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Galectins-1 and -3 and their ligands in tumor biology

Non-uniform properties in cell-surface presentation and modulation of adhesion to matrix glycoproteins for various tumor cell lines, in biodistribution of free and liposome-bound galectins and in their expression by breast and colorectal carcinomas with/without metastatic propensity

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Abstract Protein (lectin)-carbohydrate (cellular glycoconjugate) recognition is operative in biochemical information transfer. Galectins constitute a family of endogenous galactoside-binding lectins with conserved features in the binding site. The members of this lectin category are assumed to be involved in cell adhesion and growth regulation. To assess to what extent the different modes of binding-site presentation and/or carbohydrate fine-specificities will affect aspects of galectin behavior, homodimeric cross-linking galectin-1 and monomeric chimeric galectin-3, with its collagenase-sensitive stalk linked to the carbohydrate-recognition domain, were investigated. Cell-surface expression of the two galectins and accessible galectin-binding sites on various tumor cell lines was ascertained by FACScan analysis. In particular, ligand accessibility for the two galectins differed for the tested cell line types. Binding of tumor cells to laminin and plasma or placental fibronectin was generally reduced by treatment of cells or matrix with galectins. Galectin-3 was more efficient than galectin 1 at impairing laminin's potency as matrix. Cell binding of galectin-1, on the other hand, proved on average more effective for blocking cell association to fibronectins after its preincubation with cell suspensions. Differences were also apparent in the biodistribution of the galectins, where an avian homolog of galectin-1 served as the control to distinguish effects of spatial and sugar-binding features. Histopathological analysis of lymph-node-negative and -positive breast and colorectal carcinomas (n = 180 including 60 metastatic lesions) indicated a correlation of either increased galectin-1 binding and reduced galectin-3 expression or reduced binding of both galectins with the occurrence of lymph node lesions. Together with data on the heparin-binding lectin, revealing reduced expression to be associated with a positive lymph-node status in the breast cancer group, these results can be interpreted to reflect cell-type-dependent requirements of galectin ligand presentation during the metastatic cascade. By introducing mammalian lectins to lectin-histochemical studies, the detection of quantitative differences in glycosylation brings an understanding of its cell biological significance one step closer.

Key words Cell adhesion · Galectin · Lectin · Liposome · Matrix glycoproteins · Metastasis

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Introduction

An impressive wealth of evidence assigns to the extracellular matrix a large variety of functional aspects in the realm of cell sociology with relevance for tumor biology. Regulation of cell adhesiveness and migration requires an intricate interplay between determinants presented on cell surfaces and this network, most attention often being paid to peptide motifs of integrins, cadherins or cell-adhesion molecules of the immunoglobulin superfamily. When taking stock of the implications of theoretical

considerations for the coding capacity of biomolecules (Laine 1997), a pertinent lesson to be learned is that oligosaccharides are second to no other class of biochemical oligomer in this ability. Consequently, their presence in the glycocalyx should not only be considered from a physicochemical point of view, focusing on contributions to solubility or resistance against proteolytic attacks, but also with regard to information transfer (Varki 1993; Dwek 1996; Hooper et al. 1997; Kopitz 1997; Sharon and Lis 1997; Reuter and Gabius 1999). To this end, suitable receptors are expected to be expressed in tissues. Indeed, the last two decades have witnessed rapid progress in the purification and classification of mammalian lectins (Ashwell and Morell 1977; Gabius 1991, 1997a; Zanetta 1997; Kaltner and Stierstorfer 1998; Lis and Sharon 1998). Among them, galectins have the notable capacity to recognize spatially accessible terminal sections of *N*- and *O*-glycans as well as glycolipids that present galactosides, even in the presence of α2,3-sialylation (Caron et al. 1990; Barondes et al. 1994; Kasai and Hirabayashi 1996; Gabius 1997a; Hirabayashi 1997; Perillo et al. 1998). Intriguingly, structural features of their binding sites are not the only properties that can differ despite a common framework. The binding-site presentation also constitutes a further level of variability, though the purpose of this is still rather enigmatic. While galectin-1, for example, is a homodimer and a potent cross-linker by non-covalent association of the two binding sites, galectin-3 harbors a chimeric design with an N-terminal stalk as accessory module, characterized by Pro-, Gly-, Tyr-rich sequence repeats (Gabius 1997a; Hirabayashi 1997). Since cell immortalization, transformation and natural or chemically induced differentiation can markedly and differently affect the expression of galectins-1 and -3 in vitro (Crittenden et al. 1984; Gabius et al. 1985a, 1989; Leenen et al. 1986; Raz et al. 1987, 1988; Gabius and Vehmeyer 1988; Agrwal et al. 1989; Lotan et al. 1989; Nangia-Makker et al. 1993; Hébert and Monsigny 1994; Gaudin et al. 1997; Gillenwater et al. 1998), they may act as molecular switches contributing to processes during the acquisition of distinct phenotypes. Fittingly, the introduction of histopathological and blotting analysis of tumors for monitoring galectin expression in situ has fully corroborated this notion (Gabius et al. 1986a, b), and further application of these methods presently argues in favor of the two currently investigated galectins being involved in malignant phenotypes (Gabius 1997b; Itzkowitz 1997; Ohannesian and Lotan 1997).

As part of a molecular recognition system, galectins will exert their effects by binding to a cellular ligand and through the ensuing post-binding events (Villalobo and Gabius 1998). The spectrum of glycoconjugates with in vitro ability to react with galectins-1 and/or -3 has increased steadily, encompassing a variety of relevant molecules for cell adhesion and migration. Though galectins-1 and -3 were first referred to as major non-integrin laminin-binding proteins (Cherayil et al. 1990; Woo et al. 1990; Zhou and Cummings 1990; Lee et al. 1991), reactivity of one or both galectins has been reported with glycans of a cell-adhesive and secreted protein identical with human lung tumor L3 antigen (Rosenberg et al. 1991; Inohara and Raz 1995; Inohara et al. 1996; Sasaki et al. 1998), tissue fibronectin (Sato and Hughes 1992; Ozeki et al. 1995; Ellerhorst et al. 1999), immunoglobulin E glycoforms, and their high-affinity receptor (Cherayil et al. 1989; Laing et al. 1989; Robertson and Liu 1991; Frigeri et al. 1993), the $\alpha_7\beta_1$ and $\alpha_1\beta_1$ integrins as well as the α subunit (CD11b) of the CD11b/CD18 integrin (Gu et al. 1994; Dong and Hughes 1997; Ochieng et al. 1998a), with a *β-N*-acetyllactosamine-containing glycolipid and the ganglioside GM_1 (Mahanthappa et al. 1994; Kopitz et al. 1998), lysosome-associated membrane glycoproteins-1 and -2, carcinoembryonic antigen and nonspecific cross-reacting antigen (Do et al. 1990; Skrincosky et al. 1993; Ohannesian et al. 1994, 1995; Inohara and Raz 1995; Yamaoka et al. 1995), with core 2-O-glycans (Baum et al. 1995a), myelin-associated glycoprotein and tenascins-R and -C (Probstmeier et al. 1995), gastrointestinal mucin (Bresalier et al. 1996; Wasano and Hirakawa 1997) and a glycoprotein with partial similarity to the heat-shock protein hsp90 (Chadli et al. 1997). However, one must add a note of caution: the positive response in an in vitro assay will not necessarily translate into a definitive interaction in vivo. The assay conditions, involving types and densities of test substances different from in vivo conditions, have an undeniable impact on the result. This reasoning calls for independent lines of evidence to confirm unequivocally the relevance of the data obtained, as instructively discussed in a similar context for another class of lectins (selectins) recently (Varki 1994).

