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Review

Expression, localization and function of galectin-8, a tandem-repeat lectin, in human tumors

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Summary. Galectin-8 (Gal-8) is a '*tandem-repeat*'-type galectin, which possesses two carbohydrate recognition domains connected by a linker peptide. Gal-8 complexity is related to the alternative splicing of its mRNA precursor, which is known to generate isoforms. Regarding its carbohydrate-binding specificity, Gal-8 has a unique feature among galectins, since its C-terminal domain has higher affinity for *N*-glycan-type branched oligosaccharides, while its N-terminal domain shows strong affinity for α2-3-sialylated or 3'-sulfated ß-galactosides. We integrate here the available information on Gal-8 expression in different tumor types and attempt to elucidate associations of its expression and localization with tumor progression with the overarching goal of analyzing its potential applications in diagnosis and prognosis. Differential diagnosis is still a prime concern in tumor pathology, and Gal-8 could be of great value in some types of primary or secondary tumors (i.e. papillary thyroid carcinoma, advanced colon carcinoma from patients with distant metastases, or metastases from primary lung carcinoma). The prognostic value of Gal-8 has been described for laryngeal carcinoma as well as advanced colon carcinoma. Further studies are needed to explain the relevance of Gal-8 and its isoforms in tumor pathology and their different intra- or extracellular roles (cytoplasmic, nuclear or extracellular) in tumor biology.

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Galectin-8: cloning, isoforms, carbohydrate specificity and functions

Galectins (Gals) are a family of proteins first identified as galactoside-binding lectins in extracts of vertebrate tissues, and formally defined on the basis of both shared sequence and galactoside binding in 1994 (Barondes et al., 1994). The consensus sequence corresponds to the "carbohydrate-recognition domain" (CRD), which is a ß sandwich of about 135 amino acids that possesses the ß-galactoside binding activity.

To date, 15 Gals have been identified in mammals;

Abbreviations. ANAs: anaplastic astrocytomas (grade III); ASTs, diffuse astrocytomas (grade II); BGA, blood group A determinants [GalNAcß1-3(Fucα1-2)Gal-]; BGB, blood group B determinants [Galα1-3(Fucα1-2)Gal-]; BPH, benign prostatic hypertrophy; CRD, carbohydrate-recognition domain; Gals, Galectins; Gal-8S, *small* Gal-8; Gal-8M, *medium* Gal-8; Gal-8L, *long* Gal-8; Gal-8C, C-terminal domain of Gal-8 or C-CRD; Gal-8N, N-terminal domain of Gal-8 or N-CRD; Gal-8Null; protease-resistant mutant of human Gal-8 with 2 amino acids instead of the linker peptide; GBM, glioblastoma (grade IV); IHC, immunohistochemistry; LacNAc, N-acetyllactosamine (Galß1-4GlcNAc); mAb, monoclonal antibody; PCTA-1, prostate carcinoma tumor antigen 1; PILs, pilocytic astrocytomas (grade I); PIN, prostatic intraepithelial neoplasia; Po66-CBP, Po66 carbohydrate-binding protein; Po66 mAb, Po66 monoclonal antibody; PSA, prostate-specific antigen; α/ß/γ RARs: α, ß, γ retinoid acid receptors; α2-3-sialyllactose, Neu5Acα2-3Galß1-4Glc; 3'-sulfolactose, SO_3^--3Galß1-4Glc.

they are numbered according to the chronology of their discovery (Gal-1 to Gal-15) (Yang et al., 2008). Gals known so far have one or two CRDs within a single polypeptide chain. The mono-CRD Gals can be biologically active as monomers (Gal-5, -7, -10) or as homodimers (Gal-1, -2, -11, -13, -14, -15). Bi-CRD Gals (Gal-4, -6, -8, -9, 12) are active as monomers but might develop oligomers. Gal-3 is the only representative of the "*chimera*" galectin type, containing a long NH2-terminal collagen-like domain (with proline- and glycine-rich motifs) and a C-terminal domain with a single CRD (Rabinovich, 1999).

Gal-8 was initially cloned and sequenced from a rat liver cDNA library by Zick's group: the isolated clone contained an open reading frame that coded for a protein of 316 amino acids (~35 kDa) with two CRDs. These N- and C-terminal domains share 35% homology, and both contain sequence motifs that have been conserved among most CRDs of Gals (Hadari et al., 1995). Almost at the same time, Su and co-workers identified a human homologue gene, which they called *prostate carcinoma tumor antigen-1* (PCTA-1), by screening a cDNA expression library of the human LNCaP cell line derived from advanced prostate cancer with the monoclonal antibody (mAb) Pro 1.5 (Su et al., 1996). PCTA-1 gene (*LGALS8*) mapped to the chromosome region 1q42-43 (Gopalkrishnan et al., 2000). Hadari et al. (1997) also cloned another cDNA encoding for a human Gal-8 (GenBank/EMBL accession Nº X91790) isolated from a human brain hippocampus library, using the full-length rat cDNA as a probe. This lectin shares 97% homology at the protein level with PCTA-1, but both differ in eight amino acids. Po66, an mAb directed against human lung squamous cell carcinoma (Dazord et al., 1987), was also used to clone the cDNA of the protein called *Po66 carbohydrate-binding protein* (Po66-CBP), which showed high sequence similarity to the rat Gal-8 and to the human homologue PCTA-1 identified in human prostate carcinoma cells. The deduced amino acid sequence of Po66-CBP was 98.7% similar to PCTA-1 (Su et al., 1996) and 82.6% similar to the rat galectin-8 (Hadari et al., 1995), and presented two CRDs separated by a hinge peptide, which exhibited no homology to any other Gal (Bidon et al., 2001).

