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Sialyltransferases functions in cancers

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1. ABSTRACT

Abnormally elevated levels of sialylated tumor associated carbohydrate antigens are frequently described at the surface of cancer cells and/or secreted in biological fluids. It is now well established that this over-expression may result from deregulation in sialyltransferases enzymatic activity involved in their biosynthesis, but the precise molecular mechanisms remain unknown. Twenty different human sialyltransferases preside to the sialylation of glycoconjugates, either glycolipids or glycoproteins. This review summarizes the current knowledge on human sialyltransferases implicated in the altered expression of sialylated tumor associated antigens, the molecular basis of their regulated expression in cancer cells and the various tools developed by researchers and clinicians for their study in pathological samples.

2. INTRODUCTION

The oligosaccharide chains of glycoproteins and glycolipids of vertebrates are often terminated with sialic acids, a family of nine carbon monosaccharides derived from neuraminic acid (1, 2). These sialylated glycoconjugates at the cell surface mediate important biological roles and promote cell-cell interactions (3-6). In humans, sialylation of glycoconjugates is carried out by twenty sialyltransferases (ST), which catalyze the formation of different types of glycosidic linkages through an α2,3- or an α2,6-bond to galactose (Gal); an α2,6-bond to N-acetylgalactosamine (GalNAc) or N-acetylglucosamine (GlcNAc), or through an α2,8-bond to another sialic acid, forming polysialic acid (reviewed in (7-9)). During neoplastic transformation, the activity of the Golgi-localized STs is usually increased (10) and as a consequence, cancer cells express more heavily sialylated

Table 1. Sialyltransferases described in human tissues associated to the biosynthesis of sialylated TACA and with malignant transformation

Sialyltransferase	Substrate	Structures formed	TACA formed	Cancer and expression	Ref.
ST3Gal I	O-GP/ GL	Neu5Acα2-3Galβ1-3GalNAc- and G_{D1a} G_{M1b} G_{T1b}	sT	Breast ↑, Gastric, Pediatric leukemia Bladder	(25, 27) (67) (45) (29)
ST3Gal II	O-GP/ GL	Neu5Acα2-3Galβ1-3GalNAc- and G_{D1a} G_{M1b} G_{T1b}		Neuroblastoma ↓	(157)
ST3Gal III	GP	Neu5Acα2-3Galβ1-3/4GlcNAc-	sLe^a		
ST3Gal IV	GP/GL	Neu5Acα2-3Galβ1-4GlcNAc- Neu5Acα2-3Galβ1-3GalNAc-	sLe^x	Breast, Gastric	(182) (39)
ST3Gal V G_{M3} synthase	GL	Neu5Acα2-3Galβ1-4Glcβ-Cer G_{M3} and possibly G_{M4}	G_{M3}	Pediatric leukemia	(45)
ST3Gal VI	GP/GL	Neu5Acα2-3Galβ1-4GlcNAc-	sLe^x	Gastric ↓	(155)
ST6Gal I	N-GP (GL)	Neu5Acα2-6Galβ1-4GlcNAc-	CD75s ST2H	Cervical ↑ Gastrointestinal ↑ Colon	(30, 60) (183) (49)
ST6Gal II	N-GP	Neu5Acα2-6Gal(NAc)β1-4GlcNAc-			
ST6GalNAc I	O-GP	$(Neu5Ac\alpha2\text{-}3)_{0\text{-}1}(Gal\beta1\text{-}3)_{0\text{-}1}$GalNAc-Ser Neu5Acα2-6′	sTn	Breast ↑, Gastric ↑	(17) (65)
ST6GalNAc II	O-GP	$(Neu5Ac\alpha2\text{-}3)_{0\text{-}1}$Galβ1-3GalNAc-Ser Neu5Acα2-6′			
ST6GalNAc III	O-GP /GL	Neu5Acα2-3Galβ1-3GalNAc-Ser Neu5Acα2-6′ $G_{D1\alpha}$			
ST6GalNAc IV	O-GP/ GL	Neu5Acα2-3Galβ1-3GalNAc-Ser Neu5Acα2-6′ $G_{D1\alpha}$			
ST6GalNAc V $G_{D1\alpha}$ synthase	GP/GL	Neu5Acα2-3Galβ1-4GlcNAc- Neu5Acα2-6′ $G_{D1\alpha}$			(72, 73)
ST6GalNAc VI	GL	α-series gangliosides ($G_{D1\alpha}$ $G_{T1a\alpha}$ $G_{Q1b\alpha}$) and di-sialyl $Lewis^a$	Disialyl-Le^a	↓	
ST8Sia I G_{D3}synthase	GL	$(Neu5Ac\alpha2\text{-}8)_{1\text{-}2}$Neu5Acα2-3Galβ1-4Glc-Cer G_{D3} and G_{T3}	9-O-Ac G_{D3}	Melanoma	(184)
ST8Sia II STX	GP	$(Neu5Ac\alpha2\text{-}8)_n$Neu5Acα2-3Galβ1-4GlcNAc-	PSA	Pancreas Neuroblastoma ↑ Small cell lung carcinoma	(109), (185) (88)
ST8Sia III	GL/GP	Neu5Acα2-8Neu5Acα2-3Galβ Neu5Acα2-8Neu5Acα2-6GalNAc-			
ST8Sia IV PST	GP	$(Neu5Ac\alpha2\text{-}8)_n$Neu5Acα2-3Galβ1-4GlcNAc-			
ST8Sia V G_{T3}synthase	GL	G_{D1c}, G_{T1a}, G_{Q1b}, G_{T3}			
ST8Sia VI	O-GP	Neu5Acα2-8Neu5Acα2-3Galβ1-3GalNAc-			

