Demonstration of Keratin in Human Adenocarcinomas

FRANS RAMAEKERS, JO PUTS, OLOF MOESKER, ARIE KANT, PAUL JAP, and PETER VOOIJS

From the Department of Pathology, Radboud Hospital, University of Nijmegen, and the Department of Cytology and Histology, University of Nijmegen, Nijmegen, the Netherlands

The occurrence and localization of intermediate-sized filaments in 85 cases of adenocarcinoma have been examined by the indirect immunofluorescence technique as well as by the immunoperoxidase technique. Frozen sections of human tumor tissue were incubated with antibodies to keratin, vimentin, and desmin. In contrast to earlier studies by Schlegel et al,[1] this study demonstrates the presence of keratin in 64 cases of primary adenocarcinoma, including tumors of stomach, colon and rectum, lung, pancreas, bile ducts, ovary and uterus, female breast, and prostate, and in 21 cases of adenocarcinomatous metastases in lymph nodes, thoracic and abdominal wall, omentum, mesentery, testis, liver, and the pelvis. In order to establish the possibility of demonstrating intermediate filament proteins by immunohistochemical techniques in fixed, paraffin-embedded material, the authors tested seven fixation methods. It is concluded from the data that antibodies to intermediate filament proteins can be useful in the differential diagnosis of adenocarcinomas because they can distinguish them from tumors of nonepithelial origin in frozen sections. (Am J Pathol 1983, 111:213–223)

SEVERAL REPORTS in the literature have shown that antibodies directed against protein constituents of the different types of intermediate-sized filaments are useful tools in tissue recognition.[2] These proteinaceous 10-nm filaments occur in most, if not all, eukaryotic cells and represent a substantial portion of the cytoskeleton, in addition to microfilaments, microtubules, and the microtrabecular network (see, for example, Schliwa and Blerkom[3]). Five types of intermediate filaments have been distinguished so far, each type being found in a specific tissue type. From biochemical and immunocytochemical data it has become evident that in vivo and in vitro, epithelial, mesenchymal, myogenic, neural, and glial cells can be identified by the use of antibodies directed against their specific intermediate filament proteins: keratin, vimentin, desmin, the neurofilament proteins, and the glial fibrillary acidic protein, respectively.[4] Recent investigations[1,5–25] have shown that solid (human) tumors retain their original intermediate filament types and do not develop additional intermediate filament systems upon malignant transformation. Therefore, antiserums directed against intermediate filament proteins are valuable tools in the differential diagnosis of tumors and their metastases. The method is now being tested systematically for its applicability to various problems in surgical pathology. Especially in those cases where diagnostic interpretations on the basis of conventional histologic and cytologic methods give rise to doubtful results or where diagnosis is even impossible (ie, anaplastic growth, differential diagnosis of lymphoma and small cell carcinoma or anaplastic adenocarcinoma, etc.), this method can be most helpful. The present work clarifies a discrepancy in the literature concerning the presence and the nature of intermediate filaments in human adenocarcinomas. In a preliminary survey, Schlegel et al[1] have reported that, in their hands, adenocarcinomas of the colon, prostate, kidney, lung, and breast did not show any positive staining with antibodies to keratin when the immunoperoxidase technique on paraffin-embedded sections was used. Adenocarcinomas of the pancreas and sometimes those from breast and lung showed a weak reaction with keratin antibodies. On the basis of these and other results, the authors conclude that not all adenocarcinomas contain intracellular keratin, although normal, ductal cells of various tissues of ectodermal and entodermal origin could be shown to

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Address reprint requests to Dr. F. C. S. Ramaekers, Department of Pathology, Geert Grooteplein Zuid 24, University of Nijmegen, 6525 GA Nijmegen, the Netherlands.

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contain this type of intermediate filament.[24] Some reports in the literature thereafter[5-7,13,14,21,23,25] have mentioned some cases of keratin-containing adenocarcinomas. We have examined, therefore, 85 cases of human adenocarcinomas, originating from different organs, including metastatic adenocarcinomas. In this paper we report that 1) all adenocarcinomas examined here contain keratin, as detected by the indirect immunofluorescence and the immunoperoxidase technique; 2) fixation of normal and tumor tissue in formalin can prevent detection of keratin in this way; 3) keratin in many adenocarcinomas seems to be concentrated mainly at the cell periphery; 4) solid adenocarcinomas in general do not obtain an additional intermediate filament cytoskeleton in addition to that of the cell of origin as a result of malignant transformation or metastasis.

Materials and Methods

Tissues

Normal and malignant tissues were obtained immediately after surgery, and small pieces were frozen in liquid nitrogen. The rest of the tissue was used for routine diagnosis and fixed in neutral formalin for histologic studies. Paraffin sections were stained with hematoxylin and eosin (H&E). In some instances tumors were cut immediately on a cryostat. Frozen sections (4–7 μ thick) were stained for the indirect immunofluorescence and/or immunoperoxidase technique as described below. Table 1 gives an overview of the types of human adenocarcinomas used in this study.

