Biochimica et Biophysica Acta 1820 (2012) 1366–1372

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Different fractions of human serum glycoproteins bind galectin-1 or galectin-8, and their ratio may provide a refined biomarker for pathophysiological conditions in cancer and inflammatory disease ☆

Michael C. Carlsson [a,*], Crina I.A. Balog [b], Ola Kilsgård [c], Thomas Hellmark [d], Omran Bakoush [d], Mårten Segelmark [d,e], Mårten Fernö [f], Håkan Olsson [f], Johan Malmström [c], Manfred Wuhrer [b], Hakon Leffler [a,g,*]

[a] Section MIG (Microbiology, Immunology, Glycobiology), Department of Laboratory Medicine, Lund University, Lund, Sweden
[b] Biomolecular Mass Spectrometry Unit, Department of Parasitology, Leiden University Medical Center, Leiden, The Netherlands
[c] Department of Immunotechnology, Lund University, Lund, Sweden
[d] Department of Nephrology, Lund University Hospital, Lund, Sweden
[e] Department of Medicine and Health, Linköping University, Sweden
[f] Department of Oncology, Lund University Hospital, Lund, Sweden
[g] Department of Clinical Immunology and Transfusion Medicine, Skåne University Hospital, SUS, Lund, Sweden

ARTICLE INFO

Article history:
Received 11 October 2011
Received in revised form 10 January 2012
Accepted 11 January 2012
Available online 17 January 2012

Keywords:
Galectin-1
Galectin-8
Biomarkers
Cancer
IgA-nephropathy
Serum glycoprotein

ABSTRACT

Background: Changes in glycosylation of serum proteins are common, and various glycoforms are being explored as biomarkers in cancer and inflammation. We recently showed that glycoforms detected by endogenous galectins not only provide potential biomarkers, but also have different functions when they encounter galectins in tissue cells. Now we have explored the use of a combination of two galectins with different specificities, to further increase biomarker sensitivity and specificity.
Methods: Sera from 14 women with metastatic breast cancer, 12 healthy controls, 14 patients with IgA-nephritis (IgAN), and 12 patients with other glomerulonephritis were fractionated by affinity chromatography on immobilized human galectin-1 or galectin-8N, and the protein amounts of the bound and unbound fractions for each galectin were determined.
Results: Each galectin bound largely different fractions of the serum glycoproteins, including different glycoforms of haptoglobin. In the cancer sera, the level of galectin-1 bound glycoproteins was higher and galectin-8N bound glycoproteins lower compared to the other patients groups, whereas in IgAN sera the level of galectin-8N bound glycoproteins were higher.
Conclusion: The ratio of galectin-1 bound/galectin-8N bound glycoproteins showed high discriminatory power between cancer patients and healthy, with AUC of 0.98 in ROC analysis, and thus provides an interesting novel cancer biomarker candidate.
General significance: The galectin-binding ability of a glycoprotein is not only a promising biomarker candidate but may also have a specific function when the glycoprotein encounters the galectin in tissue cells, and thus be related to the pathophysiological state of the patient. This article is part of a Special Issue entitled Glycoproteomics.

1. Introduction

Since it was discovered in 1969 that cancer glycans differ from glycans on healthy cells [1], efforts have been made to try to understand the underlying molecular mechanism in order to improve diagnostics and treatment. Today, glycosylation changes are considered a universal feature of tumour progression and malignancy. This alteration is due to alterations in cellular pathways probably early in cancer development [2]. Common modifications of cellular glycosylation in cancer include an increase in β-1,6-GlcNAc branching, increased numbers of sLe^x, sialyl-Lewis^a (sLe^a), T and Tn antigens and increased sialylation of *N*–linked glycans [3–6]. These tumour-associated carbohydrate antigens

Abbreviations: CRD, carbohydrate recognition domain; Galectin-8N, N-terminal CRD of galectin-8; IgAN, IgA-nephritis

☆ This article is part of a Special Issue entitled Glycoproteomics.

* Corresponding authors at: Department of Laboratory Medicine, Section MIG (Microbiology, Immunology, Glycobiology), Sölvegatan 23, S-223 62 Lund, Sweden. Tel.: +46 46 173273; fax: +46 46 137468.
E-mail addresses: michael.carlsson@med.lu.se (M.C. Carlsson), hakon.leffler@med.lu.se (H. Leffler).

0304-4165/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbagen.2012.01.007

are central players in cancer progression, and are involved in processes such as cell detachment, proliferation, metastasis, invasion and angiogenesis [7].

In the acute phase response during inflammation, synthesis of certain acute phase proteins in the hepatocytes is upregulated, many of which are glycoproteins. In addition to the increased serum levels, alterations of the glycans of these acute phase glycoproteins also occur over time [8]. Inflammation is considered to be the seventh hallmark of cancer [9] and one common denominator is the altered glycosylation of plasma proteins. While some features are seen both in cancer and inflammation such as increased branching of N-glycans and increased expression of sLe^x epitopes, other features still remain cancer specific [2,8].

Galectins are a family of small animal proteins that bind specific carbohydrate chains containing β-galactosides, such as N-acetyllactosamine (LacNAc) commonly found in glycoproteins [10,11], and hence, have the potential to "read" the changes of glycosylation in cancer and inflammation described above. Galectins have a wide range of proposed biological roles in cancer, inflammation and immunity [12–14]. At the cellular level they are emerging as key regulators of intracellular trafficking of cellular glycoproteins, with consequences for cellular function, e.g. in cancer, as well as other pathophysiological conditions [15,16].

