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Clinicopathologic Study of Basaloid Squamous Carcinoma of the Upper Aerodigestive Tract

The clinicopathologic and immunohistochemical characteristics of nine cases of basaloid squamous carcinoma (BSC) of the upper aerodigestive tract are reported, along with the results of an in situ hybridization for human papilloma virus (HPV) DNA. The cases were selected through a review of 237 head and neck carcinomas, and were located in the supraglottic larynx (5), hypopharynx (2), and the base of tongue (2). The patients were 7 males and 2 females with the mean age of 62. BSCs were histologically characterized by lobules and nests of basaloid cells with scanty cytoplasm, comedonecrosis and adenoid features, and by concomitant presence of squamous cell carcinoma. Immunohistochemically, all BSCs showed positivity for high molecular weight cytokeratin (HMW CK) with heterogeneous or diffuse staining pattern, but lacked reactivity for neuroendocrine markers and bcl-2 oncoprotein. No HPV DNA was detected in BSCs. This study reaffirms that BSC is a rare carcinoma with a peculiar topographic distribution and distinct pathologic features.

Key Words : *Carcinoma, Basal cell; Larynx; Hypopharynx; Keratin; Papillomavirus; Immunohistochemistry; In situ hybridization*

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INTRODUCTION

Basaloid squamous carcinoma (BSC) is a recently recognized variant of squamous cell carcinoma that most commonly arises in the upper aerodigestive tract. Since the first report on 10 cases of BSC of the tongue, hypopharynx and larynx by Wain et al. in 1986 (1), over 100 cases have been described in the head and neck region (2-10). Esophagus is another predilection site (11-14), and similar neoplasms have also been reported in the lung (15). BSC is known to present at an advanced clinical stage, and follow an aggressive course in some studies (1-5). In spite of its distinct histologic features, differential diagnosis from various carcinomas can always be a problem in the field of pathology. Etiologic factors such as tobacco and alcohol abuse seem to be linked to BSC (8), however, the role of viral infection in BSC has been unexplored. Associations between human papillomavirus (HPV) and carcinomas with basaloid features have been reported mostly in the anal location (16-18). In this study, we analyzed the clinicopathologic and immunohistochemical features of 9 cases of BSC of the upper aerodigestive tract that were found through a retrospective review of head and neck carcinomas. We highlight immunohistochemical features of BSC that allow its distinction from small cell carcinoma, neuroendocrine carcinoma, and basal cell carcinoma, and its relationship with HPV infection.

MATERIALS AND METHODS

The current study involved a series of 237 laryngectomy and/or neck dissection specimens from surgical pathology files of the Korea Cancer Center Hospital from 1988 to 1995. Nine cases of BSC were selected on the basis of histologic criteria proposed by Wain et al. (1), which included small crowded cells with hyperchromatic nuclei, scant cytoplasm, small cystic spaces, foci of tumor necrosis, and prominent hyalinosis. Clinical information of 8 cases were available from the hospital records regarding the patients' age, sex, medical and social history, treatment and follow-up status. Immunohistochemical study was performed on formalin-fixed paraffin-embedded 5 μm-thick sections. After being dewaxed in xylene, sections were rehydrated in graded alcohols, distilled water, and phosphate buffered saline. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol. In order to enhance the immunostaining, sections were microwave treated. Application of primary antibody was followed by an appropriate biotinylated linking antibody and avidin-biotin-peroxidase complex,