One validated approach to testing the biological efficiency of a presumed in vivo ligand is to determine cellular responses in the absence and presence of purified substances that block access to it. The attachment to cell matrix constituents is especially important in tumor biology, as it governs adhesiveness and invasiveness. However, the available data on galectins as modulators of this activity do not appear to obey simple rules. Promotion of cell binding to laminin has been reported for F9 mouse teratocarcinoma and Chinese hamster ovary cells (Zhou and Cummings 1993), rat olfactory neurons (Mahanthappa et al. 1994) and A375/A2058 human melanoma cells (van den Brule et al. 1995a) in the presence of galectin-1, as well as for human neutrophils, but not for the two just-mentioned melanoma cell lines in the presence of galectin-3 (van den Brule et al. 1995b; Kuwabara and Liu 1996). Its molecular design as a homodimer, with the ligand-binding sites at opposing ends of the protein, make galectin-1 an efficient cross-linker (Mandal and Brewer 1992). Evidence for monomeric galectin-3 forming aggregates to attain a comparable functionality has been presented (Hsu et al. 1992; Massa et al. 1993; Ochieng et al. 1993; Mehul et al. 1994). In the presence of a sugar ligand occupying

the binding site in the C-terminal section, these contacts are established nearly exclusively via the N-terminal part of galectin-3 (Kuklinski and Probstmeier, 1998; Yang et al. 1998). In addition to a bridging function, consequences of galectin-triggered cell activation, at present ill-defined, can contribute to increased Ca^{2+}-dependent adhesiveness (Kuwabara and Liu 1996). Interestingly, galectin-3-gene-transformed breast carcinoma cells were found to display an elevated $\alpha_6\beta_1$ integrin surface expression (Warfield et al. 1997). The increased adhesion to laminin and invasion of a Matrigel barrier of this subclone is in line with the enhancement of migration of human breast cancer cells in a paracrine manner by matrix-bound galectin-3, at an early, but not at a late stage of malignancy (Le Marer and Hughes 1996). However, the presence of galectin-3 on the cell surface will not automatically bring about cell adhesion to laminin, as shown for SCM-153 human breast epithelial cells (Ochieng et al. 1992). When operative, laminin is not the only molecule playing a bridging role in adhesion. Human fibronectin has also been demonstrated to serve as a target in the case of galectin-1 and rhabdosarcoma cells (Ozeki et al. 1995). Nonetheless, positive galectin effects should not be anticipated in each case. The adhesion to the matrix of galectin-1-saturated A121 ovarian carcinoma cells is impaired (Allen et al. 1990). Likewise, negative effects are substantiated by the loss of cell-substratum (laminin) adhesion for galectin-1-secreting C2C12 mouse muscle cells (Cooper et al. 1991) as well as by the blocking of cell attachment of baby hamster kidney cells to laminin (45 μg/ml; Sato and Hughes 1992) and of A5 mouse spindle-cell carcinoma, PC-3 human prostate carcinoma and HT-1080 human fibrosarcoma cells to laminin, fibronectin and collagen IV by galectin-3 (100 μg/ml; Ochieng et al. 1998a).

Albeit confusing at present, these data unanimously underscore the potential importance of galectins and their binding capacity for functional aspects of tumor cells. Obviously, the dependence of these parameters on the tumor cell type and the ensuing consequences for adhesion to matrix constituents warrant further investigations. The cell assays should be performed under the same conditions with aliquots of the same cell batches and matrix glycoprotein preparations to avoid results being confounded by any parameter change. As matrices, we have deliberately chosen a strongly glycosylated form of laminin and tissue/plasma fibronectins, placental fibronectin containing more poly-*N*-acetyllactosamine and galectin-reactive α2,3-sialylated N-chain termini than its less reactive plasma variant (Krusius et al. 1985; Zhu and Laine 1985; Takamoto et al. 1987; Sato and Hughes 1992; Ozeki et al. 1995). To probe the effect of galectins, we have saturated cell surfaces and matrix separately, with no further addition of Ca^{2+} to the incubation buffer. The availability of purified and labeled galectins also enabled us to deduce their biodistribution in vivo and to use them in histopathological studies. Accordingly, the following issues have been addressed systematically in this study: quantification of cell-surface expression of galectins-1 and -3 and accessible binding sites in a panel of tumor cell lines with different histogenetic background, the effect of the galectins on adhesion to matrix glycoproteins with either cell or matrix saturation by preincubation with galectins, the biodistribution of free or liposome-bound galectins to assess organ uptake via contact to endothelium, and the monitoring of expression of galectins and galectin-reactive sites in primary lesions of two carcinoma types and their lymph node metastases. The results indicate that a modulation of galectin-reactive glycans is an important factor for adhesive interactions with cell-type-dependent differences.

Materials and methods

Lectin and antibody preparation and labeling

Galectin-1 was purified from human placenta and bovine heart by affinity chromatography on lactosylated Sepharose 4B, obtained by divinyl sulfone activation as the crucial step, as described in detail elsewhere (Gabius et al. 1985b; Gabius 1990). The periplasmic fraction of expression-vector(prCBP35s)-carrying *Escherichia coli* JA221 cells (Agrwal et al. 1993), kindly provided by Prof. J.L. Wang (East Lansing, Mich., USA), was the source of recombinant murine galectin-3. The chicken galectins CG-14 and CG-16 were purified from liver and intestine by affinity chromatography, employing anion-exchange chromatography on a Mono Q column (1 ml bed size; Pharmacia, Freiburg, Germany) as a suitable tool to remove residual impurities, and one- and two-dimensional gel-electrophoretic analyses were routinely performed to ascertain the purity of each batch of galectin (Schneller et al. 1995). The galectins were labeled under activity-preserving conditions with either biotinyl-*N*-hydroxysuccinimide ester or chloramine-T, as described (Kojima et al. 1990; Gabius et al. 1991a, 1992; Kayser et al. 1992, 1996). Polyclonal antibodies to the mammalian galectins were raised in rabbits, purified from serum by affinity chromatography on protein-A–Sepharose-4B and checked for specificity and lack of crossreactivity in cell and blotting assays, as described elsewhere (Bardosi et al. 1989; Kopitz et al. 1998). Antibodies against the heparin-binding lectin were raised similarly after purification of the lectin from human placenta, as described (Kohnke-Godt and Gabius 1991).

