MONOCLONAL ANTIBODIES IN LUNG CANCER PATHOLOGY

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ABSTRACT
Monoclonal antibody based immunohistochemistry is a very powerful tool for the establishment of a pathological diagnosis of lung cancer. Applying a panel of intermediate filament antisera and an antibody recognizing neuroendocrine differentiation we have tested about 240 human lung tumors and 15 human lung tumor cell lines. Our results can be summarized as follows:
1. a differential diagnosis between neuroendocrine and non-neuroendocrine lung tumors can be obtained by the application of the monoclonal antibody MOC-1 directed against neuroendocrine antigens.
2. Immunohistochemistry can lead to a better recognition of lung tumor heterogeneity within the established histologies. Examples of this phenomenon are:
   a. the presence of neuroendocrine and/or neural components within non-neuroendocrine tumors.
   b. the presence of squamous cell or adenocarcinomatous differentiation in non-SCLC can be detected by chain specific anti-cytokeratin antibodies.
   c. the degree of differentiation towards the variant type within SCLC can be detected by the monoclonal antibody directed against neurofilaments.
3. lung cancer cell lines can serve as an in vitro model for immunohistochemical studies on different lung cancer subtypes.

INTRODUCTION
Lung cancer is a major problem in oncology, since it is responsible for the highest amount of cancer deaths in western societies and shows a still rising incidence, especially among women and in developing countries (1,2). The best opportunity for curative treatment is in the first stage of disease, when cancer is limited to the lung and surgical intervention is treatment of choice. The number of patients presenting with really limited disease is small, however, and epidemiological studies have given some indication that the overall survival will not ameliorate by screening programs set up to find such early cases (3).
Lung cancer can be histopathologically typed according to a revised version of the WHO classification published in 1982 (4). In this scheme, five major lung cancer types, comprising 95% of all lung tumors, are recognized on light-microscopically evaluable, histological criteria: squamous cell carcinoma, small cell carcinoma (SCLC), adenocarcinoma, large cell carcinoma and adenosquamous cell carcinoma. Within these types, further subtyping can be performed. In 1985, the pathology panel of the IASLC proposed to use for subtyping of SCLC the classification SCLC (classic type), SCLC (mixed small/large cell) and SCLC (combined). The last subtype comprises cases showing substantial squamous or adenocarcinoma components or both (5).
The recently introduced plastic embedding technique has, as compared to the results obtained with conventional paraffin embedding techniques, resulted in a further refinement of morphological evaluation possibilities (6). As a
result, it has become apparent that more lung cancer cases show combined histologies, and that the percentage of so-called "undifferentiated" large cell carcinoma is much smaller than originally thought. Implications of these findings are indicated below.

A clinically relevant histopathological subdivision in lung cancer is that between SCLC and non-SCLC (7), since SCLC generally shows earlier and more extensive metastatic spread, more rapid tumor cell proliferation and greater sensitivity to chemo- and radiotherapy. However, despite the fact that SCLC is initially highly sensitive to therapy, untreatable recurrences often occur within a year and, also for this type of lung cancer, overall five year survival is disappointingly low.

A histopathological classification of lung cancer can provide information on the nature and possible clinical behaviour of a tumor case. Nevertheless, a purely histologically based subdivision appears not completely satisfying. As indicated above, the application of new histological techniques has shown that a considerable number of cases shows admixtures of areas with different histology. These data have confirmed earlier electronmicroscopical studies indicating that, ultrastructurally, combinations of varying degrees of squamous-, adeno- and neuroendocrine differentiation are often present within one biopsy. This heterogeneity in tumor cell composition is also reflected in tissue culture studies in which profound heterogeneity can be observed within one cell line (8). In addition to a heterogeneous histological composition, changes in histological appearance have also been reported to occur in time, both in vivo (9) and in vitro (10). These observations have led to the idea that lung cancer comprises in fact a continuum of cancer types in which the different histologically recognizable groups represent preferentially expressed, non-fixed differentiation pathways (11). The implications of such an idea for treatment and diagnostic modalities are still unclear. However, the apparent present lack of further advance in therapeutical options demands for intensive fundamental and clinical research to seek new treatment avenues. In addition, new methods in the diagnostic approach have to be developed in order to select lung cancer cases with a good prognosis for treatment.

