



The glyco-redox interplay: Principles and consequences on the role of reactive oxygen species during protein glycosylation

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ABSTRACT

Reactive oxygen species (ROS) carry out prime physiological roles as intracellular signaling agents, yet pathologically high concentrations of ROS cause irreversible damage to biomolecules, alter cellular programs and contribute to various diseases. While decades of intensive research have identified redox-related patterns and signaling pathways, very few addressed how the glycosylation machinery senses and responds to oxidative stress. A common trait among ROS and glycans residing on glycoconjugates is that they are both highly dynamic, as they are quickly fine-tuned in response to stressors such as inflammation, cancer and infectious diseases. On this account, the delicate balance of the redox potential, which is tightly regulated by dozens of enzymes including NOXs, and the mitochondrial electron transport chain as well as the fluidity of glycan biosynthesis resulting from the cooperation of glycosyltransferases, glycosidases, and nucleotide sugar transporters, is paramount to cell survival. Here, we review the broad spectrum of the interplay between redox changes and glycosylation with respect to their principle consequences on human physiology.

1. Introduction

Reactive oxygen species (ROS) are highly reactive compounds formed by redox reactions inside and outside of cells. Cellular ROS production can be either nonenzymatic or enzymatic (for review see Ref. [1]). ROS are considered to be proper radicals such as the superoxide anion ($O_2^{\bullet-}$), the hydroxyl radical ($\cdot OH$), the peroxy radical ($ROO\cdot$), and the alkoxyl radical ($RO\cdot$) or non-radicals such as hydrogen peroxide (H_2O_2), organic hydroperoxides ($ROOH$), singlet molecular oxygen (1O_2), and hypochlorous ($HOCl$) and hypobromous ($HOBr$) acids [2–4]. ROS can be both, detrimental to cells especially under conditions leading to oxidative stress, and essential cellular signaling molecules modulating physiological responses.

Another fundamental cellular component are polysaccharides or glycans. Complex glycan chains can be found on proteins and lipids to which they are added by glycosylation and where they contribute to their correct folding, proper enzymatic activity and molecular interactions [5]. There exist up to 16 protein and lipid glycosylation pathways which are defined by distinct linkages and initiating enzymes. This diversity greatly expands the glycoproteome and glycolipidome and extends their functions and their interactions [6]. Studies related to

glyco-enzyme deficiencies in diseases increased our understanding of the roles of glycosylation in physiology, yet full and reliable characterization and readouts of glycans on macromolecules remain challenging.

Cellular glycan profiles are heterogeneous and vary depending on the cell's state. They are stage specific during development and can be over- or under-represented on the cell's surface in response to molecular cues or a stressor. In addition, glycans are tissue specific whereby each cell type generally has distinct glyco-profiles that can vary depending on different stages or in disease. Therefore, glycans are considered as biological switches which alter molecular functions and turn on or off multiple signaling pathways [6] making them excellent biological readouts and assessment tools for scientists and clinical researchers.

Recently, the connection between redox signaling and functional changes of glycans was described with the term “Glyco-redox” [7]. In this review, we extend this view and centralize recent glyco-redox studies in an attempt to close the gap between two cardinal fields, glycobiology and redox biology which allows us to evaluate their inter-related functions and dysfunctions and to reflect on novel therapeutic approaches for the treatment of related diseases.

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2. ROS as signaling molecules in cellular homeostasis

In the cell, endogenous ROS sources reside within different cellular compartments. ROS levels vary with the majority of ROS produced by the mitochondrial electron transport chain (ETC: complex I, II and III) [8], localized NADPH oxidases (NOX1 to 5) [9–12], dual oxidases (DUOX1 and 2), superoxide dismutases (SOD1, 2 and 3) [11], and oxidation of polyunsaturated fatty acids [13]. Cellular ROS levels are tightly balanced by a system consisting of antioxidants such as glutaredoxin, peroxiredoxin, thioredoxin, and glutathione as well as antioxidant enzymes such as glutathione reductase (GR), and glutathione peroxidases (GPX) complemented by exogenous low molecular weight substances such as Vitamin E, or Vitamin C which altogether ensure that ROS levels are maintained within a physiological range necessary for intracellular signaling [14]. In addition to ROS generators and antioxidants, membrane proteins from the aquaporin (AQP) family contribute to ROS regulation by controlling their transport, thus providing ROS gradients in the cell [15,16]. A disbalance between prooxidants and antioxidants in favor of the oxidants is commonly referred to as oxidative stress (for reviews see Refs. [1,17,18]).

