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Detection of Cytoskeletal Proteins in Small Cell Lung Carcinoma

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Abstract. Small cell lung carcinoma (SCLC) is the most aggressive of lung tumors, metastasize widely and are virtually incurable by surgical means Therefore, the classification of lung cancer into SCLC and non-small cell lung carcinoma is essential for disease prognosis and treatment

For this purpose we have compared the immunohistochemical distribution of different cytoskeletal proteins as tumor markers Analysis was performed by using of monoclonal antibodies directed against cytokeratins, neurofilaments, βIII-tubulin, epithelial membrane antigen and neuron-specific enolase Our results indicate that keratin and epithelial membrane antigen are reliable epithelial markers for SCLC In addition, the positive staining with monoclonal antibodies TU-20 against βIII-tubulin and neuron-specific enolase was found in some cases of SCLC We suggest, that these antibodies could be a useful tool for complex immunohistochemical diagnosis of SCLC

Introduction

Small cell lung carcinoma (SCLC) is a clinicopathologically distinct form of pulmonary carcinoma characterized by specific morphological, ultrastructural, biochemical and cytogenic markers Ultrastructurally, a few dense-core neurosecretory-type granules were found in about 80% of the SCLC cells The granules are similar to those found in the APUD (Kultchitsky's) cells of the lung, which are originally supposed to be of ectodermal neural crest origin (de Leij *et al* 1985) According to the other hypothesis, SCLC originates in primitive cells of the basal bronchial epithelium, which in the process of neoplastic change undergoes partial differentiation towards neuroendocrine cells Immunohistochemically, the positivity of SCLC for keratin was described, often simultaneously with the neural markers such as neurofilaments, Leu-7, chromogranin, synaptophysin and neuron-specific enolase (Guinee *et al* 1994) In this study, we have used a monoclonal

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antibody against neuron-specific isotype of β-tubulin to compare its distribution in SCLC with other neuronal and epithelial markers

Materials and Methods

Human tissues were obtained from the Pathology Department of the Medical Faculty of Masaryk University, Brno Samples (biopsies and autopsies) were fixed in buffered formaldehyde for 24-48 h and routinely embedded in paraffin for histopatological classification For immunohistochemical staining paraffin sections were cut, deparaffinised and the endogenous peroxidase was blocked In some cases enzymatic pretreatment of sections was needed Samples were incubated for 1 h with monoclonal antibodies, washed and then incubated with biotinylated secondary antibody and streptavidine-peroxidase complex 3,3'- Diaminobenzidine was used as a chromogen and Harris's haematoxylin was used for counterstaining the nuclei

Monoclonal antibody TU-20 (Dráberová *et al* 1998) directed against neuron-specific isotype of β-tubulin and monoclonal antibody NF-01 (Lukáš *et al* 1993) directed against high molecular weight neurofilament protein were described previously Monoclonal antibodies against neuron-specific enolase (NSE), cytokeratins and epithelial membrane antigen (EMA) were from DAKO (Denmark)
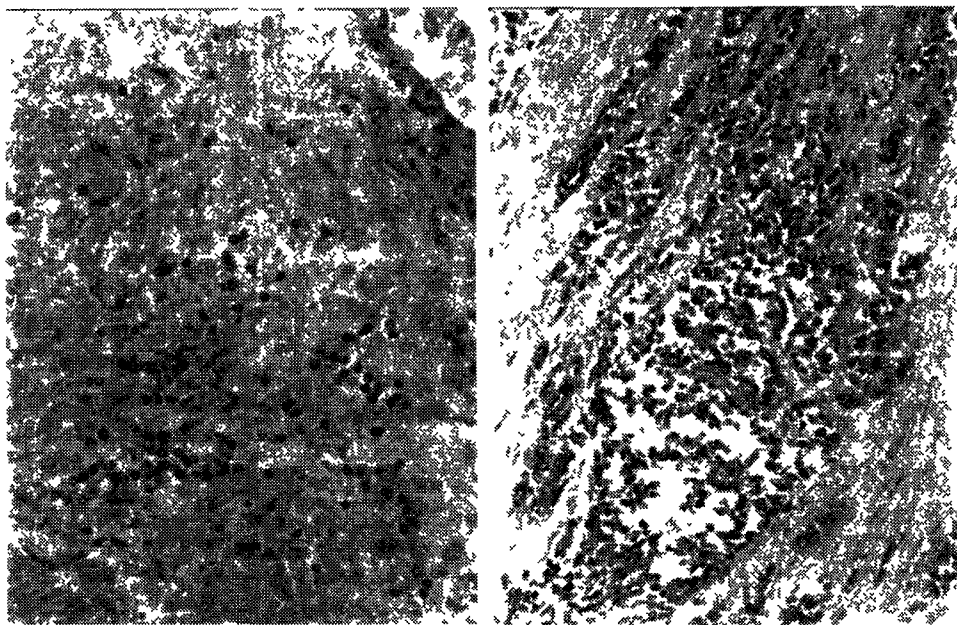
Figure 1. Immunostaining of small cell lung carcinoma with monoclonal antibodies NF-01 against neurofilaments (left) and TU-20 against class III β-tubulin (right)

Results and Discussion

All cases studied showed the positive staining with antibodies to keratins and EMA The most intense reactivity of epithelial markers was found with the antibodies against the keratins (clones AE1/AE3) This cytokeratin cocktail is directed against epitopes of high- and low-molecular weight keratins 1-8, 10, 13, 14, 15, 19 Staining for EMA was also positive, though slightly less than that for keratin Epithelial membrane antigen, however, is not a specific epithelial marker, some lymphoid cells express this antigen (Delsol *et al* 1984) Positive reaction with the antibodies to neuronal markers was found in 60–80% of SCLC cases The least intensive staining was found with the antibody NF-01 against 200 kDa subunit of neurofilament proteins (Fig 1) Other papers have reported varied results in detection of neurofilaments in SCLC, from 0–69% of cases (Shy *et al* 1990, Shah and Schlageter 1990) This variation could be due to the use of different antibodies to the various subunits of NFP The antibodies against βIII-tubulin and NSE revealed a similar diffuse staining of the cells NSE is an enolase isoenzyme that appears to be the best neuroendocrine marker for SCLC (Cooper 1994) On the other hand, the reported rates of detection range for NSE varies from 60–100% and some data indicate that NSE could be detected in as many as 60% of non-small cell lung carcinoma (Guinee *et al* 1994) Monoclonal antibody TU-20 against βIII-tubulin reacts specifically with normal and neoplastic cells of neuronal origin as described previously (Dráberová *et al* 1998) The positive reaction with SCLC (Fig 1) suggests that this antibody could be used also in the panel of antibodies – neuroendocrine markers of SCLC

In conclusions, the antibodies TU-20 and NF-01 against neuron-specific β-tubulin and neurofilaments could be a useful tool for complex immunohistochemical diagnosis of SCLC as well as for further studies of lung carcinoma histogenesis

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