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Significance of keratin 13 and 6 expression in normal, dysplasic and malignant squamous epithelium of pyriform fossa

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Summary. It has been suggested that cytokeratin 13 is a useful marker of malignancy. We examined normal squamous cell epithelia, hyperplasia, dysplasias of various grades, intraepithelial neoplasia and invasive squamous cell carcinomas of the pyriform fossa using K13 and KL1. Positive staining for K13 was seen in all normal or hyperplastic benign epithelia, was inconstant in dysplasia, and intraepithelial neoplasia and carcinoma was negative. KL1 expression is constant and non significant. These results suggest that tumour cells are unable to synthesize keratin 13 a finding which may be valuable in surgical pathology.

Key words: Epithelial neoplasia – Immunoperoxydase – Cytokeratins – K13, KL1

Introduction

Keratins are fibrous polypeptides basically composed of 10 nm thick or intermediate-sized filaments, in almost all epithelial cells and tissues (Moll et al. 1982). Different keratin patterns have been found in different epithelia and in carcinomas (Moll et al. 1982; Cooper et al. 1985). Recently, Wild et al. (1987) found a difference between normal epithelium and invasive squamous cell carcinoma (SCC) in that SSC was unable to synthesize keratin 13. Precancerous lesions and their relationship to invasive SCC and normal epithelium have not been studied for the functional significance of keratin 13. We have investigated the cell and tissue distribution of two keratin antigens in lesions of the pyriform fossa using two monoclonal antibodies designated K13 and KL1; KL1 is a control (Viac et al. 1983). KL1 (Immunotech, ref. 0128) identifies the cytokeratin proteins of 56 kD and K13 (Progen, ref. K13.1) the cytokeratin proteins of 54 kD corresponding to number 6 and 13 respectively in the catalogue of human cytokeratins (Moll et al. 1982).

Immunohistochemical studies have demonstrated that KL1 is a sensitive and specific marker for neoplastic and non-neoplastic epithelium, reacting with keratin acid polypeptides in tissue sections. It recognises the suprabasal layer of keratinocyte cytoplasm of the epidermis and mucous membranes (Viac et al. 1983). K13 is specific for several stratified squamous epithelia such as exocervix, vagina, oral mucosa, tongue, oesophagus and urothelium of bladder. This antibody is reliable for the identification of various forms of squamous metaplasia and cell type heterogeneity (Moll et al. 1982; Brozman 1978).

Material and methods

Twenty-six normal squamous cell epithelia, 6 hyperplastic lesions, 13 cases of dysplasia 11 intraepithelial neoplasia (IN) and 14 head and neck SCC (9 well differentiated keratinizing, 5 poorly differentiated non keratinizing) were analysed for keratin expression using the immunoperoxidase method, and selected for a prospective study.

Surgical specimens of total pharyngolaryngectomy were obtained from patients not previously treated with radio or chemotherapy. The tissues were fixed in 4% buffered formalin and embedded in paraffin. Haematoxylin-eosin sections were examined to determine the nature of the lesion. Paraffin slides were stained with KL1 and K13 antibodies and a three-grade classification was used: strongly positive (more than 50% of cells), weakly positive (less than 50% of cells) and negative. The data were compared with the control group for keratin expression in normal and hyperplastic epithelia using the chi-2 test.

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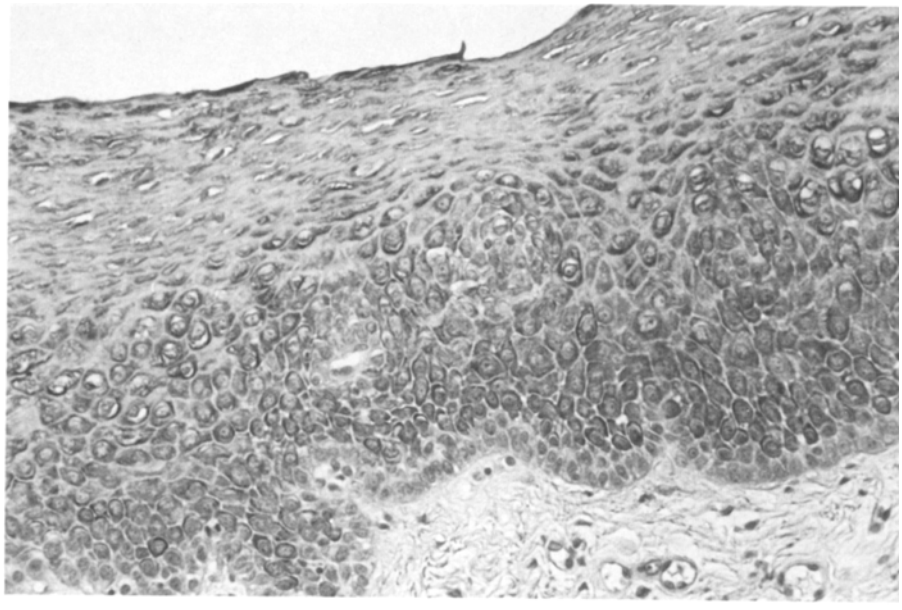