ROS signaling or redox signaling is highly dynamic, tissue specific, reversible and affects multiple levels. Thereby, ROS control a large spectrum of cellular functions [19–23] including regulation of enzyme activity, protein folding, transcription factor targeting, as well as coding and non-coding RNA regulation by targeting cysteines, tyrosines, and iron-sulfur (Fe-S) clusters. For instance, redox-sensitive targets may include among others transcription factors such as NF- κ B, hypoxia-inducible factors (HIFs), and nuclear factor E2-related factor 2 (NRF2). Thereby, cytosolic H₂O₂ could increase NF- κ B signaling by oxidizing and destabilizing its inhibitor I κ B [24,25], while nuclear H₂O₂ inhibits it by oxidizing discrete cysteines in the DNA-binding region of NF- κ B [26]. Such differential effect is crucial for the regulation of the inflammatory response and is an example for the specificity and the spatiotemporal activity of ROS. Under hypoxia, ROS can stabilize HIFs in certain cell types by inhibiting HIF prolyl-hydroxylases, which promotes changes in cellular programs such as replication, survival and stress adaptation [27,28]. Other redox-sensitive targets include energy sensing and metabolic regulators such as AMP-activated protein kinase (AMPK) [29,30] and forkhead box proteins (FOXO) [31,32], the glycolytic glyceraldehyde 3-phosphate dehydrogenase (GAPDH) [33] or the cell cycle regulator p53 [34], which are elucidated in more detail in previous excellent reviews [4,14]. While redox signaling regulates important cellular functions, dysregulated ROS production and subsequent oxidative stress lead to imbalances in various cellular processes that can result in diseases including neurodevelopmental disorders [35], circadian rhythm disruption [36], neuro-degeneration such as Parkinson's and Alzheimer's disease [37], cardiovascular dysfunction [38–40], impaired immune response and autoimmune diseases [41–43], metabolic diseases such as diabetes [44,45] as well as premature ageing and/or cell death.

3. Glycans and cellular homeostasis

The importance of glycans for cellular homeostasis is highlighted by the existence of congenital disorders of glycosylation (CDG) where mutations or absence of genes encoding enzymes involved in the glycan biosynthesis process have serious implications on development and health [46,47]. The proper biological roles of glycans are numerous and there are far too many examples to be covered in this review. To state a few, other than their role in protein folding and protein-protein interactions [48,49], they are essential for multicellular development [50], being involved in the acrosomal reaction during fertilization [51] as well as in the inflammatory response of cells by modulating leukocyte recruitment [52]. Glycans serve as binding sites for commensal and pathogenic bacteria as well as viruses [53,54]. Altered glycans are also hallmarks of cancer and in many cancers they are used to evade immune

recognition [55,56]. For instance, abnormalities and truncations of glycans are observed in the O-linked glycome accompanied by an increased synthesis of Tn, T and sialyl-T antigens. On the whole, hypersialylation (N- and O-) is associated with a poor cancer prognosis and is observed in aggressive and highly metastatic cancers [55–60].

Glycan biosynthesis requires first cellular uptake of simple sugars whereby they are activated by kinases and processed by transferases to become nucleotide sugars (e.g., UDP-Galactose, GDP-Fucose, or CMP-Sialic acid). These sugars are then imported into the endoplasmic reticulum (ER) and the Golgi via nucleotide sugar transporters (NST) (Fig. 1). Within the ER and Golgi they function primarily as donors in sequential glycosyltransferase reactions which form an elongated and branched glycan polymer (Fig. 1). Often, glycosidases trim the glycan chains by removing monosaccharides, hence forming intermediates that can be reused by glycosyltransferases. Around 17 monosaccharides are found in mammals and combining them together generates a large variety of glycan structures where stereochemical α or β conjugations add even more to structures' complexity. A total of 10¹² combinations of glycan structures is thought to exist. The complete repertoire of these sugars is called the glycome and is considered to be a third language of life beyond nucleic acids and proteins. Subsequently, the glycans produced can be used for the biosynthesis of glycolipids, glycoproteins, and proteoglycans.