Cell lines and flow-cytofluorimetric measurements

The mouse melanoma lines B16-F1 and B16-F10, the mouse monocyte/macrophage line J774 A.1, the human ovary adenocarcinoma line NIH-OVCAR 3, the human ductal breast carcinoma line T-47D and the human colon adenocarcinoma cell lines COLO 205 and SW480/SW620, the latter originating from a primary tumor and lymph node metastasis of the same patient, were purchased from the American Type Culture Collection (Rockville, Md., USA) and cultured, as recommended by the distributor. The human lung cancer lines HS-24 (epidermoid carcinoma) and SB-3 (metastasis of an adenocarcinoma to the suprarenal gland) were kindly provided by Prof. E. Spiess and Prof. W. Ebert (Heidelberg, Germany) and kept in culture, as described (Erdel et al. 1990a,b). Tumor cells were thoroughly washed with Dulbecco's phosphate-buffered saline (PBS) solution containing 0.1% carbohydrate-free bovine serum albumin to remove any potentially interfering glycoproteins and to block any non-specific protein-binding sites prior to the incubation with labeled galectins (25 μg/ml and 50 μg/ml) or anti-galectin antibodies (1:25 or 1:50 dilution) for 30 min at 4°C. Signal development with streptavidin–R-phycoerythrin conjugate or fluorescent anti-(rabbit immunoglobulin G) (1:40 dilution; Sigma, Munich, Germany) and the quantitative assessment in a

FACScan instrument (Becton-Dickinson, Heidelberg, Germany) were done, as described (André et al. 1997; Kojima et al. 1997).

Adhesion assay

To establish a matrix with adhesive properties, the adhesion molecules fibronectin and laminin were coated onto the surface of polystyrene microtiter plate wells. Human placental and plasma fibronectin were purified by gelatin-affinity chromatography as the central step, as described (Hayashi and Yamada 1982; Zhu et al. 1984). Murine Engelbreth-Holm-Swarm (EHS) laminin with its abundance of bi- to tetraantennary *N*-glycans was kindly provided by Prof. R. Timpl (München, Germany). Glycoprotein adsorption was carried out for 12 h at 4°C from a 50-µl solution of 1 µg/ml in 20 mM PBS, pH 7.2. Further steps were performed as described for cell binding to neoglycoproteins (Gabius et al. 1990a). Briefly, after thorough washing, the remaining protein-binding sites were saturated with 150 µl buffer solution containing 1% carbohydrate-free bovine serum albumin for 1 h at 37°C. Careful removal of this solution and three washing steps with buffer solution containing 0.1% carbohydrate-free bovine serum albumin finished the matrix preparation. The effect of galectins on cell adhesion to each glycoprotein was assayed by preincubation of the matrix with 50 µl galectin-containing solution (5–150 µg/ml) for 30 min at room temperature, and preincubating the cell suspensions with galectins for 15 min at room temperature. The reaction mixtures were subsequently centrifuged and the cells were washed to remove free galectins from the solution, and the two incubation steps were combined, maintaining presence of galectins during the coincubation of the cells with the matrix. This step lasted 2 h at 37°C. Brief washes to remove non-adherent cells and trypsinization of adherent cells preceded quantification of the cell number in a Neubauer chamber. Control experiments with aliquots of the same cell batch undergoing mock treatment without galectin to maintain identical conditions throughout the processing served to assess the normal level of cell adhesion (100%).

Biodistribution of radioiodinated galectins and galectin-bearing liposomes

Biodistribution in male ddY mice bearing solid Ehrlich tumor was evaluated after 1 h, 3 h and 6 h following injection of 5 µg radioiodinated protein (1×10^4–2×10^4 Bq) into the tail vein, and expressed as the percentage of the injected dose retained per gram of wet tissue or per milliliter of blood, as described in detail previously (Kojima and Gabius 1988; Kojima et al. 1990). In addition to monitoring the behavior of free galectins, that of their conjugates with liposomes was similarly analyzed. Liposomes were prepared by the modified controlled cholate dialysis method, with dipalmitoylglycerophosphocholine, cholesterol, dicetylphosphate and gangliosides at a molar ratio of 35:45:5:15 as starting material and a lipid-to-detergent ratio of 0.6, as described in detail elsewhere (Yamazaki et al. 1992, 1994). Following fractionation by diafiltration on a Diaflo PM10 membrane (Amicon, Witten, Germany) and gel-permeation chromatography on Sephacryl S1000 (superfine; Pharmacia, Freiburg, Germany) to yield a liposome fraction with a mean diameter of 100 nm, galectins were covalently coupled to the surface of the liposomes at a yield of 0.3 g/g liposome fraction by a two-step procedure involving initial periodate oxidation of sensitive carbohydrate moieties of the gangliosides and galectin attachment by sodium-cyanoborohydride-mediated reductive amination, as described in detail previously (Heath et al. 1981; Yamazaki et al. 1994).

Immuno- and lectinohistochemistry

Sections (4–6 µm thick) of formalin-fixed and paraffin-embedded specimens of primary breast cancer (mean age of patients: 62 ± 13 years) and colorectal cancer (mean age of patients: 68 ± 12 years), without and with a clinically proven capacity for metastasis to the lymph nodes (30 cases of each group with a total of 180 specimens), were processed according to an optimized protocol. This included a series of steps, such as blocking of endogenous peroxidase activity as well as biotin-binding and protein-binding capacity, prior to serial incubations with the markers, ABC kit reagents and the chromogenic substrates diaminobenzidine/H_2O_2, as described (Kayser et al. 1994, 1996; Kaltner et al. 1997). Specificity controls included the omission of the probe in the standard processing to delineate signal generation by binding reactions other than probe-dependent processes, haptenic inhibition for the galectins, and presaturation with an excess of galectin for the galectin-specific antibodies. The histochemical reaction was classified to be positive if all or at least clusters of tumor cells showed a dark brown staining, while concomitant controls revealed no or negligible deposition of insoluble dye.

Results

Cell-surface expression of galectins and accessible galectin ligands

Flow-cytofluorimetric analysis affords the opportunity to determine accessibility of surface epitopes for selected probes. Cells of different histogenetic origin and relation to metastasis were quantitatively monitored for surface expression of galectins-1 and -3 by specific non-crossreactive antibodies and of accessible galectin-binding sites by the biotinylated lectins. The scans, as shown in Fig. 1, provided evidence for expression of the respective binding sites on the cell surface with cell-type-dependent variability and no evidence for subpopulations with markedly different levels of expression (Table 1). The two lines from primary colon carcinomas (SW480, COLO 205) displayed a disparate capacity to bind

