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Aberrant protein glycosylation: Implications on diagnosis and Immunotherapy

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Highlights

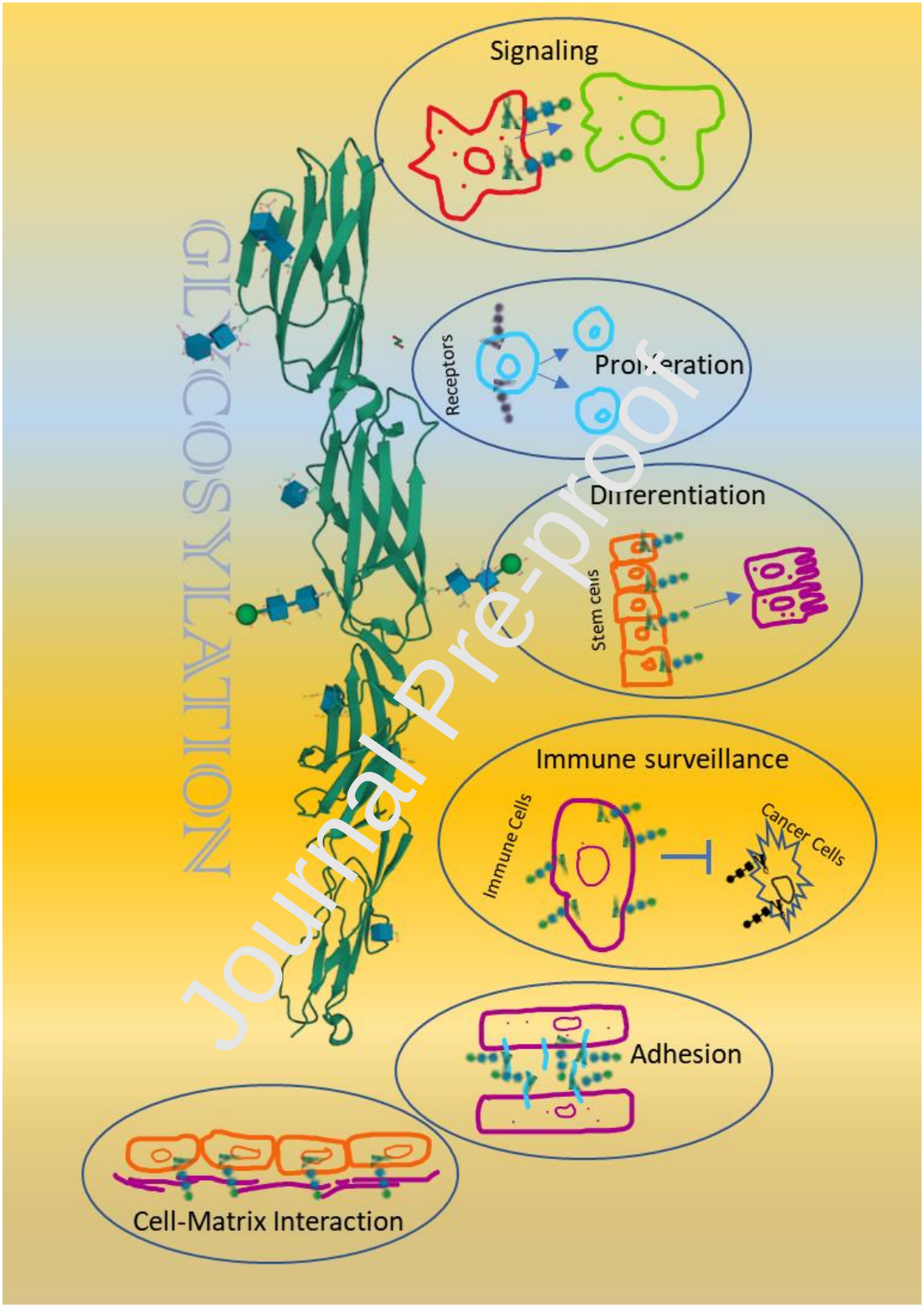
- *O*- and *N*-linked glycosylation orchestrate proteoforms and their functions
- Incomplete/neosynthesis of glycan protein lead to immune dysregulation and cancer
- Signaling via PDGF, FGFR, EGFR, MET, RON, or IGFR receptors needs glycosylation
- Multi-omics of tumors reveal heterogeneity in the glycosylation pattern
- Tumor-specific glycans interact with CD43, CD45, selectins, galectins and siglecs

Abstract

Glycosylation-mediated post-translational modification is critical for regulating many fundamental processes like cell division, differentiation, immune response, and cell-to-cell interaction. Alterations in the *N*-linked or *O*-linked glycosylation pattern of regulatory proteins like transcription factors or cellular receptors lead to many diseases, including cancer. These alterations give rise to micro- and macro-heterogeneity in tumor cells. Here, we review the role of *O*- and *N*-linked glycosylation and its regulatory function in autoimmunity and aberrant glycosylation in cancer. The change in cellular glycome could result from a change in the expression of glycosidases or glycosyltransferases like N-acetyl-glucosaminyl transferase V, FUT8, ST6Gal-I, DPAGT1, etc., impact the glycosylation of target proteins leading to transformation. Moreover, the mutations in glycogenes affect glycosylation patterns on immune cells leading to other related manifestations like pro- or anti-inflammatory effects. In recent years, understanding the glycome to cancer indicates that it can be utilized for both diagnosis/prognosis as well as immunotherapy. Studies involving mass spectrometry of proteome, site- and structure-specific glycoproteomics, or transcriptomics/genomics of patient samples and cancer models revealed the importance of glycosylation homeostasis in cancer biology. The development of emerging technologies, such as the lectin microarray, has facilitated research on the structure and function of glycans and glycosylation. Newly developed devices allow for high-throughput, high-speed, and precise research on aberrant glycosylation. This paper also discusses emerging technologies and clinical applications of glycosylation.

Keywords: Metastasis; Glycosylation; Cancer; Multi-omics; glycans; immunotherapy

Graphical Abstract



1. Introduction:

The process of translation is not enough to form functional proteins. Many proteins require post-translational modifications, including proper protein folding or the addition of functional groups. Several of these modifications, with prominent phosphorylation, participate in signal transduction and gene regulation (Lv et al., 2022). Glycosylation is a complicated process of protein or lipid modifications involving specific enzymes (glycosidases or glycosyltransferases), which helps in adding glycan moieties to provide particular functions to glycoproteins like cell-to-cell interaction, cellular differentiation, agglutination reaction in blood, etc. Most of these modifications are densely located in the outer region of the cell surface, the glycocalyx (Alymova et al., 2022). The two main categories of protein glycosylation are *O*-linked and *N*-linked glycosylation (abbreviated herein as *OLG* and *NLG*, respectively), which are structurally and functionally different from each other (Hulsmeier et al., 2011). The N- and O-glycans are typically released from glycoproteins by either enzymes or chemical methods.

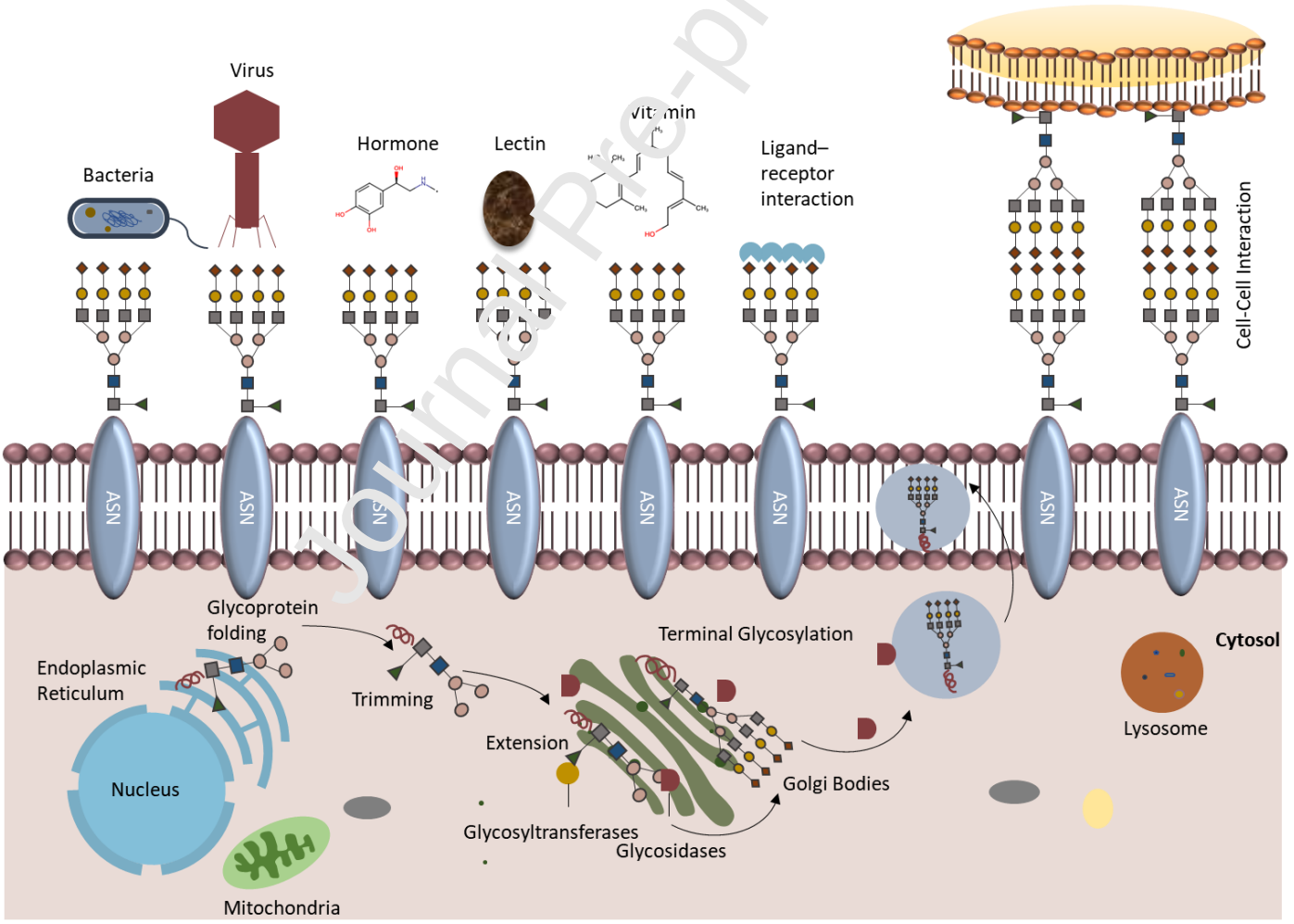


Figure 1: Illustration of N-glycosylation expression and function. The process initiates in ER, and after elongation, it matures in the Golgi body. Next, sugar nucleotides

from the nucleus go to ER. The synthesized glycoproteins with specific functions like cell-cell adhesion and microbe/ligand-receptor interaction are then transported to the cell surface.

