Glycoconjugate Journal 20, 353–364, 2004

Molecular mechanism for cancer-associated induction of sialyl Lewis X and sialyl Lewis A expression—The Warburg effect revisited

Reiji Kannagi

Molecular Pathology, Aichi Cancer Center, Chikusaku, Nagoya 464-8681, Japan

Cell adhesion mediated by selectins and their carbohydrate ligands, sialyl Lewis X and sialyl Lewis A, figures heavily in cancer metastasis. Expression of these carbohydrate determinants is markedly enhanced in cancer cells, but the molecular mechanism that leads to cancer-associated expression of sialyl Lewis X/A has not been well understood. Results of recent studies indicated involvement of two principal mechanisms in the accelerated expression of sialyl Lewis X/A in cancers; 'incomplete synthesis' and '*neo*synthesis.' As to 'incomplete synthesis,' we have recently found further modified forms of sialyl Lewis X and sialyl Lewis A in non-malignant colonic epithelium, which have additional 6-sulfation or 2 → 6 sialylation. The impairment of GlcNAc 6-sulfation and 2 → 6 sialylation upon malignant transformation leads to accumulation of sialyl Lewis X/A in colon cancer cells. Epigenetic changes such as DNA methylation and/or histone deacetylation are suggested to lie behind such incomplete synthesis. As to the mechanism called '*neo*synthesis,' recent studies have indicated that cancer-associated alterations in the sugar transportation and intermediate carbohydrate metabolism play important roles. Cancer cells are known to exhibit a metabolic shift from oxidative to elevated anaerobic glycolysis (Warburg effect), which is correlated with the increased gene expression of sugar transporters and glycolytic enzymes induced by common cancer-specific genetic alterations. The increased sialyl Lewis X/A expression in cancer is a link in the chains of these events because our recent results indicated that these events accompany transcriptional induction of a set of genes closely related to its expression.
Published in 2004.

Keywords: **selectin, incomplete synthesis, histone deacetylation, *neo*synthesis, Warburg effect, hypoxia inducible factor**

Introduction

Cell adhesion mediated by selectins and their carbohydrate ligands play important roles in hematogenous metastasis of cancer cells. Extravasation of malignant cells involves the interaction of selectins, cell adhesion molecules at the surface of endothelial cells lining the blood vessels, with corresponding carbohydrate ligands on the surface of malignant cells [1,2]. Several molecular species of carbohydrate ligands for selectins are expressed on cancer cells, including sialyl Lewis X and sialyl Lewis A [3]. Expression of sialyl Lewis X/A is markedly enhanced in solid tumors, but the molecular mechanism underlying accelerated expression of sialyl Lewis X/A in cancers was not well understood, until the glycosyltransferases and their genes involved in the step-wise synthesis of sialyl Lewis X/A determinants have extensively been characterized in recent years [4–14].

Two principal mechanisms are known for tumor-associated alteration of carbohydrate determinants; 'incomplete synthesis' of pre-existing, and '*neo*synthesis' of unusual carbohydrate determinants, as formulated by Hakomori [15–17]. Here is how both mechanisms are involved in the cancer-associated enhancement of sialyl Lewis X/A determinant expression.

Incomplete synthesis

Synthesis of complex carbohydrate determinants well-developed on normal epithelial cells tends to be impaired upon malignant transformation, predisposing the cells to express less complicated carbohydrate determinants. We have recently found further modified forms of sialyl Lewis X and sialyl Lewis A in non-malignant colonic epithelium, such as sialyl 6-sulfo Lewis X [18] and (2 → 3), (2 → 6) disialyl Lewis A [19,20] in colon cancers (Figure 1). The impairment of GlcNAc 6-sulfation and GlcNAc (2 → 6) sialylation upon malignant transformation leads to accumulation of sialyl Lewis X/A in colon cancer cells. This is typical of the involvement of the 'incomplete synthesis' mechanism in the enhanced expression of sialyl Lewis X and sialyl Lewis A in cancers.