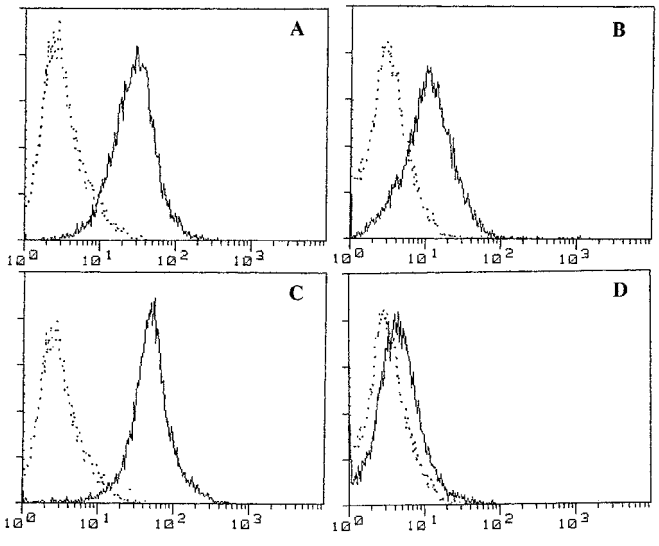

Fig. 1A–D Analysis of cell-surface expression of galectins and accessible galectin ligands by FACScan, employing non-crossreactive antibodies to galectins-1 and -3 (**A, C**) at 1:50 dilution and biotinylated galectin-1 (**B** 50 µg/ml) and galectin-3 (25 µg/ml) on aliquots of suspensions of the cell lines J774A.1 (**A, C**) and SW620 (**B, D**). Fluorescence intensity was measured in the presence (—) and in the absence (- - -) of the marker (antibody or biotinylated galectin)

Table 1 Flow-cytofluorimetric analysis of binding of galectins (25/50 μg/ml) and anti-galectin antibodies (1:50/1:25) to murine and human tumor cell lines. The median fluorescence of binding of the fluorescent probes in the absence of any marker (lectin or antibody), referred to as background binding, varied from 4.6 (B16-F10, SW480) to 11.3 (J774A.1) for the phycoerythrin-streptavidin conjugate and from 5.9 (COLO 205) to 15.4 (B16-F1) for the fluorescent anti-IgG second-step reagent

	Anti-galectin-1 (dilution 1:50/1:25)		Galectin-1 (human) (25/50 μg/ml)		Anti-galectin-3 (dilution 1:50/1:25)		Galectin-3 (murine) (25/50 μg/ml)	
Type of cell line	Median fluorescence	Positive cells (%)	Median fluorescence	Positive cells (%)	Median fluorescence	Positive cells (%)	Median fluorescence	Positive cells (%)
B16-F1	18.3/24.3	7.5/10.2	81.0/149	47.1/63.6	20.3/30.5	10.0/13.1	9.7/9.3	11.4/7.5
B16-F10	11.2/10.9	6.8/5.8	36.9/72.5	49.2/63.2	15.2/12.1	8.8/8.8	7.9/21.2	9.1/3.5
J774A.1	38.5/57.4	86.3/89.9	36.1/32.3	55.5/50.1	67.8/67.0	91.6/92.2	46.2/49.6	63.6/56.1
NIH-OVCAR 3	22.9/22.0	6.4/6.6	53.9/66.6	46.3/45.5	24.3/24.2	8.7/9.6	92.0/866	50.9/82.2
T-47D	25.7/35.1	25.3/27.7	54.3/62.1	28.1/28.0	19.6/24.2	19.9/23.1	8.3/9.5	11.6/10.5
COLO 205	9.7/12.2	10.1/15.6	28.2/33.1	45.9/63.8	13.9/17.1	23.4/37.7	6.7/6.3	7.3/6.5
SW480	6.9/19.7	8.4/16.9	4.2/13.8	5.1/2.3	13.1/19.3	28.1/42.4	8.3/6.8	40.9/35.8
SW620	8.3/11.5	19.9/26.4	4.2/4.2	54.1/63.9	8.1/13.4	32.5/37.9	4.0/4.1	27.0/62.4
HS-24	17.2/21.3	14.2/12.9	14.1/17.4	14.4/15.9	15.4/17.7	17.5/18.7	12.4/10.8	10.2/8.0
SB-3	13.8/18.6	18.9/26.8	22.2/23.7	20.3/47.5	10.4/11.8	7.2/7.9	10.2/12.5	34.4/40.3

labeled galectins, precluding the possibility that results could be generalized within a certain tumor class. Percentages of cells positive for galectin features were also different for the two colon cancer lines from primary and secondary lesions, whereas this was not apparent for the parental and metastatic murine melanoma lines. The presence of glycoinhibitors (0.5 mg asialofetuin/ml and 0.1 M lactose) drastically reduced or completely abolished cell binding of galectins, indicating the inherent sugar specificity of the process involved. Presaturation of the antibody fractions with the respective galectins led to a similar result, yielding background levels in cytofluorimetric scans. To check that the cell surface of the tumor cell populations indeed displayed the implied binding capacity for β-galactosides, a neoglycoprotein (lactosylated bovine serum albumin) and the natural glycoprotein asialofetuin were employed as suitable probes. At 50 μg (neo)glycoprotein/ml, specific binding was invariably detected (not shown). The presence of galectins, galectin-binding sites and operative β-galactoside-binding sites prompted us to study the impact of galectins on the capacity of this panel of tumor cells to adhere to the matrix glycoproteins laminin and fibronectin.

Influence of galectins on tumor cell adhesion to matrix glycoproteins

In order to assess the relevance of galectin-binding epitopes on the matrix and the cell surface separately, both assay constituents were saturated separately by a preincubation step with galectin. Moreover, adhesion assays were performed with galectin-saturated matrix and cells. Mock-treated aliquots of individual cell batches served as controls, to calculate the relative impact of galectin treatment, graphically given in Figs. 2–4 for a medium concentration. The abundantly *N*-glycosylated laminin proved to be a potent substratum for cell adhesion. As a tendency, galectin-3 was more capable of reducing cell adhesion than cross-linking galectin-1, when bound to the matrix glycoprotein (Fig. 2). Galectin saturation of reactive sites on the cell surface reduced

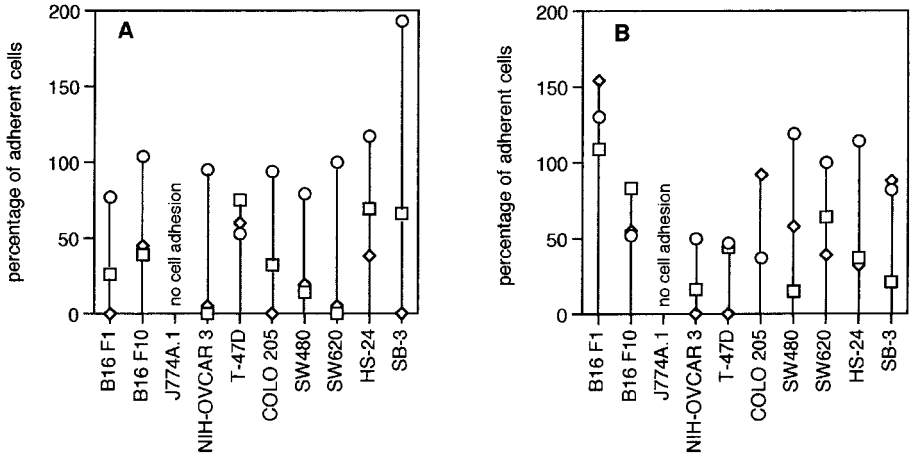

Fig. 2A, B Comparison of the effect of preincubation of surface-immobilized laminin (○), cells (□) or cells and matrix (◇) with galectin-1 (**A**) or galectin-3 (**B**) at a concentration of 25 μg/ml on the percentage of adherent cells relative to mock-treated controls