While all members of the galectin family bind β-galactosides they show differences in affinity for longer saccharides and intact glycoproteins, their so called fine specificity [11]. Consequently, galectin-1 and galectin-8 bind different partially overlapping sets of human serum glycoproteins [10,17,18]. Previously we found that galectin-1 binds on average almost twice as much glycoprotein, mainly haptoglobin, in sera from patients with metastatic breast cancer compared to healthy individuals [17]. Galectin-1 binds terminal LacNAc residues and also those carrying 2–3 sialic acid equally well, but the tolerance for 2–3 sialylation is required for its binding to serum glycoproteins [17,19]. The galectin-1 bound haptoglobin has a different glycosylation (N-linked glycans with less terminal sialic acids, and increased proportion of an additional antenna), and has a different function (altered trafficking after uptake in macrophages) [17]. In another study we found that the N-terminal carbohydrate recognition domain (CRD)[1] of galectin-8 (galectin-8N) binds significantly more glycoproteins, including haptoglobin, in sera of patients with IgA-nephritis (IgAN) compared to patients with other forms of kidney disease and healthy subjects [18]. Galectin-8N has a strong preference for 2–3 sialylated galactosides [20,21], and its binding of serum glycoproteins requires the presence of 2–3 sialic acid [18], in contrast to the case of galectin-1, suggesting a different binding site.

Here we show that galectin-1 and -8 bind mainly different glycoforms of serum glycoproteins, including different fractions of haptoglobin. Sera from metastatic breast cancer patients contain increased levels of glycoforms that bind galectin-1 but decreased levels that bind galectin-8N, whereas sera from patients with a chronic inflammatory condition such as IgA nephritis display an opposite profile. As a result, the ratio of galectin-1-bound/galectin-8-bound serum glycoproteins provides a strong biomarker to segregate the cancer patients from the other groups.

2. Materials and methods

2.1. Patient samples

2.1.1. Cancer sera

Human serum samples from 14 female metastatic breast cancer patients and 12 age matched healthy female volunteers (Indicated by C and H in Table S1) were used [17], under ethical permit to authors HO and MF and approval from the Ethical Review Board at Lund University (Now Regional Ethical Review Board Lund, http://www.epn.se/lund/om-naemnden.aspx). Written informed consent was obtained from all participants.

2.1.2. IgAN sera

Serum samples from 14 IgAN patients and 12 patients with other forms of glomerulonephritis (selected as the first non-IgAN sample in the biobank taken after the respective IgAN sample) were used (Indicated by P and K in Table S1) [18]. The patients and the controls in this study were all participants in a long-term prospective study of glomerular diseases conducted at the Department of Nephrology, Lund University Hospital, Sweden. Serum samples were taken at time of kidney biopsy. Presenting symptoms were most often hematuria. After approval by the ethical committee at Lund University (LU 47-02) we obtained written informed consent from patients with biopsy proven IgAN, diagnosed between February 1992 and November 2003. The morphological diagnoses were established by evaluation of representative percutaneous renal biopsy specimens by both light microscopy and direct immunofluorescence. The diagnosis of IgAN was based on the finding of IgA as the dominant or co-dominant immunoglobulin in a mesangial distribution pattern.

All samples were collected and stored as previously described [10]. All information and data were handled confidentially, and evaluation of information linked to patients was carried out in accordance with the Swedish Personal Data Act (Personuppgiftslagen).

2.2. Production of recombinant galectins

The recombinant galectins were produced in *Escherichia coli* BL21 Star and purified by chromatography on lactosyl-Sepharose as previously described [10]. The less oxidation sensitive galectin-1 mutant C3S was used as characterized and described [17,22].

2.3. Galectin affinity chromatography

Sera and haptoglobin (pooled from human plasma, Sigma-Aldrich) were analyzed on affinity columns with immobilized galectins as described by Cederfur et al. [10]. One serum from a cancer patient and one serum from an IgAN patient in addition to 2 mg of haptoglobin were subjected to a two-step separation. The unbound fraction of one galectin was rechromatographed on the other. There was no evidence that the column-binding capacity was exceeded, since when haptoglobin flow-through fractions were rechromatographed *on the same galectin*, no additional glycoproteins bound.

2.4. Protein identification

Serum ligands were analyzed by one-dimensional 4–20% SDS-PAGE as described previously [10] and four samples; galectin-1 and galectin-8 bound fractions from two cancer patients (C3 and C8) were analyzed by LC–MS/MS of pooled tryptic peptides to identify major protein components and estimate their relative abundance as described in Ref. [18].

2.5. Glycan analysis

2.5.1. PNgaseF release

Lactose was removed from galectin-8N by ultrafiltration performing 3 washes with PBS (10 kDa Centrifugal Concentrators; Pall, Port Washington, NY). SDS was added to the samples to final concentrations of 1.3% (w/v). Samples were heated for 10 min at 60 °C and then put on ice. NP-40 was added to the samples to a final concentration of 1.5%. Six mU of PNGase F were added to each sample followed by 24 h incubation at 37 °C. Subsequently, another 2 mU of PNGase F was added to each sample, followed by overnight incubation at 37 °C. The PNGase F released glycans were purified by C18-RP-SPE (flow-through) and graphitized carbon SPE (retentate). The C18-RP-cartridges were preconditioned with 5 ml of ACN, 5 ml of water/ACN (40/60, by vol), and equilibrated with 5 ml of water. The samples were applied to the cartridges followed by washes with 3 ml of water/ACN (90/10, by vol)