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Galectins detection for the diagnosis of chronic diseases: An emerging biosensor approach

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ABSTRACT

Galectins are an ancient family of lectins characterized by the specific binding of β-galactosides through evolutionarily conserved sequence elements of the carbohydrate recognition domain. Interest in this protein family is growing due to the crucial role of galectins not only as therapeutic agents but also as biomarkers of the inflammatory stage occurring in several diseases, including cancer, cardiovascular disease, type 2 diabetes, musculoskeletal disorders, and neurodegenerative diseases. For this reason, the biosensing of galectin becomes crucial for the evaluation of a pathological state as well as for the follow-up of a therapeutic treatment. The design of biosensors for galectin detection is becoming a reality in recent years, as complementary analytical tools to be exploited at the point of need to support laboratory setup methodologies. This review reports the latest trends in biosensing systems for galectins based on different natural and artificial bioreceptors, integrated into different transduction systems and exploiting nanomaterials to improve analytical performance.

1. Introduction

Long exposure to proinflammatory stimuli of microbial, environmental and endogenous origin could generate a chronic, low-grade, asymptomatic inflammatory state associated with aging known as inflammaging [1]. The aging-associated glycosylation changes often resemble those observed in inflammatory conditions. Several reproducible glycomic markers are associated with the calendar and biological aging. Among them, galectins reflect inflammatory processes related to diverse age-related diseases, including cardiovascular diseases, type 2 diabetes, musculoskeletal disorders, various types of cancer, and neurodegenerative diseases [2].

Galectins are mammalian lectins involved in a variety of roles, including immune regulation, cancer cell growth, and apoptosis. Galectin research is becoming increasingly important in recent years, thanks to the different roles they are able to play in many applications as disease biomarkers, therapeutic agents, and drug targets. For this reason, the biosensing of galectin represents a crucial requirement for the evaluation of the inflammatory state of patients with different pathological conditions as well as for the follow-up of disease treatment. A search for articles on "galectins biosensors" as well as on "therapeutic agents", "cancer", and "family", performed on the main web browsers for scientific publications, like PubMed, Springer, and Google Scholar, revealed increasing hits as reported in Fig. 1, indicating that the interest on galectins biosensors is growing (accessed October 2022).

The following sections describe the interest in galectin biosensing and the last trends in biosensors developed exploiting different configurations in terms of bioreceptors, transduction systems, and nanomaterials.

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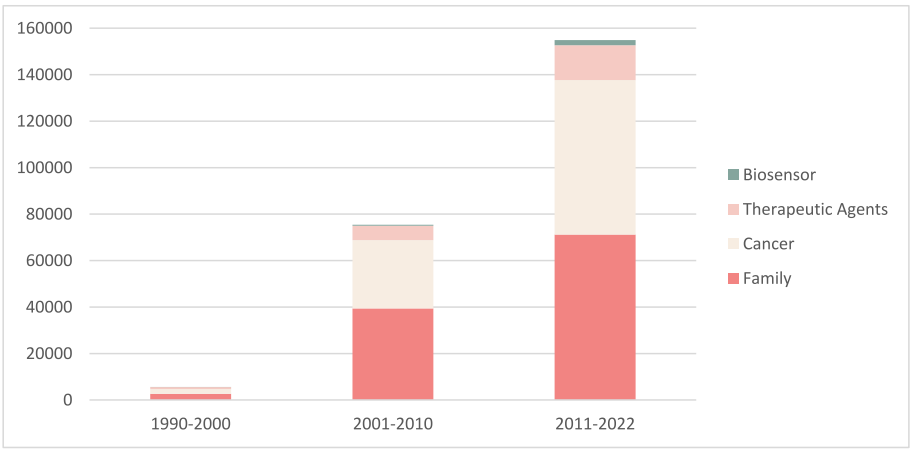


Fig. 1. Number of publications during the cited period relating to galectins biosensors, therapeutic agents, cancer, and family. Data taken on October 2022 from www.googlescholar.com.

1.1. Galectins: structures and ligand interactions

Galectins (Gal) belonging to the lectin family, are characterized by the ability to bind specifically to β-galactoside residues, such as N-acetyllactosamine (Galβ1-3GlcNAc or Galβ1-4GlcNAc). Initially called S-type lectins, due to dependence on the reductive environment to maintain the structural stability provided by disulphide bonds, galectins are now identified by sequential numbers in order of discovery. Each galectin is encoded by a specific gene belonging to the LGALS gene family, also present in early organisms. Indeed galectins have been discovered as conserved proteins in many organisms, from sponges and nematodes to humans, and are known to be produced within cells as cytosolic proteins. Although there is no signal sequence in their structure, these proteins can also be found in the nucleus, mitochondria, and extracellular space: they leverage a non-canonical secretion system that avoids the Golgi pathway [3]. Almost 16 members have been identified in mammals, while the galectins found in humans are Gal-1, -2, -3, -4, -7, -8, -9, -10, −12, −13, −14, and −16, Gal-5 and -6 are typical of rodents and Gal-11 and -15 are found in sheep and goats. All these proteins share a common Carbohydrate Recognition Domain (CRD), which is directly involved in carbohydrate binding. The CRD consists of approximately 130 amino acids organized in a typical β-sandwich fold composed of two antiparallel β-sheets of six concave strands (S1−S6, S-sheet) and five convex strands (F1−F5, F-sheet), with a short α-helix connecting S4 and F5 strands (not always present). The amino acid residues on the concave surface identify the binding groove in which specific ligands are embedded [4]. The crystal structure of the CRDs of all observed galectins highlights the extreme redundancy of this β-sandwich arrangement. Sequence identity among human galectin CRDs reported in the literature ranges from 20% to 50%, the only exception includes the C-terminal CRD of Gal-12, which is less than 20% identical to the other CRDs [5]. Many studies that have performed alignment of their CRD amino acid sequences focus attention on the high degree of identity between most of them, despite the different classifications of each protein [6,7]. However, the analysis of some subtle differences in primary and quaternary structure has been performed in order to link structure and binding specificity.