Fig. 1. Paraffin section of normal squamous epithelium immunostained with K13. Stained maturing cells and unstained basal layer can be easily identified (×400)

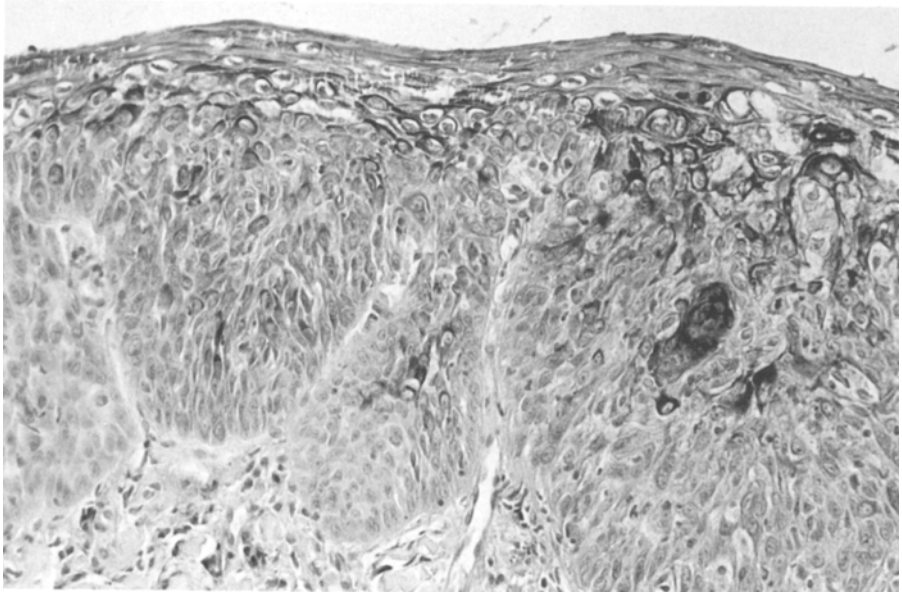

Fig. 2. Severe dysplasia. The highest K13 staining is found in normal residual cells whereas dysplasic cells are weakly positive. Several mitotic figures are seen (×400)

Results

A cases of normal epithelium and hyperplasia (32) were strongly positive in the normal suprabasal layer in K13 (Fig. 1), whereas 5 cases were weakly and 27 strongly positive in KL1.

Thirteen cases of dysplasia were studied (2 mild, 4 moderate and 7 severe) (WHO 1978). Seven were strongly positive (2 mild, 2 moderate, 3 severe), 5 weakly positive (2 moderate, 3 severe) and 1 (severe) negative in K13 (Fig. 2). Eight were strongly positive, 3 weakly positive and 2 negative (2 moderate) in KL1.

Eleven cases of IN were examined. There was no strongly positive case in K13, two cases showed weakly dispersed, single cell positivity of residual normal cells, 9 cases were negative (Fig. 3).

Staining with KL1 showed 9 strongly and 2 weakly positive cases.

Only 1 case of infiltrating SCC out of 14 was positive in K13, in well differentiated dispersed keratinizing areas. Poorly differentiated non keratinizing cells were negative. Staining with KL1 showed all cases positive (more than 80% of cells).

The probability for K13 was <0.001 for dysplasia, <0.0001 both for IN and SCC, and non significant for KL1.

