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REVIEW

Aberrant *N*-glycosylation in cancer: MGAT5 and β1,6-GlcNAc branched *N*-glycans as critical regulators of tumor development and progression

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Abstract

Background Changes in protein glycosylation are widely observed in tumor cells. *N*-glycan branching through adding β1,6-linked *N*-acetylglucosamine (β1,6-GlcNAc) to an α1,6-linked mannose, which is catalyzed by the *N*-acetylglucosaminyltransferase V (MGAT5 or GnT-V), is one of the most frequently observed tumor-associated glycan structure formed. Increased levels of this branching structure play a pro-tumoral role in various ways, for example, through the stabilization of growth factor receptors, the destabilization of intercellular adhesion, or the acquisition of a migratory phenotype.

Conclusion In this review, we provide an updated and comprehensive summary of the physiological and pathophysiological roles of MGAT5 and β1,6-GlcNAc branched *N*-glycans, including their regulatory mechanisms. Specific emphasis is given to the role of MGAT5 and β1,6-GlcNAc branched *N*-glycans in cellular mechanisms that contribute to the development and progression of solid tumors. We also provide insight into possible future clinical implications, such as the use of MGAT5 as a prognostic biomarker.

Keywords MGAT5 · *N*-glycans · Cancer · Biomarker

Abbreviations

5-AZA-dC	5-Aza-2′-Deoxycytidine
ACC	Adrenocortical carcinoma
AJ	Adherens junctions
AKT	Protein kinase b
Asn	Asparagine
BAX	BCL2-associated X protein
BCL2	B-cell lymphoma 2
BLCA	Bladder urothelial carcinoma
BRCA	Breast invasive carcinoma
CA19-9	Carbohydrate antigen 19 − 9
CA72-4	Cancer antigen 72 − 4
CAR	Chimeric antigen receptors
CEACAM6	Carcinoembryonic antigen-related cell adhesion molecule 6
CESC	Cervical squamous cell carcinoma and endocervical adenocarcinoma
CHOL	Cholangio carcinoma
CMS	Consensus molecular subtypes
COAD	Colon adenocarcinoma
CRC	Colorectal cancer
CRISPR/Cas9	Clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9
DC-SIGN	Dendritic cell-specific ICAM-3 grabbing non-integrin
DLBC	Lymphoid neoplasm diffuse large B-cell lymphoma
EGFR	Epidermal growth factor receptor
EMT	Epithelial-mesenchymal transition
ER	Endoplasmic reticulum
ERAD	Endoplasmic reticulum-associated protein degradation
ESCA	Esophageal carcinoma
ETS	Erythroblast transformation-specific transcription factor
FZD-7	Frizzled class receptor 7
Gal	Galactose
GATA	GATA-binding protein
GBM	Glioblastoma multiforme
GEPIA2	Gene expression profiling interactive analysis 2
GlcNAc	N-acetylglucosamine

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GPI	Glycosylphosphatidylinositol
GTEx	Genotype-tissue expression project
HBP	Hexosamine biosynthetic pathway
HGF	Hepatocyte growth factor
HIF1A	Hypoxia inducible factor 1
HK	Hexokinase
HNSC	Head and neck squamous cell carcinoma
IBD	Inflammatory bowel disease
IFNγ	Interferon gamma
IGF1	Insulin-like growth factor 1
IGF-1R	Insulin-like growth factor 1 receptor
IGF2BP1	Insulin Like Growth Factor 2 MRNA Binding Protein 1
IgG	Immunoglobulin G
INSR	Insulin receptor
KICH	Kidney chromophobe
KIRC	Kidney renal clear cell carcinoma
KIRP	Kidney renal papillary cell carcinoma
LacNAc	N-acetyl-lactosamine
LAML	Acute myeloid leukemia
LGG	Brain lower grade glioma
LIHC	Liver hepatocelular carcinoma
L-PHA	Phaseolus vulgaris leucoagglutinin
LUAD	Lung adenocarcinoma
LUSC	Lung squamous cell carcinoma
m6a	N6 methyladenosine
mAbs	Monoclonal antibodies
Man	Mannose
MAPK	Mitogen-activated protein kinase
MESO	Mesothelioma
MGAT	N-acetylglucosaminyltransferase
GnT-V	N-acetylglucosaminyltransferase V
miR-124	MicroRNA 124
mRNA	Messenger RNA
MSI	Microsatellite instability
MT1-MMP	Membrane-type matrix metalloproteinase-1
MYB	v-Myb myeloblastosis viral oncogene homolog
mTORC1	Mammalian target of rapamycin complex 1
ncRNAs	Non-coding RNAs
NSCLC	Non-small cell lung cancer
O-GlcNAc	O-linked β-N-acetylglucosamine
OST	Oligosaccharyltransferase
OV	Ovarian serous cystadenocarcinoma
PAAD	Pancreatic adenocarcinoma
PCPG	Pheochromocytoma and paraganglioma
PD-1	Programmed cell death protein-1
PD-L1	Programmed death ligand-1
PI3K	Phosphoinositide 3-kinase
PRAD	Prostate adenocarcinoma
PTEN	Phosphatase and tensin homolog
PTMs	Post-translational modifications
RAF	Cellular homolog of viral raf gene
RAS	Rat sarcoma viral oncogene homolog
READ	Rectum adenocarcinoma
RNA	Ribonucleic acid
SARC	Sarcoma
SCID	Severe combined immunodeficiency
Ser	Serine
siRNA	Small interfering RNA
SKCM	Skin cutaneous melanoma
SPPL3	Signal peptide peptidase like 3
SRC	v-src avian sarcoma viral oncogene homolog
STAD	Stomach adenocarcinoma
TCGA	The cancer genome atlas
TCR	T-cell receptor
TGCT	Testicular germ cell tumors
TGF-β1	Transforming growth factor beta
TGF-βR	Transforming growth factor beta receptor
THCA	Thyroid carcinoma
Thr	Threonine
THYM	Thymoma
TIM-4	T-cell immunoglobulin domain and mucin domain 4
TIMP-1	Tissue inhibitor of metalloproteinase-1
UC	Ulcerative colitis
UCEC	Uterine corpus endometrial carcinoma
UCS	Uterine carcinosarcoma
UDP-GlcNAc	Uridine diphosphate N-acetylglucosamine
UVM	Uveal melanoma
WNT	Wingless-type MMTV integration site family
β1,6-GlcNAc	β1,6-linked N-acetylglucosamine

1 Introduction

1.1 Protein glycosylation

Glycosylation consists of the binding of carbohydrates to target molecules, such as proteins, lipids, and even small RNAs [1]. Among the various post-translational modifications (PTMs) that occur in proteins, glycosylation is among the most common ones. It is estimated that approximately 20% of all proteins in a cell is glycosylated [2]. Glycosylation substantially increases the diversity of protein structures, thus playing a critical role in cellular homeostasis. The main types of protein-bound glycans are shown in Fig. 1.

1.2 *N*-glycosylation

Prior to *N*-glycosylation, the assembly of a precursor oligosaccharide linked to a dolichol molecule present in the

