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Combined analysis of tumor growth pattern and expression of endogenous lectins as a prognostic tool in primary testicular cancer and its lung metastases

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Summary. The aim of this study was to glyco- and immunohistochemically analyze expression of distinct growth/adhesion-related markers of primary testicular carcinomas and their lung metastases in relation to the risk of developing lung metastases and survival of patients, and to correlate immunohistochemical staining profile and syntactic structure analysis in order to delineate new prognostic parameters for this tumor type. Clinical features of 50 patients with primary testicular carcinomas and their corresponding lung metastases were evaluated and compared to those of a control cohort of 25 cases. The set of eight probes including labeled galectins-1 and -3, specific non-cross-reactive antibodies against galectins-1, -3, and -8 as well as anti-Ki-67, anti-bcl-2, and anti-p53 was applied to formalin-fixed, paraffin-embedded tumor sections of both primary and metastatic lesions. Syntactic structure analysis computed staining intensities and structural features of the tumor cells. These parameters were set into relation separately and in combination to clinical data including tumor stages, smoking habits, applied cytostatic therapy, disease-free interval, and survival. The risk of testis cancer patients to develop lung metastases depends in descending order on the tumor cell type (non-seminoma versus seminoma), tumor cell heterogeneity (mixed versus monomorphous cell type), age of patients, and pT stage. The extent of differential expression of galectin-related features between primary and secondary lesions was pronounced. Prognostic correlations for distinct galectin-related features were delineated in combination with data from syntactic structure analysis, for example cluster radius of galectin-3-positive tumor cells and post-surgical and total survival. Lengths of disease-free interval and total survival of patients were also correlated to characteristics obtained by syntactic structure analysis and their combination with galectin data in the first place, then to smoking habits, percentage of proliferating cells in the primary and secondary tumors, and finally to expression of certain galectins and of p53. Patients with non-seminoma testicular cancer should be thoroughly controlled for lung metastases. Regarding marker selection, our study underscores that further investigation of the growth-regulatory network of galectins is clearly warranted.

Key words: Glycohistochemistry, Lectins, Lung metastases, Survival, Syntactic structure analysis, Testicular cancer

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Introduction

Carcinomas of the testis are the most frequent malignant tumors in young men in the age range between 15 to 35 years. They contribute to about 1% of all malignant tumors in men, and their incidence has doubled within the last 40 years (Hernes et al., 1992; Swerdlow, 1993; Buetow, 1995; Bosl and Motzer, 1997). Due to the development of multimodal therapy regimens the mortality has dropped considerably. Currently, the prognosis reaches about 92% of 5 years survival for patients with normal tumor markers free of distant metastases (Aass et al., 1991; International Germ Cell Cancer Collaborative Group, 1997). However, a non-negligible percentage of patients develop lung metastases, which effectively reduce the quality of life and length of survival period. A pertinent issue to address will thus be to correlate biochemical and morphological tumor features to the secondary involvement of other organs. This analysis of primary/secondary lesions might also allow us to discern parameters for prognostic evaluation. In consequence, this study deals with the following questions: 1. Are there characteristic features of primary malignant testis

tumors, which can predict the development of lung metastases or the length of the disease-free interval? 2. To what extent does the marker profile of testis cancer cells change between primary and secondary carcinomas? 3. How effective is cytostatic therapy of lung metastases, and which molecular and structural characteristics are typical for tumors completely or partially resistant to it? 4. Are there emerging parameters to gauge the prognosis of the patients in terms of disease-free interval and total survival?

