Chapter 9
Tumor-Associated Glycans and Their Functional Roles in the Multistep Process of Human Cancer Progression

Reiji Kannagi, Keiichiro Sakuma, Bi-He Cai, and Shin-Yi Yu

Abstract Cancer develops through a multistep process of carcinogenesis. This process accompanies incremental alterations of expression of biologically functional glycans on the surface of cancer cells. A variety of glycans are expressed in nonmalignant epithelial cells, including several normal glycans serving as ligands for siglecs, the immunosuppressive molecules carried by interstitial immune cells. These normal glycans decrease or disappear and are replaced by cancer-associated glycans at the early stages of carcinogenesis. This glycan transition facilitates production by mucosal immune cells of inflammatory mediators that are known to promote cancer progression. Expression of glycans that regulate growth factor receptor functions is also affected at the early stages of cancers. The major mechanism involved in glycan alteration at the early stages is epigenetic silencing through DNA methylation and/or histone deacetylation/methylation of genes responsible for synthesis of normal glycans, leading to their incomplete synthesis. In the locally advanced stages, multiple glycan-related genes are induced transcriptionally and posttranscriptionally by tumor hypoxia and epithelio-mesenchymal transition, thus further culminating in abnormal expression of cancer-associated glycans. Some such glycans serve as specific ligands for selectins, the cell adhesion molecules carried by vascular endothelial cells, and facilitate tumor vascularization and ultimately hematogenous metastasis. Advanced cancer cells which have undergone epithelio-mesenchymal transition share biological characteristics with so-called cancer stem cells, and glycans associated with such cells are currently known to be frequently expressed in human embryonic stem cells as well.

R. Kannagi (✉) • B.-H. Cai • S.-Y. Yu
The Institute of Biomedical Sciences (IBMS), Academia Sinica,
128 Sec. 2, Academia Rd. Nankang, Taipei 11529, Taiwan, R.O.C.
e-mail: rkannagi@ibms.sinica.edu.tw

K. Sakuma
Division of Molecular Pathology, Aichi Cancer Center,
#1-1 Kanokoden, Chikusa-ku, Nagoya, Aichi 464-8681, Japan
e-mail: ksakuma@aichi-cc.jp

T. Suzuki et al. (eds.), *Sugar Chains*, DOI 10.1007/978-4-431-55381-6_9

Keywords Cancer progression • Sialyl Lewis A • Sialyl Lewis X • Epigenetic silencing • DNA methylation • Histone deacetylation/methylation • Sulfate transporter • Siglec • Selectin • Hypoxia inducible factor • Epithelio-mesenchymal transition (EMT) • Cancer stem cells • Embryonic stem (ES) cells

9.1 Introduction

Cancer develops through a multistep process of carcinogenesis. Tumor progression is caused by genetic and epigenetic alterations of a variety of key regulatory molecules, and this process accompanies an incremental alteration of expression of biologically functional glycans on the surface of cancer cells. Here we will review recent findings on the mechanisms through which genetic and epigenetic alterations affect glycan expression in cancer cells and the pathophysiological roles of altered glycan expression during the course of cancer progression.

9.2 Epigenetic Silencing of Glycan-Related Genes Causing "Incomplete Synthesis" of Normal Glycans at Early Stages of Cancer

It has long been known that glycans undergo drastic changes upon carcinogenesis. We had classified the cancer-associated changes of glycan expression into two categories almost three decades ago; one had been "incomplete synthesis" of normal glycans and the other "*neo*synthesis" of abnormal glycans (Hakomori and Kannagi 1983).

The concept of "incomplete synthesis" had referred to the accumulation of structurally simpler abnormal glycans due to disturbance of synthetic pathways for normal glycans, which mainly occurs during the course of early carcinogenesis. This concept assumed some suppression to occur in the transcription/translation of genes involved in the synthesis of normal glycans during carcinogenesis. At present, the major suppression mechanism is regarded to be epigenetic silencing. The process of "incomplete synthesis" is known to start working at relatively early stages of carcinogenesis.

On the other hand, the concept of "*neo*synthesis" had referred to the appearance of abnormal glycans in cancers, which are not present, or present only in a negligible amount, in normal cells. This had been assumed to be due to transcriptional induction of genes involved in the synthesis of abnormal glycans along with progression of cancers. Nowadays, acceleration of transcription/translation of genes involved in the synthesis of these abnormal glycans is known to occur frequently along with the multistep progression of cancers at advanced stages.

9.3 Examples of Key Glycan-Related Genes Exhibiting Epigenetic Silencing at Early Stages of Cancer

A variety of glycans are expressed in normal epithelial cells, expression of some of which is conventional in that they are also constitutively expressed in cancers. In contrast, some other glycans exhibit preferential expression in nonmalignant epithelial cells and tend to decrease or disappear and be replaced by cancer-associated glycans upon malignant transformation. These glycans are aptly named "normal" glycans, although in a narrow definition of the word (Fig. 9.1).

Such normal glycans include disialyl Lewis A, which was found to be preferentially expressed in nonmalignant epithelial cells of the digestive organs (Kannagi et al. 1988; Itai et al. 1990, 1991), and clinical evaluation of it in patients' sera was proposed to be useful for making differential diagnoses of benign and malignant diseases, especially when routine serum determination of sialyl Lewis A, a well-known cancer-associated glycan, gave false-positive results (Kannagi 2007). Soon it was found that the disialyl Lewis A glycan is a normal counterpart of the cancer-associated glycan, sialyl Lewis A, and that epigenetic silencing of *ST6GalNAc6*, a gene for α2,6sialyltransferase, is the key for diminishing expression of disialyl Lewis A and inducing sialyl Lewis A in cancers (Miyazaki et al. 2004). Downregulation of *ST6GalNAc6* was observed at the early stages of colon carcinogenesis in the normal-adenoma-carcinoma sequence (Bowden et al. 2007). This interconversion of glycans seems to be applicable to a wider range of cancers than initially expected. In addition to the cancers of digestive organs, preferential loss of disialyl Lewis A expression is also noted in prostate cancers (Young et al. 1988), and cancer-associated decrease of the *ST6GalNAc6* mRNA level is noted in breast (Potapenko et al. 2010) and renal (Senda et al. 2007) cancers, as well as glioblastoma (Kroes et al. 2007).

Another example of a "normal" glycan is sialyl 6-sulfo Lewis X, which was found to be preferentially expressed in nonmalignant epithelial cells of the colon and to disappear in colonic cancer cells (Izawa et al. 2000). This finding was in line with the classical histochemical finding on colon cancer glycans that the amount of sulfomucin is decreased in cancers compared to nonmalignant colonic tissues (Shamsuddin et al. 1981). Sialyl 6-sulfo Lewis X glycan is a normal counterpart of the well-known cancer-associated glycan, sialyl Lewis X, and epigenetic silencing of *SLC26A2*, a gene for sulfate transporter DTDST, was found to be a key mechanism responsible for diminished expression of sialyl 6-sulfo Lewis X and appearance of sialyl Lewis X in cancers (Yusa et al. 2010). Downregulation of *SLC26A2* was also observed at the early stages of colon carcinogenesis (Lee et al. 2006). It is notable that not only the genes for glycosyltransferases, which are directly involved in glycan synthesis, but also those for some transporters or enzymes in the intermediate carbohydrate metabolism are capable of playing a key role in the cancer-associated glycan alteration.

