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Lectin Histochemistry of Resected Adenocarcinoma of the Lung

Helix pomatia *Agglutinin Binding Is an Independent Prognostic Factor*

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The worldwide incidence of adenocarcinoma of the lung is rising. Unfortunately, no significant prognostic marker beyond the classical TNM staging exists to stratify these patients for appropriate therapy. Because lectins, carbohydrate-binding proteins, have been shown to be useful prognostic markers in several other adenocarcinomas, a panel of lectins [*Helix pomatia a*gglutinin (HPA), *Phaseolus vulgaris* leukoagglutinin, *Ulex europaeus* agglutinin, *Maackia amurenis* agglutinin, *Sambucus nigra* agglutinin] with different carbohydrate-binding specificities were tested for their prognostic relevance. Paraffin wax sections of 93 patients with adenocarcinomas of the lung who had undergone surgery between 1990 and 1995 were investigated by lectin histochemistry. Lectin-binding data and other known prognostic factors were correlated with survival. In univariate analysis, binding of HPA, *Phaseolus vulgaris* leukoagglutinin, and *Ulex europaeus* agglutinin to adenocarcinoma cells were prognostic indicators for overall and relapse-free survival, whereas *Maackia amurenis* agglutinin and *Sambucus nigra* agglutinin binding had no prognostic value. However, in a multivariate analysis next to stage and gender only HPA was a significant independent prognostic factor on survival. In conclusion, HPA binding was the primary marker-based predictor of prognosis in our patient population and allows to stratify patients with adenocarcinomas of the lung into a low- and a high-risk group. *(Am J Pathol , :1001–1008)*

In the United States and Western Europe lung cancer is the most common fatal neoplasm. From a histological point of view, lung cancer is a heterogeneous group of tumors, three-quarters being non-small cell lung cancer. In non-small cell lung cancer surgical resection is the therapy of choice in early stages of the disease. However, even in this selected group of patients, approximately half of the patients relapse after complete resection indicating that the tumor has already spread beyond its anatomical site at the time of surgery. At present, the prognostic gold standard is the stratification of patients according to the TMN classification.[1,2] Patients with early disease (stage I and II disease) have a 5-year survival rate between 30 and 75% after complete resection. Patients with locally advanced disease (stage IIIA/B disease) have a 5-year survival rate between 5% and 15%, and patients with metastatic disease (stage IV disease) have a survival rate of less than 2%.[2,3]

Based on their morphology non-small cell lung cancers can be subdivided into adenocarcinomas, squamous cell carcinomas, and large cell carcinomas. Adenocarcinomas have been rising in incidence during the past decades and have become the most common type of non-small cell lung cancer in Western Europe and in the United States.[4,5] Despite the clinical need to stratify adenocarcinomas of the lung with regard to prognosis beyond the classical TNM classification, no satisfactory prognostic marker has emerged as yet.

The classical TNM classification rests on the anatomical description of the tumor spread. The inherent disadvantage of such a classification is that it is purely anatomical and descriptive and thus does not allow any functional insight into the metastatic capability of the tumor, the knowledge of which could lead to innovative therapeutic strategies.

In adenocarcinomas other than lung (eg, breast, colon, stomach, and prostate) the lectin from the Roman snail, *Helix pomatia* agglutinin (HPA), has been used to define the metastatic phenotype of these tumors.[6] Lectins are carbohydrate-binding proteins and because most of the

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cellular glycoconjugates are membrane-bound glycoproteins it has been hypothesized that their altered glycosylation is functionally involved in the metastatic process thus providing a rationale for the use of HPA in metastasis research.[7] However, HPA has not been the only lectin that has provided useful prognostic information. The lectin *Phaseolus vulgaris* leukoagglutinin (PHA-L) was a useful prognostic marker in breast and colon cancer[8,9] as well as in human diffuse large B-cell lymphoma.[10] A significant correlation between the expression of PHA-L-binding oligosaccharides and the incidence of the metastasis to regional lymph nodes in oral squamous cell carcinoma has also been reported.[11] The lectin *Ulex europaeus* agglutinin (UEA-I) has been investigated in breast cancer where its staining was related to disease-free interval and survival.[12] In the study by Fenlon and colleagues,[12] sialidase predigestion was also used before HPA and UEA-I application. The results indicated that both lectins lost their prognostic significance indicating that sialic acid can play a role in masking binding sites for these two lectins. In contrast, enhanced sialylation of tumor cell glycoproteins has been considered important for the metastatic capabilities of colorectal carcinomas. Thus, the sialic acid-specific lectins *Sambucus nigra* agglutinin (SNA-I) and *Maackia amurenis* agglutinin (MAA) have been discussed as useful prognostic markers in colorectal carcinoma.[13–16]

