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MINIREVIEW

Sialyltransferases in cancer

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It has long been known that cancer cells often express more heavily sialylated glycans on their surface and that this feature sometimes correlates with invasion. It is now well established that specific sialylated structures, such as the Thomsen-Friedenreich-related antigens, the sialyl Lewis antigens, the sialyl α2-6 lactosaminyl structure, the polysialic acid or some gangliosides, can mediate cellular interactions and are altered in cancer cells. This review summarizes the current knowledge on the cancer-associated alterations in sialyltransferase expression which are often at the basis of the deranged expression of sialylated structures.

Keywords: **glycosyltransferases, sialyltransferases, polysialic acid, gangliosides, Lewis antigens**

The distal portion of the oligosaccharide chains of glycoproteins and glycolipids is often decorated by sialic acids (Sia), nine carbon sugars bearing a net negative charge at physiological pH values (for a recent review see [1]). A determinate number of principal sialyl-linkages has been described so far in mammalian sialoglycoconjugates: sialic acid may be linked either through an α2-3- or an α2-6-bond to galactose (Gal); through 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. The different sialyl-linkages are elaborated by different members of the sialyltransferase family, a class of glycosyltransferases which share the same donor substrate (CMP-sialic acid) but may differ for the glycosidic structure on which they act and for the type of glycosidic linkage they form. Despite the relatively small number of existing sialyl linkages, the number of different sialyltransferase cDNAs cloned so far approaches 20 (for a recent review see [2]). As described for other glycosyltransferases, such as galactosyltransferases and GalNAc transferases, a certain degree of redundancy exists also for sialyltransferases, in that the same glycosidic linkage can often be elaborated by different gene products (see Table 1).

Sialyltransferases are relevant in cancer for several reasons. First, sialic acids can prevent cell-cell interactions through non-specific charge repulsion effects; second, sialylated structures can be specifically bound by cell adhesion molecules such as those of the selectin [3] or the siglec [4] families; third, the addition of sialic acids may mask the underlying sugar structure, thus avoiding recognition by other lectin like molecules, such as galectins [5]; fourth, the regulatory elements of sialyltransferase genes might be a target of specific cell signaling pathways. Thus, the up-regulation of a sialyltransferase in a cancer cell might indicate that a specific signaling pathway has been activated. An example is provided by the up-regulation of ST6Gal I by *ras* oncogene overexpression [6].

Early indications on a role played by sialic acids in cancer biology came from experimental studies showing that the extent of cell surface sialylation of various murine cancer cell lines positively correlated with their invasive properties [7–9] and from clinical studies showing a relationship between serum sialyltransferase levels and tumor burden in gastrointestinal cancers [10]. More recently, it has been shown that the sialyltransferase inhibitor KI-8110 reduces the metastatic potential of murine [11,12] and human [13] cancer cells, while the sialidase activity of rat 3Y1 cells inversely correlated with their metastatic potential [14]. However, some discrepancies exist among the cited studies. For example, the enzymatic removal of sialic acid from the cell surface results in an increased binding of murine cancer cells to collagen type IV [8], but in a reduced binding of human cancer cells to the same adhesive glycoprotein [9]. Sialic acid depletion caused by KI-8110 does not modify the binding of mouse colon carcinoma cells to extracellular matrix glycoproteins but rather affects their ability to induce platelet aggregation [12]. Even though an overall altered sialylation has often been described in cancer cells, the conclusions reached by the experiments based on a complete depletion

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Table 1. Cloned sialyltransferases

Sialyltransferase[a]	*Other name(s)*	*Acceptor(s)*	*References*
ST3Gal I	ST3O, ST3GalA.1, SiaT4a	Galβ1-3GalNAc	[19,137–140]
ST3Gal II	ST3GalA.2, SAT4, SiaT4b	Galβ1-3GalNAc	[137,141–143]
ST3Gal III	ST3(N)	Galβ1-3(4)GlcNAc	[144,145]
ST3Gal IV	STZ, SAT3, SiaT4c	Galβ1-3GalNAc[b]/Galβ1-4GlcNAc	[19,137,146]
ST3Gal V	GM3 synthase	Galβ1-4Glc-Cer	[147,148]
ST3Gal VI		Galβ1-4GlcNAc	[61]
ST6Gal I	SiaT1, α2,6ST, ST6(N)	Galβ1-4GlcNAc	[64,149–151]
ST6GalNAc I		GalNAc/Galβ1-3GalNAc/Siaα2-3Galβ1-3GalNAc	[152–154]
ST6GalNAc II		GalNAc[c]/Galβ1-3GalNAc/Siaα2-3Galβ1-3GalNAc	[155–157]
ST6GalNAc III		Siaα2-3Galβ1-3GalNAc	[158,159]
ST6GalNAc IV		Siaα2-3Galβ1-3GalNAc	[158,160]
ST6GalNAc V	GD1α synthase	GM1b	[161]
ST6GalNAc VI	GD1α/GT1aα/GQ1bα synthase	GM1b, GT1b	[162]
ST8Sia I	GD3 synthase, SAT II	GM3	[163–166]
ST8Sia II	STX	Sia Galβ1-4GlcNAc	[98,167]
ST8Sia III		Siaα2-3Galβ1-4	[168,169]
ST8Sia IV	PST-1	Sia Galβ1-4GlcNAc	[99,170–172]
ST8Sia V	SAT V/SAT III	GM1b, GD1a, GT1b, GD3	[173]