The genes associated with the expression of these glycans are known as glycogenes (Angata et al., 2020). The expression profile of these genes correlates with the glycomic alterations occurring within a cell (Li et al., 2017; Lumibao et al., 2022; Shah et al., 2015; Zhou et al., 2017; Zhu et al., 2019). Various enzymatic techniques were reported for analyzing different glycans; for instance, N-glycan analysis is done by releasing glycan with PNGase F (peptide-N-glycosidase F), followed by mass spectrometric analysis. Similarly, the Pronase enzyme releases the O- glycan or mass spectrometry (Wang et al., 2022).

In eukaryotes, the *NLG* process (addition of glycan to free nitrogen of asparagine residue) follows three stages. First is the synthesis of long chains of oligosaccharide subunits on lipid molecules, known as lipid-linked oligosaccharides. In the second stage, it is transported on the targe UDP18 GalNAc polypeptides linked to the protein and, finally, the maturation (Stanley, 2007). The process starts on the Endoplasmic Reticulum (ER) surface while the protein N-glycan chains' elongation and maturation occur in Golgi-body. For Asn-linked glycosylation (ALG), the oligosaccharide moiety is synthesized by a group of enzymes working in a cascade. N-glycosylation starts with adding two N-acetylglucosamine (GlcNAc) molecules to the dolichol pyrophosphate residue (PP-Dol) embedded into the cytosolic side of the ER, as illustrated in figure 1. In the first reaction, the GlcNAc molecule, which is attached to Uridine diphosphate (UDP), is transferred to PP-Dol by the enzyme DPAGT1 (in mammals) or GlcNAc-1-phosphotransferase ALG7 (Bretthauer, 2009). After this, a complex of ALG13/ALG14 adds another GlcNAc yielding GlcNAc2-PP-Dol (Gao et al., 2005; Bickel et al., 2005), in which two molecules of GlcNAc are linked together by β (1,4) corresponding to its chitobiose core unit. Then, many enzymes add five mannose (Man) molecules to the chitobiose structure to form an intermediate complex, Man5-GlcNAc2-PP-Dol. Finally, this structure is transferred to ER surface by a flip-flop mechanism using flippases enzymes (Frank et al., 2008).

Further synthesis occurs in the luminal compartment of ER with the ligation of four residues of mannose leading to the formation of Man9-GlcNAc2-PP-Dol, in which three glucosyltransferases come into action viz ALG3, ALG12, and ALG9. These enzymes transfer three glucose molecules (Glc), yielding Glc3Man9GlcNAc2-PP-Dol (Bloch et al., 2020; Burda and Aebi, 1998; Farid et al., 2011). This oligosaccharide moiety is transferred to the Asn residue of a specific protein with a conserved Asn-X-Ser/Thr/Cys motif (Matsui et al., 2011; Schwarz and Aebi, 2011). Interestingly, this process of transferring could occur co-translationally or post-translationally in eukaryotes. Mature N-glycoproteins are expressed on the cell surface, as shown in figure 2.

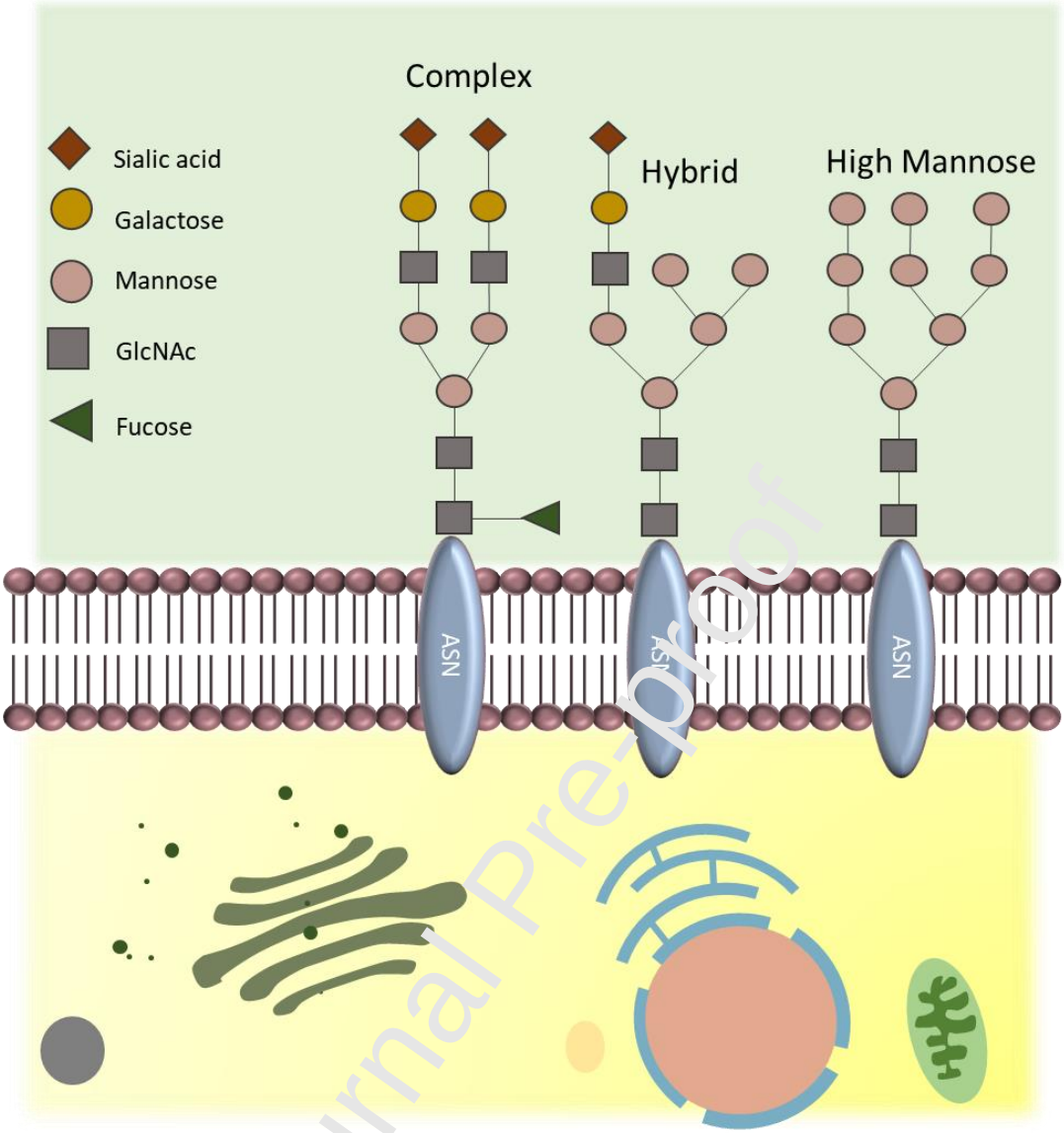


Figure 2: The structure of N-glycoproteins on the cell surface.

The *OLG* process includes oxygen-carbon bonding between the hydroxyl group of Ser/Thr residues of folded protein (figure 3) (Bennett et al., 2012). In most eukaryotes, the sequences of different O-glycans are conserved and it mainly occurs on mucins, the large glycoproteins, comprising of a cytoplasmic tail, a transmembrane domain, and outside extracellular region harboring a Pro-Ser-Thr rich peptide repeat. Mucin has one monosaccharide molecule, mostly β-GalNAc, but could have α-Man, α-GalNAc, or other sugar (Bennett et al., 2012; Schoberer and Strasser, 2018). Different UDP-GalNAc polypeptides linked with 1N-acetylgalactosaminyl transferases are responsible for the attachment of GalNAc (Hagen et al., 2003). The attachment of various sugar moieties (galactose, GlcNAc, fucose, and sialic acid) further modifies the GalNAc, which leads to the formation of varying mucin O-glycans that play an essential function in many biochemical processes (Ludovic et al., 2015; Schjoldager and Clausen, 2012). In yeasts, O-glycans comprise many units of

Man molecules attached to Ser/Thr residues (Barolo et al., 2020; Schoberer and Strasser, 2018). Depending upon the added moiety, O-glycosylation could occur either on the surface of ER (manno- or fucosylation) (Harris and Spellman, 1993; Lommel and Strahl, 2009) or in the Golgi body (mucin-type or the synthesis of glycosaminoglycan) (Bishop et al., 2007; Tian and Ten Hagen, 2009).

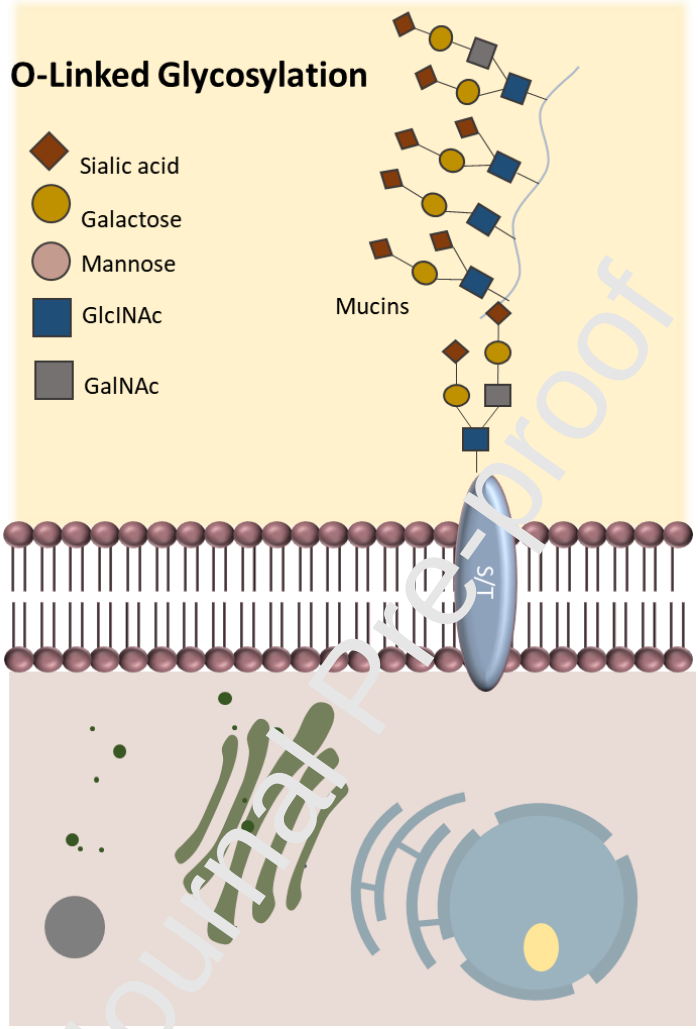


Figure 3: Structure of O- linked glycosylation core on the cell surface.