Based on the number of CRDs and the overall structural organization, human galectins are classified into three groups: prototype (Gal-1, -2, -7, -10, −11, −13, −14, and −15 and −16), tandem-repeat type (Gal-4, -8, -9 and -12) and chimera type (Gal-3) (Fig. 2). Prototypical galectins are characterized by homodimer assembly in which each monomer has a unique CRD that is generally at the farthest ends of the same surface from the other homologous domain. Tandem-repeat type represents the only class in which there are two CRDs in the same protein, located at the C- and N-termini of each chain. The chimera-type galectin, Gal-3, is characterized by a single C-terminal CRD and an N-terminal non-lectin domain, connected by a short linker.

In prototypical galectins, the orientation of the two monomers influences the spatial arrangement of the CRDs and in turn the ability to interact with different ligands. At Gal-1, Gal-7 and Gal-13 dimers interface there are the N- and C-termini, with the involvement of S1−F1 strands. In dimers as arranged, the CRDs are localized as far as possible, on the opposite sides of the complex. Among these dimers, those from Gal-1 and Gal-13 are covalently stabilized with one and two disulphide bonds respectively, while Gal-7 monomers, which appear to be a little further than other prototypical galectins (50 Å vs ~42 Å), interact only by hydrogen bonds and van der Waals interactions. On the other hand, Gal-10 and Gal-14 monomers interact at the S-face (S5−S6 strands) to identify homodimers stabilized by several non-covalent interactions [10]. In the case of S-face interaction, the two CRDs are unusually close to each other, affecting the multivalence of dimers [11]. The last discovered human prototype galectin, Gal-16, does not dimerize in solution as described in recent studies [12].

As described before, the three-dimensional conformation of CRDs is very close to one another, as well conserved concave and convex β-strands organized in the β-sandwich configuration. Also, the amino acid sequences of CRDs of prototype galectins are very similar to each other and also to those of the other galectins, with the conserved residues Asn/His/Arg/Trp/Glu involved in the ligand recognition and binding. Small shifts of the main residues involved in ligand binding justify different affinities for the same ligand; for instance, the substitution of typical His 53 with Gln 53 and Arg 57 with His 57 is responsible for the weaker binding of lactose than Gal-1 [13]. As previously described, tandem repeat-type galectins have two CRDs in the same structure, located at the opposite ends of the same chain. Among these members, outside of the conserved β-sandwich arrangement of CRDs, there is a variable but minimal contribution of α-helix, according to structural and modeling studies [14,15]. The amino acid residues of the N-CRD involved in the ligand interaction, Arg/His/Glu/Asn and a single Trp, are well conserved, except for the substitution of typical Lys with Tyr71 in Gal-9 and a key Val residue with Ile 108 in Gal-12 [16]. Also for the C-CRD, the residues involved in ligands recognition and binding are

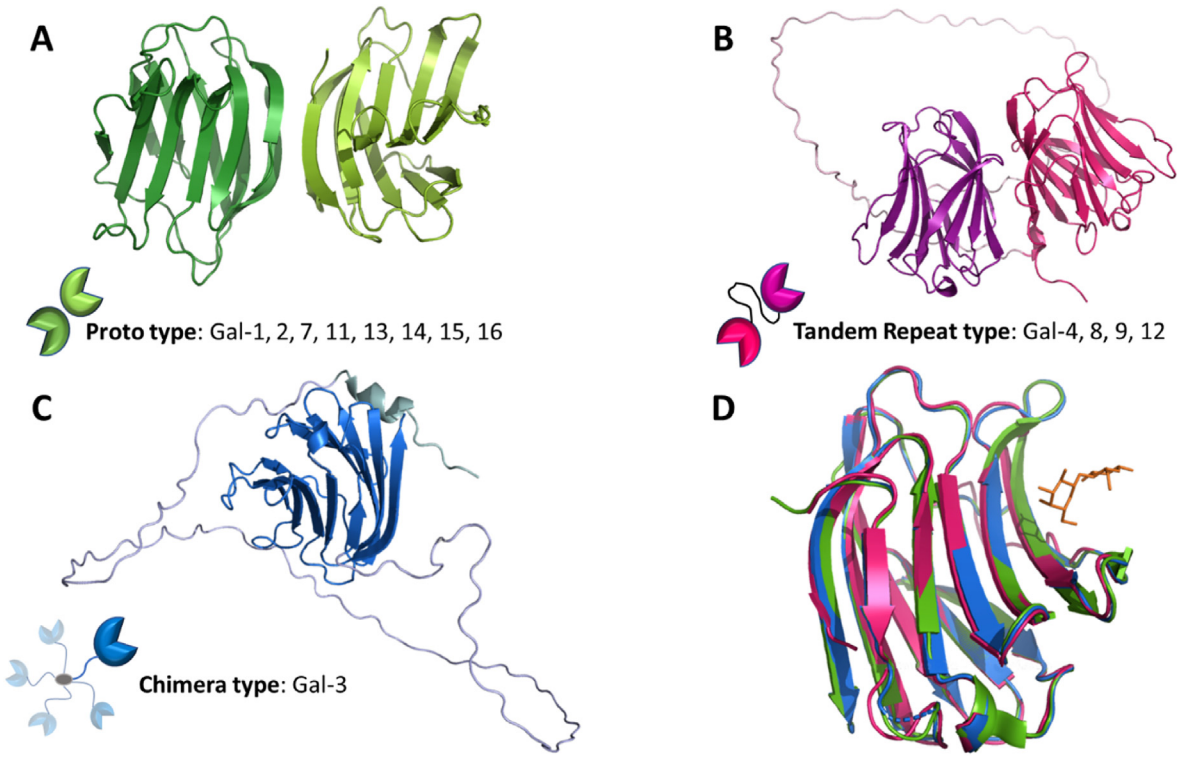


Fig. 2. Galectin classification. (A) Ribbon diagram of Gal-1 homodimer, as member of prototype class (PDB ID: 1gzw). (B) Structural model of the tandem repeat type full-length Gal-9 extracted from AlphaFold Database (UniProt ID: O00182), with N-CRD, C-CRD, and linker domain identified by different shades of colors. (C) Structural model of the full-length chimera type galectin, Gal-3, extracted from AlphaFold Database (Uniprot ID: P17931) with CRD, N-terminal non-lectin domain and linker domain identified by different shades of colors. (D) Superimposition of the CRDs of Gal-1 (green), Gal-3 (blue), and C-terminal Gal-9 (red) shows high conservation of three-dimensional arrangement of these different domains (PDB ID: 1gzm, 3ZSM, and 3NV1, respectively [8,9].

the same (Arg/His/Glu/Asn/Trp), as the exceptions described before. The two CRDs are connected by a linker domain that is usually a variable length region rich in Pro, especially that of Gal-4 and Gal-9. Increasing the length of this domain, it enhances its susceptibility to proteolytic cleavage and consequently full-length protein instability. As a rule, the proper positioning of the two CRDs allows the multivalency of each tandem repeat galectin.