tumor associated carbohydrate antigen (TACA) at their surface (11). In addition, increased sialylation and shortening of *O*-glycosylproteins glycan chains is also observed (12, 13) as well as reduced *O*-acetylation of sialic acid (14, 15). This aberrant sialylation of glycoconjugates mediate key pathological events during tumor progression, including invasion and metastasis. These specific over-expressed sialylated glycans are useful prognostic markers of malignant disease states. Cancer biomarkers like sialyl-$Lewis^a$ (sLe^a) epitope, known as the CA19-9 (Neu5Acα2-3Galβ1-3[Fucα1-4]GlcNAc) and sialyl-Tn (sTn) epitope, known as the CA72.4 (Neu5Acα2-6GalNAc) (16) have been used as targets for cancer immunotherapy in preclinical and clinical vaccine evaluation (17). However, most of the time, the mechanisms explaining observed altered sialylation in cancer cells remain unknown and no obvious correlation can be established between the enzymatic activity and the appearance of sialylated TACA. This review focuses on human ST implicated in the biosynthesis of the sialylated TACA and their role in cancer. In particular, we briefly review the tools of investigation available for researchers and clinicians and the proposed underlying mechanisms regulating aberrant expression of sialylated TACA in cancer are discussed.

3. HUMAN ST INVOLVED IN THE SYNTHESIS OF SIALYLATED TACA

Twenty sialyltransferases have been cloned from human sources (Table 1). The use of these ST cDNAs for recombinant protein production has shed light on their substrate specificity *in vitro,* although one should keep in mind that these enzymes might have slightly different enzymatic characteristics *in vivo*.

3.1. The β-galactoside α2,3-sialyltransferases (ST3Gal)

Six β-galactoside α2,3-sialyltransferases cDNAs (reviewed in (7, 8) have been identified and cloned from the human genome (Table 1). These enzymes catalyze the transfer of sialic acid in α2,3-linkage to terminal Gal residues found on glycoproteins or glycolipids.

ST3Gal I and ST3Gal II use almost exclusively type III disaccharide Galβ1-3GalNAc found onto *O*-glycosylproteins (core1) and glycolipids (asialo-G_{M1}, G_{M1a} and G_{D1b}) (18, 19). ST3Gal I has also a little enzymatic activity towards type I disaccharide (Galβ1-3GlcNAc) (20, 21). The human ST3Gal I leads to the formation of