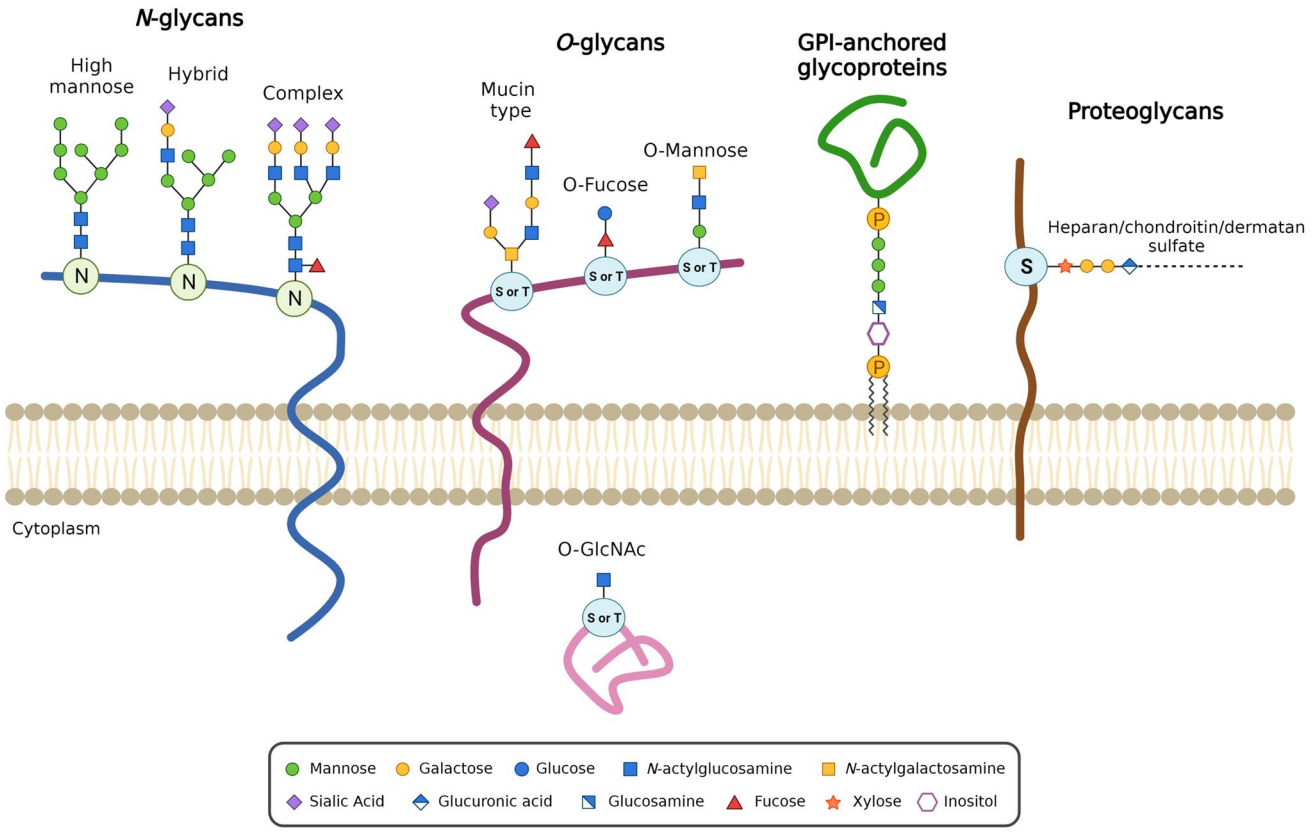

Fig. 1 Major protein-bound glycans found in mammalian cells. *N*-glycans bind to proteins through covalent binding to asparagine (N) residues and can be classified as high-mannose, hybrid, and complex. *O*-glycans are characterized by the binding of a saccharide to proteins via *O*-linkage to Ser (S) or Thr (T). Mucin-type is initiated by *N*-acetylgalactosamine, and its presence is abundant in membrane-associated glycoproteins. Other types of *O*-glycans found in the membrane include *O*-mannose and *O*-fucose. There is also a type of post-translational modification that is characterized by the addition of *O*-linked β-*N*-acetylglucosamine (O-GlcNAc) to S or T of cytosolic and nuclear proteins. Some proteins found more externally in the membrane are modified with glycolipids that present phosphatidylinositol, thus forming glycosylphosphatidylinositol (GPI)-anchored proteins. Proteoglycans are heavily glycosylated proteins modified by glycosaminoglycans (long linear polysaccharides formed by repeating disaccharide units), represented in the figure by a dotted line, that are connected to the protein core by a tetrasaccharide linker formed by a xylose, two galactoses, and a glucuronic acid

endoplasmic reticulum (ER) membrane occurs. Subsequently, through the action of oligosaccharyltransferase (OST), this precursor (a 14-sugar molecule) is transferred *en bloc* to asparagine residues (Asn-X-Ser/Thr motif, where X can be any amino acid except proline) of nascent proteins synthesized via the ER (Fig. 2, upper panel). From there on, *N*-glycan processing begins, which includes several steps of addition and removal of monosaccharides catalyzed by glycosyltransferases and glycosidases that act along the protein's passage through the ER and Golgi apparatus. As a result, three main structure types are generated: (I) high-mannose, (II), hybrid, and (III) complex (Fig. 1). During the biosynthesis of complex *N*-glycan, the addition of *N*-acetylglucosamine (GlcNAc) residues to the mannose (Man) of the *core* (Man3GlcNAc2) generates branches that can serve as a structural basis for chain elongation, thus forming so-called antennae. These extensions (bi-, tri-, or tetra-antennary structures) are commonly formed by *N*-acetyl-lactosamine (LacNAc; disaccharides formed by galactose and *N*-acetylglucosamine) or poly-LacNAc and capped with fucose and sialic acid [3].

1.3 MGAT5 and β1,6-GlcNAc branched *N*-glycans

In the early 1980s, the enzymatic activity of MGAT5 was first identified in a study that revealed a *N*-acetylglucosaminyltransferase deficiency in murine lymphoma cells. The lack of this enzyme resulted in a reduction in the formation of tri- and tetra-antennary complex-type *N*-glycans recognized by the L-PHA lectin (specific for branched complex-type *N*-glycans with β1,6-linked *N*-acetylglucosamine), in addition to the accumulation of bi-antennary *N*-glycans [4]. MGAT5 catalyzes the transfer of GlcNAc from UDP-GlcNAc to α1,6-mannose via a β1,6-linkage, thereby giving rise to β1,6-GlcNAc branched *N*-glycans. The levels of these branched structures on the cell surface are also influenced

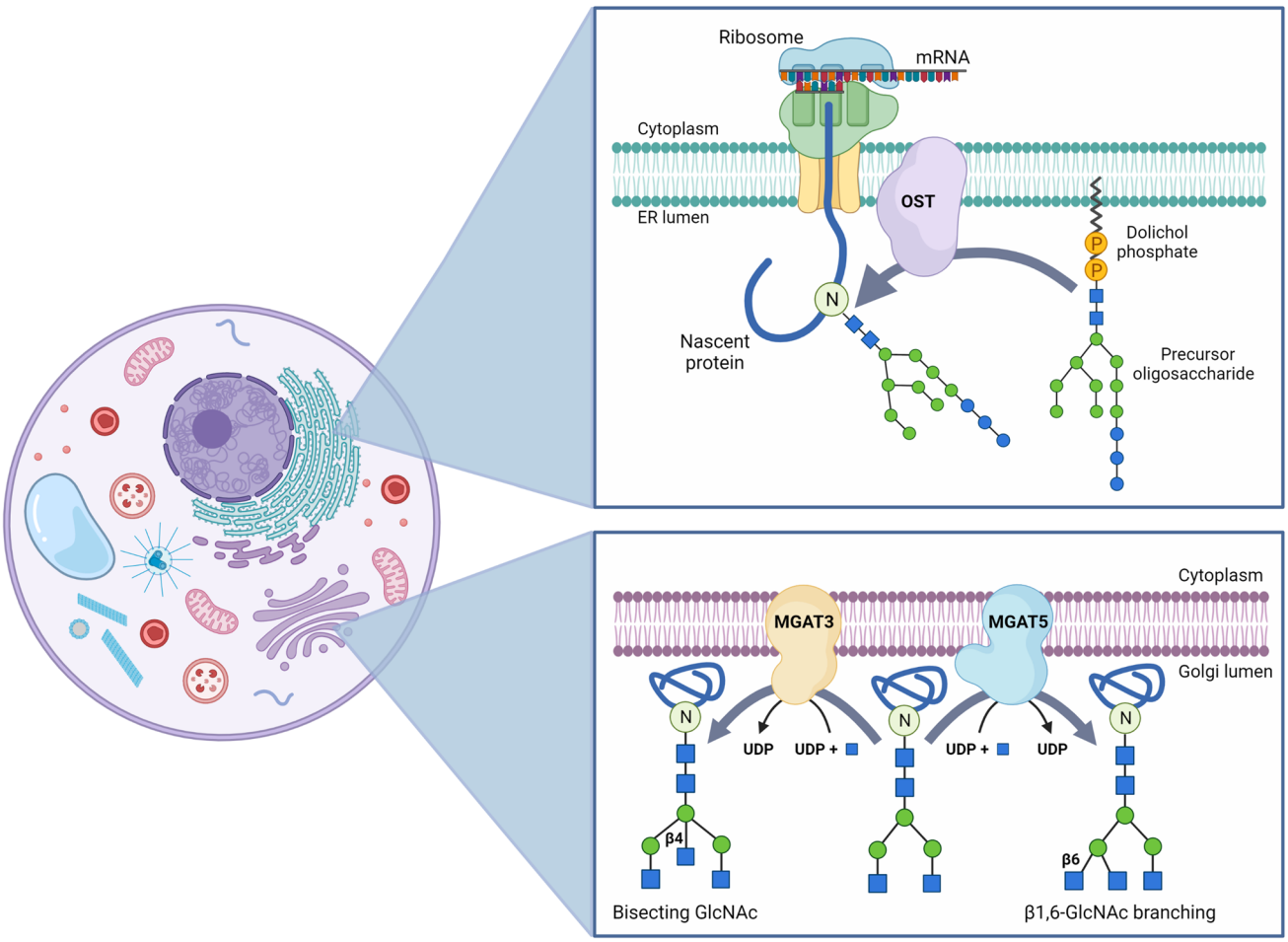

Fig. 2 Upper panel: protein *N*-glycosylation reaction. Nascent proteins synthesized within the endoplasmic reticulum are glycosylated via an *en bloc* transfer of a dolichol lipid-linked oligosaccharide consisting of 14 sugars to an asparagine. This reaction is catalyzed by OST (oligosaccharyltransferase). Lower panel: action of MGAT3 and MGAT5 enzymes on the Golgi apparatus. MGAT3 catalyzes the transfer of GlcNAc from UDP-GlcNAc to the core mannose in a β1,4 linkage, thus generating bisected *N*-glycans. In turn, MGAT5 catalyzes the transfer of GlcNAc in a β1,6 linkage, generating branched *N*-glycans. Although not shown in this figure, it is necessary to emphasize that MGAT3 products cannot be used as a substrate for MGAT5

by another *N*-acetylglucosaminyltransferase that competes with MGAT5 for the same substrates during the biosynthesis of *N*-glycans, i.e., the enzyme MGAT3 (or GnT-III), which catalyzes the transfer of GlcNAc from UDP-GlcNAc to the core mannose in a β1,4 linkage, thus generating a unique structure called bisecting GlcNAc (Fig. 2, lower panel).

1.4 Regulation of MGAT5 and β1,6-GlcNAc branched *N*-glycans

Despite the well-established importance of MGAT5 in different cellular processes, details involved in its transcriptional regulation are not fully understood and need further investigation. The first pieces of evidence in this context have indicated that NIH3T3 murine fibroblast cells, transformed with a RAS oncogene, showed increases in the levels of both MGAT5 and *N*-glycans recognized by the L-PHA lectin [5]. Pioneering work investigating potential transcriptional regulators of *MGAT5* found that this gene is expressed in a tissue- and cell-type-specific manner and, in addition, revealed putative binding sites for the ETS and MYB transcription factors in its promoter region [6]. Another study performed with human bile duct carcinoma cells also showed that *MGAT5* expression regulation is mediated by the oncogenic transcription factor ETS1 [7]. In an attempt to corroborate this finding, the same research group evaluated 16 cancer cell lines and found that the MGAT5 mRNA levels closely correlated with ETS1 expression [8]. Interestingly, transformation of baby hamster kidney fibroblasts by the SRC oncogene led to increases in MGAT5 activity and mRNA levels, which could be reversed by herbimycin A treatment, a SRC kinase inhibitor. The authors also found that the effects of SRC on the expression of *MGAT5* depended on

both the downstream protein RAF1 and the transcription factor ETS2 [9].