The aim of the present investigation was to analyze the glycoconjugate expression of adenocarcinomas of the lung with several lectins differing in their carbohydrate specificity to possibly define a prognostic marker in this clinically important tumor entity.

Patients and Methods

Patients

Tissue blocks containing tumor tissues of 93 patients with adenocarcinomas of the lung who had undergone surgery between 1990 and 1995 in the General Hospital Harburg, Hamburg, Germany, were investigated.

Histology and Histochemistry

Formalin-fixed and wax-embedded tissue blocks were used. After dewaxing, lectin histochemistry was performed using biotinylated lectins (for lectins, their abbreviation, and sugar specificity see Table 1; all lectins were obtained from Sigma, Deisenhofen, Germany) and an avidin-biotin-alkaline phosphatase complex was used for visualization (ABC Alkaline Phosphatase; Vector Laboratories, Peterborough, UK) with a slight hematoxylin counterstain.[17] For HPA only an indirect immunohistochemical technique [designated as iHPA in contrast to biotinylated HPA (=bHPA)] was also used because it has been shown that the staining methodology influences the prognostic impact.[18]

For the indirect method, dewaxed sections were treated for 15 minutes at 37°C with 0.1% trypsin dissolved in lectin buffer. After rinsing in distilled water, endogenous peroxidase was blocked at room temperature by incubating the sections in 3% H_2O_2 in methanol. After careful rinses in buffer, the sections were incubated with 10 μg/ml of HPA for 1 hour at room temperature. After rinsing in lectin buffer, sections were incubated in normal swine serum (1:5 dilution in buffer) for 30 minutes. An overnight incubation with a rabbit anti-HPA antibody (diluted 1:500; EY Lab, San Mateo, CA) followed. After careful rinsing of the slides, the sections were incubated with a biotinylated anti-rabbit antibody (1:400; DAKO, Glostrup, Denmark). After rinsing with buffer, incubation with an avidin-biotin-horseradish peroxidase complex followed. Diaminobenzidine and H_2O_2 were used as substrates for enzyme visualization.

Table 1. Origin of the Lectins and Their Sugar Specificity

Origin of the lectin	Abbreviation	Nominal sugar specificity
Helix pomatia	HPA	*N*-acetylgalactosamine/*N*-acetylglucosamine
Phaseolus vulgaris	PHA-L	β-1,6-Branched tri- and tetraantennary oligosaccharides
Maackia amurensis	MAA	Sialic acid α-2,3-galactose/*N*-acetylgalactosamine
Sambucus nigra	SNA-I	Sialic acid α-2,6-galactose/*N*-acetylgalactosamine
Ulex europaeus	UEA-I	α-L-fucose

Sections from the *in vitro* grown human HT 29 cancer cell line, which was HPA-positive and metastasized in SCID mice,[19] were used as positive controls. These cells stained intensively for HPA. Omission of the lectin or preincubation with 0.3 mol/L *N*-acetylgalactosamine resulted in an abolishment of the cancer cell reactivity.

In addition to lectin histochemistry, hematoxylin- and eosin-stained slides were used for a general overview and to identify area of the tumors.

The staining of the cancer cells was recorded as follows: negative indicated no staining or weak staining of single tumor cells (<5%), positive staining indicated that at least 6% of the tumor cells were stained (Figure 1).[20] The classification into negative or positive was done independently by two observers who agreed in more than 95% of the cases; in the remaining cases, consensus was achieved after discussion. The slides were examined under a Zeiss Axioplan photomicroscope (Carl Zeiss, Jena GmbH, Jena, Germany) and photographed with a Kodak Ektachrome 64T color film (Kodak Company, Rochester, NY).