[a]The nomenclature used is that proposed by Tsuji et al. [174].
[b]Galβ1-3GalNAc is reported to be a good acceptor of human ST3Gal IV [19], but a poor acceptor of the mouse enzyme [20].
[c]Activity of ST6GalNAc II on GalNAc, formerly excluded [155], has been successively documented by Kono et al. [18].

of sialic acid residues from the cell surface should be considered with caution. As recently demonstrated [15], overexpression of a sialidase activity in mouse colon adenocarcinoma cells results in a decreased metastatic ability which is not dependent on a reduction of the overall sialylation level but is dependent on a reduced expression of specific sialylated structures, such as sialyl Lewis^x and ganglioside GM3. Thus we will discuss the cancer-related changes of specific sialylated structures and of their cognate sialyltransferases.

Thomsen-Friedenreich-related antigens

Carbohydrate structures formed by a GalNAc or by a Galβ1-3GalNAc disaccharide O-glycosidicaly-linked to serine or threonine, as well as by their sialyl-substitutions are collectively referred to as Thomsen-Friedenreich-related antigens (TF antigens) (Figure 1). The biosynthesis of these and other simple O-linked structures depends on a coordinate expression of several glycosyltransferases [16,17] (Figure 1) and has often been correlated with cancer progression. After the addition of a peptide-linked GalNAc by a peptidyl-GalNAc transferase, originating the Tn-antigen, the sugar chain can be completed by the addition of an α2-6-linked sialic acid, yielding the sialyl-Tn antigen. Both ST6GalNAc I and -II can act as a sialyl-Tn synthase, provided that the GalNAc residue is linked to a peptide moiety [18]. Otherwise, the Tn-antigen can be elongated by the addition of a β1-3-linked galactose, yielding the core 1 structure (T-antigen) or by the addition of a β1-3GlcNAc, yielding the core 3 structure. Further processing of the T antigen include: (1) the addition of a GlcNAc β1-6-linked to GalNAc, generating the core 2 structure; (2) the addition of sialic acid in α2-3 linkage to Gal by ST3Gal I or ST3Gal II, yielding the sialyl-T antigen. The contribution of ST3Gal IV to the biosynthesis of sialyl-T antigen is controversial. In fact, the activity towards Galβ1-3GalNAc displayed by the human form is good [19], while that displayed by the mouse form is very poor [20]. Sialyl-T antigen, in turns, can be further sialylated by ST6GalNAc I, -II, -III or -IV, giving rise to the di-sialyl T-antigen; (3) the addition of a sialic acid α2-6-linked to GalNAc, by ST6GalNAc I or -II [18].

During neoplastic transformation of breast epithelium, mucin glycosylation undergoes a characteristic switch from the expression of core 2 structures to accumulation of T [21] and sialyl-T structures [22,23]. The molecular basis of this change is provided by a coordinate down-regulation of core 2 β1-6 GlcNAc transferase and an up-regulation of α2-3 sialyltransferase activities [24,25], enzymes which compete for core 1 structure. However the mere overexpression of ST3Gal I, even in the presence of unaltered levels of core 2 GlcNAc transferase, is sufficient to induce the shift from core 2 to core 1 structure in mammary cells [25,26]. Of the three sialyltransferases which can mediate the α2-3 sialylation of T-antigen, ST3Gal I and ST3Gal IV are expressed in both breast cancer tissues [27] and cell lines [28], and the former is elevated, compared with normal breast tissue [29]. A simple down-regulation of core-2 GlcNAc transferase, as occurs in some breast cancer specimens, is consistent with the overexpression of the T-antigen, which correlates with progression and metastasis in this and other epithelial malignancies [21]. A molecular basis for the relationship between T-antigen expression and metastasis is provided