Looking at the conservation of various enzymes involved in glycosylation, it seems they have evolved to perform specific and vital functions. In the current review, we will focus on variations in different glycosylated proteins. The aberrant glycosylation occurs because of cellular and metabolic variations leading to variant expressions of integrated glycans (Lv et al., 2022). Multi-omics studies of various tumors revealed tumor-associated variations in macromolecule structures, including an increase in the branching pattern of N-glycans, upregulation of specific antigens like sialyl lewis, and change in the expression of O-glycan expression or fucosylation. In addition to this, glycans are also involved in protein lysis, which directly links with cancer signal transduction, ligand interactions with the receptor, and inter and intra-cellular communication in tumor cells. Below, we will give a detailed overview of

how alteration in glycosylation promotes different cancers, including cell-cell interaction in the tumor, tumorigenic signaling cascades, cell-matrix interaction, and metastasis.

2. Biological significance of glycosylation

Glycans are found to be associated with many fundamental processes like cellular metabolism (Bassaganas et al., 2014; Dennis et al., 2009), immune surveillance, inflammation, or cell–cell adhesion (Pinho et al., 2013; Zhao et al., 2008), cell–matrix interaction (Zhao et al., 2008) and inter- and intracellular signaling (Gomes et al., 2013; Takeuchi and Haltiwanger, 2014). Therefore, alterations in glycan levels, as well as changes in their pattern, could transform the cell's behavior leading to cancer. The change in the glycan pattern on various proteoforms alters their conformation and structure, leading to a change in their activity (Bassaganas et al., 2014). Alterations in glycosylation linked to transformation were first described six decades ago (Lv et al., 2022). Cancer cells exhibit an extensive range of glycosylation variations compared to normal cells. Upregulation or downregulation in protein glycosylation increases both molecular and functional heterogeneity within cell populations.

First, we would discuss the role of glycosylation homeostasis in cellular signaling and proliferation pathways. The *OLG* of various transcription factors like cMyc, cyclin D1 or FoxM1 (forehead protein M1) is linked to cell cycle progression (Caldwell et al., 2010; Itkonen et al., 2013). *OLG* of cMyc (Master regulation of cell entry and proliferative metabolism) at Thr-58 competes with phosphorylation at this residue (a hot spot in human cancers) which stabilizes cMyc protein leading to oncogenesis (Itkonen et al., 2013). Figure 4 shows that an increase in *OLG* of cMyc increases its stability (Jóźwiak et al., 2014). As mentioned above, the degree of branching in N-glycan can affect the activity and signaling of growth factor receptors leading to alteration in the signaling pathway for cellular proliferation (Boscher et al., 2011; Jain et al., 2023; Stanley, 2007). Previously, it was shown that the change in the branching of glycans leads to a disturbance in the signaling molecules involved in cell survival during oncogenesis.

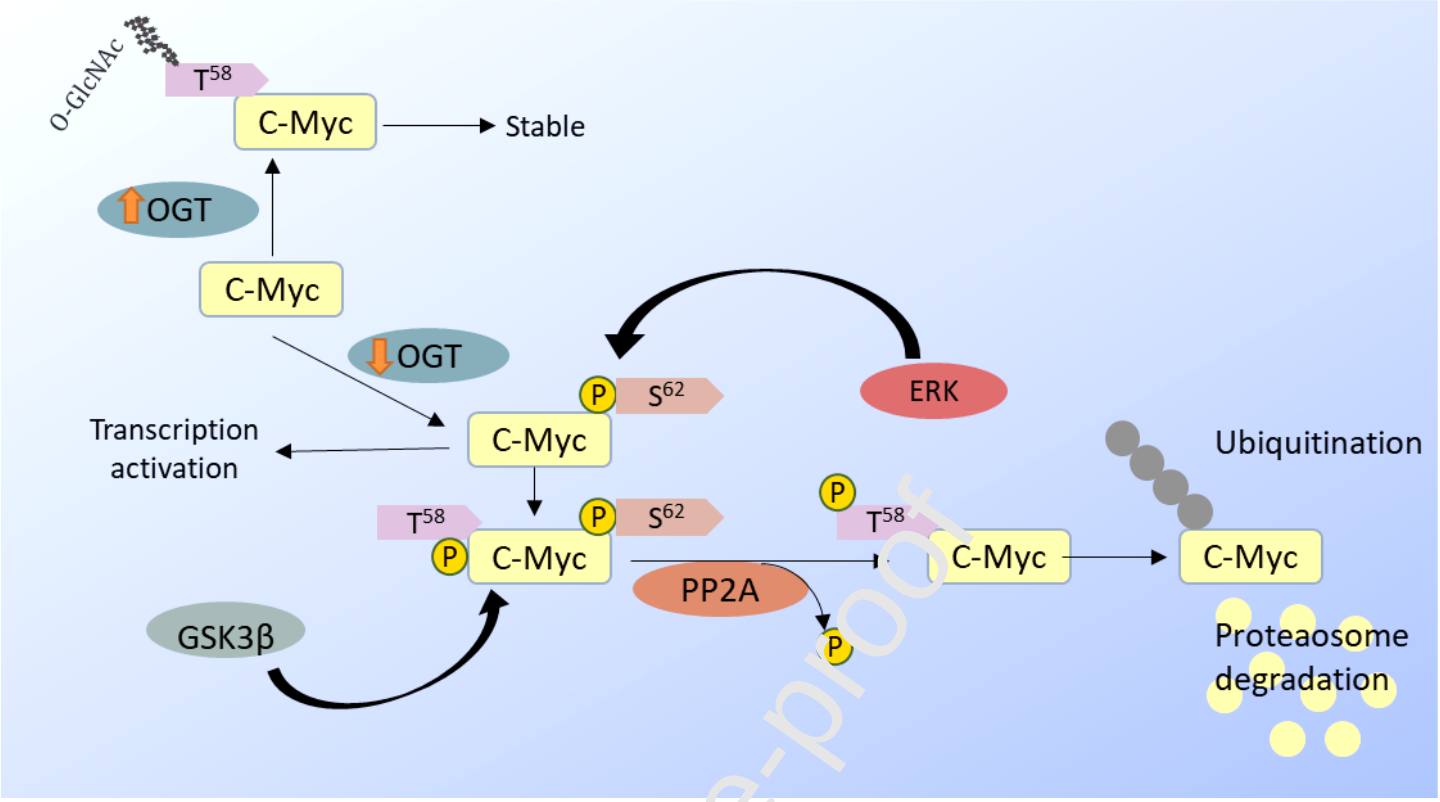


Figure 4: Impacts of increase in *OLG* of cMyc in cells. Higher levels of Transferase (OGT) produce OLG of c-Myc at Thr-58 residue which increases its stability. When OGT levels are low, the interplay of Ser-62/Thr-58 phosphorylation changes the fate of c-Myc to either degradation (Thr-58-P) or transcriptional activation (Ser-62-P).

Many cellular receptors linked to proliferation and survival are governed through glycosylation, for example, PDGF, FGFR, EGFR, MET, or IGFR (Julien et al., 2013; Todeschini et al., 2007). The extracellular matrix (ECM) activates the signaling events of different growth factor receptors, comprising a variable glycoprotein, glycosaminoglycans, collagens, or proteoglycans. Glycosylated proteins have been important in facilitating integrin-dependent signaling by promoting cell growth and survival. The glycosylation ceramide on the cell surface can activate the c-Src signaling pathway. It can increase the expression of factors important for the survival of cancer stem cells through β-catenin. On the other hand, proteoglycans play a role in the formation and recognition of exosomes (Iozzo and Schaefer, 2015).

Earlier, two major mechanisms underlying the alterations in tumor-associated carbohydrate structure have been proposed, incomplete synthesis and neosynthesis processes (Kannagi et al., 2008). The incomplete synthesis, which occurs more frequently in the early stages of the tumor involves disruption in the process of glycans synthesis as seen in epidermal cells. The incomplete synthesis observed in gastrointestinal and breast cancers is linked to sialyl Tn (STn) expression (Marcos et al., 2011). The mucin-type STn antigens, also called CD175s, were observed in

abundance in most colorectal, gastric, breast, ovarian and pancreatic cancers. STn expression on cells is an important indicator in the poor prognosis of patients with precursor and early lesions of carcinomas (Marcos et al., 2011).

Neosynthesis, on the other hand, is a cancer-associated increase in the expression of various genes for saccharide determinants like sialyl Lewis an (SLea) and Sialyl Lewis X (SLeX) antigens, which mediate the adhesion between cancer cells and endothelium through selectin ligands. Slex- or SLea-carrying glycopeptides interact with selectins present in leukocytes, endothelial cells or platelets (Kudelka et al., 2015). Metastasis is facilitated via higher expression of SLex or SLea antigens by tumor cells, possibly through increased platelets and endothelial interaction. Jeschke et al. showed that the lower expression of these Sialyl Lewis antigens was found to be linked with the higher expression of E-cadherin and maintained the expression of Cathepsin-D in carcinoma in situ (without metastases) (Jeschke et al., 2005). On the other hand, an increase in the expression of both Sialyl Lewis antigens resulted in poor expression of E-cadherin, as observed in primary carcinoma. These antigen molecules are not expressed in a healthy organism but are reported in endometrial cancer (Kolben et al., 2022), colorectal cancer (Madunic et al., 2022), and renal carcinoma (Borzym-Kluczyk et al., 2015). Overexpression of both antigens is combined with poor prognosis and malignancy in cells (Kannagi et al., 2008b). Additionally, SLex expression was seen in almost every PDAC tissue with a loss of E-cadherin expression at the cell contacts, which is otherwise seen in normal cells (Bassaganas et al., 2014). Recently, the modifications in SLex or SLea by conjugating a lactose unit showed a robust increase in immune responses (Guo et al., 2020).

