Basal-cell Keratins in Cervical Reserve Cells and a Comparison to Their Expression in Cervical Intraepithelial Neoplasia

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Expression of keratins 5, 14 and 17 in endocervical subcolumnar reserve cells was detected by means of immunohistochemical studies using polypeptide specific monoclonal antibodies. These particular keratins that were found among others in basal cells could also be detected to a variable extent in metaplastic and dysplastic cervical lesions. In some cases of immature squamous metaplasia all three keratin subtypes were expressed throughout the full thickness of the epithelium. In contrast, in mature squamous metaplasia a compartmentalization of these keratins was observed. Mature squamous metaplastic epithelium showed a keratin distribution pattern comparable to ectocervical squamous epithelium, with the exception of keratin 17, which was only sporadically found in the basal layer of ectocervical epithelium and was always present in the basal cells of mature squamous metaplastic epithelium. During progression of cervical intraepithelial neoplasia a clear increase in the expression of keratin 17 was observed. However, also keratins 5 and 14 were expressed. Our results demonstrate that a considerable number of premalignant lesions of the uterine cervix express the same keratins as found in the progenitor reserve cells. Lesions that lack expression of keratin 17 may form a distinct group, which are regressive in nature and do not progress into cervical cancer. (Am J Pathol 1992, 140:601–612)

It is believed that reserve cells play a central role in the pathogenesis of cervical intraepithelial neoplasia. Considerable efforts have been undertaken in the study of the exact nature of these cells and the precise role they play in neoplastic transformation. One of the recent approaches that has been chosen is the characterization of cytoskeletal components, in particular keratins, in reserve cells and cervical intraepithelial neoplastic (CIN) lesions. With chain specific monoclonal antibodies against various individual keratins, information concerning the keratin content of reserve cells and other cervical epithelial cells can be easily derived with immunocytochemical techniques. By comparing the keratin distribution patterns in CIN with that in reserve cells, a possible link between the various stages of cervical carcinogenesis can be studied.

In previous publications reserve cells were reported to contain keratins 5, 8, 17, 18, and 19, with the presence of keratin 13 in a subpopulation of these cells. The presence of keratins 5 and 17 in reserve cells is based on gel electrophoretic studies. Endocervical columnar cells have been shown to contain keratins 7, 8, 18, 19, with variable amounts of keratin 4. Keratin expression in mature squamous metaplastic epithelium and in ectocervical nonkeratinizing, squamous epithelium is highly comparable. Type II keratins 1, 2, 4, 5, 6, and 8 and type I keratins 10, 11, 13, 14, 15, 16, 17, and 19 have been detected with some variability in their expression. These data are partly based on one- and two-dimensional gel electrophoresis combined with immunoblotting and partly on immunocytochemical studies.

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Reserve cell proliferaions can lead to immature squamous metaplastic epithelium, which can transform into mature squamous metaplastic epithelium on the one hand. On the other hand the reserve cell proliferation may be atypical and progress into intraepithelial neoplasia. Immature squamous metaplastic epithelium has been found to express keratin characteristic of simple epithelia, such as keratins 8, 18, and 19, as well as keratins found in ectocervical and mature squamous epithelium, i.e., keratins 4, 13, and 14.5-7

Regarding the keratin expression in cervical intraepithelial neoplasias (CIN I, II, III) only little has been published. In a previous article we demonstrated that in a number of these lesions, keratin characteristic of simple epithelia, i.e. keratins 7, 8, 15, and 19 occurred in a scattered manner, but in such a way that their presence was usually more pronounced in CIN III lesions than in CIN I and CIN II lesions. Furthermore, we found that with increased severity of CIN, keratins 4, 13, and 14 were less frequently detected, but again this change was most pronounced in CIN III. The keratins were also not expressed in a uniform manner in CIN lesions but were irregularly distributed, sometimes throughout the full thickness of the epithelium.