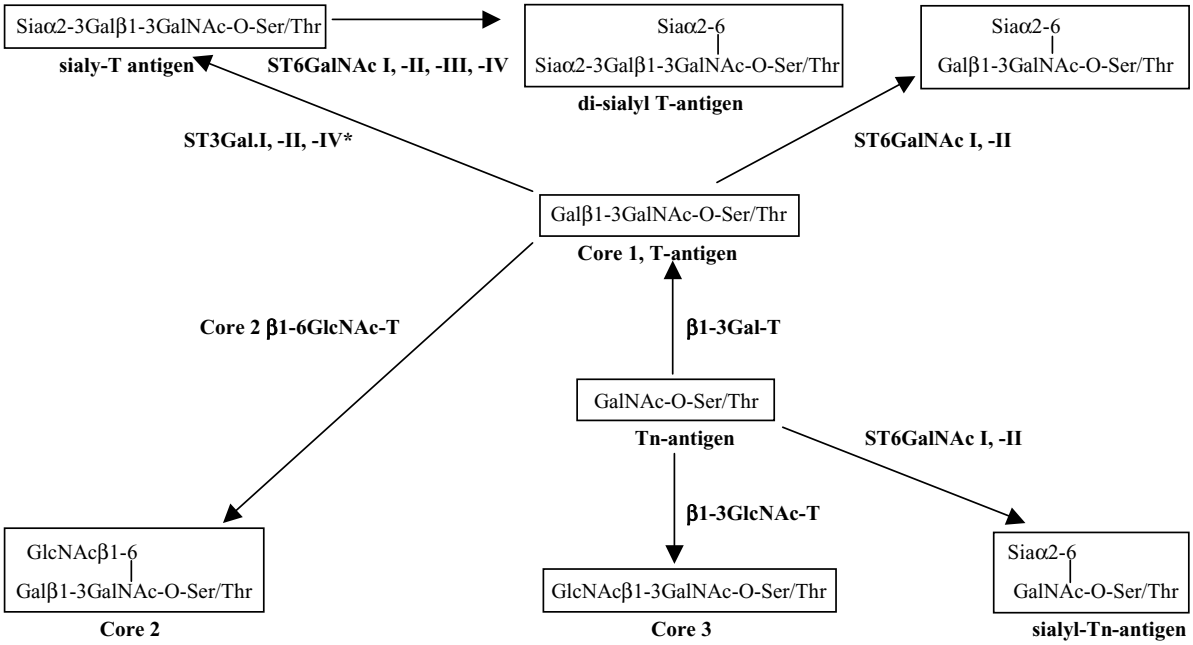

Figure 1. Schematic representation of the biosynthesis of Thomsen-Friedenreich-related antigens. The Tn antigen, originated by the addition of a GalNAc to serine or threonine residues of the polypeptide chain, can be transformed in sialyl-Tn antigen by the action of ST6GalNAc I or -II, or can be elongated by the addition of a β1-3-linked galactose, yielding the core 1 structure (T-antigen) or by the addition of a β1-3GlcNAc, yielding the core 3 structure. The T antigen can be further processed by the addition of a GlcNAc β1-6-linked to GalNAc, generating the core 2 structure; or by the addition of sialic acid in α2-3-linkage to Gal by ST3Gal I or ST3Gal II or ST3Gal IV (but the contribution of the latter enzyme is controversial, see text), yielding the sialyl-T antigen which, in turns, can be further sialylated by ST6GalNAc I, -II, -III or -IV, generating the di-sialyl T-antigen. Core 1 structure can be also directly sialylated on the GalNAc residue by ST6GalNAc I or -II.

by the observation that this carbohydrate structure can mediate docking of tumor cells to endothelial cells expressing galectin 3 [30]. Also sialyl-Tn antigen is expressed by breast cancer and correlates with a poorly differentiated state [31], but the molecular basis of this change is not clear.

