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REVIEW

Tumor galectinology: Insights into the complex network of a family of endogenous lectins

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β-Galactosides of cell surface glycoconjugates are docking sites for endogenous lectins of the galectin family. In cancer cells, primarily galectins-1 and -3 have been studied to date. With the emergence of insights into their role in growth control, resistance to or induction of apoptosis and invasive behavior the notion is supported that they can be considered as functional tumor markers. In principle, the same might hold true for the other members of the galectin family. But their expression in tumors has hitherto been a subject of attention only to a very limited extent. Pursuing our concept to define the complexity of the galectin network in cancer cells and the degree of functional overlap/divergence with diagnostic/therapeutic implications, we have introduced comprehensive RT-PCR monitoring to map their galectin gene expression. The data on so far less appreciated galectins in this context such as galectins-4 and -8 vindicate this approach. They, too, attach value to extend the immunohistochemical panel accordingly. Our initial histopathological and cell biological studies, for example on colon cancer progression, prove the merit of this procedure. Aside from the detection of gene expression profiles by RT-PCR, the detailed molecular biological monitoring yielded further important information. We describe different levels of regulation of galectin production in colon cancer cells in the cases of the tandem-repeat-type galectins-8 and -9. Isoforms for them are present with insertions into the peptide linker sequence attributed to alternative splicing. Furthermore, variants with distinct amino acid substitutions (galectin-8, Po66-CBP, PCTA-1, Cocal/II and galectin-9/ecalectin) and generation of multiple mRNA species, notably those coding for truncated galectin-8 and -9 versions with only one lectin site, justify to portray these two family members not as distinct individuals but as groups. In aggregate, the ongoing work to thoroughly chart the galectin network and to disentangle the individual functional contributions is expected to make its mark on our understanding of the malignant phenotype in certain tumor types.
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Introduction

The cell surface displays a variety of determinants which are relevant for the cells' communication with the environment and their social behavior. Alterations in the profile of cell surface epitopes are thus likely to reflect shifts in cellular parameters such as differentiation and malignancy. An illustrative example for the way how malignant transformation impinges on properties of the cells' exterior surface has been given by analyzing the carbohydrate chains of cellular glycoconjugates. This procedure to map the complete set of glycan structures (glycome) is currently referred to as glycomic profiling. Disease development and progression is often associated with changes in the glycome [1–14]. Monoclonal antibodies and plant/invertebrate lectins are instrumental to detect and localize deviations from the glycosylation patterns of normal cells [15–19]. While it has become straightforward to detect plant-lectin-reactive

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carbohydrate epitopes in relation to tumor types, their actual functional relevance remains rather elusive. The evidence for regulation of glycan expression and the documented experience with plant lectins as tools commend endogenous lectins in today's search for glycan functions [20]. As crucial step in this process lectin presence has to be detected (lectinomics).

From plant lectin histochemistry to endogenous lectins: Functional lectinomics

As epitomized by coining the term *sugar code*, oligosaccharides harbor an unsurpassed capacity to store biological information [21]. After all, plant lectin histochemistry burnishes this image with its voluminous literature on distinct staining patterns. Their detection implies strict temporal and spatial regulation of glycan epitope presentation. Its occurrence intimates a role of these sugar epitopes as signals. By serving as binding partners for endogenous lectins *in situ*, a versatile protein(lectin)-carbohydrate recognition system for information transfer can be implemented [22–25]. If this system is operative in tumor biology with ensuing implications *e.g.* for tumor growth control and invasion, characteristic glycans and distinct sugar receptors of the cells should become a new set of functional tumor markers [26]. Consequently, the development of two experimental approaches was prompted to further the progress in this area of glycosciences.

Exploiting the glycohistochemical technique with tools complementary to the recognition sites of the mentioned lectins, *i.e.* chemically prepared neoglycoconjugates with custom-made mono- to oligosaccharide derivatives as ligand part, the determination of the cells' capacity to bind glycans became feasible. Because the secondary structure of various types of endogenous lectins is very stable and can even withstand exposure to aprotic solvents [27,28], carbohydrate binding activity is maintained in routinely processed tissue sections. Specific binding and staining patterns with prognostic relevance were delineated when localizing glycan-binding sites in sections of certain tumor types and setting glycohistochemical and clinical data into relation [29–31]. The presence of the histo-blood group epitope A detected with monoclonal antibody and of the respective binding sites localized with a neoglycoconjugate exposing this tetrasaccharide as a ligand correlated with favorable prognosis of non-small cell lung cancer patients and points to the potential for a clinically relevant interactive system [32–34]. At this stage, the next step is to define the detected activity in biochemical terms. This knowledge and access to the purified protein(s) then lead to the second mentioned approach, *i.e.* to introduce the endogenous lectin to histochemical localization and biochemical characterization of accessible cellular ligands. Examples for the application of tissue lectins as probes to localize binding sites for the tissue-derived receptor histochemically have been given for various sugar epitopes, *e.g.* β-galactosides or heparin/heparan sulfate [29,31,35,36]. Conceptually, neoglycoconjugates (or lectin-specific antibodies) and labeled lectins establish the panel of probes to put the hypothesis to the test that lectins might play a tangible role for the malignant phenotype [26].

