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ORIGINAL ARTICLE

**Michael Scarpatetti · Oleksiy Tsybrovskyy**
**Helmut H. Popper**

# Cytokeratin typing as an aid in the differential diagnosis of primary versus metastatic lung carcinomas, and comparison with normal lung

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**Abstract** Due to more efficient chemotherapy protocols, the number of second and even third primary carcinomas is steadily increasing. To denominate the possible origin of a carcinoma, different markers are available as an aid, e.g. hormones, proteins and lipoproteins, secretion products and cytoskeletal proteins. Cytokeratins (CKs) have gained new popularity; however, they have not been extensively evaluated in lung tumours. In our study we evaluated the staining patterns of CK polypeptides 4–8, 10, 13, 14, and 17–20 and high molecular weight (HMW) CK polypeptides in routinely processed primary lung carcinomas and lung metastases of diverse origin. As expected, immunohistochemical investigation gave no clear-cut results, but, with statistical analysis, lung adenocarcinomas could be separated from metastatic adenocarcinomas using CK 5 and 18 and HMW CK (specificity 92.5%, sensitivity 62.5%). The different origin of the metastases could often be detected using CK 18 and CK 20. Lung clear cell carcinomas and large cell carcinomas with clear cell areas could be distinguished from metastatic renal clear cell carcinomas by the CK 7 staining reaction. Squamous cell carcinomas of the lung and metastatic squamous cell carcinomas of the larynx, pharynx and oesophagus could not reliably be separated in part due to the few number of cases available. CK polypeptide typing is thus an additional aid in the differential diagnosis of lung carcinomas versus carcinomas metastatic to the lung.

**Keywords** Cytokeratin polypeptides · Lung carcinoma · Lung metastasis · Lung epithelia

M. Scarpatetti (✉) · O. Tsybrovskyy · H.H. Popper
Institute of Pathology, Lab. Environmental and Respiratory Pathology, School of Medicine, University of Graz, Auenbruggerplatz 25, 8036 Graz, Austria
e-mail: michael.scarpatetti@kfunigraz.ac.at
Tel.: +43-316-3804450, Fax: +43-316-3853432

## Introduction

Due to better cancer-treatment protocols, the incidence of second and even third primary cancers of the lung has obviously increased. Because treatment is quite different for primary tumour or lung metastases, it is always necessary for pathologists to make that distinction. Sometimes this is an easy task that is achievable without any special staining. In other cases unique markers can be used to differentiate metastatic carcinomas from primary lung carcinomas, e.g. thyroglobulin to distinguish between metastatic papillary thyroid carcinoma and papillary adenocarcinoma of the lung. In cases like metastatic squamous cell carcinomas of the larynx or oesophagus, it might be impossible to discern them from primary squamous cell carcinomas of the lung.

Cytokeratin (CK) polypeptides are the major cytoskeletal proteins in epithelial cells. Usually two peptides are joined together, one acidic and one alkaline [3]. For more than a decade, it has been well known that different epithelia have unique sets of CK polypeptides. However, this has never been evaluated at the cellular level, especially not in the lung, nor has there been an investigation reported on CK composition in peripheral versus central bronchial epithelia.

Therefore, we studied the immunohistochemical expression of CK polypeptides in normal lung epithelia from the central bronchi to the alveolar periphery and also compared their expression in primary non-small cell carcinomas of the lung and lung metastases from carcinomas of the colon, breast, stomach, prostate, oral cavity, larynx and oesophagus, and kidney. The aim was to find subsets of CKs that might be used as an aid in the differential diagnosis of second primary versus metastatic carcinomas.