Antiserum

The following antiserums were used in this study: 1) a rabbit antiserum directed against human epidermal keratins isolated from foot calluses essentially as described by Franke et al[2]; 2) a rabbit antiserum raised against vimentin isolated from calf lenses by preparative gel electrophoresis as described before[21]; 3) a rabbit antiserum directed against chicken gizzard muscle desmin isolated by modifications of the methods used by Geisler and Weber[26] and Franke et al[27] briefly as follows. Chicken gizzard muscle tissue was minced in phosphate-buffered saline (PBS) at 4 C (all the subsequent isolation steps were performed at 4 C), stirred for 10 minutes, and homogenized with the use of an Ystral X10/20 homogenizer (Ystral GmbH, Ballrechten-Dottingen). After stirring for another 10 minutes and centrifugation at 6000 rpm in a GSA rotor for 10 minutes, the pellet was homogenized in EDTA buffer (1 mM EDTA, 0.01% beta-mercaptoethanol, 0.5% Triton X-100, and 10 mM Tris HCl, pH 7.4) and extracted for 30 minutes. After centrifugation the pellet was washed once more with EDTA buffer and thereafter homogenized in KCl buffer (1 M KCl, 0.01% beta-mercaptoethanol, 0.5% Triton X-100, and 10 mM Tris-HCl, pH 7.4) and extracted for 3 hours. After centrifugation as described, the resulting pellet was homogenized in KI buffer (0.6 M KCl, 0.01% beta-mercaptoethanol, 0.5% Triton X-100, and 10 mM Tris-HCl, pH 7.4), extracted for 3 hours, repelleted, and washed with PBS. The final pellet was treated with DNase I (Sigma) for 20 minutes at 37 C. Desmin was purified from this crude preparation by preparative gel electrophoresis as described in the preparation method of vimentin.[21] Purity of the final desmin preparation was checked by one- and two-dimensional gel electrophoresis.

New Zealand white rabbits were immunized subcutaneously at 20 sites in the back with approximately 0.4 mg of the keratin preparations, 0.2 mg of vimentin, or 0.25 mg of desmin. All preparations were dissolved in a buffer containing 2.3% sodium dodecyl sulfate, 5% beta-mercaptoethanol, 10% glycerol, and 40 mM Tris-HCl, pH 6.8, as the final concentration. An equal volume of complete Freund's adjuvant was added before the injection. Rabbits were boosted repeatedly subcutaneously at 4 sites (up to three times within three-week intervals) with equal amounts of protein in incomplete Freund's adjuvant and the serums collected 3 weeks after the last booster. Before immunization the serums of the rabbits were tested for autoimmune antibodies on frozen sections from human skin by the indirect immunofluorescence test (see below). Only those rabbits that had negative preimmune serums were used for immunization. Preimmune serums were used as controls in parallel with the antiserums described above. Furthermore, antiserums absorbed with the specific antigens were included as additional controls. The anti-keratin antiserum was absorbed with callus keratins and tested on human urothelium and rectal epithelium. The anti-vimentin antiserum was absorbed with purified vimentin from calf lenses and tested on human melanomas and a giant cell tumor. In all cases reactions with these absorbed antisera were completely negative. Serums were tested for specificity on frozen sections of human tissues. In addition, tissues from hamster and rat, as well as cultured cells, were used for quality screening as described earlier.[21-23] For reaction of the vimentin antiserum, see also Klymkowsky.[28]

Indirect Immunofluorescence and Immunoperoxidase Staining

Air-dried cryostat sections prepared as described above were fixed in methanol for 5–10 min at −20 C and thereafter dipped in cold acetone. After rehydration of the sections in PBS, the first antiserum was added (dilutions between 1:10 and 1:50), and the sections were incubated at room temperature in a moist chamber for 45 minutes. After washing with PBS (3 washes of 10 minutes each) the fluorescein-labeled second antibody (goat anti-rabbit IgG conjugated with FITC; Nordic, Tilburg, the Netherlands) was added, and the sections were incubated for another 45 minutes. After a second series of washes in PBS, the sections were mounted with 50% glycerol in PBS or in Gelvatol (Monsanto, St. Louis, Mo) and viewed with a Leitz Dialux 20 EB microscope equipped with epifluorescent illumination with appropriate filters for fluorescein fluorescence. Pictures were taken on Tri-X-film (Kodak) with an automatic camera with an ASA-setting of 400 or 800.

For the immunoperoxidase technique frozen sections were fixed and incubated for 30 minutes with 1% H_2O_2 in methanol at room temperature and thereafter rinsed in PBS, while paraffin sections were deparaffinized with xylene (3 × 10 minutes) and ethanol (3 × 5 minutes), also treated in H_2O_2 in methanol for 30 minutes and brought to PBS with a descending alcohol series. The sections were incubated with 1% normal swine serum (obtained from DAKO) for 30 minutes at room temperature, followed by the first antiserum in 1% normal swine serum/PBS. Serum dilutions used in the peroxidase technique were 1:20 to 1:40 for the keratin antiserum, 1:40 to 1:80 for the vimentin antiserum, and 1:80 to 1:160 for the desmin antiserum. The sections were incubated overnight at 4 C, followed by 1 hour at room temperature. After three washing steps in PBS of 10 minutes each, the sections were incubated 30 minutes at room temperature with swine anti-rabbit IgG (DAKO; dilution 1:25) in 1% normal swine serum/PBS. After another series of washing steps the sections were incubated for 30 minutes with the rabbit peroxidase–antiperoxidase complex (DAKO; dilution 1:100) in 1% normal swine serum/PBS. After washing, the antigens were visualized using 3,3′-diaminobenzidine tetrahydrochloride (DAB, obtained from Sigma) during a 2–3-minute incubation step. Mayer hematoxylin (1–3 minutes) was used as counterstain.

