APMIS 110: 435–46, 2002
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APMIS
ISSN 0903-4641

Primary colorectal carcinomas and their intrapulmonary metastases: Clinical, glyco-, immuno- and lectin histochemical, nuclear and syntactic structure analysis with emphasis on correlation with period of occurrence of metastases and survival

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Kayser K, Zink S, André S, Schüring M-P, Hecker E, Klar E, Bovin NV, Kaltner H, Gabius H-J. Primary colorectal carcinomas and their intrapulmonary metastases: Clinical, glyco-, immuno-. and lectin histochemical, nuclear and syntactic structure analysis with emphasis on correlation with period of occurrence of metastases and survival. APMIS 2002;110:435–46.

Background. The aim of the study was to correlate clinical factors (disease-free interval/survival) with growth pattern in terms of structural entropy of patients with primary colorectal carcinomas and secondary lung lesions. *Methods.* Proliferation and apoptosis markers as well as determinants involved in information transfer by protein-carbohydrate interactions were monitored. The clinical history, surgical and histopathological reports, tumor load, survival of the patients with a maximum follow-up of 14 years, and sections of paraffin blocks of 60 colorectal carcinoma specimens and their pulmonary metastases were examined. Measurements of the staining intensities after processing sections of primary and secondary carcinomas with the marker panel and calculations of syntactic structure and stereological parameters were performed. *Results.* The majority of primary tumors (80%, 49/60) were surgically treated at advanced tumor stages (pT3/pT4), with detectable lymph node involvement (34/60). Lung metastases were resected after a median disease-free interval of 30.5 months, an average of 3.0 metastases adding up to a mean intrapulmonary tumor load of 9.98 ccm. The median survival was calculated to be 82 months after resection of the colon/rectal carcinomas and 40 months after that of intrapulmonary metastases. It was correlated with certain structural and vascular features such as vascular circumference. The proliferation index and several textural features were strongly associated with vascularization in primary and secondary tumors. *Conclusions.* Despite intra- and interindividual variations, vascularization properties and features such as bcl-2 positivity and CEA- and galectin-3-associated structural entropy in primary tumors or metastases are described as independent prognostic features. Absence of lymph node involvement or limited tumor stages of colon/rectal carcinomas should not exclude patients from thorough postsurgical scrutiny to detect lung metastases.

Key words: Apoptosis; colorectal carcinoma; galectin; lectin; lung metastasis; structural entropy.

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Carcinomas of the colon and rectum are a major cause of cancer deaths in industrialized countries. In fact, colorectal cancer takes second place after lung cancer in men and ranks third after lung and breast cancer in women (1). Thus, colon/rectal cancer is a disease with evident socioeconomic importance (2). Whereas curative treatment of the primary tumor is possible by extensive surgery in the early stages, a not inconsiderable number of patients develop distant metastases, which significantly affects the prognosis (2, 3). In addition to the liver, the lungs are a frequent target for distant metastases of colon and rectum cancer. They are in direct vascular and lymphatic connection with the intestinal tract. Their dense capillary system has been suggested to act as a filter for circulating tumor cells and, in addition, to provide a suitable microenvironment for tumor cell implantation and growth (2, 4). Histological specimens of primary and secondary lesions from the same patient combined with detailed clinical monitoring form the basis for delineation of biochemical factors which are relevant for tumor propagation and survival.

After collecting material from 60 patients for whom duration of disease-free interval and survival were known, we started a retrospective study focusing on the following parameters. First, a detailed syntactic structural analysis and monitoring of the degree of vascularization was performed. For patients with primary lung cancer, measured parameters such as the extent of structural entropy or its current have been reported to correlate with survival (5, 6). The relevance of vascularization, for the prognosis in this tumor entity is an open question. Presence of vessels was visualized by application of a CD34-specific antibody which stains endothelial cells. In addition to these structural aspects, epitope-specific monitoring was performed. As well as the frequently tested tumor markers carcinoembryonic antigen (CEA) and the so-called colorectal-specific glycoprotein CA19/9, the proliferation marker Ki-67 (MIB-1), the apoptosis-related bcl-2 and the suppressor p53 were included to compare characteristics of primary and secondary lesions. Since the lymphokine macrophage migration inhibitory factor (MIF) has been described to be of prognostic significance in primary lung cancer (7), it was pertinent to explore its presence in this tumor class, again exploiting its binding partner sarcolectin for localization.

