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Glycomics investigation into insulin action

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Abstract

Defects in glycosylation are becoming increasingly associated with a range of human diseases. In some cases, the disease is caused by the glycosylation defect, whereas in others, the aberrant glycosylation may be a consequence of the disease. The implementation of highly sensitive and rapid mass spectrometric screening strategies for profiling the glycans present in model biological systems is revealing valuable insights into disease phenotypes. In addition, glycan screening is proving useful in the analysis of knock-out mice where it is possible to assess the role of glycosyltransferases and glycosidases and what function they have at the cellular and whole organism level. In this study, we analysed the effect of insulin on the glycosylation of 3T3-L1 cells and the effect of insulin resistance on glycosylation in a mouse model. Transcription profiling of 3T3-L1 cells treated with and without insulin revealed expression changes of several glycogenes. In contrast, mass spectrometric screening analysis of the glycans from these cells revealed very similar profiles suggesting that any changes in glycosylation were most likely on specific proteins rather than a global phenomenon. A fat-fed versus carbohydrate-fed mouse insulin resistant model was analysed to test the consequences of chronic insulin resistance. Muscle and liver N-glycosylation profiles from these mice are reported.

Keywords: Glycomics; Insulin resistance; 3T3-L1; Glycogene

1. Introduction

Following the establishment of genomics and proteomics, it is becoming increasingly apparent that a complete biological picture can only be obtained by also considering the post-translational modifications. Glycosylation is the most common post-translational modification found on proteins with up to 70% of entries in protein databases containing at least one consensus N-glycosylation site (Asn-X-Ser/Thr, X not proline) [1,2]. Although not all of these sites will be glycosylated, Apweiler et al. [1] predict that over half of all proteins will carry complex glycans. The number of glycoproteins may increase even further as more nuclear and cytosolic proteins are shown to be modified by the single O-GlcNAc residue [3].

Secreted glycoproteins coat the outer layer of a cell and are therefore a primary point of contact with the extracellular environment. The oligosaccharides provide additional recognition epitopes for protein receptors and are implicated in a variety of cell–cell and cell–matrix communication events [4–6]. N-glycans are also important for protein folding and subsequent conformational maturation [7]. A number of proteins are completely dependent on N-glycans for correct protein folding, stability and function [2,8,9]. The failure of

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glycoproteins to be processed and trafficked through the cell leads to the onset and progression of many human diseases [10].

One heterogeneous group of disorders known as Congenital Disorders of Glycosylation (CDGs) are caused by substantial deficiencies in certain enzymes in the biosynthetic steps of N-glycosylation [11]. CDGs affect multiple organs and are associated with developmental delay. Group I CDGs are caused by defects in the biosynthesis and transfer of the dolichol-PP linked oligosaccharide precursor to the nascent polypeptide [12,13], whereas Group II CDGs affect N-glycan processing and synthesis [14]. Defects in protein glycosylation have also been linked to several forms of congenital muscular dystrophy resulting in brain abnormalities. Fukuyama congenital muscular dystrophy and congenital muscular dystrophy type 1C are caused by mutations in genes that encode putative glycosyltransferases while muscle–eye–brain disease and Walker–Warburg syndrome are the result of defects in proteins responsible for O-mannosylation [11,15]. Each of these disorders is associated with defective processing and maturation of the α-dystroglycan—an extracellular peripheral membrane glycoprotein which binds extracellular proteins such as laminin and agrin [16]. Incorrectly processed α-dystroglycan disrupts the interactions with the components of the extracellular matrix, and results in muscle disease and, in many cases, a neuronal-migration disorder.