In order to obtain an enhanced representation of the keratin content and distribution in these images, we scanned sections with a Leitz Axiomat microscope at 0.5 μ resolution. Interactively, thresholds were selected in the extinction values histogram. The threshold images were then displayed on a TV screen.

Fixation Tests

Small pieces of tissue from an adenocarcinoma from colon and an oral squamous cell carcinoma were fixed in the following fixatives: 1) ethanol for 48 hours; 2) methanol for 48 hours; 3) Carnoy's solution (ethanol:chloroform:concentrated acetic acid = 6:3:1) for 8–12 hours; 4) Bouin's solution (saturated picric acid in water:40% formaldehyde:concentrated acetic acid = 15:5:1) for 8–12 hours; 5) half-strength Zenker's fixative containing 1.25% potassium dichromate, 0.5% sodium sulfate, 2.5% mercuric chloride, and 2.5% acetic acid for 8–12 hours; 6) 4% formaldehyde pH 4.5, for 8–12 hours; 7) 4% buffered formaldehyde, pH 7.3, for 8–12 hours. Tissue from a lobular breast carcinoma was fixed in Carnoy's solution only. The tissue after fixation was dehydrated with the use of alcohol and xylene and embedded in paraffin. The formalin fixative was also tested on cryostat sections from other human tumors and rat tissues.

Results

Normal Tissues

The reactions of the antibodies to intermediate filament proteins in normal human epithelial cells, either keratinizing or nonkeratinizing squamous epithelia or glandular epithelia, can be summarized as follows. With the use of an antiserum to human skin keratins, a strong staining reaction is observed in all stratified squamous epithelia, including those from epidermis, tongue, esophagus, cervix, etc., and in dermal appendages such as hair follicles, sebaceous glands, and sweat glands. In addition, epithelial cells lining the urinary tract, stomach, small and large intestines, uterine endometrium, bile ducts, prostate, kidney collecting tubules, as well as liver hepatocytes, thymic reticulum cells, pancreatic epithelium, and mesothelia show positive keratin staining.

The antibodies to vimentin and desmin do not stain these epithelial tissues but, in contrast, give strong staining reactions in cells of mesenchymal origin. Vimentin antibodies, for example, stain fibroblasts in connective tissue, endothelial cells in blood vessels, Kupffer cells, osteoblasts, chondrocytes, some vascular smooth muscle cells, melanocytes, etc. Desmin antibodies react exclusively with striated skeletal muscle, cardiac muscle, and smooth muscle.

Adenocarcinomas

Table 1 gives an overview of the types of human adenocarcinomas that were examined for the nature of their intermediate filament proteins with frozen sections. We have observed intracellular keratin in all adenocarcinomas described here, while vimentin and desmin are absent in the tumor cells. This holds true for primary adenocarcinomas as well as metastases, either well-differentiated or poorly differentiated variants. With the keratin antibodies used in this study, adenocarcinomas stain, however, significantly less intensively than, for example, squamous cell carcinomas. The fibroblasts present in the stroma accompanying the tumor cells stain with antibodies to vimentin (Figure 1b), while muscle tissue can be detected by the anti-desmin antiserum (Figure 1c).

Figures 1 and 2 present a pictorial overview of some typical examples of adenocarcinomas incubated for the indirect immunofluorescence technique with the antibodies to keratin and in one case with antibodies to vimentin and desmin (Figure 1b and c). It is obvious from several of these immunofluorographs that with the keratin antibodies, often a typical peripheral staining pattern is observed in the adenocarcinoma cells; whereas in the case of prostatic carcinoma, a granular staining pattern is observed. The nature of these dots is not yet understood but may well represent attachment points of keratin filaments at the membrane, eg, desmosomes. In several of the cases studied, the keratin was mainly concentrated at the cell membrane, with sometimes higher concentrations at the apical and basal ends of the cells. Such a submembranous distribution is also often seen in columnar epithelial cells lining ducts of glands, cells lining the respiratory, gastrointestinal, and genital tracts, as well as in hepatocytes. This phenomenon could be observed in a more pronounced way when the keratin patterns in the immunoperoxidase staining method were scanned at 0.5-μ resolution and, after thresholding, the pictures displayed on a TV screen. Figure 3 shows examples of such enhanced representations of the keratin content in an adenocarcinoma from colon.

