Glycoconjugate Journal 19, 557–563, 2004

Human galectin-8 isoforms and cancer

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Galectins are animal lectins that can specifically bind β-galactosides. Thirteen galectins have already been described. This review focuses on a specific member of this family: galectin-8. This galectin was discovered in prostate cancer cells eight years ago and has been studied extensively in the last few years. The galectin-8 gene (*LGALS8)* encodes numerous mRNAs by alternate splicing and the presence of three unusual polyadenylation signals. These mRNAs encode six different isoforms of galectin-8: three belong to the tandem-repeat galectin group (with two CRDs linked by a hinge peptide) and three to the prototype group (with one CRD). Various studies showed that galectin-8 is widely expressed in tumor tissues as well as in normal tissues. The level of galectin-8 expression may correlate with the malignancy of human colon cancers and the degree of differentiation of lung squamous cell carcinomas and neuro-endocrine tumors. Recently, the differences in galectin-8 expression levels between normal and tumor tissues have been used as a guide for the selection of strategies for the prevention and treatment of lung squamous cell carcinoma. These experiments are still under investigation, but demonstrate the potential of galectin-8 research to enhance our understanding of, and possibly prevent, the process of neoplastic transformation.
Published in 2004.

Keywords: **galectin-8, isoforms, neoplastic transformation, alternate splicing**

Abbreviations: **Mab: monoclonal antibody; IS: insertional sequence; CRD: carbohydrate recognition domain; ORF: open reading frame; Gal8: Galectin-8.**

Introduction

Galectins are a family of carbohydrate-binding proteins that recognize structural variations among beta-galactoside containing glycoconjugates. They are widely distributed in the animal kingdom from lower invertebrates to mammals [1,2]. Thirteen proteins representing a high degree of sequence identity in their carbohydrate recognition domains (CRD) have already been described [3–7]. They share an affinity for β-galactoside moieties and are classified as prototype or tandem-repeat galectins depending upon their number of CRDs [2–8]. The first group includes galectins-1, -2, -5, -7, -10 and -13 with only one CRD, while the second group contains galectins-4, -6, -8, -9 and -12 with two CRDs linked by a hinge peptide. Galectin-3 bears only one CRD, but possesses a domain consisting of Pro-Gly-rich repeating units and is thus classified as a chimeric galectin [5,8]. Widely expressed in various cells, galectins have been attributed a large number of cellular functions in normal and neoplastic tissues such as involvement in cell-cell and cell-matrix interactions and cellular transformation [3,9]. Galectins can be found inside the cell, either in the nucleus or in the cytoplasm or outside the cell, either associated with the membrane or the matrix [10–17]. As they lack a signal peptide, a non-typical secretion pathway is used [18,19].

Within this family of proteins, galectin-8 is unique in existing as many forms encoded by the same gene. Six isoforms of the mature protein harboring either one or two CRDs seem to be encoded for by only one gene as a result of alternate splicing. Moreover multiple polyadenylation signals combine with alternate splicing to generate a complex panel of variant mature messenger RNAs [20–22]. Studies showed that galectin-8 is a matricellular modulator of cell adhesion and that it needs both CRDs to be active [23,24]. This galectin is widely expressed in normal and tumor tissues and at different levels, making Gal8 an interesting tool for the understanding of neoplastic transformation [22,25–27].

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Galectin-8: From cDNA to isoforms

The first galectin-8, called galectin-8 HT, was described in 1995, when a 1.25 kb cDNA was fortuitously isolated from