The TF-related antigens are not normally expressed in colonic epithelium, while T, Tn and sialyl-Tn are expressed by adenomatous polyps and carcinomas [32–34]. The biochemical bases of the aberrant expression of these antigens in colon cancer appear to be complex; in fact marked inconsistencies between the expression of TF-related antigens and that of their cognate glycosyltransferases have been reported to occur in colon cancer tissues [35]. The reduction of core 3 GlcNAc-transferase activity, which normally compete with core 1 galactosyltransferase, reported in colon cancer tissues, [36] and cell lines [37], may "open the way" to the biosynthesis of T-antigen and, together with the up-regulation of α2-3 sialyltransferases, may explain the increased expression of T- and sialyl-T antigens. The up-regulation of the sialyl-Tn antigen, which is an highly reliable tumor marker in colon cancer [38,39], can be explained by an increased activity of sialyl-Tn synthases in rat tissues [40], but not in human colon cancer tissues or cell lines, where these activities are decreased, rather than increased, [36,37]. Another potential mechanism, decribed in a colon cancer cell clone, is based on the downregulation of core 1 β1-3Gal-T which results in the accumulation of Tn and sialyl-Tn antigens because of the inability to synthesize the core 1 structure [41]. However, the most important mechanism which explains sialyl-Tn expression in colonic tumors is probably based on the level of O-acetylation of sialic acid. The sialyl-Tn antigen is normally expressed also by the normal mucosa but cannot be detected by monoclonal antibodies because of the O-acetylation of sialic acid; a modification reduced in cancer tissues [42,43]. Other studies on the sialyltransferases of TF-related antigens in colonic tissues report sometimes conflicting results. At the mRNA level, there is a general agreement on the increased expression in colon carcinoma of ST3Gal I [44–46] and ST3Gal II [45,46]. On the contrary, ST3Gal IV has been found to be decreased in colon carcinoma tissues by a microarray analysis [47] and by RT-PCR analysis [44], while other RT-PCR studies reported either an elevation in the less differentiated carcinoma specimens [45], or a decreased expression but only in a subset of patients [48]. Recently, an overexpression of ST6GalNAc II has been reported in patients with lymph node metastasis and has been proposed as a prognostic indicator of a poor survival [46]. The same study reports also a competition between ST6GalNAc II and core 2 GlcNAc-T in determining the switch from core 2 to TF-type oligosaccharides, proposing that the balance of these two enzymes plays a key role in the regulation of mucin glycosylation in colon cancer.

Sialyl Lewis antigens

Cell adhesion molecules of the selectin family mediate fundamental processes, including lymphocyte homing, adhesion to endothelial cells of leukocytes during inflammation and of cancer cells during metastasis formation. Sialyl Lewis^a (Siaα2-3Galβ1-3[Fucα1-4]GlcNAc, sLe^a) and sialyl Lewis^x (Siaα2-3Galβ1-4[Fucα1-3]GlcNAc, sLe^x) antigens are both selectin ligands and are derived from the α2-3sialyl- and the α1-3/4 fucosyl-substitution of type 1 (Galβ1-3GlcNAc-R) or type 2 chains (Galβ1-4GlcNAc-R), respectively, by the coordinate expression of α2-3 sialyltransferases and 1,3/4 fucosyltransferases. An increased expression of sLe^x and sLe$^\alpha$ structures in various malignancies and in metastatic lesions has been well documented (reviewed in [49]). Although alternative mechanisms, like the level of O-acetylation of sialic acids, (as occurs for the sialyl-Tn antigen, see above) appear to play a role [50], the altered expression of these antigens usually depends on an altered expression of α2-3 sialyltransferases and/or of α1-3/4 fucosyltransferases. The α2-3 sialylation of type 1 chains can be catalyzed by ST3Gal III, while that of type 2 chains can be mediated by ST3Gal III, ST3Gal IV and ST3Gal VI. Normal and neoplastic colon tissues [51] and colon cancer cell lines [52] express an α2-3 sialyltransferase activity towards type 2 chains much lower than the corresponding α2-6 activity (ST6Gal I); a remarkable exception is provided by the cell line HT29 [52]. A study on α2-3 sialyltransferases and fucosyltransferases in the colon cancer cell lines HT29 and COLO205 provides important clues on the relative contribution of the two classes of enzymes to the biosynthesis of sLe^a and sLe^x antigens [53]. The first cell line expresses an α2-3ST activity towards both lacto N-biose (type 1 chain) and N-acetyllactosamine (type 2 chains) about ten times higher than the second, but the expression of ST3Gal III mRNA is not different in the two cell lines, suggesting the involvement of other α2-3 sialyltransferase encoding genes (not yet cloned at the time of the study). On the contrary, the fucosyltransferases acting on the two types of chains are much more active in COLO205. As a result, the level of expression of sLe^a and sLe^x antigens is not very different in the two cell lines, suggesting that the regulation of the biosynthesis of sialylated Lewis antigens may occur both at the α2-3 sialyl- and fucosyltransferases level. HT29 cells grown in galactose-containing medium (a condition which induces differentiation of this cell line) show, compared with the usual growth conditions, an increased sLe^a expression and a concomitant elevation α2-3 sialyltransferases [54]. Consistently, the expression of the CA19-9 (sLe^a) antigen in colon cancer homogenates was found to correlate with the expression of α2-3 sialyltransferases active on type 1 chains but not with that of fucosyltransferases [55]. An opposite conclusion was achieved by a study showing that the increased level of sLe^a antigen displayed by highly metastatic variants of a human colon cancer cell line correlates with the expression of an α1-4 fucosyltransferase, but not with the expression of the α2-3 sialyltransferases acting on type 1 chains [56]. In a study by Kudo et al. [45] the expression of several glycosyltransferases involved in the biosynthesis of Lewis-type antigens was measured by competitive RT-PCR in normal colon and colon carcinoma specimens, in parallel with the presence of sialyl-Lewis antigens. The conclusion reached was that sialyltransferase ST3Gal IV and fucosyltransferases Fuc-TIII and Fuc-TVI are the main responsible for the biosynthesis of sialylated Lewis antigens in colonic tissues but the increased expression of these antigens in cancer tissues does not depend on the up-regulation of these enzymes. As discussed in detail in the previous section, the alterations of ST3Gal IV in colon cancer are probably different in different groups of patients, but marked discrepancies exist about the reported alterations of ST3Gal III. This transcript was found to be expressed at a very low level by Kudo et al. [45], while it was significativelly increased in carcinoma tissues according to Petretti et al. [48] but unchanged according to Ito et al. [44]. The importance of the α2-3-sialylated type 1 and type 2 chains in mediating adhesion of colon cancer cells is demonstrated by the fact that transfection with either ST3Gal III or ST3Gal IV results in an increased adherence of the colon cancer cell line SW48 to endothelial cells [57].