In human ovarian cancer cells, it was recently found that increased expression levels of the gene encoding the transcription factor GATA3 correlated with increased *MGAT5* expression. The authors also found that treatment with 5-AZA-dC (a DNA methyltransferase inhibitor) led to an increase in the levels of highly branched glycans as well as an increased expression of *MGAT5* in A2780 and PEO1 cells. Interestingly, it was found that one of the *MGAT5* transcript variants harboured a CpG island, suggesting that the expression of this gene may be epigenetically regulated by promoter methylation [10]. Another level of MGAT5 regulation that has been proposed involves control over the stability of its mRNA. In a recent study, a possible mechanism was presented by which insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1) could promote MGAT5 mRNA stability through upregulation of N6 methyladenosine (m6A) modification. Additionally, it was found that this stabilization may favor maintenance of the cancer stem cell phenotype in hepatocellular carcinoma [11]. In recent years, increasing evidence has indicated that non-coding RNAs (ncRNAs) may regulate the expression of genes involved in the pathogenesis of a wide range of diseases. In breast cancer cells (MDA-MB-231 and MCF-7), it has for example been found that increased miR-124-3p levels may lead to reductions in MGAT5 protein and mRNA levels [12].

Another relevant factor regarding the regulation of β1,6-GlcNAc branched *N*-glycan levels concerns the concentration/availability of donor nucleotide sugars for MGAT5. About 2–5% of glucose taken up by cells supplies the hexosamine biosynthetic pathway (HBP), whose endproduct is the sugar nucleotide uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc), which is crucial for the biosynthesis of various glycosaminoglycans and glycans [13]. Thus, a high intracellular level of UDP-GlcNAc favors an increase in the degree of branching of *N*-glycans linked to membrane proteins [14]. In tumor cells, metabolic reprogramming is commonly observed in which, although glucose uptake is increased, there is a preference for lactate production, even under normal conditions of oxygen concentration (Warburg Effect). In this case, glycolytic pathway intermediates are used to fuel anabolic pathways, such as the pentose-phosphate pathway (PPP) and the HBP [15, 16]. Interestingly, the MGAT5 Km value for UDP-GlcNAc is considerably higher compared to that observed in other *N*-acetylglucosaminyltransferases (e.g. MGAT1, MGAT2, and MGAT4) [14, 17]. This suggests that β1,6-GlcNAc branched *N*-glycan levels may be strongly influenced by both donor availability (which reflects the nutrient flux) and variations in MGAT5 protein levels [18]. As mentioned under 1.3, ample evidence indicates that variations in the concentration of MGAT3 can influence the levels of β1,6-GlcNAc branched *N*-glycans, since these enzymes compete for the same substrate. In addition, it has been found that the products of MGAT3 cannot be used as MGAT5 substrates [19]. Thus, one may speculate that pathways capable of affecting MGAT3 levels, such as the WNT/β-catenin signaling pathway [20], may also have implications for branched *N*-glycan levels.

As our understanding of the control over the activity and structure of enzymes involved in the biosynthesis of *N*-glycans advances, the greater the chances of identifying new regulatory mechanisms. In terms of topology, MGAT5 has a short *N*-terminal cytosolic region and a transmembrane domain, which are connected to a large *C*-terminal catalytic region by a stem region [21, 22]. Recently, it was shown that the luminal portion of MGAT5 (which contains the catalytic domain and faces the lumen of the Golgi complex) harbours a specific noncatalytic domain at the *N*-terminal side (*N* domain) that is necessary for its activity toward glycoprotein substrates [21]. However, it remains to be established whether this domain is the target of MGAT5 activity control mechanisms. Yet another mechanism capable of regulating MGAT5 is through cleavage by a Golgi-resident protease called SPPL3. It has been found that overexpression of this protein may lead to a reduction in cellular levels of MGAT5. In addition, it has been shown that SPPL3−/− mouse-derived embryonic fibroblasts exhibit more abundant MGAT5 levels compared to those in wild-type control cells [23].

These multiple mechanisms involved in regulating MGAT5 and β1,6-GlcNAc branched *N*-glycans highlight the broad spectrum of possibilities that may lead to physiological and pathological changes in the *N*-glycosylation of proteins.

1.5 Physiological and pathophysiological roles of MGAT5 and β1,6-GlcNAc branched *N*-glycans

Murine models (null or overexpression) have provided relevant data on the physiological role of both MGAT5 and *β*1,6-GlcNAc branched *N*-glycans. In transgenic mice in which a β-actin promoter regulates *Mgat5* expression (although the development of spontaneous tumors in any organ has not been verified) it was observed that *Mgat5* overexpression resulted in the enhancement of an epithelial-mesenchymal transition-like phenotype in skin cells [24]. These animals were more susceptible to damage caused by removal of the stratum corneum, thus indicating impaired cell-cell adhesion. Furthermore, re-epithelialization for wound healing was found to be considerably faster in mice overexpressing *Mgat5*, thus suggesting an enhanced motility of keratinocytes [24, 25]. On the other hand, primary keratinocytes isolated from *Mgat5*-deficient mice have been shown to exhibit a lower proliferation rate compared to those in wild-type mice [26]. Together, these

findings suggest that MGAT5 and β1,6-GlcNAc branched *N*-glycans may play a key role in maintaining the cyto-architecture and homeostasis of certain tissues.

During the last decades, several studies on cells of epithelial origin have shown that MGAT5 and branched *N*-glycans are important players in regulating cell-cell adhesion and cell-matrix interactions. It has e.g. been shown that increased levels of β1,6-GlcNAc branched *N*-glycans bound to E-cadherin (the main cell-cell adhesion molecule in epithelia) can lead to cell-cell adhesion destabilization [27]. In contrast, the presence of E-cadherin-linked bisecting GlcNAc *N*-glycans (which is catalyzed by MGAT3, see Fig. 2, lower panel) has been associated with an increased stability of adherens junctions (AJ) [27, 28]. In addition, it has been found that the biological functions of integrins (proteins responsible for binding cells to the extracellular matrix) and, consequently, the migratory potential of epithelial cells, are also strongly influenced by the profile of *N*-glycans that decorate these proteins [29]. Whereas overexpression of *MGAT5* leads to a substantial increase in cell migration mediated by α3β1 integrin on the laminin 5 substrate, overexpression of *MGAT3* has the opposite effect and leads to a reduced cell migration [19]. A possible mechanism that may explain the opposing effects of MGAT5 and MGAT3 on cell-cell and cell-matrix adhesion is that the MGAT5 products carry larger glycans with more antennae, thus constituting a chemical barrier that may oppose not only the formation of more stable cell-cell adhesions, but also compromise the establishment of a stable cell-matrix interaction [30].

Evidence also indicates that metabolic homeostasis and tissue renewal depend on β1,6GlcNAc-branched *N*-glycans. *Mgat5* deletion in mice has been found to lead to a hypoglycemic condition, including resistance to weight gain and a reduced fat content even in case of a high-calorie diet. In addition, these animals appeared to be hypersensitive to fasting and to have an increased oxidative respiration [31]. Interestingly, administration of oral GlcNAc (which by itself is capable of increasing the levels of *N*-glycans recognized by L-PHA on the cell surface) in *Mgat5*$^{-/-}$ mice was able to restore lipid storage and to promote body weight gain [32]. It has also been found that lack of MGAT5 compromises the functionality of the glucagon receptor in liver cells, leading to an increase in glycogen storage, which contributes to systemic hypoglycemia [33]. In the absence of MGAT5, the levels of HBP intermediates are reduced, including UDP-GlcNAc, which is the donor substrate for the MGAT5 enzyme [33]. Conversely, in vitro studies have shown that tetracycline (tet)-induced overexpression of *MGAT5* in HEK293 cells promotes nutrient uptake, especially when glucose and glutamine are limiting, as observed by an increased uptake of amino acids. Overexpression of *MGAT5* also led to increased intracellular levels of intermediates of the glycolytic pathway, the tricarboxylic acid cycle, and HBP, in addition to promoting cell growth [34].

Evidence suggests that *N*-glycan branching may play a key role in controlling cell proliferation and, consequently, tissue renewal. In this context, it was found that *Mgat5*$^{-/-}$ mice showed an accelerated loss of both muscle and bone mass during aging. In addition, these animals showed less osteogenic activity in the bone marrow and exhibited lower amounts of muscle satellite cells [31]. A known mechanism that explains the relationship between MGAT5 and proliferation control is the stabilization of growth factor receptors (e.g. EGFR) on the cell surface via interaction of β1,6-GlcNAc branched *N*-glycans with galectin lattices, especially those formed by galectin-3 [35]. In addition to the already presented roles played by MGAT5 in metabolism and cell proliferation, it is also known that *Mgat5*$^{-/-}$ female mice, especially of the PLJ and 129 strains, show behavioral changes and fail to nest and feed their pups [36]. Although these results suggest that MGAT5 may be related to the maintenance of neuronal functions, further studies are needed to better understand this issue.

