Journal of Cellular Biochemistry, Supplement 17F:155–164 (1993)

Papillomaviruses in Head and Neck Disease: Pathophysiology and Possible Regulation

Bettie M. Steinberg, PhD and Karen J. Auborn, PhD

Department of Otolaryngology and Communicative Disorders, Long Island Jewish Medical Center, The Long Island Campus for the Albert Einstein College of Medicine, New Hyde Park, NY 11042

Abstract Human papillomaviruses (HPVs) are etiologic agents of both benign and malignant epithelial tumors. More than 60 different types of viruses are known, each associated with tissue site and lesion type specificities and differing probabilities of malignant progression. HPVs type 6 and type 11 cause benign papillomas of mucosal squamous epithelium in the aerodigestive tract, with only rare conversion to malignancy. HPV 16 is the most frequently detected HPV in the genital tract, inducing flat lesions with a significant risk of malignant conversion. In the aerodigestive tract, HPV 16 is found only rarely in benign lesions but is detected in 5–20% of squamous carcinomas.

In the aerodigestive tract, HPVs frequently cause latent infection, *i.e.*, viral DNA present in tissue but no evidence of clinical or histologic disease. Approximately 10% of the general population may have latent infections. Regulation and activation of latent infections are not well understood, although it is clear that viral functions are tightly regulated by the state of differentiation of the squamous host cell. Control of viral transcription may be the key to prevention of viral activation, and thus control of disease. Among the possible agents under investigation are retinoids, growth factors, anti-sense RNA which interferes with viral expression, and estrogen metabolites. All of these agents modulate either viral expression or cell differentiation or both. It is hoped that in the near future one or more of these agents will be useful in preventing HPV-associated disease. © 1993 Wiley-Liss, Inc.

Key words: epidermal growth factor, estrogens, HPV, laryngeal papillomas, retinoids

Human papillomaviruses (HPVs) are small DNA viruses that proliferate and cause disease in squamous epithelium. The diseases caused by HPVs include both benign lesions (papillomas and warts) and carcinomas. There are more than 60 different HPV types, and new types are still being identified [1]. Typing is based on extent of DNA homology, although all of the HPVs share the same genome organization as well as some highly conserved genes. The types can then be grouped into larger, related groups that share DNA sequences and biologic properties. The latter include tissue trophism and potential for inducing malignant disease. The HPVs of most interest in the head and neck are those types which generally infect mucosal epithelium, causing both aerodigestive and genital lesions [for review see 2]. HPV 6 and HPV 11 cause benign papillomas with a low probability of malignant progression. HPV 16 and HPV 18 are associated with lesions with a much higher probability of progression to carcinomas.

The life cycle of the HPVs is shown in Figure 1. Unlike most viruses, there is a very tight relationship between HPV functions and the stage of differentiation of its host cell. The virus infects the basal cells of the epithelium. As

Address correspondence to Dr. Bettie M. Steinberg, Department of Otolaryngology and Communicative Disorders, Long Island Jewish Medical Center, 270-05 76th Avenue, New Hyde Park, NY 11042.

© 1993 Wiley-Liss, Inc.

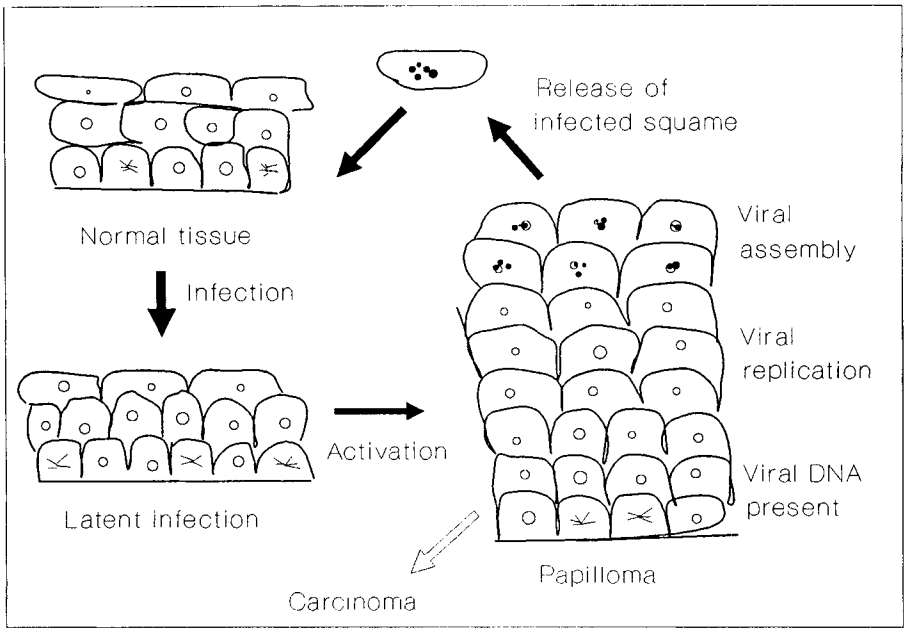

Fig. 1. Life cycle of papillomaviruses. This figure illustrates the latent period before any lesion is evident, as well as the relationship between viral DNA replication, virus production, and epithelial stratification in the papilloma.

these cells proliferate, the viral DNA also proliferates, maintaining a low copy number of episomal viral DNA molecules in the daughter cells. For some variable period of time the virus is latent, with no evidence of infection. The tissue is clinically and histologically normal. This latent state can presumably be maintained for the life of the patient, since HPV DNA can be detected in airway biopsies of patients with no history of papillomavirus-related disease [3].