Alternative splicing of the Gal-8 mRNA precursor has been shown to generate isoforms, suggesting that this lectin could be considered as a particular sub-family among Gals, similar to Gal-9 (Nishi et al., 2006; Heusschen et al., 2013). Initially, the *LGALS8* gene was proposed to encode at least six isoforms of the protein: all of them contain the N-terminal region of Gal-8, with its unique WGXEXI "signature motif". The three isoforms belonging to the *tandem-repeat* type (bi-CRDs isoforms) differ only in the size of their hinge region and share similar CRDs. Deletion of the linker peptide impaired the adhesive and signaling functions of Gal-8, demonstrating that both CRDs must be properly oriented to produce cooperative interactions between themselves and the hinge domain (Levy et al., 2006). These three isoforms have been called according to the length of the linker peptide as Gal-8S ("*small*" Gal-8, with a short linker region), Gal-8M ("*medium*" Gal-8, with an intermediate linker region), and Gal-8L ("*long*" Gal-8, with the longest linker peptide), and are produced by alternative splicing, mostly at the level of intron VIII (Bidon-Wagner and Le Pennec, 2004; Ahmed et al., 2007). Moreover, in the genomic structure of PCTA, the two exons at the extremities (exons 1 and 10) are present as partial or complete entities in the mature message due to internal processing; the 5' most exon (exon 1) is present in the mature transcript either as a single 828 nucleotide unit (unspliced) or is processed internally to give a 488 nucleotide (spliced) product (Gopalkrishnan et al., 2000). In humans, Gal-8M, the middle isoform with 317 amino acids (NCBI reference: NP_963837; NM_201543), seems to be the major naturally occurring form of human Gal-8 (Fig. 1A). The isoform with the longest linker peptide, that is Gal-8L, is highly susceptible to thrombin cleavage, whereas the predominant isoform Gal-8M is resistant to thrombin; the thrombin cleavage site (-IAPRT-) of Gal-8L resides within its long linker peptide (Nishi et al., 2006). The other Gal-8 isoforms are *prototype* Gals because they lack the C-terminal domain and contain only the N-terminal domain, fused to linker regions bearing different length insertions (Bidon et al., 2001; Bidon-Wagner and Le Pennec, 2004). Ahmed et al. (2007) identified another *prototype* isoform of Gal-8, which they called "gal8g", adopting Cooper's nomenclature (i.e. "gal8a, gal8b, gal8c", and so on; Cooper, 2002) to design all isoforms of this lectin.

As expected for all Gals, both CRDs in Gal-8 are encoded by three exons (the middle one being most conserved and containing the codons encoding the sugar residue binding site): exons 3-5 and 8-10, contain the N-CRD and C-CRD, respectively (Gopalkrishnan et al., 2000). The mRNA of most Gal-8 transcripts has a short 5'UTR, but the 3'UTR is among the 5% longest found to date, and as such it may serve a regulatory function. Strikingly, it has been postulated that the Gal-8 gene is antisense over ~1 kbp, both at its 5'- and 3'-ends, to two separate genes each encoding a protein of yet unknown function (Zick et al., 2004).

Relative to the carbohydrate-binding specificity of Gal-8, Hirabayashi's group reported that the C-terminal CRD (Gal-8C) showed lower affinity for oligosaccharides than the N-terminal CRD (Gal-8N), but had higher affinity for N-glycan-type branched oligosaccharides (K_d=47-290 μM for Gal-8N and 26-52 μM for Gal-8C); Gal-8N showed strong affinity for α2-3-sialylated oligosaccharides (K_d=0.5-30 μM for Gal-8N and 97-230 μM for Gal-8C), a feature unique to Gal-8 (Hirabayashi et al., 2002). In this sense, Ideo et al. (2003) demonstrated that Gal-8 has much higher affinity for 3'-*O*-sulfated or 3'-*O*-sialylated lactose and Lewis X-containing glycans than for oligosaccharides terminating in Galß1-3/4GlcNAc; once more, this carbohydrate binding specificity was mainly attributed to

Gal-8N and unique to this member of the Gal family. Carlsson et al. (2007) observed that the preferred sialylated/sulfated ß-galactosides for Gal-8N was Galß1-4Glc (lactose), Galß1-3GlcNAc or Galß1-3GalNAc, whereas Galß1-4GlcNAc (*N*-acetyllactosamine, LacNAc) was less preferred. Later on, the crystal structure of Gal-8N in complexes with various oligosaccharides including lactose, Neu5Acα2-3Galß1-4Glc (α2-3-sialyllactose) and SO_3^--3Galß1-4Glc (3'-sulfolactose) was reported. These structures revealed that Arg^{45}, Gln^{47}, Arg^{59} are unique amino acids in Gal-8N, and interact with the sulfate or sialic acid moieties of 3'-sialyl- and 3'-sulfo-lactose (Protein Data Bank [PDB] code: 3AP7; Ideo et al., 2011). Finally, the first X-ray structure of a *tandem-repeat* Gal containing both CRDs was reported from a protease-resistant mutant of human Gal-8 (Gal-8Null, with 2 amino acids instead of the linker peptide) and a pseudo-dimer of Gal-8N, in complexes with lactose, α2-3-sialyllactose, and α2-3-sialyllactosamine (Yoshida et al., 2012). As expected, each CRD adopts a ß-sandwich arrangement formed by two antiparallel ß-sheets of six (S1-S6) and five (F1-F5) ß-strands, with a short α-helix located between F5 and S2; oligosaccharides bind to the concave surface created by S3, S4, S5, and S6 strands. Arg^{59}, located in the long S3-S4 loop particular to Gal-8N, is responsible for the strong affinity for α2-3-sialylated oligosaccharides, and Arg^{45} and Gln^{47} also help to recognize sialic acid moieties (Fig. 1B). Thus, the protein-ligand interactions at N-CRD found in this study are almost equivalent to those in the X-ray structure of Gal-8N in complexes with α2-3-sialyllactose reported by Ideo (PDB code: 3VKM; Yoshida et al., 2012).

On the other hand, Gal-8C has a preference for non-sialylated oligosaccharides like poly-*N*-acetyl-lactosamine ($[\text{-3Galß1-4GlcNAcß1-}]_n$, poly-LacNAc) and the blood group A (BGA) and B (BGB) glycan structures (Patnaik et al., 2006; Carlsson et al., 2007; Stowell et al., 2008; Ideo et al., 2011). For instance, Carlsson et al. (2007) reported that the BGA [GalNAcß1-3(Fucα1-2)Gal-] or the BGB [Galα1-3(Fucα1-2)Gal-] determinants were well recognized by Gal-8C. Recently, Kumar et al. (2013) performed molecular dynamics simulations on the crystal structure of Gal-8C, showing the strongest binding energy for lacto-N-neotetraose (Galß1-4GlcNAcß1-3Galß1-4Glc), followed by di-LacNAc, BGB and BGA antigens, and finally the disaccharides LacNAc, lacto-N-biose (Galß1-3GlcNAc) and lactose (the last three with almost identical binding efficiency).