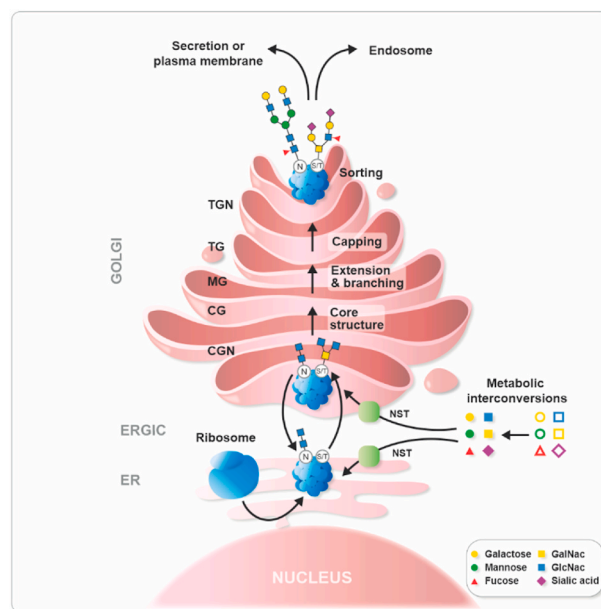


Fig. 1. Glycoprotein formation. (A) First, cytosolic enzymatic reactions convert monosaccharides to “active sugars”, which are then translocated into the ER and Golgi via nucleotide sugar transporters (NST). The most frequently occurring glycans are N-glycans and O-glycans. In N-linked glycans the glycosylation process starts in the lumen of the ER where an oligosaccharyltransferase attaches a monosaccharide precursor to an amide nitrogen of an asparagine or arginine residue residing in a specific consensus sequence of the nascent protein. Further trimming of the glycan structures and control of misfolded glycoproteins in the ER is performed before the protein exits the ER via the Golgi intermediate compartment (ERGIC). The O-linked glycosylation is a form of glycosylation that occurs in the ER or the Golgi where different sugars can be added to hydroxyl groups present in the amino acids serine, threonine, tyrosine, hydroxylysine, or hydroxyproline; alternatively oxygen present in lipids such as ceramide can be used. In the Golgi lumen, activated sugars are used as donors in sequential reactions with glycosyltransferases (GTs) which together with glycosidases (Gly) extend, and branch the glycan by involving the cis Golgi (CG), and medial Golgi (MG) before they are capped with terminal sugars such as sialic acid. Finally, the glycoprotein is sorted into a vesicle through the trans Golgi (TG) and the trans Golgi network (TGN) and transported to its final destination.

Among glycolipids are glycosphingolipids. These subtypes of glycolipids are ubiquitous, required for embryonic development and depending on the attached sugar chains exert different functions at different stages of development [50,61]. For instance, globosides are predominant in the early embryonic stages but gangliosides, a family of sialic acid-containing glycosphingolipids, are expressed during neural development and participate in neural signaling processes [62].

While glycoproteins such as collagens, mucins, transferrin, and immunoglobulins consist of oligosaccharide chains that are covalently attached to proteins, proteoglycans as chondroitin sulfate, dermatan sulfate, heparan sulfate, or keratan sulfate are made of a core protein to which one or more linear polysaccharide chains made of disaccharide building blocks [63] known as glycosaminoglycans (GAGs) are covalently attached. In both, the glycosylation, i.e. the covalent attachment to the respective protein occurs mainly via two types of glycosylation: N-linked glycosylation and O-linked glycosylation where oligosaccharides are commonly attached to a nitrogen atom of an asparagine or to an oxygen atom of serine or threonine, respectively. In addition, other hydroxyl group containing amino acids such as tyrosine, hydroxylysine, or hydroxyproline as well as oxygen present in lipids such as ceramide can be used for O-linked glycosylation. There are major differences between N- and O-linked glycosylations such as the implicated enzymes, the consensus sites, synthesis localization and glycan chain type and complexity which are elucidated in more detail elsewhere [5].

4. Glycosylation and glycan profiles under oxidative stress

As glycan synthesis occurs in the ER and Golgi it is reasonable to assume that redox changes in these compartments affect glycosylation reactions. By using a genetically encoded redox sensitive probe it was recently shown that the redox status of mitochondria, ER and Golgi is different and that the lumen of the Golgi has even a more oxidizing environment than the ER [1]. This is in agreement with the use of high oxidizing power for tail-to-tail disulfide bond formation during the assembly of secretory proteins from oligomeric or multimeric precursors in the Golgi [64,65]. This process is likely supported by members of the Quiescin-Sulfhydryl Oxidase (QSOX) family. These proteins represent an ancient fusion between PDI-like thioredoxin (TRX) domains and ERV-like oxidase domains. As a result, they can efficiently couple disulfide bond generation with reduction of O_2 to H_2O_2 . In addition, ROS generated during these processes may be used by the glutaredoxins, GRX6 and GRX7 which in baker's yeast, are located in the cis-Golgi thereby reducing disulfide bonds by the use of reduced glutathione. GRX6 and GRX7 represent closely related monothiol glutaredoxins that are found in this compartment [66].

Apart from disulfide bond formation ROS in ER and Golgi appear to affect the identity of glycans displayed on proteins and lipids via direct and indirect mechanisms.