using microprobe method. The source of primary antibody and dilution are as follows: high molecular weight cytokeratin (34 βE12, 1:50, DAKO, Denmark), low molecular weight cytokeratin (35 βH11, 1:25, DAKO, Denmark), neuron specific enolase (1:50, DAKO, Denmark), chromogranin (1:100, DAKO, Denmark), synaptophysin (1:50, DAKO, Denmark), and bcl-2 (1:100, Santa Cruz, CA, U.S.A.). Chromogenic development was accomplished with 3, 3' diaminobenzidine. In situ hybridization for HPV DNA was performed on formalin-fixed paraffin-embedded tissue sections, using DNA probes for HPV DNA type 6/11, and 16/18 (Kreatech Diagnostics, Netherlands). After dewaxing and rehydration, treatment with RNase (Sigma, MO, U.S.A.), 0.1% pepsin solution (Research Genetics, AL, U.S.A.), and formamide (Sigma, MO, U.S.A.) was successively performed at 110℃ for 2-3 minutes. Hybridization with biotin-labeled probe reagent was proceeded at 110℃ for 10 minutes, at 85℃ for 5 minute, at 65℃ for 20 minutes, and at 50℃ for 20 minutes. After washing, the sections were reacted with streptavidin-alkaline phosphatase conjugate (Research Genetics, AL, U.S.A.) for 15 minutes at 50℃, and nitroblue tetrazolium for 10 minutes at 50℃. Washing and light counterstaining followed.

RESULTS

Clinical findings

The clinical features of nine patients are as summarized in Table 1. The nine patients included 7 men and 2 women with the age ranging from 52 to 74 years (mean 62). Alcohol use was recorded for 4 patients, and 7 patients were smokers. The presenting symptoms varied including palpable mass (n=4), hoarseness, dysphagia and dyspnea. The primary site of BSC were supraglottic larynx (n=5), hypopharynx (n=2) and base of the tongue (n=2). The patients presented at stage IV (n=6), stage III (n=1), stage II (n=1) and unknown stage (n=1). The treatment consisted of laryngectomy and/or neck dissection (n=4) and combined operation and radiation (n=4). Five out of 9 patients had metastases to regional lymph nodes at the time of operation. After a mean follow-up of 18.2 months, two patients died of pulmonary metastasis 7 and 15 months after first diagnosis, respectively. Four patients were alive without evidence of active disease. Three patients were lost to follow-up.

Pathologic findings

The size of neoplasms varied, being from 1.5 cm to 7 cm in diameters (Table 1). The tumors grossly showed protruding or polypoid (n=5), ulceroinfiltrative (n=1), or solid growth pattern (n=3) (Fig. 1). They were histologically composed of 2 types of cells. Basaloid cells had

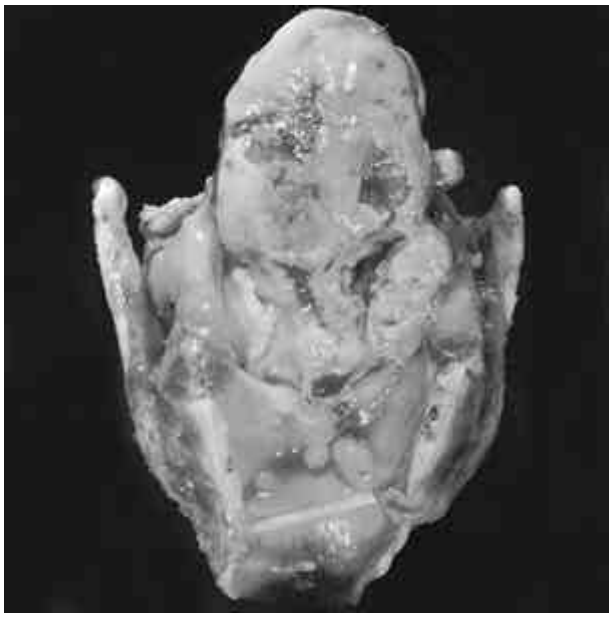

Fig. 1. Case 6, grossly showing bulky elevated tumor involving whole supraglottic larynx.