As a step toward answering these questions we turned to the analysis of a distinct aspect of intra- and intercellular communication, i. e. the information storage in glycan chains of cellular glycoconjugates and the involvement of endogenous lectins to trigger responses (Kaltner and Stierstorfer, 1998; Villalobo and Gabius, 1998; Gabius, 2000, 2001; Solís et al., 2001; Gabius et al., 2002). We focussed on those glycan components which are readily accessible at branch ends, namely ß-galactosides and derivatives thereof. The mitogenic effect of a plant agglutinin for immune and tumor cells *in vitro* and *in vivo* which binds to these carbohydrate epitopes attests the bioactivity of this class of lectin ligands (Gabius et al., 2001; Timoshenko et al., 2001). They are also targeted by a family of endogenous lectins (galectins), and this interplay is involved in cellular growth control, adhesion, migration and immunological effector functions (Gabius, 1987, 1997a,b; Bresalier et al., 1998; André et al., 1999; Hadari et al., 2000; Kopitz et al., 2001; Liu et al., 2002; Rabinovich et al., 2002). In the clinical context, a galectin (i.e. galectin-1) has already been described as major factor to eliminate CD7+ leukemic T cells in patients with Sézary syndrome and to yield invasiveness of glioblastoma cells (Camby et al., 2002; Rappl et al., 2002). Due to the emerging complexity of the galectin network of growth control including antagonistic effects on the same target, as inferred by database mining and measured by RT-PCR and immunohistochemical analysis as well as cell biological experiments (Wollina et al., 2000, 2002; Cooper et al., 2001; Kopitz et al., 2001; Lahm et al., 2001; Sheikholeslam-Zadeh et al., 2001; Danguy et al., 2002; Nagy et al., 2002), it is becoming necessary to monitor the presence of more than one galectin in a study. Thus, we measured the expression of three galectins deliberately chosen from the three subfamilies on the basis of RT-PCR profiling (Lahm et al., 2001). Besides taking this aspect into consideration, we also extended common plant lectin histochemistry in search of tumor markers (Caselitz, 1987; Rüdiger and Gabius, 2001). Following the introduction of endogenous lectins to histopathology and initial documentation of their potential to refine diagnostic procedures (Gabius et al., 1993; Francois et al., 1999; Plzák et al., 2000, 2001), we applied two mammalian lectins to visualize accessible binding sites, i. e. galectins-1 and -3. Importantly, fine-specificity differences in carbohydrate selection epitomize the assumed fine-tuning in this area of information transfer and the enormous potential of mutual regulation processes between glycans and endogenous lectins (Gabius, 2001; Ahmad et al., 2002; Plzák et al., 2002). In testicular cancer, lectin presence has been inferred by glycohistochemistry and drug targeting with neoglycoproteins (Gabius et al., 1987; Gabius and Kayser, 1989; Xu et al., 2000) and the presence of galectins-1 and -3 has been described for a cell line and testicular tumor specimen (Gabius et al., 1985a,b). In this report, we build on this experimental basis to analyze primary and secondary lesions by lectin- and immunohistochemistry.

Proceeding from the given reasoning to select a representative for each subfamily, we monitored the presence of galectins-1, -3, and -8. Accessible binding sites for galectins with homologous carbohydrate recognition domains were detected by labeled galectins using purified galectins-1 and -3 which can bind to cell surface ligands including laminin, fibronectin, integrins or tetraspanins (for review, please see André et al., 1999). In addition, we included antibodies against p53 and bcl-2 for obtaining data on apoptosis regulation and Ki-67 on proliferation. Syntactic structure analysis was applied to relate the histochemical features of individual tumor cells and tumor cell cohorts (clusters) visible at low-level magnification to topology and to compute the structural entropy of tumors. Emphasis was given to the combination of information on topological cell arrangement and marker expression to answer the question as to whether this approach might lead to prognostic predictions.

Materials and methods

Materials

The patients constituted two different groups: a) 50 patients with primary testis cancer and their lung metastases as well as detailed clinical follow-up. Tissue blocks of both primary testis tumors and their lung metastases were available in 34 cases, and b) a “control” cohort of 25 patients with primary malignant testis tumors, collected in a separate institution, of unknown cancer progression.

