The limited difference between keratin patterns of squamous cell carcinomas and adenocarcinomas is explicable by both cell lineage and state of differentiation of tumour cells

E B L van Dorst, G N P van Muijen, S V Litvinov, G J Fleuren

Abstract

***Aim*—To study the differentiation of epithelial tissues within their histological context, and to identify hypothetically, on the basis of keratin pattern, the putative tissue origin of a (metastatic) carcinoma.**

***Methods*—Using well characterised monoclonal antibodies against individual keratins 7, 8, 18, and 19, which are predominantly found in columnar epithelia, and keratins 4, 10, 13, and 14, predominantly expressed in (non)-keratinising squamous epithelia, the keratin patterns for a series of 45 squamous cell carcinomas and 44 adenocarcinomas originating from various epithelial tissues were characterised.**

***Results*—The predominant keratins in all adenocarcinomas proved to be 8, 18, and 19. In addition, these keratins were also abundantly present in squamous cell carcinomas of the lung, cervix, and rectum and, to a lesser extent, of the larynx, oesophagus, and tongue, but not in those of the vulva and skin. Keratins 4, 10, 13, and 14 were present in almost all squamous cell carcinomas, but also focally in some of the adenocarcinomas studied.**

***Conclusions*—There is a limited differential expression of distinctive keratin filaments between squamous cell carcinomas and adenocarcinomas. Apparently, squamous cell carcinomas that originate from columnar epithelium by squamous metaplasia gain the keratins of squamous cells but retain the keratins of columnar epithelial cells. However, the simultaneous expression of two of three squamous keratins (4, 10, and 13) identifies a squamous cell carcinoma, and thus might be useful in solving differential diagnostic problems.**

(*J Clin Pathol* 1998;51:679–684)

Keywords: keratin; carcinoma; differentiation

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Accepted for publication 11 March 1998

The main structural proteins of the intermediate filament based cytoskeleton of epithelial cells are the keratins.[1] The composition of these filaments is highly heterogeneous. Approximately 20 polypeptides of this multigene family have been characterised.[2 3] Findings revealing the differentiation and cell type specificity for expression of individual combinations of keratins[2] raised the question of whether the analysis of the individual keratins present in fetal and normal epithelia, carcinomas, and their metastases could serve as a basis for a histologically meaningful classification of neoplasms.[4–10]

For normal epithelia, distribution patterns of specific keratins are well established. The markers used in this study are generally distributed in the following way: keratins 8 and 18 are always present in columnar epithelia, while keratins 19 and 7 may also be found[11]; non-keratinising stratified squamous epithelia contain keratins 4 and 13 suprabasally, and keratins 14 and 19 in the basal compartments[12 13]; keratinising stratified squamous epithelia express keratin 14 in basal compartments up to the stratum corneum and keratin 10 suprabasally as a marker for phenotypic keratinisation[14 15]; and the myoepithelial cells of bronchial epithelium[16] and mammary epithelium also contain keratin 14.[17 18]

The value of monoclonal antibodies to keratin 18 for identifying adenocarcinomas has been described in several reports,[19–21] though this does not apply to keratin 18 positive focal areas of less differentiated cells in squamous cell carcinomas of the uterine cervix, the epiglottis, and lung.[5 22–25] It has been reported that keratin 7 distinguishes between colon carcinomas (negative) and other adenocarcinomas.[5 21 26–30] Recently, keratin 20 was found to distinguish colonic adenocarcinomas, mucinous ovarian tumours, and transitional cell and Merkel cell carcinomas (positive) from squamous cell carcinomas, adenocarcinomas of breast, lung, and endometrium, non-mucinous ovarian tumours, and small cell lung carcinoma (negative).[31 32]

We performed a detailed systematic study to define the keratin expression patterns in epithelial neoplasms with respect to cell lineage and differentiation, and the differential diagnostic questions for which keratin typing may be informative. Special attention was paid to the diagnostic value of keratin patterns in defining squamous cell carcinoma and adenocarcinoma. In clinical practice, for example, the differential diagnosis for squamous cell carcinoma of the cervix uteri from adenocarcinoma is highly important, since the prognosis and treatment of the two conditions are quite different. The results show that the patterns of keratin expression in adenocarcinomas and squamous cell carcinomas are dependent on both cell lineage and state of tumour cell differentiation.

