Cytokeratins in Different Types of Human Lung Cancer as Monitored by Chain-specific Monoclonal Antibodies

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ABSTRACT

The expression of cytokeratins (CKs) in human lung cancer was studied using chain-specific monoclonal antibodies to CKs 4, 7, 8, 10, 13, 18, and 19. When applied to adenocarcinomas (ACs) of the lung, high levels of CKs 7, 8, 10, and 19 were detected in all tumors, while CK 4 was detected in high concentrations in some ACs. CK 10 and 13 were completely absent, or only present in low numbers of cells. Small cell lung carcinomas (SCLCs) and lung carcinoids contained CK 18 and sometimes 8 and 19, but no CK 7 in most cases. Three out of four tumors, histologically classified as SCLC, and expressing CK 7 in a variable number of cells were found by electron microscopic studies to contain regions with AC and/or squamous cell carcinoma (SCQ) differentiation. The monoclonal antibody specific for CK 7 can therefore possibly help to distinguish AC differentiation within SCLC. CKs 10 and 13 were completely absent in SCLCs and lung carcinoids, while few CK 4-positive cells were found in some SCLCs and in one lung carcinoid. Within SQC the monoclonal antibodies revealed a pronounced heterogeneity in CK expression. CKs 4, 7, 8, 10, 13, 18, and 19 could be detected, although not evenly distributed among all tumor cells. Highly differentiated SQC expressed high levels of the CKs specific for squamous differentiation, i.e., CKs 4, 10, and 13 in variable numbers of cells. Decreasing histologically detectable SQC differentiation these markers were gradually lost, while the number of cells containing CKs 7, 8, 18, and 19 increased.

Application of this panel of monoclonal antibodies can therefore distinguish not only the main subtypes of lung cancer, but can also indicate the degree of differentiation and the degree of heterogeneity. These findings can be used as a diagnostic aid in lung tumor pathology, which may have an impact on treatment and prognosis.

INTRODUCTION

Lung cancer is now generally accepted to be a very heterogeneous type of cancer, in which a cooccurrence of the different subtypes is rather a rule than an exception. The three main subtypes are SCLC, AC, and SQC. While after histological examinations heterogeneity is found in about 10–15%, this number increases to about 50% after cytological examinations (1), electron microscopy (2, 3), or when using refined immunohistochemical techniques (4, 5). Because of differences in treatment and prognosis of the different types of lung cancer, detection of each subtype, especially SCLC, within individual lung tumors is of clinical importance.

Virtually all epithelial tissues, both normal and malignant, contain CKs, which form the intermediate filament cytokeratin within the epithelial cell. This family of human CKs consists of 19 different polypeptides, which have been numbered 1 through 19 by Moll et al. (6). These CKs are not randomly distributed in the different epithelia, but appear to be characteristic for certain types of epithelial differentiation. For instance, relatively high molecular weight CKs are characteristic for more complex, stratified epithelia and SQCs, while relatively low molecular weight CKs, such as CK 18, are specific for simple, columnar epithelia (6) and ACs (7). A possible method to detect CKs is the biochemical demonstration based on (two-dimensional) gel electrophoresis as performed previously for lung carcinomas (8–11). This integral biochemical approach, however, is limited to defining the total of CKs expressed by a carcinoma specimen as a whole. CKs which are only expressed by a small subset of cells may go undetected (12). Immunohistochemically, the presence of a certain CK, corresponding to a particular type of differentiation, is detectable at the cellular level with MoAbs to a specific subtype of CK, which have recently been developed (7, 12–22). Since the detection of type and degree of differentiation can be of clinical importance, the use of this panel of MoAbs can add to a more specific treatment for certain tumors.

MATERIALS AND METHODS

Lung Tissues. Fresh samples of normal bronchi (five cases), normal lung (three cases), and lung tumors were snap-frozen and stored in liquid nitrogen until use. Lung tumors examined in this study were classified on histological basis according to the WHO classification (23). From lung carcinoids, SCLC, AC, poorly differentiated SQC, moderately differentiated SQC, and well-differentiated SQC, 10 cases each were examined. In addition, five adenocarcinomas, three large cell carcinomas, and four combined SCLC/SQC were examined. Frozen sections approximately 5 μm thick were cut on a cryostat, air dried, and stored at −20°C before use.