Based on the fundamental importance of protein-carbohydrate recognition for cell growth regulation, cell-cell/cell-matrix adhesion and cell migration (8–11), we placed special emphasis on characterizing this aspect using three approaches. First, we employed labeled neoglycoconjugates, especially with histo-blood trisaccharides as ligand. These tools detect presence of endogenous binding sites as part of a recognitive interaction system (11–13). Notably, the detection of binding sites is a significant step towards ascribing a function to the expression of such oligosaccharides whose significance in tumor biology has so far remained unclear. By documenting presence of a specific binding capacity, the application of neoglycoconjugates can intimate that the tested oligosaccharide is not merely a tumor marker but a biologically active ligand. Indeed, in primary lung cancer and pulmonary carcinoids especially the presence of binding sites for histo-blood group H-trisaccharide was correlated with a favorable prognosis (6,7). Also, presence of binding sites for the A-trisaccharide and absence of receptor capacity for the Forssman disaccharide were favorable indicators for late occurrence of lung metastases and prolonged median survival after excision of the primary tumor and after resection of the lung metastasis in breast carcinoma patients (14). For comparison, in other tumor classes such as those of the oral cavity and brain, modulation of this parameter has likewise been described to be associated with disease progression (15, 16). This glycohistochemical evidence thus prompts investigations on endogenous lectins, a superfamily of proteins binding glycan epitopes and involved in diverse cellular activities (17). One group comprises the galectins, which recognize β-galactosyl determinants and derivatives thereof in cellular glycoconjugates (18, 19). Especially galectins-1 and -3 with their reactivity to extracellular matrix glycoproteins and pro- versus antiapoptotic and -proliferative potential could play a salient role in the metastatic cascade and evaluation of the prognosis (9, 10, 20, 21). These

Received October 29, 2001.
Accepted February 7, 2002.

aspects have led to the employment of antibodies and labeled galectins when measuring expression of receptor and accessible ligand density in an approach referred to as galectin fingerprinting (20, 22). Combined with the monitoring of other biofactors, for example MIF as determined in cholesteatomas with relevance to recurrence status (23), new insights into molecular aspects of tumor behavior could be provided. Equipped with these probes and the collected clinical data, the sections of the 60 cases with primary and secondary lesions from the same patient were processed.

MATERIALS AND METHODS

Patients and clinical data

For 60 patients who underwent potentially curative resections of colon/rectal carcinomas and their lung metastases (during the period from 1 January 1988 to 31 December 1998) clinical data and paraffin-embedded specimens of the primary tumors and their lung metastases were available. Cases with simultaneously developed metastases from the lung or other organs were excluded from this study. Maximum follow-up after excision of the primary carcinoma was 140 months (range: 13–140 months; median: 84 months) and after resection of lung metastases 100 months (range: 11–100 months; median: 43 months). Detailed follow-up was obtained for all patients included in the study. All patients died from causes directly or indirectly associated with the underlying colon/rectum cancer or its metastases.

Histology, morphometry and data processing

The tissue blocks were cut into 4–5 μm sections and stained with HE, PAS, and Feulgen for tumor classification. The staining intensities obtained after processing with each marker were subjected to quantitative and syntactic structure analysis as well as stereological computations for assessment of vascularization. The entropy (according to (24, 25)) and the current of entropy of the metastases were measured on MIB-1-stained slides using an automated image analyzing system. The self-written programs were based upon the commercially available DIAS programming language (Towersoft, Berlin) and also included syntactic structure analysis of the two-dimensional images. The construction of the minimum spanning tree (MST) readily enabled the assessment of distances between neighboring tumor cells with different staining intensities, between tumor cells and lymphocytes, the degree of cluster formation of tumor cells, the mean diffusion length within a tumor, as well as the MST entropy and the current of MST entropy (lung metastases only). The structure-related entropy is a measure of spatial homogeneity between neighboring so-called basic structural units (for example tumor cells). In ligand or immunohistochemistry, it can be calculated for the distance between and the staining intensities between neighboring tumor cells according to the equation (24, 25):

$$E(MST)=c^* \sum [(dr/dr_m)^2+(di/i_m)^2]$$

c=constant; dr=distance between neighboring tumor cells (centers of gravity); dr_m=mean distance between neighboring cells; di=difference in staining intensities of neighboring cells of the applied probe; i_m=mean staining intensity of the applied probe.