Clinical data such as smoking, TNM stages, therapeutic regimens, disease-free interval and survival of patients were known for all patients of the “metastase group”. They were obtained by responses to questionnaires sent repeatedly to the house physicians. The minimum and maximum follow-up periods were 14 and 156 months. The tumor cell types and the pTNM stages are grouped according to the rules of the UICC and WHO, respectively (UICC, 1998; WHO, 1998). Nearly all patients (47/50) were treated with cytostatic drug regimens after resection of primary testis tumors. The therapy most frequently included the PEI (Cisplatin, Etoposide, Ifosfamide) and the PEB (Cisplatin, Etoposide, Bleomycin) regimens. In total, 17/50 patients were treated after resection of lung metastases.

Histochemical analysis

Sections (4-5 μm thick) obtained from tissue blocks of the primary and metastatic tumors were processed at a concentration of 10μl/ml with the following mono- or polyclonal antibodies: anti-MIB-1 (Zymed Laboratories, San Francisco, CA, USA), anti- bcl-2 (DAKO, Hamburg, Germany), and anti-p53 (DAKO, Hamburg, Germany). Polyclonal antibodies against galectins-1, -3 and -8 had been raised using purified proteins and were tested for specificity and lack of cross-reactivity as described previously (André et al., 1999; Camby et al., 2001; Wollina et al., 2002). Galectins-1 and -3 were purified and labeled under activity-preserving conditions following an optimized protocol including quality controls to ensure lectin activity (André et al., 1999, 2001). The three commercially available antibodies and the galectin-specific antibodies were applied to histological slides after deparaffinization and rehydration of the tissue as well as after blocking of non-specific protein-binding sites, and their specific (antigen- or epitope-dependent) binding was visualized by the conventional peroxidase-anti-peroxidase technique (PAP). Commercially available avidin-biotin kit reagents were employed to localize specific binding of the biotinylated galectins. Positive and negative controls were performed as usual, e. g. by omitting the incubation step with the marker to measure the extent of probe-independent staining, by blocking binding of the reagents by suitable preincubation steps and by simultaneous staining of sections from tumors known to react positively.

Image and syntactic structure analysis

Stained slides of proliferatively active tumor compartments were subjected to digital image analysis: areas of interest were interactively selected at moderate/high levels of magnification (x40) and digitized into a 511x511 matrix using a commercially available CCD camera (TK 1070, JVC) and a Matrox (Meteor) frame grabber. Self-written programs based on the commercially available DIAS language (Digital Image Analysing System, University of Jena, Germany) classified the cases as positive/negative by measuring the overall intensity of the (brown) color with the exception of slides stained with MIB-1 and p53. The color intensity of the slides was then further grouped into the categories I: zero-weak (<34%); II: moderate (33%<I<67%); and III strong (>66%). Cases with tumor cells belonging to class I only, or those with less than 5% of moderately-intensely-stained, histologically vital tumor cells were classified as negative and the others as positive. Images of slides stained with MIB-1 and p53 antibodies were analyzed with separate programs measuring stereological features such as area fractions, numerical densities of positively stained nuclei and corresponding volume fractions. Syntactic structure analysis was performed on cases classified as positive. The following features were measured: distances between tumor cells in relation to the staining intensity, distances between tumor cells and neighboring lymphocytes, arrangement in clusters (radius and number of cluster-building tumor cells) and computation of the structural entropy and its current. The structural entropy was calculated as follows:

$$E(MST) = c\Sigma\{ii/im)^2 + (di/dm)^2\}$$

with the following abbreviations:
E(MST) = structural entropy; ii = difference in staining intensity between an individual cell and its nearest neighbor; im = mean of difference of staining intensity of all cells; di = distance between the individual cell and its nearest neighbor; dm = mean distance between all nearest neighboring cells.

The technical procedures and clinical applications demonstrating the way how to translate the experimental data into the thermodynamic parameter have been described in detail previously (Kayser et al., 1992, 1997; Kayser and Gabius, 1997, 1999).

