Epithelial markers in the diagnosis of nasopharyngeal carcinoma: an immunocytochemical study

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SUMMARY Immunocytochemical stains for three epithelial cell markers—keratin, epithelial membrane antigen (EMA) and carcinoembryonic antigen (CEA)—have been examined on paraffin-embedded material from 14 patients with nasopharyngeal carcinoma (NPC). Tumour cells staining positively for keratin were found in all cases and for EMA in eight; two tumours contained CEA-positive cells. Seven cases of Hodgkin's disease and 24 non-Hodgkin's lymphomas were uniformly negative. Keratin is the most reliable epithelial marker for identifying NPC and excluding lymphoma. The regular finding of stainable keratin in non-keratinising and anaplastic NPC supports the view that NPC is a homogeneous group exhibiting variable degrees of squamous differentiation.

Antibodies to keratins have been shown to be of diagnostic value in the assessment of the epithelial nature of both primary and metastatic poorly differentiated neoplasms.^{1,2} Anti-keratin antibodies have proved of particular value in distinguishing thymomas from lymphomas³ and as tools to study the cellular heterogeneity of both normal and malignant epithelial cells in both the lung⁴ and the breast.⁵ Another epithelial marker, epithelial membrane antigen (EMA), has also proved to be a useful reagent in the diagnostic dilemma of the undifferentiated tumour.⁶

In this paper we have studied the potential usefulness of these epithelial markers—keratin, epithelial membrane antigen and carcinoembryonic antigen—as diagnostic aids to differentiate between lymphomas and nasopharyngeal carcinomas.

Material and methods

IMMUNOCYTOCHEMICAL REAGENTS

Keratin was extracted from human carcinomas by the method of Sun and Green.⁷ Antisera were raised by dissolving 1 mg of keratin in 500 μ l 1 M urea and 5 μ l of mercaptoethanol in phosphate-buffered saline, preparing an emulsion with 1 ml Freund's complete adjuvant, and injecting it subcutaneously into the nuchal region of rabbits at monthly inter-

vals. The resultant antisera gave a single immunoprecipitation band on Ouchterlony diffusion against purified keratin. Details of antibodies to epithelial membrane antigen (EMA) and carcinoembryonic antigen (CEA) have been previously described.^{8,9} Second antibodies were obtained by affinity purification of a sheep antirabbit gamma globulin, and conjugated to alkaline phosphatase using glutaraldehyde.¹⁰

STAINING PROCEDURE

A detailed account of the method used has been reported.¹¹

Sections were cut from formalin-fixed paraffin wax-embedded material at approx 4 μ m and dewaxed in xylene. The sections were treated with 15% acetic acid for 15 min to block endogenous alkaline phosphatase activity, incubated with the appropriate antiserum diluted 1/100 for 90 min, washed and incubated with the enzyme-conjugated second antibody for a further 90 min. The stain was developed using AS:B1 phosphoric acid plus Fast Red TR. The slides were counterstained with Mayer's haemalum and mounted in buffered glycerin jelly.

Specificity of staining was confirmed by absorption of each antiserum with the appropriate antigen.

Results

The patterns of staining with keratin, EMA and

Patterns of immunocytochemical staining for epithelial markers in nasopharyngeal carcinomas and in lymphomas

	Incidence of positive markers		
	Keratin	Epithelial membrane antigen	Carcinoembryonic antigen
Nasopharyngeal carcinomas, primary n = 8	8	4	0
Nasopharyngeal carcinomas, metastatic n = 6	6	4	2
Non-Hodgkin's lymphoma* n = 24	0	0	0
Hodgkin's disease n = 7	0	0	0

*Comprise histiocytic (8), poorly differentiated lymphocytic (6), immunoblastic (5), well differentiated lymphocytic (3), Burkitt (2). Tumours classified according to a modified Rappaport system.¹⁹

Fig. 1 Non-keratinising nasopharyngeal carcinoma stained with antikeratin antibodies. There is strong positive staining of all tumour cells with strong staining of focal areas. Stroma is negative. $\times 300$

CEA in 14 patients with nasopharyngeal carcinomas are summarised in the Table: control data from 31 patients with lymphomas are also included. Positive staining was confined to NPC, the lymphomas examined being uniformly negative. All NPC contained keratin-positive tumour cells, and eight tumours also contained EMA-positive elements. CEA-stained tumour cells were seen in only two cases. Keratin staining was, predictably, most marked in tumours with keratin pearls but it was also demonstrated in non-keratinised and undifferentiated forms. Positively-staining cells showed a characteristically patchy distribution in the tumours,

and staining in individual cells was confined to the cytoplasm (Figs. 1 and 2). EMA-positive tumour cells showed a similar patchy distribution with staining concentrated along cell borders or more diffusely in the cytoplasm. In the undifferentiated NPC group the staining was weaker and focal but in all cases positive cells were identified.

Discussion

The recognition of primary and metastatic NPC can be difficult,¹²⁻¹⁴ despite the clarified diagnostic criteria proposed in the recent WHO classification of these tumours;¹⁵ the continuing problem is the distinction between anaplastic NPC and lymphoma. Electron microscopy may be helpful¹⁴ but appropriately fixed tissues are often not taken at the time of biopsy. Immunocytochemical studies of epithelial cell markers can be readily performed on formalin-fixed and paraffin wax-embedded tissues, and provide a useful diagnostic aid. Keratin staining in NPC, briefly noted by Costa,¹⁴ has now been described in more detail in a recent survey of 40 cases by Madri and Barwick.¹⁶ Our results confirm the value of keratin staining in the recognition of NPC; we also show that staining with two other epithelial markers—EMA and CEA—is less reliable. When considering the general diagnostic problem of differentiating between anaplastic carcinoma and large cell lymphoma, epithelial membrane antigen is more reliable than the anti-keratin antiserum.²⁰ In cases of poorly differentiated squamous cell carcinomas however, keratin is usually demonstrable. The fact that keratin is regularly demonstrated in the non-keratinised and anaplastic forms of NPC strengthens the view that these tumours are a homogeneous group exhibiting varying degrees of squamoid differentiation.^{17,18}

Addendum

In a separate study, one of us (RLC) has investigated EMA— and CEA— staining in biopsies of

Fig. 2 Undifferentiated nasopharyngeal carcinoma positively stained with antikeratin antibodies. $\times 450$

nasopharyngeal carcinoma (NPC) from Guangxi, a high-incidence region in the People's Republic of China. The work was carried out in collaboration with Dr Zeng, Dr Li and Dr G de Thé. Among 15 examples of NPC, nine stained positively with EMA and only one with CEA. Local *uninvolved* mucosa was frequently positive for both antigens (EMA 8/10, CEA 4/10). Mucosal staining was also positive in most of 15 control biopsies from patients with chronic nasopharyngeal inflammation (EMA 15/15, CEA 9/15). An association was noted in both tumour and control biopsies between positive staining for EMA and CEA and local chronic inflammation. Scattered foci of mild mucosal dysplasia, present in both series, did not "mark" with EMA or CEA in any distinctive fashion.

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