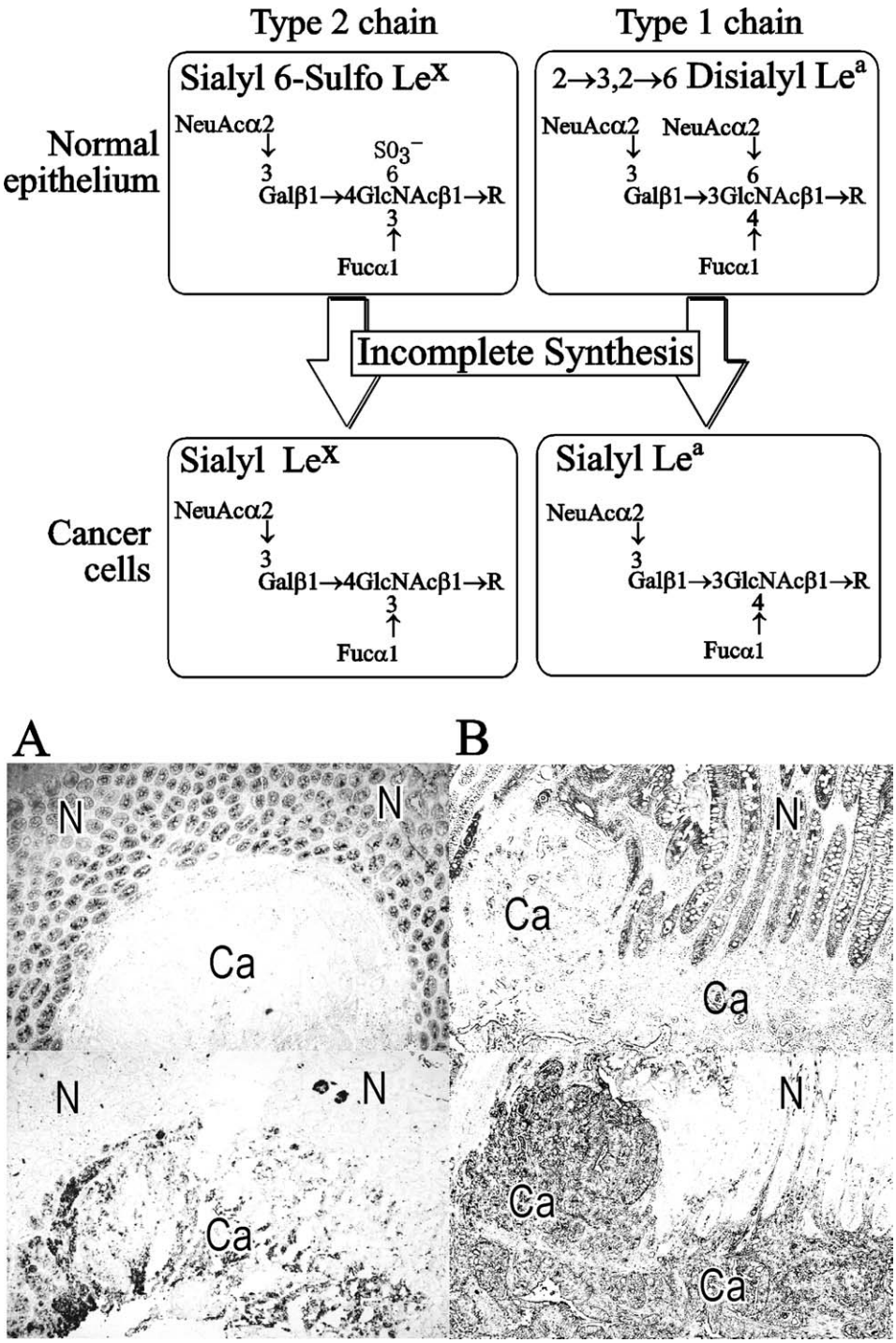

Figure 1. Scheme illustrating the induction of sialyl Lewis A or sialyl Lewis X expression in cancers as a result of 'incomplete synthesis' of more complex carbohydrate determinants, (2 → 3), (2 → 6) disialyl Lewis A or sialyl 6-sulfo Lewis X. Panel *A*, typical distribution pattern of sialyl Lewis X (upper panel) and sialyl 6-sulfo Lewis X (lower panel) determinants in colon cancer tissues, indicating that sialyl Lewis X determinant is preferentially expressed on cancer cells, while sialyl 6-sulfo Lewis X determinant is specifically localized in non-malignant epithelial cells. Adopted from reference [18]. Panel *B*, typical distribution pattern of sialyl Lewis A (upper panel) and disialyl Lewis A (lower panel) determinants in colon cancer tissues, indicating that (α2 → 3) monosialyl Lewis A determinant is preferentially expressed on cancer cells, while (α2 → 3), (2 → 6) disialyl Lewis A determinant is specifically localized in non-malignant epithelial cells. Adopted from reference [29]. Ca, cancer cells; N, non-malignant epithelial cells.

GlcNAc 6-sulfation of type 2 chains

We found that sialyl 6-sulfo Lewis X, initially described as specific ligand for L-selectin on high endothelial venules in peripheral lymph nodes [21,22], is significantly expressed in non-malignant colonic epithelial cells [18]. This determinant has one sulfate residue attached to the C-6 position of the GlcNAc moiety of sialyl Lewis X (Figure 1). Expression of sialyl 6-sulfo Lewis X was confined in non-malignant epithelial cells, and its expression was significantly decreased in colon cancers (Figure 1A, upper panel). In contrast, expression of the non-sulfated form of the determinant, sialyl Lewis X, was preferentially expressed in cancer cells rather than non-malignant epithelial cells (Figure 1A, lower panel) [18]. These findings suggested a close association of a decrease in 6-sulfation with malignant transformation of colonic epithelial cells, and this can explain the enhanced expression of non-sulfated sialyl Lewis X in cancer cells.

There are five known isoenzymes of 6-sulfotransferases [23], and I-GlcNAc6ST, the intestine-specific GlcNAc 6-sulfotransferase, is the major isoenzyme in the colon [24]. Its message shows a significant decrease upon malignant transformation of colonic epithelial cells ([25,26] and our unpublished results), and this may at least partly explain the decreased expression of sialyl 6-sulfo Lewis X in cancers. Another possibility would be a generalized decrease in sulfate utilization in malignant cells [27].

(2 → 6) sialylation at GlcNAc in type 1 chains

With regard to sialyl Lewis A determinant, we sometime ago showed that a carbohydrate determinant having a more complicated structure than sialyl Lewis A is expressed on non-malignant colonic epithelia, which is (α2 → 3), (2 → 6) disialylated Lewis A determinant [19,20] (Figure 1). This determinant has one extra sialic residue attached to the C-6 position of the GlcNAc moiety of sialyl Lewis A. The disialylated determinant was preferentially expressed on non-malignant epithelial cells, and its expression tended to decrease in cancer cells (Figure 1B, upper panel). This was in a clear contrast to the usual (α2 → 3) sialyl Lewis A, which is preferentially expressed in cancer cells (Figure 1B, lower panel). These results suggested that the (2 → 6) sialylation at GlcNAc was impaired upon malignant transformation, and that this led to the accumulation of mono-sialylated sialyl Lewis A, a cancer-associated carbohydrate determinant, in cancer cells. The enhancement of sialyl Lewis A expression by the decrease of GlcNAc (2 → 6) sialylation is a good example of the incomplete synthesis of carbohydrate determinants in cancers (Figure 1).