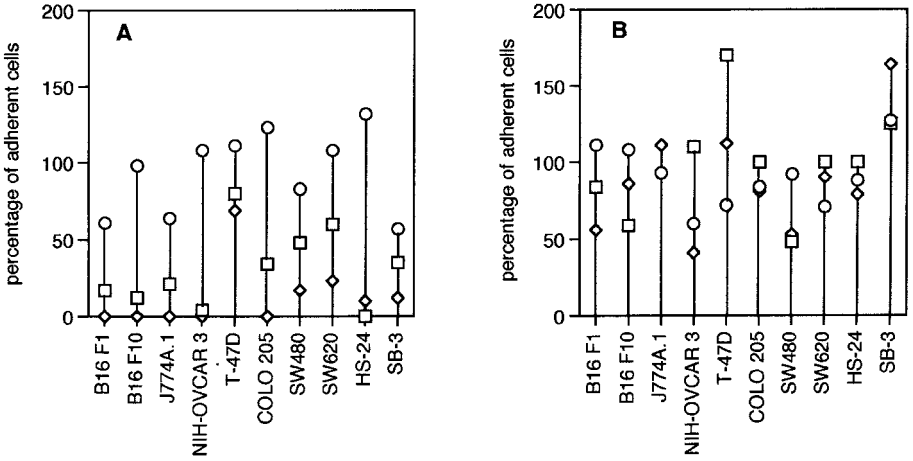

Fig. 3A, B Comparison of the effect of preincubation of surface-immobilized plasma fibronectin (○), cells (□) or cells and matrix (◇) with galectin-1 (**A**) or galectin-3 (**B**) at a concentration of 25 μg/ml on the percentage of adherent cells relative to mock-treated controls

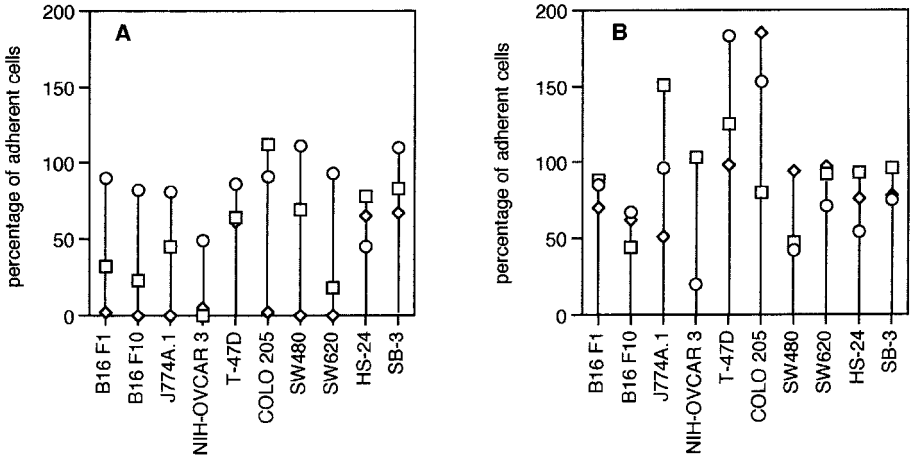

Fig. 4A, B Comparison of the effect of preincubation of surface-immobilized placental fibronectin (○), cells (□) or cells and matrix (◇) with galectin-1 (**A**) or galectin-3 (**B**) at a concentration of 25 μg/ml on the percentage of adherent cells relative to mock-treated controls

cell adhesion more effectively than presaturation of laminin. When both reactants had been exposed to galectins, generally the lowest level of adhesion was measurable for galectin-1 (Fig. 2). The potential for homotypic non-carbohydrate recognition of galectin-3 and differences in target populations may explain the differences between responses to galectin-3 and those to galectin-1. Evidently, the amount of cell-surface galectin is able to modulate cell binding to laminin. Further increases of the galectin concentration to 150 μg/ml led to progressive inhibition following preincubation of cells. However, it is unlikely that this high concentration endpoint can be reached in vivo.

Fibronectin binding is also sensitive to galectin saturation (Figs. 3, 4). As seen from a comparison of Figs. 3 and 4, the increased level of glycosylation of placental fibronectin with poly(*N*-acetyllactosamine) chains, relative to the plasma glycoform(s), frequently correlated with an increased impact. Similar to the experiments with laminin, blocking of matrix sites by galectins was less effective than blocking of cell surfaces at all concentrations tested. Nonetheless, occupation of glycans of these glycoproteins by galectins cannot be neglected in the modulation of cell adhesion. On average, galectin-1 was a better inhibitor than galectin-3 in this assay. A potential for increased adhesion by cross-linking via the two binding sites of dimeric galectin-1 was only encountered in matrix-saturating experiments at a rather low level in the case of plasma fibronectin (Fig. 3). Concerning cell type, the three colon carcinoma cell lines displayed non-uniform behavior, expressed in quantitative differences. It is noteworthy that an experimentally induced increase of surface galectins, which are normally secreted and can rebind to the external membrane side (Barondes et al. 1994; Gabius 1997a; Hirabayashi 1997), can lead to reduced matrix binding for various tumor cells and galectins-1 and -3. To probe further the functions of galectins in tumor biology during the metastatic cascade, the question was asked; could galectins accumulate at specific organ sites? For this purpose, radioiodinated galectins were injected, and their presence at different sites was monitored.

Biodistribution of galectins and galectin-coated liposomes

Intravenously injected galectins can be found in different organs with non-uniform kinetics of clearance (Tables 2–4). Preferential sites of organ uptake were the

Table 2 Biodistribution of ^{125}I-labeled mammalian and avian galectins (percentage of the injected dose per gram of tissue or per milliliter of blood ± standard deviation) in mice bearing Ehrlich solid tumor after 1 h. Galectin CG-14 was from chicken intestine, galectin CG-16 was from chicken liver; each value represents the mean ± SD of measurements with three or four mice