E(MST) is zero if all cells display the same staining intensity and all are located at the same distance from each other. It is independent of the actual structure, for example a ''circle'', a ''column'', a ''tree'' or a ''line'' (24). It should be noted that this equation is also valid for non-stained tumor cells and negatively classified cases.

The current of structural entropy is a measure of the ''produced entropy'' which is removed through the surface of a circumscribed system, for example a tumor. It is a measure of the ''growth'' of structural entropy of a system in relation to its connections with its environment, and can be calculated as follows:

$$CE(MST)=E(MST)*PA/(Sv+Ss)$$

PA=proliferation activity, i.e. percentage of proliferating tumor cells in relation to the tumor volume; Sv=inner (vascular) tumor surface calculated from the length-to-area fraction of intratumorous vessels; Ss=outer (macroscopic) tumor surface, calculated from the three maximum perpendicular diameters of the tumor assuming a ball-like tumor growth which has been shown to be a reasonable approximation for tumor volume and surface (24–26). The details, including procedures to ascertain standardized intensity measurements, have been described elsewhere (24, 25, 27). Information on survival times was obtained after repeatedly sending questionnaires to the house physicians. The survival rates were computed according to Kaplan-Meier (28) using a commercially available statistical package (Number Cruncher Statistical System, NCSS, Kaysville, UT, USA), which was also used for multivariant analysis of data. Parameters restricted to subgroups of cases have not been included in the survival analysis.

Glyco-, immuno- and lectin histochemistry

The presence of binding activities was detected glyco-, immuno- or lectin histochemically with the panel of probes already described in the introduction. Their synthetic preparation or purification, labeling under activity-preserving conditions and set of controls were as described in detail elsewhere (7, 14, 20, 22, 29). All noncommercial probes were applied at a concentration of 10 μg/ml, otherwise the recommenda-

tions of the manufactures were followed. The development of the chromogenic product was based upon the avidin-biotin-technique (ABC), as described previously (6,7,14). Immunohistochemical detection of p53, CD-34 and MIB-1 included microwave pretreatment of the sections (3×5 min at 600 W in citric acid) and was performed according to the manufacturer's recommendations (Biotrend, Cologne, Germany). In both techniques, the conventional peroxidase-anti-peroxidase method was used with horseradish peroxidase and diaminobenzidine/H_2O_2 for visualization of the specifically bound probes. All cases were classified as positive if a dark brown color was seen in all or in clusters of the tumor cells (minimum 10% of all tumor cells visible in the slide). Only slides with a positive reaction were introduced to and processed by the described quantitative analysis. Routine positive and negative controls were carried out, for example by omission of the incubation step with the primary probe to visualize staining due to binding of kit reagents, by competitive inhibition, and by presaturation of antibody fractions with purified antigens. Moreover, the anti-galectin-specific antibodies were routinely checked for lack of cross-reactivity to other homologous galectin family members to avoid false-positive results.

RESULTS

A synopsis of the material is given in Table 1. Mean age of the 39 men was 56.8 years and of the 21 women 55.4 years at excision of the primary colon/rectal cancer. Of the 29 tumors originating from the rectum, 2 cases were staged as pT1 and 7 cases as pT2, of the 31 tumors from the colon, only 2 cases were staged as pT2 and none as pT1. Postsurgical chemotherapy/radiation was performed in 22/60 patients (35%). The disease-free interval for lung metastases and in relation to the intrapulmonary tumor load is presented in Table 2. The mean number of resected metastases was 3.0 with the largest metastasis having a mean maximum diameter of 26 mm. The mean total tumor load of all metastases was computed to be 9.98 ccm. The median CEA level prior to surgery was within the normal range (3.2 ng/ml) and was elevated (>5 ng/ml) only in 24 patients (Table 2).