It is well understood that the cells of the immune system, like immune effector cells protect against carcinogenesis by inhibiting the transformed cancer cells. Recent approaches suggested that the tumor-specific glycans interact with lectins present in immune cells for modulating the microenvironment of tumors (Rabinovich et al., 2012) and could provide resistance to tumor suppression responses (Perdicchio et al., 2016; Pinho and Reis, 2015). This process involves various lectins like CD43, CD45, selectins, galectins, and siglecs, leading to B and T cell differentiation via interaction and the recognition of various glycosylated tumor antigens (MacAuley et al., 2014; Rabinovich and Toscano, 2009). Galectins modulate immune and inflammatory responses leading to tumor cells escaping immune surveillance (Liu and Rabinovich, 2005; Rabinovich and Toscano, 2009). Another mechanism to avoid immune surveillance by tumor cells is the immunosuppressive signaling pathway involving PD-L1 (programmed death ligand 1)/PD-1 (PD receptor 1), which inhibits the anti-tumor activity of T-cells. Besides other post-transitional modifications like acetylation, sumoylation and phosphorylation, PD-L1 or PD-1 harbor glycosylation (Li et al., 2016; Zhou et al., 2022). It was shown that the glycosylation of Asn residues at 192, 200 and 219 positions of PD-L1 protein was important in antagonizing its proteasomal degradation (Li et al., 2016). In the case of head and neck squamous cell cancer, the glycosylation of PD-L2, a ligand of PD-1, lead to its binding to EGFR and

PD-1, resulting in immune evasion (Xu et al., 2021). Glycosylation-targeted immune therapy is a very promising area in cancer biology.

3. Altered protein glycosylation in Cancer:

3.1 Role in cellular adhesion:

Glycans are well studied for their involvement in cell-cell adhesion (Pinho et al., 2013; Zhao et al., 2008), cell-matrix interaction (Zhao et al., 2008) and inter- and intracellular signaling (Gomes et al., 2013; Takeuchi and Haltiwanger, 2014). Tight junctions allow intercellular communication across the barriers, while adhesion junctions act as cell-cell and cell-matrix regulatory molecules between adjacent cells. For example, adherens junctions via cadherin-catenin complex between corresponding cells. In addition, calcium-dependent transmembrane protein E-cadherin interacts with actin filaments and regulates various processes like adhesion between cells, cell differentiation, and cell motility (Thomas et al., 2021). The disruption in the cadherin-catenin complex affects cell interaction and the integrity of tumor cells. In addition, pieces of evidence suggest that glycosylation influences both cadherin stability and cadherin-mediated interactions. One of the critical enzymes, N-acetyl-glucosaminyl transferase V (known as GnT-V), alters the glycosylation pattern of N-cadherin.

Overexpression of GnT-V has been shown to alter the branching of 1,6-GlcNAc on N-cadherin, resulting in decreased cell-cell adherence and leading to tumor invasion as observed with human fibrosarcoma cells (Guo et al., 2003). Interstingly, using metastasis models of lung orthotopic and tail vein, it was revealed that GnT-V overexpression is linked to epithelial to mesenchymal transition and metastasis (Khan et al., 2018; Pucci et al., 2021). The knockout of GnT-V$^{-/-}$ enhanced the N-cadherin mediated cell-cell adhesion in mouse embryonic fibroblasts (Guo et al., 2003). Adding to this, the reduced N-glycosylation of E-cadherin leads to the increased stability of adherent junctions in normal and cancer cells. Mechanistically, it could be linked to the interaction between the protein phosphatase 2A (PP2A) to hypo-glycosylated E-cadherin that enhances tight junction assembly in cancer cells (Thomas et al., 2021). On to this, it was shown that in oral cancer, hyper-glycosylated E-cadherin destabilizes adherens junction proteins (Nita-Lazar et al., 2009).

In various cancers, catenin is a crucial component in Wnt signaling, which is also involved in intercellular junction stability along with E-cadherin. It was observed that enhanced *OLG* increased catenin and E-cadherin expression leading to fibroblast cell motility. Moreover, higher *OLG* enhanced tumor metastasis and mortality rate in the murine orthotropic colorectal cancer model (Reddy et al., 2018). Asn-554 residue plays a vital role in the function of E-cadherin as alteration of N-glycan 1,6-GlcNAc at this residue inhibits the physiological functions of E-cadherin (Carvalho et al., 2015). Interestingly, previous research found that inhibiting 1,6 fucosyltransferase recovers cell-cell adhesion and reduces lung cancer invasion via E-cadherin. Similarly, fucosyltransferase 8 (FUT8) knockdown in pancreatic acinar carcinoma inhibits calcium-dependent E-cadherin facilitated cell-cell bonding (Thomas et al., 2021).

FUT8 catalyzes the formation of -1,6 linkage and transfers l-fucose from GDP—l-fucose (GDP-Fuc) onto the innermost GlcNAc of an N-glycan (shown in figure 5). Furthermore, FUT8 deficient MCF-7 cells display reduced fucosylation of E-cadherin, leading to lowered cell migration and invasion. In FUT8 deficient MCF-7 cells, the integrin-mediated focal adhesion kinase (FAK) signaling was also suppressed with diminished catenin nucleocytoplasmic localization (Liu et al., 2019).

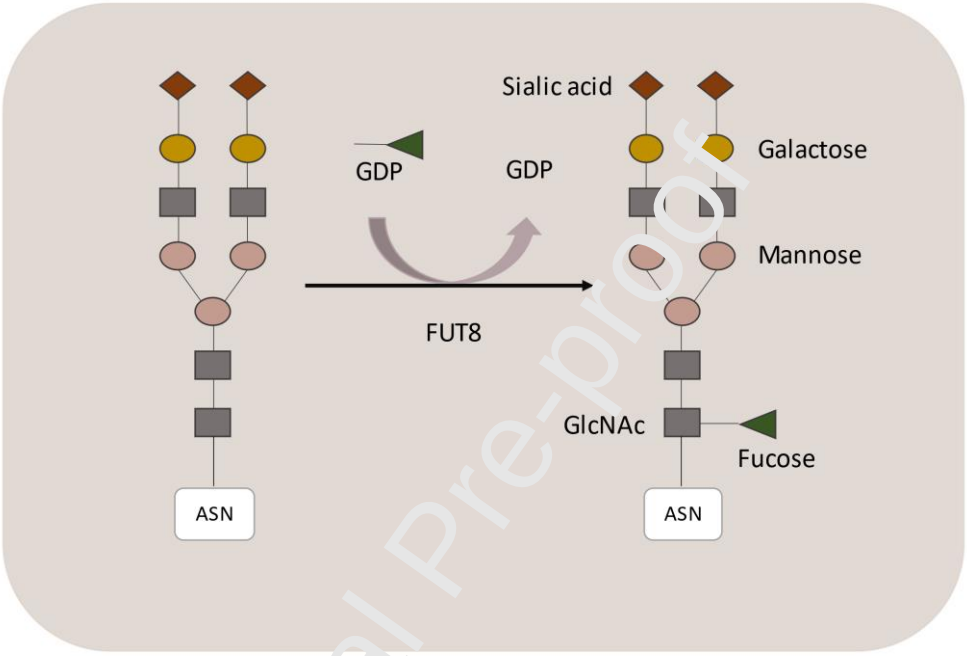


Figure:5 Fucosylation. FUT8 catalyzes the reaction. To form a -1,6 linkage, FUT8 transfers l-fucose reside from GDP—l-fucose (GDP-Fuc) onto the innermost GlcNAc of an N-glycan.

Tumor spheroid formation is caused by intercellular adhesion molecule 1 and activated leukocyte cell adhesion molecule as seen in cancer cases and is critically linked to clinical outcome and embolism (Clasen et al., 2022; Erturk et al., 2016; Ferragut et al., 2021; Wu et al., 2020). In ovarian cancer cells, it was shown that gene silencing of mannosidase (MAN1A1) of the Golgi apparatus blocked tumor formation via modifying N-glycans of ALCAM, resulting in decreased cell-cell interaction and motility (Hamester et al., 2019). Anoikis, a programmed cell death type, occurs in anchorage-dependent cells and is linked to loss of adhesion. The overexpression of GALNT3 (N-acetyl galactosaminyl transferase 3) increases *OLG* of mucin 1 (MUC1), a key player of anoikis, resulting in high stability of the E-cadherin and catenin complex and, as a result, induces cell propagation and motility in ovarian cancer cells. Furthermore, inhibiting GALNT3 destabilizes MUC1, which prevents cell proliferation and dissemination (Cheung et al., 2016). MUC16, a glycosylated transmembrane protein, promotes the interaction of epithelial cells with the extracellular matrix cytoskeleton (ECM). MUC16 genetic ablation disrupts actin

cytoskeleton binding, increasing proliferation and epithelial cell migration with altered zonula occludens-1 (tight junction protein-1) expression (Gipson et al., 2014; Thomas et al., 2021).

3.2 Role in cell-matrix interaction:

Proteins for anchoring and present in extracellular basement membrane also get affected by aberrant glycosylation, as seen in the case of laminin, integrins, collagen, and fibronectin that maintain cell-cell and cell-matrix interactions by interacting directly with ECM proteins. Changes in glycosylation patterns of the anchoring protein can cause a variety of pathological conditions, such as cardiovascular ailments (Menni et al., 2018), muscular dystrophy (Thomas et al., 2021), and cancer (Laubli and Borsig, 2019). Previously, knockout of GnT-III impeded β-1,6 branching of N-glycans affecting integrins levels and blocking metastasis in mouse melanoma cells (Thomas et al., 2021).