In the course of resolving this issue, consideration of those sugar receptors which interact with spatially readily accessible glycan determinants deserves attention. Automatically, this reasoning guides us to β-galactosides and derivatives thereof. Because the enzymatic machinery to perform β1,3(4)-galactosylation of suitable acceptors presently numbers thirteen glycosyltransferases in total [37–39], the question arises as to whether we will be faced with a comparable complexity on the level of the receptor side. The answer to this question is not only relevant for basic sciences but also with respect to relating properties of the malignant phenotype to proteins as a step to refine tumor diagnosis and to devise rational therapy modalities.

Galectins in tumor cells: A complex network?

The biochemical analysis of galectins started with the purification of an *electrolectin* from *Electrophorus electricus* in 1975 [40]. For a human tumor initial evidence for galectin presence was provided 10 years later [41], and further biochemical work, presently often guided by results of database mining and cloning, ensured that the number of galectins detected continues to grow [42]. In principle, obtaining insights into this emerging complexity will pique our interest to learn more about cell expression profiles, functionality and interrelationship of the family members. This will only then not carry a serious concern when exclusively monitoring galectins-1 and -3 in tumor histopathology, if no other members are present in tumor cells or if the different galectins showed identical activity profiles. Taking stock of the pro- and anti-apoptotic activities of galectins-1 and -3 by homing in on different extra- and intracellular targets [43–47] and functional divergence even after binding to the same ligand set, as shown for human SK-N-MC neuroblastoma cells [43–45], it becomes clear that the clinical interpretation of immunohistochemical data for only one or two galectins from a larger panel might not be free of ambiguities. Noted in the initial immunohistochemical study in this field on human breast cancer, coexpression of two of three tested galectins was not a rare event [48]. In the wake of these lines of evidence it appears likely that tumor cells are endowed with a network of galectins. Indeed, this result has been confirmed for various tumor types and galectins-1 and -3 [49–52]. In other words, the challenge needed to be taken on was to accomplish profiling of galectin expression beyond these two types (*i.e.* to define the profile of galectins expressed, the galectinome). By using a convenient and reliable method based on the available cDNA sequences we designed fingerprinting by RT-PCR analysis to determine the extent of complexity of galectin expression in tumor cells [53].

Galectin fingerprinting by RT-PCR

Flanked by adequate controls (*e.g.* excluding to amplify genomic DNA or cross-reactive cDNA, monitoring of protein production) [53,54], the RT-PCR analysis with discriminatory

