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Review

Glyco-nano-oncology: Novel therapeutic opportunities by combining small and sweet

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ABSTRACT

Recent efforts toward defining the molecular features of the tumor microenvironment have revealed dramatic changes in the expression of glycan-related genes including glycosyltransferases and glycosidases. These changes affect glycosylation of proteins and lipids not only in cancer cells themselves, but also in cancer associated-stromal, endothelial and immune cells. These glycan alterations including increased frequency of β1,6-branched *N*-glycans and bisecting *N*-glycans, overexpression of tumor-associated mucins, preferred expression of T, Tn and sialyl-Tn antigen and altered surface sialylation, may contribute to tumor progression by masking or unmasking specific ligands for endogenous lectins, including members of the C-type lectin, siglec and galectin families. Differential expression of glycans or glycan-binding proteins could be capitalized for the identification of novel biomarkers and might provide novel opportunities for therapeutic intervention. This review focuses on the biological relevance of lectin-glycan interactions in the tumor microenvironment (mainly illustrated by the immunosuppressive and pro-angiogenic activities of galectin-1) and the design of functionalized nanoparticles for pharmacological delivery of multimeric glycans, lectins or selective inhibitors of lectin-glycan interactions with antitumor activity.

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1. Mammalian glycosylation: a brief introduction

Glycosylation is a common post-translational modification by which specific glycan structures are incorporated into proteins and lipid backbones. This process involves the synchronized action of a series of glycosyltranferases and glycosidases, enzymes that contribute to selective addition or removal of specific saccharide residues [1]. The glycosylation machinery represents more than 1% of the genome and more than 100 glycosyltransferases and glycosidases have been identified to date [2]. This non-template approach capable of building a highly diverse number of glycans, allows the display of relevant information, of various order of magnitude higher than that encoded by other biological molecules such as nucleic acids and proteins, which together contribute to build the 'blocks of life' [3].

Glycans may be covalently linked to asparagines in Asn-X-Ser/Thr motif generating *N*-glycans or to hydroxyl groups of the amino acids serine, threonine or hydroxylysine generating *O*-glycans [2]. The presence of potential glycosylation sites in a protein backbone, together with the presence or absence of glycosyltransferases and glycosidases are key elements in determining the extent and nature of protein glycosylation. This post-translational modification is dynamically regulated during cellular activation, differentiation and trafficking and changes in glycosylation occur frequently in response to cellular stress and environmental cues [1]. At the cellular level, glycans can differentially regulate segregation, localization and turnover of glycoprotein receptors [4] and play essential roles in cellular recognition, communication and signaling [1].

1.1. Aberrant glycosylation in the tumor microenvironment

Cancer is a major cause of death that contributes to 15% of mortality worldwide providing 14 million estimated new cases per year [5]. During the past few years, many research groups have focused their attention in trying to elucidate a marker of poor prognosis in both primary tumors and metastatic lesions that could predict the evolution of neoplastic disease. Most of this information, arising from detailed analysis of gene and protein signatures from tumor cells or from tumor-associated microenvironment, has been used in discovery platforms to identify potential novel therapeutic targets. Interestingly, a number of studies revealed that changes in the glycosylation signature, not only of tumor cells themselves, but also of stromal cells, tumor-associated vascular cells and immune cells, are a hallmark of tumor transformation, metastasis, angiogenesis and immune escape [6]. This differential glycosylation of cancer-associated versus healthy tissues correlates with altered glycan expression in tumor cells or in circulating glycoproteins and could be potentially used for diagnostic, prognostic and therapeutic purposes.

The first demonstration of this phenomenon occurred as early as 1969, when Meezan et al. showed that membrane glycoproteins of healthy fibroblasts were significantly smaller than those exhibited by transformed fibroblasts [7]. This finding was later corroborated by histopathological evidence showing differential binding of glycan-binding proteins to cancerous versus healthy tissues. These results highlighted important changes in glycosylation during tumorigenesis and metastasis, including an increase in the size of *N*-glycans in several cancer-associated glycoproteins. Current evidence, in experimental and human tumors, convincingly demonstrated that increased size of *N*-glycans is, at least in part, due to an increase in β1–6 branching of *N*-glycans, resulting from enhanced expression of UDP-GlcNAc:*N*-glycan GlcNAc transferase 5 (GnT5; encoded by the *MGAT5* gene) [8]. In addition, augmented expression of the glycosyltransferase UDP-GlcNAc:*N*-glycan GlcNAc transferase 3 (GnT3; encoded by the *MGAT3* gene), which catalyzes the addition of the bisecting GlcNAc branch, has also been documented in certain tumors [9]. These results illustrate the importance of aberrant *N*-glycosylation during the tumorigenic process.