The only chimera galectin, Gal-3, has a unique structural organization; by its N-terminus non-lectin domain, which consists of an approximately 12 amino acid sequence rich in Pro/Gly, Gal-3 can self-oligomerize forming pentamers in solution and it defines the latter properties of the protein. The linker domain consists of almost 110 amino acids in α-like collagen arrangement with a Pro-Gly-Tyr tandem repeat sequence that identifies a region recognized by several metalloproteases. The amino acids that recognize and bind ligands are the same described for the other galectins, as Arg/His/Glu/Asn/Trp [17,18].

1.2. Galectins as disease biomarkers

Galectins are becoming increasingly relevant due to their involvement in several diseases including cancer. Very often galectins, normally expressed at low levels in the physiological processes in which they are involved, are overexpressed in many pathological conditions such as asthma, fibrosis, inflammation, cardiovascular and renal disease, and several different cancers. Thanks to galectin interaction with glycans, they are involved in tumor development and metastasis progression, i.e., in all those processes such as cell adhesion and invasion, migration, and angiogenesis. In addition, since galectins are present in the cytoplasm, released from injured cells and inflammatory cells, and secreted in biological fluids, like serum and urine, their increased levels in sick individuals make them potential biomarkers [19].

1.2.1. Gal-1

High Gal-1 levels were found in the serum of epithelial ovarian cancer patients. It is worth noting, a reduction of Gal-1 levels is used to monitor the tumor progression and reaction to treatments. Moreover, its serum levels are recognized among normal, benign, malignant, and metastatic samples [20]. Furthermore, patients with rheumatoid arthritis showed increased Gal-1 levels both in serum and synovial fluid levels compared to the healthy controls [21,22]. Gal-1 concentrations, as well as Gal-3 and -9, were found modified in the serum of chronic interstitial lung diseases, such as idiopathic pulmonary fibrosis and idiopathic non-specific interstitial pneumonia suggesting their potential utility as clinical, diagnostic, and prognostic biomarkers [23].

1.2.2. Gal-3

Many studies have revealed that Gal-3 plays an important role as a diagnostic or prognostic biomarker for heart disease [24], kidney disease [25], autoimmune disease, indicating the inflammatory status and fibrosis, neurodegenerative disorders, and different tumor stages. It has been recognized that Gal-3 is extremely useful for detecting many of these diseases in their early phases [26]. Very recently, the emergence of cytokine release syndrome has been identified as the major cause of mortality in COVID-19 patients. In this regard, a massive increase of plasma Gal-3 and Gal-9 has been revealed in COVID-19 patients compared to the normal population [27]. Accordingly, plasma galectin measurement has been defined as a surrogate diagnostic biomarker in COVID-19 patients [28].

Getting into the details of the pathologies above described, FDA has accepted Gal-3 as a soluble biomarker for myocardial fibrosis to identify ventricular remodeling [29]. Furthermore, Gal-3 is associated with different neuropathological injuries, such as traumatic brain lesions [30], ischemia [31,32], demyelination [33], and encephalitis [34], relating to a more serious fate in stroke and cerebrovascular diseases [35]. Cengiz et al. [36] indicated that Gal-3 levels in serum might be a potential marker of Parkinson's disease. Numerous studies have indicated that Gal-3 may also be used as a diagnostic or prognostic biomarker for rheumatoid arthritis (RA) and osteoarthritis [37,38], besides connective tissue diseases such as systemic lupus erythematosus (SLE) and systemic sclerosis [39].

Extensive literature is available on the role of Gal-3 as a tumor marker. Serum levels of Gal-3 were significantly higher in cancer patients with pancreatic carcinoma [40], breast, and bladder cancer with respect to the normal population [41,42]. Moreover, the serum Gal-3 level of the pre-surgical intervention patients is of diagnostic value for papillary thyroid carcinoma, as it was markedly higher in cancer patients than in healthy subjects [43,44]. More, the serum level of Gal-3 was increased 11.3-fold in patients with colorectal cancer and remarkably improved 31-fold in those with metastases [45]. In addition, non-acute promyelocytic leukemia patients' present serum Gal-3 levels significantly higher with respect to control group subjects. Importantly, this increased Gal-3 expression level was an independent poor prognostic marker [46]. These studies reveal that Gal-3 is expressed anomalously in different types of cancer, which indicates that it is not always a tumor-specific biomarker. In these cases, it could be appropriate to use Gal-3 in combination with other specific biomarkers [47,48].

1.2.3. Gal-4

It is well known that diabetes negatively influences the clinical course and compromises the therapy of patients with Heart Failure, furthermore in these patients, diabetes is associated with different inflammatory processes [49]. Since patients with diabetes showed higher levels of Gal-4, it has been suggested that Gal-4 may serve as a biomarker. Additionally, infants with intestinal injury present circulating Gal-4 concentrations significantly elevated, indicating Gal-4 may as a biomarker for neonatal gastrointestinal pathologies [50]. The LGALS4 gene, significantly downregulated in colon adenocarcinoma (COAD) and bladder urothelial carcinoma (BLCA) compared to healthy samples, represents a prognostic and diagnostic marker for COAD and BLCA and for multiple cancer types [51].

1.2.4. Gal-7

Rare diseases, such as Stevens-Johnson Syndrome (SJS) and Toxic Epidermal Necrolysis (TEN), showed widespread epidermal necrosis and sloughing of skin. Numerous proteins, including Gal-7, were identified as promising potential biomarkers for SJS/TEN, although are still in the early phases of research. Unfortunately, it is difficult to conduct statistical studies due to a paucity of patients [52].