Table 1. List of investigated tumor cell lines

Cell line	*Reference*[+]	*Cell type*
Breast cancer		
HuMI*	[108]	Immortalized mammary epithelial cells
HuMI-T*	[108]	Weakly tumorigenic HuMI cells
TTu1*	[108]	Highly tumorigenic HuMI subline
TTu2*	[108]	Highly tumorigenic HuMI subline
BT-20	HTB-19	Mammary gland adenocarcinoma
DU4475	HTB-123	Mammary gland adenocarcinoma
MDA-MB-468	HTB-132	Adenocarcinoma
T-47D	HTB-133	Ductal carcinoma
ZR-75-30	CRL-1504	Ductal carcinoma
Colorectal carcinomas		
Caco-2	HTB-37	Adenocarcinoma
Co115	[109]	Carcinoma
Colo201*	CCL-224	Adenocarcinoma (ascites)
Colo205*	CCL-222	Adenocarcinoma (ascites)
DLD-1	ACC-278†	Adenocarcinoma
HCT-15	ACC-357†	Adenocarcinoma
HCT-116	CCL-247	Carcinoma
HPR600	§	Carcinoma
HT29	HTB-38	Adenocarcinoma
Isreco-1*	[110]	Primary tumor
Isreco-2*	[110]	Liver metastasis
Isreco-3*	[110]	Peritoneal metastasis
Lisp-1	§	Carcinoma
LoVo	CCL-229	Supraclavicular metastasis
LS174T	CL-188	Adenocarcinoma
LS411N	CRL-2159	Carcinoma
LS513	CRL-2134	Carcinoma
LS1034	CRL-2158	Carcinoma
SW480*	CCL-228	Adenocarcinoma
SW620*	CCL-227	Lymph node metastasis
SW1116	CCL-233	Adenocarcinoma
WiDr	CCL-218	Adenocarcinoma
Lung carcinomas		
NCI-H69	HTB-119	SCLC, carcinoma
NCI-N417	CRL-5809	SCLC, carcinoma
NCI-N592	CRL-5832	SCLC, carcinoma (bone marrow metastasis)
SW210.5	[111]	SCLC, carcinoma
SCLC-16HV	[111]	SCLC, carcinoma
SCLC-21H*	ACC-372†	SCLC, carcinoma
SCLC-22H*	ACC-373†	SCLC, carcinoma
SCLC-24H	[112]	SCLC, carcinoma
HS-24	‡	NSCLC, squamous cell carcinoma
SB-3	‡	NSCLC, suprarenal gland metastasis
Neural tumors		
H4	HTB-148	Neuroglioma
Hs683	HTB-138	Glioma
SW1088	HTB-12	Astrocytoma
SW1783	HTB-13	Astrocytoma
T98G	CRL-1690	Glioblastoma
U87	HTB-14	Glioblastoma/astrocytoma
U118	HTB-15	Glioblastoma/astrocytoma
U373	HTB-17	Glioblastoma/astrocytoma
Renal tumors		
ACHN	CRL-1611	Renal cell adenocarcinoma
SW13	CCL-105	Adrenal gland carcinoma
293	CRL-1573	Transformed embryonic epithelial kidney cells
Ovarian cancer		
NIH-OVCAR3	HTB-161	Ovarian adenocarcinoma
OAW-42	‡	Ovarian carcinoma
Prostate cancer		
DU145	HTB-81	Prostate carcinoma (brain metastasis)
LNCaP-FGC	CRL-1740	Prostate carcinoma (supraclavicular lymph node metastasis)
PC-3	CRL-1435	Prostate adenocarcinoma (bone metastasis)
Hematopoietic tumors		
B-cells		
BLIN-1	[113]	Pre-B cell
Croco II	[114]	B lymphoblastoid
MEC-1	[115]	B-chronic prolymphocytic leukemia
Raji	CCL-86	Burkitt's lymphoma
Ramos	CRL-1596	Burkitt's lymphoma
T-cells		
CCRF-CEM	CCL-119	Acute lymphoblastic leukemia
HSB-2	CCL-120.1	Acute lymphoblastic leukemia
HUT-78	TIB-161	Cutaneous lymphoma
Jurkat	TIB-152	Acute T cell leukemia
MOLT-4	CRL-1582	Acute lymphoblastic leukemia
Others		
HL-60	CCL-240	Acute promyelocytic leukemia
KG1*	CCL-246	Acute myelogenous leukemia (bone marrow)
KG1a*	CCL-246.1	Acute myelogenous leukemia (bone marrow)
K-562	CCL-243	Chronic myelogenous leukemia (bone marrow)
M07e	ACC-104†	Acute megakaryoblastic leukemia
TF-1	CRL-2003	Erythroleukemia (bone marrow)
THP-1	TIB-202	Acute monocytic leukemia
Skin tumors		
A375	CRL-1619	Melanoma
BS1251	§	Lymph node metastasis of melanoma
BuHOM	[116]	Lymph node metastasis of melanoma
Colo38	[117]	Melanoma
Hs294T	HTB-140	Lymph node metastasis of melanoma
Mel Ju	[118]	Melanoma
MeWo	HTB-65	Lymph node metastasis of melanoma
SK-Mel25	[119]	Melanoma
SK-Mel28	HTB-72	Melanoma

[+]If not otherwise stated the numbers refer to the code of the American Type Culture Collection (Manassas, VA).
†DSMZ number (German Collection of Microorganisms and Cell Cultures, Braunschweig).
‡Tumor cell collection of the DKFZ (German Cancer Research Center, Heidelberg).
*These cell lines were established from tumor material of a single patient.
§Kindly provided by Drs. H.-P. Rutz (Lausanne, Switzerland), D. Lopez (São Paulo, Brazil) and S. Ugurel (Mannheim, Germany), respectively.