Methods

Frozen tissue specimens of 44 primary adenocarcinomas and 45 primary squamous cell carcinomas originating from various epithelial tissues were obtained from our frozen tissue collection (tables 1–3). Keratin expression was

Table 1 Keratin expression in 44 adenocarcinomas

	Stomach (n= 5)				*Colon (n = 9)*				*Pancreas (n = 5)*				*Lung (n = 6)*				*Kidney (n = 5)*			
Keratin	*7*	*8*	*18*	*19*	*7*	*8*	*18*	*19*	*7*	*8*	*18*	*19*	*7*	*8*	*18*	*19*	*7*	*8*	*18*	*19*
++++	–	4	5	5	–	9	9	9	2	5	5	5	1	5	5	6	–	5	4	2
+++	–	1	–	–	–	–	–	–	2	–	–	–	1	1	–	–	1	–	–	1
++	–	–	–	–	–	–	–	–	1	–	–	–	1	–	1	–	–	–	1	1
+	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
–	2	–	–	–	9	–	–	–	–	–	–	–	3	–	–	–	4	–	–	1
Keratin	*4*	*10*	*13*	*14*	*4*	*10*	*13*	*14*	*4*	*10*	*13*	*14*	*4*	*10*	*13*	*14*	*4*	*10*	*13*	*14*
++++	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
+++	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
++	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
+	2	–	–	2	–	–	–	–	1	–	2	1	2	–	–	1	–	–	–	–
–	3	5	5	3	9	9	9	9	4	5	3	4	4	6	6	5	5	5	5	5

studied immunohistochemically using chain specific mouse monoclonal antikeratin antibodies in serial slides of a tissue specimen (table 4). The reacted monoclonal antibodies were detected using antimouse IgG peroxidase conjugate.[40] Normal tissues from the uterine cervix that contained epithelial cells (both squamous and columnar) expressing all the keratins studied were used as a positive control. As a negative control, we used isotype matched irrelevant mouse monoclonal antibodies. The number of positive cells was determined semiquantitatively: –, no positive tumour cells, +, 0–25%, ++, 25–50%, +++, 50–75%, and ++++, 75–100% positive tumour cells. All frozen tissue samples were reviewed in paraffin embedded sections and found representative.

In addition, we compared the staining patterns for two antikeratin 18 antibodies—RGE53 and M9—and two anti-keratin 14 antibodies—CKB1 and LL002—in serial dilutions in a selected series of squamous cell carcinomas and adenocarcinomas.

The specificity of RGE53 and M9 were verified by immunoblotting, using lysates of cultured carcinoma cells. For the preparation of keratin extracts, we used MCF-7, a cell line derived from a breast carcinoma, and SCC-15, derived from squamous cell carcinoma of the tongue, in accordance with a published protocol.[12] The cells were cultured under standard conditions and harvested at confluency. According to Laemmli,[41] 10% polyacrylamide gels were used for one dimensional gel electrophoresis.

Results

KERATIN PATTERNS IN ADENOCARCINOMAS

The predominant keratins of the adenocarcinoma tissues were represented by keratins 8, 18, and 19, that were present abundantly and homogeneously. Only in renal cell carcinomas was keratin 19 not abundantly present, and it was even found to be completely absent in one case. The staining patterns of M9 and RGE 53, both antikeratin 18 antibodies, were identical.

Keratin 7 expression was variable among the types of adenocarcinoma tested. All pancreatic, oesophageal, and breast carcinomas contained variable numbers of positive cells. Three of six adenocarcinomas of the lung and two of five cervical adenocarcinomas were negative for keratin 7. Only a few groups of tumour cells expressed keratin 7 in three of the stomach carcinomas (fig 1A), and only one renal cell carcinoma expressed keratin 7, though this was present in approximately 75% of the tumour cells. No colon carcinomas were found to be positive for keratin 7.