## Materials and methods

Lung metastases and primary carcinomas of the lung were retrieved from the archives for lung pathology. Only metastases

**Table 1** List of antibodies and the protocols used

| Cytokeratin | Clone | Processing | Dilution | Antigen retrieval |
|---|---|---|---|---|
| CK 4 | MON 3015 | Manual | 1:2 | MW 30'160 W |
| CK 5 | MON 3029 | Ventana | 1:20 | Pronase 8' |
| CK 5, 8 | ICN 10521 | Ventana | 1:10 | Pronase 8' |
| CK 6 | BMS 65190 | Manual | Neat | MW 30'160 W |
| CK 7 | Dako M7019 | Ventana | 1:200 | Pronase 8' |
| CK 8 | Progen 61031 | Ventana | 1:100 | Pronase 8' |
| CK 10 | Dako M 7002 | Ventana | 1:200 | None |
| CK 10, 13 | Dako M7003 | Ventana | 1:100 | MW 30'160 W |
| CK 14 | Novo.NCL LL002 | Ventana | 1:50 | MW 30'160 W |
| CK 17 | Dako M7046 | Ventana | 1:20 | MW 30'160 W |
| CK 18 | Boehringer8143385 | Ventana | 1:10 | Pronase 18' |
| CK 19 | Dako M7010 | Ventana | 1:10 | MW 30'160 watts |
| CK 20 | Dako M888 | Ventana | 1:200 | Pronase 8' |
| High molecular weight 34βE12 | Dako M630 | Ventana | 1:50 | Pronase 8' |

**Table 2** Cytokeratin (CK) polypeptide reactions in primary lung and metastatic adenocarcinomas

| | CK 4 | CK 5 | CK 6 | CK 7 | CK 8 | CK 10 | CK 13 | CK 14 | CK 17 | CK 18 | CK 19 | CK 20 | High molecular weight CK |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Primary lung adenocarcinomas | 1/16 | 10/16 | 7/16 | 15/16 | 15/16 | 0/16 | 2/16 | 1/16 | 6/16 | 14/16 | 15/16 | 4/16 | 8/16 |
| Lung metastases from colonic carcinomas | 8/21 | 21/21 | 12/21 | 8/21 | 18/21 | 0/21 | 9/21 | 4/21 | 4/21 | 9/21 | 18/21 | 16/21 | 3/21 |
| Lung metastases from breast carcinomas | 4/19 | 17/19 | 10/19 | 18/19 | 14/19 | 0/19 | 1/19 | 5/19 | 5/19 | 11/19 | 17/19 | 0/19 | 6/18* |
| Lung metastases from prostate carcinomas | 2/8 | 7/8 | 2/8 | 4/8 | 6/8 | 0/8 | 0/8 | 1/8 | 3/8 | 6/8 | 8/8 | 0/8 | 1/8 |
| Lung metastases from gastric carcinomas | 0/5 | 5/5 | 3/5 | 5/5 | 3/5 | 0/5 | 1/5 | 2/5 | 0/5 | 0/5 | 3/5 | 0/5 | 1/5 |
| Fisher's exact test primary vs metastatic carcinoma, *P* value | 0.16 | 0.004 | 0.78 | 0.05 | 0.27 | – | 0.72 | 0.27 | 0.33 | 0.008 | 0.67 | 0.76 | 0.03 |
| $\chi^2$ test for metastatic tumours only, *P* value | 0.17 | 0.22 | 0.62 | 0.002 | 0.39 | – | 0.001 | 0.60 | 0.31 | 0.03 | 0.14 | <0.0005 | 0.61 |

* No more material was available

with a proven location of the primary tumour and only proven cases of primary lung carcinomas were selected. The specimen consisted of open and transbronchial biopsies, as well as lobectomies. Neither material from needle biopsies nor material from autopsies was used. A total of 111 different cases were investigated; these were composed of 82 carcinomas metastatic to the lung and 29 primary non-small cell lung carcinomas. In the metastases group, we investigated 21 adenocarcinomas of the colon, 19 adenocarcinomas of the breast, 8 adenocarcinomas of the prostate and 5 adenocarcinomas of the stomach. Two metastatic squamous cell carcinomas originated from the larynx, six from the oesophagus, and three from the oral cavity and hypopharynx. We also investigated 18 lung metastases from renal clear cell carcinomas. The primary lung carcinomas were divided into 16 adenocarcinomas: seven squamous cell carcinomas and six large cell carcinomas with predominant clear cell areas, including three pure clear cell carcinomas of the lung.

The tissue was routinely fixed in neutral buffered formaldehyde, paraffin embedded, cut into 5-µm thin sections and then incubated with commercially available antibodies to CK polypeptides, using our modified immunohistochemical protocols (Table 1). A reaction was regarded as positive when at least 5% of tumour cells were stained. We did not further grade staining intensity or the amount of positive tumour cells, due to the limitations in small bronchial biopsies.

