity for the growth of HPV-immortalized human keratinocytes is also revealed by the suppression of anchorage-independent growth following expression of dominant negative jun *(117)*. The fra-I gene is activated by AP-1 *(118)*. In contrast to AP-1 in the HPV promoter, this gene is also activated by TNF-α treatment *(112)* and, in addition, by retinoic acid *(119)*.

The emerging picture from this set of data implies an important role of an intercellular, cytokine-mediated control of HPV transcription in suppressing the conversion of HPV-infected human cells toward malignant growth, even at a stage that corresponds to immortalization, clinically most likely represented by low-grade intraepithelial lesions. Malignant progression obviously involves interruption of cellular genes engaged in the signaling cascade controlling this pathway. Indirect evidence points to the involvement of a gene on the short arm of chromosome 11 and of protein phosphatase 2A (PP2A) activity in this process: Human cells containing a deletion in the short arm of chromosome 11 are more readily immortalized by SV40 or high-risk HPV DNA *(120)*. Under these conditions, one regulatory component of PP2A, PR 55β, becomes activated, resulting in a suppression of the catalytic component of the enzyme and in an increase in HPV transcription. Similar effects are observed by inhibiting PP2A with ocadaic acid or by introducing SV40 small T antigen.

## Conclusions

The molecular pathogenesis of cancer caused by high-risk HPV infections is presently not fully understood. Some characteristic features, however, become evident. These viruses are, in a way, self-sufficient to induce carcinogenesis, despite the fact that infection *per se* is not sufficient to induce malignant conversion. The induction of chromosomal instability, of mutations, and of aneuploidy in previously noncommited proliferating basal and suprabasal cells emerges as one driving force to induce modifications, even in those cellular genes that, by themselves, control viral oncogene transcription and viral oncoprotein functions. The tight control of viral oncogenes and oncoproteins by intracellular and intercellular signaling cascades, obviously only active in proliferating but not in differentiated cells, underlines and supports the existence of cellular-interfering factors *(70)*. According to our present understanding, interruption of two independent signaling cascades is responsible initially for the immortalized and later for the invasive phenotype of the respective cells, although the sequence of changes may be different in clinical lesions in comparison to events occurring in tissue culture.

A scheme of events in the course of progression is outlined in Fig. 1. The development of cervical cancer and subsequent metastases clearly require more changes. They do involve additional modifications within the affected cell to escape cell-mediated immune responses and additional changes facilitating

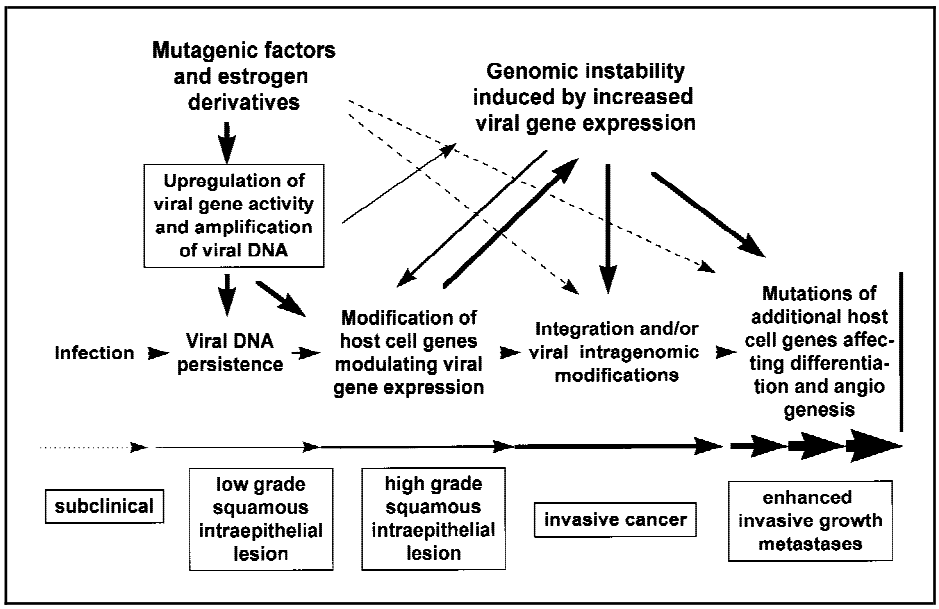


**Fig. 1.** Scheme of high-risk human papillomavirus-mediated pathogenesis [modified from *(123)*].

cell detachment and the formation of distant metastases. It is outside the scope of this review to cover these aspects.

The identification of specific types of papillomaviruses as causative agents for important human cancers was clearly a precondition for the development of new strategies in the prevention and will probably also influence the therapy for these infections. The ongoing clinical trials for HPV vaccines will, hopefully, lead to an effective prevention of one of the globally most frequent cancers in women.

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## NOTE

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