Keratins 4, 13, and 14 were sporadically detected in a limited number of cells of 30% of the adenocarcinomas studied, except for the colonic and renal cell carcinomas. These keratins were not confined to a particular type of tumour cell, for instance cells with histological signs of squamous differentiation. The two antikeratin 13 antibodies used did not show a difference in staining pattern. The selected series of 12 adenocarcinomas stained with two antikeratin 14 antibodies in several dilutions showed no differences in staining patterns. Keratin 10 was not observed in any of the

Table 2 Keratin expression in 44 squamous cell carcinomas

	Skin (n= 8)				*Vulva* (n = 5)*				*Larynx† (n = 7)*				*Lung (n = 10)*				*Cervix (n = 5)*			
Keratin	*7*	*8*	*18*	*19*	*7*	*8*	*18*	*19*	*7*	*8*	*18*	*19*	*7*	*8*	*18*	*19*	*7*	*8*	*18*	*19*
++++	–	–	–	–	–	–	–	–	2	–	3	–	5	–	9	–	–	–	–	5
+++	–	–	–	–	–	–	–	–	1	1	–	–	1	3	3	1	1	4	3	–
++	–	–	–	–	–	–	–	–	–	–	1	–	–	–	3	–	1	1	2	–
+	–	–	–	–	–	–	–	–	1	2	4	2	1	2	3	–	2	–	–	–
–	8	8	8	8	5	5	5	5	5	2	2	2	8	–	1	–	1	–	–	–
Keratin	*4*	*10*	*13*	*14*	*4*	*10*	*13*	*14*	*4*	*10*	*13*	*14*	*4*	*10*	*13*	*14*	*4*	*10*	*13*	*14*
++++	–	–	–	5	–	–	–	1	–	–	–	2	–	–	–	1	–	–	–	2
+++	–	3	–	2	–	1	–	1	–	–	–	3	1	–	–	1	1	–	–	2
++	–	2	–	1	–	–	–	–	–	–	–	2	2	1	1	–	2	1	2	1
+	–	2	–	–	2	4	1	–	5	6	5	–	4	4	4	2	1	3	2	–
–	8	1	8	–	3	–	4	–	2	1	2	–	3	5	5	1	1	1	1	–

*Two tissues stained for keratin 14; †five tissues stained for keratin 14.

Table 1 continued

Breast (n = 7)				*Cervix (n = 7)*				*Oesophagus (n = 2)*			
7	*8*	*18*	*19*	*7*	*8*	*18*	*19*	*7*	*8*	*18*	*19*
3	7	7	7	–	5	2	5	1	2	2	2
1	–	–	–	1	–	3	–	–	–	–	–
1	–	–	–	2	–	–	–	1	–	–	–
2	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	2	–	–	–	–	–	–	–
4	*10*	*13*	*14*	*4*	*10*	*13*	*14*	*4*	*10*	*13*	*14*
–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	1	–	–	–	–	–	–	–
–	–	–	–	1	–	–	–	–	–	–	–
4	1	1	3	–	–		2	1	–	–	–
3	6	6	4	3	5	5	3	1	2	2	2

adenocarcinomas. A single case of expression of this keratin in glandular tissue was a breast carcinoma specimen, where it was confined to cells in regions of squamous metaplasia present in the specimen.

Expression of vimentin was found in all cases of renal cell carcinomas and in three of the breast carcinomas. None of the adenocarcinomas studied expressed neurofilaments or desmin. The data are summarised in table 1.

KERATIN PATTERNS IN SQUAMOUS CELL CARCINOMAS

Table 2 shows that, with the exception of the squamous cell carcinomas derived from keratinising squamous epithelia of the skin and vulva, squamous cell carcinomas contained a varying fraction of cells expressing keratins 7, 8, 18, and 19 (fig 1B and 1C). Only one of 31 carcinomas derived from non-keratinising squamous epithelia (a verruceus carcinoma of the larynx) did not express one of the simple keratins. From the simple keratins, keratin 19 was most often found in all squamous cell carcinomas, except in the case of two laryngeal carcinomas and the carcinomas of the skin and vulva. Keratin 7 was found in small number of cells, and was often completely absent.

