Chapter 6

EARLY DISSEMINATED TUMOUR CELLS IN OPERABLE NON-SMALL CELL LUNG CANCER

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Abstract

Metastasis to lymph nodes or distant organs is a well-known feature of poor prognosis in potentially resectable non-small cell lung cancer (NSCLC). However, a significant number of lymph node negative patients die early of metastatic disease. Therefore, it has to be assumed that in some patients an early tumour cell dissemination has occurred which is clearly underestimated by current staging procedures. Recently, it has been shown, that an early dissemination of individual carcinoma cells to regional lymph nodes or bone marrow can be detected by using sensitive immunocytochemical techniques with monoclonal antibodies against epithelium-specific proteins. The incidence of immunohistochemically positive patients varies between 30% and 70% depending on the type of primary tumour, the immunohistochemical staining procedure used and especially on the primary monoclonal antibody. The detection of disseminated tumour cells in lymph nodes or bone marrow by immunocytochemistry is associated with a poorer prognosis in lung cancer. In conclusion, the immunohistochemical detection of early disseminated tumour cells in lymph nodes or bone marrow can help to obtain a more exact identification of patients with an unfavorable prognosis. Whether the identified patients will gain from an adjuvant therapy needs to be evaluated in further studies.

INTRODUCTION

The dissemination of malignant cells to distant organs via lymph nodes or blood vessels in solid tumours can occur at an early stage of primary tumour growth and is regularly underestimated by currently available clinical and pathological staging procedures (1). For example, approximately 40% of patients who undergo surgical resection of non-small cell lung cancer (NSCLC) without overt metastases (pT_{1-2}, N_0, M_0, R_0) relapse within 24 months after surgery (2). This is also reflected in a poor 5-year survival rate of about 60% and suggests that an occult tumour load is the major reason for the high mortality in surgically treated lung cancer patients (3).

Indeed, several groups, including ours, have shown that the early dissemination of individual lung carcinoma cells to regional lymph nodes (4–6) and

K. Pantel (ed.), Micrometastasis, 117–125.

distant organs like the bone marrow (7–9) can be detected by immunocytochemical techniques using monoclonal antibodies against epithelium-specific proteins. In bone marrow the occurrence of cytokeratin-positive cells has recently demonstrated to be indicative for a later clinical relapse (7–9) and the malignant nature of these cells has further been supported by their tumour-associated genetic characteristics and their metastatic capacity after transplantation in immunodeficient mice (10).

DETECTION OF TUMOUR CELLS IN LYMPH NODES

Methodological Aspects

Minimal tumour cell dissemination to regional lymph nodes has been previously assessed by serial sectioning of lymph nodes hematoxylin-eosin (HE) staining and routine histopathologic examination of an extensive number of consecutive sections (11). Using this approach the number of positive lymph nodes can be increased in about 8% to 30% of the specimens (12). However, the method is time-consuming and thus not practicable as a routine procedure for tumour staging. Thus, sensitive immunocytochemical assays with antibodies to epithelial antigens might be more reasonable alternatives.

Monoclonal antibodies to epithelial cytokeratins have been successfully used to identify individual metastatic cells in bone marrow of patients with various epithelial tumours (13). However, since reticulum cells express cytokeratins (14, 15), antibodies directed against these proteins are not the best choice for the identification of individual carcinoma cells in lymph nodes, because somewhat subjective morphological criteria must be imposed.

To develop an observer-independent assay solely based on the assessment of immunoreactivity we used mAb Ber-EP4 for the detection of micrometastatic tumour cells. Ber-EP4 (IgG1; Dako, Hamburg, Germany) is directed against two glycopolypeptides of 34 and 49 kD present on the surface and in the cytoplasm of all epithelial cells except the superficial layers of squamous epithelia, hepatocytes, and parietal cells (16, 17). The antibody does not react with mesenchymal tissue, including lymphoid tissue (16), and can also be used on paraffin sections.

The high sensitivity of mAb Ber-Ep4 for detection of NSCLC cells was supported by positive staining of 81 out of 82 (99%) primary tumours (45 adenocarcinomas and 37 squamous cell carcinomas). The majority of these samples (73/81) displayed a homogeneous staining. The consistent staining of 15 lymph nodes with overt metastases (Stage N_1) further indicated that the corresponding antigens remain preserved during the process of metastases (6).

In order to compare the effectiveness of the immunohistochemical analyses directly with the conventional HE-method two additional sections consecutive to those displaying Ber-Ep4 positive cells were studied. One section was stained by routine HE staining, the other was immunostained with Ber-Ep4. Both