Table 1. The clinical features of basaloid squamous carcinoma

Age/sex	Alcohol	Smoking	Site	Size (cm)	Nature	Stage	Treatment	Metastasis	Follow-up
66/F	not known	not known	supraglottic	4	ulcerofungating	not known	TL	not known	lost (immed po)
74/M	not known	not known	base of tongue	3	exophytic	$T_2N_0M_x$, 2	biopsy	liver & lung (1 mo po)	lost (1 mo po)
55/M	+	+	hypopharynx	2	ulceroinfiltrative	$T_4N_0M_0$, 4	TL+RT	–	NED (43 mo)
61/M	–	+	supraglottic	1.5	polypoid	$T_2N_1M_0$, 3	TL	–	NED (37 mo)
66/M	+	+	supraglottic	3	protruding	$T_3N_{2b}M_x$, 4	TL+RT	lung (15 mo po)	DOD after 15 mo
54/M	+	+	supraglottic	7	solid	$T_4N_3M_0$, 4	TL+ND+RT	neck (at op)	NED (24 mo)
70/M	+	+	hypopharynx	2.5	solid	$T_4N_{2b}M_x$, 4	TL+ND	lung (7 mo po)	DOD after 7 mo
59/F	–	+	supraglottic	3	elevated	$T_4N_0M_0$, 4	TL+ND+RT	neck (at op)	NED (18 mo)
52/M	–	+	base of tongue	5	solid	$T_3N_{2b}M_0$, 4	RT+ND	neck (at op)	lost (1 mo po)

TL: total laryngectomy, RT: radiotherapy, ND: neck dissection, NED: no evidence of disease, DOD: died of disease, mo: months, po: post-operatively, op: operation, immed: immediately

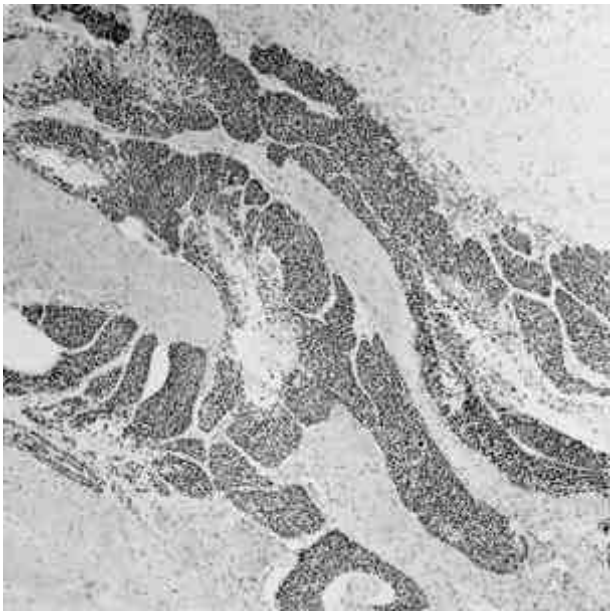

Fig. 2. Histologically, the neoplasms were composed of closely packed, moderately pleomorphic basaloid cells, forming variable sized nests with smooth margin.

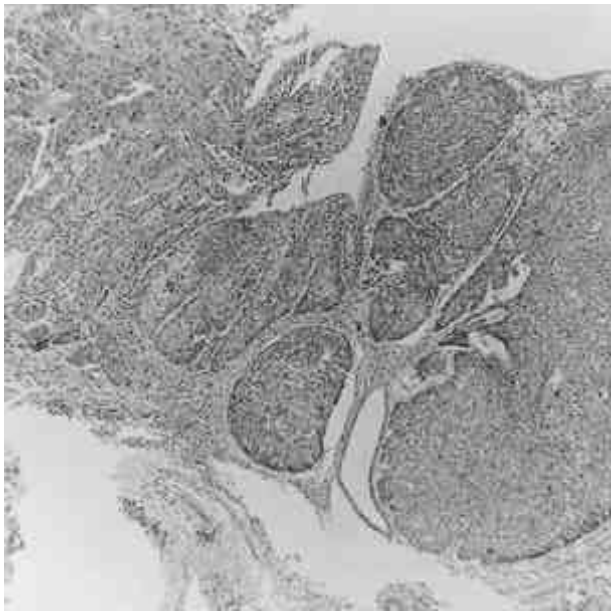

Fig. 4. Concomitant squamous cell carcinoma component neighboring basaloid squamous carcinoma.