The levels of MGAT5 and β1,6-GlcNAc branched *N*-glycans in different tissues may also play an immunomodulatory roles. It was found that *Mgat5*-deficient mice, from 12 months of age, had enlarged spleens and an increased presence of leukocytes in the kidneys. These animals are more likely to have glomerulonephritis with mononuclear infiltration and fibrin accumulation within Bowman's space, similar to what is observed in autoimmune-mediated glomerulonephritis. Furthermore, *Mgat5* deficiency makes these mice more susceptible to experimental induction of autoimmune encephalomyelitis by immunizing with myelin basic protein [37]. Together, these results indicate that MGAT5 may play a regulatory role in immune cell trafficking and recruitment and may act in the recognition of self- and non-self-antigens, which may affect antibody production during autoimmune- and inflammation-associated diseases. Above a certain threshold of T-cell receptor (TCR) clustering, complete T-cell activation can occur. In this context, it has already been demonstrated that the TCR's glycosylation profile influences its functionality. *Mgat5*-deficient mice exhibit lower thresholds for TCR clustering and, consequently, higher T-cell activation and a greater propensity to develop autoimmune diseases [37]. Similarly, mutations at *N*-glycosylation sites of TCR have been shown to enhance their clustering and activation, in addition to improving the recognition of tumor cells by T-cells. [38]. The levels of MGAT5 and β1,6-GlcNAc branched *N*-glycans also seem to be important in the dynamics of inflammatory processes. *Mgat5*-deficient mice are for example more susceptible to the development of severe forms of inflammatory bowel disease (IBD) [39]. Moreover, in transgenic mice with up-regulation of *Mgat5* submitted to a diet with high fat and cholesterol

levels (which constitutes a model of non-alcoholic steatohepatitis), a marked suppression of lymphocyte infiltration in the liver was observed compared to that in wild-type mice. This less infiltrated profile can be related to a shift from a T helper 1 (Th1) to a T helper 2 (Th2) response, as a reduction in the levels of Th1-related cytokines was observed in the livers of these transgenic mice [40]. In patients with ulcerative colitis (UC), a chronic IBD, it was found that mucosal T lymphocytes display low levels of *N*-glycans recognized by the L-PHA lectin and a reduced expression of *MGAT5* [41]. Interestingly, it was found that restoration of β1,6-GlcNAc branched *N*-glycan levels in mucosal T-cells from patients with UC (obtained through GlcNAc supplementation) led to suppression of T-cell growth in addition to inhibition of the Th1 immune response [39].

In addition to the obvious role played by MGAT5 in the generation of branched *N*-glycans, a non-enzymatic function of this glycosyltransferase has been described, where a secreted type of MGAT5 was able to promote angiogenesis in vitro and in vivo at physiological concentrations [42]. The data presented so far show how comprehensive the systemic and cellular functions performed by MGAT5 and β1,6-GlcNAc branched N-glycans are, thus providing clues as to how alterations in these players can be crucial in the development of several diseases, such as diabetes, hepatitis, IBD, and cancer. Figure 3 summarizes these findings. As mentioned under 1.2, during the maturation process in the Golgi complex, branched β1,6-GlcNAc *N*-glycans can be extended with LacNac residues and capped with fucose and sialic acid. Alterations in the levels of these terminal structures, such as terminal sialyl Lewis X antigens and α2,6-sialylation, have also been considered as cancer-associated carbohydrate antigens [43, 44].

Next, the involvement of MGAT5 and β1,6-GlcNAc branched *N*-glycans in fundamental processes related to cancer will be discussed, as well as their promising role in the development of strategies that aim to improve the diagnosis, prognosis, and therapeutic stratification of cancer patients.

2 MGAT5 and β1,6-GLCNAC branched N-glycans in cancer

2.1 Changes in *MGAT5* expression and β1,6-GlcNAc branched *N*-glycans are linked to several types of cancer and can be useful in predicting clinical outcomes

A survey made using data from the GEPIA2 platform, an online tool for analyzing RNA sequencing expression data of tumors and normal samples from The Cancer Genome Atlas (TCGA) [45] showed that, despite not being a universal

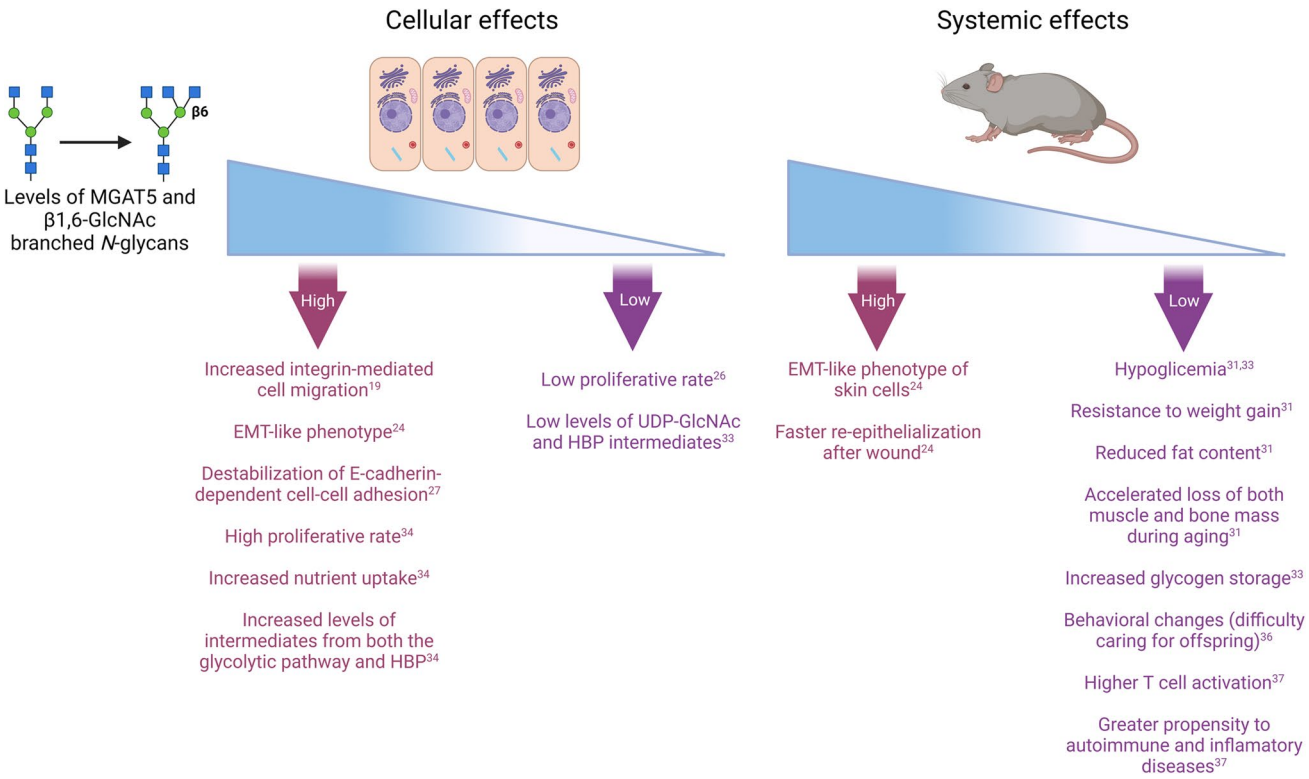

Fig. 3 Cellular and systemic consequences related to differences in the levels of MGAT5 and β1,6-GlcNAc branched *N*-glycans

feature, a wide variety of malignant tumors exhibits a high expression of *MGAT5* (Fig. 4a and b). Using this same platform, a screening to identify the usefulness of evaluating MGAT5 expression as a prognostic biomarker revealed that, except for renal clear cell carcinoma, high levels of expression of this gene are related to a worse prognosis (Fig. 5).

Another study using primary patient samples reported absence of highly branched *N*-glycans with β1,6-GlcNAc in astrocytes from normal adult brains and their high levels in glioblastoma, contributing to the hypothesis that the presence of high levels of β1,6-GlcNAc branched *N*-glycans is a characteristic of glial tissue neoplasms [46]. β1,6-GlcNAc branched *N*-glycan level evaluation in histological sections from colonic tissue revealed that adenomas exhibit a small increase in L-PHA staining compared to normal colonic epithelium, while the reactivity to this lectin was greatly increased in carcinomas [47]. In fact, it has been found that in colorectal tumors, staining with L-PHA lectin provides an independent prognostic indicator for both tumor recurrence and patient survival, and is associated with the presence of lymph node metastases [48]. Corroborating these findings, it was noted that samples from colorectal adenomas, carcinomas, and liver metastases exhibited an increased *MGAT5* expression compared with their corresponding mucosa [49]. Recently, through the analysis of data from gene expression deposited in the Genotype-Tissue Expression (GTEx) and TCGA databases, it was confirmed that colorectal tumors have an increased expression of *MGAT5* compared to normal colonic tissue [50]. *N*-glycomic analysis of colorectal cancer samples by MALDI-TOF/MS and LC-MS also showed that they exhibit increased levels of tetra-antennary *N*-glycans [51].

Based on data produced by an international molecular subtyping consortium, a classification of colorectal cancer distinguishing four consensus molecular subtypes (CMS) was established: CMS1 (immune MSI), CMS2 (canonical), CMS3 (metabolic) and CMS4 (mesenchymal), with CMS4 having the worst and CMS1 the best prognosis [52]. We found, through *in silico* analyses, that *MGAT5* expression is increased in CMS4 compared to CMS1. In addition, we observed high expression levels of *MGAT5* even in early stages of CRC development, thereby suggesting its possible use as a biomarker [53]. Overexpression of *MGAT5* has been associated with a poor prognosis, not only in colorectal cancer but also in gastric cancer [54, 55]. In breast cancer, an increase in L-PHA staining was observed compared to normal breast tissues or regions of hyperplasia [56]. Also, β1,6-GlcNAc branched *N*-glycans have been found to be increased in lymph node metastases and to predict poor clinical outcomes in this same type of cancer [57].

