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K.Y. Lam · S.L. Loke · X.C. Shen · L.T. Ma

Cytokeratin expression in non-neoplastic oesophageal epithelium and squamous cell carcinoma of the oesophagus

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Abstract The expression of cytokeratins (CK) 19, 8, 18, 13, 10 and 7 was examined in 35 cases of squamous cell carcinomas of the oesophagus (10 well-differentiated, 13 moderately-differentiated, and 12 poorly-differentiated) and the adjacent mucosa by means of a panel of monoclonal antibodies on frozen sections. The study was undertaken to assess the pattern of expression of these keratins in oesophageal tumours and its relation to the degree of differentiation. The normal oesophageal epithelia expressed CK19 in 86%, CK18 in 17% and CK13 in 14% of cases. CK8, CK10 and CK7 immunoreactivity was not observed. The tumours expressed CK19 in 86%, CK8 in 46%, CK18 in 97%, CK13 in 83%, CK10 in 34% and CK7 in 29% of cases. Thus, the so-called simple epithelial markers CK18 and CK19 occurred in the majority of oesophageal squamous cell carcinomas. CK13 (the so-called non-keratinizing squamous epithelial marker) was only infrequently demonstrated in the non-neoplastic oesophageal mucosa, and its expression was more frequent in carcinomas. CK10 was not demonstrated in non-neoplastic mucosa, but was mostly associated with well-differentiated carcinomas. We therefore conclude that the pattern of expression of cytokeratins in oesophageal carcinomas is different from that in normal oesophageal epithelia and varies with differentiation.

Key words Cytokeratins · Oesophageal carcinoma · Immunohistochemistry

Introduction

Keratins (cytokeratins) are the intermediate filament-forming cytoskeletal proteins. At present, 30 keratin polypeptides have been identified [8]. There are 20 keratins (CK1 to CK20) known as epithelial (soft) keratins, and 10 more recently discovered ones that are called hard keratins. The latter are mostly seen in hair- and nail-forming epithelia. The classification and numbering system for keratins (except those of hair and nail) is based on the catalogue of Moll [9]. The different soft keratins are divided into two subfamilies; type I (acidic) keratin (CK9 to CK20) and type II (basic) keratin (CK1 to CK8), according to molecular weight and isoelectric pH value. Keratin filaments are formed by tetrameric heteropolymers of two different sorts of keratins, two from type I and two from type II. Thus, any epithelial cell contains at least two keratins. Studies have further shown that keratins have characteristic topographic and type expression in different epithelia [3, 8]. Tumours arising from the epithelia usually retain some of the keratins of their normal progenitor cells and they may develop some new keratins [13].

Oesophageal cancers are tumours with a high mortality rate and their incidence is high in oriental populations, such as the people of China and Hong Kong [11, 12]. About 90% of these cancers are squamous cell carcinomas [6]. Keratins are the major constituent of the oesophageal epithelia [8]. Although several laboratories have described the pattern of keratin expression in human oesophageal epithelia and squamous cell carcinomas of the oesophagus, the number of cases described in these studies are low and no immunohistochemical profiles of oesophageal carcinomas have yet been performed [1, 2, 4, 5, 7, 9, 14, 15, 17]. In the present study, we report the expression of cytokeratins in the normal oesophageal epithelium and oesophageal carcinomas to assess: the ease of demonstration of these cytokeratins in normal mucosa, the difference in their expression between normal mucosa and tumours, and its relation to the degree of tumour differentiation.

Materials and methods

Fresh tissue samples were collected prospectively from primary squamous cell carcinomas of the oesophagus resected in Queen Mary Hospital in the period from 1989 to 1991. They were taken from the surgical specimens obtained at oesophagectomy from patients not previously treated with radio- or chemotherapy. One representative block from the tumour and one from the non-neoplastic

K. Y. Lam (✉) · S.L. Loke · L.T. Ma
Department of Pathology, University of Hong Kong, Queen Mary Hospital, Hong Kong

epithelial tissue at a distance from the tumour were taken in each case. These were snap-frozen in isopentane cooled in liquid nitrogen at -70°C until use. The major parts of the specimens were further processed through paraffin after routine fixation in 10% formalin for light microscopic analysis. The pathology of the tumour tissues was reviewed. The blocks chosen from the tumours were assessed histologically to ensure relative homogeneity of differentiation throughout the blocks. The carcinomas were graded according to the WHO criteria [16].

A panel of commercially available monoclonal antibodies against cytokeratins was used: CK19 (RPN 1165, 1:10, Amersham, England), CK8 (RPN1166, 1:10, Amersham), CK18 (MON3006, 1: 25, Monosan, The Netherlands), CK13 (C0791, 1:100, Sigma, USA), CK10 (MON3010, 1:10, Monosan), CK7 (MON3007, 1:10, Monosan).

