Keratin Expression in Cervical Cancer

Frank Smedts,* Frans Ramaekers,‡
Sergey Troyanovsky,‖ Maciej Pruszczynski,*
Monique Link,* Birgitte Lane,¶ Irene Leigh,#
Charles Schijf,† and Peter Vooijs*

From the Departments of Pathology* and Gynecology and
Obstetrics,† University Hospital Nijmegen, Nijmegen, The
Netherlands; the Department of Molecular Cell Biology and
Genetics,‡ University of Limburg, Maastricht, The
Netherlands; the All-Union Cancer Research Center,‖ The
USSR Academy of Medical Sciences, Moscow, Soviet Union;
the Department of Biochemistry,¶ Cell Structure Research
Group, The University of Dundee, Dundee, United Kingdom;
and the ICRF Skin Tumor Laboratory,# London Hospital
(Medical College), London, United Kingdom

Using a panel of 21 monoclonal and 2 polyclonal keratin antibodies, capable of detecting separately 11 subtypes of their epithelial intermediate filament proteins at the single cell level, we investigated keratin expression in 16 squamous cell carcinomas, 9 adenocarcinomas, and 3 adenosquamous carcinomas of the human uterine cervix. The keratin phenotype of the keratinizing squamous cell carcinoma was found to be most complex comprising keratins 4, 5, 6, 8, 13, 14, 16, 17, 18, 19, and usually keratin 10. The nonkeratinizing variety of the squamous cell carcinoma expressed keratins 6, 14, 17, and 19 in all cases, usually 4, 5, 7, 8, and 18, and sometimes keratins 10, 13, and 16. Adenocarcinomas displayed a less complex keratin expression pattern comprising keratins 7, 8, 17, 18, and 19, while keratin 14 was often present and keratins 4, 5, 10, and 13 were sporadically found in individual cells in a few cases. These keratin phenotypes may be useful in differential diagnostic considerations when distinguishing between keratinizing and nonkeratinizing carcinomas (using keratin 10, 13, and 16 antibodies), and also in the distinction between nonkeratinizing carcinomas and poorly differentiated adenocarcinomas, which do not express keratins 5 and 6. Keratin 17 may also be useful in distinguishing carcinomas of the cervix from those of the colon and also from mesotheliomas. Furthermore the presence of keratin 17 in a CIN I, II, or III lesion may indicate progressive potential while its absence could be indicative of a regressive behavior. Because most carcinomas express keratins 8, 14, 17, 18, and 19, we propose that this expression pattern reflects the origin of cervical cancer from a common progenitor cell, i.e., the endocervical reserve cell that has been shown to express keratins 5, 8, 14, 17, 18, and 19. (Am J Pathol 1992, 141:497–511)

Based on biochemical[1] and immunochemical studies[2–5] a considerable volume of information has been derived concerning keratin expression in the normal uterine cervix, in cervical intraepithelial neoplasia (CIN), and in cervical cancer. Not only have the prevalent keratins in each of these types of epithelium been characterized by gel electrophoresis, but the intraepithelial distribution pattern of many of these keratins has also been clarified immunohistochemically using polypeptide specific keratin antibodies.[3–6] The distribution of the keratins in the cervix can be summarized as follows (Figure 1).

Ectocervical epithelium (Figure 1a) has been shown to contain type I keratins 13, 14, 15, 16, and 19 with the sporadic expression of keratins 10, 11, and 17 and also type II keratins 4, 5, 6, and 8 with sporadic expression of keratins 1 and 2.[1–3]

Endocervical columnar cells (Figure 1b) contain keratins 7, 8, 18, and 19[1–3] and variable amounts of keratin 4.[3] In the direct vicinity of reserve cells, small amounts of keratins 5, 14, and 17[4] have sometimes been observed.

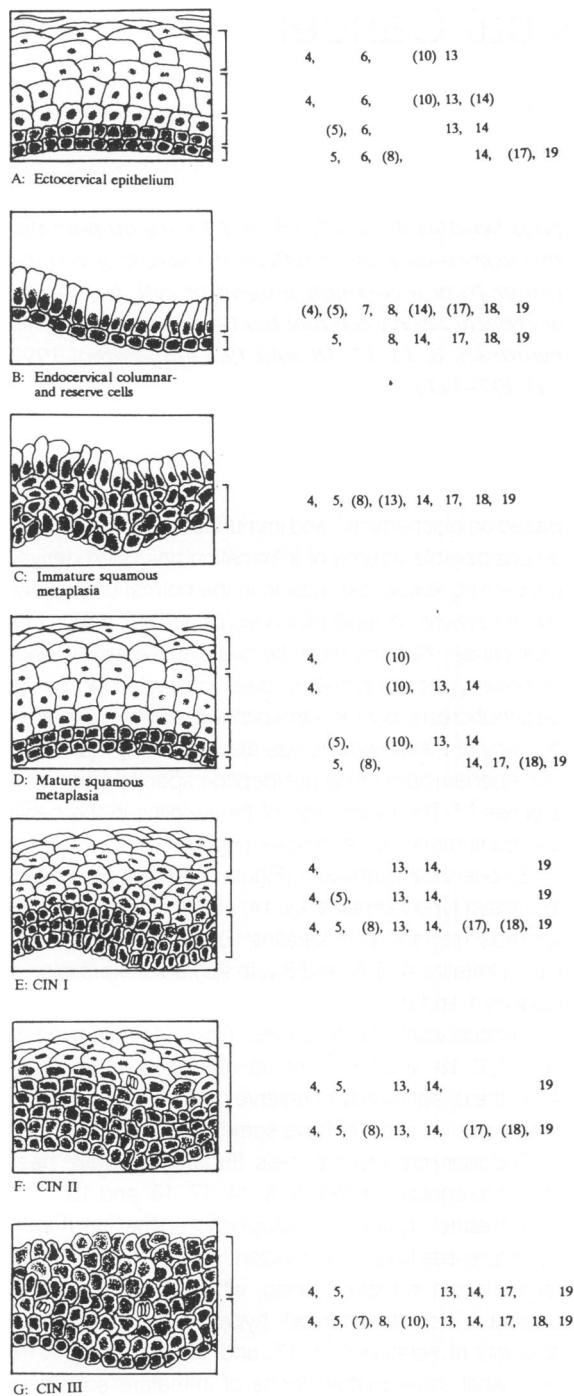Endocervical reserve cells (Figure 1b) have been shown to contain keratins 5, 8, 14, 17, 18, and 19.[3–5]

Immature squamous metaplastic epithelium (Figure 1c) expresses keratins in relation to the level of its maturation. In the immature forms, which are hardly distinguishable from reserve cell hyperplasia, considerable amounts of keratins 5, 8, 17, and 18 are found. In the somewhat more mature forms of immature squamous metaplasia, these simple keratins are expressed to a lesser extent and the expression of keratins 4, 13, and 14 appears. Keratin 19 is found throughout the full thickness of this type of epithelium.[3,4,6]

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4,   6,   (10) 13

4,   6,   (10), 13, (14)

(5), 6,   13, 14

5, 6, (8),   14, (17), 19

A: Ectocervical epithelium

(4), (5), 7, 8, (14), (17), 18, 19

5,   8, 14, 17, 18, 19

B: Endocervical columnar- and reserve cells

4, 5, (8), (13), 14, 17, 18, 19

C: Immature squamous metaplasia

4,   (10)

4,   (10), 13, 14

(5), (10), 13, 14

5, (8),   14, 17, (18), 19

D: Mature squamous metaplasia

4,   13, 14,   19

4, (5), 13, 14,   19

4, 5, (8), 13, 14, (17), (18), 19

E: CIN I

4, 5, 13, 14,   19

4, 5, (8), 13, 14, (17), (18), 19

F: CIN II

4, 5,   13, 14, 17, 19

4, 5, (7), 8, (10), 13, 14, 17, 18, 19

G: CIN III

Figure 1. *Schematic overview of the keratin expression patterns in the various epithelia of the human uterine cervix under physiologic and pathologic conditions. The keratins found in the different epithelial layers are represented by numbers on the right. Numbers in brackets indicate that the keratin in question is only found in some cells and expression is usually weak. In other cases the keratin indicated is found in the majority of cells and expression is moderate to intense.*

Mature squamous metaplasic epithelium (Figure 1d) expresses keratins in much the same way as observed in ectocervical nonkeratinizing squamous epithelium. Keratins 5, 17, and 19[4] are invariably found in the basal layer of this epithelium. Keratin 14 is found in the basal, para-basal, and intermediate epithelial layer with its intensity of expression gradually diminishing towards the surface of the epithelium. In contrast, keratin 4 is detectable only sporadically in parabasal cells, with expression increasing in the intermediate and superficial epithelial layers. Keratin 13 is found throughout the full epithelial thickness except in the basal epithelial layer, which is usually not stained. Keratins 8 and 18 are sporadically detectable in basal cells.[3] There have been a few reports on keratin expression in CIN I (Figure 1e), CIN II (Figure 1f), and CIN III (Figure 1g).[3,4] The pattern of keratin expression in these lesions is reminiscent of keratin expression in reserve cells and in immature squamous metaplastic epithelium. In CIN I and CIN II, keratins 4, 5, 13, 14, and 19 are usually found, although with somewhat erratic expression patterns. Keratins 8, 10, 17, and 18 are incidentally found, usually in the basal part of CIN I and CIN II lesions. In CIN III lesions a considerable change has taken place in keratin expression in comparison to CIN I and CIN II lesions. The keratins 5, 8, 14, 17, 18, and 19 found in reserve cells, are only irregularly present with variable staining intensity in many of the CIN III lesions. Keratins 4 and 13 are also found in CIN III although expression is usually less than in CIN I and II lesions.