The antibodies to keratin did not stain tumors of mesenchymal origin (lymphomas, rhabdomyosarcomas, or other soft-tissue tumors). These tumors, on the contrary, are positive with the antibodies to vimentin or desmin (results not shown).[21-23]

Effect of Fixatives

Since Schlegel et al[1] obtained negative results with anti-keratin antiserums in several types of carcinomas using formalin-fixed, paraffin-embedded tissue, we examined the effects of fixation and/or paraffin embedding on the antigenic determinants recognized by our keratin antibodies. Fixation of frozen sections with ethanol, methanol, and/or acetone resulted in a strong staining reaction in both immunofluorescence and immunoperoxidase. However, when these sections were fixed in formalin before incubation, the staining reaction was drastically reduced. When human tumor tissues (an adenocarcinoma from colon and an oral squamous cell carcinoma) treated with several fixatives, as described in Materials and Methods, were embedded in paraffin and used in the immunoperoxidase technique with antibodies to keratin, the reaction in all tissue sections fixed in formalin, Bouin's solution, or Zenker's solution was greatly reduced. The sections of the adenocarcinoma were completely negative when fixed by these methods, whereas the squamous cell carcinoma gave a weak reaction. When, however, the tissues were fixed in methanol, ethanol, or Carnoy's solution and examined for their reaction with the anti-keratin antibodies in paraffin sections, the squamous cell carcinoma gave a strong positive reaction. In the colonic adenocarcinoma only a minor part of the tubular structures were positive for keratin. These data suggest that destruction or masking of keratin antigenic determinants not only occurs as a result of formaldehyde fixation, but may also be a result of the procedure of paraffin embedding of the tissue. Tissue from a lobular breast carcinoma fixed in Carnoy's solution and embedded in paraffin, however, gave a strong reaction for keratin.

The fixatives that result in good reactions for the keratin antibodies are also suitable for the fixation of tissues to be tested for the presence of vimentin. Again, formalin and Zenker's solution reduced the reaction of anti-vimentin antibodies when tested on paraffin sections. The antiserum to desmin described in this paper can be used on paraffin-embedded tissue fixed in all the fixatives mentioned above.

Discussion

Epithelial cells in animal tissues have been shown to contain intermediate-sized filaments of the keratin type (for references see introduction). Not only have these filaments been detected in keratinizing squamous epithelia, but they can also be demonstrated in the nonkeratinizing epithelia from internal organs with the use of antibodies to epidermal keratins[2,29] or liver cytokeratin D,[14] for example. In addition to studies performed on animal tissues, recent efforts have concentrated on human epithelial tissues, with similar results. Special attention is now being paid to

Table 1—Immunohistochemical Detection of Intermediate Filament Proteins in Human Adenocarcinomas*

Location	Diagnosis	No.	Tumor cells K	V	D
Skin	Metastasis of a poorly differentiated ductal breast carcinoma	1	+ +/+ + +	–	–
Submandibular gland	Mucinous adenocarcinoma	1	+ +	–	–
Esophagus/cardia	Moderately well differentiated adenocarcinoma	2	+/+ +	–	–
Stomach	Moderately well/poorly differentiated mucinous adenocarcinoma of the cardia	1	+ +	–	–
	Poorly differentiated adenocarcinoma of the cardia	1	+ +	–	–
	Scirrhous carcinoma	1	+	–	
Colon	Moderately well differentiated adenocarcinoma	2	+	–	
	Mucinous adenocarcinoma from the cecum	1	+/–	–	–
	Moderately well differentiated mucinous adenocarcinoma	1	+ +	–	–
Rectum	Adenocarcinoma	2	+	–	
	Moderately well differentiated adenocarcinoma	1	+	–	
	Villous adenoma (malignant)	1	+/+ +	–	–
	Mucinous adenocarcinoma	1	+ +	–	
Abdominal wall	Metastasis of a mucinous carcinoma from the colon	1	+/+ +	–	–
Lung	Moderately well differentiated adenocarcinoma	2	+	–	–
Thoracic wall	Metastasis of a poorly differentiated ductal breast carcinoma	2	+	–	–
Pancreas	Well differentiated adenocarcinoma	1	+ + +	–	–
	Nesidiocytoma	1	+/–	–	–
Liver	Metastasis of a moderately well differentiated adenocarcinoma	1	+ +	–	–
Bile duct	Poorly/moderately well differentiated adenocarcinoma from the ductus choledochus	1	+	–	–
Ovary	Poorly differentiated adenocarcinoma	1	+ +	–	–
	Cystadenocarcinoma papilliferum serosum	3	+/+ +	–	–
	Brenner tumor	1	+ +	–	–
Uterus	Adenosquamous carcinoma of the endometrium	1	+ +	–	–
	Moderately well differentiated adenocarcinoma of the cervix	1	+/+ +	–	
	Moderately well differentiated adenoacanthoma of the corpus uteri	1	+ +	–	–
Vagina	Moderately well differentiated adenocarcinoma	1	+ +	–	
Female breast	Well differentiated invasive ductal carcinoma	3	+ +	–	–
	Moderately well differentiated invasive ductal carcinoma	15	+/+ + +	–	–
	Poorly differentiated invasive ductal carcinoma	6	+ +	–	–
	Tubular carcinoma	2	+ +	–	–
	Invasive lobular carcinoma	5	+/+ +	–	–
	Invasive small cell lobular carcinoma	1	+ + +	–	–
Prostate	Poorly differentiated adenocarcinoma	2	+ +	–	–
Bladder	Anaplastic, partially duct-forming mucinous carcinoma	1	+ +	–	–
Testis	Metastasis of a mucinous adenocarcinoma from the digestive tract	1	+	–	–
Lymph nodes	Metastasis of an invasive ductal breast carcinoma	1	+ +	–	–
	Metastasis of an invasive lobular breast carcinoma	1	+ +	–	
	Metastasis of a uterine adenosquamous carcinoma	1	+/+ +	–	
	Metastasis of a moderately well/poorly differentiated adenocarcinoma	1	+ +	–	–
	Metastasis of a moderately well differentiated mucinous adenocarcinoma of the cardia	1	+ +	–	–
	Metastasis of a poorly differentiated adenocarcinoma	1	+/+ + + +	–	–
	Metastasis of a poorly differentiated adenocarcinoma from ovary	1	+ +	–	–
	Metastasis of a papillary thyroid carcinoma	1	+ +	–	–
Omentum	Metastasis of a cystadenocarcinoma papilliferum serosum from ovary	1	+/+ +	–	–
	Metastasis of a poorly differentiated adenocarcinoma from ovary	1	+ +	–	–
	Metastasis of an anaplastic carcinoma	1	+ +	+/–	–†
	Metastasis of a poorly differentiated adenocarcinoma probably from the gall bladder (partly papillary)	1	+ +	–	–
Mesentery	Metastasis of a poorly differentiated adenocarcinoma, probably from the gall bladder (partly papillary)	1	+ +	–	–
	Invasive carcinoid	1	+ + +	–	–
Pelvis	Metastasis of a cystadenocarcinoma papilliferum serosum from the ovary	1	+/+ +	–	–
Gluteal muscle	Metastasis of a mucinous carcinoma	1	+	–	–