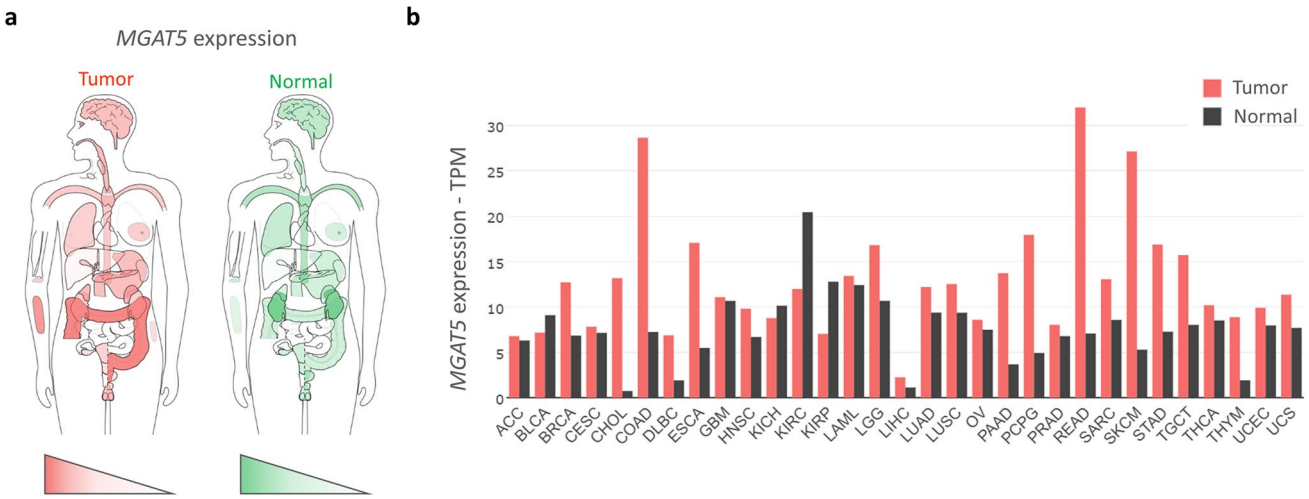

Fig. 4 *MGAT5* expression in tumor and normal samples. (**a**) Body map comparing *MGAT5* expression in non-tumor tissues, where darker tones depict higher expression levels and lighter tones reflect lower expression levels. (**b**) Bar chart showing *MGAT5* expression in different types of cancer and in normal samples. TPM, transcripts per million; ACC, adrenocortical carcinoma; BLCA, Bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangio carcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, brain lower grade glioma; LIHC, liver hepatocelular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma

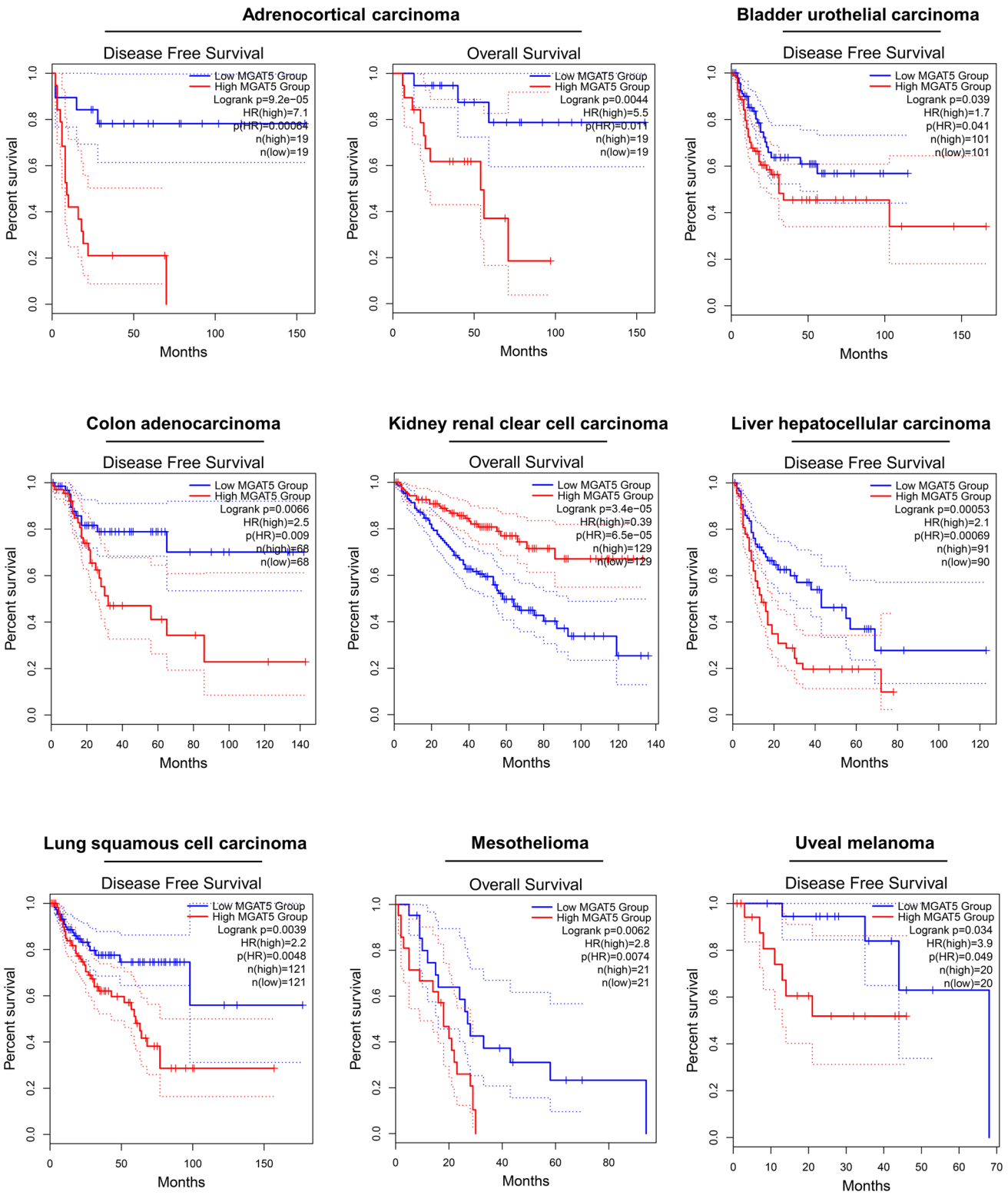

Fig. 5 Overall and disease-free survival according to *MGAT5* expression levels in different tumor types. The data are divided into four sections, and the upper and lower quartiles are considered as high and low expression groups, respectively. HR, hazards ratio calculated based on the Cox proportional-hazards model. Dotted lines represent 95% confidence intervals

Increased levels of *N*-glycans with β1,6-GlcNAc have also been observed in primary hepatocellular carcinomas compared to surrounding tissues or in non-diseased livers [58]. In this type of cancer, a high expression of *MGAT5* in tumor tissues was positively correlated with histological grade and metastasis. Overexpression of *MGAT5* also indicated a poor survival and recurrence, especially when observed in early-stage disease [59, 60]. Moreover, serum *N*-glycomic analysis of patients with castration-resistant prostate cancer revealed that they exhibit increased levels of tri- and tetra-antennary *N*-glycans [61].

Together, these findings support a potential usefulness of MGAT5 and β1,6-branched *N*-glycans as tumor markers, especially in colorectal cancer.

2.2 Regulation of cell-cell adhesion and cell-matrix interactions by MGAT5 and β1,6-GlcNAc branched *N*-glycans

Tumour progression involves alterations in cell adhesion properties that favor the acquisition of migratory phenotypes [62]. These events are most evident in epithelial-mesenchymal transition (EMT), an important phenomenon that occurs during the progression of epithelial cancers [63]. In the EMT process, post-translational modifications may regulate molecular mechanisms that contribute to phenotypic changes in tumor cells. Various studies have been conducted to understand the molecular mechanisms underlying EMT, including those related to changes in *N*-glycans [64].

Through structural modification of *N*-glycans, MGAT5 can modulate the activity of several proteins involved in cell adhesion, including matriptase, integrins, and cadherins [65]. As mentioned earlier, it has already been shown that epithelial cells with induced overexpression of *MGAT5* exhibit a loss of cell contacts and an increased cell motility [66]. E-cadherin, the main cell-cell adhesion molecule, plays an important role in suppressing tumor cell migration and metastasis, which is crucial in the EMT process [67]. Several mechanisms, such as post-translational modifications by *N*-glycosylation, have been found to downregulate E-cadherin in cancer [68]. Human E-cadherin has four potential *N*-glycosylation sites [69], and different profiles of *N*-glycans linked to this protein have been found to be related to the stability of adherens junctions, thereby playing a crucial role in the acquisition of malignant phenotypes [70, 71]. Increased modification of E-cadherin by β1,6-GlcNAc branched *N*-glycans drives E-cadherin translocation to the cytoplasm, alters cis-dimer formation and molecular assembly, and promotes destabilization of cell-cell adhesion with consequences for tumor progression [27, 72]. As mentioned before, these branched *N*-linked glycans are believed to function as a stereochemical barrier, thus compromising not only the stability of cell-cell adhesion complexes but also cell-matrix interactions [30]. On the other hand, it has been found that E-cadherin modification by bisecting GlcNAc *N*-glycan (Fig. 2. lower panel) increases the stability of adherens junctions and is associated with suppression of tumor progression [27, 28]. Interestingly, E-cadherin has also been shown to regulate *MGAT3*, resulting in its increased expression and the addition of bisecting GlcNAc *N*-glycans to the plasma membrane-bound protein [73]. This finding was corroborated by other studies, showing that the E-cadherin-catenin-actin complex plays an important role in the enzymatic regulation of MGAT3 [74, 75]. Site-specific glycosylation occupancy of Asn-554 with complex-type *N*-glycans has been shown to have a deleterious effect on E-cadherin localization and dimerization. Concordantly, preventing the presence of branched *N*-glycan at Asn-554, either by site-directed mutagenesis or by silencing *MGAT5*, resulted in a protective effect on E-cadherin, precluding its functional dysregulation and contributing to tumor suppression [76].