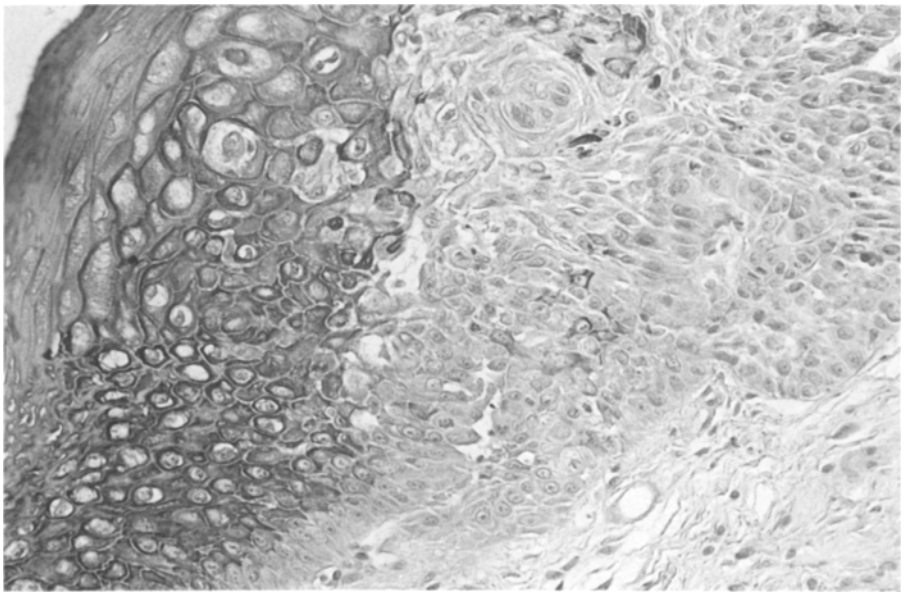

Fig. 3. Border between normal epithelium and in situ carcinoma. The normal cells are strongly stained, whereas all tumour cells are negative (K13, ×400)

Table 1. Distribution of keratins in various epithelial lesions of pyriform fossa

	Results	Antibody		K 13 *P* Value
		K L1	K 13	
Normal epithelium	+	21	26	Control
$n=26$	± and −	5	0	
Hyperplasia	+	6	6	group
$n=6$	± and −	0	0	
Dysplasia	+	11	7	<0.001
$n=13$	± and −	2	6	
In situ carcinoma	+	11	0	<0.0001
$n=11$	± and −	0	11	
Infiltrating carcinoma	+	13	1[a]	<0.0001
$n=14$	± and −	1	13	

\+ positive
± rare, weak or residual normal cell positivity
− negative

[a] in dispersed well differentiated keratinizing areas

Table 1 compares results obtained by staining a variety of lesions with KL1 and K13.

Discussion

From the results in our study we conclude that the absence of K13 in different precancerous and cancerous lesions is highly specific. The sensitivity of K13 in detecting invasive malignancy and IN may be useful for the differential diagnosis of difficult "border-line" cases. K13 expression is valuable in the differential diagnosis of malignancy, but it should not be used to confirm it. It seems that cancer cells lose some capacity for terminal keratin differentiation. The two keratins are possibly markers of differentiation, since in one case of SCC there was positivity in keratin pearls whereas non keratinizing cells were negative. However, 8 well differentiated and keratinizing SCC were negative.

Keratin expression in the normal and malignant upper aerodigestive tract squamous epithelium has been monitored by high resolution gel electrophoresis and immunohistochemical techniques (Moll et al. 1982; Cooper et al. 1985). Recently, Wild et al. (1987) found a particular difference between normal epithelium and invasive highly proliferative tumour cells, in that the latter were unable, in most cases, to synthesize keratins 4 and 13. Electrophoretic analysis (Pellegrino et al. 1988) has indicated that keratin 13 may be useful in distinguishing between normal and benign and malignant states of mammary epithelium. Chain-specific cytokeratin antibodies are useful tools for classifying epithelia and carcinomas. The K13 distribution in epithelial lesion described here may be of value in the diagnosis of metastases derived from SCC, especiallyin cases where the location of the primary tumour is not known (Van Muijen et al. 1986).

It will be interesting to study disturbances in cytokeratin composition in more dysplasias and in lymphadenopathies without primary using these antibodies.

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