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Comparison of commercially available cytokeratin antibodies in normal and neoplastic adult epithelial and non-epithelial tissues

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Abstract

Five commercially available cytokeratin antibodies (lu-5, AE1/AE3, CAM 5·2, MFN116 and anti-cytokeratin 18) were used to stain a wide range of normal and neoplastic epithelial and non-epithelial tissues to assess their potential value in diagnostic histopathology. All five showed good specificity, with some cross-reactivity in smooth muscle cells. The wider reactivity of AE1/AE3, lu-5, and MFN 116, which includes cytokeratins 8,18 (Moll's catalogue) expressed in simple epithelia and their tumours, as well as cytokeratins expressed in complex stratified squamous epithelia, permits identification of a wider range of epithelial derived tumours.

This wider spectrum of reactivity may allow these antibodies to be used in a diagnostic panel for the identification of poorly differentiated tumours.

Immunohistochemistry has become increasingly important in identifying tumours which cannot be readily classified on morphological grounds.[1-4]

Cytokeratins occur in most normal epithelial tissues and antibodies against them have been used to characterise a wide variety of epithelial tumours. For routine histopathological diagnostic use, it is essential to use an antibody which recognises an antigen on the cytokeratin molecule which survives routine tissue fixation and embedding procedures. A cytokeratin antibody that can identify all epithelial types must react with the cytokeratins found in simple epithelia (8, 18, 19—Moll's catalogue) as well as these found in complex stratified squamous epithelia (1, 4, 5, 6, 10, 11, 13, 14 predominantly Moll's catalogue). Several monoclonal antibodies are now commercially available for this purpose. Of these, CAM 5·2 (Becton-Dickinson) and AE1/AE3 (ICN Biomedicals Ltd) have become the most widely used. More recently available cytokeratin antibodies have included lu-5 (Hoffman—La Roche), MFN 116 (Dako) and anti-cytokeratin 18 (Sigma).

CAM 5·2 is a murine monoclonal antibody raised by Makin *et al* in 1984,[5] against the colonic carcinoma cell line HT29. It is an IgG2a immunoglobulin which recognises the low molecular weight cytokeratins 8, 18, and 19 in Moll's Catalogue.[6]

AE1/AE3 is a mixture of two monoclonal antibodies, raised against human epidermal keratins.[7] AE1 recognises most of the acidic (type I) cytokeratins, while AE3 recognises all known basic (type II) cytokeratins.[8] This combination shows broad reactivity and is claimed to stain almost all epithelia and their neoplasms.[9]

lu-5 recognises an intra-cytoplasmic formaldehyde resistant epitope which is located on the surface of cytokeratins but not on other cytoskeletal filaments.[10] The epitope is not reactive after sodium dodecyl sulphate polyacrylamide gel electrophoresis, but can be identified in reconstituted purified proteins, suggesting that it is conformation dependent. The epitope is present on most cytokeratin polypeptides of both the acidic (type I) and basic (type II) subfamilies. This IgG antibody was raised by von Overbeck *et al* in 1985[11] by intra-peritoneal immunisation of mice with the lung cancer cell lines A549 and A2181 (National Cancer Institute, Bethesda).

MFN 116 is a monoclonal antibody raised against MCF-7 cells in mice. As yet it is poorly characterised but immunoblotting has shown reactivity against cytokeratins 10, 17 and 18 (Specification sheets for MFN 116, Dakopatts).

Monoclonal anti-cytokeratin peptide 18 is a mouse monoclonal IgG1 antibody which recognised the KS-B172 epitope in cells, and which reacts with a wide variety of simple epithelia.[12]

Currently available data do not permit ready comparison of the relative advantages and disadvantages of these cytokeratin antibodies. We therefore analysed the staining of a wide range of normal and neoplastic epithelial and non-epithelial tissues with these cytokeratin antibodies.

Methods

Formalin fixed, paraffin wax embedded sections of normal and neoplastic tissue were obtained from the files of Addenbrooke's Hospital, Cambridge. Sections (5 μm) were stained using the five antibodies listed (table 1). Staining was performed using the streptavidin-biotin method. Endogenous peroxidase activity was inhibited using 0·5% hydrogen peroxide for 10 minutes. The sections were predigested with 0·1% trypsin solution at 37°C for 10 minutes.