In a subset of infected cells the virus becomes active, inducing the formation of a papilloma. At this time viral RNA can be detected [4,5]. Initially, only two of the viral genes (E6 and E7) are expressed. These two genes, when derived from HPV 16 or HPV 18, are the major transforming genes. They are able to transform cells *in vitro* and are the only HPV genes expressed in most carcinomas [6,7]. HPV 16 and HPV 18 E6 binds to and degrades p53, a cellular tumor suppressor protein [8], while E7 complexes with and inactivates pRB, a second tumor suppressor protein. However, benign papilloma cells are not immortalized, and the role of these two proteins from the "benign" HPV 6 and HPV 11 must still be determined. It has been suggested that in papillomas the E6 and E7 proteins induce proliferation of their host cells, or that they maintain the function of the cellular replication machinery in an active state such that the virus might later replicate its DNA to high levels.

As the infected epithelial cells begin to differentiate, moving from a basal or parabasal position to a more suprabasal position, additional viral genes are expressed and HPV DNA is replicated to high copy numbers in some of the cells. E1 and E2 code for proteins required for the productive viral DNA synthesis [9], interacting with the cellular DNA replication complex which is normally not functional in these suprabasal cells, while E2 also regulates viral transcription [10]. E5 protein has some transforming capability [11], complexes with growth factor receptors [12], and may play an important role in the formation of the benign papilloma. If so, it is surprising that it is not expressed in the basal and parabasal layers, or if it is, it is at levels that are below detection with current procedures. The function of E4 is not yet clear, but its RNA is by far the most abundant RNA in the papilloma [4]. In a subset of cells near

the surface of the papilloma, the two HPV capsid proteins (L1 and L2) are synthesized, the viral DNA is packaged into virions, and virus-laden squames are finally shed from the papilloma.

Conversion of a benign HPV-induced lesion to a carcinoma is a rare event. All evidence suggests that malignant progression requires not only the presence of the virus but also cellular mutations, usually induced by carcinogenic agents such as X-rays, ultraviolet light in sunlight, or tobacco. There is usually a prolonged lag time of decades between carcinogen exposure and appearance of the cancer. When malignant conversion occurs, the expression of the virus changes. Usually, the viral DNA integrates into the host chromosomes in such a way that only the E6 and E7 genes are expressed. There is no longer productive viral DNA synthesis, or synthesis of capsid proteins, and new virus is no longer made. Thus, induction of cancer is a "dead end" for the viral life cycle.

HPV AND AERODIGESTIVE TUMORS

Prevalence

There are a number of HPV types associated with lesions of the aerodigestive tract, including the oral cavity, nose and nasopharynx, larynx, trachea, and esophagus [for reviews see 2,13, 14]. These viruses include HPVs 2, 6, 11, 13, and 16, as well as undetermined types. It is widely accepted that recurrent respiratory papillomas, a serious and life-threatening disease, are primarily caused by HPVs 6 and 11. The viral DNA is present and expressed in the papillomas [15]. Laryngeal tissue infected with HPV 11 virions and implanted under the renal capsule of immunodeficient mice forms typical papillomas [16]. HPVs 6 and 11 are the same viral types that cause exophytic genital lesions, and it is believed that juvenile-onset papillomas result from infection of the infant as it moves through an infected birth canal. Given this, it is surprising that the prevalence of this disease is so low (approximately 1:100,000) since the prevalence of active genital tract infection is estimated to be 10–15% of sexually active adults in the United States. Brandsma *et al.* [3] reported finding HPV DNA in 4% of random, clinically normal biopsies of the airway. This would suggest that most of the airway infections remain latent. Adult-onset respiratory papillomas could reflect either activation of virus present since birth or an infection acquired in adolescence or adult life.

Although HPVs 6 and 11 are usually considered "benign," they can be found in a subset of HPV-containing carcinomas. X-irradiation of laryngeal papillomas caused squamous carcinoma of the larynx in nearly one-third of patients, with a lag of 20 to 30 years [17]. There is a low but real possibility of spontaneous malignant conversion where the putative mutagenizing event is unknown [2]. The HPV 6 or HPV 11 molecules have sometimes undergone rearrangements in the tumor cells that might have increased their malignant potential [18].