* The results presented in this table were obtained on methanol/acetone-fixed frozen sections of the tumor tissues. K, keratin; V, vimentin; D, desmin. +/+ +, +/+ + +, etc., indicate varying intensities of fluorescence.

† Some tumor cells in this metastatic anaplastic adenocarcinoma were positive for vimentin in the immunofluorescence test. In the immunoperoxidase test on frozen sections the effect was less pronounced.

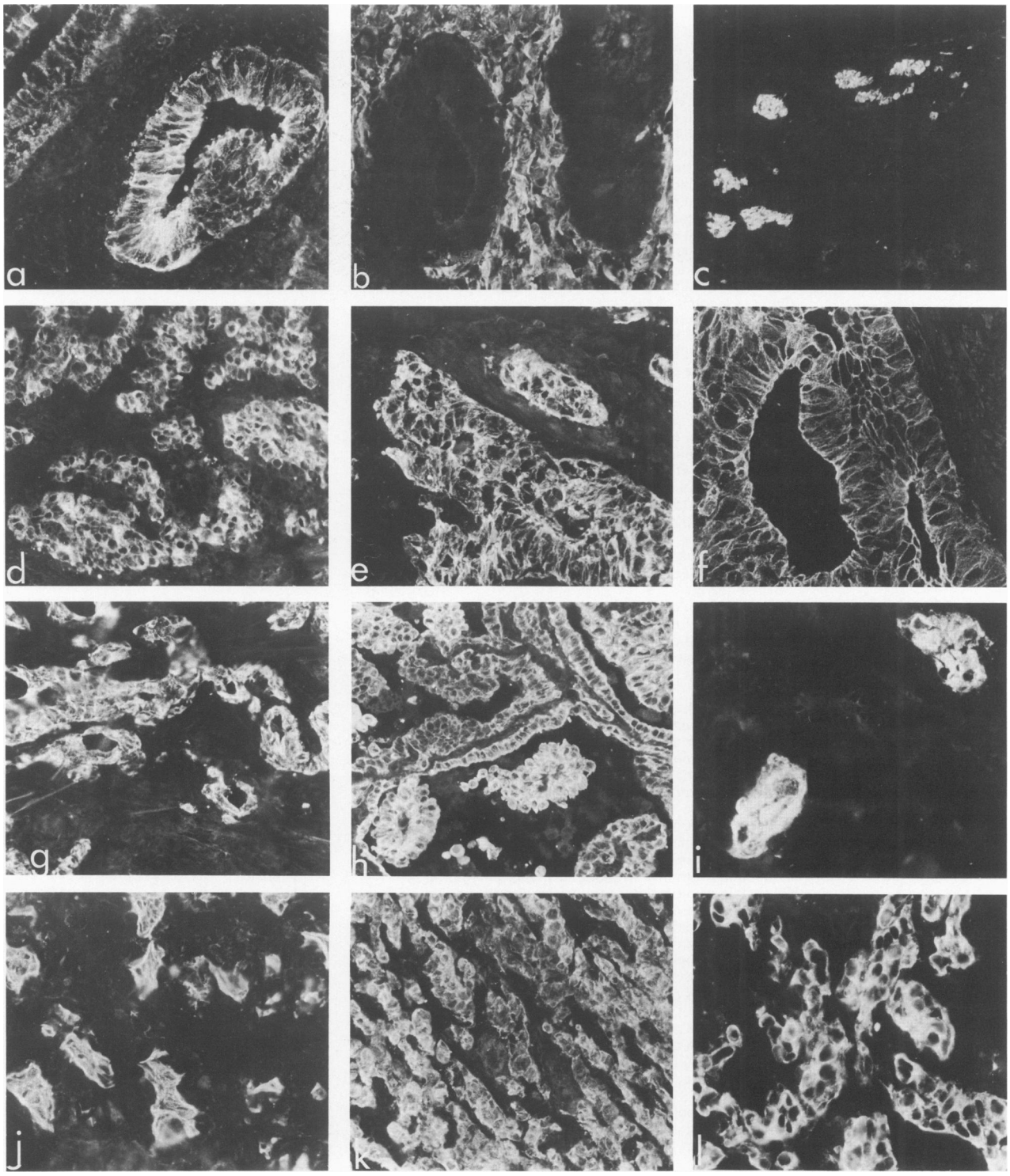

Figure 1 — Immunofluorographs showing some typical examples of human adenocarcinomas incubated with antibodies to intermediate filament proteins. **a–c** — Moderately well differentiated colon adenocarcinoma incubated with antibodies to keratin (**a**, ×240), vimentin (**b**, ×240), and desmin (**c**, ×240). **d–l** — All tumors incubated with the keratin antiserum. **d** — Moderately well differentiated adenocarcinoma in cardia and esophagus. (×150) **e** — Moderately well/poorly differentiated adenocarcinoma of the cardia. (×200) **f** — Adenocarcinoma of the rectum. (×220) **g** — Moderately well differentiated adenocarcinoma of the lung. (×160) **h** — Adenocarcinoma from thyroid, metastasis in a lymphe node. (×130) **i** — Well-differentiated adenocarcinoma from pancreas. (×320) **j** — Mucinous carcinoma in the abdominal wall. (×140) **k** — Poorly differentiated invasive ductal carcinoma in female breast. (×150) **l** — Invasive lobular small cell carcinoma. (×220)