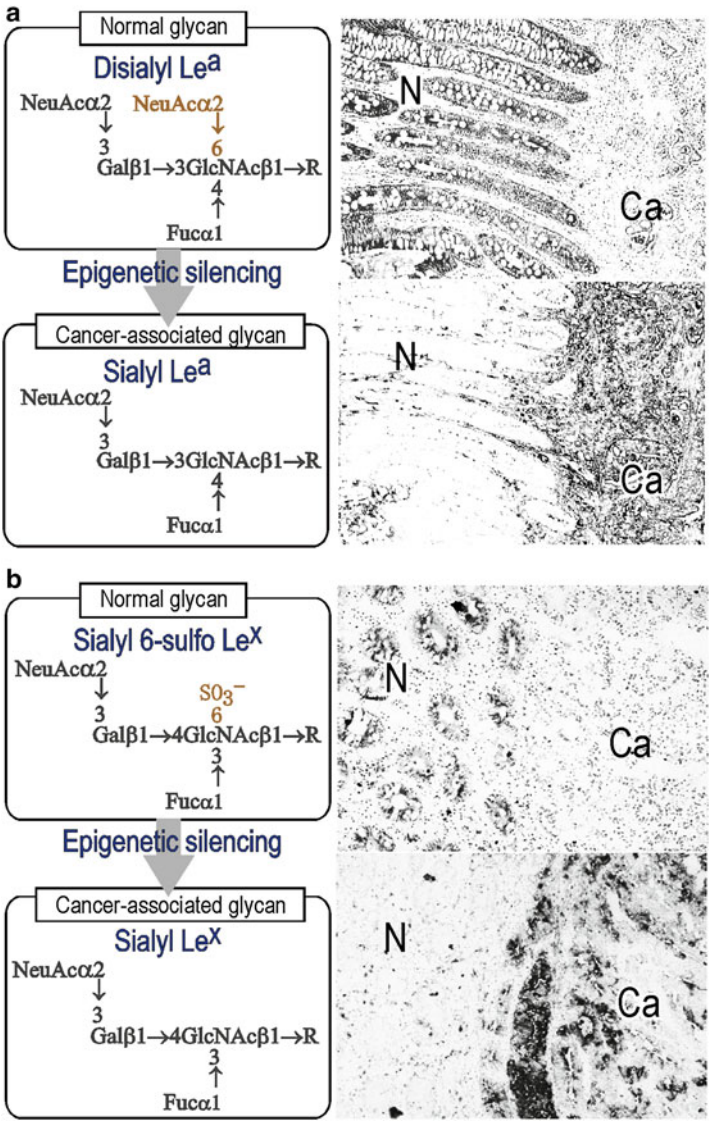

Fig. 9.1 Examples of interconversion of normal glycans into cancer-associated glycans. *Panel a*, transition of normal glycan, disialyl Lewis A, to cancer-associated glycan, sialyl Lewis A, upon malignant transformation. *Panel b*, conversion of normal glycan, sialyl 6-sulfo Lewis X, to a cancer-associated glycan, sialyl Lewis X glycan, upon malignant transformation. Typical distribution patterns shown were obtained by immunohistochemical staining using specific anti-glycan antibodies of consecutive sections prepared from colon cancer tissues. *Ca* cancer cells, *N* nonmalignant epithelial cells (Adopted from references Izawa et al. 2000; Miyazaki et al. 2004; Lim et al. 2008)

9.4 Biological Functions of Normal Glycans

The hallmark of early carcinogenesis is the acquisition of a highly proliferative activity and/or suppression of apoptosis by the transforming cells. Several glycans are known to affect cell proliferation. GM3 and other monosialogangliosides have long been known to suppress EGF receptor signaling by their direct interaction with the receptor molecule (Bremer and Hakomori 1982; Hakomori 2010). Likewise, GM2 is known to suppress c-Met kinase pathway (Todeschini et al. 2008). On the other hand, disialogangliosides, such as GD3 and GD2, are reported to enhance cell proliferation through activation of FAK and Lyn kinases (Furukawa et al. 2012). N-glycans and related genes including *MGAT5* are also known to affect growth factor receptor signaling (Matsumoto et al. 2008; Park et al. 2012; also reviewed in Lau and Dennis 2008). The glycan-related genes involved in O-glycan synthesis such as *GALNT14*, *GALNT2*, and *C1GALT1* on death receptors, and growth factor receptors such as c-Met and EGFR have recently been suggested to affect cancer cell apoptosis and proliferation (Wagner et al. 2007; Wu et al. 2011, 2013). Altered expression of these glycans may well play significant roles during the course of carcinogenesis.

There are other indications for a more indirect association of cell proliferation status with glycan expression. For instance, the decreased transcription of *SLC26A2* in cancers mediates the loss of normal glycan sialyl 6-sulfo Lewis X and induction of cancer-associated glycan sialyl Lewis X and at the same time strongly induces cell proliferation (Yusa et al. 2010). The growth suppressive effect of the *SLC26A2* gene was clearly reproduced in experiments using a Tet-off expression vector for *SLC26A2* (Yusa et al. 2010) (Fig. 9.2). It is not clear whether or not the growth suppression conferred by this gene is due to its effects on glycan sulfation, because *SLC26A2* is a sulfate transporter gene which can affect not only glycan sulfation but also sulfation of other molecules such as proteins and lipids. Still, it can at least be proposed that this is another example indicating the close link between change in glycan expression and cell proliferation status.

Cancer microenvironments also play crucial roles during the course of carcinogenesis. In the mouse model of colon carcinogenesis, mutation in the APC gene induces proliferation of epithelial cells leading to multiple benign polyp formation. Meanwhile, Taketo's group found that the malignant transformation of adenoma cells was observed selectively at the locus where interstitial cells in colonic mucosal membranes produce COX2, which is a pro-inflammatory molecule known to promote cancer progression (Oshima et al. 1996). This finding became the theoretical basis for developing COX2 inhibitors for the chemoprevention of colon carcinogenesis. Although difficulty was encountered during the development of specific COX2 inhibitors because they have intrinsic toxic cardiac effects, it is still true that inflammatory signaling pathways are activated in various cancers including colon cancer linking chronic inflammation to oncogenesis. Alternative modalities are now awaited for the chemoprevention of colon carcinogenesis.

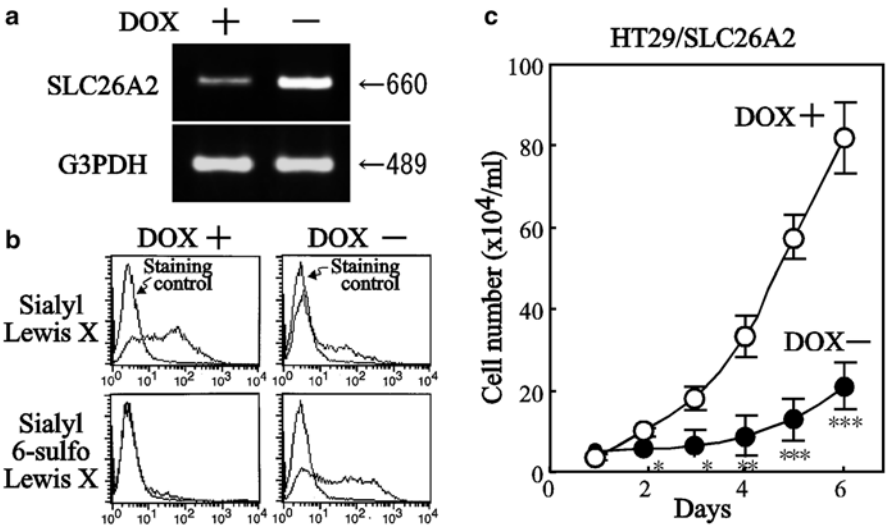

Fig. 9.2 Possible link between glycan expression change and cancer cell growth. Results of RT-PCR (*panel a*), flow-cytometry (*panel b*), and cell proliferation assays (*panel c*) are shown on colon cancer cell line HT29 that was transfected with Tet-off inducible expression vector for *SLA26A2*. When cultured without doxycycline, the *SLA26A2* gene is actively transcribed, resembling nonmalignant epithelial cells, and the normal glycan, sialyl 6-sulfo Lewis X, is strongly expressed on the cell surface. This is associated with suppression of cell proliferation. In contrast, when cultured with doxycycline, transcription of *SLA26A2* is repressed and resembles cancer cells, leading to the extinction of normal glycan expression and induction of the cancer-associated glycan, sialyl Lewis X, which is coupled with enhanced cell proliferation. The difference was statistically significant at *, $p<0.05$; **, $p<0.01$; and ***, $p<0.001$ (Adopted from reference Yusa et al. 2010)

In this context, it is noteworthy that the normal glycans in colonic epithelial cells such as disialyl Lewis A and sialyl 6-sulfo Lewis X were both shown to serve as ligands for siglecs, which are glycan-recognition molecules having immunosuppressive ITIM motifs in their cytoplasmic domains and are expressed by a variety of immune cells. The sialyl 6-sulfo Lewis X glycan was shown to be the ligand for siglec-7, and disialyl Lewis A was found to serve as a ligand for both siglec-7 and siglec-9 (Miyazaki et al. 2004, 2012) (Fig. 9.3). A significant number of tissue macrophage-like cells expressing siglec-7 or siglec-9 were present in normal colonic mucosal membranes, and ligation of either siglec had suppressive effects on the production of COX2 and IL-12 by macrophage-like cells (Miyazaki et al. 2012) (Fig. 9.3). Based on these results, it was proposed that normal glycans may play a role in maintaining immunological homeostasis and preventing cancer progression. The loss of these normal glycans due to epigenetic silencing of the key genes upon malignant transformation is expected to further facilitate carcinogenesis.

