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Expression of Monoclonal Antibody-Defined Epitopes of Keratin 19 in Human Tumours and Cultured Cells

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Abstract—*The monoclonal antibodies BA16 and BA17, reacting specifically with human keratin 19 (40 kD) have been tested by immunohistochemical staining methods for their reaction with a wide range of human tumours and cultured cells. Primary adenocarcinomas and their metastases showed a homogeneously positive reaction with > 95% of the tumour cells staining. Non-epithelial tumours, basaliomas and squamous cell carcinomas were unstained, while benign breast lesions and a thyroid adenoma show a mosaic pattern of stained and unstained (5–40%) cells. These three staining patterns were also seen in cultured cells. Positive homogeneous staining was seen in all breast cancer cell lines examined with the exception of PMC42, which exhibits stem cell characteristics, and which showed the heterogeneous pattern of staining seen in milk cell cultures. Non-epithelial lines and strains, two cell lines from cervical carcinomas and three SV40 transformed breast epithelial lines were unstained. The antibodies BA16 and 17 are potentially useful reagents for distinguishing adenocarcinomas (and their metastases) from non-epithelial tumours and from squamous carcinomas. They may also discriminate between benign and malignant breast lesions, and identify a specific differentiation phenotype in the secretory cell lineage.*

INTRODUCTION

AVAILABLE biochemical and DNA sequence data indicate that human keratins comprise a multigene family of at least 19 proteins which are expressed in different subsets in various epithelial cell types and stages of differentiation [1, 2, 3, 7]. The patterns of differential keratin expression in normal epithelia together with the tendency toward maintenance of keratin polypeptide patterns in carcinomas and their metastases suggest the possibility of improved diagnosis and subclassification of primary tumours and identification of their metastases by biochemical and/or immunohistochemical analysis of the intermediate filament composition [1, 3, 7, 8]. It has become increasingly evident that monoclonal antikeratin antibodies of two kinds can be especially useful in this respect, namely, antibodies against epitopes common to many, or to all, keratin polypeptides, and antibodies specifically recognizing only one of the keratins. The former can be used to distinguish epithelia-derived tumours from other types of neoplasms while the latter can help to further subdivide epithelia and carcinomas and thus obtain information about the origin of the tumour by simple immunohistochemical staining [4–7].

Recently we reported the development and characterisation of two mouse monoclonal antibodies (BA16 and BA17) recognizing different epitopes on the human 40 kD keratin [9] which has been classified as keratin 19 by Moll and colleagues [1]. Using these antibodies in immunohistochemical staining of sections of normal breast, we found myoepithelial cells to be unstained, while most luminal cells showed positive staining. However a subset of luminal cells were unstained and the distribution of these cells in the mammary tree was that expected for a cell compartment with the proliferative potential to give rise to the new

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growth seen at pregnancy [9]. Examination of a large series of human breast tumours with BA16 and BA17 antibodies revealed basic differences between the heterogeneous staining pattern in benign and homogeneous positivity in malignant lesions [10] and a general model was suggested to explain the presence of unstained cells in normal gland and benign proliferation and the dominance of positively staining cells in carcinomas [10, 11].

In the present study we have extended the characterization of the keratin 19 specific antibodies by (1) examining their reactivity with a range of human tumours and (2) looking at the expression of keratin 19 in cell lines and strains to see if there is any correlation to be seen *in vitro* with the patterns of expression of this keratin seen in tissue sections.

MATERIALS AND METHODS

Cell cultures

Cells from early lactation milk were cultured in medium RPMI-1640 containing 10% FCS, 10% human serum, hydrocortisone (10 μg/ml), insulin (10μg/ml) and cholera toxin (100 ng/ml) [12]. Human cell lines MCF-7, ZR75.1, T47D, MDA-MB-231, MDA-MB-157, CAMA1, HBL-100, A431, T 24, L1168, U-118-MG, HT 29, LT and LEP were cultured in DMEM supplemented with 10% FCS and antibiotics. Breast cancer lines BT20 and BT474 were grown in DMEM with 10% FCS, 10 μg/ml insulin and antibiotics, PMC-42 line in medium RPMI-1640 containing 10% FCS, 1 μg/ml insulin and antibiotics, cell lines MRC5, HeLa, N13F and ICRF-23 in MEM with 10% FCS and antibiotics. The tissue of origin of all cell lines used is shown in Table 2.

Monoclonal antibodies

Mouse monoclonal antibodies BA16 and BA17 (both IgG1) reacting with human keratin 19 were described previously [9, 10]. Monoclonal antibody CAM5.2 directed against the determinant shared by several lower mol. wt keratins [13] was used as a positive control and monoclonal antibody TF-1 against pig transferrin [14] as a negative control. All three anti-keratin antibodies strongly stain methanol/acetone-fixed frozen sections [9, 10, 13] whereas only BA17 gives strong and reliable staining on methacarn-fixed paraffin-embedded tissues [9, 10]. Good results can be obtained with CAM5.2 antibody on paraffin-embedded tissue sections pretreated with proteolytic enzymes [13]. In both immunohistochemistry and immunoblot staining undiluted hybridoma culture supernatants were used.