Statistical analysis was performed using SPSS software (SPSS, Chicago, Ill.). In univariate cross-tabulation statistics, two-sided Fisher's exact test or Chi-square test was used where appropriate. We also performed multivariate analysis using binomial and multinomial logistic regression to identify the most useful combination of CK polypeptides for distinguishing the tumour groups.

## Results

### General statements

Most primary lung adenocarcinomas did react with antibodies to CK 5, 7, 8, 18 and 19; only a few reacted with antibodies to CK 6, 17, and 20 and HMW CK; and nearly all had no reaction with antibodies to CK 4, 10, 13 and 14 (Table 2). Metastatic colonic adenocarcinomas reacted with antibodies to CK 5, 6, 8, 19 and 20. Fewer reactions were noticed with antibodies to CK 4, 7, 13, and 18, and only a weak or no reaction with antibodies to CK 10, 14, and 17 and HMW CK (Table 2). Breast adenocarcinoma metastases reacted with antibodies to CK 5–8, 18 and 19; we also noticed fewer reactions for CK 4, 14, and 17 and HMW CK (only 18 of 19 cases could be stained because of tissue limits). Very few or no reactions were seen with antibodies to CK 10, 13 and 20 (Table 2, Fig. 1). The metastases of prostate adenocarcinomas reacted with antibodies to CK 5, 7, 8, 18 and 19. Only a minority