It is intriguing that ROS or hypoxia-mediated glycosylation changes have not yet been detected in the initial synthesis of N-glycans in the ER. However, the recent finding that the redox-sensitive selenoprotein T was shown to function as a new oligosaccharyltransferase and to be indispensable for ER homeostasis as well as for maturation and secretion of glyco-hormones from endocrine glands [67] suggests that hypoxia and/or ROS could affect this process. However, further studies are needed to clarify whether and how hypoxia and/or ROS may affect ER-associated glycosylation events.

Overall, it appears that changes in ROS levels within the cell in general, but also in both, the ER and Golgi, can affect the identity of glycans displayed on proteins and lipids either by the direct interaction of ROS with carbohydrate chains or indirectly by altering protein-protein interactions, molecular signaling and genetic feedback (Fig. 2).

5. Direct

Glycosaminoglycans (GAGs) are an integral component of the

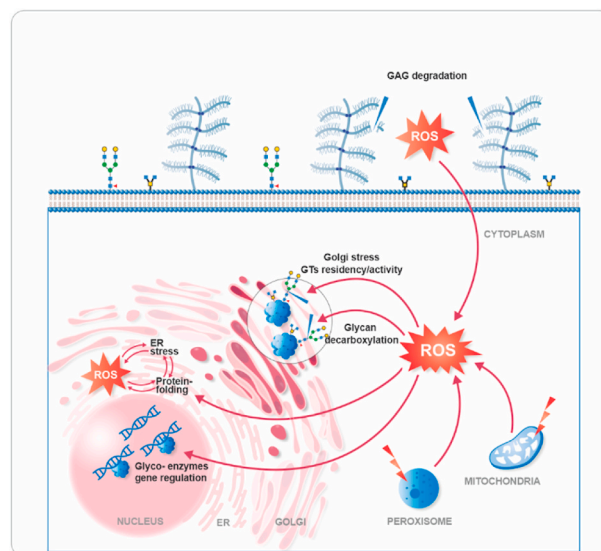


Fig. 2. The glyco-redox interplay. ROS, from exogenous sources or formed in different cellular compartments either as signal molecules or as more harmful byproducts of metabolic activities, can affect glycosylation in different ways. This can involve direct action on glycosylated proteins, e.g., on glycosidic bonds, or in a more indirect manner via the regulation of glycosylation enzyme activity, or their gene regulation.

extracellular matrix and are present throughout the entire human body with highest concentrations in skin, cartilage and bones [5,68]. As parts of proteoglycans, GAGs are involved in a wide array of biological processes and defects in GAG synthesis contribute to inflammatory diseases, connective tissue disorders and abnormal brain development [69]. GAGs can be modified or degraded by a number of ROS (Fig. 2). For instance, low reactivity ROS such as $O_2^{\cdot -}$ and H_2O_2 were shown to degrade GAGs and proteoglycan aggregates in a direct manner as well as in an indirect fashion via activation of latent collagenases and inhibition of de novo proteoglycan synthesis [70–72]. ROS that are more reactive such as HOCl and -OH depolymerized GAGs by acting directly on thiols and amino groups or, for example, on the glycosidic linkage between the glucuronic acid and the N-acetyl-glucosamine in hyaluronic acid, respectively [73,74] (reviewed in Ref. [69]). ROS and hypoxia-mimicking agents were found to directly affect cell surface localized proteoglycans (i.e. glycosaminoglycans) and N-linked oligosaccharides (i.e. N-glycans), thereby they were able to induce cleavage of the proteo-, and N-glycans in an N-acetyl-amino-sugar-specific manner by employing transition metals such as iron [75]. As a consequence, cell-cell or cell-extracellular matrix interactions were affected in a manner that coincided with inflammatory responses, atherosclerosis, and cancers [7].

6. Indirect

While the relationship between ROS and N-linked glycosylation is yet to be defined, ROS effects on O-linked glycans and GAGs as well as O-GlcNAc signaling are established [76]. Several studies have shown enhanced protein O-GlcNAcylation in response to oxidative distress [77–82]. Possible reasons behind this phenomenon would be increased O-GlcNAc transferase expression and decrease in O-GlcNAcase expression and/or activity (Fig. 3). Indeed, the O-GalNAc synthesis seems to be increased in response to oxidative stress in breast cancer due to high expression of GalNAc-transferase 6 (GalNT6) and translocation of both GalNT2 and GalNT6 from cis-Golgi towards the trans-Golgi cisternae [83]. In addition, the GDP-fucose transporter was found to be regulated by TGF- β 1 and the transcription factor SP-1, which themselves are under redox control [84].