Furthermore, highly N-glycosylated α5β3 integrin and its substrate vitronectin change cancer cell adhesion properties that promote melanoma cell invasion (Pochec et al., 2016). Overexpression of β-galactoside α-2,6-sialyltransferase 1 (ST6Gal-I) increases β1 integrin and talin α-2,6 sialylations which elevate collagen IV expressions, thereby causing invasion and migration of colon cancer cells (Thomas et al., 2021). Similarly, in hepatocellular carcinoma, enhanced ST6Gal-I expression increases the sialylations of α5β1 integrin and epithelial cell adhesion to fibronectin (Yu et al., 2013). The overexpression of β-galactoside α-2,3-sialyltransferase 3 (ST3Gal III) has been shown to modify sialylation on the α2β1 integrin, yielding reduced cellular adhesion and increased invasiveness in pancreatic cancer (Bassaganas et al., 2014). The α5β1 and α2β1 integrins also get modulated by glycosylation. It was shown that α-2,6 sialylation on these integrins reduced the binding of fibronectin and collagen IV, critical basement membrane proteins to breast cancer cells (MDA-MB-231) (Huang et al., 2022; Yuan et al., 2016). Moreover, modified MUC1 glycosylation changes α2β1 integrin the expression in pancreatic ductal adenocarcinoma, which in turn down-regulates phospho-FAK expression and its downstream signaling molecules, reducing tumor growth and metastasis (Radhakrishnan et al., 2013). The β4 integrin harboring β1,6-GlcNAc-branched N-glycans was shown to enhance interaction with gelatin-3, which improved signaling via PI3K/Akt (phosphatidylinositol 3-kinase/protein kinase B) pathway and hence cell migration, invasion, and tumor growth. Strong evidence for this comes from the GnT-III knock out, which inhibited N-glycan binding, which suppressed β4 integrin and facilitated cancer cell invasion and migration (Kariya et al., 2018).

3.3 Abberant glycosylation in signaling cascades

The exact molecular mechanism by which abnormal and altered glycosylation roots unfavorable in metabolic and cellular signaling, helping towards tumor progression, is still a subject of investigation. Several cellular factors influence

tumorigenesis, like modified glycan expression, the glycosylating enzymes mutations, and their localization. All of these abnormalities activate oncogenic signaling cascades such as PI3K/Akt, Hippo signaling, Wnt/β-catenin, JAK/STAT, TGFβ/Smad, and Notch signaling (Munkley et al., 2016).

Wnt signaling component's functional dysregulation and genetic mutations significantly impact cancer cells. One member, MUC13, a transmembrane glycoprotein regulating G1/S phase transition during cell cycle progression, phosphorylates β-catenin and destabilizes the β-catenin/ adenomatous polyposis coli /Dishvelled complex, and increases β-catenin nuclear translocation. In hepatocellular carcinoma, it was shown that the binding of β-catenin to an important transcription factor T-cell factor/ lymphoid enhancer factor increased Axin-2, c-Myc, and E-cadherin expression, promoting tumor development (Dai et al., 2018). In gastric cancer cells, overexpression of O-glycosylated MUC5AC upregulate β-catenin and its downstream molecules increasing cell division, invasion, and metastasis. MUC5AC silencing, on the other hand, reduced cancer progression by downregulating β-catenin expression (Lahdaoui et al., 2017). Furthermore, knocking out MUC16 affects the E-cadherin & β-catenin stability and increases phosphorylated Akt, ERK and EGFR expression in ovarian cancer cells, promoting tumor cell invasion and metastasis (Thomas et al., 2021). NLG of Wnt ligand, Wnt cellular receptors, and E-cadherin were shown to promote the expression and nuclear localization of β-catenin/γ-catenin, which increased the transcriptional activity of dolichyl-phosphate N-acetylglucosamine phosphotransferase 1 (DPAGT1), leading to tumor invasion and metastasis. It was shown that reducing the expression of DPAGT1 decreased E-cadherin glycosylation, suppressed Wnt signaling and determined tumor progression and metastasis (Sengupta et al., 2013).

Receptor tyrosine kinases are important N-linked glycosylated proteins expressed by both immune cells as well as cancer cells which get activated by receptor modification and impact key features of metastasis like migration, invasion etc. The genes encoding these kinases include *HER2/ErbB2*, *EGFR*, *MST1R* (encoding RON), *MET* etc. N-glycan modification on the ErbB receptor, the EGFR tyrosine kinase (Roskoski, 2014), has the potential to control the biological function and intracellular transport of these receptors, thus regulating the oncogenic signaling pathways and cancer. Bisection of GlcNAc has been shown to hinder EGFR and integrin signaling via the Mitogen-activated protein kinase (Thomas et al., 2021). The alteration in the N-glycan structure by inhibiting β1,4-N-acetylgalactosaminyltransferase III inhibited EGFR phosphorylation. Furthermore, the degradation of EGFR reduced the phosphorylation of AKT and ERK, suppressing colorectal cancer stem cells (Che et al., 2014). In another study, increased EGFR GalNAc-type-O-glycosylation by Core 1 β-1,3-galactosyltransferase (C1GALT1) enhanced tumor development. Also, the specific inhibitor (Itraconazole) of C1GALT1 reduced the O-glycosylation of EGFR, resulting in decreased tumor progression (Lin et al., 2018). Altered EGFR glycosylation by Lewisy (Ley) carbohydrate increased EGFR phosphorylation, which

in turn increased phosphorylated AKT, resulting in enhanced oral cancer malignancy; however, lack of Ley glycosylation of EGFR significantly lowered cancer cell progression. (Thomas et al., 2021). Interestingly, inhibiting the N-glycan biosynthesis in colorectal cells showed decreased in one of the critical components of tight junctions, claudin-3, possibly due to the inactivation of receptor tyrosine kinases (Perez et al., 2020).

The gene *MST1R* (Macrophage stimulating receptor-1) encodes RON (*Recepteur d'origine nantais*) protein which, after glycosylation, goes through proteolytic processing (Hunt et al., 2023). RON and its ligand, macrophage-stimulating protein (Morrison et al., 2004), are involved in different types of cancers like ovarian cancer (Zhang et al., 2010), lung and renal cancer (Ronsin et al., 1993), and gastric cancer. Aberrant glycosylation of RON leads to cancer (Zhou et al., 2016). Recently, antibodies targeting glycosylated as well as non-glycosylated RON showed promising therapeutic efficacy and imaging (Koh et al., 2019; Yu Koh et al., 2022). Besides RON, a related protein MET (MNNG HOS transforming) present in epithelial cells is involved in multiple processes, including embryogenesis and its knockout cause tumor (Wang et al., 2015). MET, a receptor for hepatocyte growth factor, is glycosylated at eleven sites which are important for its maturation and its interaction with ligands (Saitou et al., 2022). A recent study (Saitou et al., 2022) showed that suppression of N-glycosylation in cancer cells using different N-glycan mutants lead to decreased processing of MET and its downstream signaling. Taken together, the alteration in the N-glycans of receptors tyrosine kinases can be utilized not only for prognosis but also for building therapies, including immunotherapy.

The change in the expression of glycosyltransferases has been linked to tumor metastasis of oral squamous carcinoma, breast cancer, skin cancer, colon cancer, hepatocellular carcinoma., and pancreatic cancer. GnT-V mediated alteration in the glycan branches from α1,6 mannose to β1-6-linked N-acetylglucosamine in many growth factors and cell surface receptors (like EGFR, MET, RON, Src, and TGF-β family oncogenes) enhances cancer metastasis (Nagae et al., 2018; Thomas et al., 2021) (figure 6). These findings suggest that numerous gene and protein changes cause malignant transformation and neoplastic progression. According to accumulating pieces of evidence, the change in protein glycosylation machinery is associated with the attainment of cellular characteristics required for tumor cell invasion to distant regions. The remodeling of glycans that have been linked to tumor progression is primarily the result of mutations in the branching enzyme glycosyltransferases (Josic et al., 2019).

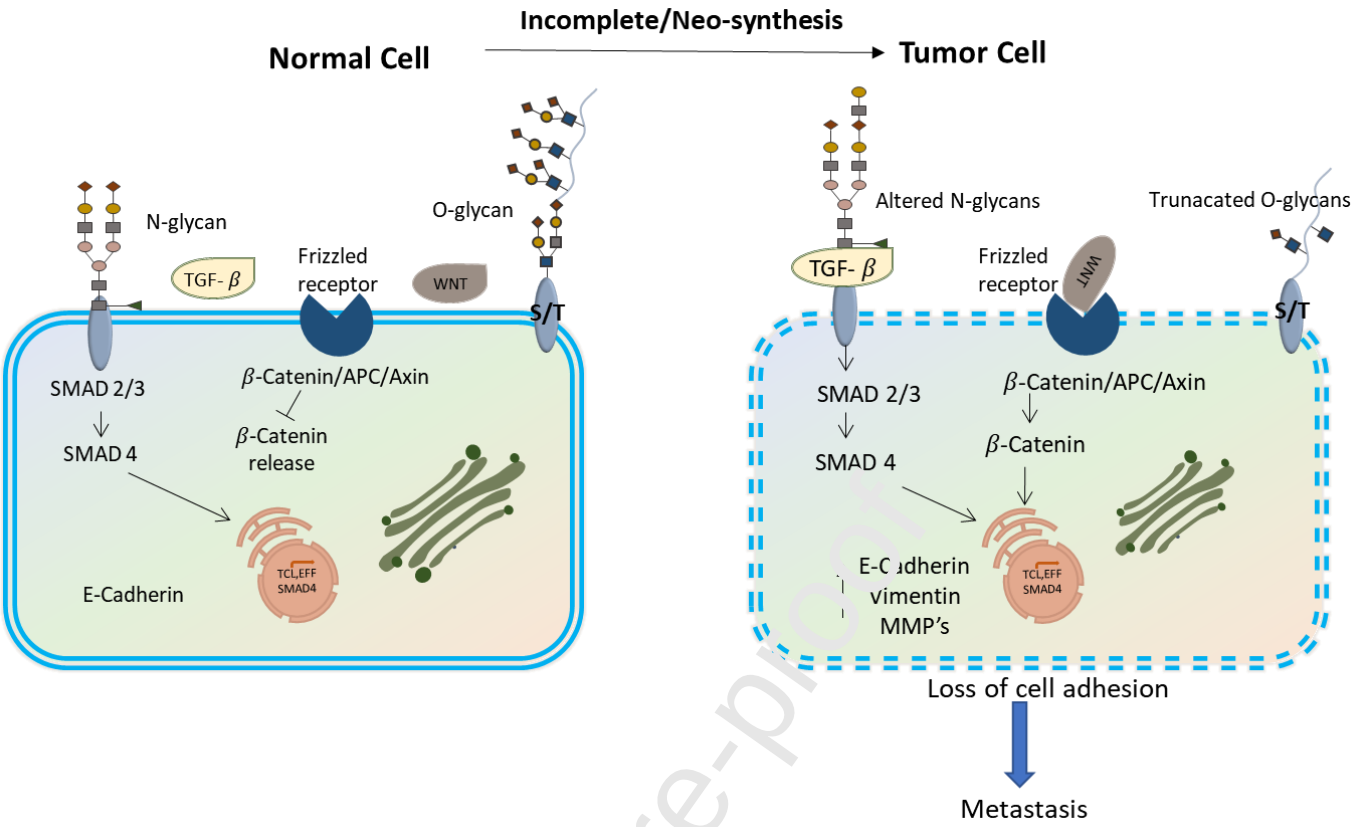


Figure 6: Function of aberrant glycosylated proteins in cancer metastasis. Changes in expressions of glycosyltransferases, fucosyltransferases, and sialyltransferases cause altered glycosylation of TGF-β and Wnt (transforming growth factor-β and wingless/integrated signaling pathway) ligands that induce EMT (Epithelial-to-Mesenchymal Transition) in tumor cells. Increased mesenchymal marker expression facilitates tumor cells' metastasis to distant organs.