Using a panel of newly developed antibodies that enable the separate immunohistochemical detection of keratins 5, 14, and 17 at the single cell level, we were able to add new data to the present knowledge on keratin expression patterns in normal endocervical and ectocervical epithelium. We also present new data regarding keratin expression patterns in CIN of various grades of severity. This newly derived information enhances our knowledge regarding the analogy between normal cervical and preneoplastic lesions and may be helpful in the diagnosis of CIN lesions and the discrimination between progressive and regressive lesions.

Materials and Methods

Tissues

The tissue specimens used in this study were taken from cervical cones removed in 40 women in whom routine cytologic examination had shown features consistent with dysplasia. Before conization, the lesions were visualized by applying a 4% solution of acetic acid to the whole cervix. Shortly after conization, each cone was divided into equal quadrants, which were again subdivided into three equal parts perpendicular to the endocervical canal. The central part of these three was fixed in 4% buffered formaldehyde and further processed through paraffin for routine light microscopic diagnosis, made independently by three qualified pathologists. The other two parts were snapfrozen in liquid nitrogen and stored at -70°C. One part of each quadrant was used for the immunohistochemical assays.

We used a total of approximately 100 tissue specimens representing normal endocervical and ectocervical epithelium, immature and mature squamous metaplasia and the three grades of CIN. Extra care was taken to select cases showing reserve cells and reserve cell hyperplasia. Furthermore, in the tissue fragments normal epithelia and or metaplastic as well as dysplastic lesions were sometimes found alongside of each other in the same fragment; 40 cases contained both ectocervical epithelium and endocervical columnar epithelium. In 20 fragments we found reserve cells, in 42, mature squamous metaplasia was found, and in 11, immature squamous metaplasia was found. CIN I, CIN II, and CIN III were diagnosed in 4, 6, and 16 cases, respectively. In cases of major discrepancies between the diagnoses of the three pathologists, the case was excluded from the study.

Antibodies

Five monoclonal antibodies were used in this study. For comparative purposes, the results with LLOO1 were taken from our previous article. The specificity of each of these reagents is described.

1) LP34,10 is a broad spectrum antikeratin antibody reacting with most epithelia, both keratinizing and nonkeratinizing. It recognizes keratins 5, 6, and 18 and possibly 4, 14, and 16 in immunoblots.

2) AE14,10 specific for keratin 5, stains the basal cells of squamous and pseudostratified epithelia. Among others, it reacts with the basal cells in the endocervical canal, some myoepithelial cells, thyric reticulum cells, certain pancreatic duct cells, a variable subpopulation of mesothelial cells and basal cells in the epithelium of the respiratory tract. This antibody was a gift from Dr. T. T. Sun.

3) LLOO1,13 specific for keratin 14, stains all stratified epithelia. Staining is most intense in the basal layer, but the parabasal and intermediate layers also react. The stratum granulosum and the stratum corneum remain negative. Outer root sheath cells of the hair follicle are positive, inner root sheath cells are negative. Sebaceous gland cells are all positive. Basal cells in complex epithelia, for example in sweat glands, mammary glands, salivary glands and the prostate gland are also positive.

4) LLOO2 recognizes keratin 14, and was used in a previous study. It has been used for comparative purposes. The staining pattern of this antibody is similar to that described earlier for LLOO1. The staining intensity is, however, stronger than found for LLOO1.13
5) Characterization of the monoclonal antibody RCK 107 was recently described.14 When tested on cultured cells containing keratins 1, 4, 5, 6, 7, 8, 10, 11, 13, 14, 15, 16, 17, 18, and 19, a filamentous staining pattern was observed. Cell lines known to contain only keratins 7, 8, 18, and/or 17 and 19 did not react with RCK 107. In a series of human tissues, RCK 107 exhibited an exclusively epithelial reaction, where it preferentially stained the basal cell compartments and other epithelial compartments described to contain keratins 5, 14 and/or 15. Similar to what had been observed in the cell lines, tissues known to contain only keratins 7, 8, 18, and/or 19 (for example, liver, colon, kidney) were negative. Cross-reactivity of RCK 107 with keratins 1, 4, 10, 11, and 13 is unlikely because of the negative results in suprabasal cells in epidermis, esophagus, as well as negativity in transitional epithelium of the urinary bladder. Finally, immunoblotting studies on cytoskeletal extracts prepared from A431 (epidermoid squamous cell carcinomas of the vulva) and HaCaT (human keratinocytes; provided by Dr. N. Fusenig, DKFZ, Heidelberg, Germany) cell cultures, revealed a protein band with a molecular weight similar to the keratin 14 band, also obtained after immunoblotting with LLOO1. Two dimensional immunoblotting further substantiated the keratin 14 character of this protein (Figure 1). These 2D-immunoblotting studies also clearly revealed that RCK 107 did not cross-react with keratin 15 or keratin 17 (Figure 1a, e).