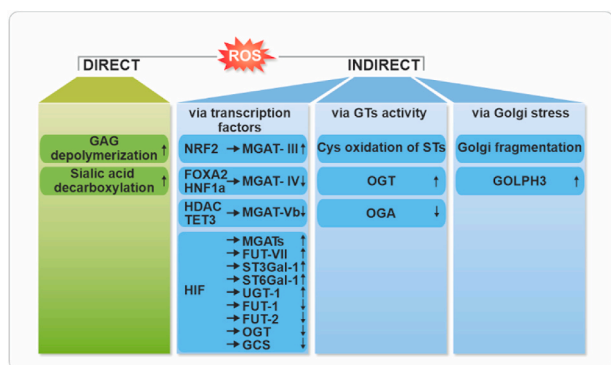


Fig. 3. Summary of the direct and indirect effects of the redox system on glycan biosynthesis. Abbreviation: FUT: Fucosyltransferase, HIF: Hypoxia-inducible Factor, HDAC: Histone deacetylase, HNF: Hepatocyte Nuclear Factors, GAG: Glycosaminoglycans, GCS: Glucosylceramide Synthase, GOLPH3: Golgi Phosphoprotein 3, MGAT: N-acetylglucosaminyltransferase, NRF2: Nuclear Factor Erythroid 2-Related Factor 2, OGT: O-GlcNAc Transferase, OGA: O-GlcNAcase, ST: Sialyltransferase, ST3GAL-1: Alpha-2,3-Sialyltransferase, ST6GAL-1: Alpha-2,6-Sialyltransferase, TET3: Methylcytosine Dioxygenase, UGT: Glucuronosyltransferase.

Whether the synthesis of other glycans containing O-mannose is influenced by ROS or oxidative stress is currently unknown as no reports are available; hence, the connections are yet to be established. However, the redox-stress mediated increase in bisecting GlcNAcs by upregulation of N-acetylglucosaminyltransferase III (MGAT-III) via Nrf2 [7] and reduced expression of N-acetylglucosaminyltransferase IV (MGAT-IV) [85] via FOXA2 and HNF1a, would reduce complex type glycans and sialic acid capping and favor high mannose type glycans which are found to be highly prevalent in cancers [86,87] (Fig. 3). Since hypoxia, which is prevalent in most solid cancers, can alter the redox state of a cell, it is ultimately linked to changes in glycosylation. However, the mechanisms involved appear to be multiple and the details therein are still not fully understood. A recent study employing redox screens was able to show that hypoxia inhibits terminal sialylation of N- and O-linked glycans. This redox state change caused loss of two surface-exposed disulfide bonds in the catalytic domain of the α -2,6-sialyltransferase (ST6Gal-I). Mutagenesis of the selected cysteine residues in ST6Gal-I also rendered the enzyme inactive. Consequently, ST6Gal-I loss of function was accompanied by the inability to form a heteromer with B4GalT-I, an enzyme adding the preceding galactose to complex N-glycans and an advantage in proliferation and migration. Interestingly, structure comparisons revealed that similar disulfide bonds reside also in other sialyltransferases such as ST3Gal-I, suggesting that redox switches at discrete disulfide bonds of sialyltransferases contribute to catalytic activation and cooperative action of those enzymes [88].

As mentioned, the redox response is linked to the hypoxia response in which hypoxia-inducible factors (HIF-1, -2 and -3) are major regulators. HIFs appear to regulate hundreds of genes required to sustain a plethora of processes necessary for cellular homeostasis [89]. Surprisingly, the number of HIF-regulated genes among the about 250 genes needed to make all glycan structures in mammalian cells is so far rather small. Among those are genes encoding enzymes that make nucleotide sugars in the cytoplasm [90], as well as in the medial-Golgi such as MGAT-II, MGAT-III, MGAT-V, MGAT-Vb, and the trans-Golgi localized ST6Gal-I [7]. Oxidative stress was shown to increase the transcription of fucosyltransferase VII (FUT7), sialyltransferase ST3Gal-I and transporters for UDP-Galactose, sialic acid (Sialin) and glucuronosyltransferase (UGT1) [91]. Further, HIF-1 α suppressed the α 1,2-fucosyltransferase genes (FUT1 and FUT2) in the pancreatic cancer cell lines Pa-Tu-8988S and Pa-Tu-8988T [92]. Likewise, the UDP-glucuronosyltransferases [93], the O-GlcNAc transferase (OGT) [94], and glucosylceramide synthase

(GCS) [95] were downregulated by HIF-1 α (Fig. 3). Thus, ROS, hypoxia and HIFs can modulate many glycosylation pathways via altering the availability of nucleotide sugars, the level of their transporters and the repertoire of transferases expressed by the cells.