Different cellular functions have been attributed to Gal-8 (Bidon-Wagner and Le Pennec, 2004; Zick et al., 2004). Extracellular Gal-8 acts as a matrix protein equipotent to fibronectin in promoting cell adhesion, for example by ligation and clustering of a selective subset of cell surface integrins (Levy et al., 2001; Zick et al., 2004). Complex formation between Gal-8 and integrins involves carbohydrate-protein interactions and triggers integrin-mediated signaling cascades such as Tyr phosphorylation of focal adhesion kinase (FAK) and

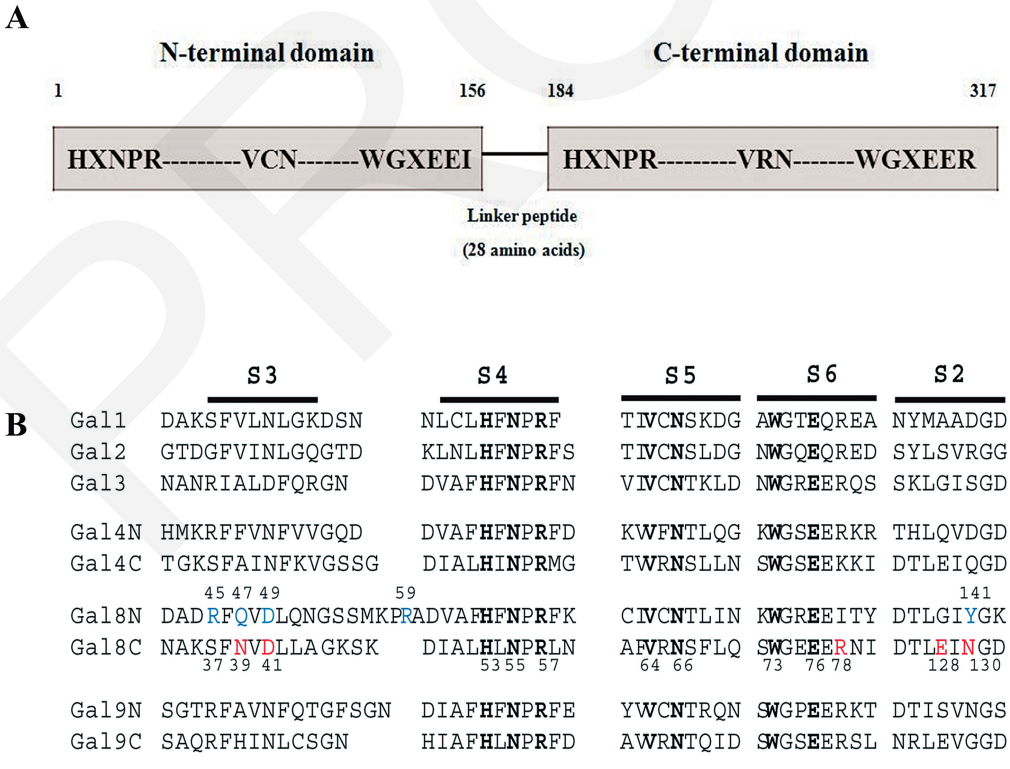

Fig. 1. Structure and sequence homologies of human galectin-8. **A.** A schematic structure of human Gal-8M (NP_963837; NM_201543) is shown. Each box represents a carbohydrate-binding domain, linked by a 28 amino acid long linker peptide. Conserved motifs HXNPR, VCN, WGXEEI, VRN and WGXEER are pointed out. **B.** Multiple sequence alignments of the human galectin members. Conserved amino acids are shown in bold, amino acids which play important roles in interactions apart from conserved residues in Gal-8C are shown in red and in blue for Gal-8N. This multiple sequence alignment was carried out by MAFFT. Taken from Kumar et al., 2013. With permission from PLoS ONE. Gal-8C amino acid numbering corresponds to the one in Protein Data Bank code 3OJB.

paxillin, and activation of the MAPK and PI3K pathways (Levy et al., 2001, 2003). In contrast, when present as a soluble ligand, Gal-8 negatively regulates cell adhesion, induces growth arrest and apoptosis (Arbel-Goren et al., 2005; Levy et al., 2006). Because of its pro-adhesive and anti-adhesive functions, Gal-8 can be considered a novel member of adhesion-modulating proteins such as SPARC, thrombospondin, tenascin, hevin, and disintegrins, collectively known as matricellular proteins (Levy et al., 2001; Zick et al., 2004; Elola et al., 2007). In fact, direct interactions between Gal-8 and fibronectin have already been documented (Levy et al., 2001; Reticker-Flynn et al., 2012).

Secreted Gal-8 selectively interacts with cell surface integrin subunits such as α_3, α_6, and $ß_1$, and to a limited extent with α_4 and $ß_3$; Gal-8 modulates integrin interactions with the extracellular matrix and regulates adhesion and survival in 1299 cells from human non-small cell lung carcinoma (Hadari et al., 2000). In Jurkat T cells, Gal-8 interacts with specific integrins, such as $\alpha_1ß_1$, $\alpha_3ß_1$, $\alpha_5ß_1$ but not $\alpha_4ß_1$, and promotes cell adhesion and asymmetric spreading through activation of ERK1/2 (Cárcamo et al., 2006). Gal-8 is a potent pro-apoptotic agent in Jurkat T cells inducing a complex phospholipase-D/phosphatidic acid signaling pathway which has not been reported for any galectin before. Gal-8 increases phosphatidic acid signaling which enhances the activity of both ERK1/2 and type 4 phosphodiesterases, with a subsequent decrease in basal PKA activity (Norambuena et al., 2009).

Tribulatti et al. (2007) showed the intrathymic expression and pro-apoptotic function of Gal-8 on $CD4^{high}CD8^{high}$ thymocytes. These data highlighted a role for Gal-8 in shaping the T-cell repertoire. Gal-8 has two distinct effects on CD4 T cells: at high concentrations it induces antigen-independent proliferation, whereas at low concentrations it co-stimulates antigen-specific T cell responses (Tribulatti et al., 2009). Gal-8 as a modulator of T lymphocyte activities strictly requires both CRDs to induce T cell proliferation, raising the idea that it might be acting by cross-linking of counter-receptors. In Jurkat T cell surface, Gal-8 induces CD45 cluster formation in a glycan-dependent fashion, suggesting that its activity on T cells might trigger lattice formation (Cattaneo et al., 2011).

Several reports highlighted different roles of Gal-8 in inflammation and autoimmunity (Troncoso et al., 2012). For instance, Gal-8 can modulate the inflammatory function of neutrophils (Nishi et al., 2003). Moreover, in rheumatoid arthritis, Gal-8 has an anti-inflammatory action, promoting apoptosis of synovial fluid cells that can be counteracted by a specific rheumatoid version of CD44 (CD44vRA) (Eshkar-Sebban et al., 2007). In systemic lupus erythematosus, an autoimmune disease, function-blocking auto-antibodies against Gal-8 have been described (Cárcamo et al., 2006). In platelets, Gal-8 can bind to the cell surface and activate different functional responses, including spreading, activation of integrin $\alpha_{IIb}ß_3$, aggregation and secretion of both dense and α granules, in a glycan-dependent manner (Romaniuk et al., 2010). In addition, Gal-8 in platelets directly interacts with coagulation factor V (FV), which is critical for the coagulation cascade. Moreover, megakaryocytic cells internalize FV at least in part through a Gal-8-dependent mechanism (Zappelli et al., 2012).