Tumour tissues

Tissues from primary and secondary tumours were obtained from the Departments of Surgery and Gynecology, Institute of Clinical and Experimental Oncology in Brno. Frozen sections fixed in a mixture of cold methanol and acetone (1 : 1) for 10 min were stained with all antibodies, sections of methacarn fixed (methanol, chloroform and acetic acid in the ratio of 6 : 3 : 1) paraffin-embedded tissues were stained with antibody BA17 and TF-1.

Immunofluorescence staining of cultured cells

The indirect immunofluorescence assay was performed either on cells cultured and subsequently fixed and air-dried in plastic Petri dishes [15] or on cells grown and fixed on coverslips as described previously [16], using FITC-conjugated rabbit anti-mouse immunoglobulin (Miles, Austria) diluted 1 : 30 as the second antibody.

Immunohistochemical staining of tissue sections

The indirect immunoperoxidase technique used in this study was performed as previously described [9] using peroxidase conjugated rabbit anti-mouse immunoglobulin antiserum (Dako, Denmark) as the second antibody, 3-3'-diaminobenzidine tetrahydrochloride (Sigma Chemical Co., U.S.A.) as chromogen and haematoxylin to counterstain nuclei. Indirect immunofluorescence staining of tissue sections was performed as described [16].

Detection of antigens in Western blots

Whole cell lysates from cultured cells were made directly in sample buffer as described by Burchell *et al.* [17] and separated by SDS–PAGE on 12.5% polyacrylamide gel with a 5% stacking gel [18]. Prestained mol. wt markers (Bethesda Research Labs., Austria) were run in parallel. Following electrophoresis the gel and nitrocellulose paper were sandwiched together between Scotch-Brite pads and the proteins transferred to nitrocellulose by diffusion in Tris–HCl buffer pH 7.6 with 20% methanol for 2 days at room temperature. The immunoenzymatic staining of nitrocellulose strips was performed as previously described [9].

RESULTS

Staining of tumour sections with antibodies BA16 and 17

To evaluate the potential usefulness of the BA16 and 17 antibodies in tumour pathology we examined tissue sections from over 100 human benign and malignant lesions of various histological origin using immunoperoxidase and immunofluorescence methods. Tumours examined and the staining results (regardless of technique used) are listed in Table 1. No significant reaction could be observed with either antibody in tumours of non-epithelial origin, and among tumours derived from various epithelia three different staining patterns could be

Table 1. *Staining patterns of human tumours with monoclonal antibodies to keratin* 19

Tumour type	No. of tumours examined	Staining pattern		
		Negative	Positive	
			Homogeneous*	Heterogeneous†
Breast tumours:				
Fibroadenoma	8	0	0	8
Fibrocystic disease	5	0	0	5
Cystosarcoma phyllodes	7	0	2	5
Infiltrating ductal carcinoma	12	0	12	0
Infiltrating lobular carcinoma	5	0	5	0
Medullary carcinoma	2	0	2	0
Metastases (lymph nodes and skin)	11	0	11	0
Male invasive carcinoma	1	0	1	0
Other tumours:				
Thyroid adenoma	1	0	0	1
Colon adenocarcinoma	5	0	5	0
Gastric adenocarcinoma	2	0	2	0
Urinary bladder carcinoma	4	0	4	0
Lung adenocarcinoma	2	0	2	0
Ovarian carcinoma	2	0	2	0
Squamous cell carcinoma of skin	4	4	0	0
Basal cell carcinoma of skin	2	2	0	0
Sarcoma	7	7	0	0
Malignant melanoma	6	6	0	0
Lymphoma	7	7	0	0
Testicular tumours:				
Seminoma	5	5	0	0
Teratoma	2	0	0	2‡
Embryonal carcinoma	4	0	0	4
Tumours of more than one histological type	8	0	0	8

*More than 95% of the lesional cells showed positive staining.
†Between 10 and 90% unstained cells, variable throughout the lesions.
‡For more detailed explanation of this complex heterogeneous pattern of staining see Results section and Bartkova *et al.* [28].

distinguished. All adenocarcinomas tested (both primary and secondary lesions) were homogeneously stained with more than 95% tumour cells being positive, although the intensity of staining was usually reduced in colon carcinomas as compared with other adenocarcinomas. Examples of positive reactions seen with an invasive breast carcinoma and a gastric adenocarcinoma are shown in Fig. 1A and Fig. 1B, respectively, while Fig. 1C demonstrates the potential usefulness of the antibodies in identifying small groups or even single metastatic breast cancer cells in a lymph node. In contrast to adenocarcinomas, tumours derived from stratified squamous epithelium of skin gave negative results with both antibodies. The third—heterogeneous or mosaic—pattern of some positive and some negative tumour cells was represented by benign breast lesions and a thyroid adenoma (Fig. 1D).