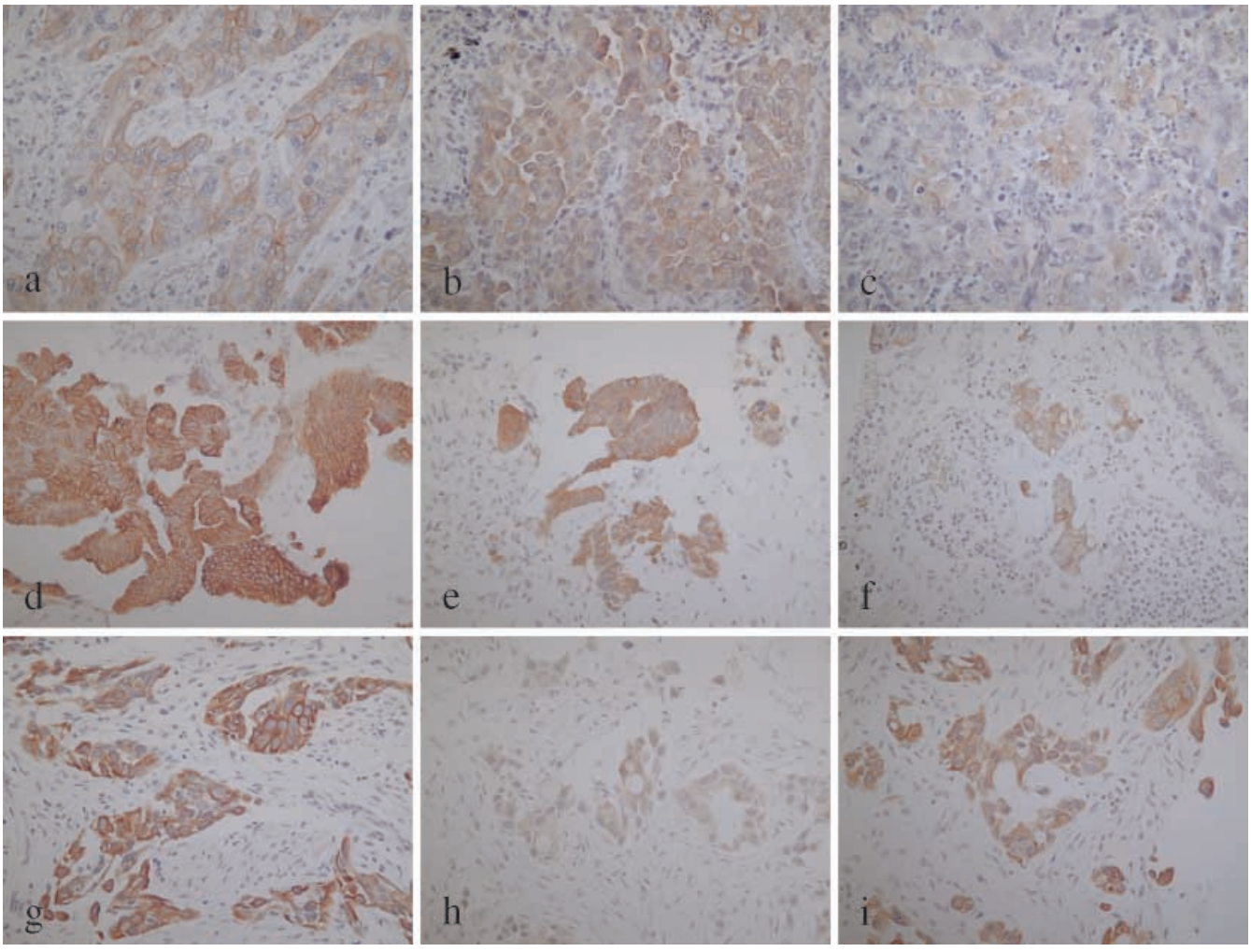



**Fig. 1** Immunohistochemical reactions of antibodies to cytokeratins 5 and 18 and high molecular weight cytokeratin in primary lung adenocarcinoma (**a**, **b**, **c**), in colon carcinoma metastases (**d**, **e**, **f**) and in breast carcinoma metastases (**g**, **h**, **i**)

reacted with antibodies to CK 4, 6, 14, and 17 and HMW CK. No reactions were seen for CK 10, 13 and 20 (Table 2). Metastatic gastric adenocarcinomas reacted with antibodies to CK 5–8 and 19. Only weak reactions were seen with antibodies to CK 13 and 14 and HMW CK, and no reactions for CK 4, 10, 17, 18 and 20 were seen.

## Primary lung adenocarcinomas versus metastatic adenocarcinomas of the lung

Statistically significant differences between primary and metastatic tumours were detected for CK 5, 7, and 18 and HMW CK (Table 2). Nearly all metastases were positive for CK 5, whereas 6 of 16 primary lung adenocarcinomas were negative. By contrast, almost all primary adenocarcinomas reacted with CK 7, whereas many metastatic tumours, especially those originating from the colon and prostate, showed only few reactions. The reaction rate for CK 18 and HMW CK was also considerably higher in primary lung carcinomas than in metastatic tumours.

By logistic regression analysis, we developed a discriminatory model based on a combination of CK 5 and 18 and HMW CK, which allowed the best discrimination between primary lung carcinoma and metastatic adenocarcinomas of the lung. Regression coefficients were 3.293 (CK 5), –2.581 (CK 18) and –2.306 (HMW CK), with a standard error of the regression coefficient of 0.945, 1.113 and 0.831, respectively ($P$=0.006, 0.003 and 0.005, respectively, which are statistically highly significant). Univariate tests for these CKs were also significant (Table 2). The coefficients for CK 18 and HMW CK were negative, indicating the higher expression of these CKs in primary lung carcinomas; by contrast, the positive coefficient for CK 5 indicated higher expression in metastatic tumours. The good agreement between uni- and multivariate statistics further proves that the model created is precise and not over-fitted.

## Discrimination of metastatic adenocarcinomas of the lung

Differences among metastatic tumours were statistically significant for CK 7, 13, 18 and 20. The significant

**Table 3** Cytokeratin (CK) polypeptide reactions in primary lung clear cell carcinomas and metastatic renal clear cell carcinomas

| | CK 4 | CK 5 | CK 6 | CK 7 | CK 8 | CK 10 | CK 13 | CK 14 | CK 17 | CK 18 | CK 19 | CK 20 | High molecular weight CK |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Large cell, clear cell lung carcinoma | 0/6 | 4/6 | 2/6 | 5/6 | 1/6 | 0/6 | 0/6 | 0/6 | 2/6 | 4/6 | 4/6 | 0/6 | 2/6 |
| Renal metastases | 6/18 | 12/18 | 5/18 | 1/18 | 11/18 | 0/18 | 3/18 | 3/18 | 2/18 | 5/18 | 12/18 | 3/18 | 0/18 |
| Fisher's exact test, *P* | 0.28 | 1.0 | 1.0 | 0.001 | 0.16 | – | 0.55 | 0.55 | 0.25 | 0.15 | 1.0 | 0.55 | 0.054 |