Importantly, another hallmark of the tumor microenvironment is the overexpression of mucins, proteins that carry many glycosylated serines and threonines in tandem-repeat regions [10]. An abnormal feature of carcinoma mucins is incomplete glycosylation. One typical consequence is the expression of T (Galβ1–3GalNAc-α1-*O*-Ser/Thr) antigen also called Thomsen–Friedenreich (TF) antigen or the expression of Tn (GalNAc-α1-*O*-Ser/Thr) or sialyl-Tn antigens. Because such *O*-glycosylated structures occur rarely in normal tissues, they have been proposed to elicit specific immune responses and have been exploited for the design of immunotherapeutic strategies and cancer vaccines [10]. Indeed, a correlation exists between the expression of the T and Tn antigens, the spontaneous production of antibodies directed against these structures, and the prognosis of cancer patients [10]. Furthermore, it has been reported that malignant cells display augmented sialylation, as demonstrated by increased frequency of α2-6-linked sialic acid attached to outer *N*-acetyllactosamine (Gal-β1–4GlcNAc units) or to inner GalNAcα1-*O*-Ser/Thr units on *O*-glycans [11]. Finally, other relevant glycosylation changes involve increases in polylactosamine elongation and exposure of sialylated Lewis structures or selectin ligands, sulfated glycosaminoglycans, hialuronans and glycosphingolipids [12]. Thus, several glycosylated structures are abnormally expressed in the tumor microenvironment and may contribute to cancer progression.

Interestingly, changes in the cancer-associated glycome have been originally characterized using monoclonal antibodies against specific glycan structures; however recent approaches have used more sophisticated technologies to identify tumor glycans, including ultra performance liquid chromatography (UPLC), mass spectrometry (MS), lectin-cytometry and lectin histochemistry [13]. By using these glycoanalytical approaches, a discrete number of glycans and glycan-binding proteins have emerged as useful diagnostic tools and prognostic markers and as key therapeutic targets in cancer. From a general standpoint, the high-throughput and reproducible nature of emerging glycomics platforms have allowed integration of glycomics with other-omics fields, such as proteomics, genomics, lipidomics and metabolomics, making systems glycobiology a reality [14].

As differential expression of glycans could be capitalized to define biomarkers that delineate malignant versus healthy tissue

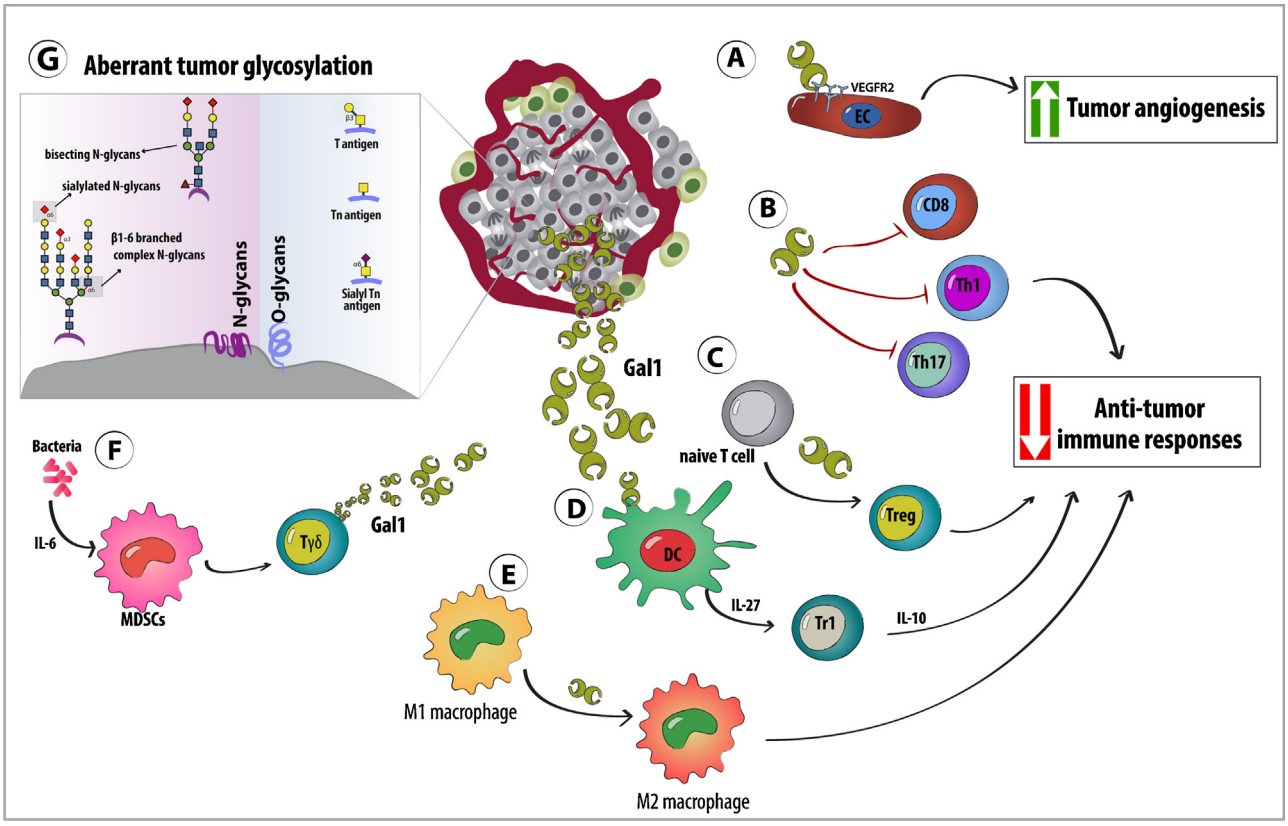

Fig. 1. Different functions of GAL-1 in the tumor microenvironment. GAL-1 is up-regulated at sites of tumor growth and metastasis in a variety of tumor types and modulates tumor progression by coupling tumor hypoxia to angiogenesis (A) and blunting anti-tumor immune responses by selectively depleting CD8, Th1 and Th17 cells (B), promoting expansion of Foxp3$^+$ regulatory T cells (Tregs) (C), inducing the differentiation of tolerogenic dendritic cells (DCs) (D) and polarizing macrophages toward an M2 phenotype (E). In addition, recent evidence suggested that GAL-1 produced by γδ T cells may link commensal microbiota, tumor-promoting inflammation and immunosuppression (F). Different glycan structures that positively or negatively regulate galectin binding are often a hallmark signature of tumor progression, including increased β1-6 branched complex *N*-glycans, bisecting *N*-glycans and sialylated *N*-glycans as well as incomplete *O*-glycans such as T, Tn or sialyl Tn antigens (G). New generation NPs are being functionalized to monitor these aberrant structures, to deliver selective inhibitors and/or to interrupt galectin-glycan interactions for therapeutic purposes.