Cytokeratin Antibodies. The following mouse monoclonal antibodies were used in this study (see also Table 1): (a) RCK102 is a broadly cross-reacting CK antibody of the IgG subclass, which recognizes CKs 5 and 8, and as a result stains virtually all epithelial tissues (13). (b) RCK105 (IgG; 13) in immunoblotting reacts only with CK 7 and stains a subgroup of glandular epithelia and their tumors, next to transitional bladder epithelium and bladder carcinomas. (c) LE41 (IgG; 14, 15) reacts with different types of epithelial tissues and in immunoblotting recognizes only CK 8. (d) RGE53 (IgG; 7, 16) and RCK106 (IgG, 13) are monospecific for CK 18 in immunoblotting. In general, these two antibodies recognize columnar epithelial cells from digestive, respiratory, and urogenital tracts, endocrine and exocrine tissues and mesothelial cells, as well as their tumors. Generally no reaction is found in squamous epithelia or squamous cell carcinomas. (e) LP32K (15) and 1BA17 (IgG; 17, 18) stain most simple epithelia and basal cells in stratified squamous epithelia that are not keratinizing, and recognize only CK 19 in immunoblotting assays. (f) RkS6a (IgG, 16, 19, 20) reacts only with keratinizing epithelial cells, and recognizes CK 10 in immunoblotting. (g) 6BI0 (IgG, 21) reacts with nonconfining squamous epithelium, and with certain ciliated pseudostratified epithelia such as cylindrical epithelium of bronchi, and recognizes only CK 4 in immunoblotting. (h) Antibodies IC7 (IgG, 21) and 207 (IgG, 21) both react with nonconfining squamous epithelia. These antibodies recognize only CK 13 in immunoblotting studies.
Immunoperoxidase Technique. In the indirect immunoperoxidase technique, cryostat sections were fixed in acetone at room temperature (3 times 5 s) and incubated with the primary monoclonal antibodies for 30–45 min at room temperature. All primary antibodies were applied as undiluted culturing supernatant. After repeated washing with 0.05 M phosphate buffered saline (pH 7.4) (PBS), sections were incubated with rabbit anti-mouse conjugate serum conjugated to peroxidase (DAKO, Denmark) diluted 1:25 in PBS with 5% human AB-serum, and incubated for 30–45 min.

After washing in PBS, peroxidase activity was detected with 3-amin-9-ethylcarbazole (Aldrich Chemical Co.). 40 mg of 3-amin-9-ethylcarbazole was dissolved in 10 ml N,N-dimethylformamide (Merck, Darmstadt, FRG) and added to 190 ml of sodium acetate buffer (0.05 M, pH 4.85). Hydrogen peroxide was added to a final concentration of 0.01%.

For an incubation for 10 min, cells were rinsed with tap water, counterstained with hematoxylin, and mounted with Kaiser's glycerol gelatin (Merck).

Gel Electrophoresis and Immunoblotting Assays. Cytoskeleton preparations from solid lung tumors were made as follows. After microdissecting tumor areas, 20-µm thick frozen sections were immediately extracted for 15 min at 0°C using a high-salt buffer (1.5 M KCl, 0.5% Triton X-100, 5 mM EDTA, 0.4 mM phenylmethylsulfonyl fluoride, and 10 mM Tris-HCl (pH 7.2)), centrifuged for 5 min at 3000 × g, and washed twice with cold (4°C) low-salt buffer (5 mM EDTA, 0.4 mM phenylmethylsulfonyl fluoride, and 10 mM Tris-HCl, pH 7.2), essentially as described by Moell and Franke (10).

After centrifugation (3000 x g for 10 min) and washing in PBS, the cytoskeleton preparation was dissolved by boiling for 5 min in SDS-sample buffer (24).

One-dimensional gel electrophoresis was performed in 10% polyacrylamide slab gels containing 0.1% SDS, as described by Laemmli (24). To compare the amounts of protein loaded on each lane, gels were stained with Coomassie Brilliant Blue R250 (Gurr, Hopkin and Williams, Chadwell Heath, Essex, U.K.) as described before (25). Two-dimensional gel electrophoresis was performed essentially as described by O'Farrell (26). In the first dimension, isoelectric focusing was performed in 4% polyacrylamide (Biorad, California, USA) rod gels containing 2% ampholine, pH 3.5–10 (LKB, Bromma, Sweden). For the second dimension, the rod gels were applied directly onto the stacking gel of SDS-polyacrylamide gels and covered with a 1% agarose solution in running buffer.