If reported prevalence studies are combined, approximately 25% of head and neck carcinomas contain HPV DNA [14]. HPV 16 is the most common HPV in carcinomas not associated with laryngeal papillomas, but HPVs 2, 18, 33, and unidentified types are also present. Until recently, studies have focused on the presence of HPV DNA and did not distinguish between active viral function and latent infection. There is a relatively high level of latent HPV 16 DNA in the aerodigestive tract [3,19, 20]. Without analysis of viral activity it is not possible to distinguish between the accidental presence of HPV DNA in a subset of tumors and a true etiologic function. A recent study by Snijders *et al.* [21] reported that the HPV in tonsillar carcinomas expressed RNA that could code for the E7 gene product, strongly suggesting that the virus does play an important role in the formation of the carcinomas.

Pathophysiology

The molecular mechanisms whereby papillomaviruses induce either benign or malignant tumors are only partially understood. We have focused our studies on laryngeal papillomas to better understand the interactions between virus and host cells that result in the initial benign lesions. Laryngeal papillomas are characterized by pedunculated masses with finger-like projections of stratified squamous epithelium supported by a connective tissue stroma (Fig. 2). Histologically, the basal layer is either normal or somewhat hyperplastic [22]. Mitotic

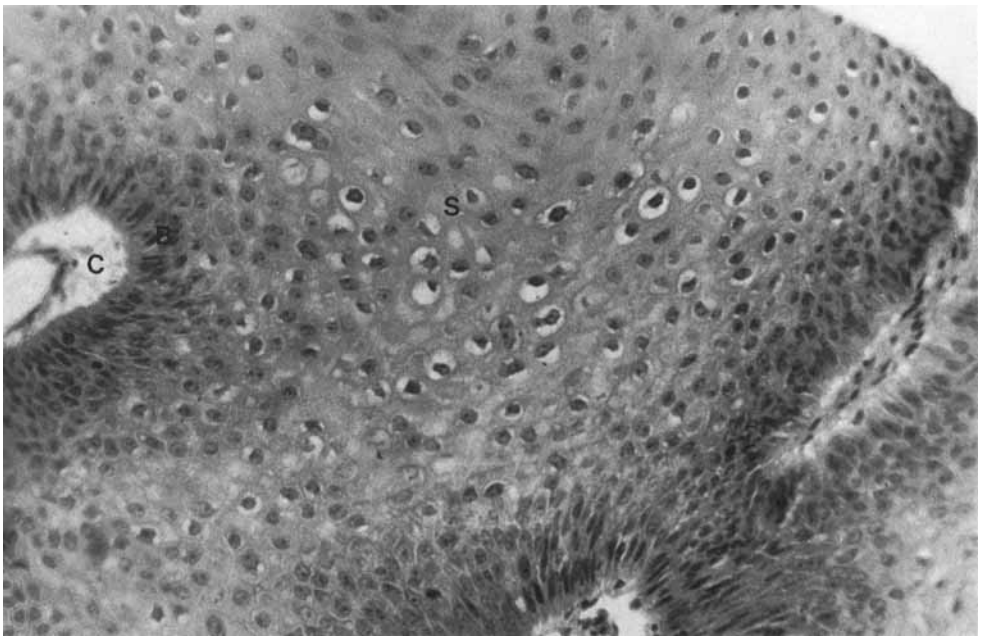

Fig. 2. Histology of laryngeal papilloma. **B**: Basal layer; **S**: Spinous layer with many cells containing perinuclear halos and abnormally large nuclei, similar to the koilocytes seen in genital tract papillomas; **C**: connective tissue core. Hematoxylin-eosin stain.

TABLE I. Proliferation and Differentiation of Papilloma and Normal Tissue

	% Proliferation[a]		Differentiation[b]	
Cell Layer	**Normal**	**Papilloma**	**Normal**	**Papilloma**
Basal	10	5.8	None	None
Suprabasal	9	3.9	Complete	None
Superficial	0	0.5	Complete	Rare Cell

[a] Percent of cells incorporating tritiated thymidine. Tissue fragments were labeled *in vitro* immediately after biopsies were taken. After labeling, the fragments were fixed with 3.7% formalin, sectioned, and autoradiographed.

[b] Determined by immunofluorescent staining of frozen sections, using antibodies that recognize keratin 13 and filaggrin, two markers of normal differentiation in mucosal squamous epithelium.

Data taken from Steinberg *et al.* [23].

figures are generally limited to the basal layer. There is marked spinous hyperplasia, maturational immaturity, and abnormal keratinization with a thin parakeratotic layer.