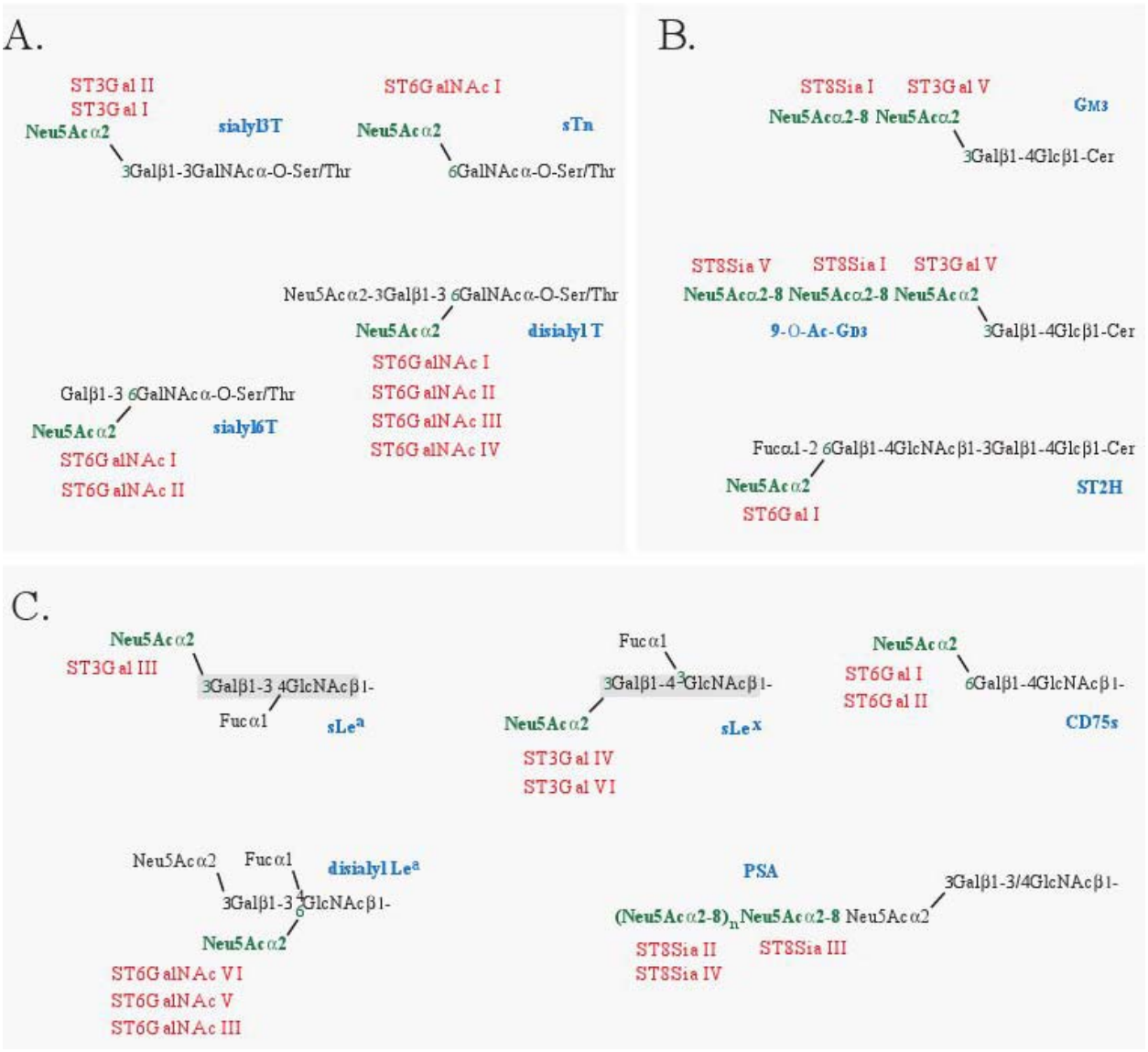

Figure 1. Sialylated tumor associated carbohydrate antigens. A) Representation of sialylated Thomsen Friedenreich antigens. Their common name is indicated in blue, the sialyltransferases involved in their biosynthesis are indicated in red characters and the transferred sialic acid residue is indicated in green bold cases. B) Representation of sialylated gangliosides up-regulated in cancer. Their common name is indicated in blue, the sialyltransferases involved in their biosynthesis are indicated in red characters and the transferred sialic acid residue is indicated in green bold cases. C) Representation of terminal sialylated tumor associated carbohydrate antigens found on *N*- or *O*-glycosylproteins or on glycolipids. Their common name is indicated in blue, the sialyltransferases involved in their biosynthesis are indicated in red characters and the transferred sialic acid residue is indicated in green bold cases. The grey shaded box indicates the type 1 or type 2 disaccharide structure recognized by the sialyltransferase.

Neu5Acα2-3Galβ1-3GalNAc, whereas the mouse ST3Gal I has a preference for glycolipid acceptors catalyzing the biosynthesis of the gangliosides G_{M1b}, G_{D1a} and G_{T1b} at least in *in vitro* assays (22). The human ST3Gal II isolated from the human T-cell lymphoblast-like cell line (CEM T-cells) shows activity towards both glycolipids and glycoproteins (23) and may have a recognition site for the ceramide moiety in addition to the one for the Galβ1-3GalNAc moiety (24). ST3Gal I enzymatic activity is elevated in breast cancer compared to normal tissues and correlates with the grade of the tumor, whereas ST3Gal II is poorly expressed in breast tumors (25-28). ST3Gal I has been implicated in the increased expression of sialylated Thomsen-Friedenreich antigen (Neu5Acα2-3Galβ1-3GalNAc, sT antigen) observed in breast cancer cell lines and bladder cancer (29), whereas ST3Gal II mRNA is decreased (Figure 1). Altered mRNA expression of these STs was also shown to be of importance in malignant epithelial ovarian cancers (30, 31), in colon carcinoma (32)

and correlated with poor prognosis in breast cancer (33, 34).

ST3Gal III, ST3Gal IV and ST3Gal VI show similar enzymatic specificity catalyzing the transfer of sialic acid on the terminal Gal residue of the disaccharide Galβ1-3/4GlcNAc of glycoproteins or glycolipids. The human ST3Gal III uses preferentially type I disaccharide Galβ1-3GlcNAc and therefore represents the most probable candidate for the *in vivo* biosynthesis of sLe^a (35). The human ST3Gal IV and ST3Gal VI use preferentially Galβ1-4GlcNAc disaccharide as acceptor substrate (36-38) probably carried by different substrates in the glycoproteome of the cell (39) leading to the formation of sialyl-$Lewis^x$ (sLe^x) epitope (Neu5Acα2-3Galβ1-4[Fucα1-3]GlcNAc). In addition, human ST3Gal IV could also contribute to the sT antigen synthesis in breast cancer cells and tumors (33, 34, 36) and ST3Gal VI would be involved in the sialyl-3-paragloboside (Neu5Acα2-3Galβ1-4GlcNAcβ1-4Galβ1-4Glcβ1-Cer) biosynthesis, a precursor of the sLe^x on ceramide (CD75s) (Figure 1) (37). Aberrant expression of Lewis-type antigens has been reported in many types of cancers, including lung, colon, kidney, stomach (reviewed in (40)), breast and gastric cancers in which their expression levels are regulated by differential expression of these α2,3-sialyltransferases (39, 41). Over-expression of ST3Gal III in pancreatic cells modulates motility and invasion and enhances metastatic potential (42). Finally, the recombinant human ST3Gal V (43) uses almost exclusively lactosyl-ceramide (LacCer, Galβ1-4Glcβ1-Cer) as an acceptor substrate giving rise to G_{M3}, and is also known as the G_{M3}-synthase (Table 1). Interestingly, Berselli *et al.* reported on the expression of N-terminal 33 amino acids extended isoform of the human ST3Gal V in placenta, which uses (LacCer) and also galactosyl-ceramide (Galβ-Cer), G_{A1} and asialoganglioside G_{A2} (GalNAcβ1-4Galβ1-4Glcβ-Cer) (44). G_{M3} is also associated with cancer and recently, ST3Gal V mRNA level was found to be increased in pediatric leukemia (45).

