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Lectin histochemistry of metastatic adenocarcinomas of the lung☆

Ina Thöm[a,*], Olaf Schult-Kronefeld[a], Iris Burkholder[b], Michael Goern[a], Birte Andritzky[a], Katharina Blonski[c], Christian Kugler[d], Lutz Edler[b], Carsten Bokemeyer[a], Udo Schumacher[c], Eckart Laack[a]

[a] *Department of Hematology, Oncology and Pneumology, University Hospital Hamburg-Eppendorf, Germany*
[b] *Department of Biostatistics, German Cancer Research Center, Heidelberg, Germany*
[c] *Institute of Anatomy II: Experimental Morphology, Universitiy Hospital Hamburg-Eppendorf, Germany*
[d] *Department of Thoracic Surgery, General Hospital Hamburg-Harburg, Germany*

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Summary
Background: Several clinical studies indicate that primary tumour cells with high metastatic potential often show aberrant glycosylation as detected by lectin histochemistry.

However, it is unclear whether aberrant glycosylation is still present in metastatic deposits. The aim of the present investigation was thus to analyse a possible association between the presence of lectin binding sites of pulmonary adenocarcinoma cells and their lymph node and haematogenous metastatic cells.
Methods: For this purpose, the expression of HPA, PHA-L and UEA-I was assessed in primary tumours, lymph node metastases and haematogenous metastases of 96 patients with metastatic adenocarcinomas of the lung that underwent surgery between 1999 and 2002. Besides, lectin-binding data and other known prognostic factors were correlated with survival.
Results: We found a significant positive correlation between the binding of the lectins HPA ($p = 0.002$), PHA-L ($p < 0.00001$) and UEA-I ($p < 0.00001$) to the cells of the primary tumour and to their lymph node metastases. There was a positive correlation between the binding of HPA to the cells of the primary tumour and the haematogenous metastases as well. Patients with tumours which did not show HPA binding sites had a median overall survival of 27.9 months (95%-CI 7.7—∞ months). Patients with a HPA binding tumour had a median overall survival of 20.9 months (95%-CI 18.5—28.7 months).
Conclusion: This is the first investigation to demonstrate a positive correlation between the binding of the lectins HPA, PHA-L and UEA-I to the cells of the primary tumour and to their

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* Corresponding author at: Department of Hematology, Oncology and Pneumology, University Hospital Hamburg-Eppendorf, Martinistraße 52, 20246 Hamburg, Germany. Tel.: +49 40 51493085.
E-mail address: i.thoem@uke.uni-hamburg.de (I. Thöm).

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lymph node metastases. Expression of HPA binding sites is also preserved in the haematogenous metastases. In summary, our results support the hypothesis that altered glycosylation of the membrane-bound glycoproteins of the tumour cells is associated with, but not sufficient for promotion of lymphogenic and haematogenous metastasis.

1. Introduction

Among all malignant diseases, lung cancer is the most frequent cause of death both in Europe and in the United States with smoking as the predominant risk factor. For a male population this has been a fact for decades, but due to increasing numbers of women smoking, females have been catching up in the last years [1]. Histologically, lung cancer contains a heterogeneous group of tumours with about three quarters being non-small cell lung cancers (NSCLC) and one quarter small cell lung cancer (SCLC). Based on histomorphology, the former group can be subdivided into adenocarcinomas, squamous-cell carcinomas and large cell carcinomas. Recent epidemiological studies have shown an increasing incidence of adenocarcinoma in women and the younger population, whereas the incidence of SCLC is slightly decreasing [2].

At the time of diagnosis, about 40% of the patients show locally advanced disease with lymph node involvement. Though surgical resection is the therapy of choice in early stages, the relapse-rate of over 50% after complete resection indicates that the tumour has already spread beyond its anatomical site even in patients with an early stage at diagnosis. This is a particular challenge to the oncologist, since the tumour-node-metastasis-classification (TNM) is still the gold standard concerning prognosis and stratification of this disease [3]. However, this merely anatomical description of tumour spread does not allow for a further insight into the biological diversity and metastatic potential of lung cancer.

The outer surface of mammalian cells including malignant ones consists of a glycocalyx, a carbohydrate rich coat. Since this outer coat is involved in cell to cell and cell to matrix interactions, which are important during malignant progression, several communications have reported an association between changes in the glycosylation and metastatic potential of the tumours indicated by the patients' prognosis [4,5].

These changes in the membrane protein glycosylation can be detected histochemically by the use of labelled lectins, which act as carbohydrate binding proteins. Several studies have demonstrated that the expression of binding sites for the lectin Helix pomatia agglutinin (HPA) on various adenocarcinoma cells such as breast, stomach, ovary, oesophagus, colorectum, thyroid and prostate is a marker of metastatic potential and poor prognosis [5,6]. Another lectin, Phaseolus vulgaris leucoagglutinin (PHA-L), is a useful marker of tumour progression in breast and colon cancer [7—9] and it has also been reported, that there is a significant correlation between the expression of PHA-L-binding oligosaccharides in oral squamous-cell carcinoma and its regional lymph node metastases [10]. Furthermore, the lectin Ulex europaeus agglutinin-I (UEA-I) was investigated in breast cancer where its binding related to disease-free interval and survival [11].

The aim of the present investigation was to analyse a possible association between the presence of lectin binding sites of pulmonary adenocarcinoma cells and their lymph node and haematogenous metastatic cells.

2. Patients and methods

2.1. Patients

Tumour tissue blocks from 96 patients with metastatic adenocarcinomas of the lung that had undergone surgery between 1999 and 2002 in the General Hospital Hamburg-Harburg, Germany, were investigated.

2.2. Histology and histochemistry

Formalin-fixed and wax-embedded tissue blocks were used. After dewaxing, lectin histochemistry was performed using biotinylated lectins (Sigma, Deisenhofen, Germany; see Table 1 for abbreviations and sugar specifity), an avidin-biotin-phosphatase-complex with a slight haematoxylin counterstain was used for visualisation.

Rehydrated sections were incubated in trypsin (0.1% w/v type II, crude, from porcine pancreas) (Sigma, St. Louis, USA), rinsed with lectin-buffer (0.05 M TRIS buffered saline, pH 7.6, with 1 mM CaCl and 1 mM MgCl added) and then incubated with the biotinylated lectin. Enzyme reactivity of the alkaline phosphate complex was visualised using Naphtol-AS-bisphosphate as a substrate and hexatozised New Fuchsine was used for simultaneous coupling (see Table 1).

The percentage of staining of the cancer cells was recorded as follows: negative indicated no or weak staining of single tumour cells (<5%), positive staining indicated that at least 5% of the tumour cells were stained. The slides were examined under a Zeiss Axioplan photomicroscope and photographed with a Kodak Ektachrome 64T colour film.

Table 1 Lectin characteristics

Origin of lectin	Abbreviation	Sugar specifity
Helix pomatia	HPA	*N*-acetylgalactosamine/*N*-acetylglucosamine
Phaseolus vulgaris	PHA-L	β-1,6-Branched tri- and tetra-antennary oligosaccharides
Ulex europaeus	UEA-I	α-1-Fucose