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# Comparative Study of the Levels of Sialyltransferases Responsible for the Formation of Sugar Chains in Glycoproteins and Gangliosides in Rat Liver and Hepatomas

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**Sialyltransferases responsible for the formation of sugar chains in glycoproteins were studied in rat hepatoma in comparison with rat liver. Hepatoma induced by feeding Wistar rats with 3′-methyl-4-dimethylaminoazobenzene (MeDAB) was more active than Wistar liver in sialylating asialo-orosomucoid, and this was due to an increased activity of Gal($\beta$1→4)GlcNAc ($\alpha$2→6) sialyltransferase, the major sialyltransferase in these tissues. Gal($\beta$1→3,4)GlcNAc ($\alpha$2→3) sialyltransferase and the sialyltransferases acting on asialo-bovine submaxillary mucin were, however, decreased in the hepatoma. A similar pattern of sialyltransferase alterations was observed in regenerating liver and other tumors such as AH-109A hepatoma and Sato lung cancer, both of which had been inoculated into Donryu rats. In contrast to these sialyltransferases, the activities of the sialyltransferases responsible for the formation of gangliosides were markedly different even between Wistar and Donryu livers. When compared with Wistar liver, MeDAB-induced hepatoma was higher in lactosylceramide- and lower in $GM_3$-sialyltransferase activity, but these two activities were both lower in AH-109A compared with Donryu liver.**

Key words: Sialyltransferase — Glycoproteins — Gangliosides — Rat liver — Rat hepatoma — Rat lung cancer

Although cell surface glycoproteins and glycolipids undergo various neoplastic alterations,[1, 2] the hypersialylation of cell surface glycoproteins[3] is of particular interest as it may be connected to cancer cell phenotypes such as decreased adhesiveness,[4] increased invasiveness,[4] metastatic potential[5] and modified immunogenicity.[6] To answer the question of how sialyltransferase (EC 2.4.99.1) is involved in this aberrant sialylation, we have previously purified Gal($\beta$1→4)GlcNAc ($\alpha$2→6) sialyltransferase from MeDAB-induced hepatoma as well as control rat liver.[7] We found that all the sialyltransferase molecules present in the hepatoma are in a sialylated form while a considerable portion of the liver transferase remains unsialylated.[7] The sialylated form would act as the active form of the enzyme since it exhibits higher affinity toward CMP-NeuAc and desialylated rat liver plasma membrane than the unsialylated form.[7, 8] As far as we are aware, however, no quantitative study has ever been made on the tissue level of this sialyltransferase.

Several studies have been carried out to elucidate the nature of neoplastic alterations in sialyltransferase,[9] but there has been no clear agreement, probably because the multiple nature of sialyltransferase has been largely ignored. In the present study, we tried to assay individual sialyltransferases in rat hepatomas using a variety of glycoproteins, oligosaccharides and glycolipids as acceptors, and the results obtained were compared with those for control liver.

## MATERIALS AND METHODS

**Materials** CMP-[4,5,6,7,8,9-$^{14}$C]NeuAc was purchased from New England Nuclear (Boston, MA) and diluted with CMP-NeuAc to give a final specific radioactivity of 1.0 or 2.5 Ci/mol.[7] Fetuin was purchased from Gibco (New York, NY) and human orosomucoid and *N*-acetyllactosamine from Sigma (St. Louis, MO). Bovine submaxillary mucin was prepared by the method of Tsuiki *et al.*[10] Asialo-fetuin, asialo-orosomucoid and asialo-

Abbreviations: MeDAB, 3′-methyl-4-dimethylaminoazobenzene; CMP-NeuAc, CMP-*N*-acetylneuraminic acid.