Stowell et al. (2008) showed that Gal-8 can induce phosphatidylserine exposure: full-length Gal-8, Gal-8N and Gal-8C bound to human HL60 cells, but only full-length Gal-8 signaled phosphatidylserine exposure, which occurred independently of apoptosis. In this study, the authors demonstrated dimerization of Gal-8, showing that the lectin dimer was composed of four CRDs and functional bivalency at each separate domain. Dimerization occurs through the Gal-8N domain and allows Gal-8 to induce phosphatidylserine exposure in leukocytes in the absence of cell death, entirely through the C-terminal domain, which recognizes cell surface poly-LacNAc, and to trigger intracellular signaling. In addition, an elegant study demonstrated that Gal-8 can recognize blood group B^+-bacteria (Stowell et al., 2010). Thus, the preference of C-CRD for blood group antigens leads to an immunoprotective effect against bacteria: Gal-8 expressed in the intestinal tract might recognize and directly kill human BGB-expressing *Escherichia coli* while failing to alter the viability of other *E. coli* strains or other Gram-negative or Gram-positive organisms. Strikingly, Gal-8 has also been shown to target damaged vesicles for autophagy in bacterial infections by physically interacting with the autophagy cargo receptor NDP52, in a protein-protein interaction. In fact, Gal-8 monitors endosomal and lysosomal integrity and detects bacterial invasion through binding to host glycans exposed on damaged *Salmonella*-containing vacuoles. Gal-8-dependent recruitment of NDP52 to *Salmonella*-containing vesicles is transient and followed by ubiquitin-dependent NDP52 recruitment and autophagy (Thurston et al., 2012). The X-ray crystal structure of the NDP52-Gal-8 complex has recently been solved, showing how NDP52 binds exclusively to Gal-8 but not to other Gals (Li et al., 2013).

Galectin-8 in human tumors

Gal-8 is one of the most widely expressed bi-CRD Gals in human cells and tissues. Gal-8 expression has been detected in lung, brain, breast, colon, head and neck, kidney, ovary, pancreas, parathyroid, prostate and uterus tumor tissues (Bidon et al., 2001). However, Gal-8 is also found in several normal tissues including brain, breast, colon, retina, kidney, pancreas, spleen, uterus, testis and vascular tissues, but only in some embryonic tissues, including brain, kidney, uterus, liver and lung (Bidon et al., 2001; Bidon-Wagner and Le Pennec, 2004). Danguy et al. (2001) evaluated the

immunohistochemical profiles of Gal-8 expression in 200 benign and malignant human tumors of different embryological origin, such as epithelial, mesenchymatic and adipose tissues, and tumors of the nervous system. Similarly, mRNA profiling of an extensive panel of human tumor cell lines showed that Gal-8 mRNA was the most abundantly expressed among Gals when 61 cell lines of different origins (breast, colon, lung, brain, skin, kidney, urogenital system, and hematopoietic system) were monitored. Expression of Gal-8 was found in all but two of the tested cells (Lahm et al., 2001). We herein integrate the available information on Gal-8 expression in different tumor types and their normal counterparts and describe the associations between the expression of this lectin, its localization and tumor progression with the overarching goal of evaluating its potential for differential diagnosis, prognosis and therapy.

Galectin-8 in lung carcinoma

Initially, Hadari et al. (1995) characterized the expression of Gal-8 in different rat tissues using Northern blot analysis, and reported that Gal-8 mRNA was highly expressed in the lungs. Later on, but prior to the knowledge that the Po66 mAb was specifically raised against Gal-8, this mAb was employed in an immunohistochemical study on human primary and secondary malignant lung tumors of various histological origins (Caulet-Maugendre et al., 2002; Henno et al., 2002). In fact, Po66 mAb has been obtained by immunizing mice with dissociated cells from a patient's lung squamous cell carcinoma (Dazord et al., 1987). Specimens of bronchopulmonary tumors included 41 primary squamous, glandular or neuro-endocrine tumors, 11 secondary tumors of glandular, connective tissue,

Table 1. Galectin-8 expression during tumor progression in different human cancers.

Organ	Tumor type	Up/down regulation	Subcellular localization with progression	Correlations and value	References
Lung	Squamous cell carcinoma	↑	ND	↑Expression/↑differentiation degree	Henno et al., 2002; Caulet-Maugendre et al., 2002
	Metastases from lung carcinoma	↑	ND	↑Expression/↑clinical stage; ↑Expression/↑metastases	Reticker-Flynn et al., 2012
Prostate	BHP and T1, T2, T3, T4 carcinoma stages	Constant levels	ND	ND	Su et al., 1996; Danguy et al., 2001; Laderach et al., 2013
Colon	Carcinoma	↓	N→C		Danguy et al., 2001
	Colon carcinoma: T1, T2, T3, T4 stages	↓	N→C		Nagy et al., 2002
Colon; rectum	Colorectal carcinoma (Dukes A, B, C, D stages)	↓		↓Expression/↓survival in Dukes C-D patients; Pronostic value	Nagy et al., 2003
Liver	Hepatocellular carcinoma	↓	N→C	ND	Danguy et al., 2001
Breast	Lobular carcinoma	↑	ND	ND	Danguy et al., 2001
Head&Neck: Brain	Pilocytic astrocytoma; Diffuse astrocytoma; Anaplastic astrocytoma; Glioblastoma	Constant levels	ND	ND	Camby et al., 2001 Danguy et al., 2001
Head&Neck: Larynge	Squamous cell carcinoma	↑	No change	↑Expression/↑metastases ↑Expression/↑tumor stage Pronostic value	Dong et al., 2009 Cludts et al., 2009
	Squamous cell carcinoma	↓		Conflicting data (but small sample)	Danguy et al., 2001
Head&Neck: Hypolarynge	Squamous cell carcinoma	↑	No change	No correlation detected	Cludts et al., 2009
Head&Neck: Thyroid	Papillary carcinoma	↑	ND	Differential diagnosis	Savin et al., 2009
Head&Neck: Parotid gland	Warthin's tumor	ND	Cytoplasmic	ND	Saussez et al., 2010
Head&Neck: Ear	Cholesteatoma	ND	ND	↑Expression/↑retinoid acid receptor ß	Sheikholeslam-Zadeh et al., 2001; Simon et al., 2001; Peng et al., 2004
Bladder	Carcinoma	Conflicting data	ND	Conflicting data	Danguy et al., 2001; Langbein et al., 2007; Kramer et al., 2011

BPH, benign prostatic hypertrophy; ND, not determined; N→C, shift from nuclear to cytoplasmic Gal-8 localization with increased malignancy; ↑, increased; ↓, reduced.