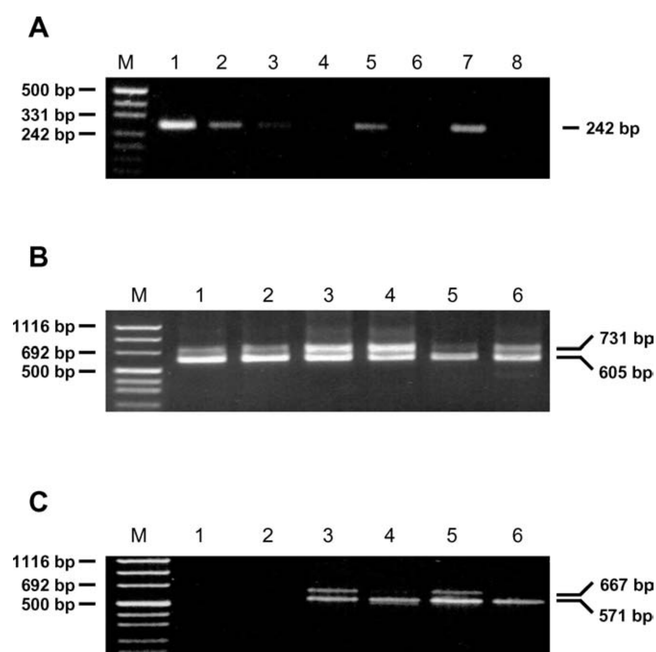

Figure 1. Detection of galectin-specific transcripts in human tumor cell lines. (A) Galectin-7 (breast cancer cell lines): HuMIp26 (1), HuMIp110 (2), HuMI-T (3), TTu1 (4), TTu2 (5), BT20 (6), DU4475 (7), MDA-MB-468 (8). (B) Galectin-8 (colorectal carcinoma cell lines): Colo201 (1), Co115 (2), HCT116 (3), Isreco-1 (4), LS1034 (5), hColon (6). (C) Galectin-9 (cell lines of different histogenetic origin): LNCaP-FGC (1), PC-3 (2), NIH-OVCAR3 (3), M07e (4), TF-1 (5), THP-1 (6) (please see Table 1 for detailed information on cell lines).

primer sets will yield amplification products of predictable size as evidence for expression of distinct galectin genes. To answer the question on influence of cell type we screened a total of 83 cell lines derived from 10 different tissue types. The characteristics of the processed cell lines are listed in Table 1. As exemplarily shown in Figure 1, galectin gene transcription is not at all confined to galectins-1 and -3. This emerging lesson is underscored in Table 2. It allows the reader to gauge the range of differential expression profiles encountered in the course of our monitoring. Moreover, when listing tumor cell lines according to their histogenetic origin, inter- and intraindividual diversity between and within defined tumor cell types becomes apparent (Table 3). Keeping a tally of reports on functional implications for galectins other than galectins-1 and -3 in tumor biology, it appears timely to let these activities come under scrutiny, too. To emphasize the importance to know about this profile in detail, the following functional correlations of galectins-4, -7, and -8 for tumor properties are noteworthy: galectin-4 belonged to a gene cluster of scirrhous gastric cancer cells prone to peritoneal dissemination, galectin-7 showed up among the 14 transcripts from a total of 7,202 tested sequences in human DLD-1 colon cancer cells upregulated by p53 prior to the onset of apoptosis and galectin-8 had a suppressor-like expression pattern in human colon cancer when monitored immunohistochemically [55–57]. In colon cancer absence of galectins-1 and -4 were indicators of favorable prognosis in Dukes A/B cases [58]. Proceeding along this route the transfer of galectin fingerprinting from the level of cDNA to that of protein expression by immunohistochemistry has so far confirmed the conclusions illustrated in Tables 1 and 2 and Figure 1 [53,59–63]. Consequently, answers to the question on prognostic relevance of galectins beyond -1 and -3 are likely to be coming in the near future. At the same time, the molecular biological analysis draws attention to the remarkable intraindividual diversity of distinct galectins. Besides allowing to decide whether a galectin gene is transcribed the RT-PCR analysis shown in Figure 1 conveys a salient message on regulatory events, *i.e.* the occurrence of isoforms in the cases of the tandem-repeat-type galectins-8 and -9 (Figure 1B and C).

Galectin isoforms: Potential for a further level of functional regulation

Tandem-repeat-type galectins are characterized by the presence of two slightly different carbohydrate recognition domains linked by a peptide spacer. This principle to achieve intramolecular spatial proximity for binding sites is also found in other lectin families such as C- and P-type lectins [64–66]. In mouse/rat galectin-9 the intestinal isoform is established by a 31/32-amino acid insertion between the N-terminal domain and the link peptide [67]. A 32-amino acid extension of the linker peptide of human galectin-9 has been described for pancreatic islets, liver, lung, tonsils, in HeLa, Jurkat, and various other tumor cell lines [68,69]. In contrast to the situation in rodents with both forms consistently being present, for example human colon cancer lines can exclusively express the extended isoform [53,69]. In addition to the isoforms with linker peptides