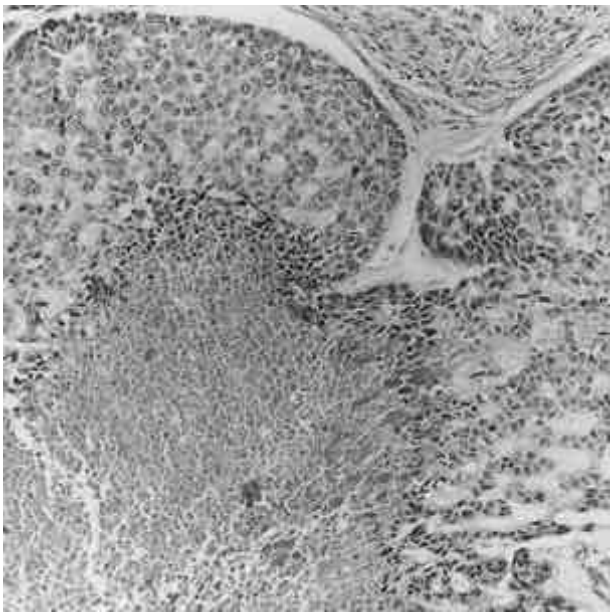

Fig. 3. Tumor cell lobules showing comedonecrosis and cribriform-like pattern.

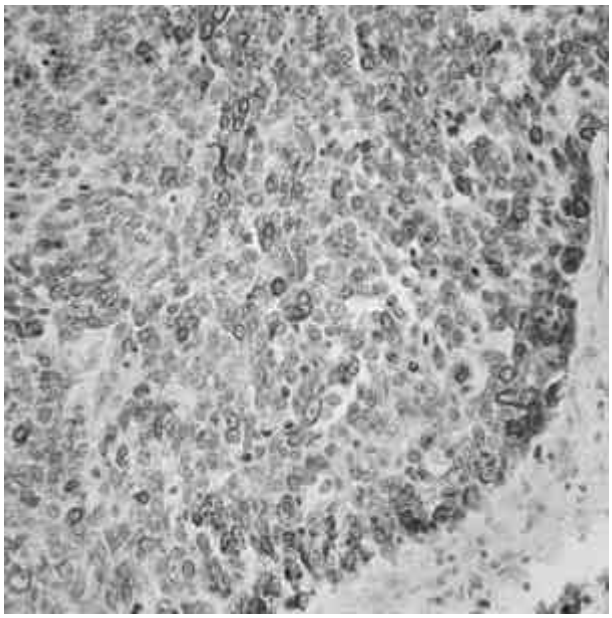

Fig. 5. Strong and heterogeneous staining for high molecular weight cytokeratin in basaloid squamous carcinoma.

high nuclear/cytoplasmic ratio, scanty cytoplasm, relatively monotonous round hyperchromatic nuclei, and formed variable sized lobules, nests and cords (Fig. 2). The lobules were smooth-margined, and frequently showed comedonecrosis, and adenoid features with gland-like spaces (Fig. 3). Concomitant squamous cell carcinoma in situ (n=1) or invasive squamous cell carcinoma (n=6) was noted (Fig. 4). In two cases including one biopsy material, we could not find any squamous components. Metastatic tumors in regional lymph nodes were either basaloid (1/5), squamous (3/5) or both (1/5).

Immunohistochemical findings and HPV DNA in situ hybridization

All cases of BSC showed diffuse or heterogeneous pos-

Table 2. Immunohistochemical findings and in situ hybridization for HPV DNA in basaloid squamous carcinoma

No. of case	HMW CK	LMW CK	Chromogranin	Synaptophysin	NSE	Bcl-2	HPV DNA
1	diffuse	–	–	–	–	–	–
2	diffuse	–	–	–	–	–	–
3	diffuse	–	–	–	–	–	–
4	heterogeneous	heterogeneous	–	–	–	–	–
5	heterogeneous	diffuse	–	–	–	–	–
6	heterogeneous	–	–	–	–	–	–
7	heterogeneous	focal	–	–	–	–	–
8	diffuse	–	–	–	–	–	–
9	heterogeneous	heterogeneous	–	–	–	–	–