Statistical Analysis

All patients were followed-up systematically for a minimum of up to 5 years. The data of diagnosis, relapse, and deaths were recorded. From these data survival curves were prepared according to the Kaplan-Meier method for time to death and time to relapse, respectively, and were compared with the log-rank test.[21,22] A chi-square test was used to analyze whether correlations existed be-

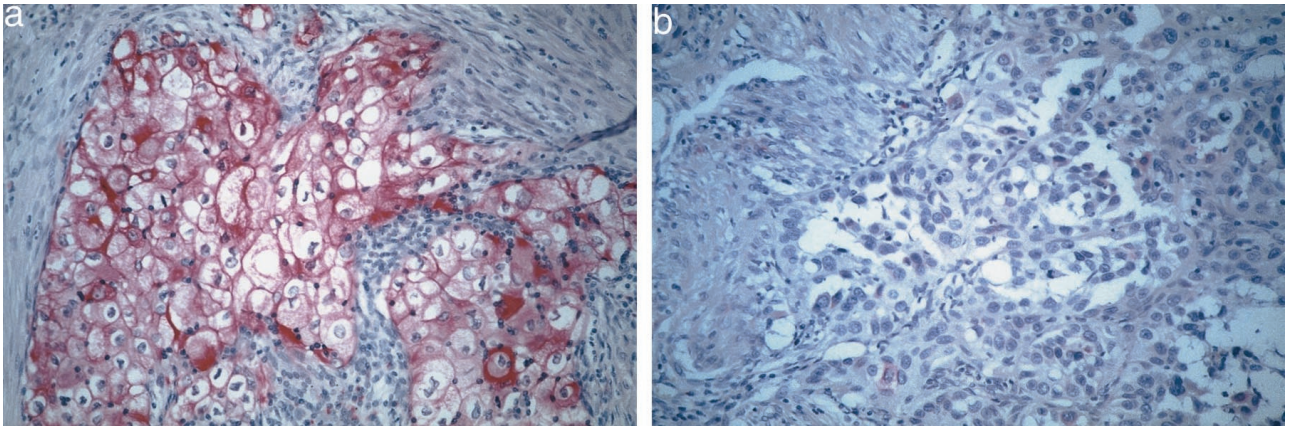

Figure 1. Biotinylated HPA-positive (**a**) and HPA-negative (**b**) adenocarcinoma of the lung. Original magnification, ×200.

tween the binding of different lectins and between each lectin and each categorical clinical factor.

The results of the lectin binding and of the clinical data were assessed for their predictive value for patient survival by the proportional hazard model (Cox regression model).[23] First the candidate factors were investigated for their prognostic value in an univariate analysis using the Cox regression model that is equivalent to the evaluation of each factor separately using the log-rank test for differences between prognostic groups determined by the respective levels of one factor. Variables with sufficient statistical prognostic power ($P < 0.1$) were further analyzed in a multivariate Cox regression model, and application of a model selection approach to determine a final parsimonious prognostic model for patient survival based on this data set. Forward and backward variable selection and the likelihood ratio statistic were used with the SAS system (SASISTAT Software, Release 6:12; SAS Institute Inc., Cary, NC). The outcome of the Cox regression was described quantitatively by the statistical estimate of the regression parameter β and its SE, its risk ratio exp (β) and the respective P value obtained from the Wald test statistic. Model uncertainty was examined in residual tests and qualitatively analyzed by the determination of the amount of collinearity in the respective sets of prognostic variables using pairwise correlation.

Table 2. Patient Characteristics

Characteristics	No. of patients
Total no. of patients	93
Gender	
Male	63 (68%)
Female	30 (32%)
Stage*	
IA	20 (22%)
IB	32 (35%)
IIA	4 (4%)
IIB	16 (17%)
IIIA	19 (20%)
IIIB	1 (1%)
IV	1 (1%)
Grading	
Well differentiated	10 (11%)
Moderately differentiated	37 (40%)
Poorly differentiated	33 (35%)
Undifferentiated	5 (5%)
Unknown	8 (9%)
Blood group type	
O	33 (35%)
A	42 (45%)
B	9 (10%)
A/B	2 (2%)
Unknown	7 (8%)

*According to the 5th edition of the TNM classification of lung cancer, 1997.