We asked whether the thickening of the basal and spinous layers reflected increased rates of proliferation, abnormalities in differentiation, or both [23]. Table I summarizes our findings. Clearly, the fraction of dividing cells in the basal and immediate suprabasal layers of the papillomas is lower than in normal tissue, although it must be remembered that the total number of basal cells is greater in papillomas. Most significantly, the papillomas do not under-

go normal differentiation, as measured by synthesis of keratin 13 and filaggrin. Rather, they go through an abnormal type of differentiation that is characteristic of hyperplastic epithelium (data not shown). We postulate that the abnormality in differentiation results in a reduction in the loss of squames at the surface, resulting in an accumulation of spinous cells and apparent hyperplasia.

As described earlier in this paper, the HPV E5 gene can alter cellular growth factor receptor functions [12]. Responses to epidermal growth factor (EGF) can modulate growth and differentiation of keratinocytes [24], and some tumors of the head and neck overexpress the EGF receptor [25]. We therefore asked whether the papilloma cells overexpressed the EGF receptor, and whether they responded to EGF in a manner different from normal laryngeal cells [26]. We found that papilloma cells do overexpress the EGF receptor. Organotypic cultures of papilloma cells showed intense antibody staining for the receptor in basal cells, which extended through the lower half of the stratified culture. In contrast, normal cells showed faint staining restricted to the basal layer. The effects of EGF on differentiation of the two types of cells were also strikingly different, while proliferation effects were similar (Table II). From these data we have postulated that HPV-induced over-responsiveness to EGF contributes to the hyperplasia in papillomas by inhibiting the normal differentiation processes.

POTENTIAL MODULATION OF HPV-INDUCED TUMORS

Ideally, chemoprevention would be able to inhibit the growth and recurrence of laryngeal papillomas, and the induction of carcinomas by papillomaviruses, by inhibiting the interactions between the virus and its host cell. Several potential modulators have been and are being investigated.

Retinoids

One interesting class of chemopreventive agents would be retinoids. These agents have been effective in preventing second primary carcinomas of the respiratory tract [27], so they have potential use against HPV-induced cancers. Retinoids have also been used on a limited basis to treat laryngeal papillomas [28]. While

TABLE II. Effects of Epidermal Growth Factor on Proliferation and Differentiation

		% Proliferation[a]				Differentiation[b]			
		Normal		Papilloma		Normal		Papilloma	
Cell Layer	(EGF)	–	+	–	+	–	+	–	+
Basal		19	52	11	23	–	–	–	–
Spinous						+	+	+	–
Superficial						+	+	+	+/–

Cells were cultured in serum-free medium (KGM) containing 1 ng/ml EGF, then re-fed with media containing either 1 ng/ml EGF or 0 EGF for 5 days before analysis. Cells were cultured on glass coverslips till near confluent for proliferation studies and on organotypic rafts at the air–liquid interface at confluence for differentiation studies.

[a] Percentage of basal cells in monolayer culture incorporating bromodeoxyuridine during a 48 hour labeling period, then stained with an antibody to bromodeoxyuridine.

[b] Presence of keratin 13 in most or all cells within a stratified layer; detected with AE8, an antibody specific for differentiation-specific keratin.

Data taken from Vambutas *et al.* [26].

TABLE III. Effects of Retinoic Acid on HPV Persistence and Cell Differentiation

Retinoic Acid Concentration	% HPV DNA Persisting[a]	Squamous Differentiation[b]	
		Normal	Papilloma
0	100	ND	ND
10^{-9} M	90	complete	complete
10^{-8} M	40	complete	partial
10^{-7} M	20	none[c]	minimal
10^{-6} M	10	ND	none

Papilloma and normal cells were cultured on organotypic rafts at the air–liquid interface in serum-free KGM supplemented with varying concentrations of retinoic acid.

[a] DNA was extracted from cultured papilloma cells, dot-blotted, probed with ^{32}P-HPV DNA, and hybridization quantified by densitometry. Normalized to HPV levels in absence of retinoic acid.

[b] Measured by immunohistochemical stains using antibody to involucrin. Involucrin is present in suprabasal squamous epithelium undergoing either normal or abnormal differentiation, but not in respiratory epithelium, thus distinguishing squamous from respiratory differentiation.

[c] Columnar epithelium with cilia.

ND: not done. Morphology in absence of retinoic acid was too poor for staining.
Data taken from Mendelsohn *et al.* [31] and Reppucci *et al.* [32].

success was limited, further studies are warranted. Retinoic acid regulates epidermal keratinocyte differentiation through pathways that are now being defined [29], and similar pathways may act to inhibit and repress HPV 18 expression in cervical carcinoma cells in culture that are exposed to retinoic acid [30].

We have investigated the effects of retinoic acid on normal laryngeal cells and papilloma cells [31,32]. These studies are summarized in Table III. Differentiation of normal cells into either squamous or respiratory epithelium can be regulated *in vitro* by the concentration of retinoic acid in the culture medium. At concentrations of retinoic acid which limit squamous differentiation as measured by expression of involucrin in the suprabasal cells, the average number of HPV DNA molecules per cell is reduced. We are currently investigating the effects of retinoic acid on expression of viral RNA to determine whether a latent state is induced, or whether retinoic acid has the potential to actually cure the cells of HPV DNA.