It has been shown that lung cancer can be subtyped not only by histological criteria, but also by immunohistological procedures using poly- or, more recently, monoclonal antibodies. Since monoclonal antibodies define lung cancer on basis of the occurrence of specific antigenic features, that need not to parallel histologically based subdivisions, it can be anticipated that new classifications might eventually emerge from the application of monoclonal antibody based immunohistopathology. The clinical relevance of such a new classification should be established, however, only after careful clinical studies and comparison with the established morphological criteria of lung cancer typing. In the following, the occurrence of specific (monoclonal) antibody defined antigens in lung cancer will be discussed. The occurrence of specific types of intermediate filament proteins will be emphasized.

Antisera to intermediate filament proteins (IFP) have proven to be powerful tools in the detection of cell lineages of both normal and neoplastic tissues. Intermediate filaments are cytoskeletal structures with diameters of about 10 nanometers, and occur in nearly all vertebrate cells. Their protein constituents have been shown to vary in a tissue-type associated way. This tissue-type associated expression of IFP is retained in most cases after malignant conversion. For instance, both normal epithelial cells and their malignant counterparts contain IFP of the cytokeratin type, whereas neural cells and some neuronal tumors contain neurofilaments. Since the derivation of certain types of lung cancer is still unclear, some investigators (12-14) have attempted to determine their origin using antisera to IFP. These investigations have led to contradictory results in the literature. For instance either
cytokeratins or neurofilaments have been shown to be present in small cell lung cancer (SCLC), a lung tumor with neuroendocrine properties, and suggested to be the malignant counterpart of normal neuroendocrine (NE) cells of the lung (15). When tested with antibodies to cytokeratins and neurofilament proteins it turned out, however, that these NE cells express cytokeratin(s) and not neurofilament proteins. This finding indicates that these cells, like many other neuroendocrine cells (such as for example Merkel cells (16)) are of epithelial origin and nature.

For the detection of certain types of IFP special attention should be given to the specificity of the antibodies used, since for instance the family of cytokeratin IFP consists of about 20 different polypeptides, which show a non-random, tissue-specific distribution throughout epithelia. Lack of cross-reactivity of cytokeratin antibodies with one or more of these cytokeratin polypeptides may result in false-negative results in certain subtypes of epithelia or epithelial tumors, such as lung tumors. Also fixation artefacts can lead to false-negative results, as has become evident from the literature. Therefore we have employed well-characterized monoclonal and polyclonal antibodies for the detection of IFP in lung tumors, whereas frozen tissue sections were used as a substrate.

MATERIALS AND METHODS
Reactivity of poly- and monoclonal antibodies on frozen tissue sections of snap-frozen tumor biopsies and surgically resected tumor specimens was assessed according to described procedures using either an indirect immunofluorescence or immunoperoxidase method (17,18). The following antibodies have been used: pker (a polyclonal rabbit antiserum to human skin keratins), RGE 53 (monoclonal antibody directed against cytokeratin 18 (19)), RKSE 60 (monoclonal antibody directed against cytokeratin 10 (20)), MNF (directed against the 210 and 68 kDa components of neurofilaments (21)) and MOC-1 (directed against a neuroendocrine related antigen (22)). All sera are available from Eurodiagnostics, Apeldoorn, The Netherlands.

RESULTS AND DISCUSSION
All lung carcinomas can be stained by generally cross-reacting cytokeratin antibodies, such as the polyclonal rabbit antiserum pker. Chain-specific monoclonal antibodies reacting with only one cytokeratin polypeptide can recognize certain directions of epithelial differentiation, i.e. adenocarcinomatous differentiation as detected by a monoclonal antibody to cytokeratin 18 (RGE 53), or (keratinizing) squamous cell differentiation as detected by a monoclonal antibody to cytokeratin 10 (RKSE 60). In the normal lung RKSE 60 is unreactive with epithelial cells, whereas RGE 53 reacts with bronchial columnar epithelial cells and type 2 pneumocytes, but not with basal cells of the bronchus.