Assessment of the staining profile using the panel of applied substances is presented in Table 3. No close association between primary carcinomas and their lung metastases with respect to the expression of analyzed binding capacities or detection of epitopes could be found, and the number of cases with intra-individual heterogeneity was fairly high. The quantitative evaluation of vascular features such as surface fraction of vessels (Av) or mean diffusion length (Table 4) revealed no differences between colon and rectum carcinomas compared to the lung metastases. They contained larger vessels and a longer diffusion distance compared to the primary tumors. The

TABLE 1. *Clinical features of the primary colon/rectum carcinomas patients, including sex, tumor stages, postsurgical radiation/chemotherapy (number of cases) as well as mean age of patients and mean maximum diameter of the tumor*

	Colon carcinoma		Rectum carcinoma		Total	
	N	Mean	N	Mean	N	Mean
- Men	21	(age: 58 yr)	18	(age: 59 yr)	39	(age: 58 yr)
- Women	10	(age: 57 yr)	11	(age: 55 yr)	21	(age: 56 yr)
- pT – stage						
- pT1	0		2		2	
- pT2	2		7		9	
- pT3	26		19		45	
- pT4	3		1		4	
- pN – stage						
- pN0	12		14		26	
- pN1	12		8		20	
- pN2	5		4		9	
- pN3	2		3		5	
Radiation/chemotherapy	9		13		22	
Max. tumor diameter		40 mm		40 mm		40 mm (range: 4–80 mm)

TABLE 2. *Median disease-free interval between resection of colon/rectal carcinomas and of subsequent lung metastases (in months), mean number of lung metastases, maximum diameter of largest metastasis (in mm), tumor load (in ccm), and number of patients with elevated CEA serum levels prior to operation of lung metastases*

Parameter	Colon carcinomas (N=31)	Rectum carcinomas (N=29)	Total (N=60)
- pT1/T2 (median)	37.5 months	30 months	35 months
- pT3/T4 (median)	33 months	27.5 months	30 months
- pN0 (median)	32 months	35 months	34 months
- pN+ (median)	39 months	28 months	29 months
Number of metastases (mean)	2.9	3.1	3.0
Maximum diameter (mean)	27 mm	25 mm	26 mm
Tumor load (mean)	10.57 ccm	9.42 ccm	9.98 ccm
Elevated serum CEA (N)	10 patients	14 patients	24 patients

TABLE 3. *Histochemical detection of marker positivity in primary colon/rectum carcinomas and their lung metastases (number of cases)*

Epitope	Primary tumor	Metastasis	Both positive	Both negative	Divergent
p53	17	26	14	31	15
Bcl-2	29	11	6	26	28
Galectin-1-Bin	50	46	38	2	20
Galectin-3-Bin	26	23	9	20	31
Galectin-1-Pres	10	15	1	36	23
Galectin-3-Pres	53	52	45	0	15
Sarcolectin	42	44	30	4	26
A-tri	37	35	20	8	32
B-tri	32	22	10	16	34
H-tri	42	40	27	5	28
Forssman-di	53	30	27	4	29
CEA	56	56	52	0	8
CA19/9	44	45	37	8	15

Explanation: A-tri, B-tri, H-tri: binding capacities for labeled histo-blood group A- (B-, H-) trisaccharide; galectin-1-Bin, galectin-3-Bin, sarcolectin, Forssman-di: binding of galectin-1, galectin-3, sarcolectin, Forssman-disaccharide (expression of galectin-1, galectin-3, sarcolectin, Forssman-di binding capacities); galectin-1-Pres, galectin-3-Pres: presence of galectin-1, galectin-3; p53, bcl-2, CEA, CA19/9: presence of p53 (bcl-2, CEA, CA19/9) detected by immunohistochemistry.