Anti-Sense RNA

Several laboratories are investigating the potential use of anti-sense RNA to limit or treat HPV-induced lesions. The basic concept behind this approach is that RNA molecules complementary to HPV mRNA can form a complex with the viral mRNA and prevent its expression. Alternatively, these molecules can form a complex with the viral DNA at the position where mRNA is initially synthesized, preventing expression of the viral genes. The anti-sense RNAs could conceivably be introduced directly into the tissues, or could be expressed from a genetically engineered virus which could infect the tissues. HPV 18 E6/E7 anti-sense RNA altered the transformed phenotype of HPV 18-containing cancer cells *in vitro*, slowing growth, reducing ability to form colonies in soft agar, and increasing serum requirements [33]. Similar anti-sense RNAs inhibited the tumorigenicity of cervical cancer cells in nude mice [34]. The potential for this approach is exciting, not only

against HPV-induced lesions, but against other types of tumors providing we can identify unique tumor proteins required for growth.

Estrogen Metabolism

The possibility exists that laryngeal papillomas and laryngeal cancers can also be prevented by altering estrogen metabolism. Estrogen metabolism can influence the risk of cancer occurring in certain hormonally sensitive tissues. Estradiol is oxidized to estrone and then hydroxylated by alternate pathways to either 16α-hydroxyestrone or 2-hydroxyestrone (Fig. 3). The relative activity of these alternate pathways of estrogen metabolism influences cancer risk. 16α-Hydroxylation is associated with increased risk of developing cancer while 2-hydroxylation is associated with a decreased risk of cancer. 16α-Hydroxyestrone prolongs estrogenic effects and causes DNA damage while 2-hydroxyestrone is anti-estrogenic. Therefore, estrogen metabolism can have profound effects on hormonally sensitive cells.

We recently established a relationship between estradiol 16α-hydroxylation and HPV infection and its possible malignant sequelae [35]. We determined that papillomavirus-induced lesions predominate in genital tissues that have high constitutive levels of estradiol 16α-hydroxylation, *e.g.*, the transformation zone of the cervix. Immortalization of cells from this tissue with HPV dramatically elevates this metabolism. Hence, a feedback mechanism exists whereby 16α-hydroxylation increases HPV expression, and HPV increases 16α-hydroxylation. Since the larynx is an estrogen-sensitive tissue, we expanded these studies to HPV lesions of the larynx [36]. We determined that the level of 16α-hydroxylation is constitutively high in normal larynx and significantly elevated above the high baseline in benign papillomas. The results of the estradiol 16α-hydroxylation from normal and HPV-infected cells of the laryngeal and genital epithelium are summarized in Figure 4. We now have data that estradiol 16α-hydroxylation is dramatically increased not only in laryngeal cancers but also in "normal" laryngeal tissue adjacent to cancerous tissue (Fig. 5). Hence, 16α-hydroxylation of estradiol apparently predisposes the larynx to early events of transformation and may play a role in the field effect of airway cancers.

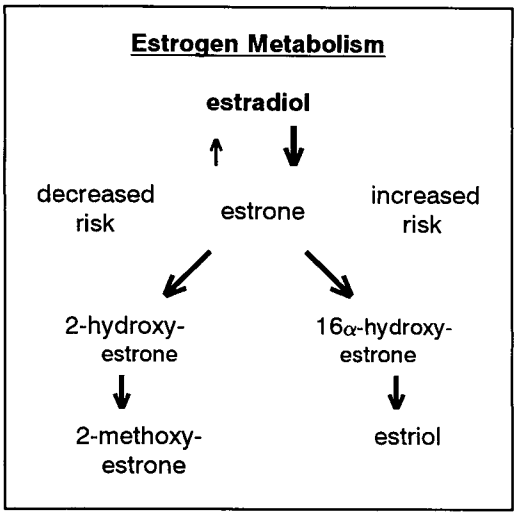

Fig. 3. The metabolism of estradiol showing the alternate pathways of hydroxylation.

Estrogen metabolism can be modulated. Feeding indole-3-carbinol (I3C), a compound present in cruciferous vegetables, increases 2-hydroxylation and reduces the formation of 16α-hydroxylation metabolites systemically [37]. Dietary I3C has been shown to decrease tumor incidence in mice bearing spontaneous mammary tumors [38]. We were able to determine that I3C has a place in preventing HPV transformation by two criteria:

In cell culture, I3C abrogated growth effects of estradiol. Estradiol or 16α-hydroxyestrone increased the number of replicating cells. I3C abrogated this proliferative effect of estradiol in both normal and HPV-infected larynx cells. I3C actually caused an anti-proliferative effect similar to that caused by 2-hydroxyestrone [36]. In a soft agar growth assay, we determined that HPV-immortalized cells acquired the ability to grow if estradiol was present. The growth in soft agar was even more dramatic if 16α-hydroxyestrone was added. Soft agar growth usually correlates with tumorigenicity. I3C abrogated the effects of estradiol in this assay (unpublished data).