1.2.5. Gal-9

Gal-9 is reported as a useful biomarker for IFN gene signatures associated with an autoimmune disease such as SLE. Serum levels of Gal-9 were significantly increased in patients with SLE compared with the healthy population. Moreover, they were significantly greater in patients with SLE-related organ involvement [53]. Shete et al. [54] recognized viremic patients detecting plasma Gal-9 levels with a sensitivity and a specificity of more than 90%, suggesting Gal-9 as a surrogate marker of viremia with an important role in HIV-infected patients undergoing antiretroviral therapy and in HIV management.

1.2.6. Gal-10

Periodontitis, a chronic inflammatory disease of tissues surrounding the teeth, showed high levels of Gal-10 in the gingival crevicular fluid of patients, electing Gal-10 as a potential biomarker for periodontitis [55].

1.2.7. Gal-13

The expression of Gal-13 was markedly increased in subjects with asthma compared to controls. Inhaled corticosteroid (ICS) treatment reduced plasma Gal-13 levels, and baseline plasma Gal-13 levels reflect the response to this therapy, so plasma Gal-13 levels may be useful for predicting responses to ICS treatment [56].

1.3. Galectin inhibitors

Due to their relevance in cancer and inflammatory processes, Galectins are crucial targets for these pathologies, and several strategies to inhibit them have been reported. The availability of structural information on galectin-ligand complexes has helped the rational design of galectin inhibitors tremendously [57].

Many chemical scaffolds have been proposed for the design of *anti*-galectin agents but the most common inhibitors of Galectins can be grouped into three categories. Firstly, carbohydrate-derived monovalent inhibitors originally consisted of either galactose, lactose, or N-acetyllactosamine scaffolds, in which their free-OH groups were modified with diverse chemical substituents. Secondly, carbohydrate-derived multivalent inhibitors, are represented mostly by the pectin derivatives or the galactomannan; thirdly, the peptide-derived inhibitors [58].

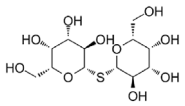
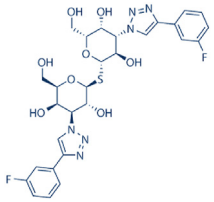
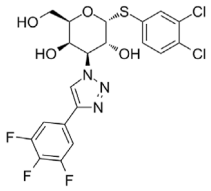
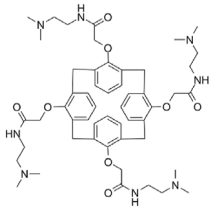
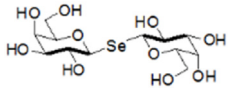
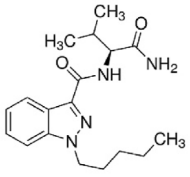
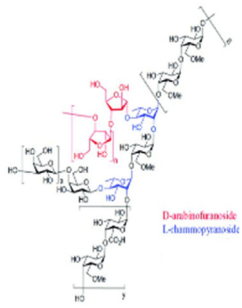
The first class belongs to a whole series of compounds with specific modifications aimed at increasing the stability and metabolic specificity of carbohydrates. The involvement of S atoms in the glycosidic bond, in particular, confers enhanced glycosidase stability and distinct stereotopic and flexibility features to the sugars generating a very interesting class of inhibitors, molecules that represent the so-called “thio-digalactoside” (**TDG**) scaffold” [59,60]. The production of asymmetrical C-3/C-3′-substituted TDG derivatives has proved very promising [61], among them, 3,3′-deoxy-3,3′-bis-(4-[m-fluorophenyl]-1H-1,2,3-triazol-1-yl)-thio-digalactoside (**TD139**; Table 1) is the most potent inhibitor of galectins-1 and -3 and has been approved by the FDA for the treatment of IPF [4].

Recently, selenium has been introduced as a bridging atom in place of sulfur, creating a new version of diglycosylated analogues characterized by a selenoglycoside bond between two galactose units (SeDG) [59,60,62–64]. Such molecules are very attractive compounds thanks to their important biological features (glycosidase stability and antioxidant and peroxidant properties) and were used as lectin binders in a lot of therapeutic applications such as antibacterial, anti-inflammatory or anticancer [63]. In particular, **SeDG**, or the benzylated derivative [60], displays anti-migration and anti-invasion activity in metastatic melanoma cells [60,64].

Another strategy involves the derivatization of a low-affinity monosaccharide with functionalities that form a combination of orthogonal multipolar fluorine-amide, phenyl-arginine, sulfur-π, and halogen bond interactions, which results in lectin ligands with affinities far surpassing those of common natural ligand fragments (for example, **GB1107**). These compounds are the smallest high affinity Gal-3 inhibitors described and thus constitute a new class of promising drug lead structures [65].

The carbohydrates are potential scaffolds for the design of galectin inhibitors; however, weak interactions make this strategy a tough challenge. To overcome this, the effectiveness of multivalent galectin inhibitors was evaluated. Two polysaccharide-based

Table 1
Galectin inhibitors widely used with proven effectiveness.

Inhibitor	Structure/Sequence	Selectivity and affinity	Activity
Small molecules			
TDG		Gal-1 K_D: 24 μM Gal-3 K_D: 43 μM (Fluorescence Polarization Assay) [67]	TDG has anti-inflammatory and anti-cancer activity. TDG reduces body weight gain in diet-induced obese rats [68]
TD-139 β-thiodigalactoside		Gal-1 K_D: 2,2 μM Gal-3 K_D: 0,036 μM Gal-7 K_D: 32 μM (Fluorescence Polarization Assay) (isothermal titration calorimetry) [4]	TD-139 is used for the treatment of idiopathic pulmonary fibrosis [69]
GB1107		Gal-3 K_D: 0,037 μM (Fluorescence Polarization Assay) [65]	GB1107 reduces human lung adenocarcinoma growth and blocks metastasis in the syngeneic model [70]
OTX008		Gal-1 [71]	OTX008 inhibits proliferation and invasion in vitro in thyroid cancer cells, reduces tumor mass in a xenograft anaplastic mouse model [72]
SeDG		Gal-1 K_D: 23,7 μM Gal-3 K_D: 20,2 μM Gal-9 K_D: 22,4 μM (isothermal titration calorimetry) [60,62]	SeDG in vitro inhibits migration and invasion of melanoma cells [60]
Polysaccharide			
GM-CT-01 Davanat™ (Galectomannan)		Gal-1 K_D: 10 μM (Miller, 2009) Gal-3 K_D: 200 μM [73] [Nuclear magnetic resonance] [IC50 − enzymatic assay]	GM-CT-01 significantly reduces liver fibrosis in rat model [74,75]
GR-MD-02 Belapectin (Modified pectin)		Gal-1 K_D: 8 μM Gal-3 K_D: 2,9 μM [Nuclear magnetic resonance] [76]	GR-MD-02 significantly reduces liver fibrosis in TAA and STZ rodent models [74,75]
Peptide			
G3-C12	ANTPCGPYTHDCVKR	Gal-3 K_D: 0,070 μM (IC50 − enzymatic assay)	GE-C12 displays anticancer activity [77]
ANGINEX	ANIKLSVQMKLFKRHLKWKIIVKLNDGRELSLD	Gal-1 K_D: 0,010 −0050 μM (Fluorescence anisotropy) [77]	ANGINEX acts as potent antiangiogenic and antitumor agent [78]