Yet another specific staining pattern was found in non-seminomatous testicular germ cell tumours. In general, those parts of teratomas and mixed tumours (see Table 1) showing simple epithelia-like differentiation reacted positively with the antibodies as did at least some cells of embryonal carcinomas. In contrast, all non-epithelial areas of these tumours as well as some regions (cysts) composed of stratified epithelium were completely unstained. In some of the stratified epithelium-containing structures the basal layer was positively stained with BA16 and BA17 antibodies while the central part of such cysts was negative.

Table 2. Reactivity of antibodies BA16 and BA17 with cultured human cells

Cells	Tissue of origin	Staining reaction*	
		BA16	BA17
BT20	Primary breast carcinoma	+	+
BT474	Primary breast carcinoma	+	+
MCF-7	Breast carcinoma metastasis†	+	+
T47D	Breast carcinoma metastasis†	+	+
MDA MB-231	Breast carcinoma metastasis†	+	+
MDA MB-157	Breast carcinoma metastasis†	+	+
CAMA-1	Breast carcinoma metastasis†	+	+
ZR75.1	Breast carcinoma metastasis†	+	+
PMC42	Breast carcinoma metastasis† (stem cell?)	+/−	+/−
Milk cells	Primary culture (see Methods section)	+/−	+/−
fR2	SV40-transformed milk epithelial cells	−	−
fR5	SV40-transformed milk epithelial cells	−	−
HBL-100	"spontaneously" SV40-transformed milk epithelial cells	−	−
N13F	Breast fibroblasts	−	−
A431	Epidermoid carcinoma	−	−
HeLa	Cervical adenocarcinoma	−	−
SVK14	SV40-transformed keratinocytes	+	+
HT29	Colon carcinoma	+	+
T24	Urinary bladder carcinoma	+	+
PC/AA‡	Colon adenoma	+	+
L1168	Rhabdomyosarcoma	−	−
U-2-OS	Osteosarcoma	−	−
U-118-MG	Malignant glioma	−	−
LT	Malignant melanoma	−	−
MRC5	Lung embryonal fibroblasts	−	−
LEP	Lung embryonal fibroblasts	−	−

*Staining reaction in an indirect immunofluorescence technique (see Materials and Methods section for details) is expressed as:

(+) positive cytoplasmic filamentous staining with no obvious unstained cells, staining intensity much weaker with BA17 than with BA16.

(−) no detectable staining.

(+/−) heterogeneous staining pattern with majority of cells being positive but some clearly unstained cells (see Results section for more details).

†Established from serous effusion.

‡Gift from C. Paraskeva.

The heterogeneity in staining with antibodies BA16 and 17 does not appear to reflect a general variability in the level of keratin expression since all the epithelia derived tumours listed in Table 1 showed a homogeneously positive reaction with antibody CAM5.2; this antibody recognizes an epitope found on several low mol. wt keratins, including keratin 19 [13].

Patterns of expression of keratin 19 by cultured cells

To investigate the expression of keratin 19 by cultured cells, a range of cell lines derived from malignant tumours were stained by indirect immunofluorescence with BA16 and 17. A wider range of lines from breast carcinomas were studied, and cultures of normal mammary epithelial cells and fibroblasts were also examined, along with SV40 transformed mammary epithelium and SV40 transformed keratinocytes [19]. The results of these studies are shown in Table 2. The first point to note is that none of the non-epithelial cell lines including fibroblasts, glioma, melanoma, osteosarcoma and rhabdomyosarcoma showed positive staining. On the other hand, most epithelia-derived lines with the exception of A431, HeLa and SV40 transformed mammary epithelium were positive with both antibodies as were the majority of colonies cultured from human milk. In agreement with our previous studies [9] antibody BA16 stained methanol/acetone fixed cells more strongly than BA17. All cells of epithelial origin listed in Table 2, including those which did not stain posi-