a rat liver cDNA library using a monoclonal antibody directed against the Insulin Receptor Substrate-1 (anti-IRS-1 Mab) [28]. The inferred amino acid sequence of this 34 kDa galectin displays two CRDs separated by a hinge peptide of 32 amino acid residues. This protein was assigned to the tandem-repeat galectin sub-class, already including galectins-4 and -6. Galectin-8 HT shares 50 and 34% identity with the nucleotide and peptide sequences of rat galectin-4, respectively. Galectin-8 HT possesses a sugar binding activity and was shown to be biologically active [28]. One year later, a unique human 3.85 kb galectin-8 cDNA was isolated from a LNCaP prostate cancer expression library. The open reading frame (ORF) obtained from this cDNA exhibits 83% nucleotide identity with the rat galectin ORF, while the inferred protein, called PCTA-1, exhibits 81% amino acid sequence identity with galectin-8 HT [29]. Simultaneously, a human lung cDNA expression library screened with a monoclonal antibody called Po66, showed that the antigen recognized by Mab Po66 corresponds to another human galectin-8, called Po66-CBP [21]. cDNA analysis revealed four mRNA species. Two of them, 2.64 and 1.8 kb transcripts, encode for Po66-CBP, while two species of approximately 1.8 kb encode for the same protein with an additional internal peptide. They were named Po66-CBP-IS1 and -IS2. Using a RT-PCR strategy, the authors confirmed the existence of these transcripts. Moreover this experiment revealed a transcript containing both IS1 and IS2 (Po66-CBP-IS1-IS2) and another transcript with IS1, IS2 and an additional sequence called IE (Po66-CBP-IS1-IE-IS2). Sequential analysis permitted the authors to understand how some mRNAs could be obtained by alternate polyadenylation signals. However, it was impossible at that time to distinguish between alternate splicing or the presence of a multigene family in the human genome for the IS1, IS2 and IE sequences (Figure 1) [21]. Within their overlapping regions Po66-CBP displays 82% nucleotide and 98.7% amino acid sequence identity with PCTA-1 [20,23]. Among these mRNAs, PCTA-1 (3.85 kb) and both mRNAs of Po66-CBP (2.64 and 1.8 kb) without any insertional sequence possess the same ORF. They only differ in their 5′ and 3′ untranslated regions [21,22,29]. The four other transcripts also have the same putative ORF partitioned into two pieces by an additional peptide sequence. It is important to notice that the four different insertional sequences interrupt the ORF at a unique insertion site and that all of the additional sequences have only been detected in the smallest messenger RNAs [21].

Six isoforms of human galectin-8 can be deduced from the isolated cDNAs and RT-PCR fragments [21,29]: Po66-CBP, Po66-CBP-IS1, Po66-CBP-IS2, Po66-CBP-IS1-IS2, Po66-CBP-IS1-IE-IS2 and PCTA-1. Po66-CBP and PCTA-1 amino acid sequences share 98.7% identity and both possess two CRDs [21,29] (Figure 1B). The presence of the insertional sequence in the Po66-CBP-IS1 transcript increases the size of the hinge peptide without modifying the ORF [21,22]. On the contrary, both of the IS2 and IE sequences contain a stop codon in their ORF, reducing the amino acid sequence of the Po66-CBP-IS2, Po66-CBP-IS1-IS2 and Po66-CBP-IS1-IE-IS2 isoforms. This gives rise to proteins with a single CRD [21,22] (Figure 1). Galectin-8 seems to be unique in the galectin family with isoforms belonging to both the prototype and tandem-repeat groups [21,22,29]. However, the prototype galectins-8 isoforms have never been isolated, but have only been deduced from the isolated transcripts.