multivalent Gal-3 inhibitors, **GM-CT-01** (Davanat) and **GR-MD-02** (Belapectin), that show low micromolar binding affinity to Gal-3 of 2.9 μM and 2.8 μM respectively, have been tested in clinical trials (Table 1). Together with other chemotherapy drugs, or tested alone, Davanat has completed its preclinical and clinical studies showing an increase in longevity of patients with colorectal cancer and a reduction of seriously adverse effects. To our understanding, the Phase III clinical trial of Davanat appears to have been accepted by the FDA [66].

Galectins were also found to be inhibited by peptide-derived inhibitors. **Anginex**, a potent antiangiogenic and antitumor peptide, is a galectin antagonist that has been shown to bind galectin-1, -2, -7, -8N and -9N, whereas no binding was observed for galectin-3, -4N, -4C and -9C. Another promising peptide-based inhibitor is **G3-C12**, a phage-display-derived peptide that is able to specifically bind Gal-3 with 70 nM affinity.

Although many questions still remain to be decrypted, such as selectivity and compound biostability, further structural exploration and modifications of promising inhibitor scaffolds will facilitate the development of galectins-targeting drugs.

2. Biosensors for galectins

2.1. Immunosensors

Immunosensors can be considered complementary diagnostics well-suited for the analysis of biomarkers of diverse diseases, thanks to their attractive features, such as rapid detection, cost-effectiveness, and high-throughput capabilities. Moreover, the possibility of analysis of complex matrices without extensive pre-treatment of the sample boosted such diagnostics as great candidates to complement chromatographic techniques in bioclinical analysis. As depicted in Fig. 1, the field of biosensors for galectin detection is at its early stage; however, the scientific community is beginning to perceive their real importance in the diagnosis of the various pathologies connected with the variation of galectins' expression. Nowadays, only a few examples are present in the literature reporting the use of antibodies for the design of Immunosensors for galectins. Tang et al. [79] reported an electrochemical immunosensor for the determination of Gal-3, as a biomarker of heart failure. In detail, a glassy carbon electrode was modified with a film of a composite made from the N-doped graphene nanoribbons immobilized Fe-based-Metal-organic frameworks deposited with gold nanoparticles (AuNPs). The primary antibody against Gal-3 was immobilized on the AuNPs on the surface of the modified electrode and then blocked with bovine serum albumin (BSA) (Fig. 3A). Methylene blue was used as an electrochemical mediator in the amperometric measurements of increasing concentrations of galectins, displaying a linear response in a range from 100 fg mL^{-1} to 50 ng mL^{-1} and a detection limit of 33.33 fg mL^{-1} (S/N = 3). Furthermore, satisfactory results were obtained in human serum samples underlining the immunosensor potential for promising application in biomarkers' monitoring.

An example of an optical immunosensor for Gal-3 was described by Primo et al. [80], based on Surface Plasmon Resonance (SPR) as a transduction system. This immunosensor was built at a thiolated Au surface modified by self-assembling four bilayers of poly(-diallyldimethylammonium chloride) and graphene oxide (GO), followed by the covalent attachment of 3-aminephenylboronic acid (3ABA) (Fig. 3B). The GO was exploited as the anchoring point of the antibody as well as a field enhancer for enhancing the sensitivity, while the 3ABA helped to orientate the antibody through the selective link to the Fc region. The immunosensor, conceived as a label-free biosensing configuration, was able to detect Gal-3 in the range of clinically relevant concentrations, with a linear range from 10.0 to 50.0 ng mL^{-1} and a detection limit of 2.0 ng mL^{-1}, with satisfactory results in enriched human serum samples.

2.2. Biosensors using artificial bioreceptors

The design of artificial molecules in the development of a biosensing system is gaining more and more impetus, to obtain novel bioreceptors with desired features in terms of affinity and selectivity; in addition, such synthetic bioreceptors are more stable, affordable, and compatible with diverse sensor surfaces [81]. Among them, Molecular Imprinting Polymers (MIPs) represent an important frontier in this sense, being such molecules highly specific towards the targets as they can be moulded from a template molecule, which can be the analyte itself or a compound with a similar structure [82]. Several electrochemical biosensors based on MIPs as bioreceptors have been reported in the literature for the detection of different targets [83].

Cerqueira et al. [84] described an electrochemical biosensor based on a specific molecular imprinting polymer (MIP) designed for monitoring Gal-3 as a melanoma biomarker. The MIP was created directly on the working electrode surface of a screen-printed electrode by electropolymerizing a mixture of analyte (Gal-3) and monomer (2-aminophenol). Then, the protein was removed from the polymeric material by oxalic acid treatment. This process formed a non-conductive polymer with recognition sites showing an affinity for the Gal-3 (Fig. 4A). The analytical performance of the MIP sensor was evaluated by electrochemical impedance spectroscopy (EIS), obtaining an analytical response towards Gal-3 in a concentration range from 0.5 ng/mL to 5000 ng/mL. Additionally, the biosensor was tested for the detection of Gal-3 in human serum (Cormay Serum HN), in a range of concentrations between 0.5 ng/mL and 5.0 μg/mL, showing satisfactory results. This biosensor displayed good sensitivity, response time, reproducibility, and affordability, demonstrating its potential to be integrated into point-of-care devices and considered a valid tool for the treatment and follow-up of melanoma.