Results

Patient Characteristics

The tumor tissues of 93 patients were investigated (for patient characteristics see Table 2). The majority of patients (68%) were male. Patients had a median age of 59 years (range, 27 to 81 years). The tumors of 72 patients (78%) were pathologically staged as early disease (stages I and II) with no signs of metastasis in mediastinal lymph nodes, and 19 (20%) were staged as locally advanced disease with tumor cell-positive lymph nodes of the ipsilateral mediastinum (stage IIIA). None of the patients showed tumor-positive lymph nodes of the contralateral mediastinum or distant metastases. Neverthe-

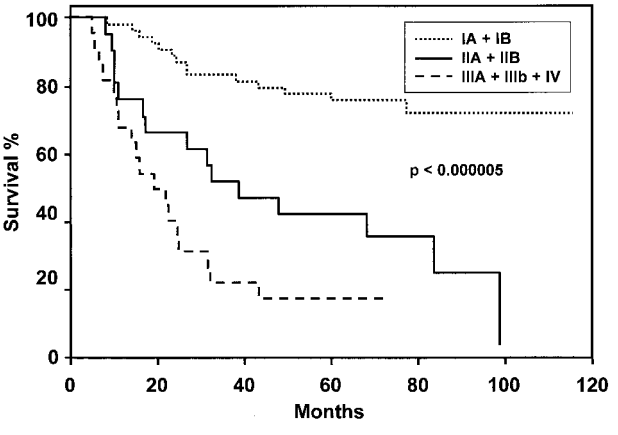

Figure 2. Kaplan-Meier plot of overall survival by tumor stage of 93 patients with resected adenocarcinomas of the lung.

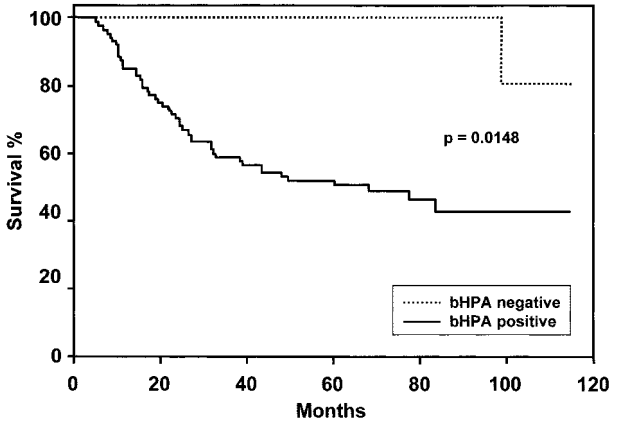

Figure 3. Kaplan-Meier plot of overall survival for biotinylated HPA (bHPA) binding. Patients ($n = 84$) whose tumors expressed bHPA-binding sites had a significantly poorer survival than patients ($n = 9$) whose adenocarcinomas were bHPA-negative.

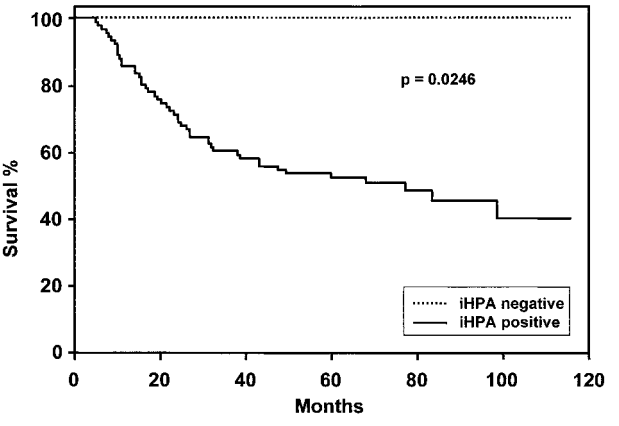

Figure 4. Kaplan-Meier plot of overall survival for iHPA binding. Patients ($n = 87$) whose tumors expressed iHPA-binding sites had a significantly poorer survival than patients ($n = 6$) whose adenocarcinomas were iHPA-negative.

less, one patient with a pulmonary metastasis in the same lobe as the primary tumor (stage IIIB disease) was included as well as one patient with a pulmonary metastasis in another lobe of the same side (stage IV disease). Patients with positive mediastinal lymph nodes received radiotherapy after surgery. The predominant grade of tumor differentiation was moderately differentiated (40%), followed by poorly differentiated tumors (35%). The most common blood group type was A (45%).

