Pharmacological Research 109 (2016) 45–54

Contents lists available at ScienceDirect

Pharmacological Research

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

Review

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

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ARTICLE INFO

Article history:
Received 12 January 2016
Received in revised form 2 February 2016
Accepted 2 February 2016
Available online 4 February 2016

Keywords:
Glycobiology
Nanotechnology
Cancer
Drug delivery
Galectins
Theranostics

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.

Contents

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http://dx.doi.org/10.1016/j.phrs.2016.02.005
1043-6618/

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