HMW CK: high molecular weight cytokeratin, LMW CK: low molecular weight cytokeratin, NSE: neuron specific enolase, HPV: human papillomavirus, diffuse: diffuse positive staining, heterogeneous: heterogeneous positive staining

itivity either in squamous or in basaloid component for high molecular weight cytokeratin (HMW CK) (Fig. 5). HMW CK was diffusely positive in squamous component, however, strong but heterogeneous staining for HMW CK was a characteristic finding in basaloid component of five BSCs. Low molecular weight cytokeratin (LMW CK) was negative in 5 cases and showed focal (1/9), heterogeneous (2/9) or diffuse (1/9) positivity in basaloid areas of 4 cases, while squamous component revealed weak and consistent positivity. None of the tumors showed immunoreactivity for neuroendocrine markers including chromogranin, synaptophysin and neuron specific enolase. The bcl-2 oncoprotein expression was not found in all cases. In situ hybridization for HPV 6/11 and 16/18 failed to reveal HPV DNA in all tumor cells. The results are summarized in Table 2.

DISCUSSION

BSC is a rare and distinct variant of squamous cell carcinoma, mostly involving upper aerodigestive tract, especially hypopharynx, base of the tongue, floor of the mouth and larynx. Prognosis of this neoplasm was reported to be worse than that of conventional squamous cell carcinoma by some investigators (1-5, 11, 12), however, others described it as the same as squamous cell carcinoma (6, 7, 13). Microscopically, BSC is characterized by growth of solid lobules and nests of basaloid cells, with frequent comedo type necrosis, and concomitant presence of squamous cell carcinoma (1-10). Because of undifferentiated features of basaloid cells, BSC could be mistaken as small cell carcinoma. It also resembles adenoid cystic carcinoma, by featuring pseudoacinar formation and cribriform-like pattern. Neuroendocrine carcinoma is another differential diagnosis because of smooth-contoured nest formation by monotonous cells of BSC. For differential diagnosis and for documentation of the nature of basaloid cells, many immunohistochemical studies have been performed. Regarding cytokeratin activity, different results have been described. Many reported a lack of pancytokeratin or HMW CK reactivity in the basaloid component of BSC of the esophagus and lung (12-15). In contrast, BSCs of the larynx, pharynx or floor of the mouth tended to show strong reactivity for basal cell-type HMW CK (5, 6). Our study showed strong but heterogeneous membrane immunoreactivity for HMW CK (34βE12) in basaloid component. Yang and Lipkin have reported the different AE1 cytokeratin reaction patterns in different differentiation status of squamous cell carcinoma (19). It is interesting to note variable HMW CK reactivity on BSCs, which may reflect complex differentiation status of the neoplasm. LMW CK immunostaining also revealed irregular staining pattern.

We attempted to evaluate bcl-2 oncoprotein expression in BSCs. *Bcl-2* is a proto-oncogene located on chromosome 18 that encodes for a protein which protects cells from programmed cell death. The bcl-2 gene product is expressed in the basal cell of epithelia (20-22) and various normal cells with proliferating activity (21). As basal cell carcinoma is thought to arise from basal keratinocytes, bcl-2 expression is expected on basal cell carcinoma, and there have been many reports about bcl-2 expression in basal cell carcinoma of the skin (21-24). In BSCs, we could not observe unequivocal bcl-2 expression, in spite of consistent expression in the basal layer of non-neoplastic epithelium. This negative result may reflect either loss of bcl-2 with neoplastic transformation or absent relationship between BSC and basal cells of squamous epithelium.