Recently, a candidate gene for sialyltransferase responsible for the (2 → 6) sialylation of the GlcNAc moiety was identified [28,29]. Transfection of the gene for this enzyme to cultured colon cancer cells induces expression of (2 → 3), (2 → 6) disialylated Lewis A, and a corresponding decrease in the expression of monosialylated Lewis A determinants. The expression of mRNA for this enzyme is found to be significantly decreased in colon cancer cells compared to non-malignant colonic epithelial cells [29].

Sialylation and serum diagnosis of cancers

The sialyl Lewis A determinant is known to be secreted from cancer cells into general circulation, and sometimes utilized as a serum tumor marker under the name of CA19-9. The (2 → 3), (2 → 6) disialylated Lewis A determinant is found in the sera of patients with non-malignant disorders more preferentially than in those with cancers. The tumor marker CA19-9 sometimes gives false positive results in patients with non-malignant disorders. In such cases, simultaneous determination of the serum levels of (2 → 3), (2 → 6) disialylated Lewis A determinant and calculation of the ratio of CA19-9/(2 → 3), (2 → 6) disialylated Lewis A determinant levels is useful for discrimination of false positive results [19,20]. Although the ratio is high in the sera of patients with cancers, it is low in the sera of patients with non-malignant disorders, because sialyl Lewis A determinant is preferentially expressed in cancer cells, while (2 → 3), (2 → 6) disialylated Lewis A determinant is preferentially expressed in non-malignant epithelial cells.

Since the structure of CA19-9 was elucidated as sialyl Lewis A [30], its serum levels have been known to depend on the Lewis blood subtype of patients. The Le^{a+b-} patients tends to have a higher serum level than Le^{a-b+} patients, and Le^{a-b-} patents are essentially devoid of the determinants [31]. This has been another inconvenience encountered in clinical application of CA19-9 as tumor markers. The calculation of the ratio of CA19-9/(2 → 3), (2 → 6) disialylated Lewis A determinant mitigates or overcomes the inconvenience because the Lewis blood group dependency of the serum level of (2 → 3), (2 → 6) disialylated Lewis A determinant is essentially the same as that of CA19-9, and the problem of Lewis blood group subtypes is resolved when the ratio is calculated.

3′-sulfation at terminal gal and substrate competition

Non-malignant colonic epithelial cells also express 3′-sulfo Lewis A and 3′-sulfo Lewis X. Expression of 3′-sulfo Lewis A is decreased in colon cancer cells [32], while the decrease of 3′-sialyl Lewis X seems to be less prominent [18]. Transcription of a candidate gene for 3′-sulfotransferase responsible for the synthesis of these determinants, namely GP3ST [33], is known to be decreased in colon cancers compared to non-malignant colonic epithelial cells, especially at the early stage of malignant progression such as Dukes' stages A and B ([34] and our unpublished results). This can also contribute to the enhanced expression of sialyl Lewis A and sialyl Lewis X in cancers [35].

However, the contribution of the decreased 3′-sulfation in the enhanced expression of sialyl Lewis X/A must be indirect and limited, because this mechanism is based on simple substrate competition. If a decrease of 3′-sulfation would occur in the cells still retaining 6-sulfation or 6-sialylation, the decrease in

3′-sulfation would result in an increased expression of sialyl 6-sulfo Lewis X and (2 → 3), (2 → 6) disialyl Lewis A, but not in an increase of sialyl Lewis X and sialyl Lewis A. The decrease of 3′-sulfation can contribute to the increased expression of sialyl Lewis X/A only in the cells where the 6-sulfation and 6-sialylation of the carbohydrate determinants are already suppressed. Another example of the similar substrate competition mechanism is the decreased synthesis of blood group A, B, and Sd^a determinant upon malignant transformation, which predispose the cells to enhance sialyl Lewis X/A synthesis by supplying more substrate acceptors for its synthesis.

Physiological function of 6-sulfated and (2 → 6) sialylated GlcNAc determinants in normal colonic mucosa

Sialyl 6-sulfo Lewis X is known to be involved not only in the homing of helper naïve T lymphocytes to peripheral lymph nodes but also in the recruitment of gut-homing helper memory T lymphocytes to Peyer's patches, appendices, and gut-associated lymphoid tissues since the determinant is significantly expressed on the high endothelial cells in these lymphoid tissues [36]. As these lymphoid tissues have no capsular structures, the recruited lymphocytes will further adhere to colonic epithelial cells expressing sialyl 6-sulfo Lewis X through L-selectin-mediated interaction. Intestinal intraepithelial lymphocytes (IEL) bearing Tcr$\gamma\delta$ are known, for the most part, to lack the expression of L-selectin, but it is accepted that a certain subset of IEL in the colon preferentially expresses L-selectin and Tcr$\alpha\beta$ [37].

The (2 → 3), (2 → 6) disialylated type 1 chain determinant was recently found to specifically bind to siglec-7 [29,38], the molecule known to function as an inhibitory receptor on human natural killer cells [39]. The physiological significance of the (2 → 3), (2 → 6) disialylated determinant, which is preferentially expressed on non-malignant epithelial cells, is the protection of epithelial cells from an accidental attack from the self NK cells.

Thus determinants on non-malignant epithelial cells having more complicated structure than sialyl Lewis X/A seem to play physiological roles in normal cell-cell interactions in the colonic mucous membrane. These determinants tend to disappear upon malignant transformation, and are replaced with simpler determinants, such as non-sulfated sialyl Lewis X and sialyl Lewis A, having no binding activity to L-selectin or siglec-7. This change would entrain a disturbance of the normal cell-cell interaction in intestinal mucous membrane.

MUC2 is acknowledged to be one of the major core proteins that carry GlcNAc 6-sulfation in non-malignant colonic epithelia [40]. A classical biochemical study indicates that (2 → 6) sialylation of GlcNAc is frequently found also in normal human colonic mucin [41], in which MUC2 is the major component [42]. In this context it is interesting to note that MUC2 knockout mice frequently develop colon cancers [43], raising the possibility that the change in the carbohydrate determinants on MUC2 may be involved in the process of colonic oncogenesis.