3.2. The β-galactoside α2,6-sialyltransferases (ST6Gal)

β-galactoside α2,6-sialyltransferases ST6Gal I and ST6Gal II catalyze the transfer of sialic acid mainly to the terminal Gal residue of type II disaccharide through an α2,6-linkage giving rise to the formation of the Neu5Acα2-6Galβ1-4GlcNAc- ($Sia_6LacNAc$) found on *N*-glycosylproteins and to a lesser extent, on *O*-glycosylproteins, glycolipids and free oligosaccharides (7, 46, 47). The recombinant human ST6Gal I produced in eukaryotic cells shows a broad substrate specificity towards Gal(NAc)β1-4GlcNAc bearing substrates (Table 1) (48). ST6Gal I is also responsible for the biosynthesis of a unique human colon cancer biomarker candidate named α2,6-sialylated blood group type 2H (ST2H) antigen (Fucα1-2[Neu5Acα2-6]Galβ1-4GlcNAcβ1-3Galβ1-4Glc-Cer) (49). Human ST6Gal II exhibits *in vitro* a more restricted substrate specificity towards a few Galβ1-4GlcNAc and GalNAcβ1-4GlcNAc bearing glycoconjugates that are not identified yet (48, 50-52). Enhanced expression of $Sia_6LacNAc$ has been reported in numerous carcinoma such as colon, breast, cervix, choriocarcinoma, acute myeloid leukemia and brain tumors (34, 53-61). Elevated ST6Gal I mRNA levels have been described in pediatric leukemia (45).

3.3. The GalNAc α2,6-sialyltransferases (ST6GalNAc)

Six different human GalNAc α2,6-sialyltransferases catalyze the transfer of sialic acid residues in α2,6-linkage to the proximal GalNAc residue of *O*-glycosylproteins (ST6GalNAc I, ST6GalNAc II, ST6GalNAc IV) or onto GalNAc residues of glycolipids like G_{M1b} (ST6GalNAc III, ST6GalNAc V, ST6GalNAc VI) (7).

Recombinant ST6GalNAc I and ST6GalNAc II proteins have similar enzymatic activity *in vitro*, catalyzing the transfer of sialic acid onto GalNAc residues of the type III disaccharide Galβ1-3GalNAc-peptide (α2,3-sialylated or not) of mucin-type glycoproteins (19) (Table 1). Their activity depends on the peptide moiety (62, 63). ST6GalNAc I is also known as the sTn synthase. Higher ST6GalNAc I and sTn antigen expression is a marker of malignancy with a poor prognosis in many epithelial cancers like gastric, pancreatic, colorectal, ovarian and breast cancers (17, 26, 41, 64-67).

The human ST6GalNAc III and ST6GalNAc IV show the most restricted substrate specificity using exclusively the Neu5Acα2-3Galβ1-3GalNAc- trisaccharide found on either *O*-glycosylproteins or ganglioside G_{M1b}, which suggests that they do not discriminate between α- and β-linked GalNAc (68, 69) (Table 1). Interestingly, Tsuchida *et al.* have proposed indirect involvement of ST6GalNAc VI in synthesizing disialyl lactotetraosyl-ceramide (Lc4), a precursor of disialyl-$Lewis^a$ (disialyl Le^a) (Figure 1) (70). Indeed, the human ST6GalNAc VI and to a lesser extent ST6GalNAc V and ST6GalNAc III also catalyze the transfer of a sialic acid residue onto GlcNAc residues (70, 71). It has been shown recently that the expression of ST6GalNAc V in breast cancer cells enhanced their adhesion to brain endothelial cells and their passage through the blood-brain barrier (72). In addition, over-expression of ST6GalNAc V in glioma cells suppresses glioma invasivity *in vivo* and tumor formation *in vitro* (73). *ST6GALNAC6* gene expression in colon cancer cells is down regulated compared to non malignant epithelial cells thereby contributing to the increased expression of sLe^a (74).

3.4. The α2,8-sialyltransferases (ST8Sia)

The six members of the human ST8Sia family catalyze the transfer in α2,8-linkages of one to several sialic acids to another sialic acid of glycoproteins or glycolipids (7).

ST8Sia I, ST8Sia V and ST8Sia VI can be viewed as mono-α2,8-sialyltransferases, since they catalyze the transfer of a unique sialic acid residue in α2,8-linkage (Table 1). ST8Sia I, which shows a strict specificity towards G_{M3} resulting in the formation of G_{D3}, is also known as G_{D3}-synthase (Figure 1). However, Nara *et al.* reported the molecular cloning of a short isoform of the human ST8Sia I (341 amino acids) using other gangliosides as acceptor substrates yielding to the formation of G_{D3} and

Table 2.Major lectins used for the study of cancer-associated sialylated structures