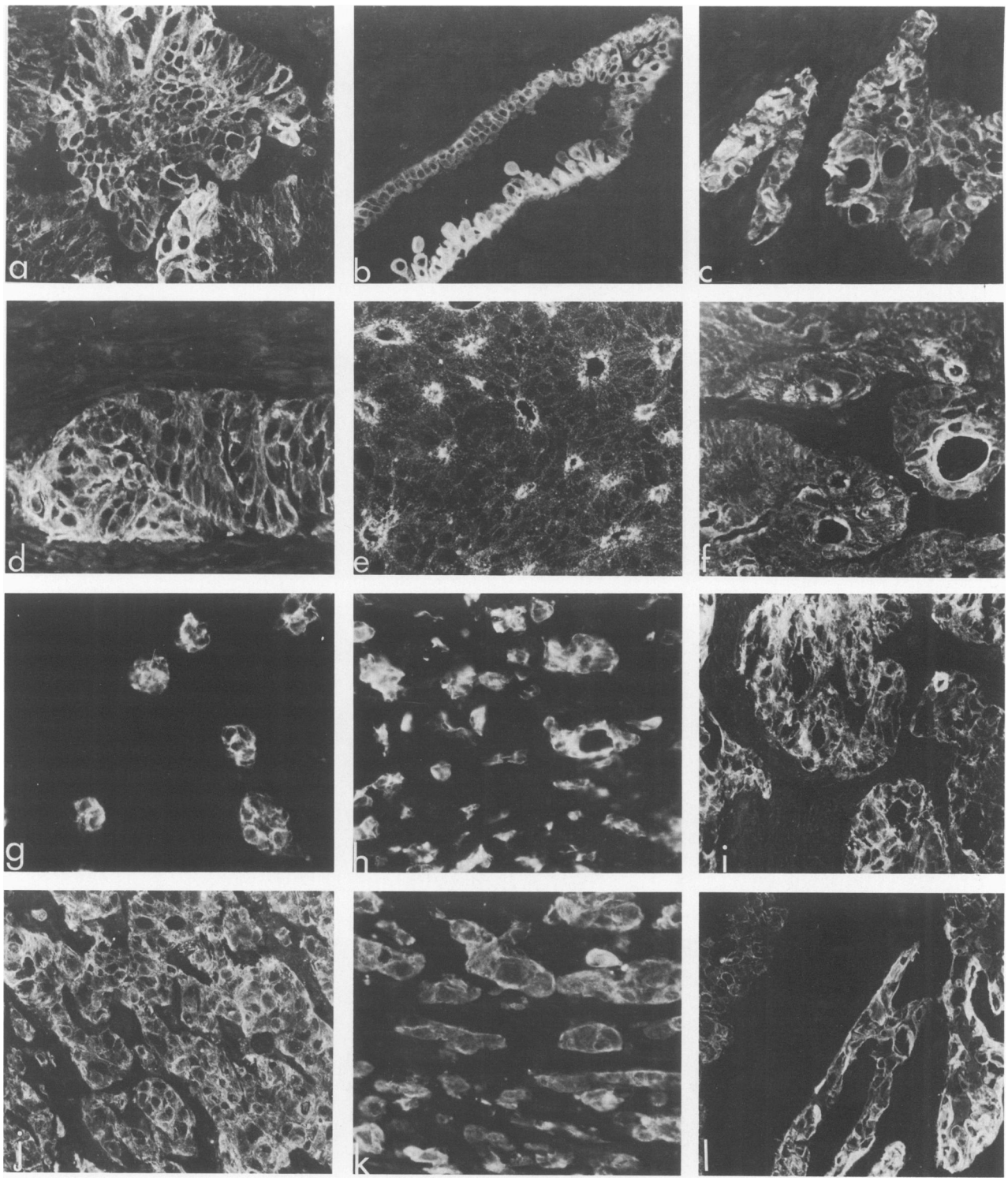

Figure 2 – Demonstration of keratin in human genital tract adenocarcinomas and in metastatic adenocarcinomas. **a** – Adenoacanthoma in the ovary. (× 230) **b** – Cystadenocarcinoma papilliferum serosum in ovary. (× 145) **c** – Moderately well differentiated adenocarcinoma from uterine cervix. (× 230) **d** – Moderately well differentiated adenocarcinoma in the vagina. (× 250) **e** – Poorly differentiated/anaplastic adenocarcinoma in prostate. (× 220) **f** – Mucinous adenocarcinoma in the testis. (× 200) **g** – Lymph node metastasis of a ductal carcinoma from female breast. (× 230) **h** – Lymph node metastasis of an invasive