Background staining was reduced by the

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Table 1 Antibodies used for the study

Antibody	*Dilution*	*Source*
CAM 5·2	1 in 10	Becton-Dickinson
AE1/AE3	1 in 30	ICN Biomedicals
lu-5	1 in 50	Hoffman-La Roche
MFN 116	1 in 50	Dako
Anti-cytokeratin 18	1 in 50	Sigma

addition of 10% human serum to the second and streptavidin stages. Negative controls, omitting the primary antibody, were performed to monitor background staining. Optimal dilutions were obtained by staining a control section of normal human breast tissue at a number of serial dilutions and the optimal dilution (table 1) used for subsequent staining.

Results

FIXED NORMAL ADULT TISSUES

The results of staining of normal adult tissues are summarised in table 2. CAM 5·2 and anti-cytokeratin 18 showed a broadly similar pattern of staining, with reactivity for simple epithelia. The intensity of staining with anti-cytokeratin 18 was less than with CAM 5·2. Neither antibody stained stratified squamous epithelia of skin, oesophagus, or ectocervix. They differed only in their reactivity with urothelium, where anti-cytokeratin 18 stained only weakly the superficial layer of the urothelium, and hepatocytes, where only weak staining was observed with anti-cytokeratin 18.

In glandular epithelia staining for cytokeratins was not uniform throughout glands, with more intense staining being seen in the base of glands and with more superficial cells showing staining in the basal portion of the cell.

The remaining three antibodies AE1/AE3, lu-5, and MFN 116 showed a strong pattern of staining with all simple epithelia, but also stained the stratified squamous epithelia. Overall, AE1/AE3 and lu-5 were more consistent in staining pattern and intensity than MFN 116.

All the antibodies, with the exception of anti-cytokeratin 18 showed some staining of smooth muscle cells, which was most pronounced in myometrium and the muscularis of the stomach wall.

Table 2 Staining of normal adult tissues

Tissue	*CAM 5·2*	*AE1/AE3*	*LU-5*	*MFN116*	*Cytokeratin-18*
Endothelium	−	−	−	−	−
Skeletal muscle	−	−	−	−	−
Smooth muscle	+/−	+/−	+/−	+/−	−
Cartilage	−	−	−	−	−
Lymphoid tissue	−	−	−	−	−
Skin					
epidermis	−	++	++	++	−
appendages	++	++	++	++	+
Breast					
ducts	++	++	++	++	+
acini	++	++	++	++	+/−
Kidney					
proximal convoluted tubules	++	++	++	++	+
distal convoluted tubules	++	++	++	++	+
Bowman's capsule	++	++	++	++	+
Oesophageal epithelium	−	++	++	++	−
Gastric epithelium (A)	++	++	++	++	+
Small intestinal epithelium	++	++	++	++	+
Large intestinal epithelium	++	++	++	++	+
Prostate epithelium	++	++	++	++	+
Urothelium	++	++	++	++	+/− (B)
Endometrium epithelium	++	++	++	++	+
stroma (A)	−	−	−	−	−
Endocervical glands	++	++	++	++	+
Ectocervical epithelium	−	++	++	++	−
Testis					
tubules	−	−	−	−	−
epididymis	++	++	++	++	+
Salivary glands					
duct	++	++	++	++	+
acini	++	++	++	++	+
Bronchial epithelium	++	++	++	++	+
Thyroid	++	++	++	++	+
Parathyroid	++	++	++	++	+
Gall bladder epithelium	++	++	++	++	+
Liver bile ducts	++	++	++	++	+
hepatocytes (C)	++	++	++	++	+/−

++ Strong staining
+ Moderate staining
+/− Weak/focal staining
− No staining
(A) Focal positive staining of smooth muscle cells in endometrium and muscular layers of stomach wall.
(B) Anti-cytokeratin 18 stained the superficial cells of transitional cell epithelium.
(C) Staining of hepatocytes was most visible in periportal areas with progressively weaker staining towards the centre of lobules.