Lectin	Structure recognized	References
Sambucus Nigra Agglutinin (SNA)	Neu5Acα2-6Gal/GalNAc-R	(141, 186, 187).
Maackia Amurensis Agglutinin (MAA) -Leukoagglutinin -Hemagglutinin	 Neu5Acα2–3Galβ1–4GlcNAcβ- Neu5Acα2-3Galβ1-3[Neu5Acα2-6]GalNAcα-	 (188) (189)
Wheat Germ Agglutinin (WGA)	GlcNAc>>Neu5Ac	(97)
Peanut Agglutinin (PNA)	Galβ1-3GalNAcα1-Ser/Thr	(29, 98)

also G_{D1c}, G_{T1a} and G_{Q1b} *in vitro* (75), whereas Nakayama *et al.* reported the molecular cloning of a long isoform of the human ST8Sia I (356 amino acids) with an extended cytoplasmic domain capable of using G_{D3} to form G_{T3} *in vitro* (76). The human ST8Sia V also known as the G_{T3}-synthase (77) sialylates different gangliosides such as G_{D3}, G_{M1b}, G_{D1a}, G_{T1b} and G_{Q1c} (Table 1) (9, 78). The human ST8Sia VI catalyzes the transfer of a single sialic acid residue mainly on α2,3-sialylated *O*-glycans of glycoproteins (Neu5Acα2-3Galβ1-3GalNAc-) and also to a lesser extent on α2,6-sialylated *O*-glycosylproteins (Galβ1-3[Neu5Acα2-6]GalNAc-) leading to the formation of diSia motifs (79). The mouse *st8sia6* gene was found to be upregulated in lung dysplasia induced by c-Raf-1 (80).

The human ST8Sia III (81) catalyzes the transfer of one to several sialic acid residues either on glycoproteins or glycolipids (82, 83) and can be viewed as an oligo-α2,8-sialyltransferase (84). ST8Sia III is thought to be implicated in the biosynthesis of G_{T3} and disialyl-motifs found on CD-166 (Table 1) (85).

The human ST8Sia II and ST8Sia IV (86-88) catalyze the transfer of several sialic acid residues on other sialylated glycoconjugates. Both enzymes are expressed in the nervous system of most vertebrates where they act mainly on the α2,3-sialylated *N*-glycans of N-CAM (89-91) resulting in an increased neuronal plasticity and migration in embryonic vertebrate embryos (92). High levels of poly-sialic acid chain (PSA) are re-expressed in tumors of neuroectodermal origin and in small cell lung carcinoma (88).

4. INVESTIGATIONS-TOOLS TO STUDY SIALYLATION CHANGES AND STs IN CANCER

Lectin histochemistry and immunohistochemistry and various combinations of these methods are widely used to discover aberrant sialylation patterns serving as markers for tumor progression and metastasis and to develop new methods for diagnosis. In addition, several strategies have been developed to investigate variations in ST enzymatic activities and level of gene expression in cancer tissues.

4.1. Linkage and sialylated products formed: Use of lectins and antibodies

Since sialylated TACA may have a low expression level or a restricted expression pattern, lectins have proven useful tools in investigating sialic acid pattern of expression as well as diversity and linkages in normal and cancer cells and tissues (5, 93). As summarized in Table 2, the *Sambuccus nigra* agglutinin (SNA) detects the Neu5Acα2-6Gal-R oligosaccharides synthesized by β-galactoside α2,6-sialyltransferases and to a lesser extent, Neu5Acα2-6GalNAc-R oligosaccharides synthesized by GalNAc α2,6-sialyltransferases. Seeds from *Maackia amurensis* (MAA) contain two lectins that exhibit different specificity towards sialylated terminal structures: the leukoagglutinin preferentially binds to the Neu5Acα2-3Galβ1-4GlcNAc- oligosaccharide, whereas the hemagglutinin shows a higher affinity for the Neu5Acα2-3Galβ1-3[Neu5Acα2-6)]GalNAc oligosaccharide (94). The animal *Limax flavus* agglutinin (LFA) and the *Limulus polyphemus* agglutinin (LPA) are specific for terminal Neu5Ac and Neu5Gc in α2,3-, α2,6-, or α2,8-linkages (95, 96). The wheat germ agglutinin (WGA) is also frequently used although its specificity has to be confirmed by sialidase treatment, since it also binds terminal GlcNAc residues (97). The peanut agglutinin (PNA) from *Triticum vulgaris* recognizes non-sialylated T antigens obtained after sialidase treatment of human normal and cancer tissues section (29, 98). In addition to lectin histochemistry on tissue sections or fixed cells, Western or dot-blot for glycoprotein analysis, as well as thin layer chromatography for detection of gangliosides are carried out with lectins (99). Other sialic acid recognizing proteins have been used such as selectins, recombinant siglecs, the cholera toxin, which is a routine reagent staining the G_{M1} ganglioside and the hemagglutinin-esterase protein of *influenza C* virus, which specifically detects 9-*O*-acetylated sialic acid residues (100).