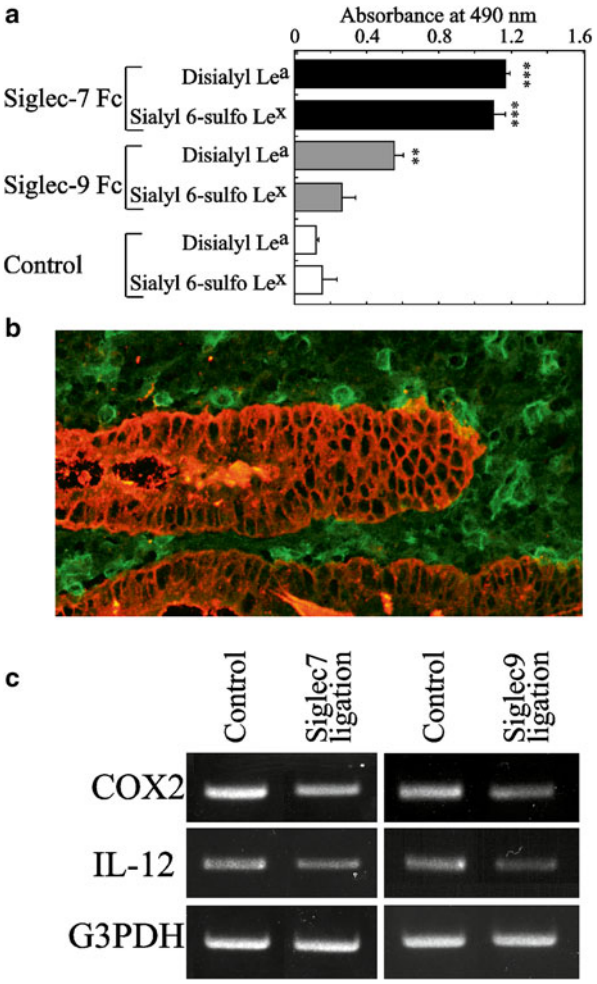

Fig. 9.3 Normal glycans serve as ligands for siglecs. *Panel a*, normal glycans such as disialyl Lewis A and sialyl 6-sulfo Lewis X, but not cancer-associated glycans, serve as specific ligands for siglecs, which are immunosuppressive receptors carried by immune cells in mucosal membranes. *Panel b*, a human colon tissue stained with anti-siglec and anti-glycan MoAbs, suggesting possible interaction between tissue macrophage-like cells expressing siglec-7 (*green*) and colonic epithelial cells expressing its ligand disialyl Lewis A (*red*). *Panel c*, suppression of LPS-induced COX2 and IL-12 in cultured macrophage-like cells by ligation of siglec-7/-9. TPA-treated U937 cells transfected with siglec-7 or siglec-9 were stimulated with LPS with or without a $F(ab')_2$ fragment of an anti-siglec-7 or anti-siglec-9 antibody (Adopted from references Miyazaki et al. 2004, 2012)

9.5 Mechanisms for Epigenetic Silencing of Glycan-Related Genes During the Course of Carcinogenesis

Aberrant promoter CpG island hypermethylation is one of the most common and well-established epigenetic abnormalities in cancer. In earlier studies, DNA methylation of A- and B-enzymes was shown to cause a decrease of normal A- and B-blood type glycans in cancers (Kominato et al. 1999; Chihara et al. 2005). Likewise, loss of the Sd^a blood group substance in colon cancer was shown to be due to DNA methylation of the *B4GALNT2* gene promoter (Kawamura et al. 2008). The decrease in all these glycan epitopes attached to the lactosamine or polylactosamine backbone structures is proposed to facilitate expression of cancer-associated glycan epitopes such as sialyl Lewis A and sialyl Lewis X, synthesized from common precursors, by leaving the surplus substrates for the enzymes responsible for synthesis of the latter two cancer-associated glycans (Kannagi et al. 2008).

Table 9.1 summarizes hitherto known glycan-related genes in cancers which represent epigenetic silencing. There are many examples of genes involved in glycan synthesis which are regulated by DNA methylation. *HS3ST2* (*3OST2*) is a typical example of genes known to be strongly hypermethylated in a variety of cancers (Miyamoto et al. 2003) and is sometimes utilized even as a positive control in DNA methylation analyses. The biological significance of this gene was not known until the heparan sulfate 3-O-sulfotransferase encoded by this gene was recently reported to suppress cell proliferation and migration (Hwang et al. 2013). The exact mechanisms behind these observed phenomena await further investigation, but this is an indication of another link between glycan expression and cancer cell proliferation. The roles of extracellular heparan sulfates as extracellular coreceptors for growth factors have been well documented (Fuster and Esko 2005).

Histone modification is also intimately involved in cancer-associated epigenetic silencing of glycan-related genes. Transcription of *ST6GalNAc6* was initially reported to be recovered either by treatment with histone deacetylation inhibitor (butyrate) or DNA methylation inhibitor (5-aza-2′-deoxycytidine) (Miyazaki et al. 2004), but the effect of the DNA methylation inhibitor was later found to be dependent on the cell lines used in experiments, and histone deacetylation turned out to be the major mechanism for silencing this gene. Histone modification was also proposed to be the major mechanism for epigenetic silencing of the sulfate transporter gene *SLC26A2*, and in the case of this gene, significant participation of histone trimethylation at H3K27 was suspected in addition to histone deacetylation (Yusa et al. 2010) (Fig. 9.4). Accordingly, addition of not only HDAC inhibitors, but histone methyltransferase inhibitor DZNep, was shown to stoichiometrically induce *SLC26A2* transcription (Fig. 9.4). Participation of DNA methylation in cancer-associated suppression of this gene was recently reported for papillary thyroid cancer (Zhang et al. 2012).

Epigenetic drugs are not yet actively utilized for therapy of cancers, but several reports suggest them to be beneficial for chemoprevention of cancers (Ravillah et al. 2014; Timp and Feinberg 2013). Assessment of normal glycan expression may be useful for monitoring therapeutic effects in such regimens.

Table 9.1 Examples of glycan-related genes in cancers which are known to represent epigenetic silencing

Genes	Glycans involved	Mechanisms	References
ABO	ABO(H) blood group substance	DNA methylation	Kominato et al. (1999)
HS3ST2 (*3OST2*)	Heparan sulfate (3S)	DNA methylation	Miyamoto et al. (2003)
GNE	CMP-sialic acid	DNA methylation	Oetke et al. (2003)
ST6GALNAC6	Sialyl Lewis A/disialyl Lewis A	Histone acetylation, DNA methylation	Miyazaki et al. (2004)
EXT1	Heparan sulfate	DNA methylation	Ropero et al. (2004)
EXTL3	Heparan sulfate	DNA methylation	Karibe et al. (2008)
FUT3	Lewis A	DNA methylation	Serpa et al. (2006)
MGAT5	*N*-glycan branching	DNA methylation	Chakraborty et al. (2006)
MGAT4A	*N*-glycan branching	DNA methylation, histone acetylation	Ide et al. (2006)
SULF1	Heparan sulfate (6S)	DNA methylation, histone acetylation	Staub et al. (2007)
B4GALNT2, *ST3GAL6*	Sd^a/sialyl Lewis A	DNA methylation	Kawamura et al. (2008)
B4GALNT2	Sd^a	DNA methylation	Wang et al. (2008)
SLC26A2 (DTDST, sulfate transporter)	Sialyl Lewis X/sialyl 6-sulfo Lewis X	Histone methylation, histone acetylation	Yusa et al. (2010)
FX, *SLC35C1* (GDP-fucose transporter), *FUT4*	Fucose in TRAIL signaling	DNA methylation	Moriwaki et al. (2010)
HS3ST1, *HS3ST2*, *HS3ST3A1* (*3-OST1*, *3-OST2*, *3-OST3A*)	Heparan sulfate (3S)	DNA methylation	Bui et al. (2010)
C4ST1, *DSE*	Chondroitin/dermatan sulfate	DNA methylation	Kalathas et al. (2010)
GMDS, *FX*, *MGAT4A*, *MGAT5*	Core fucose/*N*-glycan branching	DNA methylation	Saldova et al. (2011)
ST3GAL6	Sialyl Lewis X	DNA methylation	Chachadi et al. (2011)
HS3ST3B1 (*3-OST-3B1*)	Heparan sulfate (3S)	DNA methylation, histone acetylation	Song et al. (2011)
B4GALNT1, *ST8SIA1*	Gangliosides	histone acetylation	Suzuki et al. (2011)