In vivo, dietary I3C prevented the formation of papilloma cysts in HPV-infected human laryngeal tissue implanted under the renal cap-

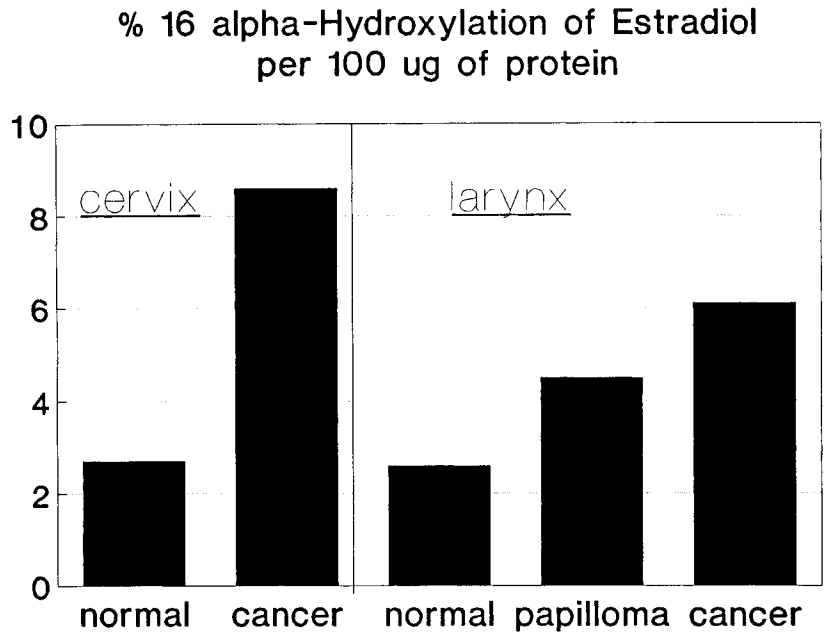

Fig. 4. 16α-Hydroxylation of estradiol is constitutively high in tissue susceptible to papillomavirus lesions, and further elevated in papillomas and cancers. Primary explants from normal, papillomatous, and cancer (larynx) tissue were grown and subcultured as described [35]. (16α-^{3}H)Estradiol was added to confluent cells and hydroxylation measured as production of tritiated water. Each assay was done with replicate samples, using multiple tissues. Data are summarized from data described in Auborn *et al.* [35] and Newfield *et al.* [36], as well as unpublished data.

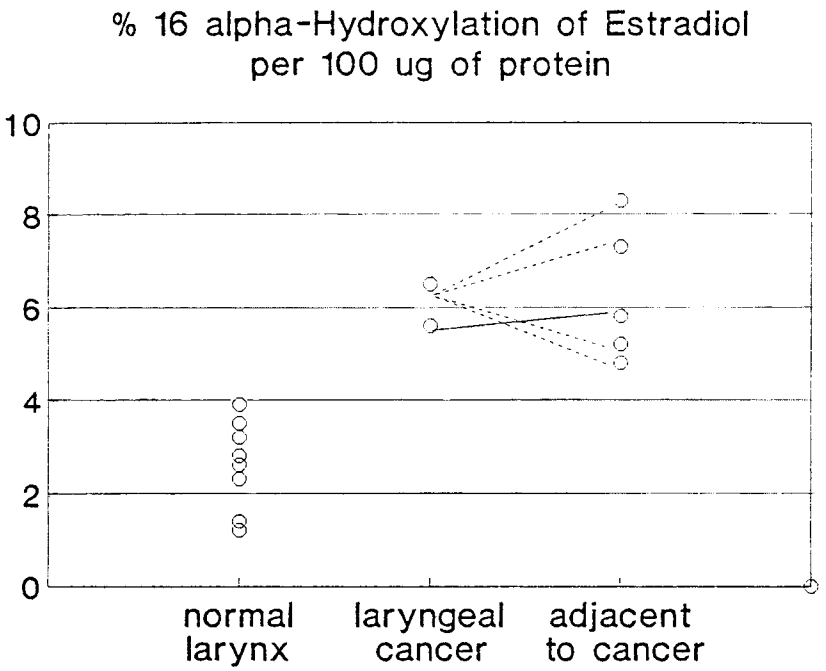

Fig. 5. 16α-Hydroxylation of estradiol is increased in both laryngeal carcinomas and normal tissues adjacent to the carcinomas. Primary explants from normal, malignant, and adjacent non-involved larynx tissue were grown and subcultured as described, and 16α-hydroxylation measured [35]. The values for the corresponding cancer and "normal" adjacent site in the same patient are connected. In the case of one patient, the four different "normal" sites were 1, 2, 2, and 4 cm from the site of the carcinoma.

sule of immunocompromised mice. Using implants of laryngeal tissue infected with HPV type 11 virions, papilloma cysts developed in 100% of surviving xenografts in mice fed a normal diet, but in only 25% of surviving xenografts in mice fed the same diet supplemented with I3C [36].