Table 2. Galectin gene expression in selected cell lines determined by RT-PCR

Cell line	*Tissue*	*Gal-1*	*Gal-2*	*Gal-3*	*Gal-4*	*Gal-7*	*Gal-8*	*Gal-9*
DU4475	Breast	+	–	+	–	+	+	–
HPR600	Colon	–	+	+	+	–	+	+
NCI-N592	Lung	–	–	–	–	–	+	–
Hs683	Brain	+	+	+	+	–	+	–
Hs294T	Skin	+	–	+	–	–	+	–
THP-1	Blood	+	+	+	+	–	+	+

Table 3. Galectin gene expression in human tumor cell lines of different histogenetic origin determined by RT-PCR[a]

Tissue	*Total*	*Gal-1*	*Gal-2*	*Gal-3*	*Gal-4*	*Gal-7*	*Gal-8*	*Gal-9*
Blood	17	11 (65%)	3 (12%)	5 (29%)	3 (12%)	0 (0%)	15 (88%)	14 (82%)
Brain	8	8 (100%)	2 (25%)	8 (100%)	3 (38%)	0 (0%)	8 (100%)	3 (38%)
Breast	9	9 (100%)	0 (0%)	9 (100%)	0 (0%)	5 (56%)	9 (100%)	0 (0%)
Colon	22	8 (36%)	7 (32%)	22 (100%)	14 (64%)	16 (73%)	22 (100%)	16 (73%)
Kidney	3	2 (66%)	0 (0%)	2 (66%)	0 (0%)	1 (33%)	3 (100%)	0 (0%)
Lung	10	4 (40%)	0 (0%)	5 (50%)	0 (0%)	2 (20%)	8 (80%)	0 (0%)
Ovary	2	2 (100%)	0 (0%)	2 (100%)	0 (0%)	0 (0%)	2 (100%)	1 (50%)
Prostate	3	2 (66%)	0 (0%)	2 (66%)	0 (0%)	0 (0%)	3 (100%)	0 (0%)
Skin	9	8 (89%)	0 (0%)	9 (100%)	0 (0%)	0 (0%)	8 (89%)	0 (0%)
$\sum$	83	54 (65%)	12 (14%)	64 (77%)	20 (24%)	24 (29%)	78 (94%)	34 (41%)

[a]number of positive cell lines and the percentage of positive cases in each group are given.

of varying length, another source of diversity was pinpointed when comparing sequences derived from cDNA libraries of a spleen from a 28-yr-old patient with Hodgkin's disease and a transformed T cell line (STO) [70,71]. It is caused by differences of the sequence. Actually, galectin-9/ecalectin were defined as a closely related pair of galectins with only four amino acid changes (R88K, F135S, L238P, G281E). By sequencing transcripts from colon cancer cell lines we confirmed the occurrence of variant transcripts. More intriguingly, a third mode for variability became visible. The sequence coding for galectin-9 was found to be subject to a frame-shift mutation [69]. This event will inevitably lead to premature termination and thus yield a truncated product with only one functional carbohydrate-binding site [69]. If translation ensues, the cross-linking galectin-9 will be turned into a monovalent module devoid of this activity. Similar to the homodimeric proto-type galectin-1 the capacity to cross-link ligands has been reckoned to be indispensable *e.g.* for the chemoattractant activity of ecalectin (please see also below) [72,73]. Having discussed galectin-9/ecalectin, the question is prompted whether the case of galectin-8 might present a similar complexity or a strict limitation to diversity by increasing linker peptide length. Also, we resolve the issue as to whether this sequence extension is a common or rare event in various tumor types.

As shown in Figure 1B and indicated in our previous report [54], the two isoform cDNAs were generally expressed. Thus, level one for variability comparable to galectin-9 is also reached. For level two to be reached, data are available on two variants of human brain hippocampal galectin-8. They are the sequences of Po66-CBP (Po66-carbohydrate-binding protein) isolated from a human lung cancer line (SK-MES-1) owing to its reactivity with the Po66 mouse monoclonal antibody, which had been raised against human squamous cell carcinoma and of PCTA-1 (prostate carcinoma tumor antigen-1) detected by surface-epitope masking [74–79]. Regarding the expression of isoforms, prolonged culture of the human lung SK-MES-1 squamous cell carcinoma line for 5 days resulted in the disappearance of the Po66-CBP transcript of the extended isoform [79]. Other authors working on PCTA-1 concluded that "results as they stand at the present time seem to indicate that regulation of the composition and abundance of isoforms appears to be stochastic in nature" [80]. In the case of Po66-CBP the theoretical molecular weights of the predicted products will be 35,647 and 40,369 Da [79]. Probing of tissue and cell extracts by Western blotting can be reconciled with the translation of two proteins in rat muscle and human 1299 non-small cell lung carcinoma cells [81]. The mouse monoclonal antibody Po66 immunoprecipitated cytoplasmic proteins of 47, 50 and 69 kDa [75], raising the suspicion for glycosylation on the two N-glycosylation sequons present in the cDNA sequence [79].