Tissues	Biodistribution of galectins (%) probed with:			
	Galectin-1 (human)	Galectin-3 (murine)	CG-14	CG-16
Blood	2.26 ± 0.13	2.74 ± 0.11	3.60 ± 0.18	2.49 ± 0.13
Liver	1.47 ± 0.05	1.50 ± 0.04	21.60 ± 0.47	2.55 ± 0.15
Kidneys	5.60 ± 0.39	7.57 ± 0.06	2.93 ± 0.37	22.41 ± 0.78
Spleen	1.45 ± 0.13	1.38 ± 0.09	22.29 ± 1.47	2.10 ± 0.14
Heart	0.64 ± 0.05	0.58 ± 0.03	0.78 ± 0.04	0.90 ± 0.07
Lung	0.97 ± 0.07	1.23 ± 0.12	2.33 ± 0.18	1.41 ± 0.08
Thymus	0.87 ± 0.05	0.92 ± 0.22	1.05 ± 0.09	1.12 ± 0.038
Pancreas	0.74 ± 0.08	0.81 ± 0.06	0.93 ± 0.09	2.65 ± 0.29
Stomach	2.76 ± 0.22	7.84 ± 0.61	2.80 ± 0.34	2.94 ± 0.22
Small intestine	0.66 ± 0.05	0.72 ± 0.11	0.46 ± 0.11	1.01 ± 0.14
Lymph node	0.73 ± 0.06	0.71 ± 0.11	0.58 ± 0.11	1.34 ± 0.13
Seminal vesicle	3.57 ± 0.45	7.20 ± 1.10	5.09 ± 0.19	4.22 ± 0.41
Salivary	5.03 ± 0.33	12.04 ± 0.28	6.71 ± 0.97	5.91 ± 0.88
Muscle	0.29 ± 0.03	0.33 ± 0.02	0.26 ± 0.04	0.63 ± 0.04
Vertebrae	0.54 ± 0.09	0.62 ± 0.04	0.68 ± 0.07	0.84 ± 0.02
Brain	0.08 ± 0.02	0.08 ± 0.01	0.07 ± 0.02	0.25 ± 0.02
Tumor	1.32 ± 0.15	1.35 ± 0.13	1.35 ± 0.13	1.98 ± 0.15

Table 3 Biodistribution of ^{125}I-labeled mammalian and avian galectins (percentage of the injected dose per gram of tissue or per milliliter of blood ± standard deviation) in mice bearing Ehrlich solid tumor after 3 h. Galectin CG-14 was from chicken intestine, galectin CG-16 was from chicken liver; each value represents the mean ± SD of measurements with three or four mice

Tissues	Biodistribution of galectins (%) probed with:			
	Galectin-1 (human)	Galectin-3 (murine)	CG-14	CG-16
Blood	1.59 ± 0.13	1.53 ± 0.18	2.07 ± 0.13	0.88 ± 0.04
Liver	1.05 ± 0.05	0.90 ± 0.08	15.18 ± 0.51	0.83 ± 0.03
Kidneys	4.13 ± 0.48	4.61 ± 0.17	1.83 ± 0.11	17.12 ± 0.72
Spleen	0.91 ± 0.09	0.86 ± 0.08	13.11 ± 1.34	0.71 ± 0.02
Heart	0.41 ± 0.04	0.38 ± 0.04	0.50 ± 0.07	0.67 ± 0.04
Lung	0.66 ± 0.06	0.79 ± 0.07	1.95 ± 0.23	1.13 ± 0.10
Thymus	0.53 ± 0.09	0.50 ± 0.13	0.71 ± 0.06	0.73 ± 0.08
Pancreas	0.52 ± 0.05	0.43 ± 0.06	0.91 ± 0.04	1.49 ± 0.05
Stomach	3.91 ± 0.23	4.80 ± 0.50	5.38 ± 0.36	2.78 ± 0.26
Small intestine	0.52 ± 0.05	0.31 ± 0.07	0.94 ± 0.10	0.67 ± 0.05
Lymph node	0.50 ± 0.04	0.30 ± 0.07	0.67 ± 0.05	0.91 ± 0.10
Seminal vesicle	2.84 ± 0.29	4.52 ± 0.65	3.60 ± 0.34	1.55 ± 0.06
Salivary	8.03 ± 1.00	7.91 ± 0.24	10.00 ± 0.12	3.74 ± 0.43
Muscle	0.18 ± 0.01	0.22 ± 0.04	0.21 ± 0.02	0.34 ± 0.01
Vertebrae	0.40 ± 0.03	0.35 ± 0.06	0.79 ± 0.05	0.48 ± 0.02
Brain	0.07 ± 0.01	0.08 ± 0.01	0.10 ± 0.02	0.09 ± 0.01
Tumor	1.03 ± 0.08	0.94 ± 0.10	1.18 ± 0.11	1.30 ± 0.05

Table 4 Biodistribution of ^{125}I-labeled mammalian and avian galectins (percentage of the injected dose per gram of tissue or per milliliter of blood ± standard deviation) in mice bearing Ehrlich solid tumor after 6 h. Galectin CG-14 was from chicken intestine, galectin CG-16 was from chicken liver; each value represents the mean ± SD of measurements with three or four mice

Tissues	Biodistribution of galectins (%) probed with:			
	Galectin-1 (human)	Galectin-3 (murine)	CG-14	CG-16
Blood	0.91 ± 0.12	1.29 ± 0.09	1.14 ± 0.06	0.41 ± 0.06
Liver	0.48 ± 0.03	0.85 ± 0.10	6.11 ± 0.45	0.47 ± 0.03
Kidneys	2.08 ± 0.17	1.94 ± 0.20	1.47 ± 0.18	10.36 ± 0.76
Spleen	0.56 ± 0.09	3.04 ± 0.56	8.48 ± 0.49	0.36 ± 0.04
Heart	0.22 ± 0.02	0.27 ± 0.05	0.35 ± 0.04	0.28 ± 0.01
Lung	0.52 ± 0.06	0.84 ± 0.17	1.14 ± 0.08	0.57 ± 0.05
Thymus	0.58 ± 0.12	0.43 ± 0.11	0.56 ± 0.04	0.33 ± 0.05
Pancreas	0.34 ± 0.06	0.44 ± 0.06	0.44 ± 0.07	0.33 ± 0.04
Stomach	3.79 ± 0.66	5.42 ± 0.46	2.98 ± 0.27	1.21 ± 0.18
Small intestine	0.30 ± 0.05	0.39 ± 0.01	0.67 ± 0.27	0.48 ± 0.07
Lymph node	0.36 ± 0.05	0.28 ± 0.17	0.43 ± 0.03	0.27 ± 0.02
Seminal vesicle	3.02 ± 0.34	2.99 ± 0.42	3.15 ± 0.33	1.03 ± 0.16
Salivary	6.30 ± 1.69	6.99 ± 0.64	7.60 ± 1.05	1.96 ± 0.10
Muscle	0.13 ± 0.05	0.13 ± 0.02	0.18 ± 0.02	0.13 ± 0.01
Vertebrae	0.26 ± 0.02	0.34 ± 0.08	0.39 ± 0.02	0.21 ± 0.01
Brain	0.05 ± 0.01	0.02 ± 0.00	0.01 ± 0.01	0.03 ± 0.00
Tumor	0.65 ± 0.07	0.67 ± 0.03	0.79 ± 0.10	0.65 ± 0.03

kidneys, stomach and seminal vesicle, with galectin-3 showing higher levels in those organs than galectin-1, especially at the first assay time of 1 h. When working comparatively with members of a certain protein family, two factors can help explain different properties in this assay: differences in the receptor properties with cellular ligands and/or differences in the overall hydrodynamic properties. Despite a similar native molecular mass, the homodimeric galectin-1 and the monomeric chimeric galectin-3 may behave differently as a result of the impact of the second parameter. To reveal whether subtle binding-site variations indeed affect biodistribution, the jelly-roll-motif-harboring galectin from chicken liver (CG-16) was tested under identical conditions. The very similar shape, as also shown by molecular modeling (Siebert et al. 1997), will render any changes in biodistribution by passive physical factors rather unlikely. The pronounced kidney localization especially can thus be attributed to the receptor features (Tables 2–4). Remarkably, the monomeric chicken galectin CG-14, with related but distinct ligand-binding specificity, was found in kidneys at a rather low level and, in contrast, preferred the spleen, underscoring the potential for organ-type differences among the closely related proteins of the galectin family.