Gene silencing by histone deacetylation and DNA methylation

The exact mechanisms involved in the suppression of genes for GlcNAc 6-sulfation and GlcNAc (2 → 6) sialylation in cancers remain unclear. We recently observed significant induction of GlcNAc 6-sulfation and GlcNAc (2 → 6) sialylation in cultured colon cancer cells by a histone deacetylase inhibitor such as trichostatin A and butyrate ([29 and] unpublished results). This would suggest that histone deacetylation is involved in the silencing of the genes responsible for GlcNAc 6-sulfation and GlcNAc (2 → 6) sialylation in cancers. GlcNAc 6-sulfation and GlcNAc (2 → 6) sialylation were not restored by treatment with 5-aza-2-deoxy-cytidine, indicating that DNA methylation is not much involved in the silencing of these genes. On the other hand, DNA methylation of transferase gene is reportedly involved in the decreased expression of blood group A and B determinant in cancers [44,45]. Gene silencing mediated by several epigenetic changes such as DNA methylation and/or histone deacetylation seems to lie behind the mechanism previously called 'incomplete synthesis.'

*Neo*synthesis

With regard to the mechanism called *neo*synthesis, cancer-associated induction of some glycosyltransferases has been assumed to influence expression of the determinants. The major four steps of glycosyltransfer reactions are involved in the synthesis of sialyl Lewis X and sialyl Lewis A: transfer of GlcNAc and galactose, followed by addition of sialic acid and fucose residues.

Step for transfer of fucose

Fucosyltransferases that transfer α-fucose residues through (α1 → 3) or (α1 → 4) linkage to a GlcNAc moiety in sialylated precursor substrates participate in the final step of sialyl Lewis X and sialyl Lewis A synthesis. Among the five genes known to encode these fucosyltransferases, only Fuc-T VII and IV show significant up-regulation of transcription in colon cancer cells compared to non-malignant epithelial cells (Figure 2). Transcription of the other fucosyltransferases remains unchanged between cancer and non-malignant epithelial cells.

Fuc-T III is the only fucosyltransferase capable of synthesizing sialyl Lewis A and is obviously involved in the synthetic process of sialyl Lewis A in cancer tissues. However, its mRNA level and enzymatic activity are not significantly altered between malignant and non-malignant colorectal tissues and do not explain the accelerated synthesis of sialyl Lewis A in cancer tissues [46–50].

The situation is more complex with regard to the synthesis of sialyl Lewis X since several fucosyltransferases can synthesize the sialyl Lewis X determinant, including Fuc-T III, IV, VI, and VII. The major isoenzymes in epithelial cells are usually Fuc-T III and VI, but the mRNA level of Fuc-T VI, as well as that of Fuc-T III, is known to show no significant difference between

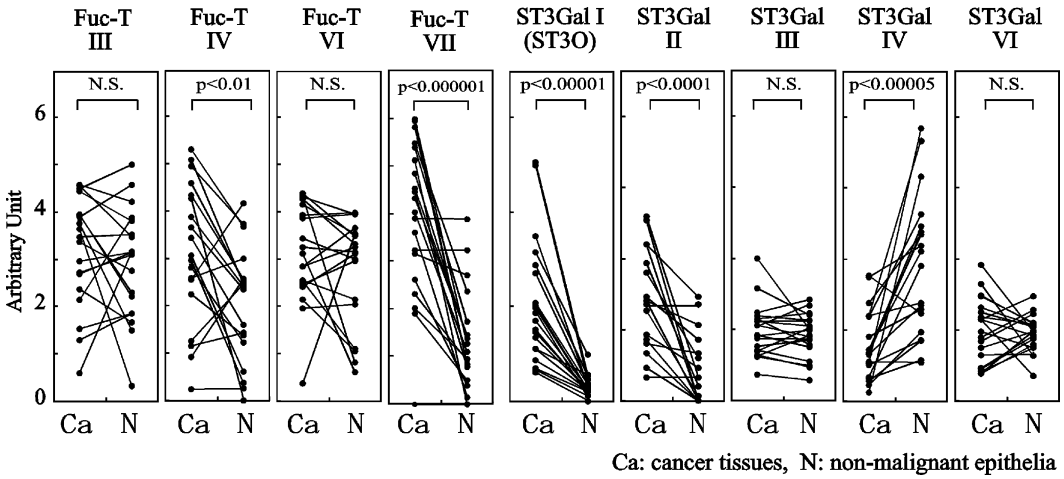

Figure 2. Expression of fucosyl- and sialyltransferase mRNA in human colorectal cancer tissues. Results of RT-PCR analyses of mRNA levels of isoenzymes for fucosyl- (Fuc-T) and ($\alpha 2 \rightarrow 3$) sialyltransferases (ST3Gal) in human colon cancer tissues and non-malignant mucosa are shown. Ca: cancer tissues; N: non-malignant mucosa prepared from the same patient. Paired *t* test was performed to ascertain statistical significance between the amount in cancer tissue and in non-malignant mucosa.

colon cancer tissues and non-malignant colonic epithelial cells [46–48]. Again, the level does not explain the increased synthesis of sialyl Lewis X in cancer.

These findings suggest that Fuc-T III or VI is not necessarily the rate limiting enzyme responsible for the cancer-associated increase in sialyl Lewis X and sialyl Lewis A synthesis. However, it does not deny that Fuc-T III and VI are major isoenzymes engaged in the synthesis of sialyl Lewis X and sialyl Lewis A in cancers. Introduction of an anti-sense DNA for Fuc-T III and VI are effective to suppress sialyl Lewis X and sialyl Lewis A surface expression in cancer cells, thus abrogating adhesion of the cancer cells to vascular endothelial cells [51]. Interestingly, the level of Fuc-T III and VI expression is reported to influence also the proliferative and tumorigenic ability of cancer cells [52].