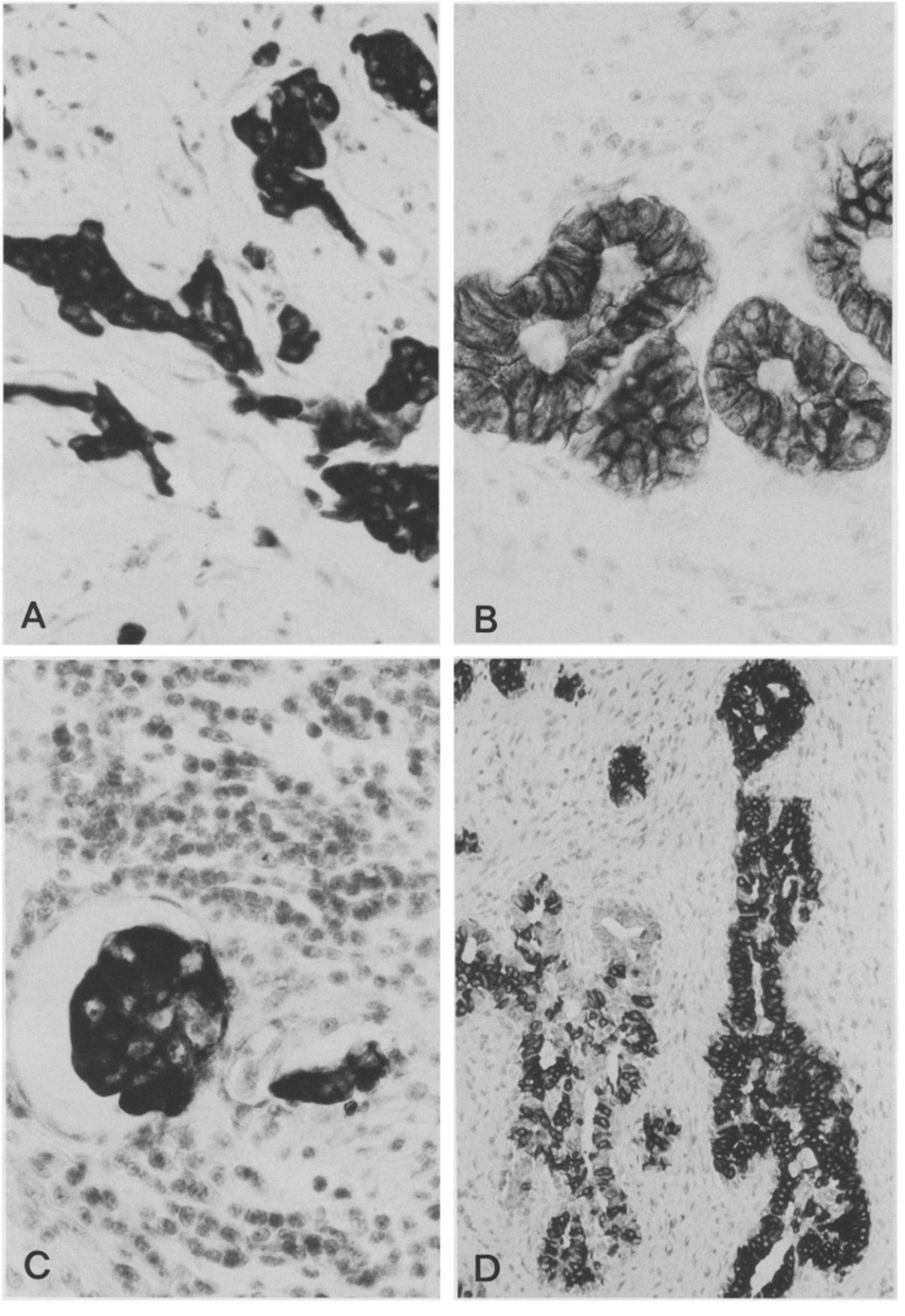

Fig. 1. *Immunoperoxidase staining of methacarn fixed sections of human tumours by antibody BA*17. A. *Primary ductal carcinoma of the breast.* B. *Gastric carcinoma.* C. *Metastatic breast carcinoma cells in lymph node.* D. *Fibradenoma of the breast.* A,B,C × 475, D × 190.