3.4 Apoptosis and glycosylation

The ability of cancer cells to avoid apoptosis is one of its a hallmark (Munkley et al., 2016). It has been shown that glycans are involved in many signaling pathways that lead to the initiation, progression, and resolution of apoptosis (Lichtenstein and Rabinovich, 2013). Glycosylation can influence the functions of TNFR1 (tumor necrosis factor receptor 1) and Fas (CD95) death receptors (Micheau, 2018). In various cells, the α2,6-linked sialylation of TNFR1 is accomplished by glycosyltransferase ST6Gal-I, and its higher expression leads to premalignant progression by reducing apoptosis in gastric cancer (Alexander et al., 2020). Recently it was shown that higher α-1,3 fucosylation of TNFR1 promotes its interaction with TNF-α and causes apoptosis (Yu et al., 2022). Mechanistically, the death receptors and their ligand's glycosylation can interfere with ligand-receptor interactions and helps in the formation of signaling molecules leading to ligand release from the effector cells (Munkley et al., 2016). Galectin-3 interaction with Fas suppresses controlled cell death signals (Fukumori et al., 2007) and enhances tumor cell growth

(Mazurek et al., 2011). The glycosphingolipid GD3 accumulates in cells, causing mitochondrial destruction and inducing apoptosis. In glioblastomas, adding an acetyl group to the terminal sialic acid (9-O-acetyl GD3) inhibits GD3-mediated controlled cell death and causes tumor survival (Munkley et al., 2016). Additionally, glycosylation of the ceramide via glucosylceramide synthase reduces the pro-apoptotic potential of the ceramide (Liu et al., 2011).

Apoptosis is also linked to telomere shortening. Telomerase activation occurs in around ninety percent of cancers and is a crucial step in carcinogenesis. A major mechanism of cancer-specific telomerase activation is the reactivation of transcription of *hTERT* (human telomerase reverse transcriptase) gene. There is no direct proof relating glycans to telomerase activation. However, glycosylation is indirectly related to telomerase activation through c-MYC glycosylation. Earlier, it has been shown that O-GlcNAc modification of c-MYC stabilizes it and contributes to carcinogenesis (Itkonen et al., 2013; Munkley et al., 2016). C-MYC transactivates TERT and influences telomerase activation indirectly by contributing toward cancer progression.

4. Immunotherapy and role of glycosylation:

One of the interventions in cancer treatment, immunotherapy, could be utilized for treatment and diagnosis. Siglecs on immune cells perform different functions, such as cell-homing receptors and antigen specific immune responses (Foffi et al., 2013). CD22, a sialoglycoprotein, is one of the 16 siglec expressed on the surface of mature B cells, where it targets the binding of α2,6-linked sialic acid-containing ligands. The presence of ligands on N-glycans is responsible for inhibiting galectin 1 binding. This interaction is crucial for the B cell receptor (BCR) signaling on the cell membrane, followed by binding with antigen (Ereno-Orbea et al., 2017). Moreover, CD22 directs the cells toward α2,6- linked sialic acids tissues (Zhou et al., 2018). It was shown that CD22-deficient mice expressed mutated IgG antibodies which were somatically generated and autoreactive. These antibodies were of higher affinity than wild-type controls (O'Keefe et al., 1999). Thus, it concludes that CD22 plays a vital role in BCR signaling for the homeostatic balance of self-tolerance in cells and could be a drug target for diseases like systemic lupus erythematosus (Marshall et al., 2018). Additionally, siglec-1 or CD169 (aka sialoadhesin), play a similar role, but it binds to sialic acid with less affinity; that means ligands are highly sialylated and multimeric for enabling efficient interactions (Fraschilla and Pillai, 2017). CD169 plays a crucial role in combating many pathogens, including viruses and several inflammations (Eakin et al., 2016; Hammonds et al., 2017; Rose et al., 2016; Sewald et al., 2015). Siglecs bind to targeted glycans and play a function in inhibiting the immune surveillance of tumor cells (Dimitroff, 2015). For instance, the change in glycans at the cell surface can alter siglec-7 mediated cell toxicity of Natural Killer cells to participate in immune evasion (Hudak et al., 2014). Glycosylation of IgG antibodies is also shown in immune surveillance of tumor cells and used as a biomarker in different carcinomas (Kazuno et al., 2016; Ruhaak et al., 2015; Saldova et al., 2007; Vuckovic

et al., 2016). Targeting the various glycosylation alterations using different anti-tumor vaccines associated with specific tumor antigens is a good weapon for cancer treatment (Julien et al., 2012; Slovin et al., 1999).

The protein family of selectins comprises E-selectin, P-selectin, or L-selectin, which in their glycosylated forms are mainly present on the inner layer of different cells as receptors, for instance, thrombocytes, endothelial cells, and leukocytes. Selectins are associated with early events of cellular adhesion mechanisms, as seen in ovarian cancer and tumor metastasis (Hassan et al., 2020). A study on selectin demonstrated that a recombinant antibody formed against the P-selectins downregulates myocardial destruction post to percutaneous coronary intervention in myocardial infarction patients (Mertens et al., 2006). On similar grounds, selectins can be beneficial targets for Immunotherapy of cancer and other diseases.

Variation in the glycosylation process is actively involved in malignancy in cancer, as suggested above (Pinho and Reis, 2015). The change in structures of specific glycan, for instance, branched N-glycans (Pinho and Reis, 2015), stage-specific embryonic antigens including terminal sialylated and fucosylated Lewis structures and immature truncated O-glycans chain structures, (Kudelka et al., 2015; Radhakrishnan et al., 2014; Williams and Stanley, 2008), relates with many aspects of development and progression of cancer by disrupting the normal functions of different protein carriers molecules (Pinho and Reis, 2015; Radhakrishnan et al., 2014; Rodrigues et al., 2018).

5. Glycoprotein as a diagnostic biomarkers tool

Several statistics showed that early cancer diagnosis increases the chances of survival tremendously. Therefore, the introduction and in-depth study of new diagnostic biomarkers for cancer are of utmost importance. A defining characteristic of cancer is abnormal glycosylation. Most research has been conducted to find variations in serum glycan composition. Fucosylation and sialylation are drastically altered in most malignancies, as mentioned above. So, it is possible to employ aberrations in glycan structures as targets to enhance current serum cancer indicators (Tuccillo et al., 2014).

Numerous studies have been carried out with a focus on uncovering diagnostic biomarkers for cancer involving glycosylation mechanisms (Table 1). One of these studies focuses on prostate cancer and its detection using a prostate-specific antigen (PSA). PSA is highly specific as a diagnostic marker, but its level rises in benign prostatic hyperplasia (BPH) along with prostate cancer. It was seen that α1,2-fucosylated (Fucα1) and β-N-acetylgalactosaminylated (βGalNAc) PSA bound to *Trichosanthes japonica* agglutinin-II (TJA-II) column. However, this property was not seen in the case of hyperplasic patients indicating the increased expression of Fucα1 and βGalNAc of PSA during tumorigenesis. Additionally, it was found with greater than 95% accuracy. Therefore, TJA-II-bound PSA is a promising biomarker

for prostate cancer to distinguish between BPH and PC (Fukushima et al., 2010). Recently, the glycosylation of growth differentiation factor-15 was also found to be linked to prostate cancer (Wang et al., 2022).

Studies showed that single-position Asn-glycosylation on GM2AP (GM2-activator protein) was more abundant in the urine of lung cancer patients than in non-cancerous individuals indicating that GM2AP unquestionably contributes to the growth of lung cancer. Lung cancer can be detected auxiliary using the level of GM2AP in serum and urine (Potprommanee et al., 2015; Scott and Salgia, 2008). The performance of GM2AP in lung cancer may be a useful prognostic indicator for non-small cell lung cancer (particularly early) that can be utilized to forecast overall and disease-free survival. The use of this protein as a lung cancer diagnostic and prognostic marker has enormous potential. It was also demonstrated that 3- or 4-antennary fucosylated and sialylated N-glycans were overexpressed in the sera of oral squamous cell carcinoma patients compared to the sera from healthy controls. Increased quantities of N-glycans of fucosylated 3-antennary-cum-di-sialylated, or fucosylated 4-antennary-cum-tetra-sialylated were discovered in serum from patients with lymphatic metastases and were proposed as prospective diagnostic biomarkers (Guu et al., 2017). Recently, gold nanocomposites were utilized to develop a label-free immunosensor to detect lung cancer in urine and serum sample (Kuntamung et al., 2021).

In a study on urothelial cancer (UC) to ascertain whether abnormal N-glycosylated blood immunoglobulins (Igs) can be used as a diagnostic indicator, it was found that that serum from 237 UC patients and 96 prostate cancer patients, when subjected to high-throughput N-glycomics showed 32 different N-glycan levels on Igs, which were then assessed using multivariable discriminant analysis (Tanaka et al., 2017). Additionally, it was discovered that Igs carrying N-glycan of bisecting GlcNAc type was considerably high in UC. They reported more than 92 percent sensitivity and 97 percent specificity in the diagnosis of UC patients using diagnostic N-glycan Score based on five N-glycans on Igs (Tanaka et al., 2017).