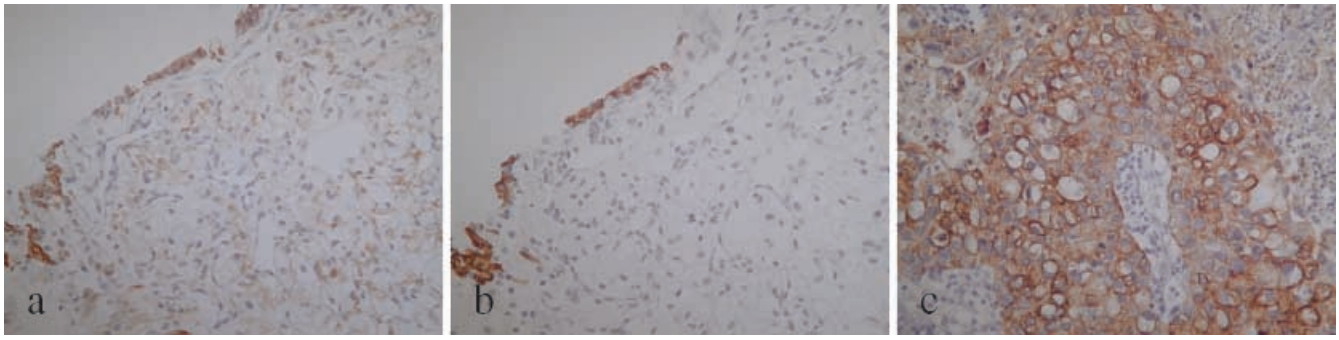



**Fig. 2** Immunohistochemical reactions of antibodies to pan-cytokeratin (CK) and CK 7 in a metastasis of a renal cell carcinoma (**a**, **b**), and reactions to CK 7 in a primary large cell and clear cell carcinoma of the lung (**c**)

result for CK 7 was due to the few reactions with antibodies to CK 7 in colonic and prostatic adenocarcinoma metastases, whereas almost all metastases from breast and stomach carcinomas, respectively, did react. CK 13 was often positive in colonic carcinoma metastases, whereas all other metastases were most often negative. A relatively high proportion of prostate carcinoma metastases reacted with CK 18 antibodies, whereas all metastases from gastric cancer did not. Finally, HMW CK was more often positive in breast cancer metastases than in metastases from other locations.

Using multinomial logistic regression, we identified the best combination of CK polypeptides to differentiate metastatic tumours with different locations of the primary tumour. Preliminary analysis showed that, apart from a minor difference in CK 7 expression, there were no systematic differences in CK polypeptides profiles between breast and prostate adenocarcinoma metastases ($P>0.05$). For CK 7, the Fisher's exact test was significant ($P=0.017$). However, it was still not possible to accurately discriminate between these tumours (prostate carcinoma: sensitivity 50%, specificity 94.5%, overall accuracy 81.5%), although this type of differential diagnosis is clinically irrelevant because breast cancer in men is extremely rare. Therefore, for the sake of subsequent analysis, these tumours were merged into one group.

The model selected for discrimination of metastatic carcinomas was based on a combination of CK 18 and CK 20. The significance values for the regression coefficients were less than 0.0005 for CK 20 and equal to 0.001 for CK 18.