Lectin-Binding Characteristics and Survival Analysis

The overall 5-year survival rate of all 93 patients was 49.5%. Distant metastases or a local relapse were diagnosed in 49 patients (53%). Tumor stage (log-rank test, $P < 0.000005$, see Figure 2), grade of tumor differentiation ($P = 0.025$), and gender ($P = 0.04$) had a significant influence on overall survival. The blood group type did not show any association with prognosis ($P = 0.36$).

The tumors of 9 patients (10%) showed no binding of biotin HPA (bHPA), whereas the tumors of 84 patients (90%) demonstrated moderate to intense bHPA binding to the tumor cells. Kaplan-Meier survival curves (Figure 3) of time to death in months for bHPA-negative patients *versus* bHPA-positive patients revealed a significant difference in survival between the two different groups ($P = 0.015$). Six tumors (6%) were classified as iHPA binding-negative and 87 (94%) as iHPA binding-positive. The patients with iHPA-negative tumors also had a significantly longer survival than those with iHPA-positive tumor tissues ($P = 0.025$) (Figure 4). There was a highly significant correlation between bHPA and iHPA binding tumors (chi-square test, $P = 0.001$).

All patients whose adenocarcinomas were either bHPA- or iHPA-negative survived 5 years (for patient characteristics see Table 3). All patients with bHPA-negative tumors showed no signs of lymph node involvement (N0-status) at the time of diagnosis. Eight of the nine bHPA-negative patients had a stage I disease [six patients: stage IA (T1N0M0); two patients: stage IB (T2N0M0)]. One patient who had a bHPA-negative and iHPA-positive tumor was staged as IIB disease (T3N0M0). This patient was diagnosed having a small cell lung cancer with distant metastases as a secondary malignancy 94 months after resection of the adenocarcinoma of the lung, and subsequently died 3 months later from this neoplasm.

Table 3. Characteristics of the bHPA- and iHPA-Negative Patients

Patients	bHPA	iHPA	Tumor stage	Status	Survival time (months)	Metastases
1	Negative	Negative	IA	Alive	64	n.d.*
2	Negative	Negative	IA	Alive	102	n.d.
3	Negative	Negative	IA	Alive	113,5	n.d.
4	Negative	Negative	IA	Alive	66	n.d.
5	Negative	Negative	IB	Alive	88	Yes†
6	Negative	Negative	IB	Alive	113	n.d.
7	Negative	Positive	IA	Alive	60	n.d.
8	Negative	Positive	IA	Alive	97	n.d.
9	Negative	Positive	IIB	Deceased‡	97	Yes‡

*n.d., not detectable.

†A single metastasis in the bone occurred after 66 months, it is still unclear if this was a late metastasis from the lung cancer or a metastasis from another cancer of unknown primary site.

‡Patient died of metastases of a secondary malignancy (small cell lung cancer) which occurred after 94 months of the diagnosis of a primary adenocarcinoma of the lung.

Table 4. BHPA Binding-Associated Factors (Chi-Square Test)

	Patients with bHPA-negative tumors	Patients with bHPA-positive tumors
Tumor stage (n = 93)	(P = 0.047)	
IA	67%	17%
IB	22%	36%
IIA	0	5%
IIB	11%	18%
IIIA	0	22%
IIIB	0	1%
IV	0	1%
Blood group (n = 86)	(P = 0.007)	
O	86%	34%
A	0	53%
B	14%	10%
A/B	0	3%
Grading (n = 85)	(P = 0.006)	
Well differentiated	50%	8%
Moderately differentiated	25%	46%
Poorly differentiated	25%	40%
Undifferentiated	0	6%

Factors showing a statistically significant association (chi-square test) with bHPA-binding pattern (negative *versus* positive) are listed in Table 4.