7. Glyco-redox feedback

Vice versa, some factors that regulate the cellular response to ROS or are themselves redox sensitive, such as hypoxia/HIF [96,97], KEAP/NRF2 [98–102], FOXO [103–105], NF- κ B [106] and p53 [107, 108], can modulate or be modulated by the levels of O-GlcNAc glycosyltransferase (OGT) and O-GlcNAcase and their crosstalk with related molecules [109,110]. Furthermore, O-GlcNAcylation on discrete residues of RelA/p65 and IKK β was found to increase the transactivation of NF- κ B RelA/p65 [111,112]. Similar modifications were found in other transcription factors and co-factors such as N-glycosylation of nuclear factor 1 C-type (NFIC) [113], O-GlcNAc and N-glycosylation of cAMP response element-binding protein (CREB) [114,115], O-GlcNAcylation of c-JUN [116], O-GlcNAcylation Ets transcription factor (ELF1) [117], O-glycosylation NOTCH [118], and O-GlcNAcylation of Yes-associated protein (YAP) [119,120]. Indicating that they play important regulatory roles in cellular signaling, metabolism and tumorigenesis.

8. Glyco-redox in cardiovascular diseases

Oxidative stress occurs during heart and vascular dysfunction caused by various pathologies including hypertension, heart insults, atherosclerosis, inflammation, and obesity, as well as during senescence. These processes are also characterized by heart and vascular remodeling, fibrosis, calcification and altered expression of matrix metalloproteinases (MMPs) affecting both contractility and endothelial functions.

It is well established that NADPH oxidases (NOX) and ROS production have a principal role in cardiovascular diseases (reviewed in Ref. [121]). However, the glyco-redox interplay here is still in its infancy. Yet, some studies have demonstrated how glycans can influence ROS levels and induce cardiovascular damage. This was especially shown in the case of O-linked glycans which are present on signaling proteins involved in vascular functions, such as glutathione peroxidase 1 (GPX-1) under hyperglycemic conditions in rat vascular smooth muscle cells [122]. Thereby, it was shown that increased levels of O-GlcNAc led to endothelial dysfunction by increasing O $_2^{\bullet}$ levels. Although the increased O $_2^{\bullet}$ levels correlated with the expression of the NADPH oxidases NOX1 and NOX4 when aortic segments and vascular smooth muscle cells were treated with 1,5-hydroxylimolactone (PUGNAc), an inhibitor of N-acetylhexosaminidases which artificially increases O-GlcNAc levels [123], mechanistic details about NOXs' involvement are still missing. In contrast, another study shows that increased O-GlcNAc levels, due to PUGNAc treatment or OGT transfection of neonatal rat cardiac myocytes, confer a protective effect by inhibiting Ca $^{2+}$ overload and ROS production. As a consequence, cells were protected from mitochondrial permeability transition pore (mPTP) opening and loss of mitochondrial membrane potential [124]. Another recent study showed that experimentally induced oxidative stress achieved by application of H $_2$ O $_2$ and buthionine sulfoximine (BSO) involves matrix metalloproteinases (MMPs) and histone deacetylases (HDACs); both are known to be associated with cardiovascular diseases via the modification of endothelial cell surface glycan structures such as syndecans. Syndecans are heparan sulfate proteoglycans which bind to a variety of ligands and are involved in tissue repair. Although the effect of ROS is not direct and details remain unknown, it appeared that MMP and HDAC inhibitors attenuated H $_2$ O $_2$ and BSO stimulated shedding and degradation of syndecans and that of the extracellular superoxide dimutase SOD3 from the endothelial glycocalyx [125]. Similar observations pointing to a correlation between oxidative stress and glycocalyx shedding through MMPs have been reported also in other studies

[126–130] suggesting a common, yet unknown, mechanism. Furthermore, oxidative stress has been shown not only to affect glycans but also glycan binding lectins. Lectins are a family of carbohydrate-binding proteins; they recognize glycans on surfaces of cells and pathogenic glycoconjugates, which make them indispensable for cell-cell recognition and immunity. The lectin pathway is part of the immune complement system and is activated by mannose-binding lectins (MBL). Oxidative stress induced by reoxygenation of hypoxic HUVEC cells induced high levels of vascular endothelial MBL and cytokeratin 1 (CK1). Binding of MBL to the kinase CK1 triggered the lectin complement pathway (LCP) and C3 deposition which normally contributes to cardiovascular dysfunction and disease [131].