## Metastatic clear cell carcinomas of the kidney versus large cell clear cell carcinoma of the lung

As can be seen in Table 3, the only highly significant result was noticed for CK 7. Only 1 of 18 metastatic renal clear cell carcinomas reacted positively with antibodies to CK 7, whereas 83% (5 of 6) primary large- and clear cell carcinomas of the lung were positive for CK 7 (Fig. 2). In addition, the test result for HMW CK was marginally significant. In multivariate analysis, however, HMW CK was not significant anymore ($P=0.11$), so only CK 7 was used to discriminate primary versus metastatic tumours. Efficiency indices relating to the primary lung clear and large cell carcinomas were as follows: sensitivity 83.3%; specificity 94.4%; positive predictive value 83.3%; and negative predictive value 94.4%. We also noticed a reaction with antibodies for CK 20 in 17% (3 of 18) of our renal cell carcinoma metastases (Table 3). In contrast to clear cell carcinomas of the kidney, pulmonary clear cell carcinomas never showed co-expression of vimentin and CK (unpublished observation, H. Popper).

## Squamous cell carcinomas: primary versus metastatic

Primary squamous cell carcinomas of the lung stained positively with antibodies for CK 7 (43%), 17 (86%), 18 (71%) and 20 (14%). In contrast, metastases from larynx carcinomas did not react with antibodies for CK 17, 18 and 20, but were positive in 50% for CK 7. Metastases from oesophageal carcinomas showed a similar positivity for CK 7, but less positivity for CK 17 and CK 18 than that from lung squamous cell carcinomas. No reactivity for CK 17 and CK 18 was found in squamous cell carcinomas of the oral cavity and the hypopharynx. Our statistical results are documented in Table 4; however, they must be interpreted with respect to the few cases available.

**Table 4** Cytokeratin (CK) polypeptide reactions in primary squamous cell carcinomas (SCCs) and SCC metastases

| Tumour | CK 4 | CK 5 | CK 6 | CK 7 | CK 8 | CK 10 | CK 13 | CK 14 | CK 17 | CK 18 | CK 19 | CK 20 | High molecular weight CK |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Primary lung SCC | 2/7 | 7/7 | 7/7 | 3/7 | 4/7 | 1/7 | 4/7 | 7/7 | 6/7 | 5/7 | 5/7 | 1/7 | 7/7 |
| Lung metastases from larynx SCC | 0/2 | 2/2 | 2/2 | 1/2 | 2/2 | 0/2 | 2/2 | 2/2 | 0/2 | 0/2 | 2/2 | 0/2 | 2/2 |
| Lung metastases from tongue SCC | 1/2 | 2/2 | 2/2 | 0/2 | 1/2 | 0/2 | 1/2 | 2/2 | 1/2 | 1/2 | 1/2 | 0/2 | 2/2 |
| Lung metastases from hypopharynx SCC | 0/1 | 1/1 | 1/1 | 0/1 | 1/1 | 0/1 | 1/1 | 1/1 | 0/1 | 1/1 | 1/1 | 0/1 | 1/1 |
| Lung metastases from oesophagus SCC | 2/6 | 6/6 | 5/6 | 4/6 | 3/6 | 0/6 | 1/6 | 5/6 | 3/6 | 2/6 | 4/6 | 0/6 | 4/6 |
| Overall Chi-square test, *P* | 0.78 | – | 0.71 | 0.46 | 0.67 | 0.80 | 0.21 | 0.71 | 0.17 | 0.29 | 0.78 | 0.80 | 0.34 |
| Fisher's exact test primary vs metastatic lung tumours, *P* | 1.0 | – | 1.0 | 1.0 | 1.0 | 0.39 | 1.0 | 1.0 | 0.07 | 0.34 | 1.0 | 0.39 | 0.49 |