A major concern with these experiments is the fact that free proteins were used, which can hardly mimic the behavior of cells. To allay this reasonable concern while maintaining the focus on galectins as a targeting device, we constructed galectin-exposing liposomes of fairly constant protein content (0.3 g/g liposome) and size (about 100 nm) as models. Taking the uptake of liposomes into the reticuloendothelial system into consideration, only a few alterations relating to free galectins, especially for kidney, were noted after 1 h (Table 2, Table 5). The differences were nearly exclusively quantitative. Having examined the influence of exogenously supplied galectin-1 and -3 on adhesion to matrix glycoproteins and on liposome distribution, it is tempting to infer a relationship of these determinants to tumor spread. We have chosen two groups of primary tumors, i.e. without and with apparent metastatic spread to lymph nodes, from two organ sites, i.e. breast and colon/rectum, to address this question.

Histopathological analysis

In addition to monitoring the presence of the galectins by specific polyclonal antibodies, labeled galectins were employed to determine the reactivity for lectin binding. This new parameter is pertinent because tumor cells can encounter galectins in homo- and heterotypic cell interactions at various steps in tumor progression and metastasis, e.g. with relevance for growth control, emboli formation and contact to endothelial and parenchymal cells. For breast cancer, multivariate discriminant analysis revealed reduced galectin-3 expression and increased binding potential for galectin-1 to be highly significant factors correlating with positive lymph node status (Table 6). The presence of the heparin-binding lectin ($P = 0.001$) and galectin-1 ($P = 0.005$) were identified

Table 5 Biodistribution of ^{125}I-labeled galectin-exposing liposomes (percentage of the injected dose per gram of tissue or per milliliter of blood ± standard deviation) in mice bearing Ehrlich solid tumor after 1 h. Galectin CG-14 was from chicken intestine, galectin CG-16 was from chicken liver; each value represents the mean ± SD of measurements with three or four mice

Tissues	Biodistribution of liposomes (%) probed with:			
	Galectin-1 (human)	Galectin-3 (murine)	CG-14	CG-16
Blood	1.60 ± 0.11	1.91 ± 0.13	2.08 ± 0.11	2.31 ± 0.19
Liver	13.26 ± 0.61	14.04 ± 0.45	15.74 ± 1.25	15.60 ± 0.47
Kidneys	4.54 ± 0.28	2.25 ± 0.14	2.73 ± 0.15	1.94 ± 0.13
Spleen	10.72 ± 0.37	21.86 ± 3.60	28.95 ± 0.66	22.76 ± 2.62
Heart	0.86 ± 0.01	0.49 ± 0.04	0.42 ± 0.06	0.42 ± 0.05
Lung	1.61 ± 0.12	1.42 ± 0.35	0.94 ± 0.06	0.96 ± 0.08
Thymus	0.55 ± 0.15	0.79 ± 0.07	0.44 ± 0.07	0.57 ± 0.07
Pancreas	1.24 ± 0.03	0.77 ± 0.06	0.69 ± 0.04	0.65 ± 0.06
Lymph node	0.70 ± 0.08	0.42 ± 0.04	0.37 ± 0.04	0.49 ± 0.06
Muscle	0.33 ± 0.02	0.23 ± 0.01	0.26 ± 0.04	0.24 ± 0.04
Vertebrae	0.94 ± 0.05	0.40 ± 0.03	0.47 ± 0.07	0.45 ± 0.06
Brain	0.14 ± 0.03	0.05 ± 0.01	0.04 ± 0.00	0.05 ± 0.00
Tumor	1.15 ± 0.08	0.91 ± 0.09	0.89 ± 0.06	1.04 ± 0.06

Table 6 Percentage of positive cases of primary lymph-node-negative (pN^-) and -positive (pN^+) breast and colorectal carcinomas and of their secondary lymph node lesions ($n = 30$ in each group). *HBL* heparin-binding lactin

Probe	Breast cancer			Colorectal cancer		
	pN^-	pN^+	Metastasis	pN^-	pN^+	Metastasis
Anti-galectin-1	100	76.7	73.3	100	83.3	83.3
Galectin-1	26.7	80.0	83.3	70.0	30.0	26.7
Anti-galectin-3	86.7	30.0	30.0	96.7	83.3	83.3
Galectin-3	60.0	60.0	60.0	96.7	36.7	36.7
Anti-HBL	86.7	46.7	43.3	100	100	100

as factors that correlate with a lack of metastatic lesions in lymph nodes. Galectin-1 binding was also found to exhibit a significant positive correlation with tumor size and stage. Features of the metastasizing primary tumors tended to be conserved in lymph node lesions. To gain evidence on how generally applicable such conclusions were for tumors from other organ sites with the same target organ, identically sized groups of colorectal cancer specimens were similarly analyzed.

On the basis of multivariate discriminant analysis, reductions in galectin-binding capacities were strong factors for the nodal status, in line with the most prominent parameter, the Dukes' stage. The presence of galectins had no statistically significant value for predicting the nodal status (Table 6). It is noteworthy that modulation of a distinct factor, e.g. the binding capacity for galectin-1, can either positively or negatively correlate with the nodal status depending on the primary tumor type (Table 6). The percentage of cases positive for node-positive primary lesions and in the groups of lymph node metastases was very similar irrespective of the type of statistical correlation, as already noted in the case of the breast cancer cohort.

Discussion

A primary concern when investigating cells and innovative reagents relates to the variable levels of expression and detection of determinants under study. This would seriously hamper the comparison of results from different laboratories working in the same field. It is therefore reassuring to note the corroborating evidence on galectin-3 expression by J774 A.1 cells (Leenen et al. 1986; Rosenberg et al. 1991), by T-47D cells (Le Marer and Hughes 1996), by COLO 205 cells (Rosenberg et al. 1991) and on expression of both galectins by SW480/SW620 and B16-F1/F10 cells (Lotan and Raz 1988; Ohannesian et al. 1995). Neoglycoproteins with lactose as an efficient galectin ligand, or Gal-β1,3(4)-GlcNAc-exposing asialofetuin as cross-linker for galactoside-dependent cell aggregate formation have furthermore been instrumental in substantiating actual sugar binding by the three colon carcinoma, the two melanoma and the macrophage/monocyte cell lines (Gabius et al. 1987, 1990a, b; Lotan and Raz 1988; Komanduri et al. 1995). Blocking of this binding of external carbohydrate ligands with anti-galectin antibodies strongly argues in favor of accessibility of the carbohydrate-recognition domain of at least some of the surface-bound galectin molecules, as shown for galectin-1 on neuroblastoma cells, for example (Kopitz et al. 1998). This situation calls for an answer to the question of how an experimental up-regulation of galectin, either on cells and/or in the substratum, will affect adhesion for different tumor cells under the same conditions. Matrix saturation by incubation with galectins will indicate the contribution of cell-bound lectins to the adhesion via *trans*-interactions with implications for invasiveness. Increasing the galectin presentation on cell surfaces by the same method will allow a positive or negative response to modulation of this parameter to be determined, mimicking elevated secretion and surface binding.