For immunoblotting experiments the electrophoretically separated polypeptides were transferred to a nitrocellulose sheet (Schleicher and Schull Membrane Filters BA 85; Dassel, FRG) by overnight blotting at 250 mA using an electrophoresis buffer containing 25 mM Tris-HCl, 192 mM glycine and 20% methanol (pH 8.3), essentially as described by Towbin et al. (27). The nitrocellulose sheets were then immunohistochemically stained essentially as described previously (25). In short, blots were preincubated for 90 min with PBS containing 0.05% Tween 20 (Sigma). All reagents were diluted in this solution, which was also used for the washing steps. After incubation overnight with undiluted culturing supernatants of the primary antibodies RCK106, ICG, RCK102, or RCK105, or with LP2K, diluted 1:10, blots were washed, incubated for 1 h with rabbit anti-mouse peroxidase (DAKO, Denmark) diluted 1:200, washed again, and stained with 4-chloro-1-naphthol.

**RESULTS**

Normal Bronchial and Lung Tissues. The cytokeratin expression patterns of normal bronchial epithelium and lung alveolar epithelium are presented in Table 2 and illustrated in Fig. 1. The cytokeratin antibodies are not reactive with other than epithelial tissues. RCK102 (Fig. 1, A and B), RCK106 (Fig. 1E), LP2K and BA17 react with all bronchial and lung epithelial tissues, while RCK105 (Fig. 1C) and RGE53 (Fig. 1G and H) recognize all bronchial and lung epithelial tissues except the basal layer in the bronchial columnar epithelium. LE41 recognizes only superficial cells of the bronchial columnar epithelium (sero-mucous glands, while again basal cells as well as lung alveoli are not recognized. 6B10 (Fig. 1D), IC7 and 2D7 (Fig. 1F) react with some cells of the bronchial epithelium, but are negative with most alveolar epithelial cells (Fig. 1I). RKS660 does not react with any type of epithelial cells in normal lung.

Lung Tumors. Results of the immunohistochemical staining procedures in lung tumor frozen sections with the different CK antibodies are summarized in Table 3 and illustrated in Figs. 2, 3, and 4. On the basis of histological examinations, SQCs were subdivided into three groups, i.e., well-differentiated SQC, moderately differentiated SQC, and poorly differentiated SQC.

In all SQCs examined, a reaction was seen with the CK 5+8 antibody (Fig. 2, A and D). The CK 10 antibody, specific for keratinizing epithelial cells, reacts more with well-differentiated SQC (Fig. 2B) than with moderately and poorly differentiated SQC, which in general do not react with this antibody (Fig. 2E). The same holds true for the two MoAbs to CK 13, which react in more cases and with more cells of well-differentiated SQC as compared to poorly differentiated SQC (compare Figs. 2B, 2C, and 2D).
Fig. 1. Immunoperoxidase staining patterns in normal bronchial and lung epithelium. The broadly cross-reacting CK antibody RCK102 reacts with all bronchial epithelium cells (4, × 240), with seromucous glands (2, × 280), as well as with lung alveolar epithelial cells (not shown). In general, antibodies to cytokeratins typical for "simple" epithelia (C, E, G, and H) reacted with more cells of normal bronchus than the antibodies to cytokeratins characteristic for stratified epithelia (D, F, and J). The CK 7 antibody RCK105 reacts with the columnar cells but not with the basal cells of bronchial epithelium (C, × 280), while the CK 18 antibody RCK106 reacts with all bronchial epithelial cells (E, × 310). In contrast, the other CK 18 antibody, RGE 53, reacted similarly to RCK105, and was also negative in basal cells of normal bronchial epithelium (G, × 210). In lung alveolar tissues, antibodies such as RCK106 (H, × 330), but also RGE53, LP2K and RCK105 recognize pneumocytes of the alveolar epithelium (arrows). The CK 4 antibody, 6B10, recognizes some basal as well as some columnar cells of normal bronchus (D, × 330), while the CK 13 antibody 2D7 only occasionally recognized some columnar cells (F, × 350). In general, no cells were stained with 6B10 in alveoli (J, × 330).