TABLE 4. *Quantitative evaluation of vascular features by Kolmogorov–Smirnov (mean values)*

Vascular feature	Colon carcinoma (N=31)	Rectum carcinoma (N=29)	Metastasis (N=60)
Area (μm^2)	200	205	238 *
Circumference (μm)	42	45	51*
Smallest diameter (μm)	8	8	8.5*
Diffusion length (μm)	33	35	37*
Perivascular matrix (μm)	1	1	2
Surface fraction (Sv) (%)	7	7	7
Area fraction (Aa) (%)	5	4	5

*) statistically significant ($p<0.01$) compared to all primary carcinomas (colon and rectum); data of colon versus rectum carcinomas were statistically indistinguishable ($p>0.05$).

percentage of nuclei with staining for MIB-1, p53 and bcl-2 and their cluster formation was similar in colon and rectum carcinomas. The percentage of proliferating tumor cells was negatively associated with the diffusion length and positively correlated with the vascular surface/area fraction at a statistically significant level (not shown). Lung metastases displayed

TABLE 5. *Quantitative evaluation of tumor cell nuclei stained for presence of MIB-1, p53 and bcl-2 by Kolmogorov–Smirnov (mean values)*

Feature	Colon/rectum carcinoma	Metastasis
MIB-1	(N=60)	(N=60)
Area fraction of proliferating nuclei (%)	13	16*
Percentage of proliferating nuclei (%)	25.2	34*
Distance between proliferating nuclei**	11	12*
Distance between non-proliferating nuclei**	13	15
Distance between proliferating/non-proliferating nuclei**	13	10
Number of proliferating nuclei/cluster	18	21.5*
Cluster radius of proliferating nuclei**	101	90.5
Number of non-proliferating nuclei/cluster	13	12
Cluster radius of non-proliferating nuclei**	88	89.5
p53	(N=17)	(N=26)
Area fraction of stained nuclei (%)	21	10.5*
Percentage of stained nuclei (%)	41	21*
Distance between stained nuclei**	13	11
Distance between negative nuclei**	12	12
Distance between stained/negative nuclei**	11	10
Number of stained nuclei/cluster	21	19
Cluster radius of stained nuclei**	86	94.5
Number of negative nuclei/cluster	17	20
Cluster radius of negative nuclei**	81	80.5
Bcl-2	(N=29)	(N=11)
Area fraction of stained nuclei (%)	3	3.33
Percentage of stained nuclei (%)	11	10
Distance between stained nuclei**	19	18
Distance between negative nuclei**	22	27
Distance between stained/negative nuclei**	17	17
Number of stained nuclei/cluster	37	41
Cluster radius of stained nuclei**	22	32
Number of negative nuclei/cluster	1	0
Cluster radius of negative nuclei**	36	0

*) Statistically significant ($p < 0.01$) compared to all primary carcinomas (colon and rectum). **) distances in μm.

an increased percentage of proliferating nuclei, albeit with nearly identical cluster formation (Table 5). The obtained entropy and current of entropy values based upon the staining intensities of the applied probes are given in Table 6. In agreement with cluster formation, the measurements and calculations yielded data which are indistinguishable between primary carcinomas and metastases for all applied probes, the only exception being staining intensity and distribution of binding sites for the Forssman disaccharide (Table 6).

The median length of the disease-free interval, an indicator of survival (Fig. 1), was not correlated with classic tumor parameters such as pT or pN stage. However, it was negatively associated with the expression of binding capacities for histo-blood group A- and B- trisaccharides as well as with the vascularization of the primary carcinomas. Load of metastases, their vascularization and bcl-2 positivity were also indicators of survival (Table 7). The median survival time was negatively related to the entropy of the distribution of CEA in primary tumors (Fig. 2). Patients with increased tumor load of the lungs and current of entropy calculated for the binding of galectin-3 had a shorter median postsurgical survival time (Fig. 3, Table 7). In aggregate, parameters of vascularization, proliferation, lectin reactivity, and tumor load, and also entropy characteristics, appeared of interest when estimating the median total survival of patients and that after resection of lung metastases (Table 7). Interest-

TABLE 6. *Entropy and current of entropy based on the staining intensities of the applied markers by Kolmogorov–Smirnov (mean values)*