Despite the possibility of *trans*-interactions, a saturation of cell surfaces or matrix glycoproteins with exogenous galectins almost always reduced binding to the matrix glycoproteins tested. These effects could be due to competitive inhibition, post-binding trigger mechanisms or simply passive spatial interference. Blocking crucial sites on the cell surface, e.g. galectin-reactive glycans of integrins such as $\alpha_7\beta_1$ or $\alpha_1\beta_1$ integrins (Gu et al. 1994; Ochieng et al. 1998a), can account for the measured response. Negative regulation also extends to the matrix, corroborating the implication of galectin-dependent changes in the invasiveness characteristic of the cell type (Le Marer and Hughes 1996). As mentioned, galectin-triggered modulation of the involvement of other factors in adhesion is also a reasonable suggestion, although such processes have so far been found to exert positive effects via enhanced $\alpha_6\beta_1$ integrin presentation or Ca^{2+}-dependent non-integrin laminin-binding mechanisms (Kuwabara and Liu 1996; Warfield et al. 1997). Interestingly, other trigger mechanisms, i.e. L-selectin binding to lymphocytes or macrophage fusion in the course of a reaction to a foreign body, are known to alter the level of presentation of ligands for galectins, presumably leading to enhanced binding to dendritic cells upon entry into the paracortex of lymph nodes in the case of activated lymphocytes (Smetana et al. 1998; Swarte et al. 1998).

What emerges from these observations are two independent routes for modulation of cell/substratum interactions by galectins: the direct or indirect blocking/cross-linking activity and the reshuffling of the presentation of these or other molecules after galectin-elicited signaling. Intriguingly, the indispensable oligomerization of galectin-3 for cross-linking is subject to a unique regulatory process. Matrix metalloproteinases-2 and -9 remove the N-terminal domain of the chimeric galectin-3, depriving it of the capacity for aggregation (Herrmann et al. 1993; Ochieng et al. 1994, 1998b). It should be mentioned that trimmed galectin-3 still able to bind sugar will escape immunohistochemical detection by monoclonal antibodies directed to the degradable domain.

The two mechanisms given by which galectins-1 and -3 engage in cell adhesion are critically dependent upon the glycosylation status of their in vivo targets, conferring a high degree of flexibility on this recognitive interplay. With knowledge accruing on stage- and cell-type-specific glycosylation of integrins and other glycoproteins (Benallal and Anner 1994; Moss et al. 1994), it is tempting to envision regulatory mechanisms involving not only the level of galectin but also that of galectin-reactive epitopes. These processes can encompass alterations of the glycan sequence by dynamic degradation/synthesis, calling primarily upon α,β-galactosyl-and α2,3/α2,6-sialyltransferases and the respective glycosidases, for example. Moreover, by imposing or

lifting spatial restrictions in the immediate vicinity of glycan chains, lectin affinity can be drastically altered as a consequence of shifting conformer equilibria (Gabius 1998; Mann and Waterman 1998; von der Lieth et al. 1998). Since an operative recognition system can only be established in the presence of a suitable ligand, this parameter must be quantified. The fine-specificity differences for glycan subgroups, despite identical monosaccharide binding, make endogenous lectins superior to commonly applied plant/invertebrate lectins, taking lectin histochemistry from glycan profiling to functional considerations (Gabius et al. 1993, 1998; Joubert-Caron 1993; Brinck et al. 1998). Only cells with accessible galectin-binding sites, assessed by labeled galectins, are expected to bind to galectin-exposing cells. Contact with microvascular cells, which are known to display this feature (Debbage et al. 1988; Cornil et al. 1990; Lotan et al. 1994a; Baum et al. 1995b; Plendl et al. 1995), can thus only be established or strengthened when ligands on the opposing cell surface are presented. Conversely, galectin-exposing cells will only have a chance to employ this type of receptor for tissue homing, when complementary binding sites are accessible. Although one adhesion mechanism without the triggered orchestration of contact stabilization may not be enough to generate sufficient attraction to withstand the shear forces during capillary flow, the biodistribution data with free and liposome-attached galectins point to a role for these molecules in organ selection by circulating cells. It is thus recommended that both parameters are measured concomitantly (galectins by immuno- or glycohistochemistry and galectin-binding sites by lectin histochemistry) in histopathological studies, as presented in this study. This type of fingerprinting (galectinomics) has already demonstrated its potential as a discriminatory diagnostic tool to distinguish lyomyomas and lyomyosarcomas (Schwarz et al. 1999). These initial data warrant the extension of this approach to other members of the galectin family with supposed relevance for tumor development and progression, such as galectins-4, -7, -8, and -9 and the galactose-specific 67-kDa non-integrin elastin/laminin receptor (Hinek 1996; Su et al. 1996; Lu et al. 1997; Rechreche et al. 1997; Türeci et al. 1997).

With respect to primary and secondary lesions, our semiquantitative immunohistochmical analysis is in line with several previous reports on primary breast, colon and gastric carcinomas and their lymph node metastases, revealing fairly constant expression at both sites or a quantitative increase in the targt organ (Gabius et al. 1991b; Lotan et al. 1994b; Schoeppner et al. 1995; Castronovo et al. 1996). Evidently, the microenvironment of the target organ, which is known to influence the cells' capacity to bind carbohydrate ligands (Glaves et al. 1989; Vidal-Vanaclocha et al. 1990; Kayser et al. 1998), will not induce major heterogeneity of galectin expression in this case. It is noteworthy that arrest and growth in lymph nodes from two primary sites is (at least phenomenologically) associated with inverse regulation of the galectin-binding capacity and galectin-3 expression. For breast cancer cells, a low galectin-3 content, reported earlier for high-grade tumors (Gabius et al. 1986a; Castronovo et al. 1996; Idikio 1998), and the abundant presence of galectin-1-binding sites can be related to an invasive phenotype, probed with Matrigel (Le Marer and Hughes 1996), with ligands also being offered to endothelial galectin-1, for example. Moreover, the expression of a heparan-sulfate-binding lectin, which could otherwise block adhesion sites in proteoglycan chains, is decreased under these circumstances. For the other cell type studied, a lower level of accessible galectin ligands can be conducive for tumor spread at other stages in the metastatic cascade.

Taken together, these results document quantitative cell-type- and galectin-type-dependent differences in the interaction with matrix glycoproteins. Their glycosylation status and the respective features of cellular ligands afford a versatile means to regulate cell/substratum interactions with implications for invasiveness. In view of these findings, it appears mandatory to employ galectin-specific antibodies and labeled galectins in histopathological studies to delineate the role of this recognition system in controlling tumor adhesiveness, growth and spread.

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