The alkaline phosphatase anti-alkaline phosphatase (APAAP) technique was used in this study. Cryostat sections 6 μm thick were cut from the frozen tissues. The air-dried and acetone-fixed sections were treated with 10% normal rabbit serum to block the non-specific binding, followed by incubation with the primary antibodies at appropriate dilutions for 30 min at room temperature. Biotinylated rabbit anti-mouse IgG (Z259, Dakopatts, Denmark) and APAAP (D651, Dakopatts) diluted at 1:50 and 1:100 respectively were each applied to the sections for 30 min. Naphthol AS–BI phosphate–new fuchsin was used as the detection system. Endogenous alkaline phosphatase was inhibited by addition of levamisole (L9756, Sigma, USA) at a dilution of 0.47 mg/ml to the phosphatase substrate solution. Negative controls were sections treated as above but with omission of the primary antibodies. Thereafter, the sections were counterstained with Mayer's haematoxylin, dehydrated, cleaned and mounted.

The distribution of positive staining was noted and the intensity of staining was recorded semi-quantitatively. The degree of cytokeratin staining was scored as -(negative), + (less than 50% of the cells were positive) or ++ (extensive staining, i.e., more than 50% of the cells positive). On some occasions, sporadic positivity was noted separately, representing less than 5% of the positive cells.

Results

In all, 35 primary squamous cell carcinomas (10 well differentiated, 13 moderately differentiated and 12 poorly differentiated) of the oesophagus were analysed. The mean age of the patients (31 men and 4 women) was 63 (ranging from 40 to 89) and the mean size of these tumours was 5 cm (ranging from 2 to 10 cm).

The results of cytokeratin staining in the oesophageal tumours and non-neoplastic oesophageal epithelia are compiled in Tables 1 and 2. In the non-neoplastic oesophageal epithelia, 86% of the cases were positive for CK19. However, the staining was restricted to the basal cells, and CK7, CK8 and CK10 staining was also not identified in the normal oesophageal epithelium in any of these cases. Though a few cases were positive for CK13 and CK18 (14% and 17% respectively), the staining in these cases was weak and focal. However, the cytokeratins were highly expressed in a high proportion of the squamous cell carcinomas. CK19, CK8, CK18, CK13, CK10 and CK7 were seen in 86%, 46%, 97%, 83%, 34% and 29% of oesophageal tumours, respectively. The staining patterns of the individual antibodies were as follows:

CK19 was frequently expressed in oesophageal squamous cell carcinomas, especially the poorly differentiat-

Table 1 The percentage of positive cases in carcinoma of the oesophagus and normal oesophageal epithelia

Tissue	Percentage of positive cells					
	CK19	CK8	CK18	CK13	CK10	CK7
WD (*n*=10)	8/10 (80%)	5/10 (50%)	10/10 (100%)	8/10 (80%)	5/10 (50%)	3/10 (30%)
MD (*n*=13)	10/13 (77%)	5/13 (38%)	13/13 (100%)	10/13 (77%)	6/13 (46%)	4/12 (33%)
PD (*n*=12)	12/12 (100%)	6/12 (50%)	11/12 (92%)	11/12 (92%)	1/12 (8%)	4/12 (38%)
T (*n*=35)	30/35 (86%)	16/35 (46%)	34/35 (97%)	29/35 (83%)	12/35 (34%)	10/35 (29%)
N (*n*=35)	30/35 (86%)	0/35 (0%)	6/35 (17%)	5/35 (14%)	0/35 (0%)	0/35 (0%)

WD: well-differentiated squamous cell carcinoma; MD: moderately-differentiated squamous cell carcinoma PD: poorly-differentiated squamous cell carcinoma; T: carcinoma as a whole; N: normal oesophageal epithelium at a distance from the carcinoma

Table 2 The degree of staining of tumour cells by different cytokeratin antibodies

Type	Number of cases positive																	
	CK19			CK8			CK18			CK13			CK10			CK7		
	–	+	++	–	+	++	–	+	++	–	+	++	–	+	++	–	+	++
WD (*n*=10)	2	2	2	5	4	1	0	3	7	2	4	4	5	5	0	7	1	2
MD (*n*=13)	3	1	9	8	5	0	0	2	11	3	4	6	7	5	1	10	3	0
PD (*n*=12)	0	2	10	6	5	1	1	0	11	1	6	5	11	0	1	8	2	2
Total	5	5	25	19	14	2	1	5	29	6	14	15	23	10	1	25	6	4

WD: well-differentiated squamous cell carcinoma; MD: moderately-differentiated squamous cell carcinoma. PD: poorly-differentiated squamous cell carcinoma; – (negative staining), + (less than 50% of the cells were positive); ++ (extensive staining, i.e. more than 50% of the cells were positive)