Epitope	Entropy primary tumor	Entropy metastases	Current of entropy (metastases only)
Galectin-1-Bin	124.5	125	5.44 * 10^{-3}/µm^2
Galectin-3-Bin	125	122	4.42 * 10^{-3}/µm^2
Galectin-1-Pres	122.5	124	6.31 * 10^{-3}/µm^2
Galectin-3-Pres	125	126.5	5.43 * 10^{-3}/µm^2
Sarcolectin	126	124	8.72 * 10^{-3}/µm^2
A-tri	123	123	4.22 * 10^{-3}/µm^2
B-tri	128.5	126.5	7.13 * 10^{-3}/µm^2
H-tri	121	118.5	4.79 * 10^{-3}/µm^2
Forssman-di	128	123.5*	5.14 * 10^{-3}/µm^2
CEA	120	119.5	5.19 * 10^{-3}/µm^2
CA19/9	119	123	5.36 * 10^{-3}/µm^2

Explanation: A-tri, B-tri, H-tri: binding capacities for labeled histo-blood group A- (B-, H-) trisaccharide; galectin-1-Bin, galectin-3-Bin, sarcolectin, Forssman-di: binding of galectin-1, galectin-3, sarcolectin, Forssman-disaccharide (expression of galectin-1, galectin-3, sarcolectin, Forssman-di binding capacities); galectin-1-Pres, galectin-3-Pres: presence of galectin-1, galectin-3; bip53, bcl-2, CEA, CA19/9: presence of p53 (bcl-2, CEA, CA19/9) detected by immunohistochemistry; * statistically significant ($p < 0.05$).

ingly, none of the classic parameters (pT, pN, tumor grading) was of statistical significance.

DISCUSSION

Colon cancer is one of the most important types of malignancy in the Western hemisphere. Its incidence is probably associated with dietary features such as fat, fibers, polyunsaturated fatty acids and beer (1, 2). In addition, genetic factors and inflammatory bowel disease place certain persons at increased risk (30–33), although gene alterations have only been found in small percentages of patients with colon/rectum cancer (34). Pulmonary metastases of colon/rectum cancer occur not infrequently and may be detected even after long disease-free periods (3, 35–39).

Whereas the predicted outcome of patients with colon/rectum carcinoma depends upon the stage of the tumor, the indices of apoptosis and mitosis, and additional factors such as mutations of the codons 12,13, and 61 in k-ras or the expression of bcl-2 (2,40–43), the parameters associated with the development of lung metastases and features for estimating the associated disease-free intervals are still not precisely known. In addition, diagnostic distinction of a primary lung carcinoma from metastatic colon/rectum carcinoma can be difficult or even impossible in specific cases, especially when details of the individual clinical history are not available (4).

The results of our study indicate that lung metastases of colon/rectum cancer can occur even in early tumor stages of the primary carcinomas, in agreement with the data of a previous study performed solely on a clinical data set for a total of 110 cases (44). The material in our study is a subset of the previous one. The clinical parameters of these two studies are indis-

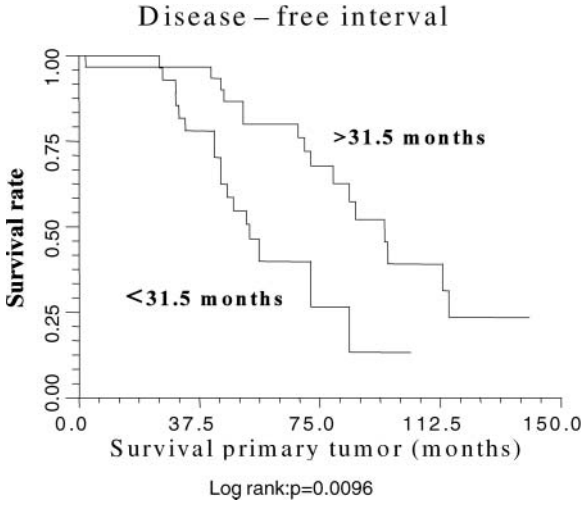

Fig. 1. Kaplan-Meier curves of total survival of patients with metastatic colon/rectum carcinomas (lung metastases) in relation to disease-free interval (N= 60).