6) E3, specific for keratin 17,16 reacts with the basal row of pseudostratified epithelium in the larynx, trachea, and bronchi; it stains the basal cell layer of the transitional epithelium in the urinary bladder and the myoepithelial cells in several tissues including the salivary gland, the sweat glands, the prostate gland and the intra- and extralobular zone in the breast. Furthermore, it stains duct epithelium of the pancreas and the outer root sheath of the hair follicle.

Immunohistochemistry

Consecutive cryostat sections were fixed in cold methanol (−20°C) for 5 minutes, rinsed in acetone and air dried. The slides were incubated with the appropriately diluted or undiluted monoclonal primary antibody for 45 minutes at room temperature.

After three subsequent washing steps with phosphate-buffered saline (PBS), the peroxidase-conjugated rabbit anti-mouse serum (Dakopatts, 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-aminoo-9-ethylcarbazole (AEC; Aldrich Chemical Comp., St. Louis, MO) as described earlier.5 The slides were counterstained with hematoxylin and mounted with Kaisers glycerin-gelatin (Boom B. V. Meppel, The Netherlands).

Results

Figure 2 shows a schematic representation of keratin phenotypes of normal ecto- and endocervical epithelium, immature and mature squamous metaplastic epithelium, and the three grades of CIN, as defined by the monoclonal antibodies. Distinct variations in the expression patterns of the various keratins will be described in detail later.

In all sections, we also carefully examined keratin expression in the subepithelial tissues of the cervix since expression of keratins has been reported in smooth muscle cells of the endocervix.16 We found no reactions in the endocervical stroma and smooth muscle cells with the antibodies used in this study.

Normal Ecto- and Endocervical Epithelium

Ectocervical squamous epithelium showed a reproducible pattern of keratin expression in all cases investigated.

LP34 stained the basai and parabasal epithelial layers moderately (Figure 3a), whereas in the intermediate layer, the staining decreased in intensity toward the periphery. In the superficial layer only some dispersed cells were weakly stained.

The antibody AE14 against keratin 5, moderately stained the basal cell compartment in most cases (Figure 3c). In the parabasal layer approximately 50% of the cells showed a reaction that varied from intense to moderate. The above-lying cells showed no reaction at all.

The keratin 14 antibodies RCK 107 (Figure 3c) and LLOO1 showed a similar staining pattern, both staining the basal layer with minor variation in staining intensity. Parabasal cells expressed this keratin polypeptide in the majority of the specimens with moderate intensity. Above the parabasal layer only some dispersed cells showed a weak reactivity. The staining intensity of LLOO1 was weaker than that of the previously described LLOO2.5

The keratin 17 antibody E3, was practically negative in all cases (Figure 3d). However, in a few cases it weakly stained a few cells in the basal layer.

Endocervical columnar cells displayed an intense staining reaction with LP34 in all cases (Figure 3e). Keratin 5, detected with AE14, was found in about 25% of the cases under investigation (Figure 3f) but was only weakly positive.

Columnar cells were usually negative with the antibodies directed against keratin 14. With LLOO1 all columnar cells were negative (Figure 3g), whereas RCK
107 which was also usually negative, sometimes stained columnar cells weakly there were they lay above reserve cells. In about one third of the cases some keratin 17 positivity was present in these columnar cells when the E3 antibody was used (Figure 3).