In breast cancer, the expression of the sLe^x antigen correlates with that of Fuc-TVI but not with sialyltransferases ST3Gal III or ST3Gal IV [58], while in lung cancer, an increased expression of sLe^x correlates with a higher expression of ST3Gal III and Fuc-TVII, but not with that of other sialyl- or fucosyltransferases [59]. Very recently, it has been shown that exposure of normal bronchial mucosa to TNF-α, a condition often occurring during lung infection, results in an up-regulation of sialylated and sulfated Lewis antigen because of an up-regulation of ST3Gal III and ST3Gal IV as long as that of fucosyl- and sulfotransferases [60]. These data confirm that the biosynthesis of the sialyl Lewis antigens may be regulated both at the level of α2-3 sialyltransferases and fucosyltransferases.

A recently cloned α2-3 sialyltransferase, ST3Gal VI [61], shows a strict substrate specificity for type 2 chains and could play a key role in sLe^x biosynthesis. Although the expression of ST3Gal VI and of sLe^x appears to correlate poorly in a panel of tumor cell lines [61] the role played by this enzyme in the biosynthesis of sLe^x epitope in cancer tissues deserves further consideration.

Sialyl α2-6-lactosaminyl antigen

The α2-6 sialylation of type 2 chains is mediated by a single enzyme: β-galactoside α2-6 sialyltransferase (ST6Gal I) [62–64].[1] Several malignancies, including colon [51,52,65], and breast carcinoma [27,28], acute myeloid leukemia [66], choriocarcinoma [67], cervical carcinoma [68] and some types of brain tumors [69] show an elevation of ST6Gal I activity or of the transcript (for a recent review see [70]). Using the α2-6-sialyl-specific lectins from *Sambucus nigra* (SNA) [71] and *Tricosanthes japonica* as probes, a marked increase of the degree of α2-6 sialylation has been demonstrated in colon cancer tissues [72–74]. Clinical studies reveal that both a high degree of SNA reactivity [75] and of ST6Gal I expression [76] are

indicators of a poor prognosis. However, the quantitative relationship between ST6Gal I expression and SNA reactivity in colon cancer specimens is not as strict as one could expect [77], suggesting that the expression of α2-6-sialylated oligosaccharides is controlled at multiple levels. At the mRNA level, several studies report a tendency towards an up-regulation in colon cancer tissues [45,48,77,78]. Transcription of the ST6Gal I gene occurs through physically distinct tissue-specific promoters which originate mRNA species different in the 5′ untranslated regions. In normal and neoplastic colonic cells at least two mRNA species are produced [77,79]: one, referred to as YZ, is expressed by several tissues and is thought to correspond to the housekeeping expression of the gene [80], the second is thought to be liver-specific [81]. It is not clear at the moment which factors affect quantitatively the expression of either isoform in colonic tissues. In breast cancer, high ST6Gal I expression was correlated with histoprognostic grade III and down regulation of progesterone receptor [27].