(continued)

Table 9.1 (continued)

Genes	Glycans involved	Mechanisms	References
MGAT5B	*N*-glycan branching	DNA methylation, histone acetylation, histone methylation	Kizuka et al. (2011)
B3GALT5	Sialyl Lewis A	DNA methylation, histone acetylation, histone methylation	Caretti et al. (2012)
Cosmc	Mucin core 1	DNA methylation	Mi et al. (2012)
B3GALT1	Sialyl Lewis A	Histone acetylation	Chachadi et al. (2013)
B3GNT7	Sialyl Lewis A/X	DNA methylation	Lu et al. (2014)

Candidate glycans which are expected to be affected by these alterations are also included

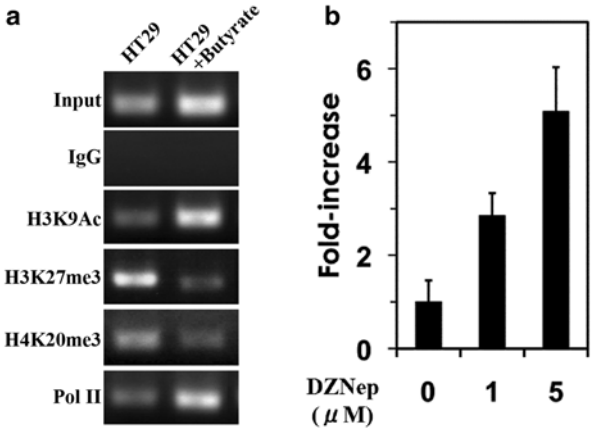

Fig. 9.4 Epigenetic silencing of *SLC26A2* gene in colon cancer cells. *Panel a*, results of ChIP assays in human colon cancer HT29 cells cultured with or without an HDAC inhibitor, butyrate. *Panel b*, effect of histone methyltransferase inhibitor DZNep on the transcription level of the *SLC26A2* gene (Adopted from reference Yusa et al. 2010)

9.6 Acquisition of Resistance to Hypoxia by Cancer Cells in Advanced Stage of Cancers

At the locally advanced stages, cancer cells must cope with hypoxic environments to survive and proliferate, and some cancer cell clones having hypoxia-resistant characteristics appear through accumulation of genetic anomalies. Such hypoxia-resistant cancer cells usually exhibit a higher proliferating rate, enhanced cell

mobility, greater angiogenic activity, and stronger multidrug resistance, thus having multiple advantages over other cancer cell clones, and will eventually occupy all cancer cell nests. The transcription factor HIF-1α plays a central role for cancer cells in acquiring hypoxia-resistant characteristics.

Intense changes of glycan expression are observed in advanced-stage cancers, and this is partly because HIF-1α induces the transcription of a variety of genes involved in the synthesis of glycans (Kannagi 2004, 2010). For instance, tumor hypoxia induces, through the action of HIF-1α, transcription of genes for sialyltransferase, fucosyltransferase, and UDP-galactose transporter, which are involved in the synthesis of sialyl Lewis A and sialyl Lewis X (Koike et al. 2004). Tumor hypoxia thus enhances expression of sialyl Lewis A and sialyl Lewis X in cancer cells, which further help cancer cells in coping with hypoxic environments, since these glycans serve as ligands for vascular E-selectin and mediate adhesion of cancer cells to endothelial cells. Interaction between E-selectins on endothelial cells and its ligands on cancer cells is known to facilitate tumor vascularization (Tei et al. 2002). HIF-1α also induces transcription of genes for several enzymes in the synthetic pathway of the lipid moiety of glycolipids, and this is expected to affect their localization in cell membrane microdomains (Yin et al. 2010).

The gene for sialin, *SLS17A5*, is also induced by HIF-1α (Yin et al. 2006). Normal cells synthesize sialic acid, usually from the de novo synthetic pathway starting from UDP-GlcNAc. Cancer cells seem to enhance the de novo synthetic pathway to some extent to meet the increased demands for sialoconjugate synthesis (Go et al. 2007), but upon progression, cancer cells tend to rely more on the salvage pathway, which reutilizes the sialic acid residues cleaved from exogenous glycoconjugates in lysosomes. Sialin is a lysosomal sialic acid transporter that pumps in free sialic acids released in lysosomes to cytoplasm and is closely involved in the salvage pathway. In humans, sialic acid species provided by the de novo synthetic pathway is limited to NeuAc but not NeuGc, because humans lack the enzyme which converts NeuAc to NeuGc. On the other hand, sialic acids transported by sialin contain NeuGc, the nonhuman sialic acid derived from fetal calf serum under cell culture conditions and from a dietary origin under in vivo conditions. Consequently, the amount of glycans containing NeuGc in cancer cells having enhanced sialin activity is usually higher than that in nonmalignant cells. A glycan containing NeuGc was sometimes known to be antigenic to humans and was termed Hanganatziu-Deicher antigen. This antigen was for a long time counted as a member of cancer-associated glycans, as cancers have a higher amount of NeuGc-containing glycans than normal tissues. The Hanganatziu-Deicher antigen occurs late during cancer progression, mainly in the advanced stages of cancers, because its appearance is driven by HIF-1α, which starts to work in the advanced stages. Recently, cultured ovarian cancer cells having an extremely high NeuGc content were reported, and other mechanisms, in addition to enhanced sialin transcription, were suggested to be involved in this extremely high NeuGc expression (Inoue et al. 2010).

9.7 Glycan Alteration by Epithelio-Mesenchymal Transition of Cancer Cells in Advanced Stages of Cancers

Epithelio-mesenchymal transition (EMT) is a critical event in the advanced stages of cancers which prepares cancer cells for metastasis. EMT is caused by a well-defined set of transcription factors and is found to induce several genes related to glycan expression such as sialyltransferases, fucosyltransferases, and galactosyltransferases, which are also involved in the synthesis of sialyl Lewis A and sialyl Lewis X glycans (Sakuma et al. 2012). Consequently, cancer cells which had undergone EMT have a higher expression of the sialyl Lewis A and sialyl Lewis X glycans and strongly bind to vascular E-selectin (Fig. 9.5). Several decades ago, it had been noticed that cancer cells in the invasion front having a mesenchymal cell-like morphology frequently express these glycans strongly (Ono et al. 1996). Judging from the recent findings mentioned above, this must have been due to the EMT-induced transcription of glycan-related genes.

The best-known function of cancer-associated glycans is that sialyl Lewis A and sialyl Lewis X glycans serve as vascular E-selectin and mediate hematogenous metastasis of cancer cells (Phillips et al. 1990; Takada et al. 1991, 1993). For adhesion to occur, however, these glycans need to be expressed in a density high

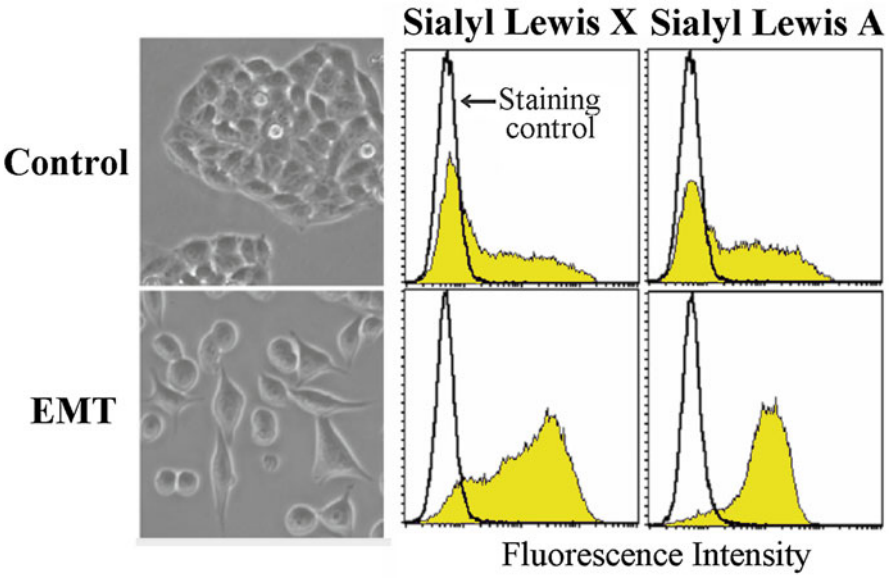

Fig. 9.5 Induction of cancer-associated glycans sialyl Lewis A and sialyl Lewis X in cancer cells underwent epithelio-mesenchymal transition (EMT). Human colon cancer cells DLD-1 were cultured with EGF/bFGF in serum-free medium to induce EMT. Morphological changes (*left panel*) and results of flow-cytometric analyses of sialyl Lewis A and sialyl Lewis X expression (*right panel*) upon EMT are shown (Adopted from reference Sakuma et al. 2012)