2, C and F). In contrast, the antibodies to CK 19 react with more cases and with more cells within each tumor in poorly differentiated SQCs (compare Figs. 2, G and J). The CK 8 antibody also seems to react with more cells in poorly differentiated SQCs. However, the overall reaction of this antibody was quite weak in all cases. The CK 18 antibodies exhibited a very strong staining reaction in most, and sometimes in all tumor cells of poorly differentiated SQCs, and staining of only a small percentage of the cells in well-differentiated SQCs (compare Figs. 2, H and K; see also Reference 4). The antibody to CK 7, which reacts with scattered cells in only a few well-differentiated SQCs, however, stains a considerable percentage of most poorly differentiated SQCs (Figs. 2, I and L).

All ACs showed a staining reaction in virtually all tumor cells with the CK 5+8 antibody (Fig. 3A), while none of these tumors reacted with CK 10. Only some scattered cells of some ACs reacted with the MoAbs to CK 13 (Fig. 3B). Using the CK 4 antibody, a very heterogeneous reaction pattern was observed in ACs, varying from a reaction in almost all tumor cells (Fig. 3C) to a completely negative reaction (not shown). The antibodies directed against CK 19 (Fig. 3D), CK 18 (Fig. 3E), CK 8 (not shown), and CK 7 (Fig. 3F) reacted with all ACs in virtually all tumor cells, although with one MoAb to CK 19 (BA17) and with the antibody to CK 8, a relatively weak reaction in less tumor cells was seen.

The cancers which were histologically classified as adenosquamous carcinomas showed also immunohistochemically this bipartite nature, as expressed in the presence of areas with cornifying epithelium which were focally CK 10 positive (Fig. 3G), and other areas which were positive with the CK 7 antibody (Fig. 3H).

The SCLCs examined for this study all contained Cks, reacting in virtually all cells with the CK 5+8 antibody (Fig. 4A). The CK 10 and 13 antibodies were negative, while the CK 4 antibody showed in some isolated cells reactivity with these SCLCs (Fig. 4B). The antibodies to CK 19 reacted with varying numbers of cells (Fig. 4C), while the CK 8 antibody reacted only very weakly in some of these tumors. The antibodies to CK 18 reacted with nearly all SCLCs (Fig. 4D). Most SCLCs did not react with the antibody to CK 7 (Fig. 4F). However, in
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* RCK102, no reaction in tumor cells.
* F+, heterogeneous focal reaction.
* D+, homogenous diffuse reaction.

Cytokeratin heterogeneity in lung cancer

Table 3: Reactivity of cytokeratin antibodies in frozen sections of human lung cancers using the indirect immunoperoxidase technique

Four cases a reaction with a varying number of cells was seen (Fig. 4E). In three of these cases, electron microscopical examination revealed the presence of cells with AC and/or SQC differentiation, next to the typical neurosecretory granules.

Carcinomas classified as combined SCLC/SQC on histological grounds showed a heterogeneous reaction pattern with the different CK antibodies in accordance with their histological heterogeneity. Most strikingly, large areas were completely positive with the antibodies to CK 18 (Fig. 4G), while other parts did not react with these antibodies, but were focally positive with the antibody to CK 4 (Fig. 4H).

The CK expression of the three large cell carcinomas examined was characterized by a positive reaction with MoAbs to the "simple" CKs 7, 8, 18, and 19, and a negative reaction with the MoAbs to CKs 4, 10, and 13 (not shown).

All lung carcinoids examined reacted with the CK 5+8 antibody (Fig. 4I), while in only one case a reaction was observed with the antibodies to CKs 4 and 13. Antibodies to CK 8 (Fig. 4J) and CK 19 (Fig. 4K) showed variable reaction patterns, while the antibodies to CK 18 were positive in all cases examined (Fig. 4L). CK 7 was detected in three lung carcinoids.

Immunoblotting Studies. Results of the one-dimensional immunoblotting experiments on cytoskeleton preparations of tissue sections of SCLC, AC, and moderately to well-differentiated SQC are shown in Fig. 5. In the three SCLCs examined the presence of CK 18 was proven using RCK106 (Fig. 5A, lanes 1–3). The two ACs reacted strongly with this antibody (Fig. 5A, lanes 4 and 5), while the three SQCs showed a relative weak reaction (Fig. 5A, lanes 6–8).