The data on the phenotypic effects of a high ST6Gal I expression are conflicting and not conclusive. Our and other groups reported the following observations, consistent with a positive relationship between ST6Gal I overexpression and malignancy: (i) a non-adherent subpopulation selected from the adherent colon cancer cell line SW948 shows ST6Gal I overexpression [82]; (ii) nude mice xenografts of colon cancer cell lines usually show an ST6Gal I activity higher than the original cell line and this enhanced activity is retained also by the cell line derived from the xenograft [52]; (iii) highly metastatic variants of colon cancer cells express stronger reactivity with SNA and higher ST6Gal I activity than poorly metastatic variants [83]; (iv) among subpopulation of *ras*-transfected fibroblasts, those expressing high ST6Gal I and strong SNA reactivity showed a more aggressive behavior *in vitro* than their SNA-negative counterparts [84]; (v) HT29 cells whose ST6Gal I expression was suppressed by antisense cDNA transfection, showed a strongly reduced anchorage-independent growth and *in vitro* invasivity [85]. On the other hand, transfection with an ST6Gal I cDNA strongly reduces the invasivity of glioma cells [86,87], while an isolated mutant cell line expressing high ST6Gal I activity turned out to be less metastatic and grew slower that the parental cell line [88]. In our lab, transfection of the human colon cancer cell lines SW48 and SW948, originally devoid of detectable ST6Gal I activity, with ST6Gal I cDNA originated cell clones expressing an ST6Gal I activity higher than that of liver cells [89,90]. The morphology of the SW48-derived clones is undistinguishable from that of control transfectants, while SW948-derived clones often display a flatter morphology and a reduced growth rate (Chiricolo and Dall'Olio, unpublished results). Neither the susceptibility to natural killer cells [91] nor the anchorage-independent growth of the SW48-derived clones appear to be dependent on ST6Gal I expression (Chiricolo and Dall'Olio, unpublished results). However, in these experiments we observed dramatic phenotypic differences among control clones, pointing to the role played by insertional mutagenesis in determining the phenotype of transfected cells. All these apparently conflicting results can be explained in the light of the following points. First, in the experiments not involving direct transfection with the sialyltransferase (or antisense) cDNA, the relationship between ST6Gal I expression and invasivity can be non-causal. It should be remembered that in rodent fibroblasts ST6Gal I is directly regulated by the *ras* oncogene [6,92,93]; thus the higher ST6Gal I expression in more invasive cancer cells might simply reflect the activation of the *ras* pathway. Second, ST6Gal I competes with ST3Gal III, ST3Gal IV and ST3Gal VI for type 2 chains and a differential expression of ST6Gal I might result in different effects on the biosynthesis of sialyl Lewis^x antigen, depending on the background of α2-3 sialyltransferases expressed by that given cell type. Third, the set of glycoprotein acceptors undergoing α2-6 sialylation is different in different cell lines and might mediate completely different biological processes. Fourth, mice whose ST6Gal I gene has been knocked out display only immunological alterations but their tissues and organs are otherwise normal [94].

Poly α2,8 sialylation

Polysialic acid (PSA) provides a unique type of modification of the N-linked chains of the neural cell adhesion molecule (N-CAM) and of a few other glycoproteins, in which tens of sialic acid residues are joined through an α2-8-linkage in a linear array [95]. N-CAM-linked PSA exists in an embryonic form with a high degree of polymerization (DP) and an adult form showing a reduced DP. A high DP results in a reduced strength of intercellular adhesion, not only those mediated by N-CAM but also those mediated by other cell adhesion molecules [96], allowing tissue plasticity. In fact, in adult brain a high DP of PSA is restricted to hippocampus and olfactory bulb, where tissue plasticity is conserved during adulthood [97]. Polysialylation is mediated by two sialyltransferases, namely ST8Sia II (also known as STX) [98] and ST8Sia IV (also known as PST) [99]. The two enzymes show distinct patterns of tissue- and developmental expression [100]; the expression of the first is strictly associated with development, while that of the second is largely development-independent [101]. On the other hand, polysialic acid chains formed by ST8Sia IV are longer than those synthesized by ST8Sia II [100,102,103]. PSA is re-expressed in a variety of human cancers mainly, but not exclusively, of neuroectodermal origin [104–109]. The contribution of the two sialyltransferase to PSA re-expression is different and strongly dependent on the histological origin of the tumor. In non-small cell lung carcinoma, PSA expression correlates with tumor stage and depends on ST8Sia II activity, while ST8Sia IV is constantly expressed in both normal and tumor lung tissues [109]. A survey of human cancer cell lines all expressing PSA, reveals that neuroblastoma cells express preferentially ST8Sia II, while rhabdomyosarcoma and acute myeloid leukemia cell lines express preferentially ST8Sia IV [110]. Another study shows that cell lines from other malignancies, such

as colon cancer, express polysialyltransferases (mainly ST8Sia IV) without a concomitant expression of PSA [111]. This is due to a lack of expression of N-CAM, the privileged acceptor of the two polysialyltransferases. Despite the fact that PSA has often been associated with malignancy [109,112], several works point to an inverse relationship between the expression of the N-CAM molecule and invasion [113–115]. This could explain why transfection of lung adenocarcinoma cells with both polysialyltransferases and N-CAM cDNAs results in transfectant cells with a reduced *in vivo* growth [111].