To further figure out the inherent complexity of galectin-8 expression, referred to as "complex subfamily of galectins" [82] we cloned the PCR products from three human colorectal cancer lines derived from primary and secondary lesions of the same patient, called Isreco-1, -2, and -3 [83], from the human U373 glioblastoma cell line and from a normal colonic cell line. While the latter two cell sources contained mRNA completely matching the Po66-CBP sequence, the three colon cancer lines consistently expressed a variant of the published sequences (Figure 2A). In direct comparison the two independently processed cDNAs from colorectal cancer lines only differed in the length of the linker peptide constituting 317 amino acid (35,808 Da) and 359 amino acid (40,363 Da) products. The internal consistency of sequencing data argues against technical errors entailing misinterpretations. In comparison with the known human galectin-8/Po66-CBP/PCTA-1 sequences these *co*lorectal *ca*rcinoma galectin-8 variant sequences I and II (Coca galectin-8I/II; GenBank accession numbers AF342815 and AF342816) harbored few but distinctive deviations from the other three accessible sequences (Figure 2B). Using a commercial *in vitro* translation system, the capacity of the cDNAs to successfully initiate protein synthesis was ascertained (Figure 3).

To answer the question as to whether the sequence deviations might be able to influence the lectin activity we performed homology-based molecular modeling using the approach as applied for human galectin-1 [84,85]. Without exception the amino acid changes do not reside in the carbohydrate