9. Glyco-redox in inflammation and immunity

ROS-mediated glycan changes closely associate with many inflammatory diseases and immune disorders. A vicious cycle appears to exist in the onset of type-2 diabetes, a disease that is due to its association with a high fat diet characterized by hyperglycemia and insulin resistance that lead to oxidative stress and inflammation. Chronic elevation of free fatty acids and increased production of ROS in mitochondria also attenuate insulin secretion in pancreatic β -cells, and thereby trigger the onset of type-2 diabetes [85]. Thereby, the excess of both glucose and free fatty acids together with high ROS levels, in particular in pancreatic beta-cells, were shown to inhibit the transcription factors FOXA2 and HNF1a. Both would normally induce expression of MGAT-IVa, a glycosyltransferase needed for the synthesis of a glucose transporter 2 (GLUT-2)-N-glycan complex that provides GLUT-2 plasma membrane retention by enabling lectin-glycan binding. Although the lectin-glycan binding is part of the immune complement system, impaired MGAT-IVa expression is not only affecting the complement pathway but also impairs GLUT-2 endocytosis that will lead to subsequent diminished glucose transport into pancreatic β -cells and reduced insulin secretion, thus linking type-2 diabetes and inflammation.

Members of the redox machinery such as NOX1, which has been associated with the innate immune system, and immune diseases appear to display also an aberrant glycosylation in the presence of ROS. For instance, hypoglycosylation of neutrophilic NOX1, *i.e.*, gp91^{phox}, has been detected in patients suffering from neutrophil dysfunction and congenital neutropenia due to mutations in the glucose-6-phosphatase 3 gene, also known as glucose-6-phosphatase beta [132]. In that study, mass spectrometry analyses of the neutrophils' N- and O-glycomes showed truncated and immature complex-type N-glycans, reduced core 2 antennae O-glycans and deficiency in sialylated and lewis X epitopes. Although the exact mechanism for the hypoglycosylation remains unknown, it is thought that glycosylation may protect NOX1 from the content of phagocytic vacuoles, including ROS, or that glycosylation of NOX1 is necessary for heterodimer formation with p22^{phox} [132,133]. Moreover, the cytochrome *b*₅₅₈ complex, consisting of NOX1 and p22^{phox} have been shown to be heavily glycosylated in Epstein Barr virus immortalized B-cells which prevents *b*₅₅₈ to be fully active through a conformational constraint [134]. At the same time, glycosylation of B-cell-produced immunoglobulins such as IgG and IgA is fundamental for their function and is necessary for Fc receptors binding [135]. Immunoglobulins' sialylation is associated with reduced inflammation [136,137] while autoimmune disease patients usually lack galactose and sialic acid and exhibit truncated glycans on their IgGs and IgAs [138, 139].

Another integral and potent component of macrophages is mannose binding lectin (MBL), which usually binds to mannoses on bacteria and viruses. It exhibits reduced levels under high oxidative stress on macrophages in the lungs due to chronic obstructive pulmonary disease (COPD) [140]. Structural analyses showed that oxidized MBL cannot form higher order oligomeric structures and disrupts macrophage activity. Although MBL is also known to be glycosylated [141], no connection to MBL oligomerization has been shown in this study. These

examples show that hypo or hyperglycosylation could alter the lifetime and activity of crucial molecules involved in the immune response.