In many specimens, groups of reserve cells were detected under the columnar cells lining the endocervical canal. These reserve cells reacted intensely with LP34 (Figure 3a). AE14 detected keratin 5 in reserve cells in about 80% of the cases under investigation. The staining varied, however, from intense to weakly positive (Figure 3f). Of the antibodies directed against keratin 14, only RCK 107 reacted with a moderate intensity with all reserve cells (Figure 3h). With the antibody E3, we observed keratin 17 in all reserve cells, the reaction usually being intense (Figure 3i).

**Immature Squamous Metaplasia**

The expression pattern of immature squamous metaplastic epithelium was in part comparable to that observed in ectocervical epithelium and in mature squamous metaplastic epithelium. The broad spectrum antibody LP34 (Figure 4a) reacted intensely with the basal part of this epithelium. The above lying layers also stained with LP34, although the number of positive cells tended to decrease towards the epithelial surface. Keratin 5 was observed in about two thirds of the cases, with AE14 staining fairly intensely through the full thickness of the epithelium (Figure 4b) with the exception of most columnar cells. Of the keratin 14 antibodies, LLOO1 reacted practically with the full thickness of the epithelium with some variation in staining intensity (Figure 4c) in most tissue fragments. RCK 107 also stained about two thirds of the cases, being strongest in the basal part but also detectable in higher epithelial layers (Figure 4d). E3 detected keratin 17 through the full thickness of the epithelium with variable intensity (Figure 4e) in 10 cases. In those cases in which keratin 17 could be detected we also found keratins 5 and 14. This means that in 70% of cases of immature squamous metaplasia keratins 5, 14 and 17 were coexpressed. Five cases were negative for keratin 5 and 17. In 2 of these specimens, however, keratin 14 was detectable.

Figure 1. Oxidization of the keratin antigen recognized by RCK 107. Two-dimensional Immunoblots, containing a cytoskeleton preparation of HuCaI cells, incubated with RCK 107 (a), recognizing keratin 14, LLO02 (b), recognizing keratin 14, and subsequently with M20 (c), recognizing keratin 8. When the Immunoblot in (c) was reincubated with RCK 107 no additional keratin spot appeared (d). Only the breakdown products of keratin 14 also seen in (a, brackets) became apparent. Reincubation of the blot with antibody E3 (e; recognizing keratin 17) showed that this keratin subtype was present in the preparation, but not recognized by RCK 107.
A: Ectocervical epithelium

B: Endocervical columnar- and reserve cells

C: Immature squamous metaplasia

D: Mature squamous metaplasia

E: CIN I

F: CIN II

G: CIN III
Mature Squamous Metaplasia

Squamous epithelium cranial to the first endocervical invagination was defined as mature squamous metaplasia. Although the tissue fragments used were small, this transitional zone could usually be identified. If there was any doubt, we used parallel paraffin sections, which had been taken for routine diagnosis. The keratin expression pattern in mature squamous epithelium demonstrated interesting differences when compared with the expression pattern in ectocervical epithelium. The broad spectrum antibody LP34 (Figure 4f) showed practically the same staining pattern in both types of epithelium with a more intense and more universal reaction in the intermediate and superficial layer of mature squamous metaplastic epithelium. Keratin 5, as detected by the AE14 antibody (Figure 4g), showed a similar expression pattern in both types of epithelium. The intensity of the reaction was, however, stronger in ectocervical epithelium. The keratin 14 antibody LLO01 (Figure 4h) stained virtually all cases of mature squamous metaplastic epithelium and ectocervical epithelium with reactivity decreasing or diminishing towards the surface. The staining intensity in the ectocervical epithelium was, however, less intense. The intermediate and superficial epithelial layers in mature squamous metaplasia usually showed some staining with LLO01, whereas in ectocervical epithelium these layers were usually negative. RCK 107 (Figure 4i) showed a similar reaction in both epithelia. Antibody E3, detecting keratin 17 (Figure 4i) showed a moderate and somewhat variable staining intensity in the basal epithelial layer in all cases of mature squamous metaplasia. The reaction was less intense and was observed only in a quarter of the parabasal cells, whereas the above-lying cells were negative. However, in the ectocervical epithelium sporadic basal cells were weakly stained.