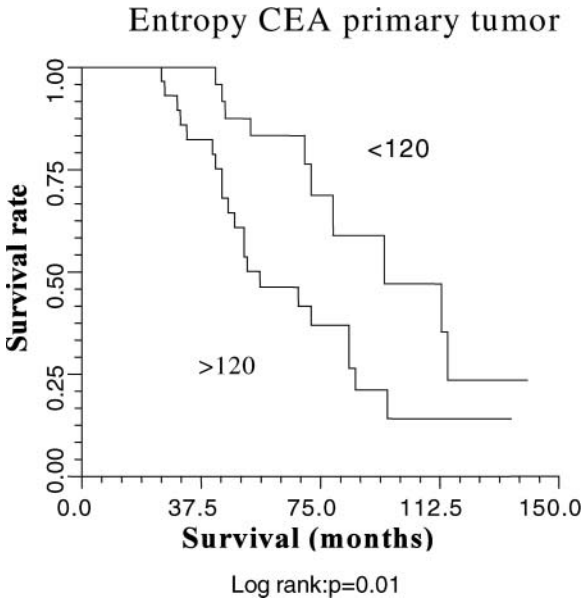

Fig. 2. Kaplan-Meier curves of total survival of patients with metastatic colon/rectum carcinomas (lung metastases) in relation to structural CEA entropy of primary carcinomas (N=60).

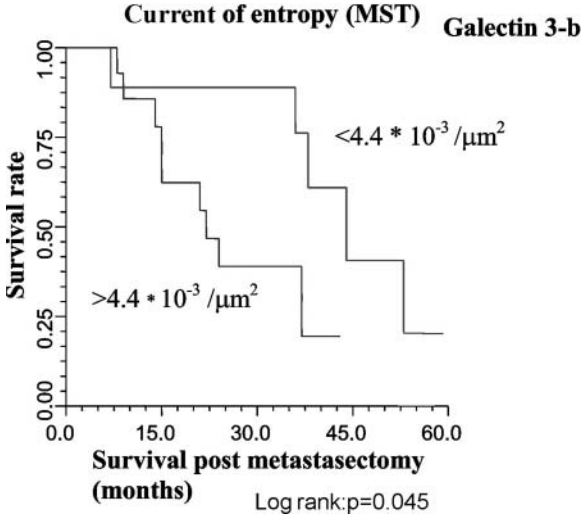

Fig. 3. Kaplan-Meier curves of survival of patients with metastatic colon/rectum carcinomas (lung metastases) after resection of metastases in relation to structural current of entropy of galectin-3 binding in metastases (N=60).

tinguishable from a statistical point of view, and major bias of our present study can be excluded. About 20% of our patients with carcinoma of the large bowel were surgically treated at pT1 and pT2 stages. The median disease-free interval (DFI) was about 31.5 months. The longest period was 99 months, the shortest 4 months only. There were no statistically significant differences in relation to tumor stage or lymph node involvement. In addition, sex or age of the patients had no influence. The percentages of detectable binding capacities of applied substances or expression of antigens did not differ markedly in most cases between the two cohorts of primary tumors and their lung metastases. However, the percentage of divergent cases was high, i.e. no correlation between the respective feature of an individual primary and its secondary cancer was seen (Table 3). These data are in agreement with the results of a study performed on primary breast carcinomas and their lung metastases, and indicate that metastases can reflect the features of their original tumors only at a limited level, e.g. that of a subpopulation (14). Thus, the phenotypic variation between primary and secondary lesions, for example also seen with classical plant lectin histochemistry (42, 43, 45–47), will make it difficult to reliably predict the metastatic phenotype based upon the determined feature of the primary tumor. Moreover, the influence of the microenvironment has also been reported to be an important factor in the modulation of glycohistochemical characteristics (48).

Within the panel of applied probes the antibody CA19–9 has been suggested to specifically bind to carcinomas of the colon/rectum. In this series, only 44/60 primary carcinomas and 45/60 lung metastases were found to react with this marker, and 15/60 cases were divergent. For comparison, CEA was present in 56/60 primary and in 56/60 secondary tumors. In this series, the proliferation and apoptosis-associated features (p53 and bcl-2) were similar in colon and rectum carcinomas. The lung metastases were found to be more active in terms of proliferation (percentage of Ki-67-positive nuclei), displaying the same basic cluster formation for proliferating and resting tumor cells (Table 5). Similarly, the primary tumors expressed p53 and bcl-2 less frequently in comparison with their metastases. Interestingly, colon carcinoma patients with positive bcl-2 expression have been reported to show an improved median survival (39). In this study, the median disease-free interval was longer in patients with detectable bcl-2 in the primary tumor than in the group negative