A different molecule was synthesized by Hashimoto et al. [85], *ad hoc* designed for the specific recognition of Gal-3 for the development of a fluorescence biosensor. In detail, the authors obtained a library of fluorophore-modified peptides folded as α-helical structures with one cysteine as probe modification site located at the center of four residues on the same face of the helix (Fig. 4B). The probe 4-nitrobenzoxadiazole (NBD) was thus conjugated to the single cysteine, whose fluorescence intensity increased with the concentration of Gal-3 and visually observed. This novel molecule demonstrated to be very useful as fast and specific probes for galectin recognition, in the view of the development of a biosensor based on visual detection, thus suitable for point-of-care applications.

2.3. Nanobased biosensors

The remarkable benefit of nanotechnology in biosensor design has been largely demonstrated in recent decades by the multiple features of nanomaterials, which are fostering further advances in the design of sensitive, miniaturized, low-cost, simple, and disposable devices for point-of-care testing. In the case of galectin biosensors, many efforts still need to be provided, being this field at an early stage. However, nanomaterials demonstrated their huge potential to enhance biosensor analytical performances also towards the cancer marker galectin-3 [86]. In particular, the authors synthesized single-walled carbon nanotubes (SWCNTs) conjugated with d-(+)-galactose as a probe for the electrochemical detection of galectin-3. Based on previous finding [87] that d-(+)-galactose was able to bind galectin-3 at a concentration of 0.5–1 μg/100 μL, they