We also found another mechanism that may influence intercellular adhesion where an interaction between E-cadherin and INSR/IGF-1R signaling was seen, modulating the levels of bisected *N*-glycans and, consequently, the invasive phenotype. In this case, exogenous E-cadherin expression in MDA-MB-435 cells (which lack endogenous E-cadherin protein levels) was found to inhibit both these receptors and the phosphorylation of downstream targets. In contrast, treating these cells with insulin or IGF1 decreased the levels of MGAT3 products (in general and specifically E-cadherin), in addition to promoting an increase in invasive capacity and in the levels of mesenchymal markers [77]. *N*-glycans also regulate the modulation of cell-matrix interactions and, therefore, the migratory potential. The biological functions of integrins can be altered by the presence of different patterns of glycans in these molecules [29]. In gastric cancer cells, α3β1 integrin-mediated cell migration has been found to be increased after *MGAT5* overexpression whereas, on the other hand, after *MGAT3* overexpression cell migration was reduced compared to what was observed in control cells [19].

The transition from an epithelial to a mesenchymal phenotype as observed during the progression of carcinomas appears to be accompanied by changes in the expression levels of *MGAT3* and *MGAT5*. Activation of the EMT program in hepatocarcinoma cells by stimulation with hepatocyte growth factor (HGF) was found to be associated with a decreased expression of *MGAT3* and an increased expression of *MGAT5* [78]. Still, in the context of EMT, it was found that in lung cancer cells TGF-β-induced EMT resulted in a concomitant overexpression of *MGAT5* and downregulation of *MGAT3* [79].

It is important to note that the role of β1,6-GlcNAc branched *N*-glycans in carcinoma cell migration and EMT

does not appear to be the same for all types of cancer. For example, in non-small-cell lung cancer cells, the suppression of β1,6-GlcNAc branched *N*-glycans by treatment with an inhibitor or by *MGAT5* silencing has been found to promote TGF-β1-induced EMT-like changes, cell migration, and invasion [80]. Despite this, most of the findings to date reinforce a pro-migratory and pro-tumor role of MGAT5 and branched *N*-glycans.

2.3 Regulation of cell growth, invasion and metastasis by MGAT5 and β1,6-GlcNAc branched *N*-glycans

Despite not being a universal feature, a wide variety of malignant tumors exhibits a high expression of *MGAT5* (Fig. 4), and this characteristic has also been related to the acquisition of malignant potential. As mentioned under 1.5, it is known that a very important mechanism through which β1,6-GlcNAc branches in *N*-glycans cooperate to regulate cell proliferation and differentiation is the differential modulation of membrane receptors. Mechanistically, the addition of β1,6-GlcNAc allows *N*-glycans to generate poly-LacNAc extensions, thereby creating high-affinity ligands for galectins. Galectins bound to these structures and, when linked together, form a molecular structure (lattice) that opposes receptor endocytosis [81]. In this way, it enhances the stabilization of receptor tyrosine kinases (e.g. IGF1R, EGFR, or TGF-βR) on the cell membrane and, consequently, favors the triggering of signaling, thus contributing to tumor growth [65, 82]. Interestingly, activation of the EMT program in retinal pigment epithelium cells has been found to lead to increased levels of β1,6-GlcNAc branched *N*-glycans, which resulted in an increased susceptibility to galectin-3 binding [83]. A majority of receptor tyrosine kinases triggers two main downstream signaling pathways, i.e., the RAS/RAF/MAPK and PI3K/AKT pathways. Opposing the PI3K/AKT signaling pathway by PTEN phosphatase (a tumor suppressor) may involve, among others, the modulation of MGAT5 levels, since PTEN heterozygosity has been found to be associated with an increase in the levels of β1,6-GlcNAc branched *N*-glycans. In the work in which this relationship was demonstrated, the authors proposed that MGAT5 and PTEN could interact in opposite ways to regulate the sensitivity of cells to growth stimuli [84].

Further evidence supporting a pro-malignant role of MGAT5 is that in NOD/SCID mice, tumors formed by colorectal cancer cells that overexpress MGAT5 grew faster than those formed by control cells. In contrast, it was found that tumors generated by the injection of MGAT5 silenced cells (siRNA) grew slower than those in the control group [85]. Corroborating these data, one study showed that tumors generated by inoculation of MC-38 cells (murine colorectal cancer) in C57BL/6J *Mgat5*-/- mice had a lower weight and growth rate than those generated in C57BL/6J *Mgat5*+/+ mice. The authors also found that treatment of MC-38 cells with kifunensin (an inhibitor of α-mannosidase I, which compromises the biosynthesis of complex *N*-glycans) potentiated the decrease in weight and growth rate observed in tumors generated in C57BL/6J *Mgat5*-/- mice [50]. Furthermore, in breast cancer models, it was seen that *MGAT5* deletion in mice resulted in decreased tumor growth and metastasis rates, while overexpression in normal mammary epithelial cells caused neoplastic changes including an abnormal morphology [86, 87].

The acquisition of an invasive phenotype is one of the essential steps in the metastatic cascade. In hepatocellular carcinoma cells, interruption of CD147 (an inducer of matrix metalloproteinase that is aberrantly expressed on the cell surface of tumor cells) through modification with β1,6-GlcNAc branched *N*-glycans led to a reduction in the expression of genes encoding matrix metalloproteinases [88]. Another study reported that matriptase (a transmembrane serine protease), which activates both urokinase-type plasminogen and HGF, is a target protein for MGAT5. In this case, it was shown that overexpression of *MGAT5* in gastric cancer cells led to severe peritoneal spread in athymic mice, which can be attributed to an increased expression of matriptase [89].

In addition to membrane-bound proteins, MGAT5 may also target proteins that are secreted by cells and play important roles in tumor progression. Through analysis of the glycoproteome of human colon carcinoma cells, it was found that aberrant glycosylation of TIMP-1 (tissue inhibitor of metalloproteinase-1), due to *MGAT5* overexpression, potentiates the malignant behavior and the rate of tumor growth [90]. Using the same cells, it was found that *MGAT5* overexpression can promote increased levels of MT1-MMP (membrane-type matrix metalloproteinase-1), thus promoting their invasive potential [91].

It is also important to emphasize the key role of MGAT5 in processes that facilitate metastasis development. In colorectal cells, MGAT5 has been found to confer resistance to anoikis (a form of apoptosis triggered by loss of cell contact with its matrix), as its overexpression protected cells from this type of cell death, while its inactivation by CRISPR/Cas9 made them more sensitive. Furthermore, it was found that *MGAT5* overexpression stimulated both anchorage-dependent and anchor-independent colony formation, even after anoikis-inducing stress [92]. Similarly, it was found that MGAT5 is involved in anoikis resistance in hepatocarcinoma cells [93]. Furthermore, in endometrial and mucinous ovarian cancer cells, a high expression of *MGAT5* seems to be related to invasion of the lymphatic vascular space [94, 95]. In oral squamous cell carcinoma cells, modification of CEACAM6 (Carcinoembryonic antigen-related cell

adhesion molecule 6) by MGAT5 has been found to promote cell invasion, migration, and metastasis [96].

Interestingly, in other tumors, the scenario is quite different. Even though the prognosis is poor for colon, mammary, and esophageal cancers that exhibit high *MGAT5* expression levels, the opposite has been observed in thyroid cancer [25]. In bladder cancer patients, high levels of MGAT5 have been found to be related to a low malignancy potential and a good prognosis [97], including a potential predictive value [98]. Immunohistochemical analysis of samples from patients with testicular germ cell tumors revealed that low levels of MGAT5 are related to malignant transformation and a significantly higher risk of recurrence compared to what is observed in MGAT5-positive tumors [99]. These data suggest that the effects triggered by changes in the levels of β1,6-GlcNAc branched *N*-glycans can be tissue/organ-specific. However, in most cases, they are related to the malignant phenotype. Recently, various genes were identified whose increased expression promoted lung metastasis, as well as several genes with well-characterized pro-metastatic roles, including *MGAT5* [100]. Interestingly, findings by this same research group revealed that *MGAT5* expression is not linked to melanoma progression, thus demonstrating once again that pro-tumor mechanisms mediated by *MGAT5* and β1,6-GlcNAc branched *N*-glycans can be cell- or tumor-specific [101].

2.4 Role of MGAT5 and β1,6-GlcNAc branched *N*-glycans in the maintenance of stemness

Cancer stem cells promote a heterogeneous and plastic population within the tumor mass [102]. Due to their characteristics of self-renewal and the generation of progenitor cells capable of differentiating, they are considered responsible for intratumor heterogeneity and maintenance of the tumor mass [103]. Therefore, cancer stem cells are considered promising targets because these cells are believed to be resistant to traditional therapies and responsible for tumor repopulation after treatment, leading to disease recurrence [104].

