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REVIEW

The cancer glycome: Carbohydrates as mediators of metastasis

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ABSTRACT

Glycosylation is a frequent post-translational modification which results in the addition of carbohydrate determinants, "glycans", to cell surface proteins and lipids. These glycan structures form the "glycome" and play an integral role in cell–cell and cell–matrix interactions through modulation of adhesion and cell trafficking.
Glycosylation is increasingly recognized as a modulator of the malignant phenotype of cancer cells, where the interaction between cells and the tumor micro-environment is altered to facilitate processes such as drug resistance and metastasis. Changes in glycosylation of cell surface adhesion molecules such as selectin ligands, integrins and mucins have been implicated in the pathogenesis of several solid and hematological malignancies, often with prognostic implications. In this review we focus on the functional significance of alterations in cancer cell glycosylation, in terms of cell adhesion, trafficking and the metastatic cascade and provide insights into the prognostic and therapeutic implications of recent findings in this fast-evolving niche.

1. Introduction

1.1. Physiological role of glycosylation

Glycosylation is a post-translational modification that occurs in the endoplasmic reticulum (ER) and results in the addition of carbohydrate motifs, "glycans", to proteins and lipids that are, in most cases, destined for the cell surface. The resultant "glycoprotein" or "glycolipid" structures at the cell surface form a carbohydrate rich layer which plays an integral role in the interaction of the cell with its surrounding environment. Of the more than 200 different types of protein PTMs, glycosylation occurs frequently and results in the addition of functional carbohydrate motifs to protein structures [1,2]. Glycans interact with carbohydrate binding proteins known as "lectins" that are specific for glycan moieties and are commonly used in purified form to study glycosylation *in-vitro*. One of the main functions of lectins in mammalian cells is to mediate cell–cell interactions and therefore interactions of glycans with their respective lectins have major implications for cell trafficking.

Glycosylation of a given protein is achieved through a complex series of post-translational enzymatic steps that lead to the formation of protein-bound glycans with specific and diverse biological functions. These carbohydrate side chains are capable of modulating the interaction of the protein with its environment influencing key factors such as protein half-life, solubility, binding activity and specificity. Proteins with the same amino acid sequence can possess different glycan structures, producing different glycoforms of the same protein. These glycoforms can differ in key properties such as stability, folding, localization and ligand specificity [3] with consequent implications for physiological processes, including protein folding and trafficking, cell–cell and cell–matrix interactions, cellular differentiation and the immune response [4–6]. Therefore, the glycosylation status of a protein can be used to differentiate protein glycoforms and molecular changes in glycosylation of proteins have been used to distinguish normal from disease states in humans [7,8]. Furthermore, as cell communication, adhesion, and signaling also play a major role in cancer, changes in glycosylation of surface proteins on malignant cells can alter interactions between cancer cells and their surrounding environment [6,9–11].

Glycosyltransferases are enzymes that regulate the process of glycosylation in humans where their action is dependent on the availability of precursor monosaccharide molecules and other parameters [12,13]. Glycosyltransferases, along with glycosidases, work to add and subtract monosaccharides to and from glycan structures, examples of these enzymes include sialyltransferases and fucosyltransferases, which are responsible for the addition of sialic acid and fucose moieties,

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respectively. The intracellular sites of action of these enzymes include the ER, golgi apparatus, cytosol and nucleus.

Two major types of glycosylation occur on proteins; 1) O-linked glycosylation refers to the addition of N-acetyl-galactosamine to serine or threonine residues by the enzyme UDP-N-acetyl-D-galactosamine transferase, this is then followed by the addition of other carbohydrates such as galactose, N-acetyl-D-glucosamine or sialic acid (Fig. 1).; 2) N-linked glycosylation occurs in the ER and refers to the process by which an oligosaccharide chain is enzymatically attached to the amide group of an asparagine in the consensus sequence Asn-X-Ser/Thr where X represents any residue except proline (Fig. 1). This sequence can be used to identify potential N-glycosylation sites in peptide sequences.

O-linked glycosylation also contributes to the production of proteoglycans by the addition of glycosaminoglycan (GAG) chains to a core protein. GAGs consist of repeating disaccharide units composed of an N-acetylated or N-sulfated hexosamine and either a uronic acid (glucuronic acid or iduronic acid) or a galactose. Examples of GAGs include hyaluronan, dermatan sulfate, keratan sulfate, chondroitin sulfate, heparin, and heparan sulfate. Heparan and chondroitin sulfate are linked to serine residues of core proteins by xylose and this process is mediated by a xylosyltransferase. Proteoglycans and their associated GAGs form essential components of the extracellular matrix where they function in cell adhesion *via* interactions between the complex carbohydrate motifs [14].