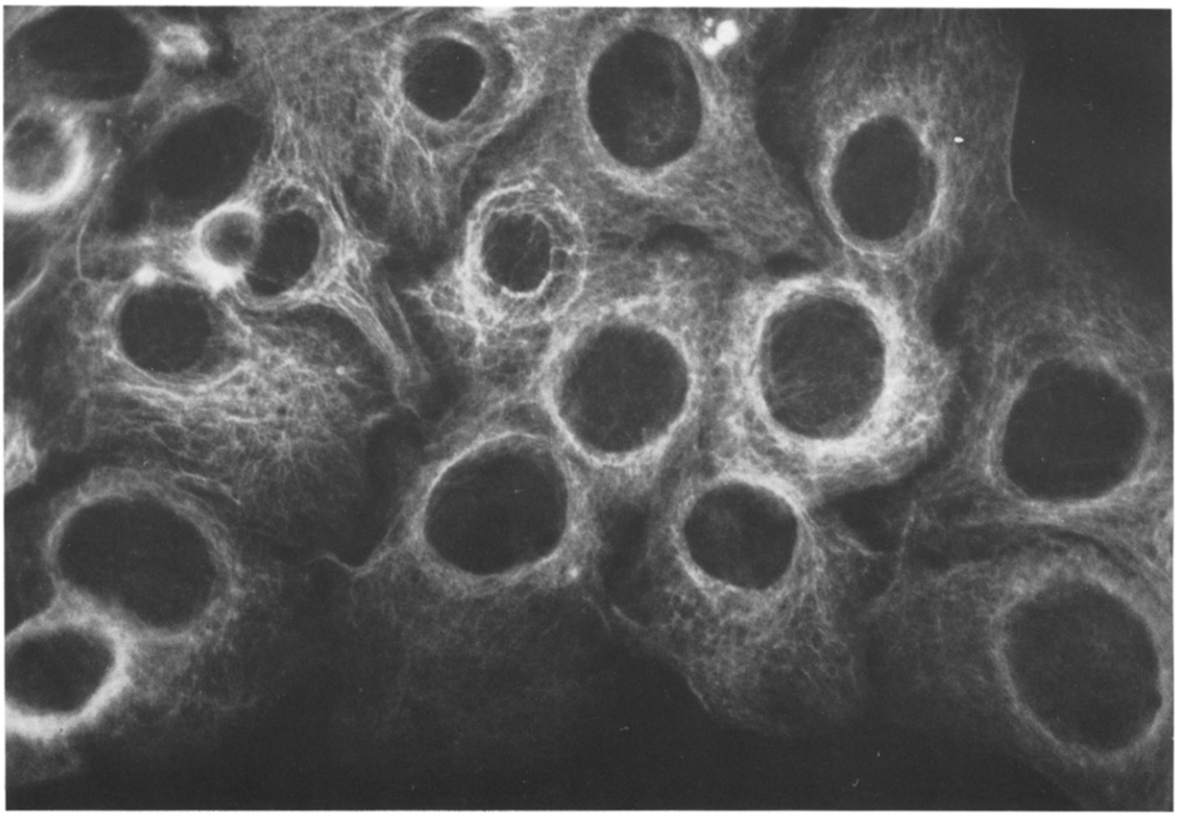

Fig. 2. Immunofluorescence staining of methanol/acetone-fixed BT20 cells with antibody BA16, × 870.