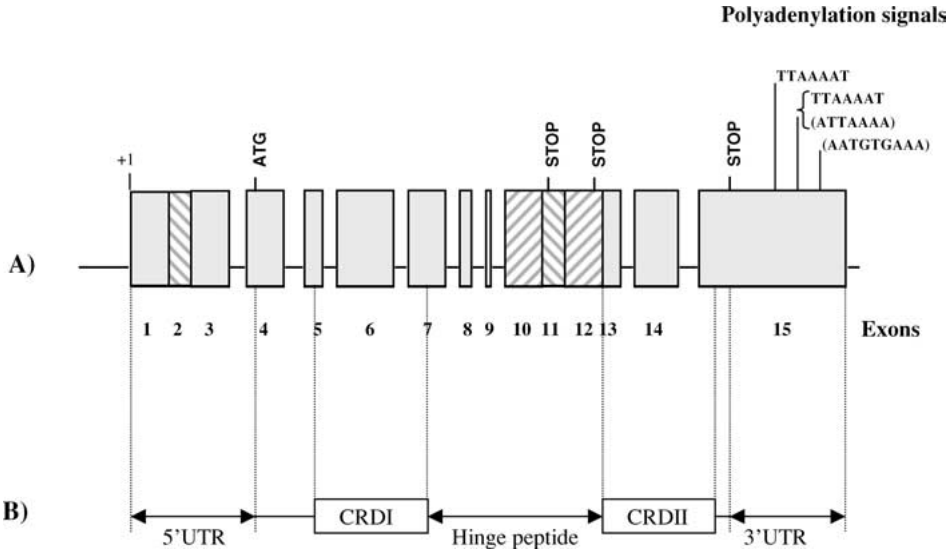

Figure 1. Genomic organization of *LGALS8*, from [20–22]. (A) *LGALS8* gene structure. Constitutive exons are in grey. Alternate exons are lined. IS1, IE and IS2 refer to alternate exons modifying the hinge peptide. Initiation codon (ATG) and stop codons are indicated. Polyadenylation signals in brackets come from [20] and the others from [21]. (B) Domain structure of the galectin-8 protein and the relationship with the exons.

The galectin-8 gene: *LGALS8*

Two teams identifyed a galectin-8 gene (*LGALS8)* in the same q42-43 region of human chromosome 1, although between different markers: the *DIS2421* and *DIS2383* markers for the prostate galectin-8 PCTA-1 gene [20,22] and the *DIS2850* and *DIS372* markers for the lung galectin-8 gene [21,22]. It is not clear if both *LGALS8* genes correspond to a unique gene with a complex transcriptional regulatory process or if they are in close vicinity, as already described for *LGALS4* and *LGALS6* [30,31]. If there is a single *LGALS8* gene encoding each galectin-8 isoform, a complex post-transcriptional regulatory process would be required (Figure 1A). Of the 15 putative exons, eleven are constitutive of any isoform, while exons 2, 10, 11 and 12 are alternatively present or absent (Figure 1A).

Exons 5, 6 and part of 7 encode for CRDI, while exons 13, 14 and the beginning of 15 encode for CRDII. Part of exon 7 and exons 8, 9, 10, 11 and 12 encode for the hinge peptide. Each insertional sequence corresponds to a specific exon: 10, 11 and 12 for IS1, IE and IS2 respectively [21] (Figure 1). A unique ATG codon is located in the fourth exon, while three different stop codons are present in exons 11 (IE sequence), 12 (IS2 sequence) and 15 (isoforms without either IE or IS2 sequences) (Figure 1).

Alternate splicing involves the 5′UTR of the messenger RNAs. Except for 36 bases upstream of the ATG in exon 4, this UTR region comes from exons 1 and 3 in the lung Po66-CBP galectin-8 [21] and exons 1, 2 and 3 in the prostate PCTA-1 galectin-8 [20] (Figure 1A). Exon 2 has never been observed in lung transcripts.

Another alternate splicing includes exons 10 (IS1 sequence), 11 (IE sequence) and 12 (IS2 sequence) [20–22]. All putative combinations seem to be possible in lung cancer tissues, giving rise to various Gal8 Po66-CBP isoforms (Po66-CBP-IS1, -IS2, -IS1-IS2 and -IS1-IE-IS2) [21,22]. This was not observed in prostate tissues [20]. A complex set of transcripts has also been observed in colon cancer cells [26] and astrocytic cancer cells [27], both by RT-PCR. These alternate splicing pathways have not yet been clearly described.

Galectin-8 expression in cancer

A number of experimental approaches have been used to determine the presence or absence of Gal8 transcript or protein in normal, embryonic or tumor tissues: RT-PCR, immunohistochemistry or Cancer Genome Anatomy Project (CGAP) library analysis [21,23,25–28,32–34]. The goal of the NCI's Cancer Genome Anatomy Project is to determine the gene expression profiles of normal, precancer, and cancer cells, leading eventually to improved detection, diagnosis, and treatment for the patient. By collaborating with scientists worldwide, CGAP seeks to increase its scientific expertise and expand its databases for the benefit of all cancer researchers (http://cgap.nci.nih.gov/). However, as shown in Table 1, CGAP analysis gave conflicting results compared to other methods (Table 1). These may have been due to the sensitivity of the technique used. The method of collecting samples could be another major problem, *e.g.* a biopsy of normal tissue is usually conducted near the tumor and later biopsies may contaminate the area. By CGAP analysis, Gal8 is widely expressed in normal tissues (brain, breast, colon, retina, kidney, pancreas, placenta, spleen, testis, uterus, vascular, esophagus and heart) as well as in tumor tissues (brain, breast, colon, germ cells, head and neck, kidney, muscles, ovary, pancreas, thyroid, placenta, prostate, uterus, lung, stomach and esophagus) [21,23,25–28,32–34]. On the other hand, Gal8 does not appear to be strongly expressed in embryonic tissues and was only detected in embryonic brain, kidney, uterus, liver and lung [21] (Table 1). Owing to the wide expression in tumor tissues, different studies have tried to demonstrate a relationship between Gal8 expression and neoplastic transformation, which would be helpful in understanding this phenomenon and determining tumor malignancy. This kind of study has been conducted using various tumor groups, particularly brain [27], colon [26] and lung [33,34].