Fuc-T VII and IV are known to be dynamically regulated at the transcriptional level [53,54]. Fuc-T VII is responsible for the increased expression of sialyl Lewis X in malignant cells in some cases, especially in certain leukemias [55,56]. The contribution of the enzymatic activity of Fuc-T VII to the total fucosyltransferase activity in epithelial cells would not be a major one, since these cells usually contain a much larger amount of Fuc-T III and VI. A line of evidence, however, suggests that Fuc-T VII is also involved in the expression of sialyl Lewis X in cancer cells of epithelial origin [57–64]. The significant difference in the substrate specificity of Fuc-T VII compared to other fucosyltransferases [65–67] implies that this isoenzyme must be at least responsible for the limited subset of sialyl Lewis X determinants, the expression of which would be increased in cancer cells. Likewise, Fuc-T IV has been regarded as mainly engaged in Lewis X synthesis and to have only a low activity in sialyl Lewis X synthesis, while recent studies suggest its synthetic activity for sialyl Lewis X is highly dependent on the structure of acceptors [68,69].

Step of ($\alpha 2 \rightarrow 3$) sialylation

α2,3-Sialyltransferases synthesize sialylated precursors for transfucosylation by fucosyltransferases. Many isoenzymes are known, and are still increasing in number. Some sialyltransferases employ type 1 chain substrates, and supply precursors for sialyl Lewis A synthesis, while others prefer type 2 chain substrates, thus supplying precorsors for sialyl Lewis X synthesis.

Earlier immunohistochemical examination of cancer tissue specimens of the digestive organs, such as the colon, revealed an increased expression of sialyl Lewis A determinant in cancer cells compared to corresponding non-malignant epithelial cells while the Lewis A antigen, the non-sialylated form of the determinant, is equally expressed on both cancer cells and non-malignant epithelial cells. Expression of the ($2 \rightarrow 3$), ($2 \rightarrow 6$) disialyl Lewis A determinant is strongly expressed on non-malignant epithelial cells, whereas its expression is significantly decreased in cancer cells [19,20,29]. These findings strongly suggest that a change in sialic acid modification is deeply implicated in the accelerated expression of sialyl Lewis A determinant in cancer cells whereas ($\alpha 1 \rightarrow 4$) fucosylation is not substantially altered between cancer and non-malignant epithelial cells.

Studies on enzymatic activities of α2,3-sialyltransferase and α1,4-fucosyltransferase for type 1 chain substrates in colon cancer [50] indicated that the α2,3-sialyltransferase activity was significantly higher in cancer tissue than in non-malignant colonic epithelia, whereas α1,4-fucosyltransferase activity showed no significant difference. The results of our study at the transcription level also indicated that the mRNA levels for ST-3O (ST3Gal-I), one of the sialyltransferase isoenzymes, was prominently increased in colorectal cancer as compared to non-malignant colonic epithelium [46]. In colon cancers, ST-3O (ST3Gal-I) and ST3Gal-II are the only

sialyltransferases that show a statistically significant elevation in gene transcription among the major α2,3-sialyltransferases (Figure 2). Supported more recently by results from other laboratories [47,48], these findings suggest the change in the α2,3-sialyltransferase activity to be at least partially involved in the enhancement of sialyl Lewis A expression in cancer.

The specific activity of sialyltransferase responsible for ($\alpha 2 \rightarrow 3$) sialylation of type 1 chain substrate was reported to be 0.26–0.30 nmoles/mg protein/h in non-malignant colonic mucosa and around 1.17 in colon cancer tissues while that for α1,4-fucosyltransferase (*i.e.*, Fuc-T III) was 14.9–22.0 nmoles/mg protein/h in non-malignant colonic mucosa and around 16.9 in colon cancer tissues [49,50]. The big difference in the specific activity between sialyltransferase and fucosyltransferase strongly suggests that the step of sialyltransferase is most likely to be rate-limiting in the synthesis of the sialyl Lewis A determinant.

The situation is obscure with regard to sialyl Lewis X synthesis. The sialyltransferase isoenzymes ST-3O and ST3Gal-II, transcription of which is increased in colon cancers, lack the ability to synthesize precursors for sialyl Lewis X [46] and can not explain the enhanced expression of sialyl Lewis X in cancer. The major ($\alpha 2 \rightarrow 3$) sialyltransferase enzymatic activities for type 2 chain substrates (ST3Gal-III and -IV) and their mRNA levels do not show significant change consistent with the enhanced sialyl Lewis X expression in cancers. At present it is difficult to explain the enhanced expression of sialyl Lewis X merely from an increase in any sialyltransferase isoenzymes.

Step for GlcNAc transfer

With regard to the GlcNAc transferases (GlcNAc-T), many isoenzymes exist for the synthesis of various O-glycan core structures in epithelial cells [70–72]. Some of them increase upon malignant transformation, while the others show a significant decrease. Enzymological studies [70] indicate that core 3 and core 4 GlcNAc-T show a significant decrease in their enzymatic activity in colon cancers compared to non-malignant colonic epithelial cells: from 1.2–1.3 in non-malignant to 0.8 in cancer for core 3, and from 7.2–8.1 in non-malignant to 3.2 in cancer for core 4 GlcNAc-Ts (nmoles/mg protein/h). On the other hand, the activity of core 2 GlcNAc-T and GlcNAc-T for elongation of polylactosamine showed no significant change: 12.7–16.0 in non-malignant and 10.3 in cancer for core 2, and 0.61–0.76 in non-malignant and 0.71 in cancer for elongation GlcNAc-Ts (nmoles/mg protein/h). The mRNA for one of the isoenzymes of core 2 GlcNAc-Ts was shown to be increased in a more recent study [73]. These changes may well explain the ups and downs of the structural variants of the core portion of O-glycans carrying the sialyl Lewis X/A determinants. At present, however, it is difficult to conclude whether the changes in such GlcNAc transferase activities really contribute to the overall accelerated expression of sialyl Lewis X/A determinants in cancer cells.