Statistical analysis

A commercially available program (NCSS, Number Cruncher Statistical System, Kaysville, USA) served for running the chi-square test, non-linear regression analysis, multivariate variance analysis, survival analysis by log rank test, and calculation of Kaplan-Meier curves.

Results

Synopsis of the patients' characteristics and the tumor material is presented in Table 1. Patients who developed lung metastases were significantly younger than those of the control group. In addition, the cell type of the testis tumors differed remarkably between the two cohorts. With the exception of only three cases, all

Table 1. Synopsis of patients' data and studied tumors (level of significance computed between the two groups of patients, cohort I versus cohort II).

FEATURE	COHORT I (n=50)	COHORT II (n=50)	LEVEL OF SIGNIFICANCE
Age	26.7±7.2	33.0±8.4	0.02
Tumor side			>0.05
Right	26	10	
Left	24	15	
Cell type			0.01
Seminoma	2	13	
Non-seminoma	47	12	
Burned-out tumor	1	0	
Stage			>0.05
pTX	1		
pT1	30	19	
pT2	11	5	
pT3	8	1	
Tumor diameter (mm)	59±36	47±26	>0.05

tumors of the lung metastases cohort are classified as non-seminomas in contrast to the control cohort with balanced frequency of the different tumor cell types.

Malignant testicular tumors are very sensitive to optimized cytostatic drug regimes. The vitality of metastases in relation to features of the primary tumors is presented in Table 2. This table also includes data on histochemical properties of metastases. The number of cases in relation to the staining is compiled in detail in Table 3. The features of the primary tumors and those of their intrapulmonary metastases displayed no association at a level of statistical significance ($p>0.5$), that is primary and secondary lesions had non-uniform expression profiles for the tested marker panel. The same observation hold true for the structural analysis of primary and metastatic tumors as shown in Table 4 and Table 5. The "width" of clusters formed by tumor cells, which were stained to a similar extent, varied broadly, and no consistently present pattern could be discerned. The entropy level of the topological arrangement revealed a relation to marker expression (Table 5). In comparison, its average extent was in the range of that measured in primary lung carcinomas (Kayser et al., 1998).

An interesting observation concerned the smoking habit. It is negatively associated with the total median survival of patients with testis cancer (Fig. 1) The length of the disease-free interval (DFI) or the total median survival (TS) of the patients were not related to galectin-dependent parameters in the primary testis tumors at a statistically significant level ($p<0.05$). Concerning the expression of accessible galectin-3-binding sites, a trend ($p<0.1$) was apparent (Fig. 2). Overexpression of wild-type p53 has been reported by Guillou et al. (1996). Patients with p53-positive tumors had a prolonged

Table 2. Characteristics of lung metastases of malignant primary testicular cancer.

FEATURE	VITAL/PARTIALLY VITAL	NON-VITAL METASTASIS
Number of cases	41	9
Mean number of metastases/case	8.2±8.3	11.7±10.3
Tumor in right or left lung	19	3
In both lungs	22	6
Central and peripheral location	23	2
Peripheral location only	18	7
Mean tumor volume (ccm)	92.3±203.1	13.1±19.7
Age of patients	27±7	26±7
Smokers	7	0
Expression of antigens	No. of cases (27)	No. of cases (7)
Gal-1	11	3
Gal-3	16	0
Gal-8	13	1
p53	11	0
Expression of binding sites		
Gal-1	3	0
Gal-3	7	0
Proliferation rate (Ki-67), in percent	35.5±28.4	0

Table 3. Classification of the cases (n=27) in primary malignant testis tumors and their lung metastases (vital and partially vital only) according to the measured histochemical characteristics.