Gal8 expression levels were quantified by immunohistochemistry in human astrocytic tumors of grades I to IV using complex computer software. Results were confirmed using RT-PCR [27]. It was shown that the level of Gal8 expression remained unchanged during the progression of the malignancy in human astrocytic tumors. However, experiments showed that Gal8 stimulated glioblastoma cell migration *in vitro*, suggesting that Gal8 could be involved in the tumor astrocytes' invasion of the brain parenchyma *in vivo*. It should be noted, however, that this effect was less pronounced with Gal8 compared to Gal1 or Gal3 [27].

A similar study was made in colon cancer [26]. A marked decrease in immunohistochemical expression of Gal8 occurred with malignancy development in human colon tissue. The more aggressive the tumor, the less Gal8 protein it harbored. Moreover, there was less Gal8 in tumorous than in normal or benign tissue colon cancer [26]. The authors also showed that Gal8 expression was inversely related to tumor growth rate and that *in vitro* Gal8 reduced the migration rate of only those human experimental models that exhibited the lowest growth rate *in vivo* [26].

An immunohistochemical study was also performed on primary and secondary malignant lung tumors of various histological types [33,34]. The authors found that Gal8 was expressed strongly in squamous cell carcinoma, very weakly in adenocarcinoma and was undetectable in small cell carcinoma [33]. In positive tissues all histological types expressed Gal8, proving that the cell origin has no influence on galectin expression [33]. The authors observed a correlation between Gal8 expression and the degree of differentiation of squamous cell carcinomas and neuro-endocrine tumors [33,34]. All of these studies showed that Gal8 expression in neoplastic transformation seems to be correlated to the type of tumor. This organ-type-dependent regulation of Gal8 expression has also been shown

Table 1. Summary of the analysis of galectin-8 transcript or protein expression in various normal, tumor and embryonic tissues using: C: CGAP library [21], RT: RT-PCR [29,32], I: Immunohistochemistry [17,23,25,32,34,35] and N: Northern blot [20]

	Normal				*Embryonic*				*Tumor*			
	C	*RT*	*I*	*N*	*C*	*RT*	*I*	*N*	*C*	*RT*	*I*	*N*
Brain	+	−	nd	+	+	nd	nd	nd	+	+	+	nd
Breast	+	nd	+	nd	−	nd	nd	nd	+	nd	+	nd
Colon	+	+	+	+	−	nd	nd	nd	−	+	+	nd
Retina	+	nd	nd	nd	−	nd	nd	nd	+	nd	nd	nd
Germ cells	−	nd	nd	nd	−	nd	nd	nd	+	nd	nd	nd
Head and Neck	−	nd	nd	nd	−	nd	nd	nd	+	nd	nd	nd
Kidney	+	nd	+	+	+	nd	nd	nd	+	nd	+	nd
Muscle	−	nd	nd	nd	−	nd	nd	nd	+	nd	nd	nd
Ovary	−	nd	nd	+	−	nd	nd	nd	+	nd	nd	nd
Pancreas	+	nd	+	+	−	nd	nd	nd	+	nd	+	nd
Thyroid	−	−	nd	+	−	nd	nd	nd	+	+	+	nd
Placenta	+	nd	nd	+	−	nd	nd	nd	+	nd	nd	nd
Spleen	+	−	nd	+	−	nd	nd	nd	−	+	+	nd
Testis	+	+	nd	+	−	nd	nd	nd	−	+	+	nd
Uterus	+	nd	nd	nd	+	nd	nd	nd	+	nd	nd	nd
Vascular	+	nd	nd	nd	−	nd	nd	nd	−	nd	nd	nd
Liver	−	+	+	+	+	nd	nd	nd	−	+	+	nd
Lung	−	−	+	+	+	nd	nd	nd	+	+	+	nd
Stomach	nd	−	+	+	nd	nd	nd	nd	nd	+	+	nd
Esophagus	nd	+	nd	nd	nd	nd	nd	nd	nd	+	+	nd
Heart	nd	+	nd	+	nd	nd	nd	nd	nd	nd	nd	nd
Prostate	+	−	+	+	−	nd	nd	nd	+	+	+	+