and/or to target tumor growth providing novel therapeutic opportunities, a number of targeted delivery systems are being designed including functionalized nanoparticles presenting glycans, glycoconjugates and/or glycan-binding proteins, thereby opening a new interdisciplinary field –'glyco-nano-oncology'– at the frontiers of glycobiology, nanotechnology and oncology.

1.2. Lectin-glycan interactions in the tumor microenvironment

As mentioned above, abnormal glycosylation has been recognized as a hallmark of the transition between healthy and neoplastic tissue [1], being appreciated not only in tumor cells themselves but also in tumor-associated stromal, immune and vascular cells [15]. In fact, glycan-related genes can be up- or down-regulated during tumor angiogenesis [16] and immunity [17]. However, the pathophysiologic relevance of these glycosylation changes is just beginning to be understood. Emerging evidence indicates that endogenous lectins are the main recognition molecules responsible of translating glycan-containing information into functional cellular responses [18].

To date, several families of lectins have been implicated in a variety of physiologic and pathologic processes [19,20]. These include, among others, the C-type lectins, galectins and, I-type lectins (Siglecs and others) [21]. This review focuses on the biological relevance of lectin-glycan interactions in the tumor microenvironment and the design of functionalized nanoparticles for delivery of immunoregulatory glycans, lectins or selective lectin inhibitors with antitumor activity.

1.3. Galectin-glycan interactions control anti-tumor immune responses

Galectins, an evolutionarily conserved family of animal lectins, recognize multiple *N*-acetyllactosamine (Galβ(1–4)-GlcNAc; LacNAc) units via a carbohydrate recognition domain (CRD) [22]. However, in spite of this general glycan specificity, fine differences exist in glycan-binding preferences among different members of the galectin family which may explain divergences in biological activities [23].

It has been demonstrated that cancer cells may usurp several glycosylation-dependent regulatory circuits to create immunosuppressive networks which contribute to thwart anti-tumor responses [22]. Interestingly, expression of galectin-1 (GAL-1), as well as other galectins, positively correlates with the aggressiveness of tumors and the acquisition of metastatic phenotypes [24]. In this regard, we and other investigators have demonstrated that GAL-1 contributes to the immunosuppressive potential of tumor cells by impairing T cell function, instructing the differentiation of tolerogenic dendritic cells (DCs) and modulating NK cell activity in a wide range of tumors including melanoma [25,26], Hodgkin's lymphoma [27], lung adenocarcinoma [28,29], pancreatic adenocarcinoma [30–32], neuroblastoma [33] and glioblastoma [34,35]. Blockade of GAL-1 expression in the tumor microenvironment augmented effector T cell responses by unleashing otherwise repressed Th1, Th17 and effector CD8 T cells [22,25]. In addition, inhibition of GAL-1 in the breast cancer microenvironment suppressed the development of lung metastasis and disarmed the suppressive

activity of Treg cells through mechanisms involving downregulation of the linker for activation of T cells (LAT), an adaptor protein involved in T-cell receptor (TCR) signaling [36]. But not only cancer cells produce GAL-1 to evade immune responses, as γδ-T cells can also suppress anti-tumor immunity through GAL-1-dependent mechanisms in a model of ovary cancer, linking commensal microbiota, tumor-promoting inflammation and distant tumor progression [37]. Moreover, other galectin members may also contribute to blunt anti-tumor immunity. For example, galectin-9 (GAL-9) increased the frequency of granulocytic myeloid-derived suppressor cells [38] and favored exhaustion of tumor-specific $CD8^+$ T cells [39]. On the other hand, galectin-3 (GAL-3) favored anergy of tumor-specific T cells [40] and dampened NK cell activity by interfering with glycosylation-dependent interactions between NKG2D and major histocompatibility complex class I-related chain A (MICA) [41]. These findings suggest that interrupting galectin-glycan interactions, using specific antibodies, soluble glycans, glycomimetics or peptidometics may contribute to stimulate antitumor immune responses.