In the past, we have hypothesized that CIN III lesions showing the keratin profile of reserve cells, may be lesions that will progress into cervical cancer. We have also suggested that adenocarcinomas may originate from CIN III lesions. These suppositions are based partly on gel electrophoresis studies that showed nonkeratinizing squamous cell carcinomas to contain keratins 5, 6, 7, 8, 13, 14, 15, 17, 18, and 19. Keratinizing carcinomas displayed a less complex keratin expression pattern, lacking keratins 7, 8, and 18. Adenocarcinomas were found to contain keratins 7, 8, 18, and 19 and sometimes 17.[1,2] There are only a few studies in which cervical carcinomas have been investigated using specific keratin antibodies.[7,8] In these studies, squamous cell carcinomas were shown to invariably express keratins 8, 14, 18, and 19 and sometimes keratins 4, 10, and 13. In adenocarcinomas, keratins 8, 18, and 19 were found and in some cases also keratin 4.

Using a large panel of monoclonal antibodies, allowing the separate detection of keratins 1, 4, 5, 6, 7, 8, 10, 13, 14, 16, 17, 18, and 19 at the single cell level, we investigated the expression of these keratins in a large number of squamous cell carcinomas and adenocarcinomas of the cervix. The data support previous speculation regarding the relationship between reserve cells, immature squamous metaplasia, and CIN lesions on the one hand and cervical cancer on the other hand. Furthermore specific keratin expression patterns may be indicators of the propensity of lesions to regress or to progress.

Materials and Methods

Tissues

The tissue specimens used in this study were taken from hysterectomies or biopsies performed because of carcinomas of the uterine cervix. The ages of the patients varied between 30 and 65 years. The tissues were brought to the pathology department immediately after removal. Small representative samples from the tumor were removed and snapfrozen in liquid nitrogen or liquid nitrogen-cooled isopentane and stored in liquid nitrogen. The rest of the specimen was routinely fixed in formalin and further processed through paraffin for light microscopic diagnosis. In total, 28 specimens representing 16 squamous cell carcinomas, 9 adenocarcinomas, and 3 adenosquamous carcinomas of the cervix were analyzed.

The tumors were classified according to the World Health Organization (WHO) classification.[9] Squamous cell carcinomas were subdivided into 5 cases of keratinizing and 11 cases of nonkeratinizing squamous cell carcinomas. Also neoplasms with a glandular component were classified according to the WHO classification.[9] Five carcinomas were of the endocervical type, they could be subdivided into three moderately and two poorly differentiated carcinomas. Four endometrioid adenocarcinomas were diagnosed, of which one was well differentiated, two moderately and one poorly differentiated. Furthermore our series included three adenosquamous carcinomas. In all cases, the slides were diagnosed independently by three pathologists.

Antibodies

Twenty-three monoclonal keratin antibodies were used in this study. Their characteristics are summarized in Table 1.[3,4,10-24]

Immunohistochemistry

Cryostat sections were fixed in acetone at room temperature for 10 minutes and air-dried. The slides were incubated with the appropriately diluted or undiluted primary antibody for 60 minutes at room temperature. After three subsequent washing steps with phosphate buffered saline (PBS), the peroxidase-conjugated rabbit-anti-mouse or swine-anti-rabbit serum (Dakopatts, Glostrup, Denmark) was applied to the sections for 30 minutes at room temperature. After a second series of washing steps with PBS, peroxidase activity was detected with 4-amino-9-ethylcarbazole (AEC; Aldrich Chemical Comp., St. Louis, MO) as described.[3] The slides were counterstained with Harris' hematoxylin and mounted with Kaisers glycerin-gelatin (Boom BV, Meppel, The Netherlands).

Results

General Considerations Concerning Immunohistochemistry

Before describing the staining patterns of the individual antibodies some general statements must be made. Al-

Table 1. *Overview of the Antibodies Used in This Study*

Antibody	Ig subtype	Keratin(s) recognized	Species	Ref	Source
AF87	polyclonal	1	Rabbit	10	D. Roop, Bethesda, MD
6B10	IgG1	4	Mouse	11	G. van Muijen, Nijmegen, NL
AE14		5	Mouse	12	T. T. Sun, New York, NY
RCK102	IgG1	5 and 8	Mouse	13	F. Ramaekers, Maastricht, NL
KA12		5 and 6	Mouse	14	R. Nagle, Tuscon, AZ
AF124	polyclonal	6	Rabbit	10	D. Roop, Bethesda, MD
RCK105	IgG1	7	Mouse	13	F. Ramaekers, Maastricht, NL
M20	IgG1	8	Mouse	15	G. van Muyen, Nijmegen, NL
CAM52	IgG2a	8	Mouse	3	Becton and Dickinson, Etten-Leur, NL
LE41		8	Mouse	16	E. Lane, Dundee, UK
RKSE60	IgG1	10	Mouse	17	F. Ramaekers, Maastricht, NL
2D7	IgG2a	13	Mouse	11	G. van Muyen, Nijmegen, NL
1C7	IgG2b	13	Mouse	11	G. van Muyen, Nijmegen, NL
LL001	IgG2a	14	Mouse	18	E. Lane, Dundee, UK
LL002	IgG3	14	Mouse	18,19	E. Lane, Dundee, UK
RCK107	IgG1	14	Mouse	4,14	F. Ramaekers, Maastricht, NL
LL025	IgG1	16	Mouse	Unpublished	E. Lane, Dundee, UK
E3	IgG2b	17	Mouse	20	S. Troyanovsky, Moscow, USSR
RGE53	IgG1	18	Mouse	21,22	F. Ramaekers, Maastricht, NL
RCK106	IgG1	18	Mouse	13	F. Ramaekers, Maastricht, NL
2C8	IgG1	18	Mouse	23	G. van Muyen, Nijmegen, NL
LP2K	IgG2b	19	Mouse	24	E. Lane, Dundee, UK
RCK108	IgG1	19	Mouse	Unpublished	F. Ramaekers, Maastricht, NL

though the staining intensity with each of the antibodies was variable within each tumor, we frequently observed a consistent pattern of compartmentalization of antibody expression to well-defined parts of the tumor islands. In general, in squamous cell carcinomas the following compartments could be defined. 1) A basal cell compartment that consisted of the layer of cells directly adjacent to the tumor stroma and at most two above lying cell layers. Morphologically these cells showed basaloid features with large nuclei and scant cytoplasm. 2) Above this layer, a second intermediate compartment, approximately three to four cells thick could be distinguished. The cells in this compartment were larger with more cytoplasm. There was some resemblance to the intermediate layer of mature squamous metaplastic epithelium or ectocervical epithelium. 3) Above this layer, there was a third layer comprising more mature squamous cells that demonstrated varying degrees of maturation and sometimes keratinization. A second feature worth mentioning is the variability of staining intensity between antibodies recognizing the same keratin. As it is a well-known fact that epitope masking may explain apparently negative results, several antibodies recognizing the same keratin were used. For reasons of clarity we will only describe the most salient features of our staining results. Table 2 shows the reaction pattern of the most strongly reacting antibody in cases where more than one reagent was used for the same keratin subtype. For reasons mentioned earlier, some antibody reaction patterns do, however, need further specification. We also refer to Figures 2–6 as illustrations of the various staining patterns.

Keratinizing Squamous Cell Carcinoma

In all keratinizing squamous cell carcinomas, keratins 4, 5, 6, 8, 13, 14, 16, 18, and 19 were detectable with the respective monoclonal antibodies. In four cases, keratin 10 was also found, and in two cases, keratin 7 could be detected. The antibody against keratin 1 was negative in all five specimens (Figure 2a). The keratin 4 antibody (Figure 2b) displayed a variable staining intensity in all cases. Part of the central compartment of the tumor always stained intensely. Staining in other compartments was variable, the basal layer often being negative. Keratin 8 detectable with the antibodies M20, CAM 5.2, and LE41 showed considerable variations in staining intensity. CAM 5.2 (Figure 2h) stained all cases, showing areas staining with moderate intensity, alternating with negative areas. Furthermore, there was some compartmentalization of staining, with one case showing stronger expression in the basal layer and other fragments giving a more intense staining reaction in the inner cell compart-

ment. The M20 (Figure 2i) antibody displayed positivity in all but one case, the staining pattern in the positive cases being much the same as CAM 5.2, although staining was sometimes more intense with M20. LE 41 was completely negative in two cases, and in three cases there was some positivity restricted to the inner most cell compartment.