(+) presence of Gal8, (−) absence of Gal8 and (nd) not determined.

by comparing Gal8 expression in normal and corresponding tumor tissues by immunohistochemistry [25]. The amount of Gal8 decreased in tumor tissue compared to normal in colon, pancreas, liver, skin and larynx. Conversely, Gal8 increased in breast and remained unchanged in lung, bladder, kidney, prostate and stomach [25].

The cellular expression of galectin-8 was also studied. In HeLa cells, Gal8 is observed exclusively, but not uniformly, within the cytoplasm. The arrangement as micro-clusters is reminiscent of proteins associated with mitochondria, Golgi apparatus or trans Golgi membrane [20]. However, Gal8 was also detected outside the cell and, thus, is secreted by human non-small cell lung carcinoma cells [17,23]. In other tumor cells, Gal8 cellular localization seems to be a function of the cellular type and of the culture time. A study made using a human squamous cell lung carcinoma cell line showed that within 48 h of culture, Gal8 accumulated on the plasma membrane; 24 h later, it was observed in the cytoplasm and on the plasma membrane. After 96 h of culture Gal8 was also present in the nucleus, where it disappeared 24 h later [17].

The role of galectin-8 in human cancer diagnosis, prevention and treatment

The studies described previously showed that the malignancy of human colon and lung cancer could be diagnosed by quantifying the Gal8 expression level [25,26,33,34]. However this approach was not helpful for human astrocytic tumors [27].

The presence of galectin-8 in lung tumor cells and its absence or very low levels in normal lung tissues permits the use of monoclonal antibodies for the prevention and treatment of lung cancer.

The Po66 monoclonal antibody, which has been used to isolate galectin-8 Po66-CBP in lung, was developed several years ago to find tumor markers in squamous cell lung carcinoma [35]. Po66 is a murine IgG1, obtained by immunization of Balb/c mice against human lung carcinoma cells obtained from a fresh human biopsy. Before knowing that this Mab was able to specifically bind galectin-8, it was used to detect human lung squamous cell carcinoma by immunoscintigraphy [36]: thirty-three patients with histologically confirmed primary non-small cell lung carcinoma were investigated. Twenty-seven of them were explored at a preoperative stage and 6 at six months after surgery. ^{131}I radiolabeled Po66 Mab was injected and detected by immunoscintigraphy. Seventy-eight percent of primary tumors and 100% of recurrences were detected. In four patients recurrence was detected by immunoscintigraphy despite being undetectable by plain chest X-ray. This clinical investigation showed that Po66 Mab was able to fix specifically, with a weak background, the lung tumor and that it was able through immunoscintigraphy to detect lung squamous cell carcinoma recurrences [34].

This specificity of Po66 Mab for squamous cell lung carcinoma has been used to treat this type of cancer by radioimmunotherapy. Studies were made on nude mice grafted with human squamous cell carcinoma cells [37–40]. The experimental biodistribution after intravenous injection showed that the fixation of Po66 Mab in the tumor was long-lasting (14 days) [37,38], but to get a better tumoral fixation of the antibody, doxorubicin chemotherapy had to be coadministrated with the radioimmunotherapy [39,40]. These preliminary studies showed that injection of ^{131}I radiolabeled Po66 Mab and doxorubicin produced a tumor size regression [40]. The major disadvantage of Po66 Mab was its very slow body clearance (8 days) generating high radioactivity-dependent toxicity for hematopoietic stem cells [40]. To decrease the unspecific irradiation related to the amount of circulating antibody, Po66 Mab has been modified to realize a two-step radioimmunotherapy using a bispecific monoclonal antibody: Po66 × anti-di-DTPA-In [41]. The first results showed that the bispecific antibody improved the tumor/blood ratio compared to the whole Po66 Mab [41,42]. This antibody is still under investigation.