submaxillary mucin were prepared from fetuin, orosomucoid and submaxillary mucin, respectively, by mild acid hydrolysis according to Spiro.[11] ($\beta$1→3) Galactosylated asialo-submaxillary mucin was prepared as described previously,[12] and its ($\alpha$2→3) sialylation at the galactosyl residue was carried out by using Gal($\beta$1→3)GalNAc ($\alpha$2→3) sialyltransferase prepared from porcine liver by the method of Bergh *et al.*[13] Asialo-submaxillary mucin and galactosylated asialo-submaxillary mucin served as substrates for GalNAc ($\alpha$2→6) sialyltransferase and Gal($\beta$1→3)GalNAc ($\alpha$2→3) sialyltransferase, respectively. ($\alpha$2→3) Sialylation of the galactosylated asialomucin provided the substrate for a type of GalNAc ($\alpha$2→6) sialyltransferase capable of sialylating only the *N*-acetylgalactosaminyl residue of NeuAc ($\alpha$ 2 → 3) Gal ($\beta$ 1 → 3) GalNAc chains.[14] Gal($\beta$1→3)GlcNAc($\beta$1→3)Gal($\beta$1→4)-Glc (lacto-*N*-tetraose), NeuAc($\alpha$2→3)Gal($\beta$1→3) GlcNAc ($\beta$1 → 3) Gal ($\beta$1 → 4) Glc (LS-tetrasaccharide *a*) and NeuAc($\alpha$2 → 3)Gal($\beta$1 → 3) [NeuAc($\alpha$2→6)]GlcNAc($\beta$1→3)Gal($\beta$1→4)Glc (disialyl-lacto-*N*-tetraose) were purified from crude oligosaccharide fraction of human milk.[15,16] To eliminate contaminating lacto-*N*-neotetraose, purified lacto-*N*-tetraose was further digested with *Streptococcus pneumoniae* $\beta$-galactosidase (kindly provided by Dr. K. Yamashita, Kobe University). Gangliosides $GM_1$ and $GD_{1a}$ were isolated from bovine brain mixed gangliosides (Sigma) as described elsewhere.[17] $GM_3$ was a generous gift from Dr. S. Handa, Tokyo Medical and Dental University. Lactosylceramide was purchased from Calbiochem (La Jolla, CA).

**Animals and Tumors** The liver was excised from male Wistar or Donryu rats (150–200 g) fed *ad libitum*. Primary hepatocellular carcinoma was induced in male Wistar rats by feeding them with MeDAB.[12] Transplantable AH-109A hepatoma was inoculated into male Donryu rats subcutaneously (for solid form) or intraperitoneally (for ascitic form).[18] Regenerating liver was obtained from partially hepatectomized Wistar rats 24 hr following the operation,[19] and fetal liver from Wistar fetuses. The lung was excised from male Donryu rats (150–200 g), and transplantable Sato lung cancer was obtained by subcutaneous inoculation into male Donryu rats.[20]

**Preparation of Particulate Fraction** Tissues were homogenized in 4 vol of 0.25*M* sucrose/25m*M* KCl /1m*M* EDTA/10m*M* potassium phosphate (pH 6.8) by using five strokes in a glass/Teflon homogenizer. The homogenate was centrifuged at 600*g* for 10 min and the supernatant was centrifuged at 78,000*g* for 70 min. The resulting pellet was suspended in 1 ml/g tissue of homogenizing buffer (the particulate fraction) and used as sialyltransferase. All operations were performed at 4°.