Keratins 4, 10, 13, and 14 were found in all squamous cell carcinomas with the exception of one poorly differentiated lung carcinoma. Carcinomas derived from non-keratinising squamous epithelia in 60% of the cases expressed keratin 4 and 13, in contrast to carcinomas derived from keratinising epithelium,

Table 2 continued

Rectum (n = 2)				*Tongue (n = 7)*				*Oesophagus (n = 4)*			
7	*8*	*18*	*19*	*7*	*8*	*18*	*19*	*7*	*8*	*18*	*19*
–	1	–	2	–	1	1	2	–	–	–	2
–	1	1	–	–	1	–	–	–	2	–	–
1	–	–	–	2	–	–	–	–	1	2	–
1	–	1	–	1	1	2	1	2	1	2	2
–	–	–	–	–	–	–	–	2	–	–	–
4	*10*	*13*	*14*	*4*	*10*	*13*	*14*	*4*	*10*	*13*	*14*
–	–	–	–	–	–	–	1	–	–	–	–
–	–	–	–	–	–	–	2	–	–	–	2
–	–	–	–	1	–	–	–	1	1	2	2
2	1	2	2	2	1	2	–	3	3	2	–
–	1	–	–		2	1	–	–	–	–	–

Table 3 Comparison of RGE53 and M9 reactivity with squamous cell carcinomas

Carcinoma type	*n*	*M9*	*RGE53*	*More positive for M9/total*
Cervix	5	25–75%*	0–50%	3/5
Lung	6	0–75%	0–50%	4/6
Larynx	7	0–50%	25–50%	5/7
Tongue	2	25%	0–25%	2/2
Oesophagus	2	25–50%	0–25%	2/2
Anus	1	75%	25%	1/1

*Range of percentage of positive tumour cells.

of which only one carcinoma of the vulva was positive for keratin 4.

Keratin 14 identified all squamous cell carcinomas with the exception of the lung carcinoma mentioned above. In 75% of squamous cell carcinomas, keratin 10 was focally expressed, usually specifically in a few cells of a tumour nest (fig 1D). The presence or absence of groups of keratin 10 positive cells did not correlate with the histological diagnosis of well or poorly differentiated squamous cell carcinoma, nor did the number of keratin 4 or 13 positive cells.

With the exception of one skin carcinoma, all squamous cell carcinomas derived from keratinising epithelia expressed keratin 10. The observed positivity for the differentiation related keratins did not, however, correlate with the absence of simple keratins.

We observed a striking difference in the keratin 18 staining patterns using both RGE53 and M9 in their preferential dilution (table 3). To exclude the possibility that the observed differences were dependent on antibody concentration (RGE53 is preferably used in a 1:30 dilution, whereas M9 is used undiluted), a series of carcinomas was stained with undiluted RGE53. Seventy four per cent of tumours tested still showed fewer positive cells after staining with undiluted RGE53 (fig 1E and 1F). The immunohistochemical staining patterns of M9 and RGE53 on normal columnar epithelia and adenocarcinomas were, however, identical (data not shown). Immunoblotting experiments showed that both antibodies reacted with one band of 45 kDa in a keratin preparation derived from MCF-7 cells (not shown).

In the selected series of eight squamous cell carcinomas stained with both CK14 and L0012 in several dilution steps, the number of positive cells found differed only slightly in two cases. The staining became less intense with antibody dilution. The two antikeratin 13 antibodies showed no difference in staining pattern.

All of the types of squamous cell carcinoma examined expressed keratins exclusively, with exception of two lung carcinomas which expressed vimentin in a few cells. No expression of desmin or neurofilaments was observed.

Discussion

Our study, performed with chain specific monoclonal antibodies against keratins in a large series of carcinomas, shows comprehensive heterogeneity of keratin expression.

The predominant keratins in adenocarcinomas of different sites of origin proved to be 8, 18, and 19. However, these keratins were also

Table 4 Summary of antibodies used in the study

Clone	*Specificity*	*Dilution*	*Source*	*Reference No*
80	All keratins	1:5	Eurodiagnostics, NL	38
V9	Vimentin	1:10	Eurodiagnostics, NL	34
D33	Desmin	1:10	Eurodiagnostics, NL	34
2F11	Neurofilament	1:10	Eurodiagnostics, NL	39
6B10	Keratin 4	1:1	Eurodiagnostics, NL	11
1C7	Keratin 13	1:1	Eurodiagnostics, NL	11
2D7	Keratin 13	1:1	Eurodiagnostics, NL	11
M9	Keratin 18	1:5	Eurodiagnostics, NL	24
M20	Keratin 8	1:5	Eurodiagnostics, NL	35
RGE53	Keratin 18	1:30	Organon, NL	19
RKS60	Keratin 10	1:10	Eurodiagnostics, NL	13
CK7	Keratin 7	1:100	Biogenex, USA	9
LP2K	Keratin 19	1:20	Amersham, UK	33
CKB1	Keratin 14	1:100	Sigma, USA	36
LL002	Keratin 14	1:100	Biogenex, USA	37