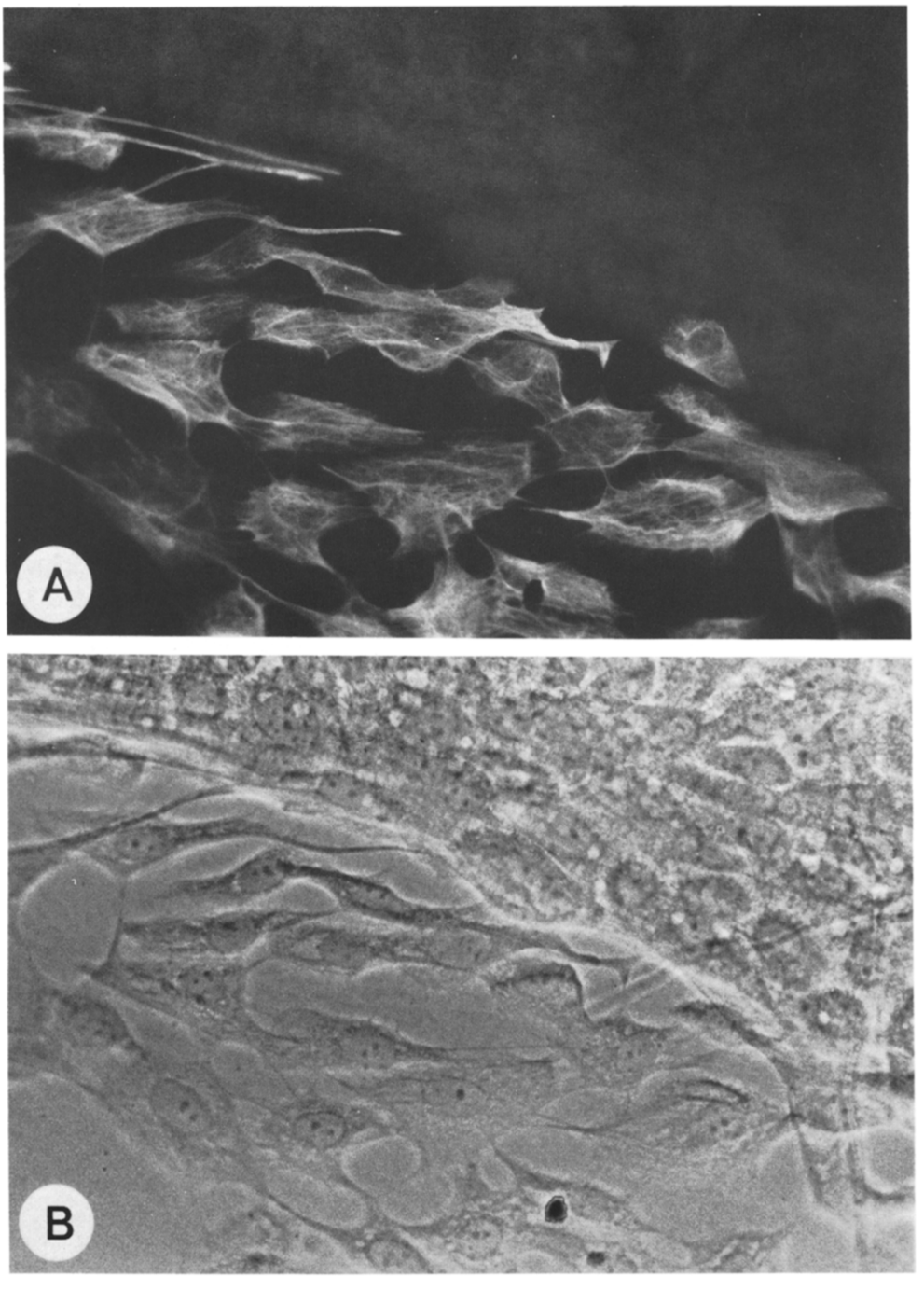

Fig. 3. *Staining of colonies of primary milk epithelial cells showing one positive and the other unstained by antibody BA*16. A. *Stained with antibody.* B. *Phase Contrast.* A,B × 870.

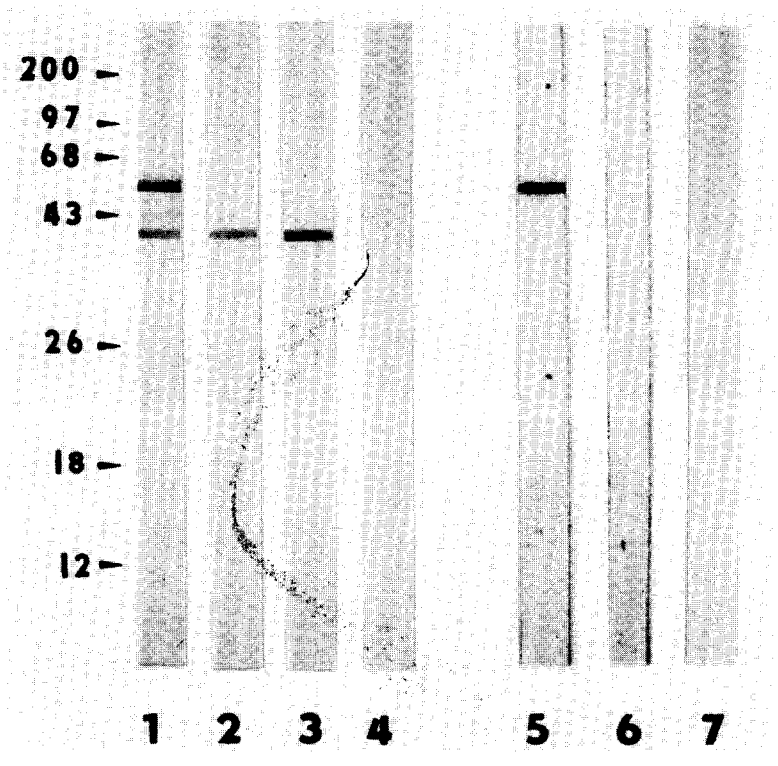

Fig. 4. *Western blots of gel-separated whole cell lysates of BT*20 *cells* (*tracks* 1–4) *and HBL*-100 *cells* (*tracks* 5–7) *stained with monoclonal antibodies* (*see Materials and Methods*). *Tracks* 1 *and* 5 *were stained with antibody CAM* 5.2, *tracks* 2 *and* 6 *with antibody BA*16, *tracks* 3 *and* 7 *with BA*17 *and track* 4 *with antibody TF*-1 (*negative control*).

tively with BA16 and BA17, showed a positive reaction with the CAM5.2 antibody. The intracellular staining pattern was, with the exception of the fR lines, that expected for the characteristic network of wavy cytokeratin filaments (Fig. 2). As has been previously reported the keratin filaments in the fR lines were condensed around the nucleus [20].

Among the epithelial colonies growing from early lactation milk about 80–90% were homogeneously positive, the remaining 10–20% of colonies being either completely negative or heterogeneously stained with some positive and some negative cells mixed together. Two colonies of milk cells one homogeneously positive and the other unstained are shown in Fig. 3. The only cell line with a clearly heterogeneous staining pattern similar to that seen in milk cultures was the PMC42 line which was shown by Whitehead *et al.* [21] to have some stem cell properties.

It is interesting to note that all three SV40 transformed breast cell lines derived from normal human mammary epithelium i.e. HBL-100 [22, 23], fR2 and fR5 [20] were negative with both BA16 and BA17 antibodies (see Table 2). This could imply that the cell lines were derived from a cell typified by the BA16 negative colonies found in cultures of normal milk. On the other hand, the SV40 transformed keratinocytes express both keratin 19 (Table 2) and keratin 18 [20] while normal keratinocytes do not, making it difficult to deduce the phenotype of the original cells from that of the SV40 transformed derivative.