PROBE	PRYMARY TUMOR POSITIVE	METASTASIS POSITIVE	PRIMARY TUMOR AND METASTASIS POSITIVE	PRIMARY TUMOR NEGATIVE	PRIMARY TUMOR AND METASTASIS NEGATIVE
Gal-1 expression	12	11	6	15	10
Gal-3 expression	5	16	3	22	8
Gal-8 expression	7	13	3	20	10
Gal-1 binding	4	3	1	23	21
Gal-3 binding	3	7	1	24	18
p53 expression	5	11	0	22	11
bcl-2 expression	1	3	1	26	24

Table 4. Distances (in μm; mean and standard deviation) between tumor cells in primary testis carcinomas and lung metastases in relation to level of expression of galectins-1, -3, and -8 and of binding sites for galectins-1 and -3.

DISTANCE BETWEEN TUMOR CELLS	EXPRESSION OF gal-1	EXPRESSION OF gal-3	EXPRESSION OF gal-8	BINDING OF gal-1	BINDING OF gal-3
Testis tumor	n=15	n=7	n=12	n=4	n=4
No staining	27.8±17.8	17.5±3.6	31.1±9.1	26.7±10.7	28.7±6.6
Moderate	9.3±1.4	12.7±2.9	9.8±1.2	12.5±1.7	9.7±1.2
Intense staining	24.6±13.9	16.1±15.4	24.2±6.7	29.5±3.1	28.0±6.7
Lung metastasis	n=14	n=17	n=14	n=3	n=7
No staining	14.2±9.8	12.4±2.6	25.5±19,7	20.3±23.4	23.8±9.7
Moderate	10.5±1.9	12.7±3.3	8.7±1.1	10.0±2.0	8.7±0.9
Intense staining	29.0±36.2	10.5±19.6	23.6±10.8	20.3±9.4	23.2±7.6

disease-free interval in comparison to their p53-negative counterparts (Fig. 3). The proliferation rate of the primary tumors measured 41.7±39.5 %, and was in between that of partially devitalized metastases (22.5±32.2%) and that of vital metastases (78.8±15.7%). The proliferation rate of primary tumor cells was significantly lower when the tumor cells expressed galectin-3 ($p<0.01$). This was not associated with the presence of any other marker. None of the reaction profiles of the applied probes displayed a relation to the patients' survival at a statistically significant level ($p>0.05$). Only survival after the resection of lung metastases was associated with the level of tumor cell proliferation rate in lung metastases ($p<0.04$). As explained in the introduction, we next proceeded to analyze staining profiles and syntactic structures in combination.

This approach revealed several parameters, which were related to the patients' total survival and to that after resection of the lung metastases (Table 6). These included percentage of intensely-stained tumor cells (presence of galectins-1 and -8 in lung metastases,

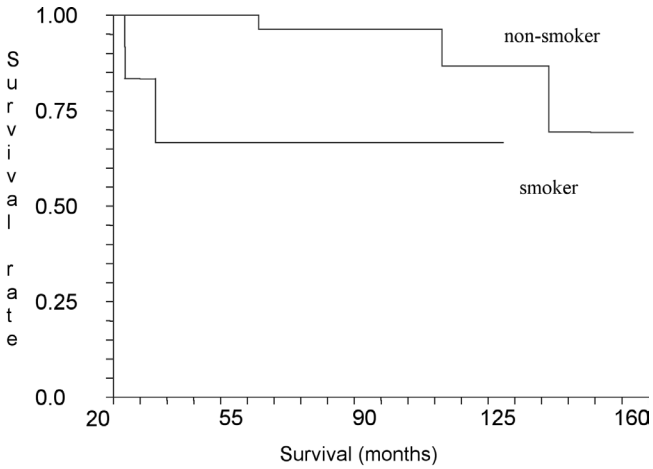

Fig. 1. Survival rate of testis cancer patients and lung metastases in relation to smoking (n=34, $p<0.05$).