The origin or histogenesis of BSC is controversial. Wain et al. (1) suggested that it may originate from a totipotential primitive cell with divergent differentiation. Others suggested that BSC represents collision tumor from two independently derived malignancies, including neuroendocrine, adenosquamous, and adenoid cystic car-

cinoma (25). Another proposal is that the cells of BSC derive from the basal cells of the squamous epithelium, which may be pluripotent (12). Our results on cytokeratin and bcl-2 immunohistochemistry suggest that the basaloid cells of BSC represent complex differentiation status of squamous cell carcinoma, and are less likely to be originated from the basal layer. Negative immunoreaction for neuron specific enolase, chromogranin and synaptophysin negates a possibility of neuroendocrine differentiation in BSC. This result is in concordance with previous reports (5, 10, 12, 13).

As causative factors of BSC, tobacco and alcohol abuse have been proposed (4). Seven and 4 out of 9 patients were smokers and drinkers, respectively, in our study. It was difficult to specifically link these factors with the BSC, since they are common risk factors in head and neck carcinogenesis. Wan et al. (9) described a case that may be related to prior radiation exposure but our study did not include patients with prior radiation history.

Human papillomavirus (HPV) is associated with a broad spectrum of epithelial cell proliferative processes. The association of HPV with neoplastic transformation of tissue has been widely explored in lesions of the uterine cervix and anorectal carcinoma. High prevalence of HPV DNA was reported in cases of basaloid carcinoma and pure squamous cell carcinoma of the anorectum (16). In situ hybridization for HPV DNA was found positive in cloacogenic carcinomas (17, 18). Our study showed negative results in all cases of BSC on in situ hybridization for HPV DNA. It suggests a negative relationship between BSC and HPV, and different carcinogenesis of BSC from anorectal and uterine cervix lesions.

Prognosis of BSC was reported to be worse than that of conventional squamous cell carcinoma by some investigators (1-5, 11, 12), however, others described it as the same as squamous cell carcinoma (6, 7, 13). Our study provided limited information about clinical course of BSC. Follow-up of larger series will be needed to determine the biologic behavior of BSC of the upper aerodigestive tract.