Keratin 10, a marker for keratinization (Figure 2j) was found in dispersed cells in the intermediate and inner most cell compartments. The basal cell compartment was only partly positive for keratin 13 (Figure 2k) and the intensity was less than in the more central compartments. Of the three antibodies that are monospecific for keratin 14 (LL001, LL002 and RCK 107), RCK 107 (Figure 2l) showed the most intense staining. The inner most cell compartment was usually negative with occasionally a few dispersed weakly staining cells. LL001 and LL002 were both completely negative in one case, the other four cases showing positivity in the basal cell compartment, which tended to decrease toward the inner cell compartment.

Keratin 16 (Figure 2m) could be found in all cases, although only a minority of cells reacted in the intermediate and central cell compartments. E3 (Figure 2n) which detects keratin 17 decorated most of the cells in the basal cell compartment with weak-to-moderate intensity. In the intermediate cell compartment and the inner cell compartment only a few dispersed cells stained.

Three antibodies against keratin 18 were used, i.e., RGE 53, RCK 106, and 2C8. Of these, the RCK 106 antibody (Figure 2o) was the strongest and stained a minority of cells in all five tumors with most of these cells located in the basal cell compartment. Virtually all cells in the intermediate and inner cell compartments were negative.

Large-cell Nonkeratinizing Carcinoma

In the 11 large-cell nonkeratinizing carcinomas, keratins 6, 14, 17, and 19 were detectable. In ten specimens, keratin 18 was detectable, whereas in nine cases, keratins 5, 7, and 8 were also expressed. In eight neoplasms, keratin 4 was found, and in seven cases keratin 13 was also found. In six carcinomas, keratin 10 was detected, whereas five tumors showed keratin 16 along with the aforementioned keratins. Keratin 1 was not found in any of these specimens.

Keratin 7 was restricted to the basal cell compartment in eight cases (Figure 3f). The three keratin 8 antibodies M20, CAM 5.2, and LE 41 showed different staining patterns. CAM 5.2 stained ten specimens, of which five cases were positive (Figure 3g) with varying intensity. Of the other five cases, two displayed reactivity in 1–2% of

Table 2. *Keratin Expression Patterns of Cervical Carcinomas as Detected by a Panel of Chain-specific Monoclonal and Polyclonal Keratin Antibodies*

| Case	Histologic diagnosis	1	4	5	5+8	5+6	6	7	8	10	13	14	16	17	18	19
1	KSQ	−	±	++++	++++	++++	++++	−	+++	+++	+++++	+++	++	+	++	+++
2		−	++	++++	++++	++++	++++	±	+++	++	++++++	+++	±	+	++	+++
3		−	±	++++	++++	++++	++++	−	+++	++	+++++	+++	+	+	+	+++
4		−	++++	++++	++++	++++	++++	−	++	+++	+++++	+++	+	++	+	+++
5		−	+++	++++	++++	++++	++++	+	+++	+++	++++++	+++	+	++		+++
6		−	+	++++	++++	++++	++++	+	+++	+++	++++	+++		++		+++
7	NKSQ	−	++	+	++++	++++	++++	±	−	+	+++	+++	+++	++	++	+++
8		−	+++	+++	++++	++++	++++	+++	+++	±	+	+++	+++	+++	++	+++
9		−	+++	+++			++++	+++	+++	−	++			++++	+	+++
10		−	+++	++		++++	++++	+++	+++	+	+++	+++	+++	++	+	+++
11		−	+	−	+	++++	−	−	−	−	−	±	−	+	+++	+++
12		−	+	−	+	++++	±	+++	+++	−	−	+	−	++	+++	+++
13		−	+++	+++	++++	++++	±	+++	+++	±	−	±	−	++++	+++	+++
14		−	±	+++	++++	++++	±	+++	+++	+	−	+	±	+	+++	+++
15		−	+	+++	+	++++	+	+++	+++	±	−	+++	+	+++	+++	+++
16		−	−	+++	+	++++	+	+++	+++	−	−	+++	−	++++	+++	+++
17	AC/MD	−	−	−	−	+	−	+++	+++	−	−	+++	−	++++	+++	+++
18		−	+++	+	+++	+++	±	+++	+++	−	+	±	−	+	+++	+++
19		−	−	−	−	−	±	+++	+++	−	−	±	−	+	+++	+++
20	AC/PD	−	−	−	−	−	±	+++	+++	−	−	−	−	+	+++	++
21		−	−	−	+	+++	±	+++	+++	−	−	+++	±	+++	+++	+++
22	ACE/GI	−	±	−	+	+++	+++	+++	+++	±	±	++	−	+	+++	+
23	ACE/GII	−	±	±	+	+++	+++	+++	+++	±	±	+++	±	+	+++	+++
24		−	−	−	−	−	+++	+++	−	−	−	−	−	+	+++	+++
25	ACE/GIII	−	±	+	+++	+++	+++	+++	+++	−	−	+	−	++	+	+
26	ASQ	−	±	+++	+++	+++	+++	+++	+++	±	+++	+++	−	++	++	++
27		−	+++	+++	+++	+++	+++	+++	+++	−	+++	−	−	+++	+++	+++
28		−	−	+	+++	+++	±	+++	+++	±	±	+	±	+	+++	+++

KSQ = keratinizing squamous cell carcinoma of the cervix; NKSQ = nonkeratinizing large-cell squamous carcinoma of the cervix; AC = adenocarcinoma of the cervix; MD = moderately differentiated; PD = poorly differentiated; ACE = adenocarcinoma of the cervix endometrioid type; G = grade; ASQ = adenosquamous carcinoma; ± = only a few scattered cells stain with variable intensity; + = 1–25% of cells stain; + + = 25–50% of cells stain; + + + = 50–75% of cells stain; + + + + = 75–100% of cells stain.
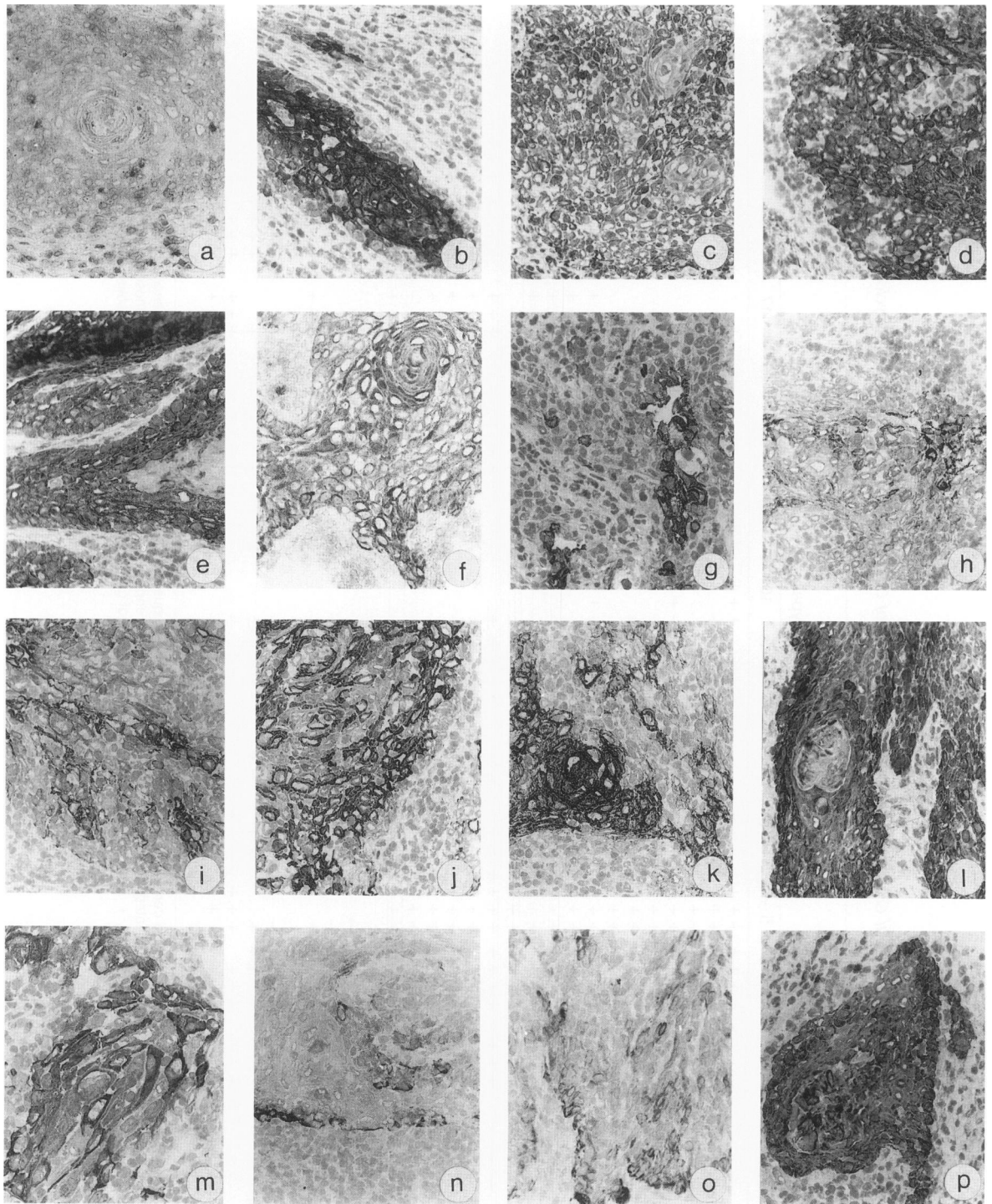
Figure 2. *Immunoperoxidase staining pattern of frozen sections from keratinizing squamous cell carcinomas after incubation with:* (a) *AF87 (keratin 1),* (b) *6B10 (keratin 4),* (c) *AE 14 (keratin 5),* (d) *RCK102 (keratins 5 + 8),* (e) *KA12 (keratins 6 + 5),* (f) *AF124 (keratin 6),* (g) *RCK105 (keratin 7),* (h) *CAM5.2 (keratin 8),* (i) *M20 (keratin 8),* (j) *RKSE60 (keratin 10),* (k) *1C7 (keratin 13),* (l) *RCK107 (keratin 14),* (m) *LLO25 (keratin 16),* (n) *E3 (keratin 17),* (o) *RCK106 (keratin 18),* (p) *RCK108 (keratin 19). Original magnification* a,c,d,f,i,k,o,p ×250; h,j,n ×300; e,g,l ×400; b,m ×500.