Ovarian malignancy can be detected by analyzing the levels of human epididymis secretory protein 4 (HE4) which is a secreted glycoprotein. HE4 from ovarian cancer. It harbors more glycosylation in cancer samples than benign on (Zhuang et al., 2013). HE4 EIA test kit, developed by Fujirebio Diagnostics in 2008 and approved by the FDA, utilizes a solid phase and is a noncompetitive immunoassay that can be considered a suitable assay for ovarian cancer (Steffensen et al., 2011). This kit utilizes two monoclonal antibodies for detecting two whey-acidic protein domains of HE4. The functionality of HE4 EIA is comparable to the MUC 16 assay, and it was also stated in a recent analysis that HE4 outperforms MUC16 in detecting early-stage ovarian cancer (Ferrarow et al., 2013).

Table 1: Glycosylation-based biomarkers and detection methods for cancer

S.No	Biomarker	Cancer type	Methods Used	Reference
1	Serum paraoxonase 1 (PON1)	Hepatocellular Carcinoma	ELISA	(Zhang et al., 2015)
2	LCA-reactive fraction of AFP (AFP-L3)	Hepatocellular Carcinoma	Radio Immuno assay	(Sato et al., 1993)
3	SLe X	Lung Cancer	ELISA	(Arnold et al., 2011)
4	WFA-positive L1CAM	Cholangiocarcinoma	ELISA, MS, Micro-array	(Matsuda et al., 2013)
5	CA15-3 (MUC1)	Breast Cancer	IRMA	(Kandylis et al., 1990)
6	CA125 (MUC16)	Ovarian Cancer	ELISA	(Bast et al., 2010)
7	TJA-II bound PSA	Prostate Cancer	Lactate levels, ELISA	(Fukushima et al., 2010)
8	α2,3-sialic acid in PSA	Prostate Cancer	Lactate levels ECLIA, ELLA, Biosensor	(Llop et al., 2016; Pihikova et al., 2016)
9	Ganglioside GM3	Breast Cancer	LC-MS, (RP)-HPLC-FTMS	(Li et al., 2019)
10	HER2 extracellular domain (ECD)	Gastric Cancer	CLIA	(Oyama et al., 2014)
11	hAGP (Human α1-acid-glycoprotein)	Pancreatic Cancer	Immunoaffinity chromatography, μZIC-HILIC-ESI-MS	(Gimenez et al., 2015)
12	RNase 1	Pancreatic Cancer	Resectable pancreatic cancer technique, MALDI-TOF	(Peracaula et al., 2008)

			MS, HPLC LC-ESI MS, ELISA	
13	CEA	Colorectal Cancer	Immunoradiometric analysis, Immunochemiluminometric assays, Biosensor	(Avelino et al., 2014; Pinho and Reis, 2015)
14	STn	Gastric Cancer	CLIA, Biosensor	(Pinho and Reis, 2015; M Luísa S Silva et al., 2014)
15	Human chorionic gonadotropin	Ovarian Cancer	ELISA, Immunoassay	(Han et al., 2018; Thomas et al., 2021)
16	CA19-9	Ovarian Cancer	Radio Immunoassay, Biosensor	(Han et al., 2018; Thapa et al., 2017; Thomas et al., 2021; Wang et al., 2016)
17	HER2	Breast Cancer	Immunohistochemistry, ELISA Western blot, Biosensor	(Chocholova et al., 2018; Hao et al., 2020; Pinho and Reis, 2015; Zaleski et al., 2018)
18	CA 27-29	Breast Cancer	ELISA, Immunoassay	(Hao et al., 2020; Pinho and Reis, 2015; Zaleski et al., 2018)
19	AFP' core' fucosylation (AFP-L3)	Hepatocellular Carcinoma	Affinity chromatography	(Zhang et al., 2020)

20	Pro PSA	Prostate Cancer	Lactate levels, ELISA	(Gilgunn et al., 2013; Oto et al., 2020)

The glycosylated proteins present in the serum of tumor tissue are used as the biomarker and also help to check the prognosis in patients and responses towards treatments, for instance, proteins such as MUC1, CEA, MUC16, and specific antigens towards prostate cancer (PSA) (Charpin et al., 1982; Frenette et al., 1994; Kumar et al., 2008; Marrelli et al., 2001; Steele et al., 1982). Carbohydrate antigen (CA 19-9) is usually detected with an antibody, which is further used to recognize SLea on a monosialoganglioside molecule, the first reported biomarker in gastrointestinal cancer. CA 19-9, a cancer antigen SLea, is often higher in the patient serum of various cancers, such as pancreatic, gastric, and colorectal. However, the mechanisms responsible for the elevation levels in serum having cancer are not clear. Possibly it is related to dysregulated sialyltransferases enzyme (Engle et al., 2019; Hartlapp et al., 2022; Lee et al., 2020; Thomsen et al., 2018). The increase in CA 19-9 across many tumors highlights that aberrant glycosylation is a main factor involved in cancer pathobiology. Another crucial *NLG* alteration seen is α2,6-linked sialic acid, mainly raised in colon and pancreatic carcinoma with concomitant high expression of ST6Gal-I as discussed above. The increase in the ST6Gal-I expression is related to pathways for pro-survival, and its sialylated variants hamper the controlled cell death of tumor cells and activate various growth factors.

Proteomics-based technologies have opened up a new horizon for utilizing novel protein biomarkers for cancer detection, as mentioned in the last section (Liang and Chan, 2007). The glycoproteomic plays an important role in this regard for the identification of cancer-specific aberrant glycosylation. As mentioned above, the crucial players are glycan biosynthesis pathways like glycosidases, glycosyltransferases which could alter glycans in cancer cells (Dennis et al., 1999; Drake et al., 2009; Dube and Bertozzi, 2005; Fukuda and Tsuboi, 1999; Fuster and Esko, 2005; Menyhart and Gyorffy, 2021; Oliveira et al., 2021). The comparison of cancer and healthy cell glycans can help in the identification of new biomarkers for diagnosis and treatment applications. Identifying such cancer-linked glycan modifications on glycoproteins may enhance the specificity of cancer biomarkers. Cancer has been linked to glycoproteins with specific glycan structures. These distinguishable molecules could be released into the bloodstream as potential biomarkers.

The mucins (MUC1, MUC4, MUC13, and MUC16) significantly impact tumor progression and have recently been suggested as cancer treatment targets (Dhanisha et al., 2018). Levels of serum mucins that are secreted (MUC5AC and MUC6) or membranous (MUC1 and MUC16) are useful in diagnosis. The tumor-associated non-mucin glycoproteins include human epidermal growth factor receptor 2 (HER2), PSA,

alpha-fetoprotein (AFP), and carcinoembryonic antigen (CEA). The aberrant glycosylation of a specific glycoprotein can be easily and specifically screened out to improve the sensitivity and specificity of diagnostic methods.

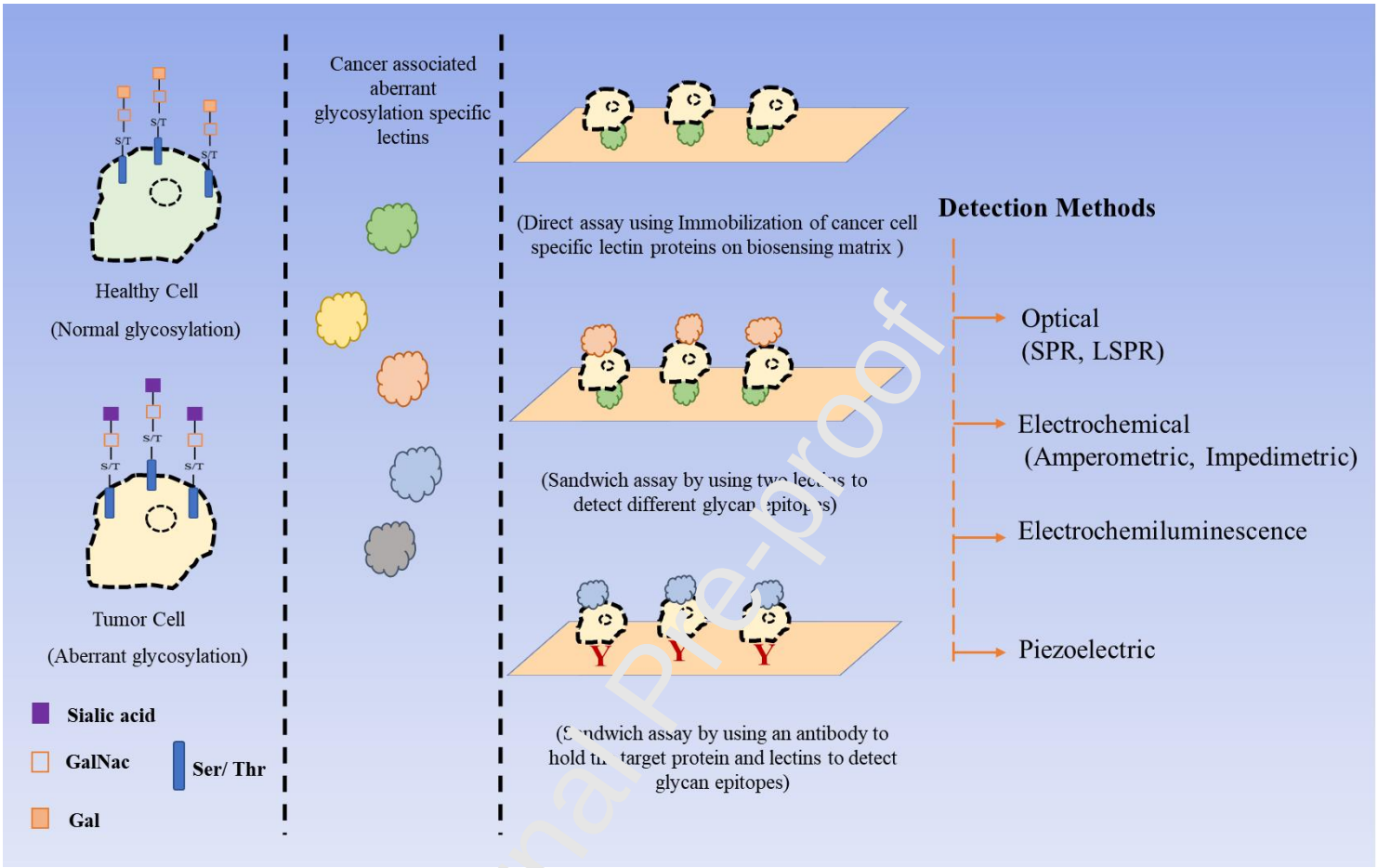


Figure 7 Lectin-based biosensors for the detection of cancer-associated glycans.

