Correspondence

Small Cell Lung Cancers Contain Intermediate Filaments of the Cytokeratin Type

To the Editor: In view of the well-established neuroendocrine nature of the pulmonary carcinoids and small cell carcinomas (4) it was of interest to note recent reports describing the presence of neurofilaments in the cytoskeleton of these cells (2, 6). Another paper, however, has appeared challenging these observations and claiming that the pulmonary neuroendocrine tumors contain cytokeratin rather than neurofilament cytoskeletal proteins (3). Stimulated by these apparently contradictory findings we have examined eight small cell carcinomas of the lung and four carcinoids. Our data obtained by using a polyclonal antiserum raised against skin keratins and two monoclonal antibodies reacting with (cyto)keratins, as well as three monoclonal antibodies to the different neurofilament proteins, indicate that these tumors indeed contain cytokeratin proteins. No staining was seen with the antibodies to neurofilaments.

In brief, cryostat sections of freshly resected tumors were examined by the indirect immunoperoxidase technique. The relevant clinical and pathologic data of the small cell lung tumors and carcinoids used in this study are summarized in Table 1. Samples of human brain, peripheral nerves, and neural tumors were also used to test the specificity of the neurofilament antibodies. The following antibodies were used (1, 5, 7, 8): (a) an affinity-purified antiserum directed against human skin keratins (pK), which reacts with virtually all epithelial tissues but not with nonepithelial tissues (8); (b) the monoclonal antibody RGE 53, directed against cytokeratin 18, which specifically recognizes columnar epithelial cells from digestive, respiratory, and urogenital tracts, endocrine and exocrine tissues, and mesothelial cells (7); (c) the monoclonal antibody RKSE 60; directed against human skin keratins and specific for keratinizing squamous cells (8); (d) the monoclonal antibodies BF10 and RT97 directed against different neurofilament polypeptides. In immunoblotting assays BF10 was shown to react only with the 155-kilodalton (kd) neurofilament protein, whereas RT97 reacts mainly with the 210-kd neurofilament protein (1); (e) a monoclonal antibody to neurofilaments (MNF) purchased from Euro-Diagnostics B.V., Apeldoorn, The Netherlands. This antibody corresponds to the monoclonal antibody 2F11 described by Klück et al. (5). In immunoblotting assays we could show that this antibody reacts strongly with the 210-kd neurofilament polypeptide and to a somewhat lesser extent with the 70-kd neurofilament polypeptide.

Using frozen sections and paraffin sections from human brain, peripheral nerves, and some neural tumors, we found a positive reaction of the monoclonal antibodies to neurofilament proteins (BF10, RT 97, and MNF) in only the neural tissue.

In the normal lung the affinity-purified polyclonal antiserum to skin keratins shows a positive reaction with bronchial, broncholar, and alveolar epithelium. A relatively strong reaction is observed in basal cells of the bronchial epithelium. No reaction was seen in nonepithelial tissues. This antibody did strongly stain squamous cell carcinomas and adenocarcinomas of the lung. The antibody to cytokeratin 18 (RGE 53) strongly stained all epithelial components except the basal cells of the respiratory epithelium. Nearly all small cell carcinomas as well as the carcinoids were positively stained with the polyclonal keratin antiserum (Fig. 1a). The monoclonal antibody to cytokeratin 18 (RGE 53) gave a strong diffuse and homogeneous staining pattern in all of the examined small cell lung carcinomas and in the carcinoids (Fig. 1b). The monoclonal antibody specific for keratinizing epithelium (RKSE 60) did not react with the small cell lung carcinomas or carcinoids. None of the neurofilament monoclonal antibodies gave a positive reaction in the small cell lung carcinomas or in the carcinoids (Fig. 1c). They did, however, strongly stain peripheral nerves present in the lung tumor sections (Fig. 1d). Also, some isolated scattered cells in normal and tumor tissue were found to be positive with all three neurofilament antibodies.

In summary, our findings contradict some published papers (2, 6) but are in agreement with data reported by Blobel et al. (3). It is not clear whether these differences are due to technical problems related to the specificity of antibodies and fixation procedures or whether we indeed have used or selected neuroendocrine tumors that express cytokeratins and not neurofilament proteins.

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Table 1. Clinicopathologic and Immunohistochemical Data of Small Cell Lung Carcinomas and Carcinoids of the Lung

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Age</th>
<th>Diagnosis</th>
<th>WHO 1982 Subtype classification</th>
<th>Clinical stage</th>
<th>Pathological stage</th>
<th>Reactivity pattern with intermediate filament antibodies</th>
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<td>70</td>
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<td>T2N0M0</td>
<td>T2N0M0</td>
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<td>F</td>
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<td>+ ++ - - - -</td>
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</table>

*Abbreviations: SCLC, small cell lung carcinoma; WHO, World Health Organisation; pK, polyclonal rabbit antiserum to human skin keratin; T1N0M0, <3 cm and no invasion; T2N0M0, >3 cm and extension to hilar region; T3N0M0, gross extension, effusion, and atelectasis.

FIG. 1. Photomicrographs of cryostat sections of small cell lung carcinomas stained by the immunoperoxidase technique using the polyclonal keratin antiserum (a), the monoclonal antibody to cytokeratin 18 (b), and monoclonal antibody to neurofilaments (BF10; c and d). Note positive reaction of BF10 with peripheral nerves but not with the tumor. a and b, x200; c, x100; d, x150.

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REFERENCES
plasms. Lab Invest 52:39, 1985