Transfection of a human medulloblastoma cell line with Pax3, a developmental transcription factor involved in neoplastic transformation, results in polysialylation of N-CAM through an increased expression of ST8Sia II but not of ST8Sia IV [116]. Even if it is not clear at the moment whether the transcriptional induction of ST8Sia II is mediated directly or indirectly by Pax3, this finding provides an important link between developmental and oncogenic processes and polysialylation.

Ganglioside sialyltransferases

Gangliosides are sialic acid-containing glycolipids, whose expression is often deranged in cancer cells [117]. These compounds may play a role in cancer biology not only as cell surface structures but also as molecules shed by the tumor. As recently shown by Finke et al. [118], gangliosides released by a tumor exert an immunosuppressive effect by sensitizing T lymphocytes to apoptosis. Key steps in ganglioside biosynthesis (Figure 2) are represented by the α2-3-sialylation of lactosylceramide, catalyzed by ST3Gal V, which results in GM3 synthesis, and by the α2-8 sialylation of GM3 by ST8Sia I, which yields GD3. GM3 and GD3 are the founders of the a- and b-series gangliosides respectively and are transformed in GM2 and GD2 respectively by the action of a β1-4GalNAc transferase (GM2/GD2 synthase). As formerly suggested by studies in which transfection of rodent fibroblasts with DNA from human tumors induced an up-regulation of ST8Sia I [119], this enzyme shows a close association with neoplastic transformation in several malignancies.

Melanoma cells express an increased level of GD3 compared with normal melanocytes; at the basis of this phenomenon there is an increased ST8Sia I expression [120–123], which results also in an accumulation of GD2, even in the presence of unaltered levels of β1-4GalNAc transferase [124]. Inhibition by antisense cDNA of the biosynthesis of GD3 and of its O-acetylated derivative in hamster melanoma cells, induces a decreased rate

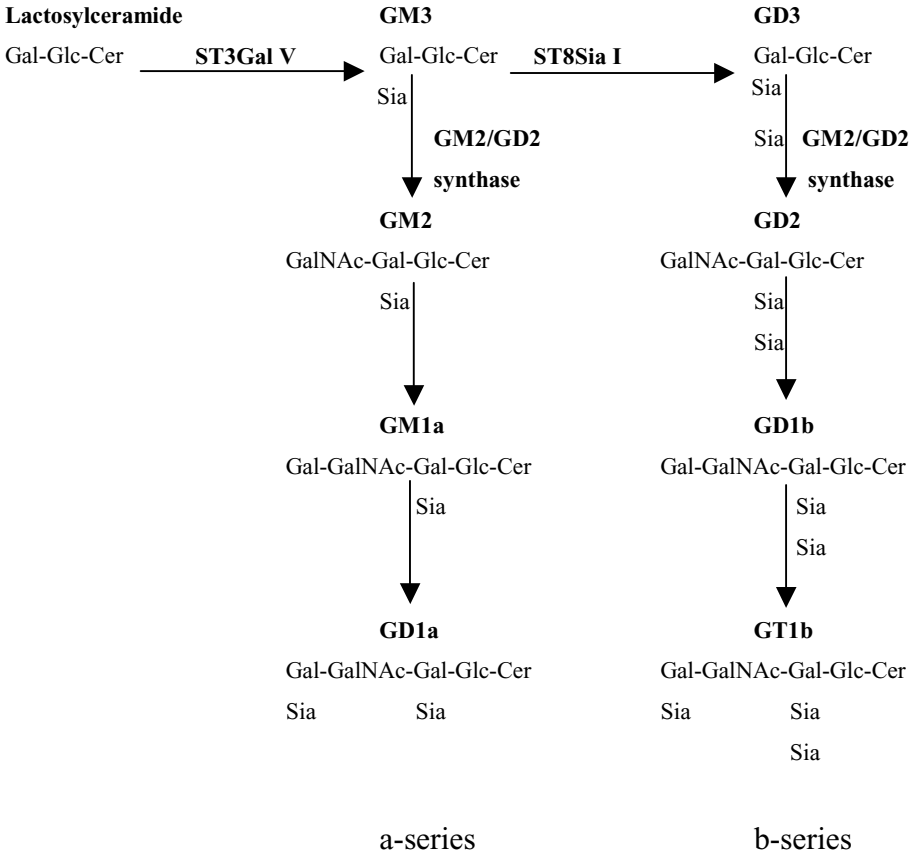

Figure 2. Simplified representation of ganglioside biosynthesis. Sialylation of lactosylceramide, mediated by ST3Gal V, is the first step of a-series gangliosides biosynthesis. The addition of a second, α2-8-linked sialic acid by ST8Sia I, yields GD3, which is the first member of b-series gangliosides. The addition of GalNAc on both GM3 and GD3 yields GM2 and GD2, respectively and is mediated by the same enzyme: GM2/GD2 synthase. The pathway leading to c-series gangliosides is omitted for simplicity.