**Assay of Sialyltransferases** When asialo-glycoproteins were the acceptors, the assay mixture contained 0.04 $\mu$mol of CMP-[$^{14}$C]NeuAc (1 Ci/mol), 50–100 $\mu$mol (expressed in terms of acceptor site) of acceptor, 5 $\mu$mol of sodium cacodylate (pH 6.3), 0.2 mg of Triton X-100 and enzyme in a final volume of 0.1 ml. After incubation at 37°, protein-bound radioactivity was determined as described previously.[7] With oligosaccharides as acceptors, the assay mixture contained 0.02 $\mu$mol of CMP-[$^{14}$C]NeuAc (2.5 Ci/mol), 40–80 $\mu$mol of acceptor, 2.5 $\mu$mol of sodium cacodylate (pH 6.3), 0.1 mg of Triton X-100 and enzyme in a final volume of 0.05 ml. After incubation at 37°, the reaction product was separated from CMP-[$^{14}$C]-NeuAc by high-voltage paper electrophoresis in 0.1% (w/v) sodium tetraborate and counted. The detailed procedure was described previously.[7] When *N*-acetyllactosamine was the acceptor, the reaction mixture was paper-chromatographed with ethyl acetate/pyridine/acetic acid/water (5:5:1:3 by volume) as described below so that individual counting of the two isomeric sialyl *N*-acetyllactosamines was possible. When glycolipids were the acceptor, the assay mixture was the same as for oligosaccharide sialyltransferase except that 20–50 $\mu$mol of the acceptor was used. After incubation at 37°, the assay was conducted as described by Chien *et al.*[21] In short, the reaction mixture was first paper-chromatographed with 1% sodium tetraborate to eliminate CMP-[$^{14}$C]NeuAc; the reaction product remaining at the origin was then paper-chromatographed with chloroform/methanol/water (60:30:6); and the appropriate area was counted. In the studies described below, sialyltransferase activity was expressed in terms of nmol of sialic acid transferred to the acceptor.

**Identification of Reaction Products** When oligosaccharide products were being identified, the reaction mixture was applied to Whatman 3MM paper together with appropriate standards and developed in a descending fashion for 3–5 days with ethyl acetate/pyridine/acetic acid/water (5:5:1:3). For the identification of gangliosides formed, the reaction mixture was lyophilized, taken up in 1 ml of chloroform/methanol (2:1) and centrifuged. The supernatant was evaporated to 10 $\mu$l, applied to a thin layer plate and co-chromatographed with standards in chloroform/methanol/water (60:35:8). The standards were visualized by the use of silver nitrate for oligosaccharides or a resorcinol reagent for oligosaccharides and gangliosides, and the radioactivity of the corresponding areas was counted.

**Other Assay Methods** Protein was assayed using a phenol reagent.[22]

## RESULTS

**Sialyltransferase Activities with Glycoproteins as Acceptors** The particulate fractions prepared from rat liver and hepatomas were assayed for sialyltransferase activity using a variety of asialo-glycoproteins as acceptors; in these experiments, endogenous activities were always insignificant. The results obtained are shown in Table I. When MeDAB-induced hepatoma was compared with normal Wistar liver, the activity toward asialo-orosomucoid, whose sugar chains are all *N*-linked, was found to be higher. In confirmation of the previous observation,[7] on the other hand, the activity toward the asialo form of fetuin, also a serum glycoprotein, was somewhat lower in the hepatoma. We are now able to attribute this decreased activity to the presence in fetuin of *O*-linked sugar chains, since the activities toward three types of asialo-bovine submaxillary mucin, whose sugar chains are all *O*-linked, are markedly decreased in the hepatoma. Increase in the sialyltransferase activity toward asialo-orosomucoid, together with decreased activity toward asialo-submaxillary mucins, has also been found in regenerating and fetal Wistar livers. Sialyltransferase alterations found in AH-109A hepatoma inoculated subcutaneously into Donryu rats were less marked than those in MeDAB-induced hepatoma. In Donryu liver, the levels of sialyltransferase activities were lower than those in Wistar liver, although the relative activities toward various glycoproteins were not very much different between the two strains.