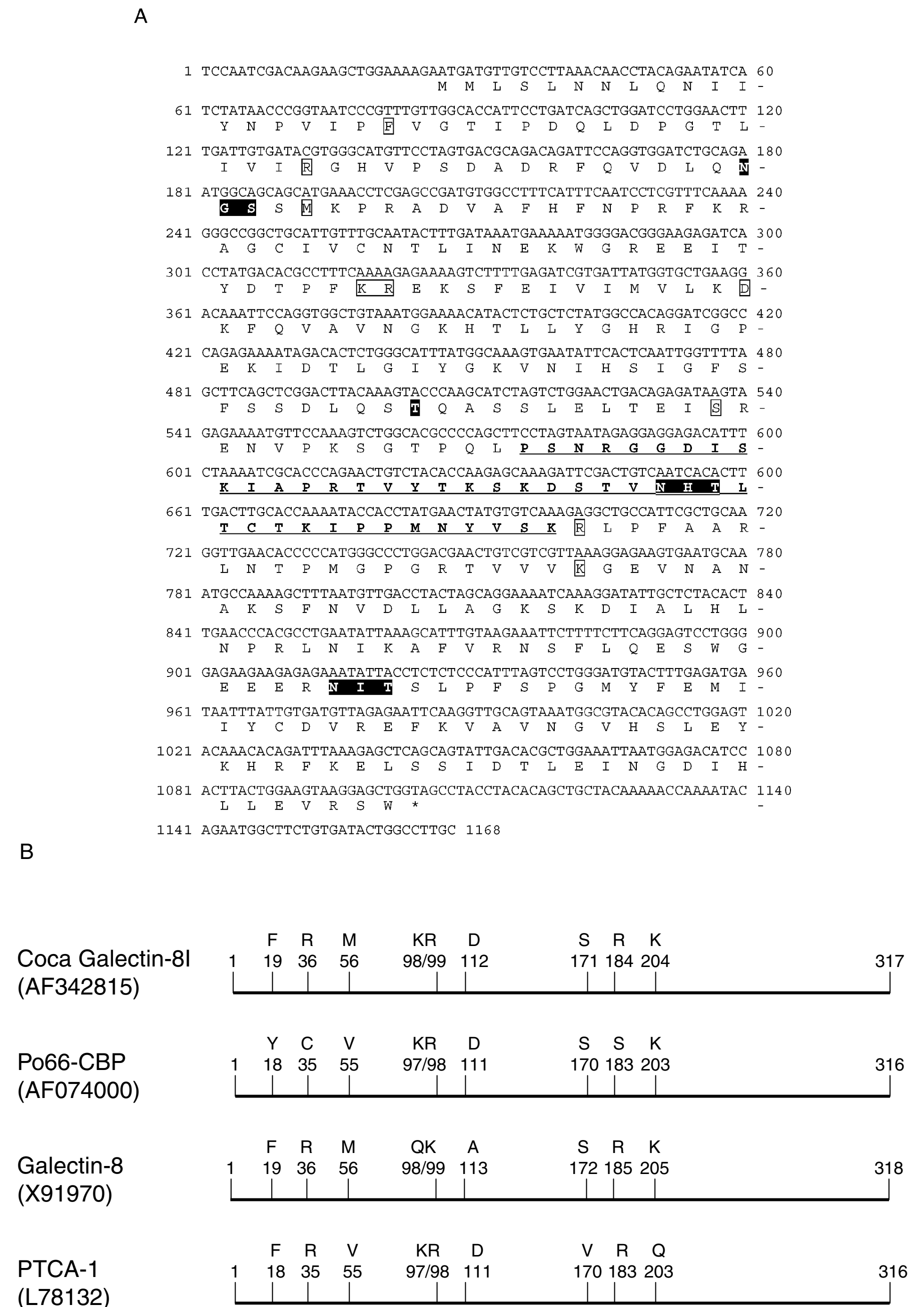

Figure 2. Sequence of colorectal carcinoma galectin-8 isoform and comparison with the other sequences in the galectin-8 group. (A) Complete sequence of Coca galectin-8II (GenBank accession no. AF342816). The 42-amino-acid insertion that separates it from the sequence of the Coca galectin-I isoform is underlined and indicated in bold letters. Positions that discriminate the Coca isoforms from other members of the galectin-8 group are indicated by boxes. Black boxes highlight potential O- (Thr160) and N-glycosylation sites determined by using the NetNGlyc 1.0 and NetOGly 2.0 Prediction Servers. (B) For detailed amino acid sequence comparison to Po66-CBP, galectin-8 from a brain hippocampus library and PTCA-1 (accession numbers given in parentheses) the type of amino acid changes and their positions are compiled.

recognition domain but in loop regions of the β-strand network and in the linker peptide (Figure 4). Although a long-range impact of single-site mutations on structural features of the lectin site cannot definitively be excluded *a priori* [84], potential functional implications might more likely be considered for emerging protein-protein interactions with relevance for growth control. In view of the intracellular occurrence of galectin-8 [59,61,78–81,86,87], the cytoplasmic interaction of galectin-1 with oncogenic H-Ras which entails guiding the oncogenic protein to its proper membrane localization and imparting selectivity to signaling into the direction of Raf-1 at the expense of phosphoinositide 3-kinase could serve as a role model also for

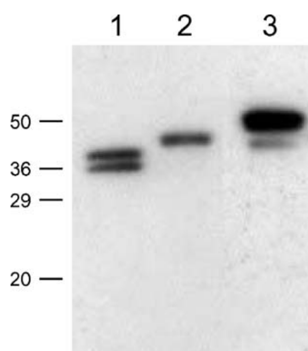

Figure 3. Gel electrophorectic analysis of products from *in vitro* translation using cDNA of human Coca galectin-8I (1), Coca galectin-8II (2), and γ-globulin (3) as control.

this tandem-repeat-type galectin [46,88,89]. Equally intriguing, the functional relevance of the presence of the variant ecalectin for galectin-9, also present in colon cancer cells [69], remains to be clarified. Albeit hypothetical, it seems fair to assume that a common theme might eventually be discerned for these closely related family members.

In relation to the homodimeric galectin-1 with rigid interprotomer distance the availability of variants with variable linker lengths and thus interdomain flexibility might be interpreted functionally in the context of target interactions. The reduced colony formation of HeLa and 1299 lung cancer cells and the induction of apoptosis in 1299 cells document galectin-8 functionality relevant for tumor biology [80,81]. As for the mentioned chemoattractant activity of ecalectin [72], bivalency is required, as it is for modulating cell adhesion [81,90]. In this respect, another feature of product generation of galectin-8 by alternative splicing deserves to be added, *i.e.* the presence of a stop codon in an insertional sequence to the linker peptide [79]. The resulting mRNA will code for a 25,976 Da protein with a single carbohydrate recognition domain. Hereby, a cross-linking device is turned into a monomeric version. Interestingly, this consequence can likewise be reached by insertion of a single nucleotide establishing a frame-shift mutation, as detected in galectin-9 cDNA from two human colon cancer cell lines [69]. Whereas multiple genes encoding ecalectin/galectin-9 transcripts have been reported based on genomic blot analysis [71], the question on galectin-8 gene frequency on chromosome 1 mapping to the region 1q42-43 (unique gene with allelic variation or more than one gene?) is still not yet finally answered [79,80]. Nevertheless, the described levels of variability including alternative splicing endorse a detailed subcategory classification. Recent results underscore that it is expedient not to consider the occurrence of various lectin versions from alternatively spliced transcripts as a rare event. Looking at other galectin subfamilies, the chimera-type chicken galectin-3 gene is expressed in two further forms with a 70-amino acid transmembrane section inserted in front of the lectin domain or an insert into the lectin domain leading to truncation [91]. Remarkably, within the family of C-type lectins alternative splicing can even be dubbed to be common with impact on transmembrane, stalk and lectin modules, for example in the cases of dectins-1/2 or DC-SIGN1/2 [92–95].