lobular breast carcinoma. (× 250) **i** – Lymph node metastasis of a moderately well differentiated mucinous adenocarcinoma. (× 145) **j** – Metastasis of an anaplastic carcinoma on the omentum. (× 145) **k** – Metastasis of a poorly differentiated gall bladder adenocarcinoma in the mesentery. (× 230) **l** – Metastasis from a cystadenocarcinoma papilliferum serosum on the omentum. (× 145)

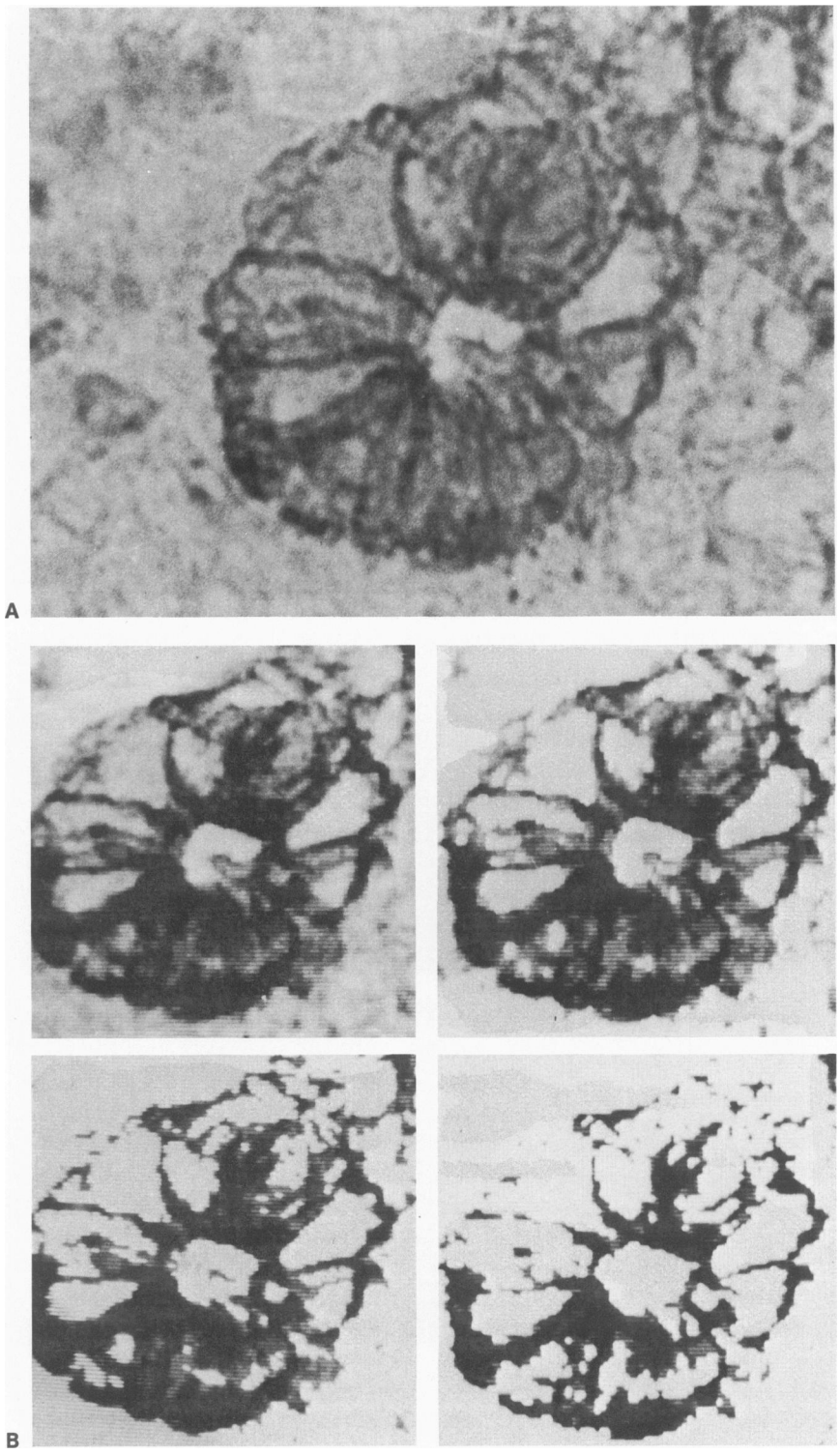

Figure 3—Peripheral localization of keratin in colon adenocarcinoma cells, visualized with the immunoperoxidase technique with the use of an antiserum to human skin keratins. (**a**, ×700) **b**—Display of digitized images at different thresholds.