melanocytic or germinal origin as well as 9 extra-pulmonary primary tumors (lung metastases). Gal-8 was strongly expressed in squamous cell carcinoma, very weakly in adenocarcinoma, and was undetectable in small cell carcinoma. A correlation between Gal-8 expression and the differentiation status of squamous cell carcinomas and neuroendocrine tumors was detected (Caulet-Maugendre et al., 2002; Henno et al., 2002; Bidon-Wagner and Le Pennec, 2004). Po66 mAb was also shown to accumulate in human lung tumors grafted into nude mice through intravenous injection (Desrues et al., 1995). Moreover, for immunoscintigraphic detection of human lung squamous cell carcinoma, Po66 mAb was injected intravenously in 33 patients with histologically confirmed primary non-small cell lung carcinoma: ^{131}I-radiolabeled mAb detected 78% of the primary tumors and 100% of recurrences by immunoscintigraphy; in four patients recurrence was detected despite being undetectable by plain chest X-ray. This clinical investigation showed that Po66 mAb was able to fix specifically to lung tumors and to detect lung squamous cell carcinoma recurrences (Bourguet et al., 1990). Biodistribution of Po66 mAb after intravenous injection in tumour-bearing mice was also analyzed and showed that the fixation of the antibody in the tumor was long-lasting (14 days); in order to get a better fixation, doxorubicin chemotherapy was co-administrated. Preliminary results showed that injection of ^{131}I-radiolabeled Po66 mAb and doxorubicin generated tumor regression, although the very slow clearance of the antibody caused high toxicity (Desrues et al., 1995). Later on, Bidon et al. (2001) cloned the cDNA of the protein recognized by Po66 mAb from a cDNA expression library of SK-MES-1 human lung squamous carcinoma cells and identified this protein as Gal-8.

Conflicting data about the expression levels of Gal-8 in normal versus tumoral lung have been reported. Bidon et al. (2001) analyzed Gal-8 expression in healthy, tumoral and embryonic lung using the Cancer Genome Anatomy Project (CGAP) library database and observed that Gal-8 is not expressed in healthy lung, but transcripts are found in tumoral and embryonic tissue. On the other hand, Danguy et al. (2001) analyzed Gal-8 expression by immunohistochemistry (IHC) in normal and tumoral human lung tissues, detecting high levels of the lectin. When comparing the immunopositivity between 5 squamous cell carcinomas plus 5 adenocarcinomas versus 4 normal samples, no statistically significant differences were detected, because of the high content of the lectin in normal tissues and the staining variability in the tumors, although a larger number of cases still need to be analyzed.

Seeking to understand the mechanistic pathways underlying progression and metastatic spread of human lung cancer, Gal-8 was identified as one of the proteins that is up-regulated in human lung cancer as well as in local and distant metastases (Reticker-Flynn et al., 2012). By using a genetic dataset analysis, it was possible to study the correlation between gene expression and disease severity, revealing that increased expression of the gene LGALS8 correlated with advanced stages and/or the presence of metastases. *In vivo* experiments in mice confirmed these findings as Gal-8 was detected within tumor metastases, both in lymph nodes and liver metastases (but not in primary tumors). Hence, Gal-8 has been proposed to be a metastasis-associated molecule, such as fibronectin, laminin or Gal-3. Furthermore, an extracellular matrix microarray and *in vitro* adhesion experiments were used to compare the adhesion profiles of murine lung adenocarcinoma cell lines (from non-metastatic primary tumors, primary tumors that metastasized, or lymph node and liver metastases). Combinations of fibronectin with Gal-8 generate adhesion patterns that differentiate metastatic cell populations from primary tumor cells. Thus, Gal-8 is a molecular mediator that influences metastatic spread of lung carcinomas (Reticker-Flynn et al., 2012). In this regard, in human 1299 cells derived from a non-small cell lung carcinoma, Hadari et al. (2000) identified high levels of Gal-8, whose secretion interacted selectively with cell surface integrins such as α6ß1 and α3ß1, modulated integrin interactions with the extracellular matrix, and regulated cell adhesion and survival.

Galectin-8 in prostate carcinoma

As mentioned above, Su et al. (1996) identified the gene PCTA-1 as human Gal-8 by screening a human prostate cancer cDNA expression library from LNCaP cells with the Pro 1.5 mAb. To determine if Pro 1.5 mAb could react with patient-derived prostate cancer specimens, frozen sections were prepared from normal (males <40 years of age), benign prostatic hypertrophy (BPH), and carcinomas of the prostate. Normal prostate showed limited reactivity with Pro 1.5, whereas the tumor marker *prostate-specific antigen* (PSA) readily stained normal prostate epithelial cells. As expected, PSA also stained prostate cells in tissue sections of benign prostatic hypertrophy (BPH) and prostate carcinomas. Pro 1.5 mAb reacted strongly with prostate carcinoma cells present in frozen tissue sections, but not with adjacent benign glands or tissue sections containing normal prostate. However, some reactivity with Pro 1.5 was found in prostatic intraepithelial neoplasia (PIN). These studies seemed to indicate that the Pro 1.5 mAb can distinguish between prostate carcinoma and PIN versus normal prostate epithelial cells. The authors proposed that Pro 1.5 might have a discriminatory value as a surrogate biomarker for the detection of prostate cancer that exceeds that of the nonspecific prostate epithelial cell marker PSA. Using RT-PCR analysis, PCTA-1 mRNA was detected in seven out of seven prostate carcinomas, one of four BPH, and one of four putative normal prostate tissue samples. In the BPH sample displaying PCTA-1 expression, histological analysis indicated that it was a PIN case; however in the

putative normal prostate tissue sample expressing PCTA-1, no tissue was available for histological analysis. Although the sampling size was small, these results indicated that PCTA-1 expression is found in prostate carcinomas and subsets of BPH displaying early stages of cancer (i.e., PIN). In contrast, Gal-8 expression was not evident in normal prostate tissue (Su et al., 1996).

Danguy et al. (2001) evaluated Gal-8 expression in human prostate specimens by IHC from benign hyperplasia (5 cases) versus adenocarcinoma (5 cases), and could not detect significant differences in the expression levels of this lectin. Accordingly, Gal-8 was found to be expressed by prostate primary tumors and benign hyperplasia (Laderach et al., 2013). Paraffin sections from prostatectomy samples were obtained from 61 patients with newly diagnosed previously untreated disease, and classified according to TNM classification including a large spectrum of prostate carcinoma stages (T1, T2, T3 and T4) in addition to BHP. By IHC, Gal-8 was detected at moderate levels in lesions corresponding to all stages, including BHP, without statistically significant differences among them. Regarding BHP samples, differences obtained between this study and that of Su et al. (1996) could be due to modifications of the IHC protocol (i.e. saponin use) and/or the distinct primary antibody employed (Laderach et al., 2013).