Whole cancerous cells have been used to immunize mice to generate monoclonal antibodies (mAbs) to various sialylated TACA, which serve for the detection of different types of sialic acid residues and linkages. Most of these antibodies are extremely specific and discriminate between various types of sialic acids (Neu5Ac or Neu5Gc) when carried by a given underlying oligosaccharide chain. Some mAbs are raised against sialylated *O*-glycans, like the sTn antigen overexpressed in cancers and their clinical usefulness for evaluating its levels in cancer patients is well documented (for review: (17)). MAbs recognizing cancer-associated terminal sialylated structures are also available, such as those against sLe^x and sLe^a tetrasaccharides carried by *N*- or *O*-glycosylproteins and some glycolipids. CA19-9 is the most common serum tumor marker for diagnosis of cancers of the gastro-intestinal tract (101). Noteworthy, sialic acid modifications such as *O*-acetylation might prevent mAb recognition. Since *O*-acetylation is significantly reduced in colon cancer, it contributes to the marked increase of detected sialic acid. Anti-gangliosides mAbs are widely used to probe their distribution in cells and tissues (102) as for the disialoganglioside G_{D3}, which is over-expressed in neuroblastoma, melanoma and also

breast cancer (103). Other gangliosides, i.e. 9-*O*-acetyl-G_{D3} and *N*-glycolyl-G_{M3}, are also abnormally expressed in breast tumors (104). Specific antibodies against all these sialylated glycolipids are available and allow their detection and quantification in cancers, which is the first step to understand their function.

4.2. ST enzymatic activities and ST mRNAs: Use of enzymatic assays, Q-PCR, microarrays

In vitro enzymatic assays, using exogenous acceptor and radiolabeled donor (CMP-Neu5Ac) substrates are carried out to compare ST activities in tumor samples and in healthy tissues. For example, increased level of ST6Gal I activity and expression of its product, the CDw75 antigen Neu5Acα2-6Galβ1-4GlcNAc- have been described in malignant and transitional tissue in human colorectal cancer compared to healthy mucosa with asialotransferrin and N-acetyllactosamine as exogenous acceptors (105). In gastric cancer, high levels of ST3Gal III and ST6Gal I activities correlated with secondary local tumor recurrence (106). However, some discrepancies might be observed between ST activities measured *in vitro* and the sialylated products detected in cancer versus healthy tissues. This can be explained at least in part by overlapping ST activities towards the same acceptor acquired during vertebrate STs evolution (8, 47, 107).

Relevance of STs in cancer is frequently evaluated at the mRNA level. Multiplex RT-PCR analysis of five human ST genes in breast cancer biopsies showed that *ST3GAL3* and *ST6GAL1* gene expression was associated with a poor prognosis (34). Moreover, a high *ST3GAL3*/*ST6GAL1* gene expression ratio associated to a high sE-selectin concentration correlated with reduced relapse-free and overall survival of node-negative patients (108). These data suggest that sLe^a and sLe^x motifs at the surface of cancer cells could be recognized by endothelial cells E-selectin, favoring metastasis formation. Mondal *et al.* demonstrated that *ST6GAL1* and *ST3GAL5* mRNAs were up-regulated in pediatric acute leukemia lymphoblasts and positively correlated with a higher risk of disease, whereas their expression was negligible in non-malignant donors (45). Similarly, *ST8SIA2* gene is highly expressed in various stages of neuroblastoma tumors suggesting its potential clinical relevance as a molecular marker of metastatic neuroblastoma (109). In most of these studies, authors correlated ST mRNA levels to the detection of specific sialylated TACA in healthy *vs*. cancer tissues. The increased *ST3GAL1* expression contributes to the higher level of α2,3-sialylation found in ovarian serous carcinoma (31). Similarly, over-expression of *ST3GAL1* involved in T antigen sialylation appears to be part of the initial oncogenic transformation of bladder cells thus predicting cancer progression and recurrence (29). However, correlation between ST gene expression and sialylated determinants is not always observed. For example, sTn is over-expressed in 30 % of breast tumors and considered as a bad prognosis factor (17) whereas the expression of *ST6GALNAC1* involved in the sTn biosynthesis has been associated with a better prognosis in breast cancer (110).

Microarrays followed by Q-PCR validation provide another method for monitoring ST mRNA expression levels simultaneously in primary tumors and cell lines. A Human Whole Genome Oligo Microarrays was used to investigate genes differentially expressed in early- and late-stage oral squamous cell carcinoma. *ST6GAL1* was up-regulated in early stages of the disease and could be involved in local tumor invasion and metastasis (111). Differences in the expression of *ST8SIA1* in breast cancer cells were investigated using microarray data of 1,581 tumor samples. Among estrogen receptor (ER) positive breast cancers, a significant worse prognosis was found for patients with tumors showing a low *ST8SIA1* expression (112). The GeneChip analysis of colonic tissue of healthy individuals compared with early staged colonic carcinomas proved a high expression level of *ST6GAL1* in colonic carcinomas, and showed that *ST3GAL4* was the most abundantly expressed ST gene in healthy tissues (113). A more focused microarray analysis of glyco-gene expression was performed in human glioma showing a low level of *ST6GALNAC5* in glioma compared to normal brain (73, 114).