enough to be recognized by E-selectin. As disialyl Lewis A and sialyl 6-sulfo Lewis X are only minor components among the glycans in nonmalignant epithelial cells, their interconversion through the "incomplete synthesis" mechanism, i.e., epigenetic silencing of *ST6GalNAc6* or *SLC26A2*, would confer, although significant, only a weak expression of sialyl Lewis A and sialyl Lewis X glycans. The high-density expression of these cancer-associated glycans could be achieved only after further enhancement of their synthesis through transcriptional induction by hypoxia and/or EMT of additional glycan-related genes, the mechanism defined as "*neo*synthesis" in our previous proposition. The two mechanisms, "incomplete synthesis" and "*neo*synthesis," are not mutually exclusive; they sometimes work on the same glycans in a stepwise manner during the multistep progression of cancers. In contrast, appearance of the NeuGc-containing glycans (Hanganatziu-Deicher antigens) in cancer cells can be regarded to have stemmed exclusively from the "*neo*synthesis" mechanism, which specifically occurs in the advanced stages of cancer progression.

9.8 Glycans Associated with Cancer Stem Cells and Embryonic Stem Cells

Characteristics of EMT-induced cancer cells are known to be very similar to those of so-called cancer stem cells (Mani et al. 2008). Expression of sialyl Lewis A and sialyl Lewis X was enhanced in cancer cells after EMT, while a paradoxical decrease was noted in the expression of some other glycans that had been assumed to be cancer associated, such as Lewis Y glycan (Sakuma et al. 2012). This unexpected finding suggested that some hitherto known cancer-associated glycans, exemplified by sialyl Lewis A/X, are linked to a more malignant population of cancer cells such as cancer stem cells, whereas others are not. Examples of glycan-related genes which have been reported to exhibit altered transcription levels in EMT-induced cancer cells and/or cancer stem cells are shown in Table 9.2, together with the candidate glycan species which are expected to be affected by these alterations.

Cell surface glycans are known to be good markers for embryonic stem (ES) cells in the field of stem cell research. Good examples are SSEA-1 for murine ES cells and SSEA-3/-4 for human ES cells. However, the mechanisms of how these glycans appear specifically on the surface of ES cells have not been elucidated yet. It is well known that a combination of several transcription factors such as OCT3/4, Nanog, and Sox-2 plays critical roles in maintaining the stemness in these cells (Takahashi et al. 2007), while transcriptional regulation of ES cell-associated glycan expression still remains largely unknown. There were sporadic publications reporting that SSEA-3/-4 glycans sometimes appear in human cancer cells (Schrump et al. 1988; Suzuki et al. 2013; Gottschling et al. 2013; Lou et al. 2014), and it was recently reported that these glycans are specifically expressed in cancer stem-like cells (Chang et al. 2008; Noto et al. 2013).

Table 9.2 Examples of glycan-related genes reported to exhibit altered transcription levels in EMT-induced cancer cells and/or cancer stem cells

Genes	Glycans involved	Mechanisms	References
B3GALT4 (decrease)	Gg4	EMT induced by hypoxia	Guan et al. (2010)
HAS2 (increase)	Hyaluronan	EMT induced by TGFβ	Craig et al. (2010)
HAS2, *HAS3* (increase)	Hyaluronan	EMT induced by EGF and IL-1β	Chow et al. (2010)
GCNT2 (increase)	I-branching	EMT induced by TGFβ	Zhang et al. (2011)
ST3GAL1/3/4, *FUT3* (increase); *FUT2* (decrease)	Sialyl Lewis A/sialyl Lewis X	EMT induced by EGF/bFGF	Sakuma et al. (2012)
MGAT3 (decrease)	*N*-glycan	EMT induced by TGFβ	Xu et al. (2012)
MGAT3 (decrease)	*N*-glycan	EMT induced by TGFβ	Pinho et al. (2012)
GCNT1 (increase)	*O*-glycan	Breast cancer tumor-initiating cells	Kim et al. (2012)
ST8SIA1 (increase)	GD2	Cancer stem cells induced through EMT	Battula et al. (2012)
UGCG (increase)	Glycolipids, Gb3	Breast cancer stem cells	Gupta et al. (2012)
HAS2 (increase)	Hyaluronan	Breast cancer stem cells	Okuda et al. (2012)
MGAT5, *FUT8*, and *B3GALT5* (increase); *MGAT3* (decrease)	Lectin binding	EMT induced by HGF	Li et al. (2013)
ST6GAL1	*N*-glycan	Colon cancer stem cells	Swindall et al. (2013)
FUT8 (increase)	*N*-glycan	EMT induced by TGFβ	Chen et al. (2013)
ST3GAL5 (increase)	GM3	EMT induced by TGFβ	Kim et al. (2013)
ST3GAL5, *B4GALNT1*, *ST8SIA1*, *ST3GAL2* (increase)	GD2, GD3, GM2, GD1a	Cancer stem cells induced through EMT	Liang et al. (2013)

Candidate glycans expected to be affected by these alterations are also included

SSEA-3/-4 glycans are classified into the globo-series glycolipids (Kannagi et al. 1983), which compose a unique series of glycolipids having glycan structures confined to glycolipids, and are not easily detectable in glycoproteins. Recently, another classical stem cell-specific glycan, TRA-1-60, was reported to be a type 1 chain glycan, having common backbone structures with glycans carried by both glycolipids and glycoproteins (Natunen et al. 2011). This glycan is structurally very similar to that of sialyl Lewis A, sharing the same enzymes in most steps of their synthetic pathways.

The recently introduced fucosylated stem cell-associated glycan, SSEA-5, again had the backbone structure of the type-1 chain glycan (Tang et al. 2011). Finally, sialyl Lewis A itself was shown to be expressed in human ES cells and to disappear upon ES cell differentiation, thus behaving as a stem cell-specific glycan (Tang et al. 2011). These findings strongly suggest that there are some common features between glycans specific to ES cells and those associated with cancer stem cells.

References

Battula VL, Shi Y, Evans KW, Wang RY, Spaeth EL, Jacamo RO, Guerra R, Sahin AA, Marini FC, Hortobagyi G, Mani SA, Andreeff M (2012) Ganglioside GD2 identifies breast cancer stem cells and promotes tumorigenesis. J Clin Invest 122:2066–2078

Bowden NA, Croft A, Scott RJ (2007) Gene expression profiling in familial adenomatous polyposis adenomas and desmoid disease. Hered Cancer Clin Pract 5:79–96

Bremer EG, Hakomori S (1982) GM3 ganglioside induces hamster fibroblast growth inhibition in chemically-defined medium: ganglioside may regulate growth factor receptor function. Biochem Biophys Res Commun 106:711–718

Bui C, Ouzzine M, Talhaoui I, Sharp S, Prydz K, Coughtrie MW, Fournel-Gigleux S (2010) Epigenetics: methylation-associated repression of heparan sulfate 3-O-sulfotransferase gene expression contributes to the invasive phenotype of H-EMC-SS chondrosarcoma cells. FASEB J 24:436–450

Caretti A, Sirchia SM, Tabano S, Zulueta A, Dall'Olio F, Trinchera M (2012) DNA methylation and histone modifications modulate the β1,3 galactosyltransferase β3Gal-T5 native promoter in cancer cells. Int J Biochem Cell Biol 44:84–90

Chachadi VB, Cheng H, Klinkebiel D, Christman JK, Cheng PW (2011) 5-Aza-2′-deoxycytidine increases sialyl Lewis X on MUC1 by stimulating β-galactoside: α2,3-sialyltransferase 6 gene. Int J Biochem Cell Biol 43:586–593

Chachadi VB, Ali MF, Cheng PW (2013) Prostatic cell-specific regulation of the synthesis of MUC1-associated sialyl Lewis a. PLoS One 8:e57416

Chakraborty AK, Sousa JF, Chakraborty D, Funasaka Y, Bhattacharya M, Chatterjee A, Pawelek J (2006) GnT-V expression and metastatic phenotypes in macrophage-melanoma fusion hybrids is down-regulated by 5-Aza-dC: evidence for methylation sensitive, extragenic regulation of GnT-V transcription. Gene 374:166–173