**Sialyltransferase Activities with Oligosaccharides as Acceptors** In order to obtain further insights into the nature of the neoplastic alterations of sialyltransferases, the enzyme activity was assayed with well-defined oligosaccharides as acceptors. With *N*-acetyllactosamine as the acceptor, we may obtain two isomers of sialyl *N*-acetyllactosamine resolvable by a paper chromatographic procedure (Fig. 1). Figure 1 illustrates that for MeDAB-induced hepatoma as well as control and regenerating livers, there is a preferential formation of the ($\alpha 2 \rightarrow 6$) isomer catalyzed by Gal ($\beta 1 \rightarrow 4$) GlcNAc ($\alpha 2 \rightarrow 6$) sialyltransferase, the major sialyltransferase species in these tissues.[7] Rat mammary glands, on the other hand, produced mainly the ($\alpha 2 \rightarrow 3$) isomer thereby confirming the report of Carlson *et al.*[23] When the hepatoma was compared with control liver, the yield of the ($\alpha 2 \rightarrow 6$) isomer was 160% while that of the ($\alpha 2 \rightarrow 3$) isomer was only 40% (Table II). Gal($\beta 1 \rightarrow 4$)-GlcNAc ($\alpha 2 \rightarrow 6$) sialyltransferase activity is therefore higher in the hepatoma than in liver, and this is probably the reason why asialo-orosomucoid sialyltransferase activity is increased in the hepatoma (Table I), since asialo-orosomucoid is a good acceptor for Gal-($\beta 1 \rightarrow 4$)GlcNAc ($\alpha 2 \rightarrow 6$) sialyltransferase.[7]

Table I. Sialyltransferase Activities of Rat Liver and Hepatomas with Asialoglycoproteins as Acceptors

| Acceptors | Sialyltransferase activities (nmol/hr/mg protein)[a] | | | | | |
|---|---|---|---|---|---|---|
| | Wistar | | | | Donryu | |
| | Liver | | | MeDAB hepatoma | control liver | AH-109A |
| | control | regenerating | fetal | | | |
| Asialo-fetuin | 25.01 ±2.14 | 23.41 ±3.44 | — | 19.76 ±0.84 | 17.83 ±2.10 | 15.60 ±1.70 |
| Asialo-orosomucoid | 26.79 ±1.90 | 37.29 ±1.65 | 36.85 ±4.48 | 33.29 ±2.81 | 20.02 ±1.58 | 19.72 ±2.02 |
| Asialo-submaxillary mucin (I) | 1.97 ±0.17 | 1.16 ±0.11 | — | 0.90 ±0.26 | 0.99 ±0.10 | 0.50 ±0.10 |
| Galactosylated I (II) | 2.32 ±0.71 | 0.90 ±0.15 | 1.03 ±0.41 | 0.81 ±0.34 | 0.79 ±0.22 | 0.44 ±0.10 |
| Sialylated II[b] | 1.75 | 0.70 | 1.31 | 0.54 | 0.84 | 0.34 |

*a*) The values are means ± SD of 3–6 experiments; 3 rats were killed for each experiment.
*b*) The values are means of two experiments.

With *N*-acetyllactosamine, AH-109A hepatoma (solid form) exhibited a similar pattern of sialyltransferase alterations but to a lesser extent (Table II). In regenerating liver, the activity of Gal($\beta$1→4)GlcNAc ($\alpha$2→6) sialyltransferase was increased as in MeDAB-induced hepatoma, but there was little deviation from the control value in the yield of ($\alpha$2→3) sialyl-*N*-acetyllactosamine.

Paulson *et al.*[24, 25] have reported that lacto-*N*-tetraose and LS-tetrasaccharide *a* are good acceptors for Gal($\beta$1→3)GlcNAc ($\alpha$2→3) sialyltransferase and *N*-acetylglucosamine ($\alpha$2→6) sialyltransferase, respectively. In our hands, when lacto-*N*-tetraose and LS-tetrasaccharide *a* were used as acceptors, the major product co-migrated on paper chromatography with LS-tetrasaccharide *a* and disialyl lacto-*N*-tetraose, respectively, thereby confirming the reports of Paulson *et al.*[24, 25] For the routine assays, however, the paper chromato-