Conclusion

Human galectin-8 was independently isolated from prostate (PCTA-1) and lung (Po66-CBP) tissues [21,29]. Both proteins share 98.7% identity in terms of amino acid sequence. The differences between them may be explained by cDNA cloning rearrangements or sequencing errors. It could also be the result of the transcription of two genes in very close vicinity, as is the case for *LGALS4* and *LGALS6*, probably arising by gene duplication and resulting in two closely related protein isoforms [30,31].

With one or more genes the most unexpected result concerning the galectin-8 family is the very complicated transcription process. First of all, the termination of transcription is multiple. This is mainly due to the presence of three weak polyadenylation signals, quite far away from the canonical sequence AAUAAA [21]. All of them seem to be used, but not in the same tissue. The longest messenger RNA observed in prostate cells has never been detected in lung cells. However, in any tissues where it has been searched for, numerous messenger RNA species were observed. Secondly the mature messenger RNAs are generated by alternate splicing, which again seems to be tissue specific. It is noteworthy that this splicing can modify either the 5′UTR portion of the mature mRNA or its coding region. By combining 5′UTR modifications with alternate splicing, or 3′URT modifications with alternate polyadenylation signals, it might be very interesting to see if any of these modifications are involved in messenger RNA stability. It should also be noticed that very small ORFs are present in the 5′UTR of various mRNAs and this can be involved in their translation efficacy [21].

Thirdly, the alternate splicing of the coding region may be the most surprising result since it gives rise to alternate forms of the protein with either one or two CRD domains. Again it is very astonishing to see at least in the lung that the insertional peptides always come from the same small messenger RNAs. It looks like if the relationship relies on these processes. The presence of one or two CRDs is important in terms of protein function and/or localization and could play a major role in the involvement of the various isoforms in neoplastic transformation. Of course, crystallographic studies could provide structural information in this regard. It is already known that galectins-4 and -9 possess isoforms belonging in both cases to the tandem-repeat galectin group, with the same sequence except a variation in the length of the hinge peptide. In the first case, the isoforms are encoded for by two linked genes *LGALS4* and *LGALS6* [30,31]. In the second, it is not known if the proteins are generated from a unique gene or not [43]. The *LGALS3* gene can also give rise by alternate splicing to two isoforms with one or two additional sequences in a process that mimics what is observed with *LGALS8* [16]. Galectin-3 is a chimeric galectin that bears a single CRD but an unusual Pro-Gly-rich domain [5,8]. One of its isoforms presents the same structure while the second one harbors a truncated CRD domain, similar to what is observed with galectin-8 [16,21]. Studies showed that the presence of the insertional sequence modifies the cellular localization of the protein: galectin-3 is a soluble protein and both isoforms are transmembrane proteins [16]. No galectin-8 cellular localization studies have been conducted with regard to the isoforms and it might be of interest to focus on this aspect. Galectin-8 is widely expressed in normal and tumor tissues [21,23,25–29,32–34] and again it might be interesting to know exactly which isoform is expressed during neoplastic transformation. Immunohistochemical studies on colon [26], brain [27] or lung tissues [33,34] did not discriminate between galectin-8 isoforms, and the synthesis of antibodies directed against each isoform could be a challenge. Regulation of *LGALS8* expression is undoubtedly complex and may be a function of the cellular environment. Preliminary results show that galectin-8 may be useful for cancer prevention or treatment by using Po66 Mab [26,40,41]. Even if studies made with this Mab are still under investigation, they have produced promising decreases in tumor size by means of immunotherapy [40,41].

Galectin-8 expression in cancer cells and tissues is probably the most complicated within the galectin family. With such complex gene regulation, giving rise to numerous messenger RNAs and at least six isoforms, this galectin could be revealed as an interesting tool to understand neoplastic transformation and perhaps reinforce our weapons against some cancers.

Acknowledgments

We gratefully thank Dr. Sarah Wardlaw for English improvement.

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