cells. In the remaining three specimens, varying numbers of cells stained for CAM 5.2 with variable intensity. M20 decorated nine specimens. In four of them, all cells

stained with variable intensity. The remaining five neoplasms showed intense positivity of most of the cells in the basal cell layer. In three of these cases, cells in the
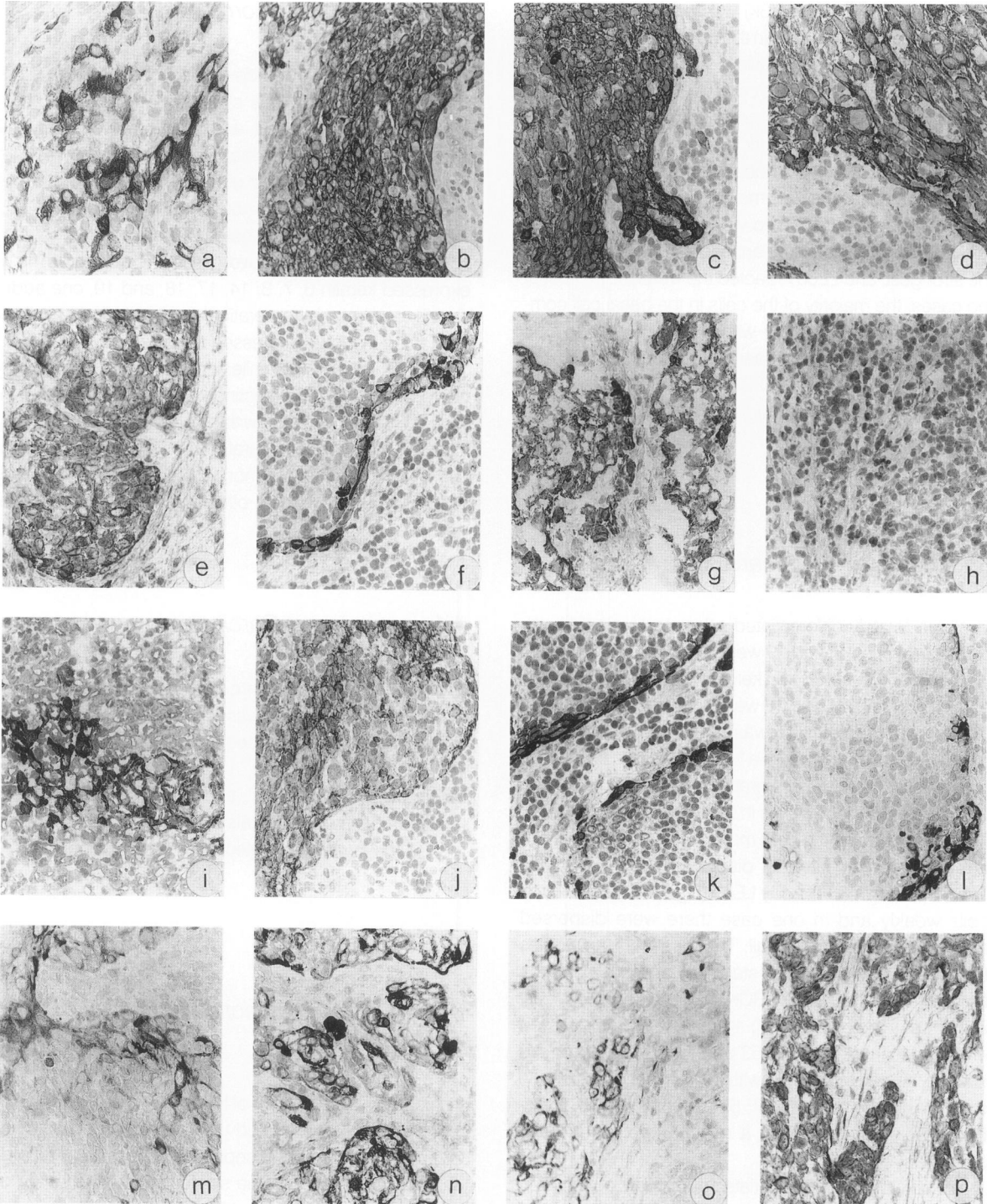
Figure 3. *Immunoperoxidase staining pattern of frozen sections from large-cell nonkeratinizing squamous cell carcinomas after incubation with:* **(a)** *6B10 (keratin 4),* **(b)** *AE14 (keratin 5),* **(c)** *RCK102 (keratins 5 + 8),* **(d)** *KA12 (keratins 5 + 6),* **(e)** *AF124 (keratin 6),* **(f)** *RCK105 (keratin 7),* **(g)** *CAM5.2 (keratin 8),* **(h)** *LE41 (keratin 8),* **(i)** *RKSE10 (keratin 10),* **(j)** *1C7 keratin 13,* **(k)** *RCK107 (keratin 14),* **(l)** *LLO25 (keratin 16),* **(m)** *E3 (keratin 17),* **(n)** *RCK106 (keratin 18),* **(o)** *RGE53 (keratin 18),* **(p)** *RCK108 (keratin 19). Original magnification* **b,c,f,j,k,l** *×250;* **e** *×300;* **d,g,h,i,m–p** *×400;* **a** *×500.*

intermediate and inner cell compartments also stained with weak intensity. Seven specimens were weakly positive with LE41 (Figure 3h). The staining intensity of CAM 5.2 and M20 was comparable; however, the reactivity of LE41 was significantly less. Also LL001, LL002, and RCK 107, which detect keratin 14, showed a variable staining

pattern. RCK 107 (Figure 3k) was positive in all cases with the majority of the cells in the basal cell compartment usually staining intensely. Cells in the other two compartments were also sometimes positive. LL002 was positive in nine cases, with obvious compartmentalization of the staining reaction mainly restricted to the basal cell compartment. LL001 showed moderate positivity in eight carcinomas, and staining was mainly restricted to the basal compartment with dispersed weak positivity in the other compartments. Of the three antibodies, RCK 107 reacted the strongest, and LL001 reacted the weakest. In most of the cases, the majority of the cells in the basal cell compartment stained intensely with RCK 106 (Figure 3n), whereas in the other compartments considerably less cells stained for keratin 18. 2C8 was the least reactive of the three keratin 18 antibodies since only four cases showed weak positivity with this reagent in dispersed cells

Endocervical Adenocarcinoma

In all moderately differentiated adenocarcinomas, keratins 7, 8, 14, 17, 18, and 19 were detected. In two cases, we also found keratin 6 and keratin 4 (Figure 4a). Keratins 1, 10, (Figure 4i) 13, and 16 were not detectable in any of the specimens. Keratin 5 was negative in two carcinomas, while in one specimen a few dispersed cells stained weakly (Figure 4b). The keratin 8 antibodies M20 and CAM 5.2 decorated all cells in the three specimens. M20 (Figure 4g) stained the cells most intensely. The CAM 5.2 antibody staining intensity displayed some variations. The third keratin 8 antibody LE41 (Figure 4h) stained the cells weakly and in one case there were dispersed cells that did not stain at all. The keratin 14 antibodies LL001 and LL002 did not stain any of the tumors, the third keratin 14 antibody, RCK 107 displayed positivity in all three cases (Figure 4j). Keratin 18 was expressed in all cases in which RGE 53 and RCK 106 (Figure 41) stained all cells intensely with only minor variations in staining intensity for the RGE 53 antibody. The 2C8 antibody only weakly stained a minority of cells in each specimen.

In the two poorly differentiated adenocarcinomas, keratin expression was the same as in the moderately differentiated adenocarcinomas. The only difference was that keratin 14 was expressed in one tumor and keratin 4 was not detectable at all. In both poorly differentiated adenocarcinomas, keratin 6, 7, 8, 17, 18, and 19 were detectable, and in one case also keratin 14. Keratins 1, 4, 5, 10, 13, and 16 were not detected in these carcinomas.