Extension of these data to clinical use would be the prophylactic use of dietary I3C or a diet rich in cruciferous vegetables. Such dietary supplements might help prevent recurrent laryngeal papillomatosis and/or recurrent laryngeal cancer.

CONCLUSIONS

In this brief review we have discussed the evidence for a clear role of human papillomaviruses in both benign and malignant tumors of the upper aerodigestive tract. We have also reviewed recent studies and presented new data that suggest possible chemopreventive or chemomodulatory approaches to the management of these diseases.

ACKNOWLEDGMENTS

This work was supported in part by grant P50 DC00203 from the National Institute on Deafness and Other Communication Disorders. We thank Dr. H. Leon Bradlow, Strang Cornell Cancer Center, for help with the estrogen metabolism assays.

REFERENCES

1. Pfister H: General introduction to papillomaviruses. In Pfister H (ed): "Papillomaviruses and Human Cancers." Boca Raton: CRC Press, 1990, pp 1–9.
2. Shah KV: Papillomavirus infections of the respiratory tract, the conjunctiva, and the oral cavity. In Pfister H (ed): "Papillomaviruses and Human Cancers." Boca Raton: CRC Press, 1990, pp 73–90.
3. Brandsma JL, Abramson AL: Association of papillomavirus with cancers of the head and neck. Arch Otolaryngol Head Neck Surg 115:621–625, 1989.
4. Stoler MH, Whitbeck A, Wolinsky SM, Broker TM, Chow LT, Howett MK, Kreider JW: Infectious cycle of human papillomavirus type 11 in human foreskin xenografts in nude mice. J Virol 64:3310–3318, 1989.
5. Iftner T, Oft M, Bohm S, Wilczynski SP, Pfister H: Transcription of the E6 and E7 genes of human papillomavirus type 6 in anogenital condylomata is restricted to undifferentiated cell layers of the epithelium. J Virol 66:4639–4646, 1992.
6. Kaur P, McDougall JK, Cone R: Immortalization of primary human epithelial cells by cloned cervical carcinoma DNA containing human papillomavirus type 16 E6/E7 open reading frames. J Gen Virol 70:1261–1266, 1989.
7. Wettstein FO: State of viral DNA and gene expression in benign versus malignant tumors. In Pfister H (ed): "Papillomaviruses and Human Cancers." Boca Raton: CRC Press, 1990, pp 155–179.
8. Schneffner M, Werness BA, Huibregtse JM, Levine AJ, Howley PM: The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. Cell 63:1129–1136, 1990.
9. Ustav M, Stenlund A: Transient replication of BPV-1 requires two viral polypeptides encoded by the E1 and E2 open reading frames. EMBO J 10: 449–457, 1991.
10. Hirochika H, Broker TR, Chow LT: Enhancers and *trans*-acting E2 transcriptional factors of papillomaviruses. J Virol 61:2599–2606, 1987.
11. Leptak C, Ramon y Cajal R, Kilke R, Horwitz BH, Riese DJ, Dotto GP, DiMaio D: Tumorigenic transformation of murine keratinocytes by the E5 genes of bovine papillomavirus type 1 and human papillomavirus type 16. J Virol 65:7078–7083, 1991.
12. Leechanachai P, Banks L, Moreau F, Matlashewski G: The E5 gene from human papillomavirus type 16 is an oncogene which enhances growth factor-mediated signal transduction to the nucleus. Oncogene 7:19–25, 1992.
13. Steinberg BM: Human papillomaviruses and upper airway oncogenesis. Am J Otolaryngol 11:370–374, 1990.
14. Steinberg BM: The role of human papillomaviruses in benign and malignant lesions. In Hong WK, Weber RS (eds): "Head and Neck Oncology 4." Norwell, MA: Kluwer Academic Publishers, 1993 (in press).
15. Ward P, Mounts P: Heterogeneity in mRNA of human papillomavirus type 6 subtypes in respiratory tract lesions. Virol 168:1–12, 1989.
16. Kreider JW, Howett MK, Stoler MH, Zaino RJ, Welsh P: Susceptibility of various human tissues to transformation *in vivo* with human papillomavirus type 11. Int J Cancer 39:459–465, 1987.
17. Majores M, Parkhill EM, Devine KD: Malignant transformation of benign laryngeal papillomas in children after radiation therapy. Surg Clin North Am 43:1019–1061, 1963.
18. DiLorenzo TP, Tamsen A, Abramson AL, Steinberg BM: Human papillomavirus type 6a DNA in the lung carcinoma of a patient with recurrent laryngeal papillomatosis is characterized by a partial duplication. J Gen Virol 73:423–428, 1992.
19. Bryan RL, Bevan IS, Croker J, Young LS: Detection of HPV 6 and 11 in tumours of the upper respiratory tract using the polymerase chain reaction. Clin Otolaryngol 15:177–180, 1990.