Chang WW, Lee CH, Lee P, Lin J, Hsu CW, Hung JT, Lin JJ, Yu JC, Shao LE, Yu J, Wong CH, Yu AL (2008) Expression of Globo H and SSEA3 in breast cancer stem cells and the involvement of fucosyl transferases 1 and 2 in Globo H synthesis. Proc Natl Acad Sci U S A 105:11667–11672

Chen CY, Jan YH, Juan YH, Yang CJ, Huang MS, Yu CJ, Yang PC, Hsiao M, Hsu TL, Wong CH (2013) Fucosyltransferase 8 as a functional regulator of nonsmall cell lung cancer. Proc Natl Acad Sci U S A 110:630–635

Chihara Y, Sugano K, Kobayashi A, Kanai Y, Yamamoto H, Nakazono M, Fujimoto H, Kakizoe T, Fujimoto K, Hirohashi S, Hirao Y (2005) Loss of blood group A antigen expression in bladder cancer caused by allelic loss and/or methylation of the ABO gene. Lab Invest 85:895–907

Chow G, Tauler J, Mulshine JL (2010) Cytokines and growth factors stimulate hyaluronan production: role of hyaluronan in epithelial to mesenchymal-like transition in non-small cell lung cancer. J Biomed Biotechnol 2010:485468

Craig EA, Austin AF, Vaillancourt RR, Barnett JV, Camenisch TD (2010) TGFβ2-mediated production of hyaluronan is important for the induction of epicardial cell differentiation and invasion. Exp Cell Res 316:3397–3405

Furukawa K, Hamamura K, Ohkawa Y, Ohmi Y (2012) Disialyl gangliosides enhance tumor phenotypes with differential modalities. Glycoconj J 29:579–584

Fuster MM, Esko JD (2005) The sweet and sour of cancer: glycans as novel therapeutic targets. Nat Rev Cancer 5:526–542

Go S, Sato C, Yin J, Kannagi R, Kitajima K (2007) Hypoxia-enhanced expression of free deaminoneuraminic acid in human cancer cells. Biochem Biophys Res Commun 357:537–542

Gottschling S, Jensen K, Warth A, Herth FJ, Thomas M, Schnabel PA, Herpel E (2013) Stage-specific embryonic antigen-4 is expressed in basaloid lung cancer and associated with poor prognosis. Eur Respir J 41:656–663

Guan F, Schaffer L, Handa K, Hakomori SI (2010) Functional role of gangliotetraosylceramide in epithelial-to-mesenchymal transition process induced by hypoxia and by TGF-β. FASEB J 24:4889–4903

Gupta V, Bhinge KN, Hosain SB, Xiong K, Gu X, Shi R, Ho MY, Khoo KH, Li SC, Li YT, Ambudkar SV, Jazwinski SM, Liu YY (2012) Ceramide glycosylation by glucosylceramide synthase selectively maintains the properties of breast cancer stem cells. J Biol Chem 287:37195–37205

Hakomori SI (2010) Glycosynaptic microdomains controlling tumor cell phenotype through alteration of cell growth, adhesion, and motility. FEBS Lett 584:1901–1906

Hakomori S, Kannagi R (1983) Glycosphingolipids as tumor-associated and differentiation markers. J Natl Cancer Inst 71:231–251

Hwang JA, Kim Y, Hong SH, Lee J, Cho YG, Han JY, Kim YH, Han J, Shim YM, Lee YS, Kim DH (2013) Epigenetic inactivation of heparan sulfate (glucosamine) 3-O-sulfotransferase 2 in lung cancer and its role in tumorigenesis. PLoS One 8:e79634

Ide Y, Miyoshi E, Nakagawa T, Gu J, Tanemura M, Nishida T, Ito T, Yamamoto H, Kozutsumi Y, Taniguchi N (2006) Aberrant expression of N-acetylglucosaminyltransferase-IVa and IVb (GnT-IVa and b) in pancreatic cancer. Biochem Biophys Res Commun 341:478–482

Inoue S, Sato C, Kitajima K (2010) Extensive enrichment of N-glycolylneuraminic acid in extracellular sialoglycoproteins abundantly synthesized and secreted by human cancer cells. Glycobiology 20:752–762

Itai S, Nishikata J, Takahashi N, Arii S, Tobe T, Tanaka O, Kannagi R (1990) Differentiation-dependent expression of I- and sialyl I-antigens in the developing lung of human embryos and in lung cancers. Cancer Res 50:7603–7611

Itai S, Nishikata J, Yoneda T, Ohmori K, Tsunekawa S, Hiraiwa N, Yamabe H, Arii S, Tobe T, Kannagi R (1991) Tissue distribution of sialyl 2-3 and 2-6 Lewis a antigens and the significance of serum 2-3/2-6 sialyl Lewis a antigen ratio for the differential diagnosis of malignant and benign disorders of the digestive tract. Cancer 67:1576–1587

Izawa M, Kumamoto K, Mitsuoka C, Kanamori A, Ohmori K, Ishida H, Nakamura S, Kurata-Miura K, Sasaki K, Nishi T, Kannagi R (2000) Expression of sialyl 6-sulfo Lewis x is inversely correlated with conventional sialyl Lewis x expression in human colorectal cancer. Cancer Res 60:1410–1416

Kalathas D, Triantaphyllidou IE, Mastronikolis NS, Goumas PD, Papadas TA, Tsiropoulos G, Vynios DH (2010) The chondroitin/dermatan sulfate synthesizing and modifying enzymes in laryngeal cancer: expressional and epigenetic studies. Head Neck Oncol 2:27

Kannagi R (2004) Molecular mechanism for cancer-associated induction of sialyl Lewis X and sialyl Lewis A expression – the Warburg effect revisited. Glycoconj J 20:353–364

Kannagi R (2007) Carbohydrate antigen sialyl Lewis a – its pathophysiological significance and induction mechanism in cancer progression. Chang Gung Med J 30:189–209

Kannagi R, Cochran NA, Ishigami F, Hakomori S, Andrews PW, Knowles BB, Solter D (1983) Stage-specific embryonic antigens (SSEA-3 and -4) are epitopes of a unique globo-series ganglioside isolated from human teratocarcinoma cells. EMBO J 2:2355–2361

Kannagi R, Kitahara A, Itai S, Zenita K, Shigeta K, Tachikawa T, Noda A, Hirano H, Abe M, Shin S, Fukushi Y, Hakomori S, Imura H (1988) Quantitative and qualitative characterization of human cancer-associated serum glycoprotein antigens expressing epitopes consisting of sialyl or sialyl-fucosyl type 1 chain. Cancer Res 48:3856–3863

Kannagi R, Yin J, Miyazaki K, Izawa M (2008) Current relevance of incomplete synthesis and neo-synthesis for cancer-associated alteration of carbohydrate determinants-Hakomori's concepts revisited. Biochim Biophys Acta 1780:525–531

Kannagi R, Sakuma K, Miyazaki K, Lim K-T, Yusa A, Yin J, Izawa M (2010) Altered expression of glycan genes in cancers induced by epigenetic silencing and tumor hypoxia: clues in the ongoing search for new tumor markers. Cancer Sci 101:586–593

Karibe T, Fukui H, Sekikawa A, Shiratori K, Fujimori T (2008) EXTL3 promoter methylation down-regulates EXTL3 and heparan sulphate expression in mucinous colorectal cancers. J Pathol 216:32–42

Kawamura YI, Toyota M, Kawashima R, Hagiwara T, Suzuki H, Imai K, Shinomura Y, Tokino T, Kannagi R, Dohi T (2008) DNA hypermethylation contributes to incomplete synthesis of carbohydrate determinants in gastrointestinal cancer. Gastroenterology 135:142–151

Kim J, Villadsen R, Sorlie T, Fogh L, Gronlund SZ, Fridriksdottir AJ, Kuhn I, Rank F, Wielenga VT, Solvang H, Edwards PA, Borresen-Dale AL, Ronnov-Jessen L, Bissell MJ, Petersen OW (2012) Tumor initiating but differentiated luminal-like breast cancer cells are highly invasive in the absence of basal-like activity. Proc Natl Acad Sci U S A 109:6124–6129

Kim SJ, Chung TW, Choi HJ, Kwak CH, Song KH, Suh SJ, Kwon KM, Chang YC, Park YG, Chang HW, Kim KS, Kim CH, Lee YC (2013) Ganglioside GM3 participates in the TGF-β1-induced epithelial-mesenchymal transition of human lens epithelial cells. Biochem J 449:241–251