of cell growth and stimulates dendrite outgrowth [125], suggesting a negative relationship between the expression of this complex ganglioside and cell differentiation. On the contrary, highly metastatic variants of melanoma cells show a reduced expression of complex gangliosides because of a reduced activity of ST3Gal V [126]. Unlike melanoma cells, neuroblastoma cells are characterized by an accumulation of GD2 [127] and/or GM2. The accumulation of GM2/GD2 appears to be driven by an up-regulation of GM2/GD2 synthase [121], while the activity of ST8Sia I is low, indicating that even a basal expression of this enzyme is sufficient to ensure a strong GD2 expression [121,123]. However, neuroblastoma cells, grown as nude mice xenografts express increased ST8Sia I levels [128], suggesting a role for its ganglioside products for *in vivo* growth. Consistently, rat neuroblastoma cells whose ST8Sia I expression was suppressed by antisense cDNA showed reduced tumor growth in athymic nude mice [129], reduced cell migration and invasion *in vitro* [130] and induce angiogenesis less efficiently, due to a reduced production of vascular endothelial growth factor (VEGF) [131]. On the other hand, a single clone of neuroblastoma cells forced to overexpress ST8Sia I by cDNA transfection, shows an altered morphology and a slower growth rate, compared with parental and mock-transfected cells [121].

While a-series gangliosides such as GM2 and GM1 are expressed by both non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), GD3 and other b-series gangliosides are expressed only by SCLC [132]. The importance of this alteration in the biology of this tumor is indicated by the fact that transfection with ST8Sia I cDNA of a low GD2-expressing SCLC cell line results in increased growth and invasion ability. Interestingly these GD2 expressing cells become sensitive to apoptosis induced by anti-GD2 antibodies, suggesting a potential therapeutic application for this reagent [132].

An up-regulation of ST8Sia I as been reported in other instances of neoplastic transformation or cell differentiation, such as in chemical carcinogenesis of rat liver [119], in T-lymphocyte activation and in HTLV-positive adult T-cell leukemia cell lines [123], in human meningiomas [133] and in mouse embryonic carcinoma cells differentiating along the neuronal lineage [134]. Gastrointestinal cancers, on the contrary, often show a down-regulation of ST8Sia I and a concomitant up-regulation of GM2/GD2 synthase, which results in an accumulation of GM2/GD2 gangliosides [135].

Many of the above mentioned papers indicate a positive relationship between expression of transfected ST8Sia I cDNA and invasive behavior in cancer cells of different tissue origin. A mechanism which could provide a molecular basis for this association is indicated by a study showing that transfection of rat pheochromocytoma cells with ST8Sia I cDNA results in enhanced proliferation and no response of neurite extension upon NGF stimulation [136]. ST8Sia I-transfected cells show a continuous dimerization of tyrosine kinase receptors which could depend on the accumulation of higher gangliosides, (such as GD1b and GT1b that are downstream products of ST8Sia I) and results in the continuous stimulation of the Ras/MEK/ERK signal transduction pathway [136].

Concluding remarks

As occurs for other glycosyltransferases, the expression of sialyltransferases is often deregulated in cancer. This may be due to a more or less direct effect of cell-growth genes on sialyltransferase genes, as documented for ST6Gal I, ST8Sia I and ST8Sia II. In some cases, the altered sialyltransferase expression results in a concomitant modulation of the cognate sialylated structures which may, in some cases (gangliosides), mediate cell growth and inhibit host immune response. However, in many cases, the structure/function relationship is uncertain, while the enzyme/product relationship does not appear to be direct. Transfection experiments aimed at establishing the relationship between the overexpression of a given sialyltransferase and cancer-associated phenotypes provide results sometimes conflicting. This may depend on the fact that the set of potential glycosidic acceptors is very different in different tumor types and also in different cell lines derived from the same type of tumor and from the powerful (but often underestimated) effect of insertional mutagenesis in determining the phenotype of transfected cells. The enzyme/structure relationship and the control of glycosyltransferases by cell-growth genes are problems which should be faced with an integrated approach, taking into consideration the large number of different glycosyltransferase gene products which contribute to the biosynthesis of a glycosidic structure. The large number of glycosyltransferase genes cloned in the past years, a more detailed comprehension of the molecular mechanisms of cell transformation and the availability of the microarray technology, which allows the simultaneous analysis of the expression of thousands of genes, will made possible this new approach.

Note added in proof

During the editorial process of this manuscript, a second β-galactoside α2–6 sialyltransferase (ST6Gal II) has been cloned (Takashima et al., *J Biol Chem* **277**, 45719–28 (2002). This enzyme sialylates oligosaccharides preferentially and is generally expressed at a much lower level than ST6Gal I.

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