Keratin Expression in Cervical Cancer

Frank Smedts,* Frans Ramaekers,‡ Sergey Troyanovsky,† Maciej Pruszczyński,* 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 often 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† and immunohistochemical studies2–5, 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 cases been characterized by gel electrophoresis, but the intracellular distribution pattern of these keratins has also been clarified immunohistochemically using polypeptide specific keratin antibodies.3–5 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

Ectocervical columnar cells (Figure 1b) contain keratins 7, 8, 18, and 193–5 and variable amounts of keratin 4.3 In the direct vicinity of reserve cells, small amounts of keratin 5, 14, and 173 have sometimes been observed.

Endocervical reserve cells (Figure 1b) have been shown to contain keratins 5, 8, 14, 17, 18, and 193–5.

Immature squamous metaplastic epithelium (Figure 1c) expresses keratin 1 in relation to the level of its maturation, in the immature forms, which are rarely 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.2–5

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Address reprint requests to Dr. F. Smedts, Department of Pathology, Diagnostic Centrum SSDZ, Reitier de Graafweg 7, 2526 AD Delft, The Netherlands.

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Mature squamous metaplastic epithelium (Figure 1c) expresses keratins in much the same way as observed in ectocervical nonkeratinizing squamous epithelium: Keratins 5, 17, and 19 are invariably found in the basal layer of this epithelium. Keratin 14 is found in the basal, parabasal, 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. There have been a few reports on keratin expression in CIN I (Figure 1e), CIN II (Figure 1f), and CIN III (Figure 1g). 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. There are only a few studies in which cervical carcinomas have been investigated using specific keratin antibodies. 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 85 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. 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. Five carcinomas were of the endocervical type, they could be subdivided into three moderately and two poorly differentiated carcinomas. Four endometroid 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, Gloseurgy, 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 (Boon 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-
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 2c) 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 compartment. The M20 (Figure 2d) 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 2e) 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 2f) 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 2g) 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 2h) could be found in all cases, although only a minority of cells reacted in the intermediate and central cell compartments. E3 (Figure 2i) 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 63, RCK 106, and 2CB. Of these, the RCK 106 antibody (Figure 2a) 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

| Keratin subtype | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
| Case            | KSQ| NKSQ| AC/MDO| 1   | 4 | 5 + 8 | 5 + 6 | 6 | 7 | 8 | 10 | 13 | 14 | 16 | 17 | 18 | 19 |
| Diagnosis       | ± | ± | ± | ±+ | + | ++ | ++++ | ++ | + | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| Histo-logic     | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |

**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 the cells stain; ++++ = 75–100% of cells stain.
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
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

Figure 3. Immunoperoxidase staining pattern of frozen sections from large-cell nonkeratinizing squamous cell carcinomas after incubation with: (a) B510 (keratin 4), (b) AI14 (keratin 5), (c) RCK102 (keratin 5 + 6), (d) RA12 (keratin 5 + 6), (e) AI124 (keratin 6), (f) RCK105 (keratin 7), (g) CAM5.2 (keratin 8), (h) LE41 (keratin 8), (i) RSE10 (keratin 10), (j) ICH keratin 12, (k) RCK107 (keratin 14), (l) LLO25 (keratin 16), (m) I3 (keratin 17), (n) RCK105 (keratin 18), (o) BGE53 (keratin 18), (p) RCK108 (keratin 19). Original magnification b,c,f,j,k,l x250; e x300; d,g,h,i,m-p x400; a x500.
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.

**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
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 keratins 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...
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 adenocarcinomas 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) AL124 (keratin 6), (f) RCK105 (keratin 7), (g,h) M20 (keratin 8), (i) K53350 (keratin 10), (j) 2117 (keratin 13), (k) RCK107 (keratin 14), (l) 15025 (keratin 16), (m) R5 (keratin 17), (n) RCK105 (keratin 18), (o) RGE53 (keratin 18), (p) RCK108 (keratin 19). Original magnification a–h ×250; |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 2CB 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. and Moll et al. 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. The immunohistochemical study of Ivanov et al. 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 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 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. and Moll et al. 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. 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-
Table 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 group 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; IC = adenocarcinoma of the cervix; ASQ = adenosquamous carcinoma of the cervix.

Keratins in Cervical Cancer

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. 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 RK5E60, 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 comitiveness 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 discriminant 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, 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,34,35 in all squamous cell carcinomas of the lung and usually not in adenocarcinomas of the lung.31 Moll and Czernobilsky1,2 did not detect keratin 17 in adenocarcinomas of the endometrium by gel electrophoresis. Troyanovsky et al.39 have shown that keratin 17 is not found in normal intestinal epithelium, the epithelium of the ovicud, or mesothelial cells. Quinlan et al.30 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.

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