To confirm the staining results and to demonstrate the mol. wt of the target component, the whole cell lysates of several cell lines were separated by SDS–PAGE and transferred to nitrocellulose, which was then stained with the antibodies by an immunoperoxidase technique (see Materials and Methods). As shown in Fig. 4 both BA16 and BA17 reacted only with a 40 kD component in the lysate from BT20 cells and showed no reaction with the blots of the HBL-100 cell line thus confirming the immunofluorescence data. The monoclonal antibody CAM5.2 was used as a positive control and also revealed the presence of a 40 kD keratin in the BT20 cell lysate and its absence from the lysate of the HBL-100 line (Fig.4). The staining of Western blots of several other breast carcinoma cell lines, milk cells, HT29, fibroblasts, A431 and HeLa cells always agreed with the positive or negative staining pattern seen in immunofluorescence (data not shown).

DISCUSSION

Keratin 19 is the smallest human keratin, with a mol. wt of 40 kD. It is found in most simple and non-keratinizing stratified epithelia, but not in adult interfollicular epidermis, hepatocytes and a few types of glandular epithelia such as pancreatic parenchymal cells and myoepithelial cells of the breast [1, 9, 33]. Like all cytokeratins, keratin 19 is not expressed by cells of non-epithelial origin. Although epidermal cells may express the 40 kD keratin in culture [25] or during embryonic development [24, 34–36] this keratin has not been detected in neoplasma (basal and squamous cell carcinomas) derived from the skin ([37] and this paper). The presence or absence of keratin 19 is therefore a potential diagnostic indicator for classification of tumours (carcinoma vs. others) and for subclassification of carcinomas. The presence of keratin 19 in human tumours and cell lines has usually been detected biochemically by several authors [1, 24–26]. However the development of keratin 19 specific monoclonal antibodies [9] has enabled us to study the expression of this keratin at the single cell level. In the current study we have examined the patterns of expression of two epitopes of the human 40 kD cytokeratin in a broad spectrum of tumours and cultured cell types using the monoclonal antibodies BA16 and BA17 [9].

When using monoclonal antibody immunohistochemistry potentially false negative staining results can be obtained due to masking of antigenic determinants (see [7] for discussion). However we consider this possibility unlikely in our present study because antibodies BA16 and BA17 recognize different epitopes on the 40 kD keratin and in all the cases examined so far they were either both positive or both negative showing the same pattern on parallel sections. Furthermore, whenever we compared the staining data with the detection of the 40 kD on Western blots following SDS–PAGE there was always correlation between the results of these two independent techniques ([9, 10], and this study).

The lack of staining of non-epithelial tumours including lymphomas, sarcomas and melanomas is not surprising as they contain vimentin as the intermediate filament protein (see [3, 8] for reviews). Similarly, no positive reaction could be detected in any of the cultured cells of nonepithelial origin. The presence of keratin 19 in most simple epithelia and their tumours together with the lack of reactivity of BA16 and BA17 with non-keratin classes of intermediate filament proteins [9] suggest that these antibodies could be useful in differential diagnosis between adenocarcinomas and non-epithelial tumours.

Among testicular tumours we found seminomas consistently negative while all nonseminomatous neoplasms were at least focally positive with both BA16 and BA17 thus confirming and extending the results of a recent study by Battifora and

colleagues [27]. A more detailed analysis of the staining patterns of BA16 and BA17 and other antibodies on testicular germ cell tumours has been published elsewhere [28].

One of the potential benefits of employing single-keratin-specific monoclonal antibodies is the possible subdivision of carcinomas and their metastases according to the keratin spectrum expressed. The validity of this approach has been demonstrated by Ramaekers *et al.* [6] and Debus *et al.* [5] who could clearly distinguish adenocarcinomas (positive) from squamous cell carcinomas (negative) using different monoclonal antibodies specific for keratin 18. We found a similar distinction between adenocarcinomas, which stained positively, and a small series of squamous cell carcinomas and basal cell carcinomas of the skin, which did not stain with antibodies against keratin 19. The availability of specific reagents for detection of keratin 18 and 19 should now allow further subdivision of carcinomas between for example, hepatocarcinomas which are reported to express keratin 18, but not 19, and other adenocarcinomas which express both these keratins [1, 29]. That this may indeed be feasible is supported by the observation that hepatocytes were unstained by the keratin 19 specific antibodies [9]. The updated list of monospecific anti-keratin monoclonal antibodies includes another against keratin 19 [30] and those referred to by Cooper *et al.* against the 51 kD, 58 kD and 64 kD human keratins [7] thus opening the way to even more subtle immunohistochemical distinctions among various epithelia and their tumours.