In epidermoid lung cancers, especially poorly differentiated squamous cell carcinomas, a profound heterogeneity was observed with these chain-specific antibody preparations. With respect to the presence of cytokeratin 18, staining with RGE 53 revealed that this could range from a nearly complete absence in well-differentiated to an abundant presence in poorly differentiated squamous cell carcinomas. With respect to the presence of cytokeratin 10, it appeared that staining with RKSE 60 occurred only in keratinizing areas of well-differentiated squamous cell carcinomas. Interestingly, some (about 7%, see table 1) of the poorly differentiated squamous cell carcinomas proved to contain neuroendocrine components and even cells with neural characteristics, as indicated by a reactivity with antibodies to neuroendocrine (MOC-1), and neurofilament proteins.
Table 1. Reactivity of specific antibodies with major "non-endocrine" lung tumors

<table>
<thead>
<tr>
<th>Histological diagnosis</th>
<th>squamous cell carcinoma</th>
<th>adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>completely positive</td>
<td>focally positive</td>
</tr>
<tr>
<td>polyclonal anti-</td>
<td>83/85</td>
<td>0/85</td>
</tr>
<tr>
<td>cytokeratin</td>
<td>RGE 53</td>
<td>95/151</td>
</tr>
<tr>
<td></td>
<td>RKSE 60</td>
<td>3/151*</td>
</tr>
<tr>
<td>VIM</td>
<td>0/77</td>
<td>9/77</td>
</tr>
<tr>
<td>MNF</td>
<td>0/112</td>
<td>8/112**</td>
</tr>
<tr>
<td>MOC-1</td>
<td>4/144**</td>
<td>22/144**</td>
</tr>
</tbody>
</table>

*only keratinizing areas show a positive reaction
**reactivity only in poorly differentiated tumors

Adenocarcinomas of the lung were always positive with antibodies broadly reacting with cytokeratins as well as with the monoclonal antibody to cytokeratin 18 (RGE 53). Our results furthermore show that all SCLCs and lung carcinoids also express cytokeratins (Table 2). These results are in accord with several reports in the literature (14,23,24). Carcinoids exhibit a characteristic staining pattern, with dot-like concentrations of cytokeratin-positive reactions close to the nucleus. We have found four SCLCs, and four carcinoids, which expressed neurofilaments next to cytokeratins. Although both cytokeratins and neurofilaments could be demonstrated in the same SCLC case, double-immunofluorescence studies showed no overlap in expression of cytokeratins and neurofilaments. Therefore, we cannot sustain results described by Lehto et al. (12), who found extensive staining with an anti-neurofilament antibody in all SCLC tumors examined, but no reactivity with an anti-cytokeratin antibody. In double-immunofluorescence studies of the lung carcinoids reacting with anti-neurofilament antibodies a co-expression of cytokeratins and neurofilaments in all tumor cells could be clearly observed. These last results are in accord with those of Lehto et al. (25).

Table 2. Reactivity of specific antibodies with major endocrine lung tumors

<table>
<thead>
<tr>
<th>Histological diagnosis</th>
<th>SCLC</th>
<th>Carcinoid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>completely positive</td>
<td>focally positive</td>
</tr>
<tr>
<td>polyclonal anti-</td>
<td>19/22</td>
<td>0/22</td>
</tr>
<tr>
<td>cytokeratin</td>
<td>RGE 53</td>
<td>24/28</td>
</tr>
<tr>
<td></td>
<td>RKSE 60</td>
<td>1/22</td>
</tr>
<tr>
<td>VIM</td>
<td>0/21</td>
<td>2/21</td>
</tr>
<tr>
<td>MNF</td>
<td>0/20</td>
<td>4/20</td>
</tr>
<tr>
<td>MOC-1</td>
<td>79/81</td>
<td>0/81</td>
</tr>
</tbody>
</table>
The observations described above could be of clinical relevance. In a recent electronmicroscopic study (26) it has been indicated that histologically undifferentiated large cell tumors, showing neuroendocrine differentiation features, have a clinical behavior similar to SCLC. Although a similar clinical study with tumors having immunohistochemically detectable neuroendocrine features has to be done yet, the same could apply to this group. If so, we should like to suggest, in parallel with treatment schedules already applied to combined SCLC, that poorly differentiated squamous cell carcinomas containing either MOC-1 or neurofilament-positive cells should be treated as combined SCLC, using irradiation and combination chemotherapy after surgery.