In human prostate cancer cell lines such as LNCaP, DU-145 and PC-3, RT-PCR analysis revealed the occurrence of considerable amounts of Gal-8 (Su et al., 1996; Lahm et al., 2001). By Northern blot analyses using total RNA from human prostate cell lines as well as normal prostate RNA, higher overall expression levels of all isoforms in metastatic cell lines (i.e. PC-3 cells) versus normal tissues were observed (Gopalkrishnan et al., 2000). Ahmed et al. (2007) found Gal-8 isoforms expressed in PrEC and LNCaP cells, and little or no expression of these isoforms was observed in BPH-1 cells. In fact, transcripts for Gal-8 were expressed at moderate levels in all prostate cancer cell lines tested, for example in the hormone-responsive LNCaP cell line and the castration-resistant 22Rv1 and PC-3 cell lines (Laderach et al., 2013).

Galectin-8 in colon carcinoma

Danguy et al. (2001) studied Gal-8 expression in malignant colon tumor tissues from paraffin sections by IHC. The 18 colon tissues examined included 7 cases of normal mucosa, 5 of low to moderate dysplasia and 6 adenocarcinomas. A statistically significant decrease in reactivity was observed in cancerous tissue from colon, with a change in the subcellular location of Gal-8: many nuclei exhibited marked staining in the normal tissues and benign tumors, while the nuclear location of Gal-8 disappeared in malignant colon. Accordingly, Nagy et al. (2002) also determined Gal-8 expression by IHC in normal, dysplastic and cancerous human colon tissues obtained after surgical resections or endoscopy; epithelial cells from 41 human cancer samples were subjected to computer assisted microscope analysis and compared with those from normal control samples. Gal-8 expression increased from the normal to dysplastic stage, and then decreased markedly in colon carcinomas. Furthermore, malignant colon tissue with augmented invasiveness exhibited significantly less Gal-8 expression than colon carcinomas with limited invasion capacity. Once again, while Gal-8 was located both in the cytoplasm and the nucleus of normal and benign colon tissues, it was located exclusively in the cytoplasm of malignant colon cells. To analyze the prognostic value of Gal-8 in colon carcinoma, Nagy et al. (2003) selected 55 colorectal carcinomas staged according to the Dukes classification, adding a "D" stage to label the tumors resected from patients with distant metastases (10 Dukes A, 16 Dukes B, 15 Dukes C and 14 D metastatic cases). No significant variation was observed in Gal-8 expression as tumors progressively increased from Dukes state A to C. When the percentages of Gal-8-expressing epithelial cells (labelling index) were evaluated as a function of the patient survival period, a Gal-8 labelling index lower than 33% was associated to short survival in patients included in Dukes C and D stages. In fact, Gal-8 expression showed a prognostic value in late clinical stages Dukes C and D in colorectal carcinoma.

Nagy et al. (2002) analyzed four human colon cancer models, using HCT-15, CoLo201, LoVo and DLD-1 human cancer cell lines and their *in vivo* corresponding xenografts by IHC. They found a decrease in Gal-8 expression in the *in vivo* models as compared with the expression observed *in vitro* in the original tumor cells. In addition, the subcutaneous grafting procedure revealed that LoVo and DLD-1, the two rapidly growing models, exhibited significantly lower amounts of Gal-8 than HCT-15 and CoLo201, the two slowly growing models. Moreover, as a substrate for migration of human colon cancer cells, Gal-8 proved to markedly reduce migration of the slowly growing HCT-15 and CoLo-201 cells, an effect which was significantly neutralized by anti-Gal-8 antibodies. In contrast, neither Gal-8 nor its antibody modified migration of other rapidly growing cell lines (Nagy et al., 2002). Interestingly, Gal-8 was identified in 22 different colon cancer cell lines tested by RT-PCR (Lahm et al., 2001), and the presence of two transcripts was described in HCT-116 and HT-29 cells (Satelli et al., 2008). cDNA cloning of Gal-8 from three human colorectal cancer cell lines (derived from primary and secondary lesions) and from a normal colonic cell line was also conducted (Lahm et al., 2004). In serum samples, Barrow et al. (2011) assessed Gal-8 levels in 51 samples from patients with colorectal cancer and 31 from healthy individuals by ELISA. Gal-8 concentration was 1.8-fold higher in patients with colorectal cancer and 5.6 higher in those with metastases, with respect to healthy individuals. Furthermore, they assessed the effects of Gal-8 on cancer cell adhesion to vascular endothelium, pre-

incubating human colon cancer HT29-5F7 cells with recombinant Gal-8. This lectin induced a dose-dependent increase of HT29-5F7 (a clone of HT29 cells with resistance to 5-fluorouracil) cell adhesion to HUVEC, which was completely abolished by lactose. In addition, Gal-8 also induced an increased adhesion of HT29-5F7 and SW620 cells, but not HT29 cells, to human HMVEC-L microvascular endothelial cells. Thus, increased circulating Gal-8 in colon cancer patients may contribute to promote metastasis by modulating heterotypic interactions between tumors and endothelial cells.

Galectin-8 in hepatocellular cancer

Expression of Gal-8 was also analyzed in different human liver specimens. Twenty liver samples were examined, including 5 normal cases, 5 cirrhotic livers, 5 hepatoblastomas and 5 hepatocarcinomas. Similar to the colon, a dramatic decrease in immunoreactivity was observed between the malignant and benign tissues. In fact, in normal and cirrhotic livers, the staining intensities of positive cells appeared to be moderate to strong and the percentage of positive cells per specimen varied between 80 and 100%, while in hepatoblastomas and hepatocarcinomas the percentage of positive cells per specimen varied between 40 and 100% and the staining intensity of positive cells was between weak to moderate. Moreover, the authors found changes in the subcellular distribution of Gal-8, with its nuclear localization disappearing with malignancy (Danguy et al., 2001).

Galectin-8 in breast cancer

Danguy et al. (2001) evaluated Gal-8 expression in breast cancer: higher expression of Gal-8 in the malignant as opposed to the benign breast tissues were mainly detected in lobular breast carcinomas. In fact, 17 specimens were analyzed by IHC, which included 4 cases of fibrocystic dysplasia, 4 tubular adenomas, 4 invasive ductal carcinomas and 5 lobular carcinomas. The staining score for Gal-8 in lobular carcinomas indicated statistically significant differences versus the one from benign tissues.

In addition, Gal-8 was identified as a tumor antigen in a neu transgenic mouse model of estrogen receptor-negative breast cancer that has significant similarity to human premenopausal breast cancer. These transgenic animals usually developed spontaneous tumors, and by using pooled sera from 10 tumor-bearing mice, a screening of recombinant cDNA expression library was performed which allowed the identification of a tumor antigen repertoire. In fact, Gal-8 was one of the five most frequently identified genes among 15 tumor antigens detected. It was proposed that these tumor antigens present in transgenic mice might predict immunogenic human homologues (Lu et al., 2006).