Kizuka Y, Kitazume S, Yoshida M, Taniguchi N (2011) Brain-specific expression of N-acetylglucosaminyltransferase IX (GnT-IX) is regulated by epigenetic histone modifications. J Biol Chem 286:31875–31884

Koike T, Kimura N, Miyazaki K, Yabuta T, Kumamoto K, Takenoshita S, Chen J, Kobayashi M, Hosokawa M, Taniguchi A, Kojima T, Ishida N, Kawakita M, Yamamoto H, Takematsu H, Kozutsumi Y, Suzuki A, Kannagi R (2004) Hypoxia induces adhesion molecules on cancer cells-a missing link between Warburg effect and induction of selectin ligand carbohydrates. Proc Natl Acad Sci U S A 101:8132–8137

Kominato Y, Hata Y, Takizawa H, Tsuchiya T, Tsukada J, Yamamoto F (1999) Expression of human histo-blood group ABO genes is dependent upon DNA methylation of the promoter region. J Biol Chem 274:37240–37250

Kroes RA, Dawson G, Moskal JR (2007) Focused microarray analysis of glyco-gene expression in human glioblastomas. J Neurochem 103(Suppl 1):14–24

Lau KS, Dennis JW (2008) N-Glycans in cancer progression. Glycobiology 18:750–760

Lee S, Bang S, Song K, Lee I (2006) Differential expression in normal-adenoma-carcinoma sequence suggests complex molecular carcinogenesis in colon. Oncol Rep 16:747–754

Li S, Mo C, Peng Q, Kang X, Sun C, Jiang K, Huang L, Lu Y, Sui J, Qin X, Liu Y (2013) Cell surface glycan alterations in epithelial mesenchymal transition process of Huh7 hepatocellular carcinoma cell. PLoS One 8:e71273

Liang YJ, Ding Y, Levery SB, Lobaton M, Handa K, Hakomori SI (2013) Differential expression profiles of glycosphingolipids in human breast cancer stem cells vs. cancer non-stem cells. Proc Natl Acad Sci U S A 110:4968–4973

Lim K-T, Miyazaki K, Kimura N, Izawa M, Kannagi R (2008) Clinical application of functional glycoproteomics – dissection of glycotopes carried by soluble CD44 variants in sera of patients with cancers. Proteomics 8:3263–3273

Lou YW, Wang PY, Yeh SC, Chuang PK, Li ST, Wu CY, Khoo KH, Hsiao M, Hsu TL, Wong CH (2014) Stage-specific embryonic antigen-4 as a potential therapeutic target in glioblastoma multiforme and other cancers. Proc Natl Acad Sci U S A 111:2482–2487

Lu CH, Wu WY, Lai YJ, Yang CM, Yu LC (2014) Suppression of B3GNT7 gene expression in colon adenocarcinoma and its potential effect in the metastasis of colon cancer cells. Glycobiology 24:359–367

Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M, Campbell LL, Polyak K, Brisken C, Yang J, Weinberg RA (2008) The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 133:704–715

Matsumoto K, Yokote H, Arao T, Maegawa M, Tanaka K, Fujita Y, Shimizu C, Hanafusa T, Fujiwara Y, Nishio K (2008) N-Glycan fucosylation of epidermal growth factor receptor modu-

lates receptor activity and sensitivity to epidermal growth factor receptor tyrosine kinase inhibitor. Cancer Sci 99:1611–1617

Mi R, Song L, Wang Y, Ding X, Zeng J, Lehoux S, Aryal RP, Wang J, Crew VK, Van D, Chapman AB, Cummings RD, Ju T (2012) Epigenetic silencing of the chaperone Cosmc in human leukocytes expressing tn antigen. J Biol Chem 287:41523–41533

Miyamoto K, Asada K, Fukutomi T, Okochi E, Yagi Y, Hasegawa T, Asahara T, Sugimura T, Ushijima T (2003) Methylation-associated silencing of heparan sulfate D-glucosaminyl 3-O-sulfotransferase-2 (3-OST-2) in human breast, colon, lung and pancreatic cancers. Oncogene 22:274–280

Miyazaki K, Ohmori K, Izawa M, Koike T, Kumamoto K, Furukawa K, Ando T, Kiso M, Yamaji T, Hashimoto Y, Suzuki A, Yoshida A, Takeuchi M, Kannagi R (2004) Loss of disialyl Lewis^a, the ligand for lymphocyte inhibitory receptor Siglec-7, associated with increased sialyl Lewis^a expression on human colon cancers. Cancer Res 64:4498–4505

Miyazaki K, Sakuma K, Kawamura YI, Izawa M, Ohmori K, Mitsuki M, Yamaji T, Hashimoto Y, Suzuki A, Saito Y, Dohi T, Kannagi R (2012) Colonic epithelial cells express specific ligands for mucosal macrophage immunosuppressive receptors, siglec-7 and -9. J Immunol 188:4690–4700

Moriwaki K, Narisada M, Imai T, Shinzaki S, Miyoshi E (2010) The effect of epigenetic regulation of fucosylation on TRAIL-induced apoptosis. Glycoconj J 27:649–659

Natunen S, Satomaa T, Pitkanen V, Salo H, Mikkola M, Natunen J, Otonkoski T, Valmu L (2011) The binding specificity of the marker antibodies Tra-1-60 and Tra-1-81 reveals a novel pluripotency-associated type 1 lactosamine epitope. Glycobiology 21:1125–1130

Noto Z, Yoshida T, Okabe M, Koike C, Fathy M, Tsuno H, Tomihara K, Arai N, Noguchi M, Nikaido T (2013) CD44 and SSEA-4 positive cells in an oral cancer cell line HSC-4 possess cancer stem-like cell characteristics. Oral Oncol 49:787–795

Oetke C, Hinderlich S, Reutter W, Pawlita M (2003) Epigenetically mediated loss of UDP-GlcNAc 2-epimerase/ManNAc kinase expression in hyposialylated cell lines. Biochem Biophys Res Commun 308:892–898

Okuda H, Kobayashi A, Xia B, Watabe M, Pai SK, Hirota S, Xing F, Liu W, Pandey PR, Fukuda K, Modur V, Ghosh A, Wilber A, Watabe K (2012) Hyaluronan synthase HAS2 promotes tumor progression in bone by stimulating the interaction of breast cancer stem-like cells with macrophages and stromal cells. Cancer Res 72:537–547

Ono M, Sakamoto M, Ino Y, Moriya Y, Sugihara K, Muto T, Hirohashi S (1996) Cancer cell morphology at the invasive front and expression of cell adhesion-related carbohydrate in the primary lesion of patients with colorectal carcinoma with liver metastasis. Cancer 78:1179–1186

Oshima M, Dinchuk JE, Kargman SL, Oshima H, Hancock B, Kwong E, Trzaskos JM, Evans JF, Taketo MM (1996) Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell 87:803–809

Park JJ, Yi JY, Jin YB, Lee YJ, Lee JS, Lee YS, Ko YG, Lee M (2012) Sialylation of epidermal growth factor receptor regulates receptor activity and chemosensitivity to gefitinib in colon cancer cells. Biochem Pharmacol 83:849–857

Phillips ML, Nudelman E, Gaeta FCA, Perez M, Singhal AK, Hakomori S, Paulson JC (1990) ELAM-1 mediates cell adhesion by recognition of a carbohydrate ligand, sialyl-Le^x. Science 250:1130–1132

Pinho SS, Oliveira P, Cabral J, Carvalho S, Huntsman D, Gartner F, Seruca R, Reis CA, Oliveira C (2012) Loss and recovery of Mgat3 and GnT-III Mediated E-cadherin N-glycosylation is a mechanism involved in epithelial-mesenchymal-epithelial transitions. PLoS One 7:e33191

Potapenko IO, Haakensen VD, Luders T, Helland A, Bukholm I, Sorlie T, Kristensen VN, Lingjaerde OC, Borresen-Dale AL (2010) Glycan gene expression signatures in normal and malignant breast tissue; possible role in diagnosis and progression. Mol Oncol 4:98–118

Ravillah D, Mohammed A, Qian L, Brewer M, Zhang Y, Biddick L, Steele VE, Rao CV (2014) Chemopreventive effects of an HDAC2-selective inhibitor on rat colon carcinogenesis and APCmin/+ mouse intestinal tumorigenesis. J Pharmacol Exp Ther 348:59–68

Ropero S, Setien F, Espada J, Fraga MF, Herranz M, Asp J, Benassi MS, Franchi A, Patino A, Ward LS, Bovee J, Cigudosa JC, Wim W, Esteller M (2004) Epigenetic loss of the familial tumor-suppressor gene exostosin-1 (EXT1) disrupts heparan sulfate synthesis in cancer cells. Hum Mol Genet 13:2753–2765