Cervical Intraepithelial Neoplasia I

Keratin expression patterns in CIN I showed differences when compared with the progenitor lesion, i.e., immature squamous metaplasia.

The broad spectrum antibody LP34 (Figure 5a) stained the basal epithelial layer intensely; the parabasal and intermediate cell layers were less intensely stained with the superficial layer not staining at all. Staining resembled the staining pattern in immature squamous metaplasia. Keratin 5 (Figure 5b) was detected in the basal cell layer and sometimes a few above-lying cells. The intensity of staining was moderate. LLO01 and RCK 107 (Figure 5c) both showed some dispersed positivity throughout the full thickness of the epithelium in one of the cases. The other cases were negative. These two antibodies showed a distinctly diminished staining intensity with also a decrease in the number of cells staining, when compared with immature squamous metaplasia. Keratin 17 antibody E3 was only mildly positive in the basal layer of one case, which also demonstrated full thickness positivity for keratin 14. The other cases were negative with this antibody (Figure 5d).

Cervical Intraepithelial Neoplasia II

Keratin expression in CIN II lesions is in part similar to that observed in CIN I lesions. The broad spectrum keratin antibody LP34 (Figure 5e) showed a similar reaction, although the superficial layer showed some staining. Keratin 5 (Figure 5f) was found in all but one case, reactivity was intense throughout the full thickness of the epithelium decreasing somewhat towards the superficial layer. Of the keratin 14 antibodies LLO01, stained one third of the fragments in a dispersed and weak manner, the superficial layer being negative. RCK 107 (Figure 5g) stained basal cells and above-lying cells weakly, whereas the superficial cells were completely negative. One case was negative. LLO02 showed expression with variable intensity in all layers. Half of the fragments remained negative. Keratin 17 antibody, E3 (Figure 5h) weakly stained the basal and above-lying cells in a single case, which was also positive for keratins 5 and 14. In all cases in which keratin 5 could be detected, keratin 14 could also be found with one of the antibodies, which meant that in one case no keratin 5 and 14 could be demonstrated.

Cervical Intraepithelial Neoplasia III

Keratin expression in CIN III was in part similar to that in CIN I and CIN II lesions, and also showed similarities to
the pattern in immature squamous metaplasia and in ectocervical epithelium. LP34 (Figure 5i) stained the lower two thirds of the epithelium with moderate-to-strong intensity, whereas the upper third was usually less intensely stained, in some fragments, while in others intense staining of all layers was observed. Keratin 5 (Figure 5j) was exclusively present in the lower two thirds of the epithelium, whereas the above lying cells were weakly positive and superficial cells did not react. The basal staining reaction showed some variation but was usually fairly intense. Of the keratin 14 antibodies RCK 107 (Figure 5k) stained the lower two thirds of the epithelium with variable intensity, whereas LLOO1 stained about half of the cases with mild intensity, in all except the superficial layers. LLOO2 showed partial positivity in all epithelial layers, alternating with negative areas in most cases, while some fragments were completely negative. E3 detecting keratin 17 (Figure 4l) showed weak dispersed single cell positivity throughout the full thickness of the epithelium in about half of the cases.

Discussion

In this study we examined the specific distribution of keratins in epithelia lining the cervical canal, i.e., in normal epithelia, reserve cells and reserve cell hyperplasia, immature and mature squamous metaplasia, and CIN I, CIN II, and CIN III lesions. This was done by using recently developed monoclonal antibodies, most of which specifically interacted with only one of the 20 known keratins.17

These monoclonal antibodies have been tested in extended series of normal tissues and malignant tumors and have to a large extent been shown to exhibit predictable reactivity patterns. Not only do these antibodies allow differentiation between different types of epithelium, they also specifically stain certain compartments in complex epithelia. We were especially interested in the keratin expression pattern in cervical reserve cells as these cells play a central role in the development of CIN lesions.11 Comparing the keratin expression patterns of reserve cells with those of metaplastic and dysplastic cervical epithelial cells will give us a more profound insight into the cell biological processes that determine normal and abnormal differentiation of these precursor cells. In the future, this knowledge may serve as a diagnostic aid and indicate whether a reserve cell proliferation or CIN lesion will progress into a malignancy.