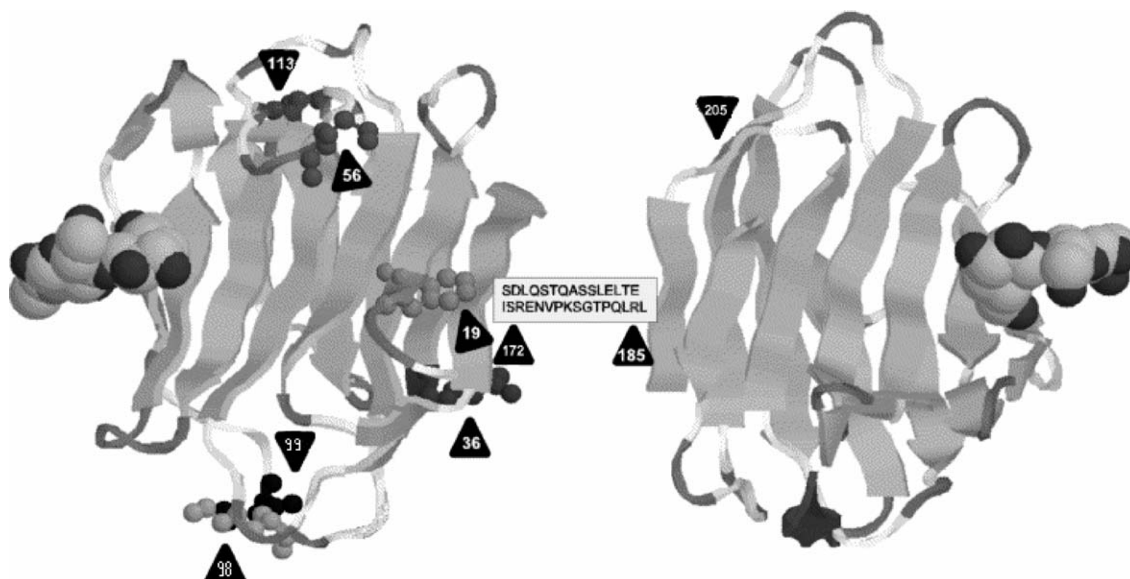

Figure 4. Homology-based molecular modeling of the galectin-8 molecule. Due to the close structural similarity to human galectin-7 with 49 identical amino acid positions (36%) and only one gap of four amino acids its crystal structure coordinates (PDB-Entry 1BKZ) were taken as a template to run modeling with the program MODELLER and further validations with standard PROSAII, What_Check and ProCheck routines. The positions of the amino acid changes distinguishing Coca galectin-8I/II from the other galectin-8 group members are indicated by black arrowheads. Numbers refer to the position within the galectin-8 sequence. The contact site with the ligand is indicated by appropriately docking the disaccharide (please see Figure 2A and B for details).

Conclusions

The presented information describes the levels of complexity of galectin expression in cancer cells. The knowledge acquired by RT-PCR analysis and initial immunohistochemical fingerprinting intimates that activities beyond commonly studied galectins-1 and -3 can matter functionally to a marked extent. The persistence to subject them to the same experimental scrutiny as galectins-1 and -3 is likely to pay off by the insights into functional overlap/divergence of galectins. Along this route, the further described levels of regulation of galectin expression in the cases of numbers -8 and -9 should be given heed to. They comprise the modulation of peptide linker lengths, the occurrence of variants with few but distinct amino acid substitutions and the potential to turn tandem-repeat-type galectins into monomeric modules.

A salient issue to be kept in mind is the cell-type specificity of the responses to a certain galectin. To give a telling example, the potency of galectin-1 to induce apoptosis by classical or non-classical pathways in leukemic T cells of the Sézary syndrome or cultured human neuroblastoma cells contrasts with the observation that galectin-1 is positively linked with proliferation, migration and invasiveness of glioma/glioblastoma cells and tumors [43,45,60,96–100]. The combination of studying clinically defined tumor sections and cell models is shaping the framework for deliberate modulation of galectin expression *in vitro* by transfection to explore therapeutic implications. Having defined a galectin as key factor for tumor invasion or metastasis, as noted already for galectins-1 and -3, the design of galectin-(type-)specific blocking reagents, too, can break new ground for modes to limit these attributes of malignancy. In this area, custom-made ligand derivatization and suitable topological presentation of high-affinity ligands are currently tested parameters *en route* to enhance target affinity and selectivity [101–107]. It is in this context that certain cancer-associated alterations of cell surface carbohydrates, still largely interpreted phenomenologically, might acquire a meaning as functional markers by the study of galectins (and other endogenous lectin families).

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