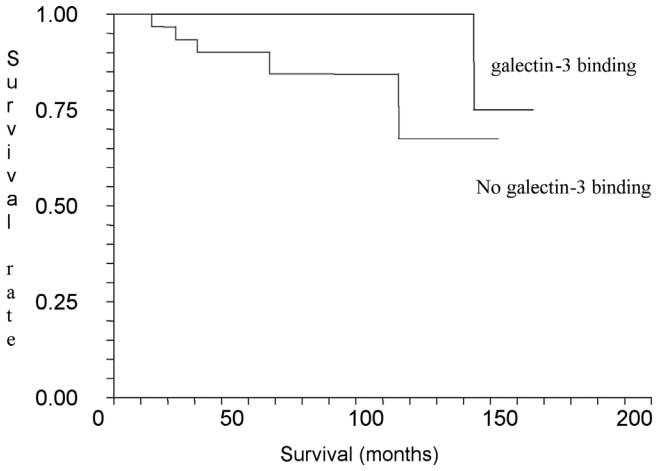

Fig. 2. Survival rate of patients with testis cancer in relation to extent of galectin-3-binding capacities (n=34, $p<0.1$).

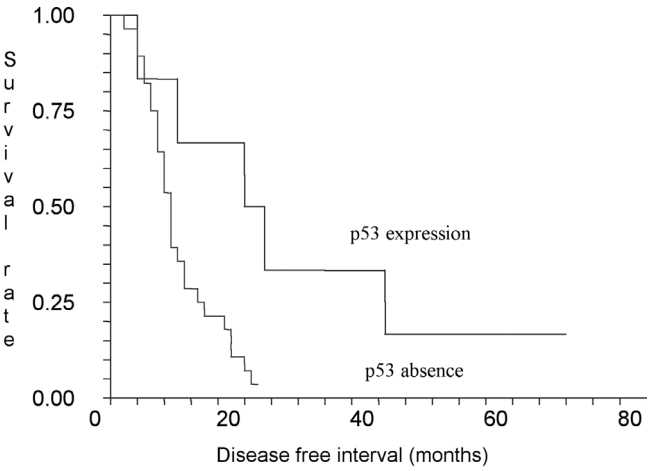

Fig. 3. Disease-free interval of testis cancer patients with lung metastases in relation to expression of p53 in the primary tumor (n=34, $p<0.04$).

Table 5. Cluster radii (in μm; mean and standard deviation) of tumor cells in primary testis carcinomas and lung metastases in relation to level of expression of galectins-1, -3, and -8 and of binding sites for galectins-1 and -3.

CLUSTEER RADIUS OF TUMOR CELLS	EXPRESSION OF gal-1	EXPRESSION OF gal-3	EXPRESSION OF gal-8	BINDING OF gal-1	BINDING OF gal-3
Testis tumor	n=15	n=7	n=12	n=4	n=4
No staining	29.8±44.9	52.8±17.8	30.0±39.2	67.2±16.3	47.7±34.1
Moderate	24.6±5.6	37.8±24.3	47.2±10.6	37.7±18.8	33.2±28.2
Intense staining	55.2±34.3	8.7±23.0	39.4±39.6	-	43.0±50.7
Entropy	122.4±6.8	133.0±13.3	139.7±15.0	133.2±14.8	147.5±24.6
Lung metastasis	n=14	n=17	n=14	n=3	n=7
No staining	33.7±24.4	34.2±14.8	37.2±34.6	30.0±51.9	41.0±39.7
Moderate	37.8±25.1	32.4±19.7	28.0±10.0	27.3±6.8	34.4±15.8
Intense staining	7.0±21.1	4.9±20.3	48.7±38.1	30.3±6.8	16.0±21.7
Entropy	133.5±7.2	132.2±7.4	132.0±24.0	124.3±6.1	138.8±11.6

positive correlation for galectin-1, negative correlation for galectin-8), distance between lymphocytes and intensely- stained tumor cells (parameter dependent on galectins-8 in primary testis tumors), distance between intensely- stained tumor cells (expressing galectin-1 in lung metastases) and cluster radius of moderately-stained tumor cells expressing galectin-3 in lung metastases (the smaller the cluster radius the longer the survival time). Proliferation parameters were mainly associated with the disease-free interval (median distance of proliferating tumor cells, distance between proliferating/non-proliferating cells. Small distances were associated with prolonged disease-free interval). The complete set of statistically significant correlations from the multivariate analysis is given in Table 6.