Sixty-eight patients had a tumor (73%) that reacted with PHA-L. Those patients who had a PHA-L-positive tumor had a significantly poorer prognosis than those whose tumors were PHA-L-negative (P = 0.017, Figure 5). Seventy-five tumors (81%) were UEA-I-positive and 29% were UEA-I-negative. Again, this lectin positivity was also associated with a poorer prognosis (P = 0.022, see Figure 6).

The predictive value of bHPA, PHA-L, and UEA-I was not limited to the overall prognosis but was also related to relapse-free intervals. Patients whose tumors did not bind bHPA, PHA-L, or UEA-I had a significantly longer relapse-free survival than those patients whose primary tumors bound these lectins [bHPA (P = 0.015), PHA-L (P = 0.028), UEA-I (P = 0.039)].

The sialic acid-binding lectins MAA and SNA-I did not have any significant influence on overall survival [MAA (P = 0.82), SNA-I (P = 0.68)] or on the relapse-free survival [MAA (P = 0.86), SNA-I (P = 0.35)].

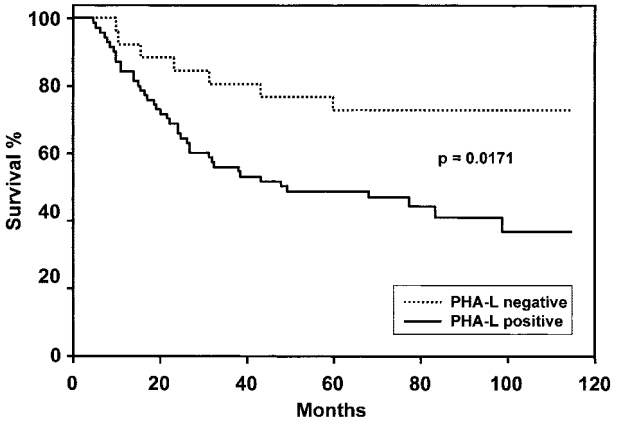

Figure 5. Kaplan-Meier plot of overall survival for PHA-L binding. Patients with PHA-L-positive tumors (n = 68) had a significantly poorer prognosis than those whose tumors were PHA-L-negative (n = 25).

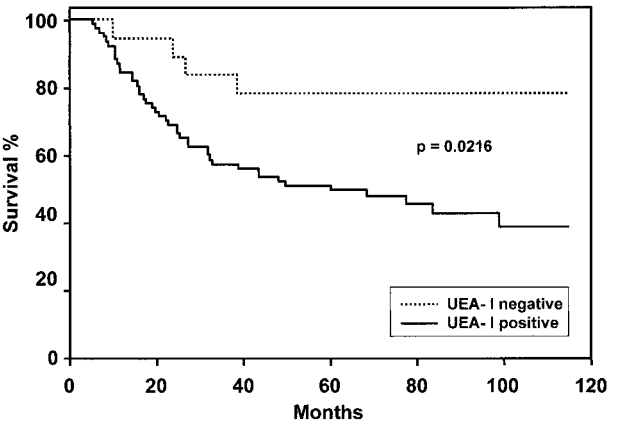

Figure 6. Kaplan-Meier plot of overall survival for UEA-I binding. Seventy-five tumors were UEA-I-positive and 18 were UEA-I-negative. The lectin positivity was associated with a significantly poorer prognosis.

Prognostic Impact

When tumor stage, grading, age, gender, blood group, bHPA, PHA-L, UEA-I, MAA, and SNA-I were included in a Cox regression model, next to stage and gender only bHPA-binding pattern was identified as a significant independent prognostic factor in this multivariate analysis (Table 5 and Table 6, A and B). iHPA was not evaluable because of nonconvergence of the maximum likelihood statistic of the Cox regression. Therefore and because of its high correlation with bHPA, only bHPA was used for further analysis.

Patients in the bHPA-negative group showed a considerably better survival than those in the bHPA-positive group. Because bHPA was the leading factor in multivariate analysis with a relative risk of mortality of 8.75, the prognostic power of the two other factors tumor stage and gender in the bHPA-positive subgroup was further investigated (Table 7). The independent prognostic power of tumor stage and gender in this subgroup and the risk estimates were very similar to those in the full set of patient data.