1.4. Galectin-glycan interactions modulate tumor angiogenesis

Although the importance of protein glycosylation in immune-related processes has largely been appreciated, our awareness of the impact of glycosylation in tumor angiogenesis is much more limited. In recent years our efforts were focused at investigating the function of GAL-1-glycan lattices in modulating endothelial cell biology. In this regards, we identified an unexpected link between tumor hypoxia and neovascularization. In human and mouse Kaposi's sarcoma cells, we found that tumor hypoxia induces GAL-1 expression and influences the development of aberrant vascular networks [42]. Moreover, we recently identified a glycosylation-dependent mechanism, mediated by GAL-1-glycan interactions that mimics vascular endothelial growth factor (VEGF) signaling and preserves vascularization in anti-VEGF refractory tumors [43]. We found that immunosuppressive and hypoxic stimuli induced significant changes in endothelial cell glycosylation, favoring the exposure of specific glycans, that are critical for GAL-1 binding, including increased β1-6 *N*-glycan branching, higher poly-*N*-acetyllactosamine extension and decreased α2,6 sialylation. At the molecular level, we found that GAL-1 promotes vascular endothelial growth factor receptor 2 (VEGFR2) signaling through binding to complex *N*-glycans on this receptor. These glycosylation-dependent interactions promoted segregation of VEGFR2 into membrane microdomains and prolonged residency of this receptor on the surface of endothelial cells [43]. This effect preserved angiogenesis in tumors with limited sensitivity to anti-VEGF treatment. Interestingly, lack of β1–6GlcNAc-branched *N*-glycans on endothelial cells or silencing of tumor-derived GAL-1, converted refractory into anti-VEGF-sensitive tumors. This effect involved GAL-1-VEGFR2 interactions, as it was prevented when GAL-1 was silenced in tumor cells or when mice were treated with anti-VEGF mAb plus axitinib, a RTK inhibitor that preferentially perturbs VEGFR signaling [43]. Administration of an anti-GAL-1 mAb promoted tumor growth inhibition and circumvented compensatory angiogenesis induced by VEGF blockade. Interruption of GAL-1-*N*-glycan interactions promoted transient normalization of the tumor-associated vasculature *in vivo* and contributed to alleviate tumor hypoxia, thus facilitating the influx of immune cells into the tumor microenvironment. This effect resulted in augmented T-cell proliferation and enhanced IFN-γ and IL-17 production by tumor-draining lymph node cells. Collectively, these findings emphasize the dual effects of targeting GAL-1-*N*-glycan interactions, which influence tumor growth by attenuating aberrant angiogenesis and by evoking T cell-mediated responses. These results offer novel opportunities for circumventing resistance to VEGF-targeted therapies and stimulating antitumor immune responses. A summary of the multifunctional roles of GAL-1 in the tumor microenvironment is summarized in Fig. 1. Thus, targeting the GAL-1-glycan axis may synergize with other immunotherapeutic or anti-angiogenic strategies to promote tumor regression [44]. For this purpose, different strategies have been designed including GAL-1-specific neutralizing antibodies, glycomimetics and peptidomimetics.

Current efforts are being made to generate appropriate vehicles, including functionalized nanoparticles (NP), to deliver these agents directly to sites of tumor growth and metastasis. In this regard, in the past few years, the tailored construction of complex NP-based systems that carry multiple chemical or biochemical functions has become a mature field. These nanosystems are able to bind to selected tissues or organs, emit fluorescent signals, respond to a change in the environment (pH, redox potential, presence of target molecules) and are also being externally controlled by magnetic fields or radiation [45]. Designed nanosystems have evolved from early polymer or liposome-based drug delivery technology to sophisticated self-assembled arrangements of inorganic, organic and biologically active building blocks that present well controlled and tailorable functional domains [46]. This new generation of active functional nanosystems, still under development, opens a new path to combine therapeutics and diagnostics into one single yet complex entity with exciting potential in cancer treatments.

2. Nanotechnology in cancer

The knowledge, techniques and approaches in nanotechnology have spilled over numerous disciplines such as physics, materials science, chemistry, biology and medicine. Nanotechnology, in the form of nanomedicinal platforms, has been recognized as a Key Enabling Technology (KET) with a significant impact on three main areas: therapeutics, diagnostics/imaging and regenerative medicine [47]. In recent years, there has been an unprecedented expansion in the field of nano-oncology with the development of nanosized systems for the diagnosis and treatment of cancer, fueled by the rapid expansion of complex nanomaterials [48]. In this context, newly developed nanosystems will have a decisive influence in cancer treatment in the next decade through new formulations for early disease detection, highly sensitive and selective imaging agents, targeting tumors with minimized side effects and development of minimal invasive techniques [49].

2.1. Why nano?

Researchers have developed a wide array of soft and hard nanoparticulate species that can circulate through the bloodstream and target tumors. In contrast to standard low molecular weight drugs, NP are essentially multifunctional, and several properties can be coded at the same time either into the NP core or their surface, taking advantage of the large surface:volume ratio. Unique biological properties arise by combining the luminescence, magnetism, plasmonic heating or payload delivery related to the NP composition and size with the controlled tailoring of the external functional groups. Functional NP can be precisely tailored to bind, absorb, and carry a variety of compounds and probes, including small molecule drugs, fluorescent groups or molecules, DNA, RNA, proteins and glycans with high efficiency, and to be compatible with different administration routes [48].