In blood vessels from human breast tissues, we have studied Gal-8 expression and localization in normal and tumor specimens. Our studies on paraffin-embedded samples revealed that blood vessels in normal breast tissues showed strong nuclear staining, which was also observed in ductal carcinoma *in situ* and invasive ductal carcinoma, whereas diffuse cytoplasmic reactivity was always detected. In summary, Gal-8 is localized in both nuclear and cytoplasmic compartments of normal and tumor-associated endothelial cells from human breast tissues, and its endothelium staining profile does not change with tumor progression (Delgado et al., 2011).

In serum samples, Barrow et al. (2011) quantified Gal-8 levels in 40 cases from patients with breast cancer in comparison with control samples by ELISA. The concentration of Gal-8 was 1.8-fold higher in sera from patients with breast cancer as compared with healthy individuals. Moreover, Gal-8 induced adhesion to vascular endothelium of HBL-100 human breast epithelial cells transfected with the Thomsen-Friedenreich (Galß1-3GalNAc) disaccharide expressed by the cancer-associated transmembrane mucin protein 1. Thus, increased circulation of Gal-8 might promote dissemination of tumor cells by promoting heterotypic adhesion of tumor cells to vascular endothelium, at least in part via the Thomsen-Friedenreich/ mucin protein 1.

Galectin-8 in head and neck tumors

Brain tumors

Gliomas are the most frequent primary brain tumors in adults, and among them astrocytomas are the most common, which are classified as pilocytic astrocytomas (PILs, grade I), diffuse astrocytomas (ASTs, grade II), anaplastic astrocytomas (ANAs, grade III) and glioblastoma (GBM, grade IV). Glioblastomas are characterized by a very poor prognosis, which can be at least in part explained by the fact that glioma cells diffusely infiltrate the brain parenchyma and exhibit decreased levels of apoptosis (Louis et al., 2007; Le Mercier et al., 2010). By IHC, Camby et al. (2001) quantitatively characterized the levels of Gal-8 expression in 116 human astrocytic tumors of grade I to IV. The 116 cases included 22 grade I (PILs), 26 grade II (ASTs), 29 grade III (ANAs) and 39 grade IV (GBMs) tumors. For each specimen, 3 types of histological structures were analyzed: the tumor body without blood vessels, the blood vessel walls, and 2-3 layers of perivascular tumor astrocytes. The percentage of Gal-8 immunopositive tissues remained near 100% and unchanged in the four histopathological groups, regardless of the histological structure considered. Comparable Gal-8 staining intensity was found in the 3 histological structures in all the tumor groups analyzed. Of note, Gal-8 expression was significantly higher in the vessel walls than in the tumor bulk for the grade I to III astrocytic tumors.

Danguy et al. (2001) also analyzed Gal-8 expression in other central nervous system tumors, including 4

benign schwannomas which were compared with 4 malignant nerve sheath tumors (tumors of cranial and paraspinal nerves), 4 primitive neuroectodermal tumors (tumors of the meninges), 5 medulloblastomas and 8 astrocytic tumors (3 grade II astrocytomas, 2 grade III anaplastic astrocytomas and 3 grade IV glioblastomas) (both neuroephitelial tumors), all of them classified according to Louis et al. (2007). The authors found that astrocytic tumors showed higher staining scores as compared to benign tissues. Interestingly, Camby et al. (2001) investigated whether the pattern of Gal-8 expression differed or not in high-grade ANAs and GBM, depending on patient survival. No significant differences were observed for Gal-8 in the two groups of patients with different prognoses. Expression of Gal-8 in xenografts was also analyzed by grafting H4, U87 or U373 astrocytic tumor cells into the left temporal lobe of nude mice, evaluating the less invasive central part versus the most infiltrating part contacting the brain parenchyma in each xenograft. In fact, Gal-8 expression was higher in the most invasive part of U87 and U373 xenografted glioblastomas compared to the less invasive part. In astrocytic cell lines, the Gal-8 profile was also analyzed by RT-PCR, and strong signals for lectin transcripts were found, which was also reported by Lahm et al. (2001). Regarding cell migration, Gal-8 significantly increased migration in T98G and U373 glioblastoma cells; these results are particularly relevant as tumor astrocyte migration constitutes the major phenotypic hallmark of malignancy in astrocytic tumors (Camby et al., 2001).

Laryngeal tumors

Gal-8 expression in laryngeal squamous cell carcinoma was first reported by Danguy et al. (2001), who performed comparative IHC studies on specimens from 4 normal and 4 squamous cell carcinoma tissues: a significant decrease in immunoreactivity was observed when comparing the malignant to the benign samples. Conflicting data were reported in more recent studies, which included a larger number of cases. Dong et al. (2009) analyzed paraffin-embedded specimens from 77 patients, which were classified by clinical stage, nodal stage, T-stage and histopathological differentiation. Based on the clinical data, the cases were divided into two groups: one that had no metastases to the cervical lymph nodes and the other that exhibited metastases; the results showed a significant increase in Gal-8 expression in cases with metastasis. The same was true when the cases were divided according to the clinical stage into two groups: early and advanced stages, or when T-staging was used for evaluation. Positive correlations between Gal-8 expression and metastasis to the lymph nodes, clinical stage or tumor stage were found. The authors concluded that the expression of Gal-8 could be used as a prognostic factor for patients with laryngeal squamous cell carcinoma. In this regard, Cludts et al. (2009) described the up-regulation of Gal-8 during hypopharyngeal and laryngeal tumor progression. The level of Gal-8 expression was determined by IHC in a series of 18 and 16 cases of tumor-free epithelium, 24 and 10 cases of low-grade dysplasia, 22 and 15 cases of high-grade dysplasia localized in peri-tumoral area and 74 and 37 hypopharyngeal and laryngeal carcinomas, respectively. Both staining intensity and percentage of immunopositive area were higher in hypopharingeal carcinoma cases as compared with tumor-free epithelium or cases with low degree of dysplasia. In contrast to hypopharingeal cancer, the staining intensity showed no evidence of a significant difference between the tumor-free epithelium, dysplasia and larynx squamous cell carcinoma, but the percentage of Gal-8 immunopositive cells was higher in carcinoma cases as compared to dysplasia and tumor-free epithelium tissue. This difference was significant when comparing laryngeal squamous cell carcinoma to tumor-free epithelium or tissue with low degree of dysplasia. No correlation with recurrence was detected and a shift between cytoplasmic and nuclear localization was not observed. These results are consistent with the up-regulation of Gal-8 expression during hypopharyngeal and laryngeal tumor progression, but they are in disagreement with those obtained by Danguy et al. (2001).