A study on colorectal cancer cells showed that high levels of MGAT5 promoted an increase in the stem cell population (aldefluor-positive), while its silencing (siRNA) had the opposite effect. The same study showed that colon cancer cells with stem cell-like properties exhibited a significant increase in *MGAT5* expression, which was also accompanied by increased levels of *N*-glycans recognized by L-PHA. The mechanism by which MGAT5 might regulate stemness and other events related to tumorigenesis could be modification of the FZD-7 membrane receptor (where the WNT protein binds) by β1,6-GlcNAc branched *N*-glycans [85]. Also, it has been reported that *MGAT5* is expressed at a relatively high level in CD133 + cells from lung adenocarcinoma, and that its knockdown results in inhibition of cancer cell growth in vitro and in vivo [105]. Another possible role of β1,6-GlcNAc branching *N*-glycans in stemness maintenance was recently noted in glioblastoma stem cells, in which CRISPR-Cas9 mediated knockout of *MGAT5* resulted in both a decreased migration as well as a reduction in protein levels associated with focal adhesion and mesenchymal-epithelial transition [106]. Moreover, as mentioned under 1.4, it has been concluded that IGF2BP1 can promote the liver cancer stem cell phenotype by regulating MGAT5 mRNA stability through m6A RNA methylation [11].

2.5 Role of MGAT5 and β1,6-GlcNAc branched *N*-glycans in anti-tumor immune responses

Some cancer biology studies have highlighted the importance of glycosylation in the immunological context. In breast cancer cells it has, for example, been seen that *MGAT5* knockout can promote a decrease in their growth and promote a better response of CD4 + T-cells and macrophages [107]. One of the immune escape strategies of tumor cells is the interaction between programmed death ligand-1 (PD-L1) and programmed cell death protein-1 (PD-1), since the PD-1/PD-L1 axis is an important inhibitory pathway involved in the regulation of T-cell responses [108]. In triple-negative breast cancer, glycosylation of PD-L1 has been found to be essential for the PD-L1/PD-1 interaction and to be vital for immunosuppression [109]. Recently, it has been found that colorectal cancer cells may use branched *N*-glycans as an escape strategy from immune recognition, instructing the creation of immunosuppressive networks through the inhibition of IFNγ (interferon gamma). By removing this "mask" formed by branched *N*-glycans, immunogenic mannose residues are exposed, enabling and potentiating immunological recognition by cells of the immune system that express DC-SIGN (C-type lectin receptor present on the surface of macrophages) and dendritic cells. As a result, an effective anti-tumor immune response occurs. This study thus revealed a new glyco-immunological checkpoint in CRC, highlighting how a tumor-specific glycosignature may serve as a biomarker for the identification of individuals at risk of disease progression [50].

2.6 MGAT5 and β1,6-GlcNAc branched *N*-glycans in the context of metabolic changes in cancer

As already discussed under 1.4, metabolic alterations can modulate the levels of branched *N*-glycans. In this context, it has been shown that exposure to high glucose concentrations may increase UDP-GlcNAc biosynthesis and potentiate characteristics related to the malignant phenotype of MC38 murine colon adenocarcinoma cells. The same study revealed a significant increase in the levels of glycans

recognized by L-PHA after exposure of 4T1 murine mammary carcinoma cells to high levels of glucose, suggesting that both aberrant glycosylation and potentiation of the malignant phenotype could be related to exacerbation of the hexosamine biosynthesis pathway [110].

2.7 Involvement of cancer-related epigenetic modifications on the levels of MGAT5 and β1,6-GlcNAc branched *N*-glycans

Aberrant glycosylation impacts oncogenesis at several levels, and although it is influenced by multiple mechanisms, recent data suggest that those underlying altered expression may include epigenetic regulation (through e.g. miRNAs and DNA methylation) [111]. Thus, as mentioned earlier, it has been found miR-124 can target *MGAT5*, thereby inhibiting its expression. Conversely, overexpression of *MGAT5* may attenuate the inhibitory effects of this miRNA on proliferation and migration in breast cancer cells. Thus, modulation of *MGAT5* by miR-124 may explain why miR-124 downregulation promotes the development of breast cancer [12]. A later study underscored this regulatory role of miR-124. In chemoresistant lung cancer cells, a lower expression of *MGAT5* was observed when miR-124 was upregulated, and this effect was reversed when *MGAT5* was exogenously overexpressed [112].

In addition, in breast and ovarian cancer it has been found that in cells exposed to hypoxic conditions the levels of branched *N*-glycans, as well as the expression of *MGAT5*, could be modulated through changes in the global level of DNA methylation. Hypomethylation promoted expression of the gene encoding the transcription factors GATA2 and GATA3, which was accompanied by an increase in the expression of *MGAT5*. This reduction in DNA methylation also led to an increase in the levels of complex *N*-glycans (branched and sialylated), overexpression of EMT markers, and a greater migratory capacity [113]. In Table 1 evidence linking MGAT5 and β1,6-GlcNAc branched *N*-glycans to cellular and molecular mechanisms in different types of cancer is listed.

3 Translational aspects and future directions

The development of alternative therapeutic strategies to traditional methods of cancer management, such as chemotherapy, radiotherapy, and surgery, is warranted to enhance patient survival, especially when detected at advanced stages. Although glycan-mediated cellular mechanisms are not completely deciphered yet and much remains to be understood, several physiologically or pathologically regulatory functions related to the role of branched *N*-glycans are dealt with here. Functional glycobiological studies have provided several translational opportunities, such as (1) the recognition of glycosylation profiles for improving clinical diagnosis or prognosis, (2) the development of vaccines or drugs targeting a glycan or glycosylation pathway, (3) the enhancement of immunity by editing glycosylation, and even (4) the glycosylation of pharmaceuticals for delivery improvement [119–122]. Aberrant glycosylation of membrane-bound or secreted proteins associated with neoplastic transformation is a proven hallmark of cancer, and some carbohydrate antigens are already utilized as serological biomarkers for diagnosis or monitoring of malignant disease progression, and also as prognostic biomarkers of disease recurrence (e.g. CA19-9 and CA72-4) [123]. As discussed here, MGAT5 and β1,6-GlcNAc branched *N*-glycans play important roles in tumor development, so studies on inhibitors that may be drug candidates for cancer treatment are justified. One drug that has been tested is swainsonine, a potent inhibitor of Golgi alpha-mannosidase II, which acts as an inhibitor of complex *N*-glycans [124]. Although in vitro and in vivo assays have shown that swainsonine exhibits anti-tumor activity [125, 126], when evaluated in a clinical trial of locally advanced or metastatic renal cell carcinoma, it did not yield satisfactory results, since all patients discontinued treatment due to disease progression or toxicity [127]. Therefore, more recent studies have sought alternatives for *N*-glycan inhibition aimed at minimizing possible adverse effects. Recently, it has been shown that inhibition of the mevalonate pathway with fluvastatin (a drug clinically approved for patients with high serum cholesterol levels) reduces the levels of branched *N*-glycans, including those associated with activation of the EMT program. This same work showed that in MGAT5-deficient MDA-MB-231 cells (human breast adenocarcinoma), the IC50 for fluvastatin treatment was significantly lower than that observed in wild-type cells [128]. Encouraging results showed that a glycomimetic compound that specifically inhibits MGAT5 activity, named PST3.1a, was able to affect the microtubule and microfilament integrity of glioblastoma multiforme stem cells, leading to the inhibition of migration, invasiveness, and proliferation of initiating cells [117]. Very recently, several UDP-GlcNAc analogs have been developed and tested as inhibitors of *N*-acetylglucosaminyltransferase. Interestingly, although their inhibitory potency turned out to be modest, these compounds showed a greater preference for MGAT5 than other MGATs, showing that using chemically modified substrates may be a promising strategy for specific inhibition of MGAT5 [129]. Moreover, improvements in the design of specific inhibitors may also come from studies that showed in more detail, from a structural point of view, how MGAT5 recognizes its substrates [21, 130].

The deficiency of anticancer strategies aimed at overcoming chemo/radioresistance often translates into high

Table 1 Contribution of MGAT5 and β1,6-GlcNAc branched *N*-glycans in cancer-related cellular and molecular mechanisms

Type of cancer	Sample	Glyco-phenotypic change	Cancer-related effect	Reference
Colorectal cancer	Cell line	Increased levels of β1,6-branched *N*-glycans	Mislocalization of E-cadherin and increased in the proliferative rate	[70]
	Patient-derived specimens	High expression of MGAT5	Correlation with distant metastasis	[49]
	Cell line and mouse model	Increased levels of β1,6-branched *N*-glycans and high expression of MGAT5	Suppression of antitumor immune response and increased tumor growth	[50]
	Cell line and mouse model	Reduced levels of β1,6-branched *N*-glycans and knockdown of MGAT5	Reduction of both cancer stem cell subpopulation and tumor growth. Decreased expression of Wnt target genes	[85]
	Cell line	Overexpression of MGAT5	Confers resistance to anoikis	[92]
Oral cancer	Cell line	Knockdown of MGAT5	Reduces CEACAM6-induced cell migration	[96]
Leukemia	Cell line	Increased levels of β1,6-branched *N*-glycans	Hyperglycosylation of CD19 leads to CAR T cell failure	[114]
Pancreas cancer	Tumor xenograft	Knockout of MGAT5	Boosts CAR T cell therapy efficacy	[115]
Melanoma	Mouse model	High expression of MGAT5	Metastasis development	[101]
Lung cancer	Tumor xenograft	Knockdown of MGAT5	Inhibits the proliferation rate of $CD133^+$ cells	[105]
	Cell line	Knockdown of MGAT5	Reduces anchorage-dependent colony formation	[112]
	Cell line and Tumor xenograft	Increased levels of MGAT5	Activation of EMT program, radioresistance, and increased growth and migration rate	[116]
Glioblastoma	Cell line	Knockout of MGAT5	Reduces migratory capacity	[106]
	Cell line and Tumor xenograft	Inhibition of MGAT5 enzymatic activity	Inhibits proliferation, migration, invasiveness, and clonogenic capacities	[117]
Breast cancer	Mouse model	Knockdown of MGAT5	Suppresses tumor progression, stimulates Th1 cytokine production, and enhances opsonophagocytic capability of macrophages	[107]
	Mouse model	Knockout of MGAT5	Delayed skin inflammation induced by either arachidonic acid or phorbol ester	[81]
	Cell line and Tumor xenograft	Restauration of MGAT5 levels	Attenuation of the inhibitory effects of miR-124 on proliferation and metastasis	[12]
	Patients-derived specimens	Increased levels of β1,6 branched *N*-glycans	Association with nodal metastasis	[57]
	Cell line	Knockout of MGAT5	Deficiency in fibronectin-dependent cell spreading	[29]
Gastric cancer	Cell line	Overexpression of MGAT5	Impairment of E-cadherin-mediated cell-cell adhesion	[27]
	Cell line	Knockdown of MGAT5	Decreased cytoplasmic localization of E-cadherin concomitant with its greater localization in the plasma membrane	[118]
	Cell line	Increased levels of β1,6-branched N-glycans and overexpression of MGAT5	Increased α3β1 integrin-mediated cell migration on laminin 5	[19]
Ovary cancer	Cell line	Reduced levels of β1,6-branched *N*-glycans	Reduces migratory capacity	[94]
Liver cancer	Cell line	High expression of MGAT5	Resistance to anoikis through EGFR/PAK1 activation	[93]