Endometrioid Carcinoma

In four specimens, an endometrioid carcinoma was diagnosed. One tumor was classified as a grade I, two were classified as grade II, and one tumor was grade III. The keratin expression in these carcinomas showed differences as compared with endocervical carcinoma. The grade I endometrioid carcinoma expressed keratins 4, 6, 7, 8, 14, 17, 18, and 19, whereas keratins 1, 10, 13, and 16 were not found. The two grade II carcinomas both expressed keratin 6, 7, 8, 14, 17, 18, and 19, one additionally expressed the keratins 4, 10, 13, and 16. The grade III carcinoma expressed keratins 4, 5, 6, 7, 8, 10, 13, 14, 17, 18, and 19, while keratins 1 and 16 were not expressed. RCK 107 detecting keratin 14 was present in all specimens (Figure 5j) with a minority of cells staining intensely. The other two keratin 14 antibodies LL001 and LL002 both stained a minority of cells intensely in the grade III carcinoma. The other three carcinomas were negative.

Adenosquamous Carcinoma

The three adenosquamous carcinomas showed a somewhat different keratin expression pattern when compared with the previously described tumors. All carcinomas expressed keratins 5, 6, 14, 17, 18, and 19 while in two carcinomas, keratins 4, 7, and 8 were additionally expressed and in one case also keratins 10 and 13. Keratins 1 and 16 were not detected in these tumors.

Discussion

Keratin Epitope Detection and Antibody Affinity

In this study the presence of keratins in 28 carcinomas of the cervix was investigated using an extended panel of 23 antibodies capable of separately detecting 13 individual keratin subtypes at the single cell level. The use of more than one keratin antibody directed against the same keratin protein allowed us to detect structural alterations in keratin filament organization probably resulting from epitope alterations due to biologic behavior during neoplastic transformation. This phenomenon, commonly referred to as epitope masking, was frequently observed. For example, keratin 8 could be detected by three different antibodies, of which the M20 antibody reacted with
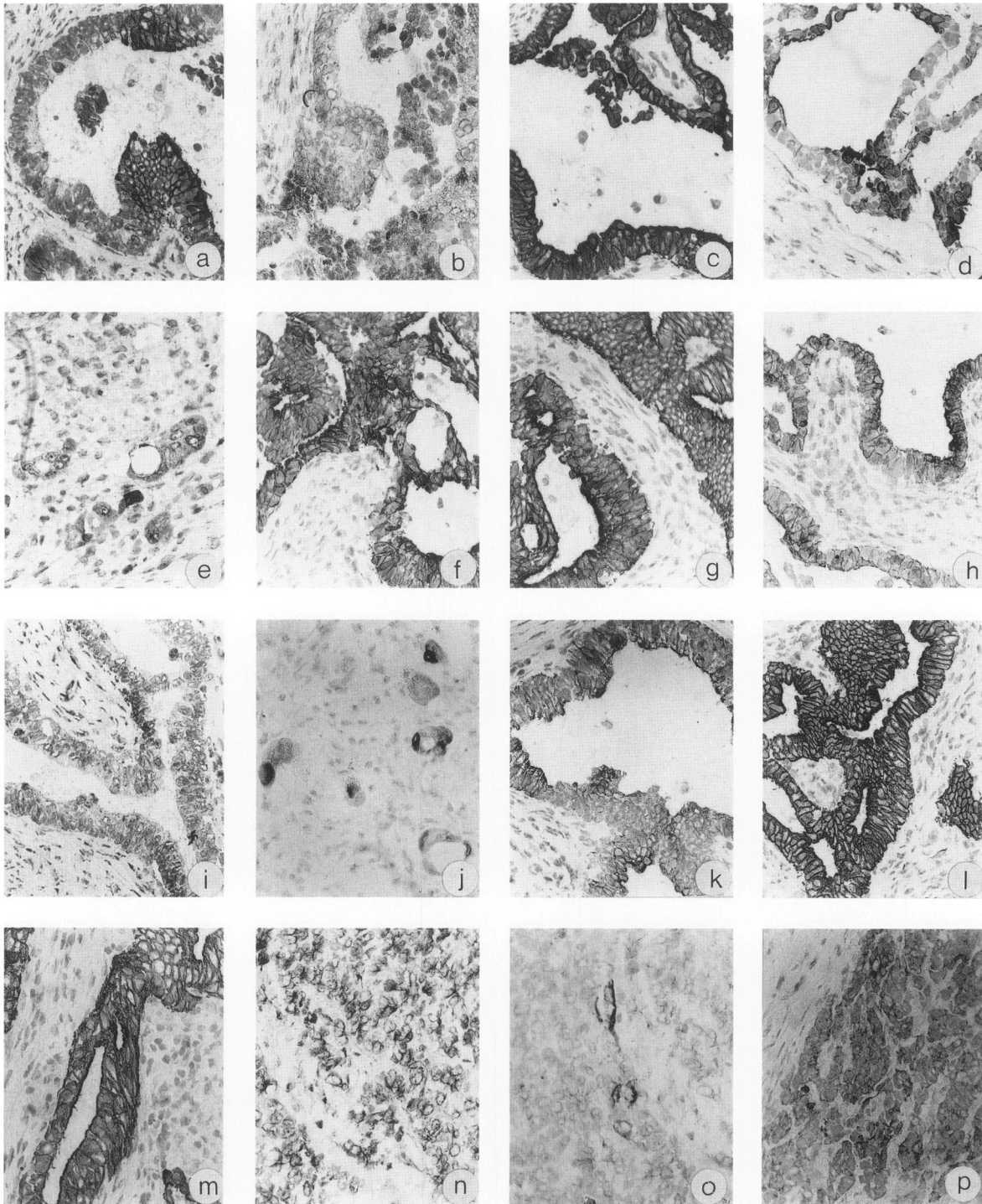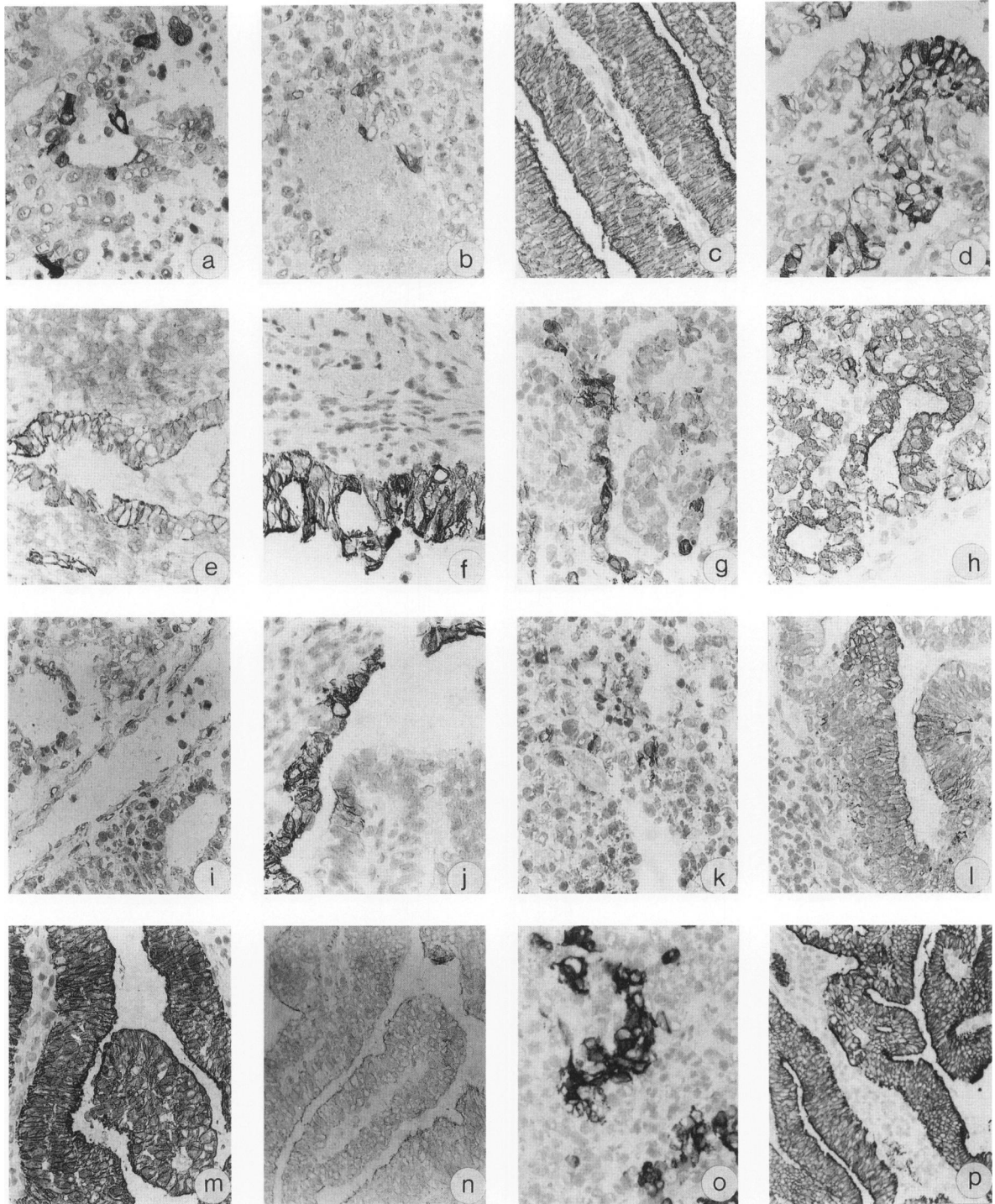
Figure 4. *Immunoperoxidase staining patterns of moderately differentiated endocervical adenocarcinomas (a–m) and poorly differentiated endocervical adenocarcinomas (n–p) after staining with:* (a) *6B10 (keratin 4),* (b) *AE14 (keratin 5),* (c) *RCK102 (keratin 5 + 8),* (d) *KA12 (keratin 5 + 6),* (e,n) *AF124 (keratin 6),* (f) *RCK105 (keratin 7),* (g) *M20 (keratin 8),* (h) *LE41 (keratin 8),* (i) *RKSE60 (keratin 10),* (j,o) *RCK107 (keratin 14),* (k) *E3 (keratin 17),* (l) *RCK106 (keratin 18),* (m) *RCK108 (keratin 18),* (p) *2C8. Original magnification,* n, o, p *×400;* a–d, f–i, k, l, m *×500;* e,j *×600.*

more cells than CAM 5.2 in all carcinomas while also the intensity of staining had increased. The 2C8 antibody reacted with less intensity. When comparing the two ker- atin 13 antibodies, this effect was observed only once and the staining patterns of both antibodies was usually the same. Keratin 14 was monitored by three antibodies