Step for galactose transport and transfer

Usually epithelial cells contain an excess amount of galactosyltransferases far beyond the activity of GlcNAc transferases, making it difficult to consider this enzymatic activity as a rate-limiting factor for the synthesis of carbohydrate determinants. For instance, an enzymatic study on colon cancer tissues [74] indicate that β1,4-galactosyltransferase activity shows no significant difference between cancer cells and non-malignant colonic epithelial cells (1.58 in non-malignant and 1.70 in cancer), while β1,3-galactosyltransferase activity evidences a drastic decrease in cancer cells (from 11.6 in non-malignant to 2.0 in cancer). These values are in terms of nmoles/mg protein/min, indicating that even the decreased activity of β1,3-galactosyltransferase in cancer cells is still nearly 10 times more than the sum of GlcNAc transferase activities supplying acceptor substrate for the galactosyltransferase. The decrease of β1,3-galactosyltransferase in cancer cells was recently confirmed by a significant down regulation of a putative β1,3-galactosyltransferase gene expression [75]. The decrease of β1,3-galactosyltransferase is not consistent with the increased expression of sialyl Lewis A in cancer cells.

Interestingly, the Km value for the donor substrate UDP-galactose is reported to be extremely high for β1,3 galactosyltransferase (ca. 200 μM) [74]. This was recently confirmed also with the product of a candidate gene encoding β1,3-galactosyltransferase [75]. Such a high Km value for UDP-galactose suggests a tight regulation of its activity by the availability and concentration of the sugar nucleotide. We found that mRNA for the UDP-galactose transporter, which is located at the Golgi membrane and limits the supply of the sugar nucleotide donor for the galactosyltransferases, is significantly increased in colon cancer cells compared to non-malignant colonic epithelial cells [76]. Transfection of the gene for UDP-galactose transporter conferred a remarkable enhancement of sialyl Lewis A expression on the cells and increased binding activity to E-selectin (Figure 3) [76]. In some cells, introduction of the gene induced an accelerated expression of even sialyl Lewis X [76]. These results indicate that transfer of galactose is accelerated in cancers compared to non-malignant epithelial cells at least partly due to the increased availability of UDP-galactose in Golgi apparatus, and this leads to increased expression of sialyl Lewis A and eventually of sialyl Lewis X.

Alteration of intracellular galactose metabolism in cancer

Clearly the relationship between the accelerated galactosylation of carbohydrate determinant due to the increased UDP-galactose transporter and the enhanced expression of sialyl Lewis determinants is an indirect one. An increase of UDP-galactose transporter can enhance sialyl Lewis X/A expression only when the synthetic pathway for glycoconjugates in the given cells is already oriented towards the preferential synthesis of sialyl Lewis X/A. In this context, it is rather surprising that induction of UDP-galactose transporter gene (UDP-GalT1)

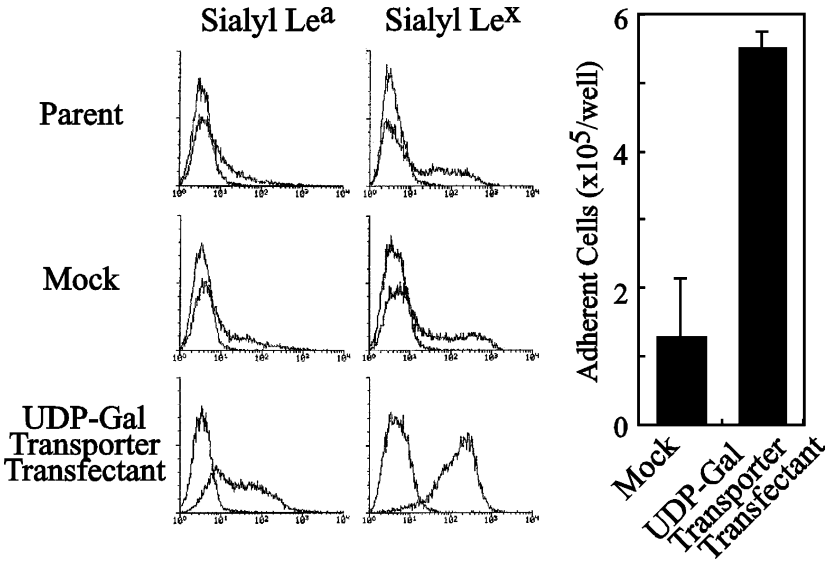

Figure 3. Induction of sialyl Lewis X and sialyl Lewis A expression (left panel) and binding activity to E-selectin (right panel) by transfection of UDP-galactose transporter gene to cultured colon cancer cells. SW480 cells were transfected with UDP-galactose transporter-1 cDNA and analyzed by flow cytometry and non-static monolayer cell adhesion assay using 300.19 cells transfected with human E-selectin cDNA [76].

could confer a clear induction of sialyl Lewis X/A and cell adhesion mediated by these determinants. This reminds us of the classical concept on the relationship between the galactose metabolism and cell sociology [77].