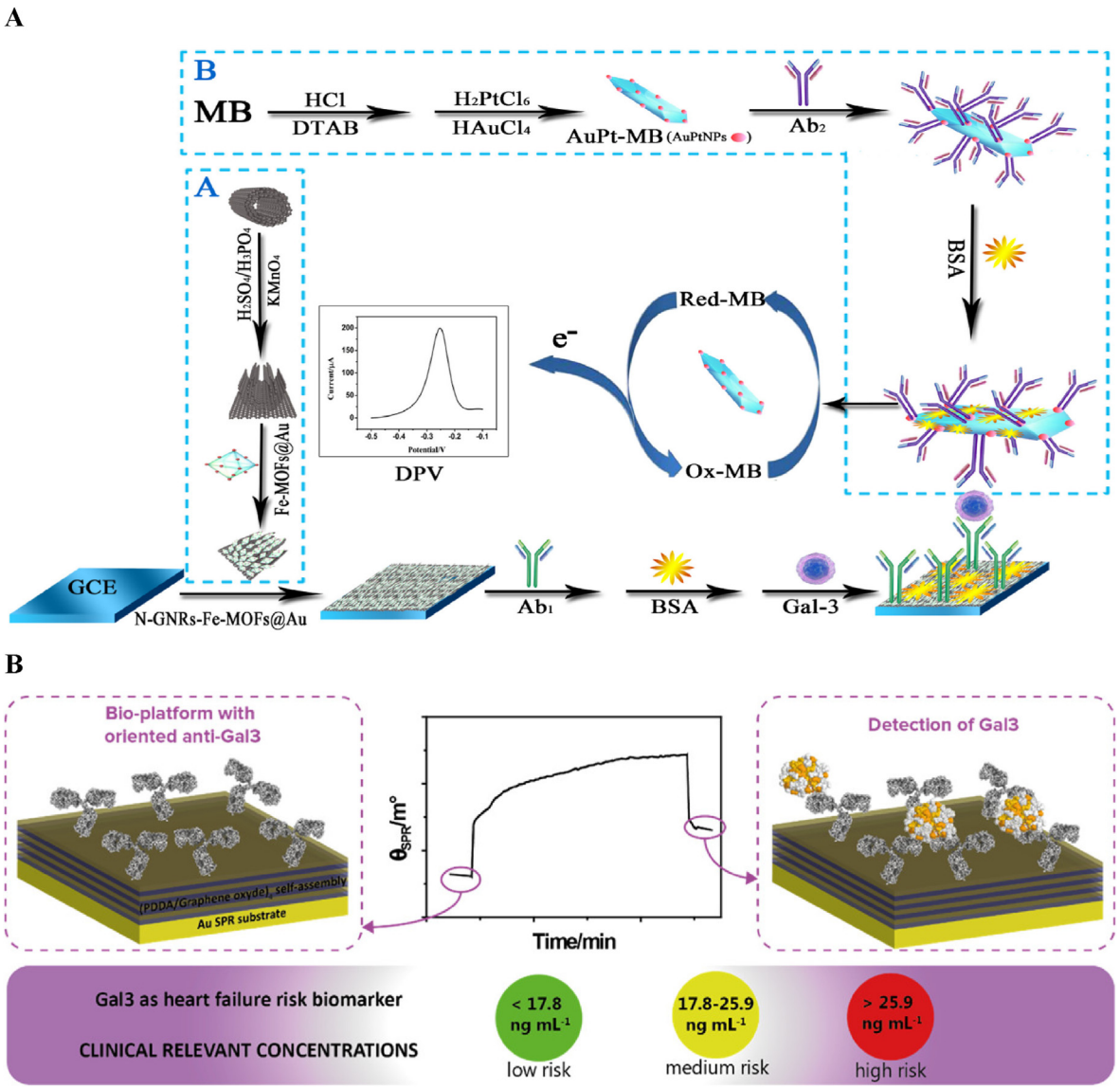


Fig. 3. A) Schematic illustration of the stepwise assembly procedure of the electrochemical immunosensor interfaces: the preparation procedures of (A) N-GNRs-Fe-MOFs@AuNPs and (B) AuPt-MB-Ab_2 bioconjugates. N-GNRs-Fe-MOFs@AuNPs was prepared using a mixture solution of N-GNRs and Fe-MOFs@AuNPs, sonicated for 30 min and stirred for 12 h at room temperature, followed by centrifugation and washing with ultrapure water. Finally, the composites were dispersed again in 2 mL deionized water and kept at 4 °C for the following experiments. AuPt-MB-Ab_2 was prepared adding 100 μL Gal-3-Ab_2 into 2 mL of prepared AuPt-MB composites solution and stirred for about 12 h at 4 °C. For fear of nonspecific adsorption, 40 μL BSA (1 wt%) was mixed with the bioconjugates for 6 h at 4 °C after incubation. MB Methylene blue; DTAB dodecyltrimethylammonium bromide; BSA bovine serum albumin; Ab antibody; GCE glassy carbon electrode; DPV differential pulse voltammetry. Reprinted with permission from Tang, Z., He, J., Chen, J., Niu, Y., Zhao, Y., Zhang, Y., & Yu, C. (2018). A sensitive sandwich-type immunosensor for the detection of galectin-3 based on N-GNRs-Fe-MOFs@ AuNPs nanocomposites and a novel AuPt-methylene blue nanorod. Biosensors and Bioelectronics, 101, 253–259. Copyright (2018) Elsevier [79]. B) Scheme of the surface plasmon resonance (SPR) immunosensor built at a thiolated Au surface modified by self-assembling four bilayers of poly(diallyldimethylammonium chloride) and graphene oxide (GO), followed by the covalent attachment of 3-aminephenylboronic acid (3ABA). Reprinted with permission from Primo, E. N., Kogan, M. J., Verdejo, H. E., Bollo, S., Rubianes, M. D., & Rivas, G. A. (2018). Label-free graphene oxide-based surface plasmon resonance immunosensor for the quantification of galectin-3, a novel cardiac biomarker. ACS applied materials & interfaces, 10(28), 23,501–23508. Copyright (2018) ACS [80].

investigated the binding affinity of galectin-3, using the d-(+)-galactose conjugated SWCNTs dropped onto the surface of a SiO_2 substrate to fabricate a molybdenum (Mo) electrode. Galectin analysis was based on differences in the resistance of samples in the presence or absence of the target and occurred in a concentration range from 0.0156 to 0.03125 μg/100 μL.

2.4. Biosensor based on galectin ligands

Recently, novel biosensing configurations have been described for the detection of proteins, which do not require protein structural changes upon ligand binding. These biosensors consist of a tag labeled with a small molecular tether bearing a ligand specific for the interaction with the target protein [88]. These systems can be realized by conjugating fluorescent proteins with a ligand-binding domain or by site-directed modification of an introduced cysteine residue near the ligand-binding site with a small fluorophore [89]. Kucińska et al. [90], for example, employed Fibroblast growth factor receptors (FGFR1) as interaction partners of galectins, demonstrating their interaction with galectin-1 and galectin-3 through multiple experiments, including pull-down performed with resin-immobilized galectins and cell lysate. Based on these findings, the idea of using receptors that interact with galectins for the development of optical biosensors becomes more and more real.

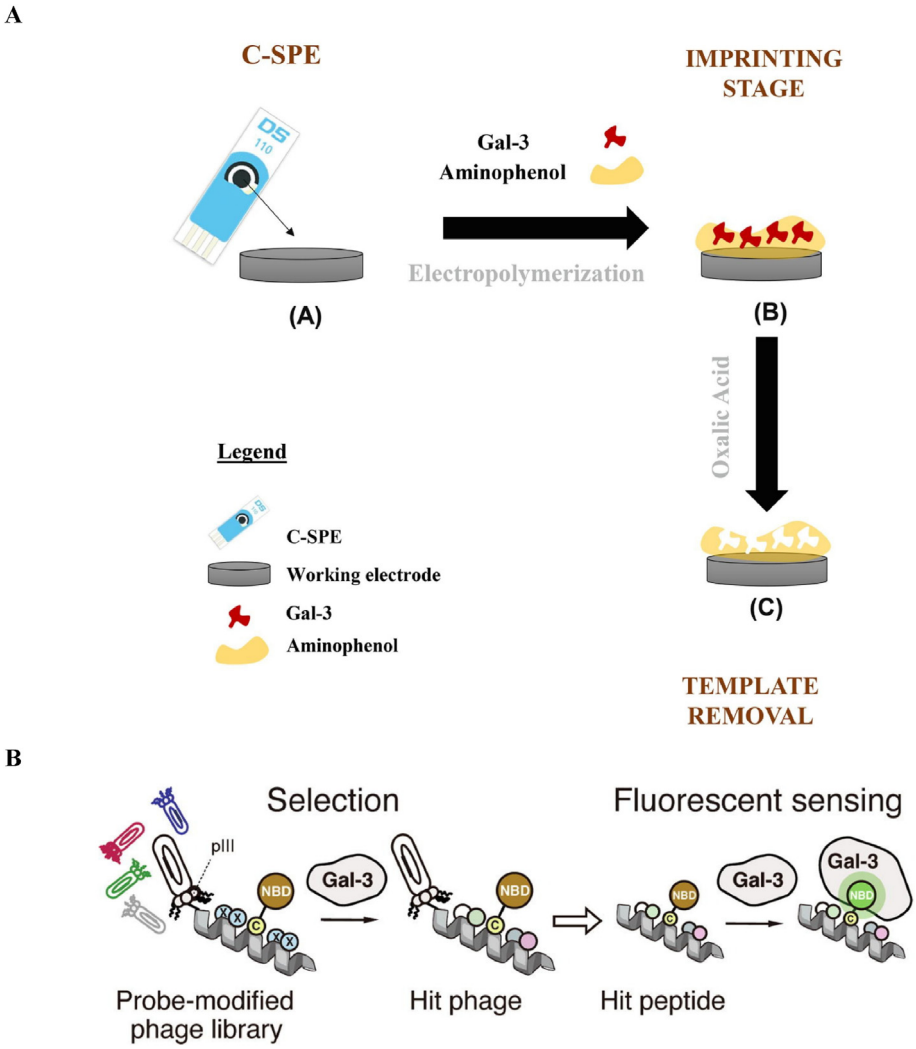


Fig. 4. A) Schematic representation of the synthetic process: (A) Working area of C-SPE; (B) Imprinting stage after electropolymerization of Gal-3 with aminophenol (C) Binding site after template removal by oxalic acid. C-SPE carbon screen-printed electrode. Reprinted with permission from Cerqueira, S. M., Fernandes, R., Moreira, F. T., & Sales, M. G. F. (2021). Development of an electrochemical biosensor for Galectin-3 detection in point-of-care. Microchemical Journal, 164, 105,992. Copyright (2021) Elsevier [84]. B) Schematic illustration of the construction of a 4-nitrobenzoxadiazole (NBD) modified phage library and selection of NBD-modified peptide biosensors against Gal-3. Reprinted with permission from Hashimoto, M., Miki, T., Chang, I. V., Tsutsumi, H., & Mihara, H. (2021). Selection of fluorescent biosensors against galectin-3 from an NBD-modified phage library displaying designed α-helical peptides. Bioorganic & Medicinal Chemistry Letters, 37, 127,835. Copyright (2021) Elsevier [85].