CELL CULTURES OF LUNG TUMORS

The high heterogeneity of lung tumors is reflected in established cell lines derived from patients with these cancers. Cell lines with characteristics of each subtype (squamous cell carcinomas, adenocarcinomas, large cell and small cell lung carcinoma) have been obtained and characterized. Investigations on these cell lines have led to the conclusion that they represent indeed certain (sub-)types of lung tumor and can be used therefore as model systems to study the cell biology of the different pathways of lung cancer differentiation. For instance "classic" SCLC cell lines have retained biological and biochemical features typical for the classic subtype of in vivo SCLC, while the "variant" SCLC cell lines represent the large cell component of the mixed small cell/large cell subtype of SCLC (27,28).

Applying antisera to cell differentiation and cell-lineage markers has shown that, also in vitro, such probes can discriminate between SCLC and non-SCLC, and even between "classic" and "variant" SCLC. Thus, using antibody preparations against IFP, all classic SCLC cell lines tested were shown to contain cytokeratins (7/7), while the variant cell lines did not contain these cytokeratin IFP (0/6). Some of the latter cell lines, however, contained neurofilaments (3/6), whereas the others (3/6) did not contain cytokeratins nor neurofilaments. These data were obtained after immunocytochemical studies using a large panel of polyclonal and monoclonal antibodies directed against cytokeratins and neurofilaments, and were confirmed by gel-electrophoretic and immunoblotting assays (29).

Cell lines derived from squamous cell carcinomas and adenocarcinomas contained cytokeratin (8,17,29) and did not react with anti-neurofilament antibodies. One cell line (GLC-2) showed a heterogeneous staining pattern when incubated with several monoclonal anti-cytokeratin antibodies (8), indicating that this cell line, which was derived from a patient with SCLC, had adapted both an adenocarcinomatous (RGE 53 positive, presence of microvilli at an electronmicroscopic level), as well as a squamous (RKSE 60 positive) differentiation pathway. The neuroendocrine antigen defined by MOC-1 could be demonstrated in both variant-and classic-type SCLC cell lines.

In conclusion, our results concerning the cell lineage (IFP) as well as cell differentiation protein phenotype (MOC-1) of SCLC derived cell lines suggest different lineages of (de-)differentiation within SCLC, with classic cells having more epithelial characteristics and variant cells having lost these epithelial characteristics but having acquired some neuronal characteristics. Since a distinction of the variant type of SCLC from the classic type may be of clinical importance (30), it is of special interest to obtain a better understanding of the carcinogenesis of these two different types of SCLC. At this moment it is not clear whether "in situ" the variant cells differentiate spontaneously from a classic cell type or from a common neuroendocrine progenitor cell.

Expression of cytokeratins and neurofilament proteins within one tumor seems to be restricted to a limited number of neuroendocrine tumors. Neurofilaments
have been found next to cytokeratins in neuroendocrine skin carcinomas (Merkel cell tumors; 31) and in some pancreatic islet tumors (32). The possible cell of origin for these tumors (Merkel cells in skin and pancreatic islet cell, respectively) express only cytokeratins and do not contain neurofilaments. Therefore it was of interest to study the intermediate filament expression in normal neuroendocrine cells of the lung. The finding that these cells contain only cytokeratins and not neurofilaments supports the hypothesis that pulmonary neuroendocrine cells are of epithelial origin. However, apparently, neuroendocrine cells do have the potential to express neurofilaments as a result of malignant transformation and growth as shown both by in vitro and in vivo findings with SCLC. A possible clinical relevance of this has to be established yet.

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REFERENCES