It is clear that alterations in gene expression and protein expression are not the sole factors responsible for phenotype determination in cancer cells, where not only the cell itself is affected, but also the microenvironmental components such as the extracellular matrix (ECM). The impact of post-translational modifications (PTMs) on proteins and lipids has identified a layer of complexity, beyond the amino acid sequence, which has the consequence of greatly altering the function and even the purpose of that protein in a given context. Although the protein sequence is governed by the relevant genomic code, many properties of functional cell surface proteins, and circulating glycoproteins, are governed by the modification of glycans and therefore consideration must be given to the glycosylation status of a protein when considering its activity within a biological system.

This rapidly developing field has provided new cancer biomarkers and potential targets recently in a variety of solid and hematological cancers [15–17]. In this review we focus on the enzymes involved in this process and the cell surface proteins that become modified as a result of their action, with an overall focus on the implications for cell trafficking and metastasis of cancer cells.

2. Carbohydrates and the cancer cell

2.1. Glycosylation and cancer

The normal process of glycosylation is disrupted during malignant transformation of cells [18,19]. These changes result in alterations in tumor cell surface glycans and therefore interactions with endogenous lectins are impacted, which influences the metastatic potential of the tumor cells. Complex carbohydrate structures that can be found attached to proteins and lipids on the surface of cancer cells have a major influence on their phenotype and the interactions that they have with the surrounding environment [20] (Fig. 2). In parallel with the changes in glycosylation, expression and levels of carbohydrate-binding proteins also change during malignant transformation leading to altered overall presentation of glycans and their cognate receptors, *i.e.*, lectins.

Alterations in glycosylation of malignant cells can take a variety of forms, including changes in the amount, linkage and acetylation of sialic acids, and changes in the branching of N-glycans mediated by glycosyltransferases, alterations in expression of glycosaminoglycans such as heparan sulfate, and altered glycosylation of mucins, which are heavily glycosylated epithelial-derived proteins known to be implicated in certain cancers [14]. Studies of the mechanisms by which alterations in glycans are able to bring about changes in cancer cell biology have been impeded by the complexity and heterogeneity of glycans, however recent advances in glycomics, including glycogenome analysis, HPLC, mass spectrometry and lectin profiling have facilitated comprehensive characterization of the glycome of several tissues [21].

The mechanisms by which glycosylation changes mediate tumor metastasis and invasion are mostly unknown, however roles of specific cell surface glycoproteins and their carbohydrate motifs have emerged and will be reviewed in the following sections.

2.2. Implications of glycosylation in cellular metastasis

Both solid and hematological malignancies begin the process of metastasis from the primary niche by escaping to the systemic circulation,

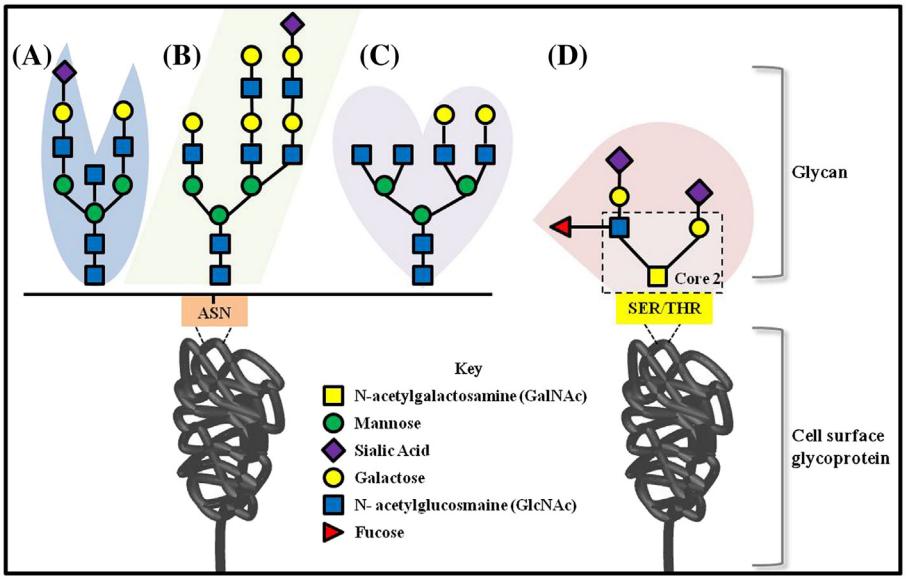

Fig. 1. O- and N-linked protein glycosylation. A–C: N-linked glycosylation; A — bisecting GlcNAc, B — Tri-antennary glycan, C — Tetra-antennary glycan. D: O-linked glycosylation, example shown is alpha 2, 3 sialylated glycan.

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