Discussion

According to the classification of the IGCCCG (International Germ Cell Cancer Collaborative Group) testis tumors are divided into the seminoma and non-seminoma cell- type cases. In both groups, prognosis-associated parameters comprise serum tumor markers (AFP, ß-HCG, LDH) and detection of extra-pulmonary and extra-visceral metastases (International Germ Cell Cancer Collaborative Group, 1997; Foster and Nichols, 1999). The 5-year survival rates range between 90% (good prognosis) to 48% (poor prognosis) (Albers et al., 1995, 1997; Schmoll and Beyer, 1998). The development of lung metastases in patients with testis tumors is not a rare event, and 40 men out of our 50 patients suffered from tumor involvement of the lungs during the pre-operative staging examinations. Presence/absence of lung metastases is not considered to be a prognosis-associated parameter (International Germ Cell Cancer Collaborative Group, 1997). Our study included two different cohorts of patients: the metastasis group (i.e. patients with lung metastases and complete clinical follow-up) and the primary group (patients who represent the control cohort, i.e. without apparent development of secondary lesions). The comparison of histological and clinical features of these two groups leads to the calculation of the relative risk of testis cancer patients to develop lung metastases. From these data, the major risk factors of testis cancer patients for developing lung metastases were computed as follows in descendent order: 1) cell type (non-seminoma versus seminoma; risk: 25.4:1); 2) tumor cell heterogeneity (mixed versus monomorphous; risk: 14.6:1); 3) age of patients (older than 30 years versus younger than 30 years; risk: 4.1:1); 4) pT stage (pT2/pT3 versus pT1; risk: 2.0:1); 5) tumor size (<5 cm versus > 5 cm; risk: 1.2:1).

Young patients with monomorphous seminomas and limited tumor stage apparently possessed a negligible risk for lung involvement in contrast to those with mixed non-seminoma cell type of older age and advanced pT stage.

Having assessed the relative risk of patients with lung metastases, the question arose as to how lung metastases would respond to applied cytostatic drug regimens. Whereas several reports confirmed the remarkable to excellent response of primary testis cancer lesions to the applied cytostatic regimes (PEI or PEB) (Steyerberg et al., 1997), lung metastases are not necessarily prone to exhibit the same response. In our cohort, impairment of viability of the metastatic tumor tissue occurred in only 9 patients, and 41 patients harbored at least partially viable metastatic tumor tissue. Thus, lung surgery will continue to play an important role in a potentially curative therapy in patients with lung metastases of testis cancer. These data reinforce the

Table 6. Multivariate analysis of disease-free interval (DFI), post-surgical survival (PSS, after resection of metastases) and total survival (TS) in 34 patients with testis cancer and lung metastases.

FEATURE	RANK	SIGNIFICANCE
Disease-free interval (DFI)		
Distance between proliferating and non-proliferating cells, (-)*	1	0.0005
Distance between proliferating cells, (-)	2	0.01
Distance between tumor cells expressing galectin-8, (-)	3	0.03
Presence of p53, (+)	4	0.04
Post-surgical survival (PSS)		
Cluster radius of tumor cells expressing galectin-3, (-)	1	0.01
Clean resection of metastases boundary, (+)	2	0.02
Smoking, (-)	3	0.03
Percentage of tumor cells expressing high level of galectin-1, (+)	4	0.03
Percentage of tumor cells expressing high level of galectin-8, (-)	5	0.04
Total survival (TS)		
Distance between tumor cells expressing galectin-1 (metastasis), (+)	1	0.01
Cluster radius of tumor cells expressing galectin-3 (metastasis), (-)	2	0.01
Distance between lymphocytes and tumor cells expressing galectin-8 (metastasis), (+)	3	0.02
Distance between lymphocytes and tumor cells expressing high level of galectin-8 (primary cancer), (-)	4	0.03