abundantly present in squamous cell carcinomas of the lung, cervix, rectum, and to a lesser extent the larynx, oesophagus, and tongue, but not in those of the vulva and skin. These keratins can therefore be of value in the differential diagnosis of carcinomas from keratinising and non-keratinising epithelia. Unfortunately they will not be of value in the immunohistochemical differentiation of squamous cell carcinomas from adenocarcinomas, in spite of previous reports to the contrary.[2 5-7 20] As more antikeratin 18 antibodies have become available, data from previous studies using single monoclonal antibodies (such as RGE53 or CK1-CK4) as markers for adenocarcinomas and squamous cell carcinomas[5 19 21-25] have proved unreliable.

In our hands, no single variable high molecular weight keratin—such as keratin 4, 10, or 13—could be used to discriminate between squamous cell carcinomas and adenocarcinomas. The best discriminative marker was keratin 10, identifying 32 of 44 squamous cell carcinomas, with only one case of squamous metaplasia in glandular tissue (breast) and no adenocarcinomas being positive. This confirms earlier studies in which no keratin 10 positive adenocarcinomas were found.[21] Keratin 13 was reported downregulated in squamous cell carcinomas.[42] In our studies, three of 44 squamous cell carcinomas were indistinguishable from adenocarcinomas if reliance was placed on the keratin 13 staining. The predominant keratin of squamous cell carcinomas was keratin 14, and only one lung carcinoma found to be negative. However, this filament was also present in a certain fraction of cells in 20% of the adenocarcinomas. According to our findings, the absence of keratin 14 in a tumour suggests an adenocarcinoma. We could find no previous reports of any large series of carcinomas being stained by an antikeratin 14 antibody. More antikeratin 14 antibodies are now available, especially those not showing basal reaction, and it may be that these antibodies are more widely expressed than was thought.

A combination of keratins appears to be useful for diagnosis than individual keratins. Thus our data show that the presence of a