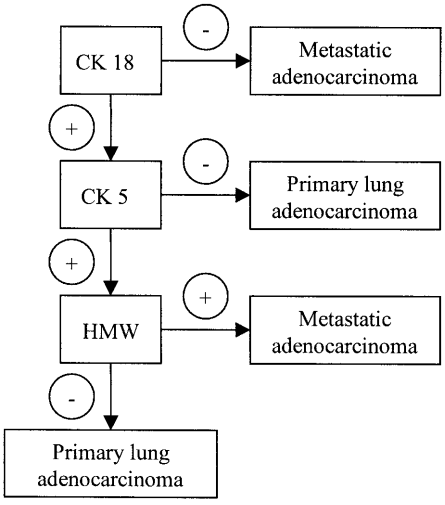
### Normal lung epithelia

Primarily we tried to attribute different CK subsets to different cell types. However, ciliated, goblet and secretory columnar cells in large bronchi all shared the same CK composition, as did Clara cells, ciliated and secretory columnar cells in peripheral bronchioli. The same was seen for basal and reserve cells in their respective location. There was a difference when central and peripheral epithelia were compared: peripheral bronchiolar epithelia were all negative for CK 4, 6 and 20, whereas epithelia of large bronchi were positive for these CKs in differing amounts. With CK 20 antibodies, we noticed a positive stain in 20% of centrally located columnar cells and in 80% of the bronchial gland cells. There was no reactivity for CK 10, centrally or peripherally; with CK 13, we noticed focal positivity in central bronchi, especially in the bronchial glands, and in the periphery only in pneumocyte types I and II. Within central bronchi, the differentiated columnar cells were positive for CK 7, whereas basal and reserve cells were negative. In the alveolar periphery, the most striking finding was CK 17 positivity in pneumocyte II, which was not found in bronchiolar epithelia.

## Discussion

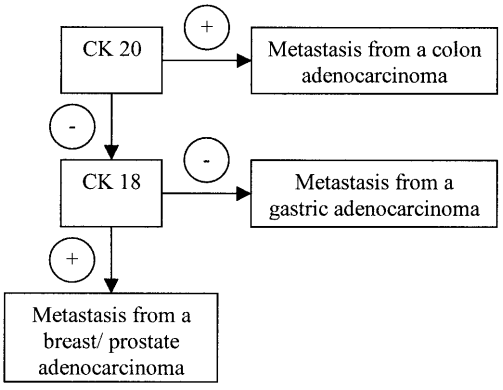
### Adenocarcinomas

According to the results of logistic regression analysis, three CK polypeptides turned out to be important with respect to a differential diagnosis: CK 5, CK 18 and HMW CK. Based on the analysis of the model coefficients, we worked out a discrimination set-up for primary and metastatic carcinomas (Scheme 1). Using this scheme, we noticed for primary lung carcinomas a sensitivity of 62.5%, a specificity of 92.5%, a positive predictive value of 71.4% and a negative predictive value of 89.1%. Previous studies reporting on CK 7 and CK 20 patterns [11, 13, 14, 15] stated that CK 20 positivity would exclude primary lung adenocarcinoma. However,




**Scheme 1** A discrimination set-up for primary and metastatic carcinomas

4 of 16 lung adenocarcinomas in our study showed positive reactivity for CK 20, which was also seen by Savera et al. [15] and other investigators [8, 11, 12, 14]. Our four cases were centrally located. This correlated well with our findings of positively stained columnar and bronchial gland cells in normal mucosa of large bronchi and might explain the CK 20 positivity. CK 7 positivity, discussed in various articles [6, 13, 14, 15, 17, 18], was not that helpful in our series. Most pulmonary adenocarcinomas and metastases from breast adenocarcinomas and all metastases from gastric adenocarcinomas reacted, but there was also a substantial amount of reacting lung metastases of colonic adenocarcinomas, consistent with the findings of many other authors [2, 4, 7, 20]. In metastatic adenocarcinomas of the prostate, we found CK 7 positivity, which is confirmed by two other studies [12, 18]. Almost all metastatic adenocarcinomas of the prostate did not stain for HMW CK, consistent with the findings of Yang et al. [19]. We noticed in the univariate statistic analysis a significancy with CK 7, but in the




**Scheme 2** Classification tree for metastases of different origin

multivariate analysis, it did not bring any further information to that gained by using CK 5 and 18 and HMW CK.

Considering the results of multinomial logistic regression, we developed a classification tree (Scheme 2) for metastases of different origin by using only CK 18 and 20. Using this scheme, the sensitivity for colonic carcinoma metastases was 76.2%, with 100% specificity; the positive predictive value was 100%; and the negative predictive value 86.5%. For breast and prostate carcinoma metastases, we noticed a sensitivity of 63%, a specificity of 88.5%, a positive predictive value of 85% and a negative predictive value of 69.7%. The sensitivity for gastric cancer metastases was 100%, the specificity 75%, the positive predictive value 29.4% (due to the few number of cases) and the negative predictive value 100%.