A subsequent incubation of this immunoblot with LP2K showed the presence of CK 19 in one SCLC (Fig. 5B, lane 2), in the two ACs (Fig. 5B, lanes 4 and 5), and in the three SQCs.
Fig. 2. Immunoperoxidase staining patterns in well-differentiated squamous cell carcinomas (SQC) A, B, C, G, H; and I) and in poorly differentiated SQCs (D, E, F, J, K, and L) of the lung. The broadly cross-reacting CK antibody RCK102 reacts with all tumor cells in both well-differentiated (A, × 270) and poorly differentiated SQCs (D, × 290). Only in well-differentiated SQCs keratinizing areas reacting with the CK 10 antibody RKSE60 are seen (B, × 250), while no reaction is seen with this antibody in poorly differentiated SQCs (E, × 360). Large areas of well-differentiated SQCs are stained with the CK 13 antibody 2D7(C, × 340), while in poorly differentiated SQCs only scattered positive cells can be found (F, × 270). The CK 19 antibody LP2K reacts with only a few cells in well-differentiated SQCs (G, × 340), while large parts of poorly differentiated SQCs show a completely positive reaction (J, × 250). In most well-differentiated SQCs some cells react with the CK 18 antibody RGE53 (H, × 250). In poorly differentiated SQCs, however, the percentage of CK 18-positive tumor cells increases to almost 100% (K, × 250). The CK 7 antibody RCK105 does not react with most well-differentiated SQCs (I, × 250), but shows a completely positive reaction in some poorly differentiated SQCs (L, × 250). H and I, staining patterns with RGE53 and RCK105 in parallel sections of the same region of a well-differentiated SQC. Note the positive reaction of CK 18 and no staining for CK 7.

(Fig. 5B, lanes 6–8). Subsequent incubation of this immunoblot with 1IC7 showed the presence of CK 13 in two SQCs (Fig. 5B, lanes 6 and 7), and the absence of this CK protein in the other SQC (Fig. 5B, lane 8). Also, the other lung tumors shown here were negative (Fig. 5B, lanes 1–5).

Incubation of a similar immunoblot with the antibody RCK105 showed a weak reaction for CK 7 in two SCLCs (Fig. 5C, lanes 1 and 2), a much stronger reaction in the two ACs (Fig. 5C, lanes 4 and 5), and only trace amounts in two of three SQCs (Fig. 5C, lanes 7 and 8). One SCLC and one SQC were negative (Fig. 5C, lanes 3 and 6, respectively). The findings of the immunoblotting match very well with the reactivity of the individual tumors with the CK antibodies in the immunoperoxidase staining.

Two-dimensional immunoblotting data on SCLC and AC are shown in Fig. 6. From these immunoblottings it is clear that this SCLC (showing AC differentiation on the basis of electron microscopy as well as on the basis of immunohistochemistry) contains small amounts of CK 19 (Fig. 6A), next to CK 18 (Fig. 6B) and CK 7 (Fig. 6C). Additional immunostaining with RCK102 (not shown) resulted in a spot in the CK 8 region. The AC contains CK 19 and CK 18 (Fig. 6D), while additional bands were stained at the CK 8 (Fig. 6E) and CK 7 (Fig. 6F) level. Note that especially in the AC immunoblot breakdown products of CK 18 and CK 19 (more acidic spots with a lower molecular weight, see also Ref. 28) are also stained with their respective antibodies.