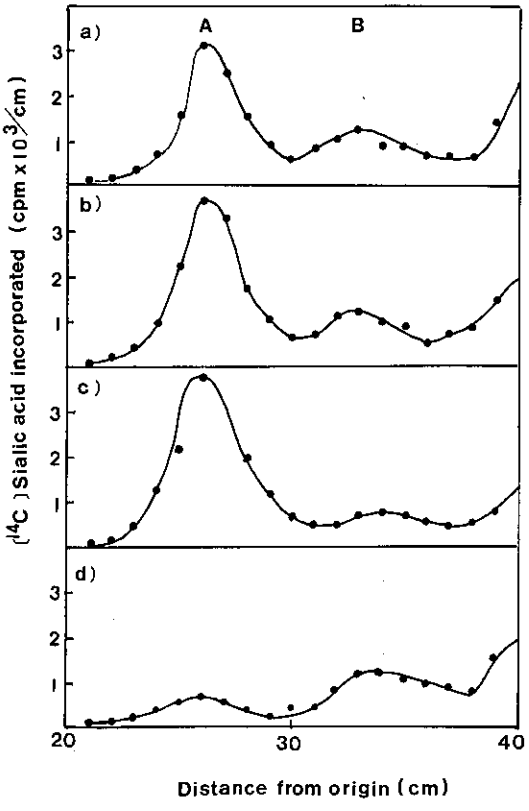


Fig. 1. The identification and assay of the isomers of sialylated *N*-acetyllactosamine. The sialyltransferase fractions of control Wistar liver (a), regenerating Wistar liver (b), MeDAB-induced rat hepatoma (c) and Wistar mammary glands (d) were separately incubated with *N*-acetyllactosamine as described in the text. Triton X-100 was omitted when the mammary gland enzyme was employed. The reaction mixture was then subjected to paper chromatography, the details of which are described in the text. After chromatography, the paper was cut into 1 cm segments and counted. A and B signify the positions of ($\alpha$2→6)- and ($\alpha$2→3)-sialyl-*N*-acetyllactosamine, respectively. The fastest-moving peak, only the tailing portion of which is seen, represents residual CMP-NeuAc and sialic acid.

Table II. Sialyltransferase Activities of Rat Liver and Hepatomas with Oligosaccharides as Acceptors

| Acceptors | Products | Sialyltransferase activity (nmol/hr/mg protein)[a] | | | | |
|---|---|---|---|---|---|---|
| | | Wistar | | | Donryu | |
| | | control liver | regenerating liver | MeDAB hepatoma | control liver | AH-109A |
| LactNAc[b] | sialyl($\alpha$2→3) LactNAc | 5.70 | 5.60 | 2.21 | 2.40 | 1.39 |
| | sialyl($\alpha$2→6) LactNAc | 13.97 | 23.86 | 22.40 | 11.80 | 14.50 |
| Lacto-*N*-tetraose | | 2.20 | 3.15 | 1.15 | — | 1.05 |
| LS-tetrasaccharide *a*[c] | | 0.55 | 0.55 | 0.40 | — | 0.55 |

*a*) The values are means of 3 experiments; for each experiment, at least 3 rats were used.
*b*) *N*-Acetyllactosamine.
*c*) One $\mu$mol of $MnCl_2$ was included in the assay mixture.

graphic isolation of the products was apparently unnecessary. Table II shows that when lacto-*N*-tetraose is the acceptor, the sialyltransferase activity of MeDAB-induced hepatoma is lower than that of control liver. This lower activity of Gal($\beta$1→3)GlcNAc ($\alpha$2→3) sialyltransferase may explain why the yield of ($\alpha$2→3) sialyl-*N*-acetyllactosamine is lower in the hepatoma, since the enzyme described here is capable of synthesizing ($\alpha$2→3) sialyl-*N*-acetyllactosamine thereby acting as a Gal($\beta$1→3,4)GlcNAc ($\alpha$2→3) sialyltransferase rather than Gal($\beta$1→3)GlcNAc ($\alpha$2→3) sialyltransferase.[24] The activity of this sialyltransferase is not decreased in regenerating liver (Table II), and this corresponds to the yield of ($\alpha$2→3) sialyl-*N*-acetyllactosamine, which is not below the control level in regenerating liver. The results obtained with LS-tetrasaccharide *a*, on the other hand, suggest that the hepatic activity of *N*-acetylglucosamine ($\alpha$2→6) sialyltransferase is low and hardly affected by hepatocarcinogenesis (Table II).