Complex multifunctional NP are actually sophisticated nanosystems, which have been reported to target and deliver therapeutic molecules to cancer cells, decreasing toxicity on healthy organs and tissues and lowering side effects. As tumor tissues display leaky and irregularly dilated blood vessels, nanosystems can easily extravasate from blood vessels to tumor tissues and are accu-

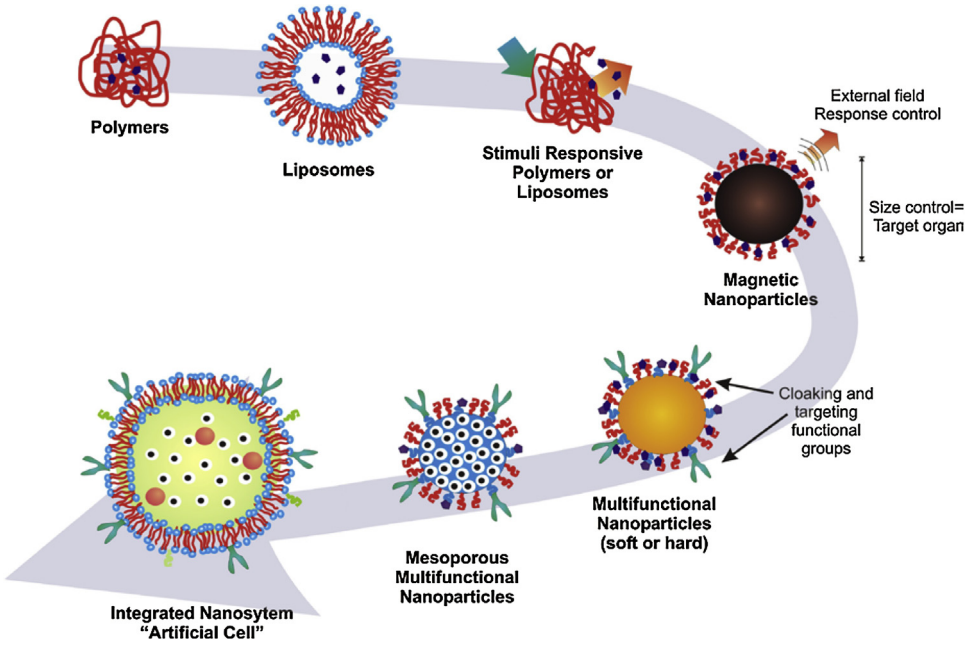

Fig. 2. Scheme of the evolution of nanosystems used for diagnosis, therapeutics (drug delivery) and theranostics, from the simplest and available conventional polymer, micelle and liposome-based delivery systems (approved for use) to the progressive development of more complex systems, leading to integrated cell-like nanosystems with multiple domains.

mulated at those sites due to the poor lymphatic drainage [50]. Selective accumulation of NP within the tumor microenvironment is referred to as 'Enhanced Permeability and Retention (EPR) effect' [51,52], allowing their therapeutic targeting and imaging. Alternatively, functional molecules located at the NP surface (antibodies, antigens, peptides, etc.) can act as targeting groups. In addition, nanosystems have tunable optical or magnetic properties that provide a means to image tumors at their earliest stages of development, or to receive an external request to trigger a response. Size, shape, and surface features of nanoscopic objects can be modified practically at will, enabling them to remain with high stability in cellular environments and with high carrier capacity. These features of nanosystems explain why the application of nanotechnology to drug delivery and early detection of tumors is expanding as a subject of research and begins to have significant impact in many areas of medicine [53].

2.2. Types of nanosystems

In principle, NP designed for cancer treatment incorporate primarily both hydrophilic and hydrophobic substances in different regions, and can be modified to achieve tumor targeting. The first generation of anticancer NPs includes mostly soft matter-derived carriers such as liposomes and polymeric platforms that have already been approved for clinical use. The main advantage of these well-established carriers is to increase the solubility of hydrophobic drugs, improving circulation time and delivery [52,54–56]. In a second generation of soft matter-based nanosystems, targeted delivery has been imparted by adding a variety of antigens or antibodies to different formulations [57]. More recently, inorganic NP-based systems are gaining momentum, due to the possibility of adding nanoparticulate cores with magnetic or optical properties, which improve localization and handling from outside the body by applying external magnetic fields or irradiation. A well-known example is the use of an alternating magnetic field on superparamagnetic NPs [58] or optical excitation of metallic NPs with NIR [59]. Both excitation pathways lead to a local increase of temperature (hyperthermia) that can cause necrosis of adjacent cells by disrupting their membrane. Hyperthermia may provide a suitable means to treat chemotherapy-resistant tumors, as well as an improved response when combined with chemotherapy and radiation [59].

A new generation of sophisticated nanosystems is coming into sight, combining responsive nanostructured cores with precisely tailored pore systems that act as controlled delivery reservoirs [60], or adding molecular fragments (polymers, biomolecules) that allow a controlled gating as a response to external stimuli [61,62]. A scheme of a variety of nanosystems that present different components is presented in Fig. 2. This third generation of nanobioactive species implies the integration of delivery, targeting and tracking in complex nanosystems, leading to the new field of *theranostics*.

2.3. Theranostics

The key concept of theranostics is the convergence of diagnosis disease and simultaneous targeted therapy in a single functional entity. This integrated system carries a sensing module for biomarkers detection, which localizes the organ or tissue under risk, while a functional component triggers the therapeutic action for medical treatment [63]. Obviously, the targeted organs or tissues will determine the synthetic approach and construction of the theranostic object. The integration of therapeutic and diagnostic capability in a single system has set a new scenario for medical treatment, and may contribute significantly to the ever-growing field of personalized medicine [63,64]. In this new paradigm proposed for disease treatment, the combination of biotechnology and advanced nanomaterials allows implementation of medical treatments tailored to the specific characteristics of the individual patients. The main goals of this approach are to maximize the efficiency and selectivity of the pharmaceutical compounds and to minimize the side effects [65,66]. Current strategies for the use of theranostic systems rely on a careful balance between the imaging quality/sensitivity and the therapeutic efficiency of the designed nanosystems. However, the power of theranostic systems relies in the direct visualization of pharmacokinetics that can provide important fundamental insights into the heterogeneities between tumors and patients, helping physicians to make informed deci-

sions about timing, dosage, drug choice, and treatment strategy, leading to improved "personalized medicine" [67].