Several proteomics techniques like HPLC, 2D-electrophoresis, capillary electrophoresis and mass spectrometry are available for detecting and characterizing cancer-linked abnormal glycans. These techniques are not beneficial for rapid point-of-care diagnostics due to the requirements of sophisticated instrumentation facilities and trained personnel to conduct the test. Several advanced detection methods have been developed for the detection of a biomarker such as AFP, PSA, and glycans, including immunofluorescence assay, electrochemiluminescence immunoassay (ECLIA), and electrochemical immunoassay. Each method has advantages and drawbacks.

Among all, electrochemical immunoassays are better in terms of their capabilities, such as lower detection limit, rapid response, small sample volume, and minimal manipulations (Nagaraj et al., 2010; Yang et al., 2013). At present, cancer diagnosis by targeting aberrant glycosylation is attracting researchers across the globe due to its

accuracy and ability to detect cancer at its early stages. In this regard, lectin-based biosensors are getting the attention of researchers across the globe due to their specific binding affinities for the cancer-linked specific lectin structures (figure 7). Lectins are natural biomolecules with specific affinities for specific glycan structures and bind to them to form a strong complex. The affinity of lectins has proven to be a valuable asset for analytical methods for the selective separation of specific glycans in complex samples and for the characterization of glycosylation profiles using lectin microarrays in various clinical conditions. The development of Lectin-based biosensors has been reported to detect specific aberrant and cancer-associated glycostructures to aid in the diagnosis, prognosis, and treatment of these patients. Biosensors' appealing characteristics, such as portability and ease of use, make them ideal for point-of-care testing. Besides this, Tn and STn antigens can be detected by graphene-based glycan biosensors and lectine-based gold electrodes, respectively (Kveton et al., 2019; Silva et al., 2014). The α2,3-, and α2,6-sialylated glycans can also be detected in serum using electrochemical-based biosensors with high precision (Niu et al., 2016). The ultrasensitive glyco-biosensing analytical tools could help in the point-of-care detection of cancer biomarkers.

6. Multi-Omics analysis for altered Glycosylation

Multi-omics approach, involving genomics, transcriptomics, and proteomics along with clinical and pathological markers, have advanced our knowledge of the genetic landscapes of tumor. Multi-omics offers clear advantages for translational cancer research and reveals vital interactions through simple co-relations (Menyhart and Gyorffy, 2021; Oliveira et al., 2021). There are two ways to analyse omics data: top-down and bottom-up integration strategies. The hypothesis-driven bottom-up strategy states that integrating several data first, then manually integrating individual clusters. Strong top-down techniques, in contrast, integrate all data types concurrently and permit both dimensionality reduction and data integration. Unsupervised, exploratory, supervised, predictive, regression, or semi-supervised analysis is all possible with integrative approaches. Although many tools combine several methods, data integration algorithms can also be roughly categorized as fusion-based, network-based, Bayesian, resemblance, correlation-based, and other multivariate methods (non-negative matrix factorization, Joint and Individual Variation Explained and MoCluster etc.). Yet, the bulk of multi-omics integration tools are insufficiently reliable, prone to mistakes, and only accessible to experienced users' knowledge of programming.

Initial studies involving mRNA profiling or whole proteome analysis releveled some level of clustering or subtyping of cancer (Cancer Genome Atlas Research Network, 2011; Du and Lovly, 2018; Zhang et al., 2016). In the recent past, there has been an advancement in clinical proteomics by measuring the differential changes in glycosylation patterns between cancer and normal cells/tissue. The techniques used for the detection include western blotting, mass spectrophotometry and radiolabeling using isotopes (Rossin et al., 2013). The mass spectrophotometry is most efficient tool

for detection of relevant peptides, but because of low ionization efficiency and stoichiometry of phosphopeptides, it is very difficult to detect them. Therefore, various methods like immobilized metal affinity and metal oxide affinity chromatography are widely used for enrichments of polypeptides.

Glycoproteomic involving analysis of glycosite-containing peptides in cancer tissue could help in understanding the macro and micro-heterogeneity between various tumors and as well as with benign tissue (Li et al., 2017; Shah et al., 2015; Zhou et al., 2017; Zhu et al., 2019). Glycoproteomic approach involving the mass spectrometry study of ovarian carcinoma revealed that the tumors could be delineated into various clusters and subtypes (Pan et al., 2020). Wang et al. quantified N-glycopeptides with a good accuracy score which was consistent with other multi-omic approaches taken to study differences between cancer cells and their stem cells (Wang et al., 2019). Cancer cells were studied by site- and structure-specific relatively quantitative N-glycoproteomics which revealed various glycosylation sites in HepG2 and MCF-7 cells (Xiao and Tian, 2019; Xue et al., 2020). Using SugarQuant and its GlycoBinder processing tool for data analysis, glycosylation changes at specific sites were determined in Burkitt's lymphoma cells after fucosylation inhibition (Fang et al., 2020). Similarly, the glycoproteomics approach revealed many site-specific glycosylation changes in cells expressing oncogenic mutations (Saraswat et al., 2022). Combining this information with site of glycosylation, mRNA levels of glycotransferases and glycosidases enzymes and other parameters, it was found that their expression level plays a critical role in inter-tumor heterogeneity and affect the clinical outcome also. Utilizing the similar approach for prostate cancer, the glycosylation of growth differentiation factor-15 is linked to castration-resistance (Wang et al., 2022). These studies could improve the disease's prognosis because it helps further our understanding related to the plasticity created by glycosylation on cancer marker proteins. Combining this information with molecular docking and pharmacology networking could further improve the prognosis and possibly cancer treatment(Lu et al., 2022).

Table 2: Abbreviations Used

S.No.	Abbreviation	Full form
1.	AFP	Alpha-fetoprotein
2.	ALCAM	Activated leukocyte cell adhesion molecule
3.	ALG	Asn-linked glycosylation
4.	BCR	B cell receptor
5.	CEA	Carcinoembryonic antigen
6.	ECLIA	Electrochemiluminescence Immunoassay
7.	ECM	Extracellular matrix
8.	EGFR	Epidermal growth factor receptor
9.	ELISA	Enzyme-linked immunosorbent assay
10.	EMT	Epithelial-to-Mesenchymal Transition
11.	ER	Endoplasmic Reticulum

12.	FAK	Focal adhesion kinase
13.	FoxM1	Forehead protein M1
14.	Fucα1	α1,2-Fucosylated
15.	GALNT3	N-acetyl galactosaminyl transferase 3
16.	Glc	Glucose
17.	GlcNAc	N-acetylglucosamine
18.	GM2AP	GM2-activator protein
19.	GnT-V	N-acetyl-glucosaminyl transferase V
20.	HER2	Human epidermal growth factor receptor 2
21.	ICAM-1	Intercellular adhesion molecule 1
22.	Igs	Immunoglobulins
23.	Man	Mannose
24.	MUC1	Mucin 1
25.	MYC	Master regulation of cell entry and proliferative metabolism
26.	NLG	*N*-linked glycosylation
27.	OLG	*O*-linked glycosylation
28.	PD-1	Programmed cell death protein 1
29.	PD-L1	Programmed death ligand 1
30.	PP2A	Phosphatase 2A
31.	PP-Dol	Pyrophosphate residue
32.	PSA	Prostate cancer antigen
33.	Ser	Serine residue
34.	SLea	Sialyl Lewis a
35.	SLeX	Sialyl Lewis X
36.	ST6Gal-I	β-Gal α-2,6-sialyltransferase
37.	STn	Sialyl Tn
38.	TGF-β	Transforming growth factor-β
39.	Thr	Threonine residue
40.	TJA-II	*Trichosanthes japonica* agglutinin-II
41.	UC	Urothelial cancer
42.	UDP	Uridine diphosphate
43.	Wnt	Wingless/integrated signaling pathway
44.	βGalNAc	β-N-acetylgalactosaminylated
45.	LC-MS	Liquid chromatography–mass spectrometry
46.	HPLC-FTMS	HPLC-fourier-transform mass spectrometry
47.	HPLC	High-performance liquid chromatography
48.	MALDI-TOF	Matrix-assisted laser desorption/ionization-time of flight
49.	LC-ESI MS	Liquid Chromatography Electrospray Ionization Mass Spectrometric
50.	CLIA	Chemiluminescence Immunoassay
51.	μZIC-HILIC-ESI-MS	Zwitterionic hydrophilic interaction capillary liquid chromatography electrospray mass spectrometry
52.	IAC	Immuno affinity Chromatography

7. Conclusions and Future perspectives

The glycosylation variations in a glycoprotein and their specific tumorigenic pathways must be considered as a diagnostic biomarker and a new target for therapeutic applications. In Immunotherapy, antibodies and glycan initiate CAR-T cells, triggered by tumor-linked glycans or glycopeptides have potential tools for tumor treatment. The earlier developments in the area also give insight into the applications based on inhibitory effects either to targeted glycosylation-linked enzymes or to blocking glycan recognizing molecules. The glycome of tumor cells, its regulation on oncology and metastasis, and the interplay of cancer with the immune response will set a range of novel and improved therapies for various types of cancers.

Author credit

AKS, RVS and VKG: conceptualization, supervision. All authors have contributed to writing, reviewing, and drafting the article.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

- *O*- and *N*-linked glycosylation orchestrate proteoforms and their functions
- Incomplete/neosynthesis of glycan protein lead to immune dysregulation and cancer
- Signaling via PDGF, FGFR, EGFR, MET, RON, or IGFR receptors needs glycosylation
- Multi-omics of tumors reveal heterogeneity in the glycosylation pattern
- Tumor-specific glycans interact with CD43, CD45, selectins, galectins and siglecs