Based on gel electrophoretic studies, Weikel et al.6 suggested that cervical reserve cells contain keratins 5 and 17. In their study a reserve cell-enriched cell fraction was used, which also contained other epithelial components. After monoclonal antibodies to keratins 5 and 17 recently became available, we were able to test their hypothesis by means of immunohistochemical methods. Using the specific antibodies AE14 (directed against keratin 5) and E3 (directed against keratin 17) we could confirm the gel electrophoretic results, meaning that we were able to demonstrate keratin 5 at the single cell level in about two thirds of the reserve cells seen and keratin 17 in all reserve cells. Also Mol, Dhouraily, and Sun10 in an immunohistochemical study recently demonstrated keratin 5 in subcolumnar reserve cells.

Since keratin 14 is generally coexpressed with keratin 5 in basal cells13 it is surprising that in both the gel electrophoretic studies of Weikel et al. as well as our own previous study keratin 14 could not be demonstrated in reserve cells. Having experienced epitope masking as a common pitfall in the evaluation of keratin expression,5,18,19 we used the additional keratin 14 antibody LLOO1 and our own recently developed keratin 14 antibody RCK 107 in this study. This last reagent has been shown to be exclusively reactive with keratin 14 which was concluded from immunoblotting studies and reactivity patterns of this antibody with human tissues and human cell lines.14 A possible crossreactivity with keratins 15 or 17 is unlikely since these proteins were not detected in the immunoblots of cell extracts containing these cytoskeletal constituents. Moreover negative indirect immunofluorescence results with cell lines described to contain keratin 17 (for example Hela) or positive results in a cell line containing keratin 14 and 17 but not keratin 15 (TR 148) support this conclusion. Furthermore comparison of staining patterns of E3 and RCK 107 on frozen sections of human tissues (for example ectocervical epithelium) revealed profoundly different reactivity spectra for both antibodies. With this antibody we could demonstrate keratin 14 in reserve cells thus confirming recent results by Ivanyi et al.20 Based on our findings the keratin profile for reserve cells comprises the keratins 5, 8, 14, 17, 18, and 19. We could not find keratin 13 expression as stated by Levy et al.9 We must also stress that only a subpopulation of reserve cells seem to contain keratin 5 contrary to what has been suggested by Weikel et al.6 The prominent differences in staining patterns between the different keratins 14 antibodies may have the following explanations. First, the different antibodies are directed against different epitopes. LLOO1 and LLOO2 were raised against the last carboxylterminal amino acids of human keratin 1419 whereas RCK 107 was obtained after immunization with a total cytoskeletal preparation of

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Figure 4. Immunoperoxidase staining patterns of frozen sections with immature squamous metaplasia (a-e) and mature squamous metaplasia (f-f) after incubation with (a, f) LP34; (b, g) AE14; (c, h) LLOO2; (d, i) RCK 107; and (e, j) E3. Original magnification, a-d, ×250; e, ×600; f-j, ×150.
T24 cells. From Figure 1, it is obvious that LLCO2 reacts with a broader spectrum of keratin 14 variants, whereas RCK 107 reacts stronger with several of the breakdown products of keratin 14. These different keratin 14 epitopes may be exposed differently under several conditions. Apparently the keratin 14 epitope recognized by RCK 107 is available for immunohistochemical detection in subcoluminar reserve cells, whereas the carboxyterminus is probably masked under the conditions used in our studies. Finally, different affinities of the antibodies for their respective epitopes may explain the variability of our keratin 14 staining results. The keratin 14 antibodies may be ranked according to staining intensity as follows: LLCO1 stains less intensely than RCK 107, which in turn stains less intensely than LLCO2. Therefore if the amount of keratin 14 is low in a given cell, a negative reaction with LLCO1 may be due to its low affinity and therefore high threshold of sensitivity. Furthermore, as observed in our previous study, there was no expression of these basal cell keratins in the endocervical stroma, which once again is powerful proof that reserve cells do not originate in the endocervical stroma. This statement is further substantiated by recent observations of Weitzels et al. in which reserve cells are found above the basement membrane.