10. Glyco-redox in cancer and other redox-associated diseases

It is well established that enhanced ROS levels and oxidative stress are associated with tumorigenesis. High levels of ROS in cancer are a consequence of a changed metabolic activity, altered mitochondrial homeostasis and differential gene expressions. DNA damage, protein and lipid oxidation all increase the metastatic and proliferative cellular profiles which in turn can lead to further generation of ROS and activation of oncogenic pathways [1,142]. Glycosylation changes in cancer have been well documented especially changes in the tumor glycocalyx and their effects on adhesiveness, metastasis, immune evasion, growth factors and signaling pathways [6,143](Fig. 2). However, the glyco-redox link in cancer is not clearly defined. Hypoxia is considered a hallmark of cancer and HIF-1 α promotes malignancy by triggering gene expressions involved in angiogenesis, glycolysis and proliferation [144]. High levels of α -2-6 sialylation, ST6Gal-I levels and ROS in cancers have been associated with poor prognosis and both were shown to increase HIF-1 α levels [145,146]. Thus, there exist a cancer-associated interplay between redox, HIF-1 α and ST6Gal-I, whereby a tumor in a hypoxic microenvironment protects itself by upregulating HIF-1 α and α -2-6 sialylation which in turn increase cell migration, reduce cell-cell adhesion and promote cancer cell survival and invasiveness [147]. Another possible link between redox stress and glycosylation in cancers can be found through Golgi stress (onco-Golgi) [148]. Golgi stress is manifested by Golgi fragmentation which has been shown to cause glycosyltransferases mislocalization and relocation, loss of oligomerization and activity and subsequently aberrant glycosylation (reviewed in Ref. [149]). For instance N-acetylcysteine (NAC) inhibiting compounds such as Geoditin, SOD1 mutations and microtubule oxidative damage, all contribute to Golgi damage and fragmentation (reviewed in Ref. [150]). In addition, inhibition of NOX1 and NOX4 by NOXi inhibitor combined with Golgi stressing compounds such as Brefeldin A (BFA) partially rescued Golgi fragmentation in HeLa or MDA-MB-231 cells [151]. Even though Golgi fragmentation in cancers have been attributed to altered Golgi pH [149], it is thus safe to propose that accumulation of ROS in cancers can distort the Golgi structure and integrity which usually results in glycosylation defects. Golgi fragmentation is not only seen in cancers but also in other diseases namely neurodegenerative diseases that are believed to be triggered by oxidative stress such as the Alzheimer's disease [152] in which decrease in sialylation and increase in high mannose glycan chains are observed [153–155]. Moreover, it has been shown recently that Golgi phosphoprotein-3 (GOLPH3), an enzyme implicated in the secretory pathway, is responsible for recycling of specific glycosyltransferases and retention is highly overexpressed in human cancer samples [156]. Interestingly, GOLPH3 was also linked to ROS as it was reported to be upregulated in neural cells upon oxygen-glucose deprivation and reoxygenation [157]. This indicates that GOLPH3 could act as a Golgi oxidative stress sensor. Consequently, its elevated expression in cancer could then increase oncogenic-related glycosyltransferases such as globoside synthase-3 (Gb3) [156]. The latter is known to contribute to uncontrolled cellular growth by modulating receptor tyrosine kinases and integrin receptors [158]. These findings are linked to changes in core fucosylation of growth factors such as TGF- β 1 and increased MMP levels that are implicated in several cancers, such as hepatocellular, gastric, pancreatic, prostate, and colorectal cancers [159,160]. Thereby, the addition of fucose in α -1,6-linkage to the innermost N-acetyl glucosamine of N-glycans carried out by FUT8 seems to control TGF- β 1 signaling and MMP function. Moreover, changes in core fucosylation would not only affect growth of cancer cells, but also normal development as knockout of FUT8 in mice leads to impaired lung development and to an emphysema-like phenotype [159, 160].

To name a few more examples of the glyco-redox interrelation; there

is accumulating evidence that glycans such as sialic acids and O-GlcNAcs are responsible for maintaining kidney functions and protection against ROS induced damage and which have been illustrated in multiple studies [161–170]. For instance, N-acetylneuraminic acid has been shown to consume H₂O₂ and hydroxyl radicals in cells and consequently become oxidized into a decarboxylated product, 4-(acetylamino)-2,4-dideoxy-D-glycero-D-galacto-octonic acid (ADOA) [167,168]. Aberrant glycans also correlate with higher oxidative stress in diabetic and hypertensive patients and patients suffering from myopathies and neurodegenerative diseases [171,172].

11. Conclusion

Given their pan distribution on proteins and lipids as well as their diverse roles in development and basic cellular functions, glycans are involved in almost all essential pathways. ROS not only can trigger subtle genetic reprogramming and switch on or off signaling pathways affecting glycan synthesis but can also directly interact and degrade saccharide moieties. However, their interrelationship comes at a cost, that is, aberrant glycan synthesis gives rise to many congenital and complex disorders. *Vice versa*, glycans affect the redox-proteome by altering redox-proteins folding, interactions and activities. Although the relationship between glycans, ROS and oxidative stress is dynamic and extensive, it lacks still knowledge about detailed mechanisms involved.

12. Perspectives

The glyco-redox field is still in its infancy and requires a novel outlook to understand regulatory mechanisms and the effects ROS can cause through glycans or vice versa. From an omics perspective and with the robust development of mass spectrometry techniques, a glyco-redox MS concept could be developed to map the glycome, glycoproteome and the redoxome together in diseases [173,174]. Consequently, it would be necessary to investigate molecular mechanisms especially with the evolution of fluorescent, electron microscopes and single cell techniques [175,176]. In short, bridging the gap between glycobiology and redox biology will most likely reveal unexplored biological mechanisms that will clarify the relationship between the stressors such as free radicals and glycosylation-based diseases and promote the search for novel medical interventions.

Declaration of competing interest

The authors declare that there is no conflict of interest.

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