Figure 5. *Immunoperoxidase staining patterns of endometrioid adenocarcinomas of the cervix, grade I (c,e,j,l–n,p), grade II (a,d,f) and grade III (b,g–h,k,o) after staining with:* (a) *6B10 (keratin 4),* (b) *AE14 (keratin 5),* (c) *RCK102 (keratin 5 + 8),* (d) *KA12 (keratin 5 + 6),* (e) *AF124 (keratin 6),* (f,g) *RCK105 (keratin 7),* (h) *M20 (keratin 8),* (i) *RKSE60 (keratin 10),* (j) *RCK107 (keratin 14),* (k,l) *E3 (keratin 17),* (m) *RCK106 (keratin 18),* (n) *2C8 (keratin 18),* (o,p) *RCK108 (keratin 19). Original magnification* i,j,k ×400; a–e,g,h,l–p ×500; f ×600.

that showed considerable variations in their staining patterns. RCK 107 stained varying numbers of cells in all tumors intensely, while LL001 and LL002 were less reactive, LL001 stained less tumors, and the staining intensity was also considerably lower than that found for the two aforementioned keratin 14 antibodies. An effect much like
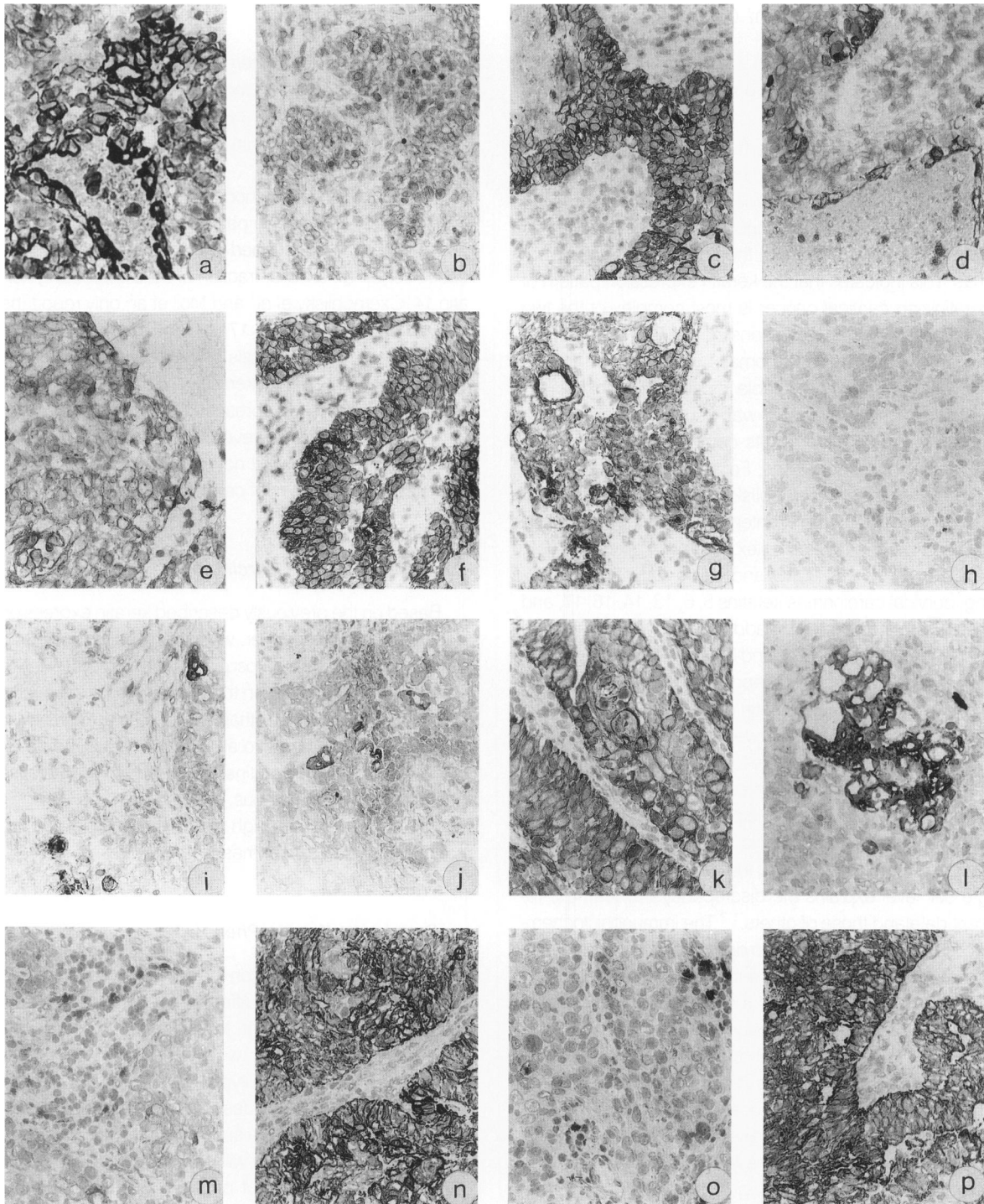
Figure 6. *Immunoperoxidase staining patterns of adenosquamous carcinomas of the cervix after staining with:* (a) *6B10 (keratin 4),* (b) *AE14 (keratin 5),* (c) *RCK102 (keratin 5 + 8),* (d) *KA12 (keratin 5 + 6),* (e) *AF124 (keratin 6),* (f) *RCK105 (keratin 7),* (g,h) *M20 (keratin 8),* (i) *RKSE60 (keratin 10),* (j) *2D7 (keratin 13),* (k) *RCK107 (keratin 14),* (l) *LLO25 (keratin 16),* (m) *E3 (keratin 17),* (n) *RCK106 (keratin 18),* (o) *RGE53 (keratin 18),* (p) *RCK108 (keratin 19). Original magnification* a–h *×250;* i,j,m–p *×400;* k,l *×500.*

this was observed with the keratin 18 antibodies of which the antibody RCK 106 was most reactive, staining cells in practically every carcinoma, RGE 53 was slightly less reactive than RCK 106 and 2C8 was least reactive with only a minority of cells staining weakly. The two keratin 19 antibodies gave similar results

Keratin Composition of Cervical Carcinomas

Squamous Cell Carcinoma

Based on the underlying study, the keratins common to all squamous cell carcinomas included 6, 14, 17, 18, and 19. In all keratinizing carcinomas, keratins 4, 5, 8, 13, and 16 were additionally found. These keratins were only detected in a subfraction of the nonkeratinizing carcinomas. This indicates that the keratin expression pattern of keratinizing cervical cancer is most complex of the two subtypes, especially if we consider the fact that in four of the five keratinizing carcinomas, we also found keratin 10, which was only detectable in half of the nonkeratinizing carcinomas in which it was usually expressed to a lesser degree. These results complement and partially contradict previous reports. For example in gel electrophoretic studies, Czernobilsky et al[1] and Moll et al[2] showed that the keratin content of nonkeratinizing carcinomas was the most complex, comprising keratins 5, 6, 7, 8, 13, 14, 15, 16, 17, 18, and 19 while during keratinizing, cervical carcinomas keratins 5, 6, 13, 14, 16, 17, and 19 were found. Our study additionally demonstrates the presence of keratins 4, 8, and 18 in all keratinizing carcinomas, the frequent presence of keratin 10 and sometimes keratin 7. To the keratin content of nonkeratinizing carcinomas known to date, we may add the presence of keratins 4 and 10 which were found in a number of cases. Our interpretation of the expression of simple keratins 7, 8, and 18 will be discussed later. The fact that the present immunohistochemical techniques using specific antibodies are capable of detecting individual keratins at the single cell level explains the discrepancy between our recent data and those of others.[1,2] The immunohistochemical study of Ivanyi et al[8] also demonstrates the presence of keratins 4 and 10 in nonkeratinizing carcinomas.