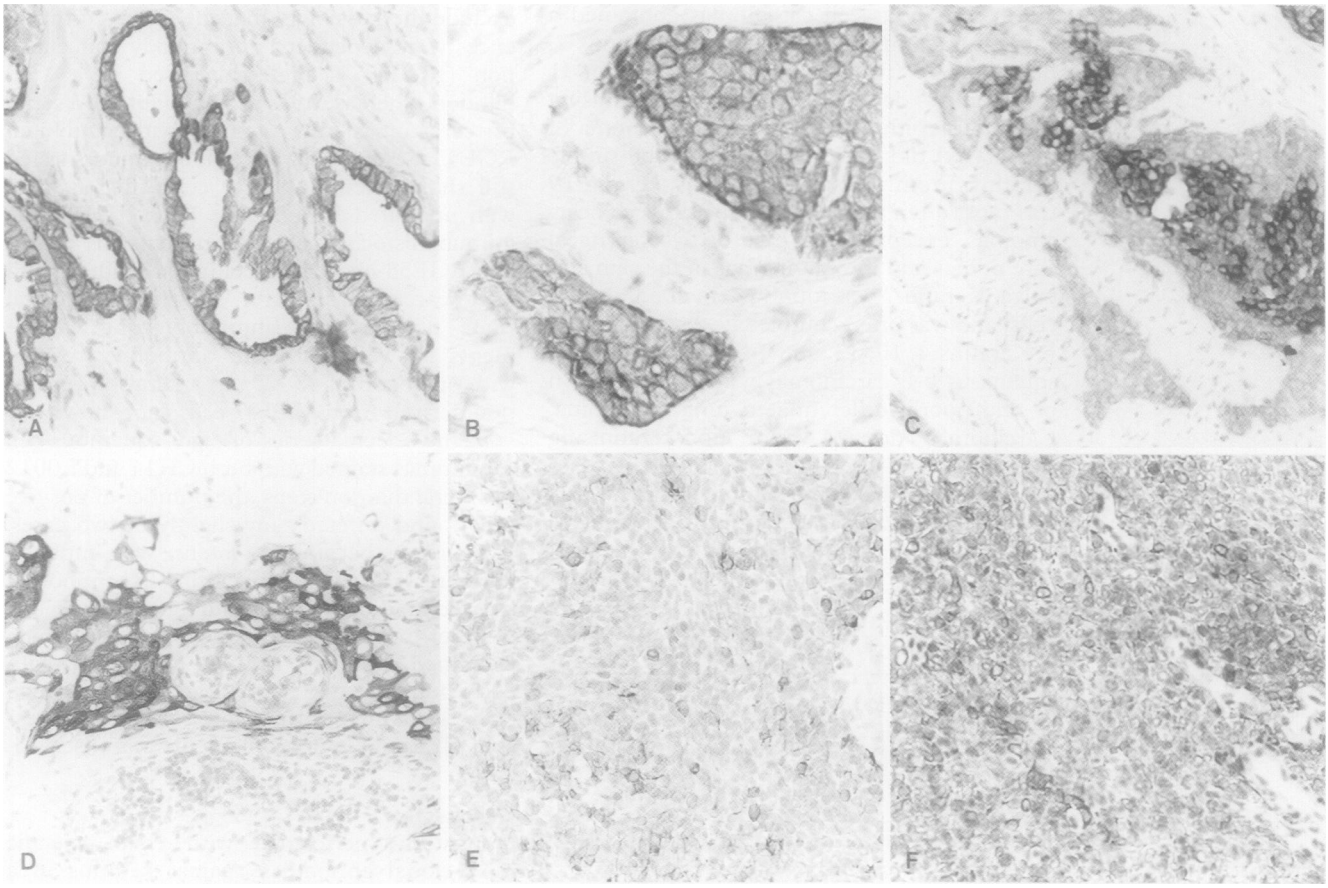

Figure 1 Immunohistochemical localisation of keratins in frozen sections of human tumours. (A) Adenocarcinoma of the stomach. Example of a group of tumour cells positively stained for keratin 7 (×20). (B) Squamous carcinoma of the lung. Strong staining for keratin 19 in tumour cells (×40). (C) Squamous cell carcinoma of the oesophagus. Prominent staining for keratin 8 in some of the tumour cells (×20). (D) Moderately differentiated squamous cell carcinoma of the larynx. Keratin 10 identifies some of the large differentiated tumour cells (×20). (E) and (F) Comparison of staining patterns of squamous cell carcinoma of the cervix by immunohistochemical staining using two undiluted antibodies directed against keratin 18, RGE53 (E), and M9 (F) (×20). M9 shows an intense staining of all tumour cells, compared with 25% of cells stained weakly positive by RGE53.

combination of at least two of keratins 4, 10, and 13 confirms squamous cell carcinoma. In this study we concentrated mainly on differentiation related markers of squamous epithelia, such as these three keratins. The detection of keratins associated specifically with keratinocyte lineage (such as of keratins 5 and 14), using monoclonal antibodies recognising their shared epitope in filaments, may be also helpful and provide additional information.

The adenocarcinomas in this study could be subdivided into keratin 7 positive tumours (breast, pancreas, and oesophagus) and keratin 7 negative tumours (colon), as in previous reports.[5 11 21 26-30] We found keratin 7 positive cells in gastric and renal cell carcinomas, in agreement with data from Ramaekers *et al* and Bartek *et al*,[27 28] but not Osborn *et al*.[26]

Keratin patterns of carcinomas are thought to be primarily determined by the cell type of origin and to be conserved through the multistage process of carcinogenesis.[4-6] We found that this conservation was usually apparent in adenocarcinomas—although with certain exceptions such as the keratin 7 expression pattern. One might speculate that a certain subpopulation of glandular carcinomas do not express keratin 7 because they originate from keratin 7 negative stem cells, as in colonic tumours. Such stem cells may express keratin 7 only if they have completed their differentiation pathway, which explains the lack of expression in poorly differentiated carcinomas.