The staining pattern in endocervical columnar cells was surprising in that these cells, in addition to the previously described presence of keratins 7, 8, 18, 19, also expressed some keratin 5, 14, and 17 in a number of cells. This was usually observed in areas in which columnar cells lay on top of reserve cells which supports the hypothesis that endocervical columnar cells develop from the underlying reserve cells. If so, reserve cells partly pass on their keratin profile during differentiation into columnar cells. As columnar cells mature keratins 5, 14, and 17 are lost in part, which is accompanied by the initiation of keratin 7 expression. In the case of dedifferentiation into an adenocarcinoma, the tumor cells may maintain part of the keratin profile of the progenitor reserve cells, which may also be concluded from the ob-
ervation by Czernobilsky et al.\textsuperscript{11} who demonstrated keratin 17 in cervical adenocarcinomas by gel electrophoretic studies.

Stratified ectocervical epithelium in gel electrophoretic studies was shown to contain keratins 5, 6, 7, 8, 13, 14, 15, 16, 17, 18, and 19.\textsuperscript{11} Other studies\textsuperscript{8,9-10} have proven the presence of keratins 4, 8, 10, 13, 14, and 19 at the single cell level using monoclonal antibodies. The present study demonstrates additionally keratin 5 and to a much lesser extent keratin 17 in the ectocervical epithelium. The observation that keratin 17 is only detected in a restricted number of cases explains why Trayanovska et al.\textsuperscript{10} did not detect keratin 17 in ectocervical epithelium in their two cases.

Taking into account the keratin expression pattern in reserve cells, it is not surprising to observe that keratins 5, 14, and 17 are coexpressed in 10 cases with immature squamous metaplastic epithelium. This article is the first report on the presence of keratins 5 and 17 in this type of epithelium. The process of squamous metaplasia is a dynamic one, with reserve-cell hyperplasia at one end of the spectrum and at the other end fully matured squamous epithelium. The presence of keratins 5, 14, and 17, and more particularly the fact that these cytoskeletal proteins show a random distribution throughout the cell layers rather than a specific compartmentalization, indicates that their expression correlates closely to the stage of maturation of the squamous metaplastic epithelium.

Only a limited number of studies on keratin expression in CIN lesions have been published.\textsuperscript{8,11,12} In a previous study\textsuperscript{8} we demonstrated that keratin expression patterns in CIN lesions could be compared with those of immature squamous metaplasia. Some keratins characteristic of simple epithelia and reserve cells, i.e., keratins 8, 18, and 19 were detected in a number of cases of CIN lesions in an irregular manner. We also observed that the number of cells, the intensity of staining as well as the number of positive cases increased with progression of the lesion. This observation led us to the hypothesis that CIN lesions that express these markers of simple epithelia may progress into cervical cancer.\textsuperscript{5}

This hypothesis is further supported by the present study that shows that keratin 17 is sporadically found in the basal cell layer of epithelium with CIN I and CIN II lesions, while it is present in a higher percentage of the CIN III lesions. We also noted that the staining intensity in CIN III had slightly increased when compared with CIN I and CIN II lesions and that in CIN III epithelium staining is often seen dispersed throughout the full thickness of the epithelium. We would like to stress that approximately 50% of the CIN III lesions contain keratin 17. Keratin 17 is present in reserve cells from which these lesions originate as well as in immature squamous metaplasia. We, however, comment that in the tissue fragments with immature metaplasia there was frequently also a dysplastic lesion present. This may be indicative of the malignant potential of immature metaplasia.

Since Czernobilsky et al.\textsuperscript{11} on the basis of gel electrophoretic studies, described keratin 17 in all of ten cases of cervical squamous cell carcinoma, and our recent results on cervix carcinomas using antibody E3 (to be published) support these findings, we hypothesize that progression of CIN II to carcinoma is restricted to a subpopulation of keratin 17-positive CIN III lesions.

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