4.3. Use of chemical inhibitors of ST activities and ST antisense oligodeoxynucleotides

Chemical inhibitors of STs are used to analyze the role of sialylation in cancer progression and metastases and serve as new potent anti-inflammatory, immunosuppressive and anti-metastatic agents for future therapeutic applications. Soyasaponin I, a natural molecule isolated from soybean, is the first described CMP-Neu5Ac competitive inhibitor of ST3Gal I *in vivo* (115). Over the past two decades, efforts have been made to design and synthesize specific ST inhibitors such as donor- and acceptor-substrate based analogs or compounds with a structural mimetic of transition-state (for review: (116-118)). Although these compounds are effective inhibitors *in vitro*, they are less efficient *in vivo* due to a poor permeability across cell membranes. Only a few of them with a cell-permeable property have been reported so far (119, 120). Among these compounds, the ST inhibitor KI-8110 (5-fluoro-2',3'-isopropylidene-5'-O-(4-N-acetyl-2,4-dideoxy-3,6,7,8-tetra-O-acetyl-1-methoxycarbonyl-D-glycero-α-D-galactooctapyranosyl) uridine), a donor-analog, efficiently lowered pulmonary metastatic potential of murine NL-17 colon adenocarcinoma cells (121) by inhibiting platelet-derived growth factor-(PDGF) dependent growth of cancer cells (122). Lithocholic acid analogues with a steroid-related structure similar to that of Soyasaponin I, were found to be noncompetitive inhibitors of Galβ1-3GalNAc α2,3-sialyltransferases (123). Among the lithocholic acid derivatives, AL10 inhibits adhesion, migration and invasion of ST3Gal I over-expressing A549 and CL1.5 human lung cancer cells, associated with reduced integrin signaling and it also significantly suppresses experimental lung metastasis (124). Lith-O-Asp, another lithocholic acid derivative, suppresses cell tumor metastasis /colonization *in vitro* and *in vivo* through degradation of ST proteins (125). Finally, competitive inhibitors of *O*-glycosylation such as benzyl-*N*-acetyl-α-D-galactosaminide (BGN) inhibit *O*-linked oligosaccharide sialylation in cancer cells (126). BGN treatment results in a decreased CD44 *O*-glycan sialylation and enhanced metastatic ability of B16BL6 melanoma cells (127).

Specific inhibition of STs by antisense or small hairpin RNA is useful to analyze their role in cancer. Antisense silencing of ST8Sia I in F-11 rat neuroblastoma cells greatly reduces cell proliferation, angiogenesis, vascular endothelial growth factor (VEGF) production and tumor growth in nude mice (128, 129). Similarly, ST8Sia I antisense knockdown reduces cell growth in the hamster melanoma AbC-1 cell line (130). Significant decrease of cell growth and invasion activity is also observed in lung cancer cells, when stably transfected with a ST8Sia I RNAi expression vector (131). In parallel, ST3Gal V silencing in 4T1 highly metastatic mouse mammary tumor cells significantly inhibits cell migration, invasion and anchorage-independent growth *in vitro*, and lung metastasis *in vivo*, through the activation of PI3K/Akt pathway and nuclear factor of activated T cells (NFAT) inhibition (132). ST6Gal I is known to be up-regulated in several human cancers, including breast and colon cancers. Transfection of MDA-MB-435 cancer cells with ST6Gal I cDNA increases cell surface α2,6-sialylation and cell migration and reduces cell–cell adhesion. Antisense ST6Gal I RNA significantly decreased collagen IV and cell-extracellular matrix adhesion (133), suggesting that cell surface α2,6-sialylation contributes to extracellular matrix adhesion of tumor cells. It has been also reported that ST6Gal I silencing strongly reduces the ability of HT-29 human colon cancer cells to form colonies in soft agar and to invade the extracellular matrix (134).

5. MECHANISMS OF ST REGULATION

The relative enzymatic activity of STs influences the expression of sialylated TACA at the cell surface and contributes to the definition of the glycosylation pattern of normal and tumor cells. STs spatio-temporal expression appears to be regulated mainly at the transcriptional level although epigenetic and posttranslational controls might be implicated.

5.1. Genetic regulation: transcriptional regulation of ST

Multiple approaches including Q-PCR, microarray analysis, enzymatic assays, as well as Northern blot and *in situ* hybridization have demonstrated a correlation between ST mRNA expression and enzymatic activity, suggesting that the synthesized sialylated structures mostly depend on transcriptional regulation of ST genes (135). In the past decades, several studies have documented the structure, genomic organization as well as transcriptional regulation of human ST genes in cultured cells (for review see (7, 136)). Multiple mRNA isoforms that differ essentially in their 5'-untranslated (5'-UT) regions have been described for several ST genes, most of which being tissue or cell type-specific. These mRNA isoforms usually arise from a combination of alternative splicing and promoter use, leading to a complex tissue or cell-specific transcriptional regulation of ST genes. Among these, *ST6GAL1* genetic regulation has been the most studied. Mammalian *st6gal1* genes share a common genomic organization with five coding exons (137-140). Sequence analysis of the 5'-flanking genomic region revealed heterogenous transcriptional start sites (TSS), at least three physically distinct promoters named P1, P2 and P3 and the absence of the canonical TATA and CCAAT boxes. These promoters govern and regulate the expression of multiple human *ST6GAL1* mRNAs resulting from the combinatory use of seven 5'-UT exons named E1', EX, EY, EZ, EU, EV and EW (reviewed in (7)). RT-PCR analysis of carcinoma samples with an increased ST6Gal I activity compared to normal mucosa revealed the presence of at least two transcripts: the ubiquitously expressed transcript with the EY and EZ 5'-UT exons arising from P3 promoter and the hepatic specific transcript with the E1' 5'-UT exon arising from the P1 promoter. Both ST6Gal I transcripts were detectable in normal and cancer tissues, but the hepatic specific transcript had a marked tendency to accumulate in cancer (141, 142). This might be explained by the fact that P1 promoter shows binding site for the hepatocyte nuclear factor HNF-1 transcription factor, which is expressed not only in liver, but also in colon cancer tissues (143). In cervical squamous carcinoma cells, an increased level of *ST6GAL1* hepatic transcripts has been described that may have an impact in cancer transformation (144). Similarly to *ST6GAL1* gene, the *ST6GAL2*, *ST3GAL2*, *ST3GAL4*, *ST3GAL5* and *ST3GAL6* genes show also several 5'-UT exons and heterogeneous TSS leading to several mRNA isoforms. It is interesting to note that the other ST genes like *ST3GAL1*, *ST3GAL3*, all the *ST6GALNAC* and all the *ST8SIA* genes described up to date display a unique promoter region and ubiquitous transcription factors binding sites. These regulatory units drive the expression of unique mRNA or alternatively internally spliced RNA (reviewed in (7)) as described recently for the human *ST8SIA1* gene (145-147)).