the identification of intermediate filament proteins in human tumor cells, because their immunochemical identification may be a diagnostic marker that can be useful for differential diagnosis in surgical pathology. For example, antiserums directed against keratin recognize carcinomas, whereas antiserums to vimentin recognize lymphomas and soft-tissue tumors.[13,23] Desmin antibodies are useful tools in the recognition of rhabdomyosarcomas.[7] The reliability of this method is, however, strongly dependent on studies with large series of the different tumor types, so that one can avoid misinterpretations in future routine diagnosis with these serums. When reviewing the literature and taking into account the data presented above, one can see two potential problems with respect to the use of keratin antibodies in the detection of (human) epithelial tumors.

The first problem concerns the tissue cross-reactivity of the different types of keratin antibodies described so far by several investigators. For example, several antiserums directed against human stratum corneum or bovine hoof keratin have been described as not reacting with hepatocytes, acinar cells in the pancreas, and lactating mammary gland cells, nor with tumors derived from these tissues.[1,14,24,29-32] However, the keratin antiserum used in this study, directed against a keratin preparation from human foot calluses, does show (weak) cross-reactivity with keratin filaments in hepatocytes from different species (rat, guinea pig,[33] man) and in cultured hepatocellular carcinoma cells from man[21] and guinea pig,[33] giving results comparable to those found by Franke et al[34,35] and Denk et al[36] with guinea pig antibodies directed against the cytokeratin polypeptide D from mouse liver. Antibodies directed against desmosome-attached tonofilaments from bovine muzzle epidermis have also been described to stain these latter tissues.[34-36] Therefore, these results indicate that sometimes antibodies to keratin from heavily keratinizing epidermis may fail to recognize some of the cell types from internal epithelial organs. On the other hand, the data with our keratin antiserum show that it is possible to raise antibodies to skin keratin that cross-react with hepatocytes and other types of "internal" epithelia, as well as with tumors therefrom.

A second problem is caused by fixation of the tissue with formalin and subsequent paraffin embedding, which seems, under some conditions, to destroy or mask antigenic determinants otherwise recognized by antiserums to these keratin proteins.[6,37] In their study of human neoplastic tissues, Schlegel et al[1] used formalin-fixed, paraffin-embedded material and obtained negative results with an antiserum to epidermal keratins in several types of adenocarcinomas (see introduction). Our data, obtained by the use of several types of fixatives on tissues used for cryostat sectioning or paraffin embedding clearly show that the antigenic determinants recognized by the keratin antiserum used in this study are either masked or destroyed by formalin fixation. For this reason, Altmannsberger et al[6] have used ethanol-fixed material for paraffin embedding. Furthermore, it is obvious that the process of paraffin embedding of the material itself can also partly destroy or mask part of these determinants. Preincubation of the paraffin sections with proteolytic enzymes may help in "unmasking" these antigens or at least some domains of their polypeptide chains (see, for example, Madri and Barwick[17]).

It is therefore most likely that differences in observations with respect to the keratin content of adenocarcinomas reported so far by Schlegel et al[1] and those reported by other investigators[5,6,7,13,14,21,23,25] are a result of differences in fixation and embedding techniques. However, when taking into account some preliminary observations reported by Krepler et al,[14] who used antibodies to hepatocytic cytokeratin on formalin-fixed, paraffin-embedded tissue, we must conclude that differences in keratin subtype recognition of the antibodies can also be responsible for negative results described so far.

Except for some cells in a metastatic anaplastic adenocarcinoma (which may have originated from ascitic fluid; see Ramaekers et al[38]) we have not observed the expression of vimentin in the solid carcinomas described in this paper. It therefore seems that in general cells in solid tumors do not contain more than one type of intermediate filament (there are exceptions[7,12,15]).

The typical peripheral distribution of keratins in many adenocarcinomas may still reflect the localization of these filaments in the polarized cells from several normal glandular epithelia. Such a typical peripheral organization of keratin is observed in several types of columnar epithelial cells as well as in liver hepatocytes. Franke et al,[31,32] for example, have shown a preferential staining of the apical and basal region in intestinal epithelium. This typical distribution may in future studies prove to be of some help in differential diagnosis between epithelial tumors, for example, mesotheliomas (see also Corson and Pinkus[39]) and adenocarcinomas of various origins. Furthermore, ultrastructural observations have shown mesotheliomas to contain higher concentrations of intermediate-sized filaments than adenocarcinomas,[40] a phenomenon that may be reflected in quantitative differences in the indirect immunofluorescence assay.

We conclude from the data presented here that antibodies to epidermal keratin are useful tools in the differential diagnosis of human tumors and can also be used in the detection of adenocarcinomas. They can be helpful in distinguishing adenocarcinomas from nonepithelial tumors, which is especially important in cases where poorly differentiated variants have to be diagnosed and adenocarcinoma has to be differentiated from nonepithelial malignancies, for example, malignant lymphoma.

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