2.4. Nanovehicle design

In this section, we will briefly point out current strategies for developing oncology therapies based on NP systems, including future challenges in the area of nano-oncology. Design and synthesis of nanoparticulate carriers involves a new paradigm, where multiple functions and characteristics are combined in a single entity. Multifunctional hybrid nanosystems are thus designed to exploit the advantages of nano-enabled properties in the NP core such as superparamagnetism, plasmons or luminescence, display targeting groups such as antibodies, antigens or peptides at their surfaces, and carry treatment drugs at the surface or in designed reservoirs. These complementary qualities have to be carefully tuned in order to produce active, traceable, non-toxic systems with higher retention times for *in vivo* imaging and therapy. In order to be able to introduce NP into an organism, it is extremely important to control the particle size and/or impart "cloaking" functional groups and to avoid, if possible, recognition by the host immune response. The internalization and biodistribution of the nanovehicles depends mostly on the chemical surface identity. In the last few years, a wide variety of nanosystems such as liposomes, micelles, protein NPs, polymers, viruses, magnetic particles, semiconductor quantum dots, mesoporous silica, gold-based NPs and core–shell systems have demonstrated the ability to perform multiple functions in biological systems [66,68,69]

Moreover, at present, early clinical detection of cancer mostly relies on histopathology, cytology or imaging techniques including X-ray derived approaches, magnetic resonance imaging, computed tomography and endoscopy. However, these methods cannot detect cancer in early stages, minimizing the chances for survival. To overcome these limitations, multifunctional nanosystems that can be tracked from the outside provide new tools to understand cellular processes related to cancer development [70]. Recent developments incorporate several tools for *in vivo* non-invasive imaging, by combining radioactive, magnetic, image contrast or optically active components in the nanosystems [71].

In summary, the desired features for a theranostic nanosystem can be summarized as follows: (a) integration of probes for external tracking (radioactivity, fluorescence, magnetic), (b) optimization and protection of the pharmacological payload, (c) combination of multiple biotargeting functional groups, (d) design and addition of responsive functional groups or biochemical actuators, (e) strategies to avoid recognition by the immunological system, and (f) biocompatibility. In this context, SiO_2 is one of the matrices showing an excellent perspective of application for nanovehicle design and disease treatment. Traditionally, colloidal silica NPs have been produced and employed on an industrial scale, with multiple uses as viscosifiers, abrasives, fiber treatment, fillers, binders, etc [72]. The biocompatibility and chemical stability of SiO_2 surfaces and the ample library of mild chemical procedures (e.g., sol–gel chemistry, polyelectrolyte layer-by-layer assembly) facilitate biomolecules encapsulation and anchoring in a rigid, but benign matrix [73–75]. Moreover, SiO_2 NPs can easily host biomolecules such as enzymes, proteins, DNA or lipid layers [74,76] can be designed with uniform and interconnected pores [77], can be deposited as shells (porous or not) onto other nanoparticulate materials [78,79], configuring a highly versatile chemical "multitool". An avenue of opportunities to tailor nanosystem behavior and performance is open. In order to achieve such tailoring, it is essential to control the main bio-physicochemical properties of the nano-vehicle, such as size, charge, surface hydrophilicity, as well as the nature and density of the ligands on their surface. All these features impact the circulating half-life of the particles as well as their biodistribution and ultimate performance [54].

The next generation of therapeutic nanosystems based in a rational approach involves a number of challenges summarized as follows: (a) a detailed understanding of the physicochemical properties of nanosystems and their proper characterization (which will involve sophisticated and cutting edge techniques) [80]; (b) an adequate drug delivery and release system that could avoid undesired uptake by nontarget organs (e.g., spleen and liver); (c) improvement of drug release systems that are programmable or triggered by different stimuli (e.g., light, electric current, enzyme, ultrasound, pH, temperature and magnetic field) [61,81]; (d) progress in the understanding of the biological bases of disease and its microenvironment, the biological obstacles that hamper drug delivery, and endosomal trafficking pathways; and (e) the identification of biological markers indicative of pathologic settings [69].

2.5. Glyconanoparticles

To date, most of the molecular recognition modules for nanosystems relied in the attachment of antibodies or antigens to the NP surface. In opposition to antigen-antibody interactions, carbohydrate-based ligands and glycan-binding proteins (lectins) show relatively weak affinities [82]. This apparent complication can be easily solved using multivalent binding sites, an essential requisite for increasing affinity in glycobiological interactions. It is clear that natural living biological systems have evolved and adapted to fulfill this condition. On the other hand, nanotechnology provides a "molecular toolbox" where building blocks can be assembled following sequential procedures, with synthetic control at each step and logical modules [61,83]. NP surfaces can be modified with multiple and different carbohydrate units, resembling glycoclusters present on the cell surfaces. This opens the path to glyco-NP-based nanosystems.