Sakuma K, Aoki M, Kannagi R (2012) Transcription factors c-Myc and CDX2 mediate E-selectin ligand expression in colon cancer cells undergoing EGF/bFGF-induced epithelial-mesenchymal transition. Proc Natl Acad Sci U S A 109:7776–7781

Saldova R, Dempsey E, Perez-Garay M, Marino K, Watson JA, Blanco-Fernandez A, Struwe WB, Harvey DJ, Madden SF, Peracaula R, McCann A, Rudd PM (2011) 5-AZA-2′-deoxycytidine induced demethylation influences N-glycosylation of secreted glycoproteins in ovarian cancer. Epigenetics 6:1362–1372

Schrump DS, Furukawa K, Yamaguchi H, Lloyd KO, Old LJ (1988) Recognition of galactosylgloboside by monoclonal antibodies derived from patients with primary lung cancer. Proc Natl Acad Sci U S A 85:4441–4445

Senda M, Ito A, Tsuchida A, Hagiwara T, Kaneda T, Nakamura Y, Kasama K, Kiso M, Yoshikawa K, Katagiri Y, Ono Y, Ogiso M, Urano T, Furukawa K, Oshima S (2007) Identification and expression of a sialyltransferase responsible for the synthesis of disialylgalactosylgloboside in normal and malignant kidney cells: downregulation of ST6GalNAc VI in renal cancers. Biochem J 402:459–470

Serpa J, Mesquita P, Mendes N, Oliveira C, Almeida R, Santos-Silva F, Reis CA, LePendu J, David L (2006) Expression of Lea in gastric cancer cell lines depends on FUT3 expression regulated by promoter methylation. Cancer Lett 242:191–197

Shamsuddin AK, Weiss L, Phelps PC, Trump BF (1981) Colon epithelium. IV. Human colon carcinogenesis. Changes in human colon mucosa adjacent to and remote from carcinomas of the colon. J Natl Cancer Inst 66:413–419

Song K, Li Q, Jiang ZZ, Guo CW, Li P (2011) Heparan sulfate D-glucosaminyl 3-O-sulfotransferase-3B1, a novel epithelial-mesenchymal transition inducer in pancreatic cancer. Cancer Biol Ther 12:388–398

Staub J, Chien J, Pan Y, Qian X, Narita K, Aletti G, Scheerer M, Roberts LR, Molina J, Shridhar V (2007) Epigenetic silencing of HSulf-1 in ovarian cancer: implications in chemoresistance. Oncogene 26:4969–4978

Suzuki Y, Yanagisawa M, Ariga T, Yu RK (2011) Histone acetylation-mediated glycosyltransferase gene regulation in mouse brain during development. J Neurochem 116:874–880

Suzuki Y, Haraguchi N, Takahashi H, Uemura M, Nishimura J, Hata T, Takemasa I, Mizushima T, Ishii H, Doki Y, Mori M, Yamamoto H (2013) SSEA-3 as a novel amplifying cancer cell surface marker in colorectal cancers. Int J Oncol 42:161–167

Swindall AF, Londono-Joshi AI, Schultz MJ, Fineberg N, Buchsbaum DJ, Bellis SL (2013) ST6Gal-I protein expression is upregulated in human epithelial tumors and correlates with stem cell markers in normal tissues and colon cancer cell lines. Cancer Res 73:2368–2378

Takada A, Ohmori K, Takahashi N, Tsuyuoka K, Yago K, Zenita K, Hasegawa A, Kannagi R (1991) Adhesion of human cancer cells to vascular endothelium mediated by a carbohydrate antigen, sialyl Lewis A. Biochem Biophys Res Commun 179:713–719

Takada A, Ohmori K, Yoneda T, Tsuyuoka K, Hasegawa A, Kiso M, Kannagi R (1993) Contribution of carbohydrate antigens sialyl Lewis A and sialyl Lewis X to adhesion of human cancer cells to vascular endothelium. Cancer Res 53:354–361

Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131:1–12

Tang C, Lee AS, Volkmer JP, Sahoo D, Nag D, Mosley AR, Inlay MA, Ardehali R, Chavez SL, Pera RR, Behr B, Wu JC, Weissman IL, Drukker M (2011) An antibody against SSEA-5 glycan on human pluripotent stem cells enables removal of teratoma-forming cells. Nat Biotechnol 29:829–834

Tei K, Kawakami-Kimura N, Taguchi O, Kumamoto K, Higashiyama S, Taniguchi N, Toda K, Kawata R, Hisa Y, Kannagi R (2002) Roles of cell adhesion molecules in tumor angiogenesis

induced by co-transplantation of cancer and endothelial cells to nude rats. Cancer Res 62:6289–6296

Timp W, Feinberg AP (2013) Cancer as a dysregulated epigenome allowing cellular growth advantage at the expense of the host. Nat Rev Cancer 13:497–510

Todeschini AR, Dos Santos JN, Handa K, Hakomori SI (2008) Ganglioside GM2/GM3 complex affixed on silica nanospheres strongly inhibits cell motility through CD82/cMet-mediated pathway. Proc Natl Acad Sci U S A 105:1925–1930

Wagner KW, Punnoose EA, Januario T, Lawrence DA, Pitti RM, Lancaster K, Lee D, von Goetz M, Yee SF, Totpal K, Huw L, Katta V, Cavet G, Hymowitz SG, Amler L, Ashkenazi A (2007) Death-receptor O-glycosylation controls tumor-cell sensitivity to the proapoptotic ligand Apo2L/TRAIL. Nat Med 13:1070–1077

Wang HR, Hsieh CY, Twu YC, Yu LC (2008) Expression of the human Sd^a β-1,4-N-acetylgalactosaminyltransferase II gene is dependent on the promoter methylation status. Glycobiology 18:104–113

Wu YM, Liu CH, Hu RH, Huang MJ, Lee JJ, Chen CH, Huang J, Lai HS, Lee PH, Hsu WM, Huang HC, Huang MC (2011) Mucin glycosylating enzyme GALNT2 regulates the malignant character of hepatocellular carcinoma by modifying the EGF receptor. Cancer Res 71:7270–7279

Wu YM, Liu CH, Huang MJ, Lai HS, Lee PH, Hu RH, Huang MC (2013) C1GALT1 enhances proliferation of hepatocellular carcinoma cells via modulating MET glycosylation and dimerization. Cancer Res 73:5580–5590

Xu Q, Isaji T, Lu Y, Gu W, Kondo M, Fukuda T, Du Y, Gu J (2012) Roles of N-acetylglucosaminyltransferase III in epithelial-to-mesenchymal transition induced by transforming growth factor β1 (TGF-β1) in epithelial cell lines. J Biol Chem 287:16563–16574

Yin J, Hashimoto A, Izawa M, Miyazaki K, Chen G-Y, Takematsu H, Kozutsumi Y, Suzuki A, Furuhata K, Cheng F-L, Lin C-H, Sato C, Kitajima K, Kannagi R (2006) Hypoxic culture induces expression of sialin, a sialic acid transporter, and cancer-associated gangliosides containing non-human sialic acid on human cancer cells. Cancer Res 66:2937–2945

Yin J, Miyazaki K, Shaner RL, Merrill AH Jr, Kannagi R (2010) Altered sphingolipid metabolism induced by tumor hypoxia – new vistas of glycolipid tumor markers. FEBS Lett 584:1872–1878

Young WW Jr, Mills SE, Lippert MC, Ahmed P, Lau SK (1988) Deletion of antigens of the Lewis a/b blood group family in human prostatic carcinoma. Am J Pathol 131:578–586

Yusa A, Miyazaki K, Kimura N, Izawa M, Kannagi R (2010) Epigenetic silencing of the sulfate transporter gene *DTDST* induces sialyl $Lewis^x$ expression and accelerates proliferation of colon cancer cells. Cancer Res 70:4064–4073

Zhang H, Meng F, Wu S, Kreike B, Sethi S, Chen W, Miller FR, Wu G (2011) Engagement of I-branching β-1, 6-N-acetylglucosaminyltransferase 2 in breast cancer metastasis and TGF-β signaling. Cancer Res 71:4846–4856

Zhang L, Shi J, Ji M, Liu W, Wang N, Guan H, Xu L, He N, Shi B, Hou P (2012) Methylation analysis of drug metabolism and transport genes in papillary thyroid cancer. Adv Sci Lett 17:243–250