STAINING OF EPITHELIAL TUMOURS

The results of staining of epithelial tumours are summarised in table 3. All five antibodies showed a similar pattern of staining, with tumours arising from simple epithelia. The intensity of staining was less with anti-cytokeratin 18 than with the other four antibodies. One prostatic and one breast carcinoma did not stain with anti-cytokeratin 18. One case of carcinoid tumour from the appendix showed no reactivity with any of the antibodies. As with the normal transitional cell epithelium, anti-cytokeratin 18 in transitional cell carcinomas stained only the superficial layers. There was some variation in the number of cases of hepatocellular carcinoma stained by the antibodies; only lu-5 stained all four cases, CAM 5·2 MFN 116 and anti-cytokeratin 18 stained three cases, and AE1/AE3 two cases.

Within tumours the intensity of staining was not always uniform throughout, but in all cases cytoplasmic staining was seen in most tumour cells.

Substantial differences were observed in the staining of squamous cell carcinomas. Only MFN116 stained all the cases. AE1/AE3 and lu-5 stained all but one case of bronchial squamous carcinoma. CAM 5·2 stained only eight of the 14 cases, and no cases were stained by anti-cytokeratin 18.

STAINING OF NON-EPITHELIAL TUMOURS

The five antibodies were also applied to several non-epithelial tumours. The results are summarised in table 4. Staining was seen in only a small proportion of smooth muscle tumours. CAM 5·2, lu-5, and MFN 116 each stained one case of leiomyosarcoma; AE1/AE3 stained two cases. No cases of leiomyosarcoma were stained by anti-cytokeratin 18.

Discussion

The importance of immunohistochemistry in histopathological diagnosis has been extensively reviewed.[1-4] As the number of commercially available antibodies increases it is important for pathologists to be familiar with them and to identify which best suit their needs. CAM 5·2 and AE1/AE3 have been in

Table 3 Staining of epithelial tumours

Carcinoma	*CAM 5·2*	*AE1/AE3*	*lu-5*	*MFN 116*	*Anti-cytokeratin-18*
Colorectal	4/4	4/4	4/4	4/4	4/4
Gastric	3/3	3/3	3/3	3/3	3/3
Breast	4/4	4/4	4/4	4/4	4/4
Prostatic	3/3	3/3	3/3	3/3	3/3
Renal cell	3/3	3/3	3/3	3/3	3/3
Hepatocellular	3/4	2/4	4/4	3/4	3/4
Transitional cell	3/3	3/3	3/3	3/3	3/3 (A)
Carcinoid of appendix	2/3	2/3	2/3	2/3	1/3
Teratoma-epithelial elements (well differentiated)	2/2	2/2	2/2	2/2	2/2
Pleomorphic adenoma (epithelial elements)	2/2	2/2	2/2	2/2	0/2
Squamous cell carcinoma					
Epidermis	5/6	6/6	6/6	6/6	0/6
Cervix	1/3	3/3	3/3	3/3	0/3
Bronchus	2/5	4/5	4/5	5/5	0/5

(A) superficial layers of transitional cell epithelium.

routine use for several years and have become a "gold standard" against which newer arrivals will be judged. In this study we compared the staining of CAM 5·2 (Becton Dickinson) and AE1/AE3 (ICN Biomedicals) with the newer antibodies lu-5 (Hoffman-La Roche), MFN 116 (Dako) and anti-cytokeratin 18 (Sigma). All are suitable for use on formalin fixed, routinely processed, paraffin wax embedded material.

The detection of cytokeratin intermediate filaments is widely used to identify tumours of epithelial origin. Alternative markers of epithelial differentiation such as epithelial membrane antigen (EMA) or human milk fat globule 1 and 2 (HMFG 1 and 2) are also used. Often a cytokeratin and epithelial membrane antibody are used together in a diagnostic panel.