Discussion

The present comprehensive lectin-binding analysis of adenocarcinomas of the lung was undertaken to identify possible new prognostic markers that could be used to stratify the patients for adjuvant therapy. Using univariate analysis, we have found that binding of the lectins HPA, PHA-L, and UEA-I to adenocarcinoma cells of the primary tumors can be regarded as prognostic indicators for overall and relapse-free survival after the tumor was surgically removed. In contrast, the binding of MAA and SNA-I to the primary tumors was of no prognostic value in our study. However, in multivariate analysis only HPA binding remained as a significant independent prognostic indicator having a relative risk of mortality of 8.75, next

Table 5. Potential Prognostic Factors and the Results of the Univariate Cox Regressions Analysis (n = 93)

Factor	Levels	n (%)	Hazard ratio	P value
Tumor stage	IA + B	55.9	1*	—
	IIA + B	21.5	4.05	0.0002
	IIIA + B + IV	22.6	7.89	0.0001
Grading (n = 85)	Well differentiated	11.8	1*	—
	Moderately differentiated	43.5	2.50	0.29
	Poorly differentiated	38.5	5.21	0.03
	Undifferentiated	5.9	5.36	0.07
Age	Years		1.01	0.61
Gender	Female	32.3	1*	—
	Male	67.7	2.0	0.05
Blood group type	O	38.4	1*	—
(n = 86)	A	48.8	1.53	0.20
	B	10.5	0.64	0.47
	A/B	2.3	1.14	0.90
bHPA	Negative	9.7	1*	—
	Positive	90.3	8.07	0.04
PHA-L	Negative	26.9	1*	—
	Positive	73.1	2.57	0.02
UEA-I	Negative	19.4	1*	—
	Positive	80.6	3.13	0.03
MAA	Negative	53.8	1*	—
	Positive	46.2	1.07	0.82
SNA-I	Negative	6.5	1*	—
	Positive	93.5	0.81	0.68

*Reference group in univariate analysis.

to stage and gender. Analysis of only the patients with HPA binding-positive tumors, revealed an independent prognostic power of stage and gender in this group of patients with very similar estimates to those obtained in the full set of patient data. Patients who had a HPA-negative, well-differentiated tumor, and tumor stage IA disease had the best prognosis. All patients with HPA-negative tumors did not show signs of lymph node involvement (N0-status) at the time of diagnosis. These findings are similar to those in breast cancer in which a strong association between HPA staining in primary tumor and the presence of metastases in the local draining lymph nodes was observed.[24]

However, only 10% of patients had a HPA-negative tumor, which demonstrates the high malignant potential of adenocarcinomas of the lung and this observation is concurrent with the high rate of relapses in our study group. From this we conclude that for our patient population it is reasonable to use HPA as the primary predictor of prognosis and that in the larger part of the HPA-positive population the factors stage and gender represent further important prognostic factors. However, these findings should be validated in further studies.

Our results are comparable to those that showed an association between HPA binding and prognosis in breast, colon, and gastric cancer,[6,7] whereas no prognostic significance for HPA could be detected in squamous cell carcinoma of the head and neck.[25] Taking the results of these studies together it becomes apparent that HPA is particularly well suited to recognize a glycotope on the histological entity adenocarcinomas, where it is equal or even superior to other classical markers of prognosis. The metastatic potential of HPA-positive tumor cells has also been confirmed in xenograft models of

Table 6. Multivariate Cox Regressions Analysis Using Factors with Sufficient Statistical Power (P <0.1) in Univariate Analysis and (Part A). Results of a Forward and Backward Variable Selection Procedure (Analysis of Likelihood) for Those Factors (Part B)

Factor	Parameter estimate	Standard error	Hazard ratio	P value
A. Tumor stage				
IIA + B	1.76	0.40	5.83	0.0001
IIIA + B + IV	1.93	0.40	6.92	0.0001
Gender	1.33	0.37	3.78	0.0004
bHPA	2.23	1.06	9.34	0.03
PHA-L	0.64	0.43	1.88	0.14
UEA-I	0.63	0.54	1.88	0.24
B. Tumor stage				
IIA + B	1.79	0.39	6.00	0.0001
IIIA + B + IV	2.14	0.39	8.53	0.0001
Gender	1.29	0.37	3.65	0.0004
bHPA	2.17	1.06	8.75	0.04