## Renal clear cell carcinomas

Negative CK 7 reactivity was most likely consistent with the diagnosis of metastatic clear cell carcinoma of the kidney, whereas primary large cell carcinomas of the lung (clear cell variant and large cell lung carcinomas with clear cell areas) predominantly showed CK 7 positivity (Table 3). Obviously there is some overlap: 1 of our 18 cases of metastatic renal cell carcinomas reacted positively for CK 7. In their cases, Ramaekers et al. [13] noticed no reaction of renal cell carcinomas with antibodies for CK 7, with a few exceptions. These findings were confirmed by Blobel et al. [1] and Moll et al. [10]. Recognising CK 7 of Moll's catalogue, Gown and Vogel [5] showed that renal adenocarcinomas had a positive reaction to the antibody 35βH11. Also Shah et al. [16] found one autopsy renal cell carcinoma positive for CK 7 in the primary as well as in the metastasis. Savera et al. [15] noticed positivity in 59% (10 of 17) of their primary renal clear cell carcinomas, but no reaction in the investigated metastases.

With antibodies to CK 20, we noticed positivity in 17% (3 of 18) of our renal cell carcinoma metastases, which was, however, not significantly higher than in primary lung carcinomas. Moll et al. [11] had only one case (1 of 25) of primary renal cell carcinoma (clear cell type) that focally positively stained for CK 20. Savera et al. [15] also noticed positivity for CK 20 in 12% (2 of 17) of primary renal clear cell carcinomas. No reaction was noticed in two investigated metastases. We think that a distinction between renal metastasis and lung primary should be possible in most cases by using CK 7 antibodies.

## Squamous cell carcinomas

We did not observe any statistically significant differences in the staining pattern of primary squamous cell carcinomas and the metastatic squamous cell carcinomas of the lung (Table 4). We also did not notice a significant difference among the metastases. This might also be due to the small number of cases investigated. The only CK that might be of some value for differentiating primary versus metastatic lung carcinoma is CK 17. In primary lung carcinomas, CK 17 expression seems to be higher than in most metastatic tumours (the Fisher's exact test for this finding is marginally significant). We found three of six oesophageal carcinoma metastases positively stained. Miettinen et al. [9] noticed no reaction in normal oesophagus epithelium for CK 17 but found significant reactivity in oesophageal carcinomas, as well as in laryngeal carcinomas. However, neither in laryngeal, oral cavity, nor hypopharynx carcinomas was the number of cases large enough to give a definite answer.

## Normal lung epithelia

Bronchial epithelia and bronchiolar epithelia did not share an identical distribution of their respective CK polypeptides. The different CK composition in bronchial and bronchiolar cells was unexpected but might reflect steps in the embryogenesis and function. Large bronchi are conducting airways, and their main function is stability in allowing undisturbed airflow. Bronchioli, especially respiratory bronchioli, are flexible structures, which must follow lung expansion and collapse during breathing; parts of them are also integrated into gas exchange. The differences of CK polypeptide composition might reflect these differences in function and stability. Another interesting fact is the unique CK 17 positivity of pneumocyte type II. It would not be surprising, therefore, if bronchioloalveolar adenocarcinomas of predominant Clara and pneumocyte types might also be positive for CK 17.

Cytokeratin typing, in addition to other markers, can aid in the differential diagnosis of metastatic versus primary non-small cell carcinomas of the lung. Finding the primary site of the tumour reduces the time period until the patient can be treated and the cost of diagnosis. Our results suggest antibodies to CK 5, 18 and 20 and HMW CK are fairly efficient for the discrimination between primary non-small cell lung carcinomas and metastatic adenocarcinomas of the lung of different ori-

gin. CK 7 appears to be very useful in distinguishing primary large- and clear cell lung carcinomas and metastases from renal cell carcinomas. We admit, however, that some of our results might be too data-driven and should therefore be tested in a larger series of tumours.

Besides CKs, other markers – thyroid transcription factor 1, different mucins, hormones, cell-specific proteins such as surfactant apoprotein and Clara cell proteins, and many others – can also be used in combination to detect the primary site of a carcinoma. Also, tissue- or carcinoma-specific genetic alteration may be important. However, this is beyond the scope of this investigation.

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