Lactose is another example of a receptor described in the literature for galectin binding. Long et al. [91] realized a label-free electrochemical biosensor based on gold nanoparticle (AuNP) loaded octahedral Cu_2O nanocomposites as binding sites for the lactose ligand, which specifically binds galectin-1 (Fig. 5). The AuNPs are also able to enhance the electrochemical signals of the

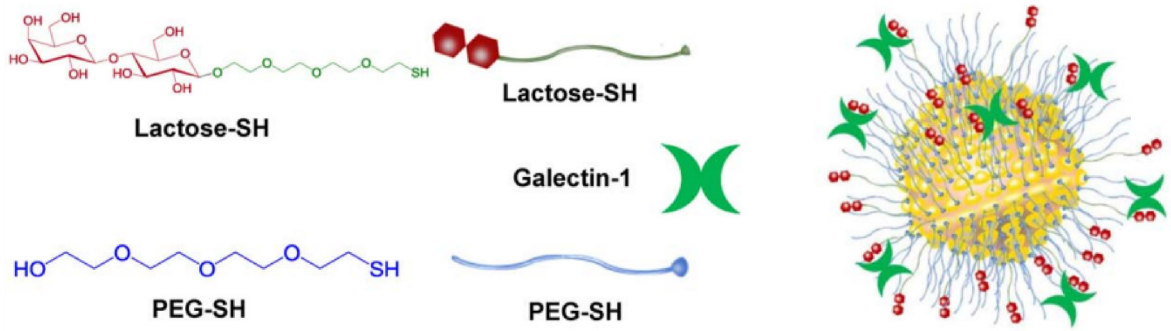


Fig. 5. Schematic illustration of the Cu_2O@Au/Lactose-SH/PEG-SH biosensor for Gal-1 recognition. PEG polyethileneglycol. Reprinted with permission from Long, F., Li, W., Chen, W., Liu, D., Chen, Y., Zhou, R., & Li, P. (2019). An amperometric biosensor based on Cu2O@ Au nanocomposites for the detection of galectin-1 via lactose–galectin interactions. Nanotechnology, 30(48), 485,706. Copyright (2019) IOPScience [91].

Table 2
Biosensing systems reported from the literature of the last years for galectins detection.

Target	Biosensor configuration	Transducer	LOD	Linear range	Sample analyzed	Ref.
Gal-3 as a biomarker of heart failure	Immunosensor based on a nanomodified glassy carbon electrode	Amperometric using methylene blue as electrochemical mediator	33.33 fg mL^{-1}	100 fg mL^{-1} - 50 ng mL^{-1}	Human serum	[79]
Gal-3	Immunosensor based on nanomodified chip	Surface Plasmon Resonance	2.0 ng mL^{-1}	10.0 −50.0 ng mL^{-1}	Human serum	[80]
Gal-3 as a melanoma biomarker	Molecular imprinting polymer directly designed on a screen-printed electrode	Electrochemical impedance spectroscopy	–	0.5 ng/mL to 5000 ng/mL	Human serum	[84]
Gal-3	4-nitrobenzoxadiazole-modified phage library conjugated with dye into the binding interface	Fluorescence	–	–	–	[85]
Gal-3 as a cancer marker	d-(+)-galactose conjugated -walled carbon nanotubes dropped onto a SiO_2-molybdenum electrode	Electrochemical	–	0.0156 −0.03125 μg/ 100 μL	–	[86]
Gal-1	Gold nanoparticle loaded octahedral Cu_2O nanocomposites modified glassy carbon electrode	Cyclic voltammetry and differential pulse voltammetry	–	0.1 pg mL^{-1} - 10 ng mL^{-1}	–	[91]

fg: femtogram; pg: picogram; ng: nanogram; mL: milliliter.

proposed biosensor, which exhibited a variation of electrochemical responses to different concentrations of Gal-1 ranging from 0.1 pg mL^{-1} to 10 ng mL^{-1}. This study demonstrated not only the suitability of galectin ligands as specific bioreceptors but also the potential of nanomaterials to improve biosensor performances.

3. Conclusions and future perspectives

Research on galectins has been increasing more and more in recent years, as shown by the literature. Galectins act as biomarkers and therapeutic agents of several diseases, including cancer, cardiovascular disease, type 2 diabetes, musculoskeletal disorders, and neurodegenerative diseases. In recent years, galectins have also been considered in the development of biosensors for the evaluation of a pathological state as well as for the follow-up of a therapeutic treatment. However, this research is still in its infancy, even if the literature shows a first step towards the expansion of this topic. Certainly, the biosensing systems described up to now turn out to be very fascinating from the point of view of the structural and functional study of the interaction of galectins with the target analyte (Table 2). What is currently an urgent requirement is to obtain a selective biosensor that can recognize between one galectin and another, with the difficulty caused by the high conservation of CRD. In addition, since galectins display a broad affinity toward β-galactosides, therefore glycan-based (nano)biosensors lack the required selectivity and affinity. A way to overcome this problem was found by Richards et al. [92] who, using a polymer-stabilized nanoparticle biosensing platform, demonstrated that the specificity of immobilized lacto-N-biose towards galectins can be 'turned on/off' by using site-specific glycan fluorination, and, in some cases, reversal of specificity can be achieved. In particular, these results showed that the incorporation of non-natural and fluorinated glycans into nanomaterials can determine an unprecedented selectivity, particularly towards galectins 3 and 7, that is not possible using native glycans, with clear potential applications in biosensing. In addition, this approach proves the translation potential of glyconanomaterials in therapeutic and biosensing applications.

Consequently, more widespread research may warrant the implementation of galectin biosensors, which may be crucial for obtaining a disease-specific, early-stage diagnosis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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