DISCUSSION

We have examined a selected group of lung carcinomas for their CK expression using a panel of MoAbs specific for one CK polypeptide. Until a few years ago, the presence of CKs in SCLCs and ACs of the lung was a matter of discussion. For instance, some investigators (29–31) could detect CKs in formalin-fixed, paraffin-embedded squamous cell carcinomas, but not in some ACs or in most SCLCs. Since then, the use of other fixation techniques such as cold methanol fixation, or even better, the examination of unfixed, frozen sections in immunohistochemistry, has improved the detection level for CKs in tissue sections considerably. In addition, better defined CK antibodies, not only recognizing the “larger” more acidic CK polypeptides, but also recognizing the CKs occurring in simple epithelia, have become available. Investigations using these techniques and antibodies have led to a general consensus that (nearly) all lung carcinomas express CKs (4, 5, 8–10, and 32–35). A biochemical examination by Blobel et al. (8, 9) and
Moll and Franke (10) using two-dimensional gel electrophoresis suggested the presence of CKs 7, 8, 18, and 19 in ACs, the presence of CKs 4, 5, 6, 8, 13, 14, 15, 17, 18, and 19 in SQCs, the presence of CKs 7 (minor relative amounts), 8, 18 and sometimes 19 in SCLCs, and of CKs 7 (minor relative amounts), 8, 18, and 19 in lung carcinoids. The CK distribution within each tumor at the cellular level, however, could not be examined by this integral gel electrophoretic technique.

In this study we have used chain-specific MoAbs for CK polypeptides 4, 7, 8, 10, 13, 18, and 19, each of them recognizing only one single CK polypeptide. This panel of MoAbs enabled us to examine the presence of CKs within each type of lung tumor at a cellular level, and to detect a possible heterogeneous CK expression within each tumor. Such a heterogeneity has become apparent from earlier studies, which have shown that many, if not most, lung carcinomas contain more than one type of differentiation, when examined with other conventional (histological) detection techniques.

When using MoAbs one should always be aware of false-negative reaction patterns as a result of epitope masking. For instance immunoblotting experiments, using RCK102, seem to prove the presence of CK 8 in all lung cancers. However, the reaction pattern with LE41, a monospecific antibody for CK 8, is in general very weak, while even in many cases no reaction is seen. Therefore it seems likely that in these tumors the CK 8 epitope recognized by LE41 is largely masked. Similarly, masking of epitopes recognized by one of the CK 19 antibodies, BA17, may explain why this antibody in general reacted with less tumors and with less tumor cells than LP2K, the other MoAb to CK 19. Epitope masking may also explain why the CK 18 antibody RGE 53 did not recognize basal cells of normal bronchial epithelium, while the other CK 18 antibody, RCK 106, showed a clear staining reaction in these cells.

From our results described here, it is obvious that a large heterogeneity in the expression of the different CK polypeptides within most lung carcinomas can occur. For instance the presence of CK 7, normally occurring in ACs, varied in SQC from no positive cells in well-differentiated SQCs, to a positive reaction in all tumor cells for poorly differentiated SQCs. This suggests the presence of varying degrees of AC differentiation within most SQCs.
Fig. 4. Immunoperoxidase staining patterns of small cell lung cancers (SCLCs, A–F), combined small cell/squamous cell carcinomas (G and H), and lung carcinoids (I–K). Most SCLCs are completely positive with the broadly cross-reacting antibody RCK102 (A, × 290). In some cases a focal reaction is seen with the CK 4 antibody 6810 (B, × 370). Varying numbers of cells are positive with the CK 19 antibody LP2K (C, × 370). Most SCLCs react with the CK 18 antibody RCK106 (D, × 335), but do not react with the CK 7 antibody RCK105 (E, × 335). In some SCLCs, in which at the electron-microscopic level a combined SCLC was demonstrated, focal areas reacting with CK 7 were present (E, × 250). Combined SCLC/SQC showed the presence of CK 18, as detected by RCK106, in large parts of the tumor (G, × 370), while also areas not reacting with this antibody were seen. These areas, on the other hand, contained CK 4 in some cells (H, × 370). All lung carcinoids are completely positive with RCK102 (I, × 370), while about half of the cases react with LE41 (J, × 250), and with LP2K (K, × 290). In addition, all lung carcinoids react with RCK106 (L, × 370). Note the typical dot-like appearance of CK 19 (K).

Another example of heterogeneous CK expression is the presence of CK 7 in some SCLCs, suggesting the presence of AC components in these malignancies. This finding is supported by the fact that in three of four SCLCs in which CK 7 is found, also at the electron-microscopic level AC differentiation could be observed. On the basis of many other studies, comparable findings were reported. For instance, at the electron microscopic level in up to 50% of all histologically pure SQCs, AC differentiation can also be detected (2), while in some tumors even tripartite (i.e., SQC, SCLC, and AC) differentiation can be found within the same tumor cell (36). This heterogeneity can also be found in the expression of peptide hormones produced by SCLCs and some non-SCLCs (37), the heterogeneity in expression of neuron-specific enolase and bombesin within SCLC (38), and even at the light microscopical level when whole tumors are examined (39).