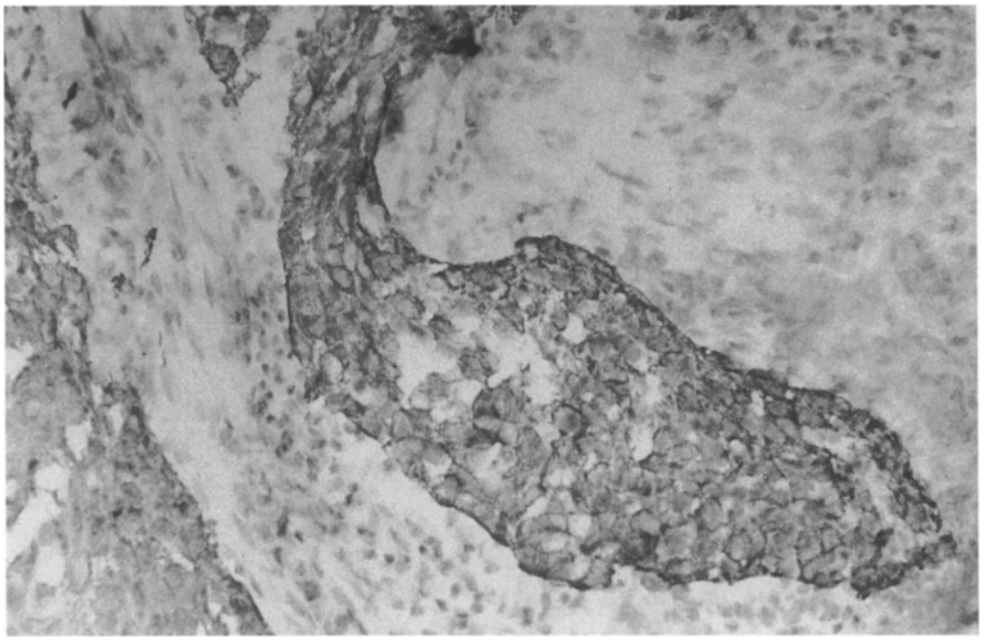

Fig. 1 CK 13 expression in a moderately-differentiated squamous cell carcinoma, showing that the tumour cells were extensively stained. ×125

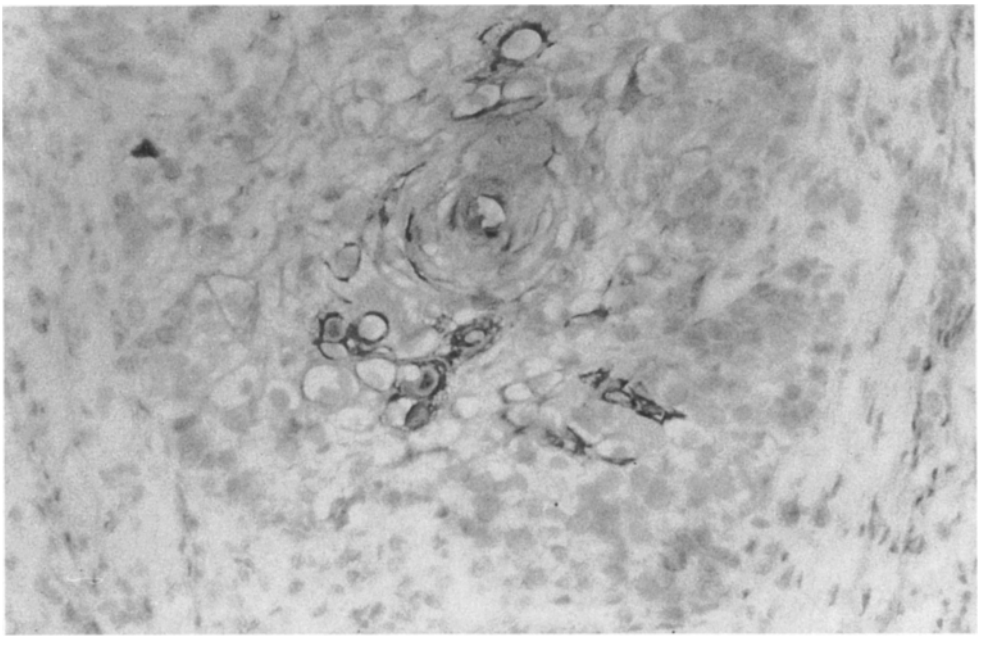

Fig. 2 CK 10 expression in keratin pearls of well-differentiated squamous cell carcinoma. ×250

ed ones (80% in well-differentiated, 77% in moderately differentiated and 100% in poorly differentiated carcinomas). Most of the positive cases (25 out of 30 positive) were extensively stained by the antibody.

CK8 was demonstrated in less than half (46%) of the tumours. Also, most of the positive cases (14 out of 16 cases) were only focally positive.