Table 7. Results of a Forward and Backward Variable Selection Procedure for Tumor Stage and Gender by only HPA-Binding Positive Patients ($n = 84$)

Factor	Parameter estimate	Standard error	Hazard ratio	*P* value
Tumor stage				
IIA + B	1.66	0.40	5.24	0.0001
IIIA + B + IV	2.05	0.38	7.76	0.0001
Gender	1.25	0.37	3.48	0.0007

breast and colon cancer. When transplanted into severe combined immunodeficient (SCID) mice, HPA-positive human breast and colon cancer cells metastasized whereas HPA-negative cancer cell lines in general did not.[19]

The molecular basis why HPA binds preferentially to metastasizing adenocarcinomas has not been elucidated completely. HPA has a specificity for *N*-acetylgalactosamine (GalNac) and as this carbohydrate is part of the blood group A carbohydrate determinant, one could speculate that it is the blood group A determinant that is recognized by HPA. However, this is not the case in our study as 84% of the tumors showed HPA binding whereas only 45% of the patients were of blood group A indicating that GalNac containing glycoconjugates other than blood group A substance are responsible for HPA positivity in the adenocarcinoma cells. This apparent discrepancy between HPA binding to the cancer cells and the blood group of the patients was also noted in breast cancers. The monoclonal antibody BRIC 66 directed against blood group A substance and HPA were both used on tissue sections and on Western blots of extracted breast cancer glycoproteins. The results indicated that HPA recognizes blood group A substance if it is expressed by the cancer cells, but its binding pattern is much broader and more heterogeneous than that of the monoclonal anti-A antibody.[26]

The precise structure of the HPA binding oligosaccharide associated with metastasis has not been identified yet, however, a monosialylated oligosaccharide of 4.58 glucose units termed HPAgly-1 has been identified in HPA-positive breast cancers, whose functional role has to be elucidated in future studies.[27]

Two different methods were used to study HPA binding to primary tumor cells. This was undertaken as much controversy had arisen concerning the predictive value of HPA in metastasis research in the early years of its usage.[28,29] In the original contribution by Leathem and Brooks,[20] HPA-binding sites were detected by an indirect method. The lectin was applied to the tissue section and subsequently localized by an anti-lectin antibody and an anti-antibody peroxidase anti-peroxidase complex (PAP-technique). Using this methodology, a significant difference in survival was seen between the HPA-positive and -negative patients. In contrast, Gusterson and colleagues[29] who were unable to see any prognostic significance for HPA binding, used HPA covalently linked to horseradish peroxidase (direct method). This discrepancy in the results was later resolved by Brooks and colleagues,[18] who used both techniques in one patient cohort. Again, only the indirect method gave good results indicating that the technique for HPA binding is crucial. In the present study of adenocarcinomas of the lung, a slightly modified indirect technique (=bHPA) was used. Here, HPA was linked to the small molecule biotin, which was subsequently detected by an avidin-alkaline phosphatase complex. Using this method, Thies and colleagues[30] could show that biotinylated HPA (=bHPA) was able to identify those malignant melanomas, that metastasized. Because the indirect and the biotinylated HPA method were both suitable to identify the metastasizing tumors,[30] we wanted to compare the relative merits of both methods in adenocarcinomas of the lung. However, the staining results of both methods do not dramatically differ in this study. From our results we cannot conclude that bHPA-negative patients differ in their prognosis from iHPA-negative patients. Therefore further investigations into the prognostic power of both methods are needed at least in adenocarcinomas of the lung. In this tumor entity it seems likely that a large number of patients needs to be examined before one would be able to find differences between bHPA and iHPA as negative cases seem to occur at a rate of less than 10%.

In summary, using the appropriate methodology, HPA binding to the cells of primary adenocarcinomas of the lung is a significant independent prognostic factor. Our results add weight to the importance of HPA as a prognostic marker and could be an important further step toward the stratification of patients with adenocarcinomas of the lung into low- and high-risk populations.

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