5.2. Epigenetic regulation

The transcriptional control of ST gene expression is not sufficient to fully explain the observed induction of sialylated TACA in cancer (25, 148-152). Recently, DNA hypermethylation or histone modifications have been recognized as one of the important mechanisms for gene inactivation in cancer (153). Indeed, addition of a histone deacetylase inhibitor trichostatin A and the DNA methylation inhibitor 5-azacytidine (5-aza-C) to the culture medium of sLe^a positive human colon cancer cells induces significant transcription of *ST6GALNAC6* and surface expression of disialyl-Le^a epitope (74, 101). These data suggest an epigenetic silencing mechanism for the *ST6GALNAC6* gene expression leading to the decreased expression of the disialyl-Le^a epitope in cancer cells, but resulting in an enhanced accumulation of sLe^a in cancer cells (154). Kawamura and co-workers have shown that among gastric cancer-associated down-regulated glycogenes, the *ST3GAL6* gene was hypermethylated in most gastric cancer cell lines and in gastric cancer tissues causing silencing of the enzymatic activity of this enzyme (155). In addition, colon carcinoma HCT15 cells treated with Decitabine (5-aza-2'-deoxycytidine: 5-Aza-dC) showed enhanced production of sLe^x on MUC1 through induction of *ST3GAL6* gene leading to increased binding to E-selectin and higher metastatic potential of these cells. These recent data raise a concern about the safety of 5-Aza-dC used for cancer treatment (156). Finally, data from microarray analysis showing candidate genes with greatest fold changes in expression after treatment with 5-aza-C in

neuroblastoma described *ST3GAL2* gene as one of these genes, although no CpG methylation could be detected (157).

5.3. Post-translational modifications and Golgi pH influence on ST intracellular location and activity

A number of studies have also suggested that post-translational modifications of STs such as phosphorylation or *N*-glycosylation and Golgi microenvironment could influence ST enzymatic activities (158). Resulting perturbations of sialylation efficiency might be secondary to disturbances in the assembly line of ST in the Golgi. The human ST6Gal I is expressed as two differentially phosphorylated protein isoforms, ST-Tyr and ST-Cys, which exhibit differences in catalytic activity and trafficking through the secretory pathway (159, 160). The enzymatic activity of STs implicated in the biosynthesis of gangliosides can be modulated by protein kinase C and phosphoprotein phosphatase (161). *N*-glycosylation of STs is also known to influence their enzymatic activity and subcellular location (162). Indeed, depletion of *N*-glycan by site directed mutagenesis of the sequon NXS/T or by treatment with glycosylation inhibitors like tunicamycin or castanospermine often leads to a reticulum re-distribution, an increased turn-over and hence a loss of enzyme activity of STs (163-170). Recently, it has been proposed that variations in the Golgi pH as observed in cancer cells (171, 172) might be partly responsible for abnormal sialylation observed in cancer cells (173) as for unsialylated MUC1 found in breast cancer cells (27, 150, 152) despite elevated levels of ST3Gal I (174). The link between Golgi pH and mislocalization of Golgi STs was recently highlighted by Rivinoja *et al*. especially for the α2,3-sialyltransferase ST3Gal I where a severe mislocalization into endosomal compartments was observed after drug-induced Golgi pH increase. At the opposite, ST6Gal I subcellular location and enzymatic activity remained almost unaffected (175). Another interesting parameter that could regulate ST activity in cancer is the interaction of STs among themselves with the formation of active homodimers or as complexes of several partners (oligomerization) through cysteine residues, as already shown for ST6Gal I (176-179).

6. CONCLUSION

Increased expression of sialylated TACA is a common feature observed at the surface of cancer cells that correlates most of the time to an altered expression of the corresponding STs involved in their biosynthesis. The molecular mechanisms governing sialylated TACA biosynthesis are not yet fully understood. Besides genetic and epigenetic regulation of STs, the leading causes of STs up- and down-regulation, posttranslational modifications of STs themselves and their arrangement along the secretory pathway appear to be essential factors controlling sialylated TACA biosynthesis. The resulting changes in sialylation are known to play key roles in cancer cell growth, invasion and metastasis. TACA provide a major source of cancer and prognostic biomarkers characterized by clinicians through the use of a panel of powerful tools such as monoclonal antibodies or lectins.

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Footnotes: The nomenclature of sialyltransferases is based on Tsuji *et al.* (180) and the nomenclature of glycolipids is based on Svennerholm (181).

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