In spite of the large heterogeneity, our results still indicate that SQC differentiation, AC differentiation, and SCLC differentiation can be distinguished on the basis of expression of different CK polypeptides. Histological squamous cell differentiation is found in parallel with the presence of CKs 10 and 13 and to a lesser extent CK 4, while pure ACs always express CKs 7, 18, and 19. SCLC differentiation is characterized by the presence of CK 18 and the absence of CKs 10 and 13, and CK 7 in most cases. These findings suggest that the presence of CK 7 may be used to detect AC differentiation. This hypothesis is supported by investigations on the cytokeratin expression in AC and SCLC cell lines, which showed that CK 7 was present in all five AC cell lines examined, while only one out of 17 SCLC cell lines showed a reaction in part of the tumor cells.

In summary, the use of a panel of monospecific CK antibodies can immunohistochemically distinguish the main subtypes of lung carcinomas, but can also detect the degree and type of heterogeneity within each tumor. Therefore, application of this panel of CK antibodies is a useful aid in tumor diagnosis.

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Fig. 5. One-dimensional immunoblotting assays using different chain-specific CK antibodies on cytokeratin preparations from three small cell lung cancers (SCLCs; lanes 1–3 of each panel), two ACs (lanes 4 and 5 of each panel), and three moderately to well-differentiated squamous cell carcinomas (SQCCs; lanes 6–8 of each panel). Comparative amounts of cytokeratin proteins from each sample were applied in A, reaction patterns of these lung tumors with the CK 18 antibody RCK106. Note the very strong reaction of the ACs (lanes 4 and 5), the weaker reaction in the SCLCs (lanes 1–3), and the weak but evident reaction in the SQCCs (lanes 6–8). The arrow denotes a breakdown product of CK 18. In B, the same immunoblot was subsequently incubated with the CK 13 antibody 1C7 and the CK 19 antibody LP2K. Note the presence of CK 13 in two SQCCs (lanes 6 and 7), and the absence of this protein in the other SQCC (lane 8), as well as in the SCLCs and ACs (lanes 1–5). In lane 7 some degradation bands of CK 13 are clearly visible (arrows). CK 19 appears to be present in one SCLC (lane 2), in the two ACs (lanes 4 and 5), and in all three SQCCs (lanes 6–8). Again, also breakdown products of CK 19 are detected (arrows). A parallel immunoblot was incubated with the CK 7 antibody RCK105, as shown in C. Two of three SCLCs show a weak reaction at the CK 7 level (lanes 1 and 2), while the third SCLC is negative (lane 3). Interestingly, the two SCLCs in which CK 7 is present, show, at the electron microscopic level, evidence for focal AC differentiation. The two ACs react very strongly with this antibody (lanes 4 and 5), while in two SQCCs trace amounts of CK 7 can be detected (lanes 7 and 8). Arrows, breakdown products of CK 7.

Fig. 6. Two-dimensional immunoblot of a cytokeratin preparation from a small cell lung cancer (SCLC, A–C) with AC differentiation, and of a cytokeratin preparation from an AC (D–F). The immunoblot of the SCLC preparation was first incubated with LP2K (A, d), resulting in a weak reaction with CK 19, followed by an incubation with RCK106, resulting in a clear recognition of CK 18 (B, e). Thereafter the same blot was incubated with 1C7, not reacting with this preparation, and with RCK105, resulting in a clear reaction at the CK 7 region (C). Additional staining with RCK102 resulted in a weak reaction at the CK 8 region (not shown). The immunoblot of the AC preparation was first incubated with LP2K, followed by an incubation with RCK106 resulting in clear CK 19 and CK 8 spots, respectively (D). Note that breakdown products of these two CKs, migrating towards the acidic side of the blots, are also immunostained. Subsequent incubation with RCK102 resulted in a weak CK 8 band (E), while additional staining with RCK105 resulted in a clear CK 7 spot (F). Incubation of this immunoblot with 1C7 did not result in additional spots.

REFERENCES
cytokeratin heterogeneity in lung cancer