20. Maitland NJ, Cox MF, Lynas C, Prime SS, Meanwell CA, Scully C: Detection of human papillomavirus DNA in biopsies of human oral tissue. Br J Cancer 56:245–250, 1987.
21. Snijders PJF, Cromme FV, Van den Brule AJC, Schrijnemakers HFJ, Snow GB, Meijer CJLM, Walboomers JMM: Prevalence and expression of human papillomavirus in tonsillar carcinomas, indicating a possible viral etiology. Int J Cancer 51:845–850, 1992.
22. Abramson AL, Steinberg BM, Winkler B: Laryngeal papillomatosis: Clinical, histopathologic and molecular studies. Laryngoscope 97:678–684, 1987.
23. Steinberg BM, Mead R, Kalinowski S, Abramson AL: Abnormal differentiation of human papillomavirus-induced laryngeal papillomas. Arch Otolaryngol Head Neck Surg 116:1167–1171, 1990.
24. Cohen S: The stimulation of epidermal proliferation by a specific protein (EGF). Dev Biol 12:394–407, 1965.
25. Rikimaru K, Tadokoro K, Yamamoto T, Enomoto S, Tsuchida N: Gene amplification and overexpression of epidermal growth factor receptor in squamous cell carcinoma of the head and neck. Head Neck 14:8–13, 1992.
26. Vambutas A, DiLorenzo TP, Steinberg BM: Laryngeal papilloma cells have high levels of epidermal growth factor receptor and respond to epidermal growth factor by a decrease in epithelial differentiation. Cancer Res 53:910–914, 1993.
27. Hong WK, Lippman SM, Itri LM, Karp DD, Lee JS, Byers RM, Schantz SP, Kramer AM, Lotan R, Peters LJ, Dimery IW, Brown BW, Goepfert H: Prevention of second primary tumors with isotretinoin in squamous cell carcinoma of the head and neck. N Engl J Med 323:795–801, 1990.
28. Alberts SD, Coulthard SW, Meyskens FL: Regression of aggressive laryngeal papillomatosis with 13-*cis*-retinoic acid. J Biol Response Mod 5:124–128, 1986.
29. Vollberg TM, Nervi C, George MD, Fujimoto W, Krust A, Jetten AM: Retinoic acid receptors as regulators of human epidermal keratinocyte differentiation. Mol Endocrinol 6:667–676, 1992.
30. Bartsch D, Boye B, Baust C, zur Hausen H, Schwarz E: Retinoic acid-mediated repression of human papillomavirus 18 transcription and different ligand regulation of the retinoic acid receptor β gene in non-tumorigenic and tumorigenic HeLa hybrid cells. EMBO J 11:2283–2291, 1992.
31. Mendelsohn MG, DiLorenzo TP, Abramson AL, Steinberg BM: Retinoic acid regulates, *in vitro*, the two normal pathways of differentiation of human laryngeal keratinocytes. *In Vitro* Cell Dev Biol 27A: 137–141, 1991.
32. Reppucci A, DiLorenzo TP, Steinberg BM: *In vitro* modulation of human laryngeal papilloma cell differentiation by retinoic acid. Otolaryngol Head Neck Surg 105:528–532, 1991.
33. Steele C, Sacks PG, Adler-Storthz K, Shillitoe EJ: Effect on cancer cells of plasmids that express antisense RNA of human papillomavirus type 18. Cancer Res 52:4706–4711, 1992.
34. Doeberitz MvK, Rittmuller C, zur Hausen H, Durst M: Inhibition of tumorigenicity of cervical cancer cells in nude mice by HPV E6–E7 anti-sense RNA. Int J Cancer 51:831–834, 1992.
35. Auborn KJ, Woodworth C, DiPaolo J, Bradlow HL: The interaction between HPV infection and estrogen metabolism in cervical carcinogenesis. Int J Cancer 49:867–869, 1991.
36. Newfield L, Goldsmith A, Bradlow HL, Auborn KJ: Estrogen metabolism and human papillomavirus-induced tumors of the larynx: Chemo-prophylaxis with indole-3-carbinol. Anticancer Res, 1993 (in press).
37. Michnovicz JJ, Bradlow HL: Induction of metabolism by dietary indole-3-carbinol in humans. J Natl Cancer Inst 82:947–949, 1990.
38. Bradlow HL, Michnovicz JJ, Telang NT, Osborne MP: Effect of dietary indole-3-carbinol on estradiol metabolism and spontaneous mammary tumors in mice. Carcinogenesis 12:1571–1574, 1991.