Apparently a number of the nonkeratinizing carcinomas have a keratin expression phenotype normally observed in keratinizing squamous cell cancers, although there are no histologic signs of cornification. We consider these to be examples of cancers that based on morphologic criteria would be classified as nonkeratinizing. Based on their keratin expression patterns, however, they should be classified as being of the keratinizing variety. The implications of this statement are far reaching since this means that these carcinomas could have a worse prognosis than the true nonkeratinizing variety.

The fact that all keratinizing carcinomas usually display a more complex keratin expression pattern than all nonkeratinizing carcinomas is not surprising as expression of keratins 4, 10, and 13 accompanies squamous cell differentiation and keratinization.

Adenocarcinoma

In the moderately differentiated, as well as in the poorly differentiated, endocervical adenocarcinomas keratins 7, 8, 17, 18, and 19 were expressed in all cases, and frequently Keratin 14. In one case, keratins 4 and 5 were found.

The endometrioid adenocarcinomas of the endocervix showed an expression pattern that was similar to that of the previously described adenocarcinomas, but all cases contained a minor fraction of cells expressing keratin 14. Czernobilsky et al[1] and Moll et al[2] only report the presence of keratins 7, 8, 17, 18, and 19 in cervical adenocarcinomas on the basis of their gel electrophoretic studies but did not detect keratins 4, 6, 10, and 13, which we find sporadically expressed in this type of malignancy. More striking, however, is the fact that these authors and Yvani et al[8] did not find keratin 14, which we detected in most cases of cervical adenocarcinoma.

Adenosquamous Carcinoma

Based on the previously described keratin expression patterns in cervical cancer, we expected the keratin expression pattern of adenosquamous carcinomas to be complex. To our surprise in these carcinomas the overall pattern was less complex than expected with keratins 14, 17, 18, and 19 common to all, while two specimens additionally expressed keratins 4, 7, and 8. The keratins characteristic of squamous differentiation, i.e., 10, 13, and 16 were oddly enough only found in one of three adenosquamous carcinomas.

Consensus Keratin Phenotypes

The keratin composition of cervical carcinomas is complex with up to 12 different keratins being coexpressed in the same tumor. In an attempt to simplify the high degree of complexity, we propose the definition of a "consensus keratin phenotype" for a given tumor type. This consensus will solely describe the most frequent keratin composition found in a specific tumor type (Figure 7). This idealized description will only in part match the determined keratin profile of an individual tumor. In this study, we were able to identify differences between distinct carcinoma groups. Thus, the most differentiated cervical tumors of the keratinizing squamous cell type exhibit the most stable keratin phenotype. The five tumors in our study varied only slightly from the consensus phenotype, i.e., positive for keratins 4, 5, 6, 8, 10, 13, 14, 16, 17, 18, 19, and mostly negative for keratin 7. The determination of the consensus for nonkeratinizing squamous cell carci-
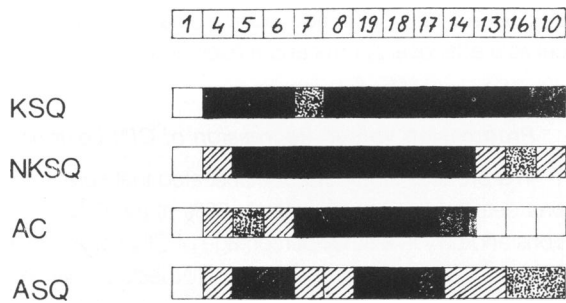
Figure 7. *Schematic representation of the "consensus keratin phenotypes" of the different cervical carcinomas. These are represented as the probability of expression of a single keratin in a each of the subgroups of carcinomas. A completely filled box means the probability of expression of a particular keratin is between 75% and 100%. A hatched box denotes a probability of expression between 50 and 75%. A box containing dots represents probability of expression between 25 and 50%. An open box represents a probability of expression between 0 and 25%. KSQ = keratinizing squamous cell carcinoma of the cervix; NKSQ = nonkeratinizing squamous cell carcinoma of the cervix; AC = adenocarcinoma of the cervix; ASQ = adenosquamous carcinoma of the cervix.*

nomas is more difficult. The most obvious difference from the first group is the presence of keratin 7 in most cases. As discussed, the keratins 10, 13, and 16 were only detected in about 50% of tumors. The phenotype of cervical adenocarcinomas is again rather uniform, i.e., positive for keratins 7, 8, 14, 17, 18, and 19. The most frequent difference from the first two groups is the absence of keratin 5. Some doubt still remains concerning the expression of keratin 6 in these tumors because of the weak reactivity. In addition, there are significant differences in the probability of deviation from the "consensus" phenotype for individual cytokeratins. We may divide these nonobligatory keratins into two groups — highly variable keratins and unusual ones. Thus, the high variability in the detection of keratins 10, 13, and 16 in nonkeratinized squamous carcinomas may be caused by the difference in tumor origin, level of differentiation, or inaccuracies in the pathologic diagnosis. The same reasons may be responsible for unusual keratin expression patterns, for example, expression of keratins 4 and 5 in one adenocarcinoma (case 18).

Reserve-cell Keratins Occur in Cervix Carcinomas

The information we have collected regarding keratin expression in cervical carcinoma allows the following hypothesis on the etiology of cancer in the uterine cervix and also allows a firmer basis to previous speculation regarding which CIN lesions are progressive and which are regressive. From the literature,[25] it is known that the progenitor cell of the majority of squamous cell carcinomas of the cervix is the subcolumnar reserve cell. From these cells a squamous cell carcinoma may develop via squamous metaplastic transformation going through pro-

gressive intraepithelial dedifferentiation. Adenocarcinomas of the cervix are believed to develop from the columnar cells lining the endocervical canal through grades of of atypia, ultimately developing into cervical adenocarcinoma. The keratin expression pattern we have described, however, does not support this theory. As a matter of fact, the keratin expression pattern of adenocarcinomas of the cervix is more reminiscent of the keratin expression pattern of reserve cells, which contain keratins 5, 8, 14, 17, 18, and 19. In squamous cell carcinomas, these keratins were also found. On the basis of these findings, we suggest that both adenocarcinomas and squamous carcinomas of the cervix develop from a common progenitor cell, namely the reserve cell. This also explains the persistence of simple keratins, i.e., keratins 8, 17, and 18 in squamous cell carcinomas.

In a recent publication, Dallenbach-Hellweg et al[26] state that keratin 8 is found in the reserve cell type of squamous cell carcinoma of the cervix and not in the squamous cell type. We found significant expression of keratin 8 in both keratinizing and nonkeratinizing squamous cell carcinoma of the cervix, as well expression of keratins 5, 14, 18, and 19. We therefore consider the more comprehensive theory regarding the origin of both squamous and adenocarcinomatous cervical cancer to be the subcolumnar reserve cells as the most likely explanation on the basis of cell biology.

Application of Keratin Antibodies in Cervical Carcinoma Diagnosis

Keratinizing Versus Nonkeratinizing Squamous Cell Carcinoma

The exact classification of a squamous cell carcinoma into the keratinizing or nonkeratinizing subtype may be rather difficult, since cornified cells that mark the distinction between these two subtypes may be difficult to detect. When keratin pearls are not found, a squamous cell carcinoma will usually be subclassified as being of the nonkeratinizing variety, which according to some authors is expected to have a better prognosis.[28] Immunohistochemical methods may be of considerable help to differentiate between these two subtypes. Keratinizing squamous cell carcinomas contain keratins 13 and 16, and usually keratin 10, which are found considerably less frequently in the nonkeratinizing variety. On the basis of the presence of these keratins, a cervical carcinoma should be classified as keratinizing. Antibodies RKSE60, 1C7, 2D7, and LLO25 may become important tools for this differential diagnosis. This means that considerably more squamous cell carcinomas will be classified as keratinizing than is presently the case. Whether the tumors expressing keratin 10, 13 and 16 but without morphologic

signs of cornification will have a poorer prognosis than the subtype in which keratins 10, 13, and 16 are not should be the subject of future research.

Squamous Cell Carcinoma Versus Adenocarcinoma

A panel of keratin antibodies may also allow distinction between poorly differentiated squamous cell carcinomas and poorly differentiated adenocarcinomas. The presence of keratin 5 is unusual in an adenocarcinoma and when present is only detectable in a few scattered cells. On the other hand, it is found in most nonkeratinizing and all keratinizing squamous cell carcinomas of the cervix. Also keratin 6 may serve as a discriminator since it is present in all squamous cell carcinomas and found only in a minority of cells in adenocarcinomas. A similar effect was also observed for keratin 14 when the RCK 107 antibody was used. In line with a previous report[28] the results presented in this article demonstrate that antibodies to keratins 8 and 18 can not be used reliably to distinguish between squamous cell and adenocarcinoma, since the latter of the two when originating from the cervix or the lung, express these simple keratins.