Table 1 (continued)

Type of cancer	Sample	Glyco-phenotypic change	Cancer-related effect	Reference
	Cell line	Knockdown of MGAT5	Reduces cell migration and invasion and decreases expression of genes encoding metalloproteinases	[88]
	Cell line	Knockdown of MGAT5	Decreases the invasion and metastasis abilities induced by Barx1 knock-down	[59]
	Cell line	Increased levels of β1,6-branched N-glycans	Correlation with activation of the HGF-induced EMT program	[78]
Fibrosarcoma	Cell line	Overexpression of MGAT5	Decreased calcium-dependent cell-cell adhesion and increased motility	[72]

mortality rates. In prostate and nasopharyngeal cancer cells, inhibition of MGAT5 increased their radiosensitivity due to, among others, an increase in irradiation-induced apoptosis [131, 132]. Radiotherapy is an important medical procedure adopted for patients with small cell lung cancer, but many patients do not benefit partly due to acquired resistance to the treatment [133]. Interestingly, overexpression of MGAT5 in cells of this type of cancer conferred an increased resistance to irradiation concomitant with a decrease in BAX/BCL2 ratio, an increase in migration, and the expression of EMT markers [116].

In recent decades, the use of small-molecule agents, such as specific inhibitors, and monoclonal antibodies (mAbs), has become a molecular-targeted anticancer approach available in clinical practice [134]. In this context, it has been found that insight into the glycosylation in monoclonal antibodies (mAbs) is important in the development of these molecules for therapeutic purposes, since the stability, immunogenicity, and biological activity can be further optimized through glycoengineering [135]. Indeed, alterations in the *N*-glycosylation of IgG class mAbs may crucially affect their structural and functional properties [136, 137]. However, it is still unclear whether β1,6-GlcNAc branched *N*-glycans play any role in this respect.

During tumor progression, cancer cells may develop strategies to evade the immune response against the tumor. Although several studies point to the potential use of immunotherapies to treat the disease, only a small proportion of patients benefit [138, 139]. Thus, further studies are needed to elucidate the mechanisms involved and to search for new therapeutic targets. In this regard, MGAT5 and β1,6-branched *N*-glycans represent new possibilities. One of the great successes of immunotherapy was the blockade of PD-1 and PD-L1, which revolutionized the clinical treatment of cancer (e.g. melanoma, non-small cell lung cancer, renal cell carcinoma, and head and neck cancer) and provided significant survival benefits to patients [140, 141]. It is known that PD-L1 is highly *N*-glycosylated [142] and it has been found that deglycosylation of the cell surface of cancer cells via enzymatic digestion may result in a significant improvement in the binding affinity of anti-PD-L1 antibodies [143]. A recent study also showed that inhibition of complex-type *N*-glycan with swainsonine together with anti-PD-L1 treatment in melanoma and lung cancer models led to suppression of tumor growth, whereas each treatment alone had little effect [144]. These results underscore that manipulating glycosylation can be a promising strategy to overcome the limitations of cancer treatment and improve the therapeutic efficacy of immune checkpoint blocking strategies. Additional recent work has pointed out that editing *N*-glycosylation can improve CAR T-cell therapy in solid tumors. Engineering T-cells to express chimeric antigen receptors (CARs) targeting cancer-associated antigens has been found to be an effective tool to treat hematological neoplasms [145, 146], but to a lesser extent to treat solid tumors [147]. Several obstacles may contribute to this latter result, including inefficient infiltration and trafficking of the CAR T-cells and a lack of specific tumor antigens. Even when these difficulties are overcome, the therapeutic benefit of CARs depends on the formation of a lytic immune synapse for these cells to carry out their biological functions [148]. It has been pointed out that one of the possible resistance mechanisms that tumor cells use to escape death induced by CAR T-cells is aberrant glycosylation of proteins on their surface [114, 149]. Very encouraging recent results revealed that both knocking out of *MGAT5* and inhibition of *N*-glycan synthesis with 2DG (a glucose/mannose analog) enhanced CAR T-cell activity in different xenograft mouse models of pancreatic adenocarcinoma. This strategy of combining 2DG and CAR T-cell therapy has also been successfully applied in other solid tumor models, such as bladder, lung, and ovary cancer [115]. These results highlight modulation of *N*-glycosylation as a potential strategy to improve immunotherapy.

4 Conclusions and perspectives

The data gathered here show how relevant the physiological and pathophysiological roles played by MGAT5 and β1,6-GlcNAc branched N-glycans are. It is clear that these

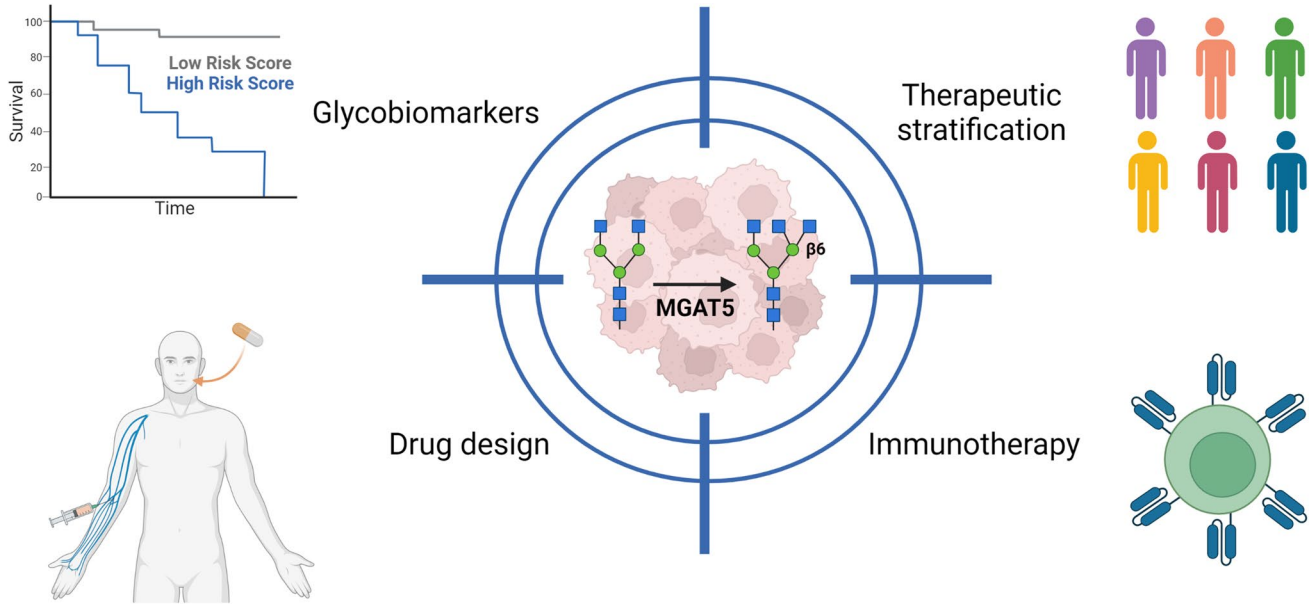

Fig. 6 Translational aspects derived from studies on MGAT5 and β1,6-GlcNAc branched *N*-glycans in the context of cancer. *MGAT5* expression or β1,6-GlcNAc branched *N*-glycan levels can be used as biomarkers capable of predicting clinical outcomes. The MGAT5 enzyme can be considered a target for developing new drugs (e.g. specific inhibitors). Information regarding the expression of *MGAT5* or levels of β1,6-GlcNAc branched *N*-glycans can discern molecular subtypes of different tumors, thus improving the therapeutic stratification of patients. Modulation of MGAT5 or the levels of β1,6-GlcNAc branched *N*-glycans may potentiate the recognition of tumor cells by the immune system

players act on cellular mechanisms that contribute to the development and progression of solid tumors. Encouraging data show that the knowledge accumulated in this area could, in the coming years, be translated into clinical applications that will bring effective benefits to cancer patients. Figure 6 illustrates several translational aspects derived from studies focused on the role of MGAT5 and β1,6-GlcNAc branched *N*-glycans in the context of cancer.

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Authors' contributions Study design: MdSF, EEF, and JCMdFJ. Literature search and data collection: MdSF, EEF, and JCMdFJ. Data analysis: MdSF and JCMdFJ. Manuscript drafting: MdSF, EEF, and JCMdFJ. Manuscript editing and revision: MdSF and JCMdFJ. Supervision: JCMdFJ.

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Declarations

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