REFERENCE

1. Wain SL, Kier R, Vollmer RT, Bossen EH. *Basaloid squamous carcinoma of the tongue, hypopharynx and larynx: report of 10 cases. Hum Pathol 1986; 17: 1158-66.*
2. Luna MA, Naggar AE, Parichatikanond P, Weber RS, Batsakis JG. *Basaloid squamous carcinomas of the upper aerodigestive tract. Clinicopathologic and DNA flow cytometric analysis. Cancer 1990; 66: 537-42.*
3. Klijanienko J, El-Naggar A, Ponzio-Prion A, Morandas P, Micheau C, Cailland JM. *Basaloid squamous carcinoma of the head and neck. Immunohistochemical comparison with adenoid cystic carcinoma and squamous cell carcinoma. Arch Otolaryngol Head Neck Surg 1993; 119: 887-90.*
4. Raslan WF, Barnes L, Krause JR, Contis L, Kileen R, Kapadia SB. *Basaloid squamous carcinoma of the head and neck: A clinicopathologic and flow cytometric study of 10 new cases with review of the English literature. Am J Otolaryngol 1994; 15: 204-11.*
5. Coppola D, Catalano E, Tang CK, Elfenbein IB, Harwick R, Mohr R. *Basaloid squamous cell carcinoma of floor of mouth. Cancer 1993; 72: 2299-305.*
6. Banks ER, Frierson HF Jr, Mills SE, George E, Zarbo RJ, Swanson PE. *Basaloid squamous cell carcinoma of the head and neck. Am J Surg Pathol 1992; 16: 939-46.*
7. Ereno C, Lopez JI, Sanchez JM, Toledo JD. *Basaloid squamous cell carcinoma of the larynx and hypopharynx. A Clinicopathologic study of 7 cases. Path Res Pract 1994; 190: 186-93.*
8. Barnes L, Ferlito A, Alitavilla G, MacMillan C, Rinaldo A, Doglioni C. *Basaloid squamous cell carcinoma of the head and neck: clinicopathologic features and differential diagnosis. Ann Otol Rhinol Laryngol 1996; 105: 75-82.*
9. Wan SK, Chan JKC, Tse KC. *Basaloid squamous carcinoma of the nasal cavity. J Laryngol Otol 1992; 106: 370-1.*
10. Saltarelli MG, Fleming MV, Wenig BM, Gal AA, Mansour KA, Travis WD. *Primary basaloid squamous cell carcinoma of the trachea. Am J Clin Pathol 1995; 104: 594-8.*
11. Takubo K, Mafune K, Anaka Y, Miyama Y, Fujita K. *Basaloid-squamous carcinoma of the esophagus with marked deposition of basement membrane substance. Acta Pathol Jpn 1991; 41: 59-64.*
12. Abe K, Sasano H, Itakura Y, Nishihira T, Mori S, Nagura H. *Basaloid-squamous carcinoma of the esophagus. A clinicopathologic, DNA ploidy, and immunohistochemical study of seven cases. Am J Surg Pathol 1996; 20: 453-61.*
13. Sarbia M, Verrect P, Bittinger F, Dutkowski P, Heep H, Willers R, Gabbert H. *Basaloid squamous cell carcinoma of the esophagus. Diagnosis-Prognosis. Cancer 1997; 79: 1871-8.*
14. Tsang WYW, Chan JKC, Lee KC, Leung AKF, Fu YT. *Basaloid-squamous carcinoma of the upper aerodigestive tract and so-called adenoid cystic carcinoma of the esophagus: the same tumor type? Histopathology 1991; 19: 35-46.*
15. Brambilla D, Moro D, Veale D, Brichon PY, Stoebner P, Paramelle B, Brambilla C. *Basal cell (basaloid) carcinoma of the lung: A new morphometric and phenotypic entity with separate prognostic significance. Hum Pathol 1992; 23: 993-1003.*
16. Shroyer KR, Brookes CG, Markham NE, Shroyer AL. *Detection of human papillomavirus in anorectal squamous cell carcinoma. Correlation with basaloid pattern of differentiation. Am J Clin Pathol 1995; 104: 299-305.*
17. Heino P, Goldman S, Lagerstedtu U, Dillner J. *Molecular and serological studies of human papillomavirus among patients with anal epidermoid carcinoma. Int J Cancer 1993; 53: 377-81.*
18. Aparicio-Duque R, Mittal KR, Chan W, Schinella R. *Cloaco-*

genic carcinoma of the anal canal and associated viral lesions. An in situ hybridization study for human papillomavirus. Cancer 1991; 68: 2422-5.

19. Yang K, Lipkin M. *AE1 cytokeratin reaction patterns in different differentiation states of squamous cell carcinoma of the esophagus. Am J Clin Pathol 1990; 94: 261-9.*
20. Hockenbery DM, Zutter M, Hickey W, Nahm M, Korsmeyer SJ. *Bcl-2 protein is topographically restricted in tissues characterized by apoptotic cell death. Proc Natl Acad Sci USA 1991; 88: 6961-5.*
21. Lu QL, Poulsom R, Wong L, Hanby AM. *Bcl-2 expression in adult and embryonic non-haematopoietic tissue. J Pathol 1993; 169: 431-7.*
22. Rodriguez-Villanueva J, Colome MI, Brisbay S, McDonnell TJ. *The expression and localization of bcl-2 protein in normal skin and non-melanoma skin cancers. Path Res Pract 1995; 191: 391-8.*
23. Cerroni L, Kerl H. *Aberrant bcl-2 protein expression provides a possible mechanism of neoplastic cell growth in cutaneous basal-cell carcinoma. J Cutan Pathol 1994; 21: 396-403.*
24. Morales-Ducret CR, van de Rijn M, LeBrun DP, Smoller BR. *Bcl-2 expression in primary malignancies of the skin. Arch Dermatol 1995; 131: 909-12.*
25. McKay MJ, Bilous AM. *Basaloid-squamous carcinoma of the hypopharynx. Cancer 1989; 93: 2528-31.*