TABLE 7. *Ranking of parameters influencing disease-free interval, total survival and survival after resection of lung metastases based upon non-hierarchic multivariant analysis*

	Rank	p	Power (Z-value)
Total survival			
Disease-free interval	1	0.00030	+3.61
Tumor load of metastases	2	0.00258	−3.01
Minimum vascular diameter in metastases	3	0.00548	−2.78
Entropy of CEA in primary tumors	4	0.0315	−2.15
Presence of bcl-2 in metastases	5	0.048	+1.95
Disease-free interval			
Features of primary carcinomas			
Vascular circumference (μm)	1	0.008	−2.76
B-tri binding	2	0.018	−2.67
Cluster radius negative nuclei (MIB-1)	3	0.025	−2.62
A-tri binding	4	0.04	−1.36
Survival after metastasis resection			
Current of entropy Gal-3-binding (metastases)	1	0.0085	−2.63
Entropy B-tri binding (metastases)	2	0.0216	−2.30
A-tri binding (primary tumors)	3	0.0469	−1.99
Tumor load (metastases)	4	0.048	−1.83

for this parameter, and the survival time after resection of lung metastases was again improved in comparison with their negative counterparts.

Several investigations analyzing the survival of patients with lung metastases of various origins reported that the number of resected metastases is significantly (negatively) associated with the prognosis (4, 14, 35, 37, 39). The tumor load of the host organ, i.e. the total volume of all resected metastases, is probably a more accurate measure. In agreement with these data, this parameter has a highly predictive value when estimating the patients' total survival and, although marginal, that after resection of lung metastases in our material.

The extent of tumor vascularization has been reported to be an additional prognosis-associated parameter in colon cancer patients (2, 36, 49), although this parameter is frequently not quantitatively determined. Within the panel of measured vascularization-associated features of the primary tumors, the absolute value of the vascular circumference had the strongest association with the disease-free interval, and the smallest vascular diameter measured in the metastases a high correlation with the total survival. In absolute terms, the mean diffusion length is rather long in colon/rectum carcinoma metastases. Compared to in primary lung carcinomas and tumors derived from the carcinoid family (50) this parameter is distinctive for colorectal cancer lesions. In addition, the percentage of proliferating cells and their area fraction was strongly (negatively) associated with diffusion length and (positively) with vascular surface fraction. These data indicate that highly vascularized tumors possess increased numbers of proliferating tumor cells. With respect to primary lung carcinomas, the reported data on vascularization and prognosis do not yet allow any definitive conclusions to be reached (51).

Calculations to determine prognosis-associated features by use of multivariant analysis showed that especially measures of structural entropy can correlate both with disease-free interval and patients' survival after resection of lung metastases. This is in line with data on carcinoids and common lung carcinomas (6, 14, 50). These measurements indicate that not only the expression of certain binding capacities in tumor cells, but, in addition, the intratumoral arrangement of tumor cells with different staining intensities and the derived architecture has clinical relevance. In tumors with substantial differences in comparison with the structural organization of their host organ the energy transport through the inner and outer surfaces is high. Owing to less efficient energy use highly malignant tumors will transfer large amounts of energy through their surfaces, a phenomenon

which has been suggested especially in the case of bronchial malignancies (24).

In aggregate, patients with colon/rectum carcinomas can develop lung metastases both in the early tumor stages and after a long disease-free interval. Therefore, a thorough follow-up of these patients with close inspection for potential lung metastases is indicated. Predictive parameters include the binding capacities of two histo-blood group antigens, i.e. A-, and B-trisaccharides, the density of vascularization, and structural tumor features. These parameters also appear to be of interest for predicting the survival period of the patients after excision of the lung metastases. When examining characteristics of metastases it should be noted that when averaged the level of interindividual variation will set limits to the achievement of reliable predictions derived from properties of metastases.

The generous financial support of the Dr.-M.-Scheel-Stiftung fuer Krebsforschung, the Wilhelm Sander-Stiftung and the Verein zur Foerderung des biologisch-technologischen Fortschritts in der Medizin e.V. is gratefully acknowledged. We thank S. and B. B. Namirha for helpful discussions.

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