* (+): positive; (-): negative correlation; DFI: features relat to properties in primary testicular cancer; PSS: features to those in metastases only.

conclusion of a previous report, documenting that 13% of the examined patients with non-seminomatous lung metastases had viable metastatic tissue (Steyerberg et al., 1997). Of major importance is thus the application of effective chemotherapy to patients after tumor resection. Patients who received a post-surgical cytostatic therapy (after resection of lung metastases) survived significantly longer than those patients, who did not undergo a cytostatic therapy after resection of lung metastases data (not shown).

Referring to the questions given in the introduction, our material has provided a so far rather rare insight as to whether and to what extent selected biochemical features will be maintained in lung metastases of primary testis cancer. The analyzed parameters included quantitative glyco- and immunohistochemical assessment of binding sites for galectins-1 and -3, and of expression of galectins-1, -3 and -8, of p53 and bcl-2 as well as the analysis of tumor proliferation measured by immunohistochemical detection of the Ki-67 antigen. In comparison to the primary cancer, none of these features remained constant in the lung metastases at a statistically significant level (Table 3). This result can be interpreted as to reflect the extent of the influence of the microenvironment yielding site-associated modulation of lectin expression (Vidal-Vanaclocha et al., 1990). Thus, the origin of intra-pulmonary lesions cannot be reliably derived from these features present in testis cancer cells of primary tumors in an individual patient. Although the percentage of marker positivity in primary and secondary testis cancer is indistinguishable at a statistically significant level, individual variations occurred, as we had previously seen in the case of primary breast and colorectal cancer (Kayser et al., 1998, 2002; André et al., 1999). To continue addressing this issue, the option to explore other carbohydrate-binding activities provides a reasonable perspective, as has been documented for lung metastases and mesothelioma (Gabius and Kayser, 1989; Kayser et al., 2001).

Regarding functional aspects of galectins, the routine immunohistochemical detection provided no direct clues for prognosis. This observation also held true for detailed independent morphometric and textural analysis. As explained in the introduction, we introduced a combined analysis of these two data panels. Our report sets an example by showing that detailed texture analysis can reveal several accompanying galectin-related features, which are associated with the median survival of the patients at a statistically significant level (Table 6). These include cluster formation of tumor cells with distinct properties as well as distances between tumor cells and neighboring lymphocytes. Interestingly, these features can pertain to primary and also secondary lesions as well as to different galectins. These results underscore the assumption of a complex network of regulation, which should prompt further cell biological studies. With respect to galectin-8 and its suppressor-like expression profile in colon cancer and proapoptotic activity on the 1299 non-small cell lung cancer model in vitro (Hadari et al., 2000; Nagy et al., 2002), measured expression parameters fit rather readily to an assumed role in prostate cancer progression (Su et al., 1996). In this case, the cell type can apparently play a major role for the regulation of galectin-8 expression in malignancy (Danguy et al., 2001). Concerning the parameter of structural entropy, notably it was in the same range when compared to that of common lung carcinomas and did not differ between primary and secondary testis tumors. Thus, a detailed analysis of the tumor cell growth pattern combined with marker analysis has provided access to a convenient tool to refine prognostic evaluation in this case.

In aggregate, the development of lung metastases of testis cancer is strongly dependent upon the cell type, the cellular composition, and the age of the patient. The survival period is favorable in comparison to that of patients with common lung cancer and is shown to depend on the smoking habit and the proliferation rate of the primary and secondary tumors. This study has explored the potential of adding textural features combined with assessment of galectin-related parameters to this panel. Our data prompt a continuing analysis of different galectin functionality in the context of the "cell sociological" behavior in testicular cancer and beyond this tumor class.

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