The profile of glycosylation on the surface of the cell can also be affected by disease state. For example, lectin studies have shown that cancerous cells tend to have different glycans on their surface compared to non-cancerous versions [17]. Changes in the expression pattern of glycosyltransferases within the Golgi cause cancer cells to produce types of glycans usually found in the embryo, or to under-express or over-express glycans found in non-diseased cells [18]. In particular, up-regulation in the expression levels of the sialyltransferases and the branch-forming enzyme, N-acetylglucosaminyltransferase V (MGAT5), results in cancerous cells with a large increase in sialic acid on their surfaces [19,20]. Similarly, over-expression of fucosyltransferases and sialyltransferases can also lead to an increase in the level of terminal groups such as sialyl Tn, sialyl Lewis x and Lewis y [18,21]. Recently, Iwai et al. [22] demonstrated that levels of O-glycan core 3 synthase (β1,3-N-acetylglucosaminyltransferase 6) were markedly reduced in cancerous gastric and colorectal cells. The resulting decrease in the levels of core 3-type mucin O-glycosylation gave rise to phenotypic changes of cancer cells such as metastasis. Increase in the amount of the eptiope sialyl Lewis x and its sulphated version also occur in response to inflammation [18,23].

Another disease that has been linked with glycosylation is insulin resistance. Insulin resistance syndrome consists of a myriad of metabolic abnormalities including type 2 diabetes, central obesity, dyslipidaemia and hypertension, which are all well known risk factors for cardiovascular disease [24]. Although the causes of this disease are still unclear, it has been shown that increased flux through the hexosamine biosynthetic pathway (HBP) leads to insulin resistance in peripheral tissues [25,26]. The

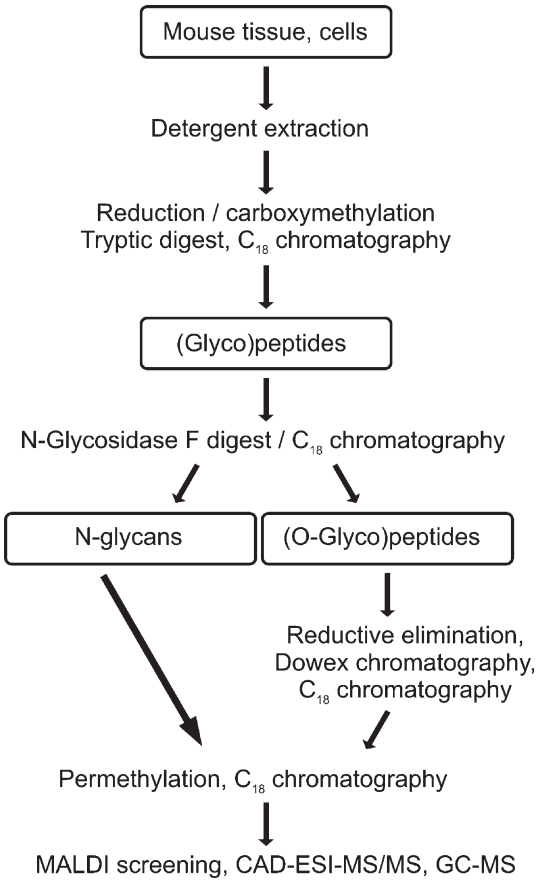

Fig. 1. Strategy for analysis of N- and O-glycans by mass spectrometry.

HBP converts fructose-6-phosphate to UDP-GlcNAc, a sugar nucleotide for lipid and secretory protein complex glycosylation, GPI anchor synthesis, and O-GlcNAc modification of cytoplasmic and nuclear proteins [3]. Addition of glucosamine which enters the pathway downstream of the rate-limiting enzyme glutamine:fructose-6-phosphate transaminase (GFAT) induces insulin resistance [26–28]. Patti et al. [29] demonstrated that the combined action of insulin and glucosamine resulted in a significant increase in rat muscle UDP-GlcNAc compared to rats infused with insulin alone. Interestingly, when 3T3-L1 adipocytes were treated with insulin and glucose, only a marginal increase (approximately 40%) in UDP-GlcNAc levels were observed over a wide range of external glucose concentrations [30], suggesting tight regulation of the HBP. One mechanism for mediating insulin resistance in cells appears to be through O-GlcNAc modification of intracellular proteins. Insulin resistance caused by increased flux through the HBP results in an increase in O-GlcNAc modification of intracellular proteins including some transcription factors and members of the insulin signalling pathway [31–37].

To date, no rigorous analysis has been carried out to determine whether the structures of the N- and O-glycans are also affected by insulin resistance. Several lines of evidence suggest that changes may exist. For example, it has been shown that alterations in the activity of enzymes involved in glycosylation result in changes in sialic acid and fucose content in insulin resistant patients and animals [38–40]. Chronic