Most cases of squamous cell carcinoma (especially carcinomas derived from non-keratinising epithelia) showed a keratin pattern that was not representative of that of normal squamous epithelia. An explanation for these results might be found in theories on the development of cancer from a possible stem cell by a certain route of (de-)differentiation. The fact that those simple keratins are present in the majority of squamous cell carcinomas derived from non-keratinising epithelia is intriguing. It is conceivable that (re-)expression of simple keratins is induced by such processes as malignant transformation[4 43] or tumour progression.[13 44] However, it is remarkable that some of these squamous cell carcinomas have a common histogenesis—that is, they arise from a "reserve cell" in columnar epithelium by squamous metaplasia. Squamous metaplasia is a reversible process in which squamous epithelial cells replace columnar epithelial cells in a chronic inflammatory process. These metaplastic cells probably arise from undifferentiated columnar stem cells through an abnormal differentiation programme or even from division of pre-existing differentiated cells. Columnar cells undergoing active metaplasia can be transformed towards premalignant conditions and perhaps eventually to invasive cancer.[45] On this basis, the keratin patterns found in the cervical and lung squamous cell carcinomas could be explained by assuming a columnar cell of origin that retains expression of its simple keratin pattern, and the induction of differentiation-type keratins 4, 10, and 13.[5 13 46-48] The large amounts of keratin 8, 18, and 19 found in laryngeal carcinomas derived from the transformation zone of the epiglottis can also be explained by the mechanism of squamous metaplasia of pseudostratified columnar epithelium.[49]

In published reports, squamous cell carcinomas of the tongue, pharynx, oesophagus, and rectum were reported to be negative for simple keratins.[22] Surprisingly, we found these carcinomas to be positive for keratins 8, 18, and 19, and sometimes for keratin 7 as well. It is likely that these carcinomas arise from cells in the basal layers of the normal squamous epithelia expressing keratin 19 and possibly also a low level of other simple keratins. It was shown recently that keratins 8 and 18, together with 19, are indeed present at the mRNA level in many stratified epithelia in the most basal layers.[50 51] More studies have been performed using mRNA that have identified keratin 4 in basal compartments as well.[52] Comparison of results identifying mRNAs with positive staining for an antibody is difficult. In squamous cell carcinomas arising directly from cornifying squamous epithelia, no simple keratins (7, 8, 18, or 19) were found, either in this or in other studies.[7 11] There have been some reports, however, that the basal compartment of cornified epithelium contains cells that may have retained the ability to produce simple keratins,[50 53] just as embryonic epithelial cells express simple keratins,[33 54] and basal cell carcinomas of the skin do indeed express keratins 8, 18, and 19 (data not shown[55]).

On the basis of previously published reports, we found that more carcinomas than expected were positive for simple keratins; this may be explained by the fact that more antibodies are now available—more even than were used in this study. This is supported by conclusions drawn by Markey *et al*,[53] that the expression of simple keratins in many squamous carcinomas in their studies might reflect the availability of new antibodies and greater sensitivity of the detection methods used. Differences in staining patterns of squamous cell carcinomas with RGE53 and M9, as reported here, could be the result of the accessibility of epitopes expressed on keratin 18.[35 56] Such a phenomenon was previously described for a monoclonal antibody against keratin 18, which appeared to interact only with antigen occurring in the heterotypic coiled coil complex, notably with keratin 8.[57]

CONCLUSIONS

To summarise, the qualitative differences in keratin patterns between normal squamous and columnar epithelia largely disappear in carcinomas. This hampers the use of monoclonal antibodies against keratins for diagnoses based on histological principles, although differences in the expression of combinations of certain keratin filaments—for example 4, 10, and 13—might be useful in solving problems of differential diagnosis. It also seems possible to state that a carcinoma negative for keratin 14 is an adenocarcinoma. The question of whether expression of a particular keratin is an intrinsic or an extrinsic property of an epithelial cell is yet to be resolved. In the light of the results presented here, we think it is likely to be extrinsically determined.

We thank Dr S Lavrijssen, Department of Dermatology, Leiden University Hospital, The Netherlands, for the tissue specimens of skin carcinomas, Mrs C B de Graaf-Reitsma for excellent technical assistance, and K van der Ham and R Heruer for preparing the photographs.

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