Compared to other molecules with biological importance that can be produced artificially in large quantities (DNA, proteins, peptides, enzymes) [84], tailored carbohydrates have been so far disregarded due to the complex procedures involved in their preparation [85]. Issues like stereochemistry (chirality), conformation, different linkages and the presence of the anomeric carbon, determine that for just one monosaccharide there is a vast number of oligomeric structural variations [86]. Minimal differences in monomer linkage type can produce even highly branched oligosaccharides, contrasting with linear biomolecules such as DNA/RNA, lipids and proteins. On the other hand, natural polysaccharides (dextrans, alginates, chitosan, hyaluronic acid, cellulose) have been used to decorate NP surfaces with multiple, but simple, purposes: increase the hydrophilicity of the nanoparticulate carrier, biocompatibility, bioavailability, antifouling and bioadhesive properties [87].

As mentioned in previous sections, protein-carbohydrate interactions (as well as carbohydrate-carbohydrate interactions) are involved in several pathophysiologic settings, including immunity, angiogenesis, metastasis and tumorigenesis, thus representing an inspiration toward developing a nano-glycomics platform. The simplest way to block these biorecognition systems is to design glyconanoparticles (GNP) that mimic the carbohydrates present in target cells. Glyco-based nanomaterials can control the biological adhesion processes, competing on the interaction reactions that take place at the host cell surface, having deep implications for anticancer strategies. It is extremely interesting to note, that most of the work on inorganic based GNP has been carried out on Au NP, while reports on GNP based SiO_2 materials are relatively scarce [88,89]. Au GNP synthesis is centered on the nature of the Au–S chemistry, fundamental in the design of self-assembled surfaces, molecular electronics, catalysis and sensors [90]. The thiol-derived

compounds adsorb on the Au surface with high affinity, leading to well-packed monolayers or strongly adsorbed molecules, exposing functional end-groups to the liquid interface (carbohydrates). A possible explanation may be attributed to the fact that $NaBH_4$ reduction, an essential step for Au NP synthesis, is compatible with non-reducing glycosides. Brust et al. set a scientific milestone in 1995, when they produced ultrasmall thiol-stabilized AuNPs (with a mean diameter of 5 nm) in a single phase methanolic system based on $HAuCl_4$, *p*-mercaptophenol and $NaBH_4$ [91]. This opened an extremely simple approach for the synthesis of AuNPs stabilized by a variety of functional thiol ligands, like highly designed thio-derived carbohydrates [92]. As a matter of fact, lactose anchored Au-1.8 nm NP have been tested as antitumoral agents in mice, reducing significantly metastasis *via* lactosylceramide interactions [93]. This is a promising result for seizing changes that occur at the surface of endothelial cells, such as glycosphingolipid (GSL) overexpression, which have been recognized as initial steps of tumor spreading.

Nonetheless, glycan clusters are expressed at the cell surface involving host-cell receptors with multivalent carbohydrate interactions. This is an extremely important detail that has to be tackled combining multiple areas such as NP synthesis, surface chemistry, synthetic carbohydrate chemistry and polymer chemistry. As an example, the precise control on the anchoring of multiple ligands, or copies of the same carbohydrate on surfaces of AuNP, has been exploited for binding assays where multivalent interactions enhance the aggregation process. Moreover, it is possible to co-assemble "silent" molecules in order to tune the local concentration of the biologically active carbohydrate units [94] for maximizing the sensitivity of the bioassay [94–97]. There are many examples in the literature that demonstrate the importance of parameters such as NP size, surface ratio of the active carbohydrate and the "silent" molecule (i.e., alkyl groups, PEG) and the spacer length of the anchored molecules [98]. This issue applies also for magnetic GNP (MGNP) and paramagnetic Gd(III)-AuGNP that have been used for targeting, labeling and imaging *in vitro* and *in vivo* cells. MRI is one of the most important noninvasive techniques for *in vitro* and *in vivo* imaging, the use of Gd-base contrast agents and superparamagnetic Fe_3O_4 NP have potential as imaging probes for diagnosis. The modification of MNPs (Fe_3O_4) with multiple copies of the biological active glycoside sLe^x, was used to target the endothelium molecules E-/P-selectin (CD62E/CD62P) present during inflammation processes, and NMR images allowed direct detection of sLe^x MNP *in vivo* [99]. Recently, Ei Boubbouet al. used MGNP with simple sugar monomers, such as D-Gal, L-Fuc, sialic acid, *N*-acetylglucosamine, for detecting and profiling cancer cells by NMR imaging, based on their carbohydrate-binding abilities [100]. Moreover, Marradi et al. combine DO3A-Gd complex and simple sugars with thiol ending linkers to functionalized Au nanoclusters. The paramagnetic Gd(III) GlycoAu NP were used as NMR probes for imaging brain tumors and was compared with Dotarem® (Gd-DOTA) contrast agent [101].

Previously, we have shown that inorganic-based GNPs have a remarkable application potential in cancer diagnosis and treatment due to unique physical properties of the core GNP (luminescence, scattering, magnetism). Conversely, polymeric and organic-based materials offer other kind of opportunities for the design of advanced glyconanocarriers [102]. State of the art carbohydrate and organic chemistry techniques are becoming essential and exceptional tools for the design and synthesis of multivalent glycoreceptors [103]. In this context, the use of glycosylated dendrimers (glycodendrimers) with tree-like structures having repeating sugar units should be highlighted [102,104]. Moreover, dendritic objects have evolved to structures that are more complex; joining two chemically distinct and opposite dendritic building blocks results in amphiphilic Janus dendrimers [105]. The controlled tailoring of the hydrophilic and hydrophobic branched segments generates self-assembled objects resembling phospholipid-based liposomes, known as dendrimersomes. Combinatorial approaches in the synthesis of multivalent carbohydrate dendrimers led to glycodendrimersomes libraries from Janus-glycodendrimers [106]. These supramolecular units mimic biological membranes for targeted drug delivery, while exposing highly controlled carbohydrate ligands for biomolecular recognition.