In our previous study of a large series of breast tumours from New Cross Hospital in London we found basic differences between benign lesions, which showed a mosaic staining pattern of positive and negative cells, and malignant tumours which generally showed a homogeneously positive reaction with antibodies BA16 and BA17 [10]. The evaluation of the present series of breast tumours from the Research Institute of Clinical and Experimental Oncology in Brno confirmed and extended our earlier results. Thus, all cases of fibroadenoma and cystic disease were heterogeneous with about 5–40% cells being negative while the samples of ductal, lobular as well as medullary carcinomas showed homogeneously positive staining patterns as described previously [10]. Furthermore, the male breast carcinoma examined stained homogeneously positive with both antibodies thus suggesting a similar pattern of keratin 19 expression in breast tumours of both sexes. The epithelial component of all but two lesions classified as cystosarcoma phyllodes showed the mosaic staining pattern characteristic of benign tumours. The epithelium of the two exceptional cases consisted of large ducts dilated and deformed by proliferating mesenchymal cells and the homogeneously positive staining pattern of their luminal epithelial cells was similar to that seen in large ducts of the resting breast [9, 10]. The only other example of a heterogeneous staining pattern was seen with a coloid adenoma of the thyroid which contained more than 50% unstained cells. Further experiments, and in particular the comparison of normal tissues and benign tumours with malignant lesions of various epithelia, need to be done to show whether the staining pattern of antibodies against keratin 19 can be used to differentiate between malignant and benign tumours, at least in some locations.

We reported previously that the heterogeneity of expression of keratin 19 seen among normal luminal epithelial cells in the human breast was maintained *in vitro* in both primary cultures of cells from human milk and reduction mammoplasty organoids cultured in collagen gels [9, 11]. In the present study we examined the staining pattern of a panel of human breast cancer and breast epithelium-derived cell lines to see whether there is any *in vitro* parallel to the patterns found in the human breast tissues and tumours even after a long period in culture. Interestingly, among 12 permanent human breast cell lines examined (see Table 2) we observed representative examples of all three staining patterns seen in tumours and tissues (i.e. homogeneous positivity, negativity and the mosaic pattern). All but one cell line established from primary and secondary breast cancers showed homogeneous positivity when stained with both antibodies, thus corresponding to the pattern seen in clinical material. The only exception was the PMC42 cell line established from a pleural effusion metastasis [21] which behaves biologically differently from the above mentioned lines. This interesting cell line which has been repeatedly reported to have stem cell characteristics showed a heterogeneous staining pattern with BA16 and BA17 antibodies with groups of unstained cells surrounded by a majority of positive cells. Whether the negative cells correspond to any of eight morphologically different cell types known to differentiate in cultures of PMC42 line [21] remains to be established.

Three out of the 12 breast derived cell lines examined showed no staining with either BA16 or BA17. The features shared by these lines and distinguishing them from the above lines which stained positively are (1) their origin from cultured milk epithelial cells and (2) the fact that they are SV40 transformed. The lines fR5 and fR2 were established by SV40 virus transformation of cultured milk epithelial cells by Chang *et al.* [20] while HBL-100 is a 'spontaneously' immortalized cell line derived from milk [22] which has been

recently shown to contain integrated SV40 virus genomes [23]. It seems likely that transformation occurred *in vivo*, possibly by virus administered inadvertantly with a polio virus vaccine. The epithelial origin of the fR and HBL100 lines has been confirmed by various criteria including the expression of some keratins ([20, 22] and this study). In view of our hypothesis that the keratin 19 negative cells represent a principle proliferative compartment in human breast it is tempting to speculate that it is this minor keratin 19 negative phenotype among milk colonies shown to have a good proliferative potential *in vitro* [9] which is the target favoured by at least some routes of immortalization and transformation. However, in contrast to the situation in malignant change, where the keratin profile of the cell, at least *in vivo* appears to be stable, SV40 transformation can affect the pattern of keratin expression, both in the specific keratins which are expressed and the way in which the keratin network is assembled in the cell. Thus, although we observed a lack of staining of squamous cell carcinomas of the skin, we found 100% of cells of the SV40-transformed keratinocyte cell line SVK14 positive with both BA16 and BA17. The SVK14 cell line has also been shown to react with monoclonal antibodies specific for keratin 18 [19] and keratin 8 [31] i.e. polypeptides found in simple epithelia and fetal skin but not in adult epidermis [24]. It could be argued that the induced expression of keratins 18 and 19 is related to the culture situation, since the expression of a 40 kD keratin has been described in some cultured squamous cell carcinoma cell lines [26] as well as in non-transformed human epidermal cells forming nonkeratinized colonies *in vitro* [25]. However profound chromosomal alterations are seen in SV40 transformed lines [32] and it would not be surprising to find that these resulted in profound changes in gene expression.

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