Adenocarcinomas of the Cervix Versus Adenocarcinomas from Other Origins

Keratin 17 was detected in variable numbers of cells in all cervical carcinomas irrespective of their subclassification. Preliminary studies have indicated that this keratin subtype is present in approximately one third of invasive ductal carcinomas of the breast,[14] in all squamous cell carcinomas of the lung and usually not in adenocarcinomas of the lung.[31] Moll and Czernobilsky[1,2] did not detect keratin 17 in adenocarcinomas of the endometrium by gel electrophoresis. Troyanovsky et al[20] have shown that keratin 17 is not found in normal intestinal epithelia, the epithelium of the oviduct, or mesothelial cells. Quinlan et al[29] demonstrated by means of gel electrophoresis that no keratin 17 could be detected in several subtypes of adenocarcinomas including those of the ovary, stomach, gall bladder, as well as mesotheliomas. In all adenocarcinomas of the cervix, however, keratin 17 was detectable. In a previous study we demonstrated that carcinomas of the colon did not contain this keratin type,[30] meaning that this antibody may be extremely helpful in the distinction between adenocarcinoma of the cervix and colon. This differential diagnosis may pose difficult problems because the close anatomical relationship of both organs may not always allow an easy distinction on the basis of topography alone. We therefore propose that keratin 17 may be potentially important for the distinction between adenocarcinomas of the cervix

and these other types of adenocarcinomas, and between cervical adenocarcinoma and mesothelioma.

Progression Versus Regression of CIN Lesions

In a previous study, we demonstrated that keratin 17 was detectable in approximately 50% of the CIN III lesions and only in a small percentage of CIN I and CIN II lesions.[3] We proposed that those CIN lesions that expressed keratin 17 may be of the progressive type and that keratin 17 could indicate whether a CIN III lesion will become malignant or not. The fact that in the underlying study we were able to detect keratin 17 in all carcinomas further supports this hypothesis and means that keratin 17 could be a marker for progression in cervical intraepithelial neoplasia.

Acknowledgments

References

1. Czernobilsky B, Moll R, Franke WW: Intermediate filaments of normal and neoplastic tissues of the female genital tract with emphasis on problems of differential tumor diagnosis. Pathol Res Pract 1984, 179:31–37
2. Moll R, Levy R, Czernobilsky B, Hohlweg-Majert P, Dallenbach-Hellweg G, Franke WW: Cytokeratins of normal epithelia and some neoplasms of the female genital tract. Lab Invest 1983, 49:599–610
3. Smedts F, Ramaekers F, Robben H, Pruszczynski M, Muijen G van, Lane B, Leigh I, Vooijs P: Changing patterns of keratin expression during progression of cervical intraepithelial neoplasia. Am J Pathol 1990, 136:657–668
4. Smedts F, Ramaekers F, Troyanovsky S, Pruszczynski M, Robben H, Lane B, Leigh I, Vooijs P: Basal cell keratins in reserve cells and a comparison to their expression in cervical intraepithelial neoplasia. Am J Pathol, 1991
5. Weikel W, Wagner R, Moll R: Characterization of subcolumnar reserve cells and other epithelia of the human uterine cervix. Virchows Arch B 1987, 54:98–110
6. Gigi-Leitner O, Geiger B, Levy R, Czernobilsky B: Cytokeratin expression in metaplasia of the human uterine cervix. Differentiation 1986, 31:191–205
7. Angus B, Kiberu S, Purvis J, Wilkinson L, Horne CHW: Cytokeratins in cervical dysplasia and neoplasia: A comparative study of immunohistochemical staining using monoclonal antibodies NCL-5D3, CAM 5.2 and PKK1. J Pathol 1988, 155:71–77

8. Ivanyi D, Groeneveld E, Doornewaard G van, Mooi W, Hageman PC: Keratin subtypes in carcinomas of the uterine cervix: Implications for histogenesis and differential diagnosis. Cancer Res 1990, 50:5143–5150

9. Poulsen HE, Taylor CW, Sobin LH: Histological typing of female genital tract tumors. Geneva, WHO 1975:55–59

10. Roop DR, Cheng CK, Titterington L, Meyers CA, Stanley JR, Steinert PM, Youspa SH: Synthetic peptides corresponding to keratin subunits elicit highly specific antibodies. J Biol Chem 1984, 259:8037–8040

11. Muijen GNP van, Ruiter DJ, Franke WW, Achtstaetter T, Haasnoot WHB, Ponec M, Warnaar SO: Cell type heterogenity of cytokeratin expression in complex epithelia and carcinomas as demonstrated by monoclonal antibodies specific for cytokeratins nos. 4 and 13. Exp Cell Res 1985, 162:97–113

12. Moll R, Dhouailly D, Sun T-T: Expression of keratin 5 as a distinctive feature of epithelial and biphasic mesotheliomas: An immunohistochemical study using the monoclonal antibody AE 14. Virchows Archiv B Cell Pathol 1989, 58:129–145

13. Ramaekers F, Huijsmans A, Schaart G, Moesker O, Vooijs P: Tissue distribution of keratin 7 as monitored by a monoclonal antibody. Exp Cell Res 1987, 179:235–249

14. Wetzels RHW, Kuijpers HJH, Lane EB, Leigh IM, Troyanovsky SM, Holland R, Haelst JGM van, Ramaekers FCS: Basal cell specific and hyperproliferation-related keratins in human breast cancer. Am J Pathol 1991, 138:751–763

15. Schaafsma HE, Ramaekers F, Muijen GNP van, Ooms ECM, Ruiter DJ: Distribution of cytokeratin polypeptides in epithelia of the adult urinary tract. Histochemistry 1989, 91:151–159

16. Lane EB: Monoclonal antibodies provide specific intramolecular markers for the study of epithelial tonofilament organization. J Cell Biol 1982, 92:665–673

17. Puts JJG, Moesker O, Kenemans P, Vooijs GP, Ramaekers FCS: Expression of cytokeratins in early neoplastic epithelial lesions of the uterine cervix. Int J Gynecol Pathol 1985, 4:300–313

18. Purkis PE, Steel JB, Mackenzie IC, Nathrath WJB, Leigh IM, Lane EB: Antibody markers of basal cells in complex epithelia. J Cell Sci 1990, 97:39–50

19. Leigh IM, Pulford KA, Ramaekers FCS, Lane EB: Psoriasis maintenance of an intact monolayer basal cell differentiation compartment in spite of hyperproliferation. Br J Dermatol 1985, 113:53–64

20. Troyanovsky SM, Geulstein VI, Tchipysheva TA, Krutovskikh VA, Bannikow GA: Patterns of expression of keratin 17 in human epithelia: dependancy on cell position. J Cell Sci 1989, 93:419–426

21. Ramaekers F, Huijsmans A, Moesker O, Kant A, Jap P, Herman C, Vooijs GP: Monoclonal antibodies to keratin filaments specific for glandular epithelia and their tumors: Use in surgical pathology. Lab Invest 1983, 49:353–361

22. Ramaekers F, Huijsmans A, Schaart G, Moesker O, Vooijs GP: Cytoskeletal proteins as markers in surgical pathology. Application of monoclonal antibodies in tumor pathology. Edited by Ruiter DJ, Fleuren G, Warnaar SO. Dordrecht/Boston/Lancaster, Marius Nijhoff Publishers, 1987, pp 65–85

23. Schaafsma HE, Ramaekers FCS, Muijen GNP, Lane EB, Leigh EB, Leigh IM, Robben H, Huijsmans A, Ooms ECM, Ruiter DJ: Distribution of cytokeratin polypeptides in human transitional cell carcinomas with special emphasis on changing expression patterns during tumor progression. Am J Pathol 1990, 136:329–343

24. Broers JLV, Carney DN, Klein-Rot M, Schaart G, Lane EB, Vooijs GP, Ramaekers F: Intermediate filament proteins in classic and variant types of small cell lung carcinoma cell lines: a biochemical and immunohistochemical analysis using a panel of monoclonal and polyclonal antibodies. J Cell Sci 1986, 83:37–60

25. Vooijs GP: Benign proliferative reactions, intraepithelial neoplasia and invasive cancer of the uterine cervix. Edited by Bibbo M. Philadelphia, WB Saunders, 1991, pp 153–230

26. Dallenbach-Hellweg G, Lang G: Immunohistochemical studies on uterine tumors 1. Invasive squamous cell carcinomas of the cervix and their precursors. Pathol Res Pract 1991, 187:36–43

27. Reagan JW, Fu YS: Histologic types and prognosis of cancers of the uterine cervix. Int J Radiat Oncol Biol Phys 1979, 5:1015–1020

28. Broers JLV, Ramaekers FCS, Klein-Rot M, Oostendorp T, Huysmans A, Muijen GNP van, Wagenaar S Sc, Vooijs GP: Cytokeratins in different types of human lung cancer as monitored by chain specific monoclonal antibodies. Cancer Res 1988, 48:3221–3229

29. Quinlan RA, Schiller DL, Hatzfeld M, Achtstätter T, Moll R, Jorcano JL, Magin TM, Franke WW: Patterns of expression and organization of cytokeratin intermediate filaments. Ann NY Acad 1985, 455:282–306

30. Ramaekers F, Niekerk C van, Poels L, Schaafsma E, Huijsmans A, Robben H, Schaart G, Vooijs GP: Use of monoclonal antibodies to keratin 7 in the differential diagnosis of adenocarcinomas. Am J Pathol 1990, 136:641–655

31. Wetzels RHW, Schaafsma HE, Leigh IM, Lane EB, Troyanovsky SM, Wagenaar SS, Vooijs GP, Ramaekers FCS: Type VII collagen distribution in different types of human lung carcinomas. Correlation with expression of keratins 14, 16, 17 and 18. Histopathology 1992, 20:295–303