A further increase was observed in sialyl Lewis A expression when the cells transfected with cDNA for UDP-galactose transporter were cultured in galactose-rich medium. For this gene transfection experiment, we have chosen a cell line, SW480, with a very low endogenous expression of UDP-galactose transporter gene, and the parental cell line showed no change in sialyl Lewis A expression by culture in a high galactose medium. This finding suggested the presence of another rate-limiting step for sialyl Lewis determinant synthesis somewhere in the intracellular galactose metabolism if the cells are supplied with a sufficient amount of UDP-galactose transporter. Studies on the gene transcription of the major components of the intracellular galactose metabolism in colon cancer tissues indicate that mRNAs for GLUT-1 and galactokinase-1 (GALK1) were significantly increased in cancer cells compared to non-malignant epithelial cells, while those for galactose-1-phosphate uridyl transferase (GALT) and UDP-galactose 4′-epimerase (GALE) exhibited no significant difference (Figure 4). These changes are compatible with the accelerated utilization of exogenous galactose in cancer cells.

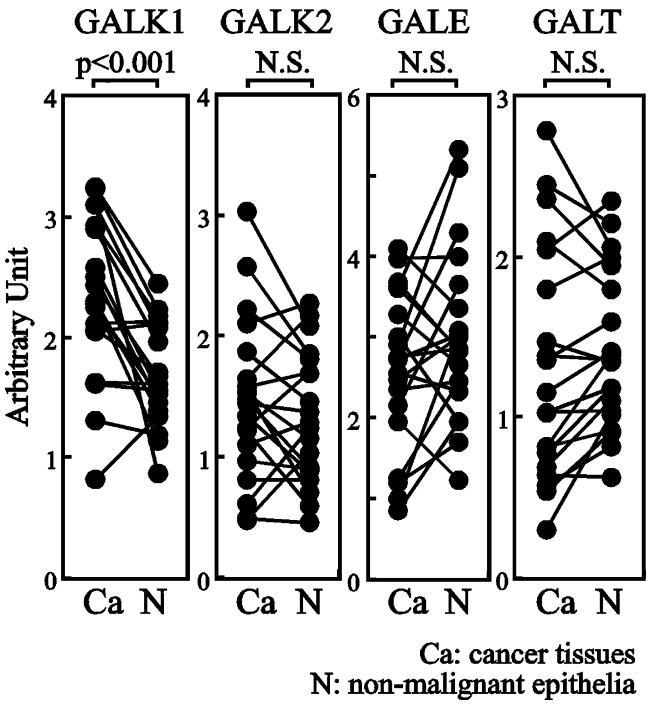

Figure 4. Expression of genes involved in intracellular galactose metabolism in human colorectal cancer tissues. Results of RT-PCR analyses of mRNA levels of galactokinases (GALK1 and GALK2), galactose-1-phosphate uridyl transferase (GALT) and UDP-galactose 4′-epimerase (GALE) in human colon cancer tissues and non-malignant mucosa are shown. Ca: cancer tissues; N: non-malignant mucosa prepared from the same patient. Paired *t* test was performed to ascertain statistical significance between the amount in cancer tissue and in non-malignant mucosa.

Molecular biological background for '*neo*synthesis' of sialyl Lewis X/A expression

Cancer cells are known to display a particular deviation in the intracellular carbohydrate metabolism, a metabolic shift from oxidative to elevated anaerobic glycolysis (Warburg effect) [78], which is correlated with the increased expression of glucose

transporters including GLUT-1 and some glycolytic enzymes [79,80]. Activation of various oncogenes, including *src*, *ras,* and *fps*, or loss of anti-oncogenes such as *VHL* is known to produce this metabolic shift. These changes are mediated by transcription factors including hypoxia-inducible factors (HIFs). Many common cancer-specific genetic alterations result in an augmented HIF expression and/or activity. This leads to survival or even increased proliferation of cancer cells within hypoxic tumor microenvironments, achieved by a metabolic shift to anaerobic glycolysis and/or by promoting the interaction of cancer cells with vascular endothelial cells. Our recent results indicated that these events accompany transcriptional induction of a set of genes for glycosyltransferase and sugar transporters closely related to sialyl Lewis determinant expression, suggesting that the increased sialyl Lewis determinant expression in cancers is a link in the chains of these events. The significant increase of GLUT-1 expression in colon cancer cells is already well known [81]. Moreover, a significant increase of hypoxia inducible factor (HIF) α chain expression in colon cancer cells is also well documented [82,83].

GLUT-1 plays a central role in the transport of not only glucose, but also galactose in epithelial cells [80]. When colon cancer cells are cultured under hypoxic conditions, we could observe a significant induction of sialyl Lewis A and sialyl Lewis X expression (Figure 5, upper panel) and a concomitant increase of E-selectin binding activity [84,85]. Moreover, we could detect a significant induction of transcription of GLUT-1, UDP-galactose transporter, Fuc-T VII, and ST3O by hypoxic culture of the cells (Figure 5, lower panel). Participation of HIF in the process, direct or indirect, is clear since the introduction of dominant negative HIF to the cells in advance completely abrogated these effects [84].

Besides the transcription of the genes GLUT-1, UDP-galactose transporter, Fuc-T VII and ST3O, the transcription of GALK1, Fuc-T IV, and ST3Gal-II is known to be elevated in colon cancer tissues compared to non-malignant epithelial cells, among the genes closely related to the synthesis of sialyl Lewis determinants. It is noteworthy that HIF can explain the cancer-associated upregulation of as many as 4 genes out of 8 sialyl Lewis determinant-related genes in colon cancers. The transcription of the latter set of genes (GALK1, Fuc-T IV and ST3Gal-II) was not induced by hypoxic culture. Obviously, genetic alteration mediated by transcription factors other than HIF must be involved in the *neo*synthesis of the latter set of the genes upon malignant transformation.