Epithelial membrane markers, although widely expressed in normal and neoplastic epithelial tissues,[13 16 17] have limitations in the range of tissues stained, and results in increasingly anaplastic tumours become unpredictable.[14] Expression is also not restricted to epithelial cells.[15]

In a first line panel of antibodies, designed to establish the nature of a tumour, a marker of epithelial differentiation needs to show high degrees of sensitivity and specificity. The three monoclonal antibodies AE1/AE3, lu-5, and MFN 116 show a higher degree of sensitivity than CAM 5·2 and anti-cytokeratin 18 because they all recognise both the cytokeratins (8, 18), found in simple epithelia, as well as those expressed in stratified squamous epithelia. In the original study describing CAM 5·2,[5] Makin *et al* showed positive staining of all nine of the squamous carcinomas to which the antibody was applied. In our current study, CAM 5·2 stained only eight of the 14 cases. This discrepancy may be explained by the well documented changes in the pattern of expression of cytokeratins in tumours.[18] In a proportion of squamous carcinomas there is presumably a change in the expression of specialised cytokeratins normally found in squamous epithelium to those found in simple epithelia. Cytokeratin 18 has been found in some squamous cell carcinomas and may be associated with invasion. These changes, however, are not universally seen.[19]

Table 4 Staining of non-epithelial tumours

	CAM 5·2	*AE1/AE3*	*lu-5*	*MFN 116*	*Cytokeratin-18*
Melanoma	0/7	0/7	0/7	0/7	0/7
Lymphoma	0/4	0/4	0/4	0/4	0/4
Malignant fibrous histiocytoma	0/3	0/3	0/3	0/3	0/3
Leiomyoma	0/6	0/6	0/6	0/6	0/6
Leiomyosarcoma	1/6	2/6	1/6	1/6	0/6
Liposarcoma	0/3	0/3	0/3	0/3	0/3
Chondrosarcoma	0/6	0/6	0/6	0/6	0/6
Ewing's sarcoma	0/5	0/5	0/5	0/5	0/5

The only tumour not stained by the panel of antibodies was one case of carcinoid tumour of the appendix. Non-expression of cytokeratins in unequivocal carcinomas has been reported previously,[20 21] most commonly in renal cell carcinomas. In this study all three cases of renal carcinoma expressed cytokeratins.

All five antibodies seem to be highly specific for epithelial tissues, with the notable exception of normal and neoplastic smooth muscle cells. Staining of smooth muscle is well documented.[22-24] Although in this study only a small proportion of smooth muscle tumours expressed cytokeratins, other studies have shown an 80–100% expression.[23 24] It has been suggested that the source of this reactivity is a cross-reaction with nuclear lamins, a nuclear associated protein, with a keratin-like amino acid composition.[25] The pattern of staining is cytoplasmic rather than nuclear as might be expected if there was cross-reaction with nuclear lamins. Cytokeratins, however, have also been isolated from smooth muscle cells by two dimensional gel electrophoresis.[26]

Cytokeratin antibodies which show a reactivity to a wide spectrum of cytokeratins have an advantage over those with a more restricted reactivity, in that they recognise a wider range of epithelial derived tumours. CAM 5·2 retains a good reactivity, although it may fail to recognise squamous carcinomas expressing high molecular weight cytokeratins. AE1/AE3 and lu-5 are well characterised and produce good reproducible results. MFN 116, while showing a similar reactivity, is less well characterised, and in our hands produced less consistent results.

Anti-cytokeratin 18, overall, produces less consistent and less intense staining patterns. As cytokeratins are obligate hetero polymers, the weaker staining observed may in part reflect less available epitope for antibody interaction, or may be due to lower affinity of the antibody. It is limited in its usefulness in a diagnostic panel because of its monospecific nature. It may, however, prove a useful tool in showing changes in cytokeratin expression in tumours when compared with normal epithelia.

We have found that the optimal working dilution of the supplied antibodies is greatest with lu-5 and MFN 116 which may make them more cost effective in routine use.

Our study has examined the staining patterns of normal and neoplastic tissues with a number of commercially available cytokeratin antibodies. The tumours studied have all been identifiable on morphological grounds and would not have posed diagnostic problems. The value of immunohistochemistry is in the

identification of those tumours, such as small and large cell anaplastic and spindle cell carcinomas, whose epithelial nature is not apparent morphologically. In this paper we established the spectrum of reactivity and specificity of these monoclonal antibodies. Further work is being undertaken to establish the value of these antibodies in tumours that are not classifiable on morphological grounds alone.

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