2.6. Glyconanoparticles for artificial antitumor vaccine development

As mentioned above, it is well accepted that tumorigenesis and metastasis are highly related to aberrant expression of *N*-glycans (branched and bisecting) on the tumor cell surface [107]. Moreover, some aberrant *O*-glycans on tumor mucins (incomplete *O*-glycans such as T, Tn and sialyl-Tn) are considered tumor-associated carbohydrate antigens (TACA) [108,109]. These aberrantly glycosylated tumor antigens constitute plausible targets for vaccine design. However, one of the greatest obstacles in developing anticancer vaccines lies in choosing the right antigen, given the heterogeneity between tumor types and the fact that some carbohydrate antigens may also be a component of normal tissues. Recently, Au NP modified with multicopy-multivalent tumor-associated Tn glycans evoked significant T cell responses *in vivo* in New Zealand white rabbits [110]. This highly attractive feature allowed the creation of fully synthetic protein- and peptide-free glycoconjugate vaccines. As carbohydrate multivalency is a critical issue in vaccine candidates, glycan dendritic scaffolds constitute another option for promising vaccine platforms [111]. Nonetheless, overcoming their excellent antigenic, but variable immunogenic responses requires rationalizing glycan presentation for systematic studies [112].

Since interruption of galectin-glycan interactions may contribute to suppress tumor growth by stimulating antitumor immunity and attenuating aberrant angiogenesis, NPs could be functionalized to deliver glycan-based galectin inhibitors or glycan ligands to sites of tumor growth. In this regard, a recent study showed the delivery to pancreatic cancer tissue of magnetic, biodegradable NPs prepared using recombinant human serum albumin and iron oxide (maghemite, γ-Fe_2O_3). Because GAL-1 expression is preferentially upregulated in pancreatic cancer and precursor lesions, but it does not appear to be elevated in inflammatory pancreatitis or in healthy pancreatic tissue, this protein has been selected as a target receptor. Tissue plasminogen activator-derived peptides (t-PA-ligands) which display high affinity for GAL-1 have been chosen as target moieties and have been covalently attached onto the surfaces of NPs [113]. Moreover, Danhier and colleagues recently showed the local administration of chitosan lipid nanocapsules (LNCs) containing the anti-epidermal growth factor receptor (EGFR) and anti-GAL-1 siRNAs, which prolonged survival of nude mice bearing orthotopic U87MG glioblastoma cells [114]. In this regard, other studies documented the design of three novel gold NPs containing multiple long, flexible linkers decorated with lactose, β-cyclodextrin or both, which interacted efficiently with human GAL-3 [115]. Finally, Reynolds et al., prepared a nanotechnology-based approach that used gold nanorod (GNR)-GAL-1 siRNA complexes (nanoplexes) to inhibit gene expression driven by this lectin [116]. These examples illustrate the feasibility of targeting, silencing and/or monitoring galectin or glycan expression using NP-based platforms.

3. Conclusions and future perspectives

Glycan structures present on glycoproteins, glycosphingolipids and proteoglycans together with specific glycan-binding proteins

(or lectins) are essential partners for cellular communication, adhesion, migration, angiogenesis and immune escape during tumorigenesis and metastasis. In the present review we summarized: (a) the relevance of the cancer-associated glycome, (b) the critical importance of glycan-binding proteins as chemical translators of glycan-containing biological information; (c) the relevance of lectin-glycan interactions in shaping the tumor microenvironment by re-wiring immune and vascular signaling programs; and (d) the possibility to use lectin- or glycan-based functionalized NPs for diagnostic, prognosis and therapeutic purposes in cancer settings. Nanotechnological engineering of the glycosides patches on GNPs combining carbohydrate chemistry, spatial occupation and self-assembly, as well as tumor biology, is an uttermost requirement for mimicking the glycocalix of tumor cell surfaces. Interestingly, NP-based silencing or delivery of glycan-related enzymes (glycosyltransferases or glycosidases) or glycan-binding proteins (including galectins) to manipulate the formation of lectin-glycan complexes may contribute to reset the tumor microenvironment by circumventing tumor-associated immunosuppression, preventing epithelial mesenchymal transition (EMT) and metastasis and overcoming aberrant angiogenesis. Glycans, though being complex species, are without doubt a very interesting nanobuilding block for a new generation of therapeutic, diagnostic or theranostic systems. From the point of view of the construction of integrated nanosystems, a better understanding of glycan structures, their interactions with other nanocomponents (i.e., other dangling bioactive groups, cloaking polymers, surfaces), their behavior under confinement (i.e., in nanopores or restricted soft environments), and their possibility of spatial self assembly, interaction control and migration on surfaces (including patching or clustering) are yet to be explored, in order to design efficient GNP-based nanosystems. However, it is clear that GNP-based theranostics represents a potentially fruitful yet unexplored field, with a bright future ahead. New opportunities arise at the frontiers of glycobiology, nanotechnology and oncology (glyco-nano-oncology) by combining small and sweet.

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