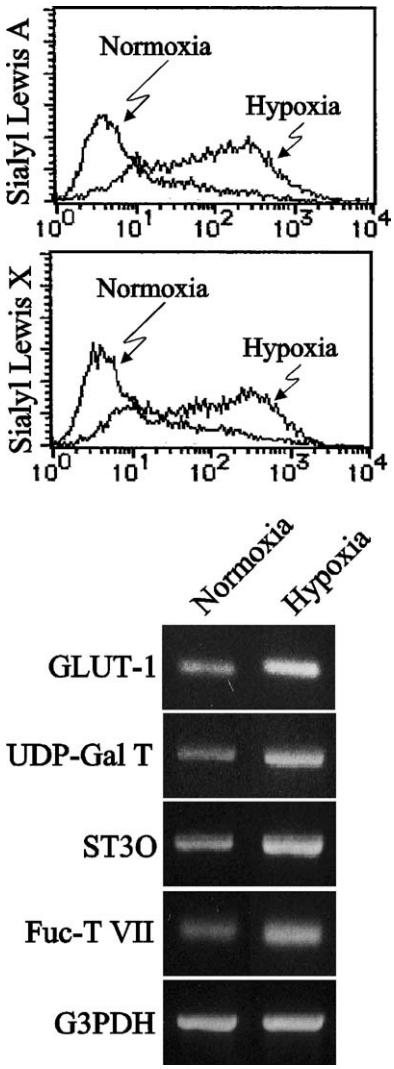

Figure 5. Induction of sialyl Lewis X and sialyl Lewis A expression (upper panel) and transcription of genes for GLUT-1, UDP-galactose transporter, Fuc-T VII and ST3O (lower panel) by hypoxic culture of colon cancer cells. SW480 cells were cultured for 7 days in 0.1% O_2 for 7 days and analyzed by flow cytometry and RT-PCR. UDP-Gal T, UDP-galactose transporter [84].

Conclusion

The mechanisms formerly characterized as 'incomplete synthesis' and '*neo*synthesis' both appear to be involved in the accelerated expression of carbohydrate ligands for selectin in cancers (Figure 6). Gene silencing due to histone deacetylation and/or DNA methylation, previously called 'incomplete synthesis,' seems to be involved in the relatively early stage of oncogenesis since decreases of GlcNAc 6-sulfation and GlcNAc ($2 \rightarrow 6$) sialylation are both observed even in adenoma, as well as in adenocarcinoma of the colon (unpublished results). This mechanism directs the glycoconjugate synthetic pathway of the cell toward the synthesis of sialyl Lewis X/A determinants by suppressing GlcNAc 6-sulfation and GlcNAc ($2 \rightarrow 6$) sialylation. Suppression of the competing synthetic pathways for 3′-sulfated determinants and blood group substances would also help to increase the synthesis of sialyl Lewis X/A determinants in the cells where GlcNAc 6-sulfation and GlcNAc ($2 \rightarrow 6$) sialylation are decreased. At later stages of malignant progression, expression of some genes related to synthesis of sialyl Lewis X/A determinants is newly induced through the changes in some oncogenes and anti-oncogenes (the mechanism previously called '*neo*synthesis'). A good

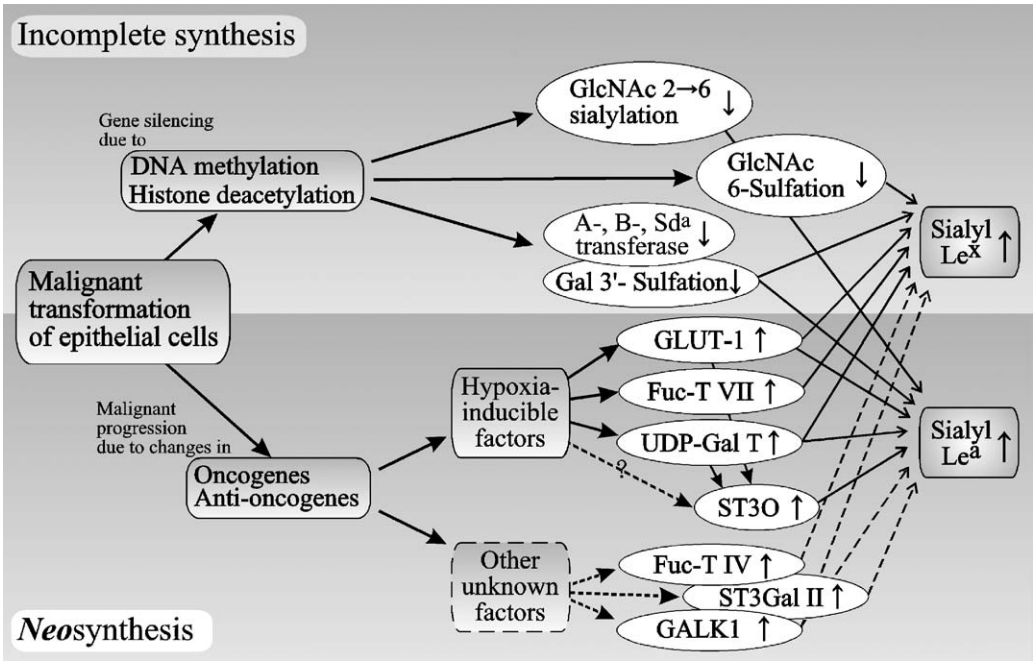

Figure 6. Suggested mechanisms for increased expression of selectin ligands in cancer cells. The schema shows participation of both 'incomplete synthesis (upper panel)' and '*neo*synthesis (lower panel)' in the accelerated expression of sialyl Lewis X and sialyl Lewis A determinants in cancers. Effect of HIF on transcription of ST3O could be indirect, as transcription of ST3O is induced also by introduction of UDP-galactose transporter gene to the cells, or culturing the cells in high galactose medium [86] and our unpublished results. UDP-Gal T, UDP-galactose transporter.

example is the induction of GLUT-1, UDP-galactose transporter, and Fuc-T VII gene transcription by hypoxia-inducible factor. This mechanism will contribute to further enhancing the sialyl Lewis X/A expression on cancer cells, already predisposed to express these determinants by silencing of the genes for GlcNAc 6-sulfation and GlcNAc (2 → 6) sialylation.

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