CK18 was demonstrated in all except one cases of squamous cell carcinomas. Most of the positive cases were extensively stained by the antibody (29 of the 34 cases).

CK13 was only infrequently demonstrated in non-neoplastic mucosa, often in the suprabasal regions, and its expression increased in carcinomas (8 of the 13 well-differentiated, 10 of the 13 moderately differentiated and 11 of the 12 poorly differentiated carcinomas), irrespective of the degree of tumour differentiation. Both focal (14 cases) and extensive (15 cases) staining by the antibody was observed (Fig. 1).

CK10 was not demonstrated in the non-neoplastic mucosa, but was noted mostly in the well-differentiated squamous cell carcinomas. It was demonstrated in 5/10

(50%) well, 6/13 (46%) moderately, and 1/12 (8%) poorly differentiated carcinomas. The positive staining was mostly associated with keratin pearls (Fig. 2) and was mostly focal (in 10 of the 11 positive cases under 50% of tumour cells were positive).

CK7 was not demonstrated in the non-neoplastic mucosa, but was seen in the glandular ducts of the oesophagus. It was noted in only 29% (10 out of 35) of the oesophageal squamous cell carcinomas. Both extensive (6 cases) and focal staining (4 cases) was identified.

Discussion

In the present study, we investigated the expression of different cytokeratins in oesophageal tissues using a panel of monoclonal antibodies in order to extend our understanding of the expression patterns of cytokeratins in squamous cell carcinomas of the oesophagus. We hoped this might also yield some insight into the programme of differentiation of carcinomas, as the immunohistochemical demonstration of cytokeratins forms an important basis for assigning tumour histogenesis. Studies have shown that cytokeratins are not consistently demonstrable in formalin-fixed paraffin sections because the keratin epitopes can be irreversibly masked [8]. Thus, we used frozen tissues in this study in order to avoid false-negative results.

Markers for simple epithelia include low-molecular-weight cytokeratins CK19, CK8, CK18 and CK7 [3]. CK 19 is normally present in simple epithelia and occasionally in minor amounts in the basal layer of some squamous epithelia. In the present study, we demonstrated that it was confined to the basal layer of the normal oesophageal epithelium and was frequently expressed in high intensity in oesophageal squamous cell carcinomas, especially the poorly-differentiated ones. Therefore, diffuse staining by CK19 is a marker of high-grade oesophageal squamous cell carcinomas. CK8 and CK18 are largely co-expressed as a pair in simple epithelium [13]. They are the first keratins to appear in embryonic development. It has been stated that very low levels of these keratins can be detected in the basal cells of the oesophageal epithelia [13]. CK8 and CK18 were also found to be expressed in 5 and 6 out of the 6 cases of oesophageal carcinomas reported by Schaafsma's group [14]. In the present study, CK18 was found in 17% of the normal oesophageal epithelia, while CK8 was not detected. However, expression of both CK18 and CK8 was more intense in oesophageal squamous cell carcinomas. This was especially true for CK18; nearly all (97%) of the tumours were found to express this keratin. The carcinoma staining was mostly focal for CK8 but very extensive for CK18. The high expression of CK18 probably also suggests that it might be a useful marker for oesophageal squamous cell carcinomas. Furthermore, CK7 expression was not seen in any case of normal oesophageal epithelia and was infrequently noted in the tumours (29%). These results indicate that oesophageal cancers show variable expression of these markers to simple epithelia.

CK10 is a high-molecular-weight keratin. The keratin was chosen in this study as an epidermis-related cytokeratin, as it is a marker for keratinizing squamous epithelia [8]. It was not demonstrated in the normal oesophageal epithelia in the current survey. In carcinomas, the expression was found to be related to the degree of differentiation, and staining was seen to be mostly focal and in the keratinizing areas. It could thus be used as a marker for keratinizing squamous carcinomas. In addition, this suggests that the programme of differentiation recognized and graded on histology is not towards normal oesophagus but resembles the epidermis.

CK13 is a keratin for non-keratinizing squamous epithelia of internal organs such as the oesophagus [8]. However, the marker was not consistently demonstrable by immunostaining in the present study, probably indicating the limitation of sensitivity of the technique. In squamous cell carcinomas, CK13 was expressed in a variable manner (with both diffuse and focal staining noted). In our experience the marker was found in 83% of tumours, while Malecha found focal positivity for CK13 in four out of the six cases in their study [7].

Our data show that the expression of the cytokeratins is tightly controlled in oesophageal epithelia and that the expression of cytokeratins in carcinomas lacks the zonal pattern, suggesting that it is less tightly controlled in tumours. In addition, the cytokeratin expression pattern in the tumours may resemble both simple and complex epithelia. Thus, we conclude that pattern of expression of cytokeratins in oesophageal carcinomas is different from that in normal oesophageal epithelia and varies with differentiation.

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