Thyroid tumors

Gal-8 expression in normal and pathological human thyroid tissue was also analyzed: 41 archival tissue samples (5 follicular adenomas, 31 papillary carcinomas, 5 follicular carcinomas) together with 36 adjacent hyperplastic or normal tissues were evaluated by IHC. Gal-8 was expressed in the majority of papillary carcinomas (87%). Positive but weaker staining was also found in some of the follicular thyroid carcinomas (40%) and adenomas (40%). It was not detectable in five normal thyroid tissue samples, whereas hyperplastic areas adjacent to the tumor were weakly positive in 9 out of 31 cases (29%). High Gal-8 immunostaining in papillary thyroid carcinoma suggested that Gal-8 may potentially serve as a marker with diagnostic value for papillary thyroid carcinoma. However, it does not seem to be helpful in the differential diagnosis of follicular carcinoma and adenoma (Savin et al., 2009).

Parotid tumors

Gal-8 was also detected in Warthin's tumor of the parotid gland, which is assumed to originate from the proliferation of epithelial inclusions within parotid lymph nodes. In that case, these cells are supposed to retain characteristics similar to common salivary gland ductal cells. Using IHC, 42 cases of Warthin's tumor and 29 cases of adjacent tumor-free tissues were evaluated: Gal-8 was localized in intralobular ducts with moderate cytoplasmic and weak nuclear immunopositivity; both layers of interlobular ducts and of Warthin's tumor presented strong cytoplasmic immunostaining.

Reactivity for Gal-8 in Warthin's tumor significantly correlated with reactivity of intralobular ducts and with reactivity of interlobular ducts (Saussez et al., 2010).

Cholesteatoma

Cholesteatomas are benign tumors characterized by the presence of an unrestrained growth and the accumulation of keratin debris in the middle ear cavity, at least in part as a result of p53-mediated apoptosis of excessive keratinocytes; cholesteatomas with the highest apoptotic indexes recur more rapidly, and also exhibit high levels of p53 immunopositive cells (Choufani et al., 1999). Sheikholeslam-Zadeh et al. (2001) investigated whether Gals were expressed in human cholesteatomas and whether they correlated or not with the levels of apoptosis. They analyzed 52 cholesteatomas, including 2 congenital and 50 acquired cases (33 primary and 19 recurrent cases). The levels of expression of Gal-8 were significantly lower in connective than in epithelial tissue, and no correlation was seen between Gal-8 expression and apoptosis (as determined by nuclear DNA fragmentation). Since retinoid acid controls the differentiation processes in keratinocytes, Simon et al. (2001) studied the levels of expression of α, ß and γ retinoid acid receptors (RARs) and Gals in a series of 70 human cholesteatomas, classified according to Sheikholeslam-Zadeh et al. (2001). RARß expression correlated with the level of Gal-8 expression, which also correlated with RARα and RARγ expression. The association between RARß and Gal-8 was also described by Peng et al. (2004), who analyzed 42 samples of acquired middle ear cholesteatoma and 18 cases of external ear skin. They showed that RARß was mainly expressed in the cytoplasm and the plasma membrane of the epithelial cells, and that Gal-8 expression was detected in the basal cell layers and in keratinocytes of the suprabasal cell layers. Again, Gal-8 expression highly correlated with RARß expression, suggesting that undifferentiated populations of keratinocytes could lead to the cholesteatoma formation, and that a functional relationship may exist between retinoid acid activity and this lectin.

Galectin-8 in bladder carcinoma

In bladder carcinoma, conflicting data have been documented regarding Gal-8 staining patterns. Langbein et al. (2007) studied 61 bladder carcinoma versus 6 normal samples and observed that Gal-8 reactivity was moderate in the cytoplasm of normal tissue and low-stage carcinoma, whereas it was intense in high-stage tumors. Significant correlations between T-staging and Gal-8 expression were found. Later on, Kramer et al. (2011) analyzed Gal-8 expression patterns in bladder cancer and normal specimens: 162 samples of non-muscle-invasive transitional cell carcinoma, 25 samples of muscle-invasive transitional cell carcinoma, and 10 samples of normal urothelium were investigated by IHC using tissue microarrays. When Gal-8 staining patterns and tumor recurrence were analyzed, opposite results were obtained as compared to those obtained by Langbein et al. (2007): loss of Gal-8 was significantly associated with the likelihood of tumor recurrence, although no significance was observed regarding tumor progression. Patients whose specimens showed weak Gal-8 expression had a shorter recurrence-free interval. All of the 10 normal urothelium samples showed high Gal-8 expression, and decreased staining was found to be associated with higher tumor stages and grades. Moreover, significant differences were found comparing normal urothelium with any tumor stage, and non-muscle-invasive versus muscle-invasive tumors. In summary, controversial data are available on Gal-8 expression in bladder carcinoma, which need to be clarified in future studies.

Conclusion

In summary, Gal-8 can be up or down regulated in some types of tumors, and its expression could positively or negatively correlate with tumor progression or recurrence, suggesting it might have diagnostic, prognostic and/or therapeutic relevance in particular cases. Differential diagnosis is still a main concern in tumor pathology, and Gal-8 could be of great value in some types of primary or secondary tumors (i.e. papillary thyroid carcinoma, advanced colon carcinoma from patients with distant metastases, or metastases from primary lung carcinoma). For instance, Gal-8 has been proposed to be of prognostic value for laryngeal carcinoma and advanced colon carcinoma with distant metastases.

With such complex gene regulation, giving rise to at least seven isoforms, this lectin could serve as an interesting tool to understand neoplastic transformation and as a possible therapeutic target in cancers. Correlations between malignant transformation and differences in subcellular localization of Gal-8 have been documented. Shuttling of Gal-8 between the nucleus and the cytoplasm is still a mystery, but loss of nuclear Gal-8 expression might be associated with tumor progression in some types of tumors (i.e. colon and hepatocellular carcinomas). In this regard, loss of nuclear Gal-3 expression was associated with tumor progression, for example in colon and prostate carcinoma, and in squamous cell carcinoma of the tongue (Davidson et al., 2002; Li et al., 2006).

Some biomarkers are already being used as clinical tools, but the field is still in its early stages. More biomarkers need to be developed and their prognostic values should be ascertained in larger cohorts of patients and contrasted with already accepted biomarkers. In this regard, Gal-8 determination in a wider range of tumors may help to delineate its value for differential diagnosis and/or prognosis purposes. Further studies are needed to clarify the potential applications of Gal-8 and its isoforms in tumor pathology and their different intra- or

extracellular roles (cytoplasmic, nuclear or extracellular) in tumor biology.

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