**Sialyltransferase Activities with Glycolipids as Acceptors** When sialyltransferase responsible for the formation of gangliosides was assayed without added acceptors, endogenous values of 0.018, 0.025, 0.225, 0.026 and 0.089 nmol/hr/mg protein were obtained for control Wistar liver, regenerating Wistar liver, MeDAB-induced hepatoma, control Donryu liver and AH-109A solid form, respectively. The relatively large values obtained for the two hepatomas are noteworthy. For all these tissues, thin layer chromatographic analysis identified the major product as $GM_3$. This means that the endogenous substrate is mainly lactosylceramide.

Exogenously added lactosylceramide, $GM_1$, $GD_{1a}$ and $GM_3$ were then used as acceptors; the thin layer chromatography revealed that the major products were $GM_3$, $GD_{1a}$, $GT_{1a}$ and $GD_3$, respectively. These reactions were apparently catalyzed by independent enzymes.[26,27] The method of Chien *et al.*[21] yielded the sialyltransferase activities shown in Table III. The values were corrected for the endogenous values except for those with lactosylceramide as an acceptor. Perhaps the most striking finding to emerge from Table III is the observation that the relative activities of the sialyltransferases are quite different between Wistar and Donryu livers. Furthermore, how the hepatoma pattern deviates from the liver pattern also differs according to the animal strain. Thus while the most remarkable alteration in MeDAB-induced hepatoma is the elevation of lactosylceramide sialyltransferase, the same enzyme is markedly decreased in AH-109A. The only alteration common to the two hepatomas is the decrease in $GM_3$ sialyltransferase activity. As far as Wistar rats are concerned, however, the elevation of lactosylceramide sialyltransferase appears to be associated with cell proliferation, since regenerating and fetal livers from this strain also exhibit this elevation.

Table III. Sialyltransferase Activities of Rat Liver and Hepatomas with Glycolipids as Acceptors

| Acceptors | Sialyltransferase activity (nmol/hr/mg protein)[a] | | | | | | |
|---|---|---|---|---|---|---|---|
| | Wistar | | | | Donryu | | |
| | liver | | | MeDAB hepatoma | control liver | AH-109A | |
| | control | regenerating | fetal | | | solid | ascitic |
| LacCer[b] | 0.051 ±0.010 | 0.192 ±0.013 | 0.430 | 0.478 ±0.068 | 0.383 ±0.110 | 0.094 ±0.014 | 0.130 |
| $GM_1$ | 0.384 ±0.111 | 0.532 ±0.022 | 0.430 | 0.260 ±0.082 | 0.274 ±0.058 | 0.382 ±0.008 | 0.110 |
| $GD_{1a}$ | 0.023 ±0.006 | 0.034 ±0.002 | 0.055 | 0.033 ±0.022 | 0.042 ±0.013 | 0.027 ±0.010 | 0 |
| $GM_3$ | 0.080 ±0.028 | 0.135 ±0.013 | 0 | 0.023 ±0.030 | 0.594 ±0.171 | 0.027 ±0.015 | 0 |

*a*) The values are means±SD of 3–6 experiments except for fetal Wistar liver and AH-109A ascitic form, where means of two experiments are given.
*b*) Lactosylceramide.

Table IV. Glycoprotein Sialyltransferase Activities in Rat Lung and Lung Cancer

| Acceptors | Sialyltransferase activity[a] (nmol/hr/mg protein) | |
|---|---|---|
| | control lung | Sato lung cancer |
| Asialo-fetuin | 6.58±1.27 | 2.40±0.20 |
| Asialo-orosomucoid | 3.35±0.59 | 4.27±0.70 |
| Asialo-submaxillary mucin (I) | 1.07±0.15 | 0.38±0.11 |
| Galactosylated I (II) | 1.24±0.17 | 0.21±0.05 |
| Sialylated II[b] | 1.35 | 0.28 |

*a*) The values are means±SE of 3–6 experiments; 3 rats were killed for each experiment.
*b*) The values are means of 2 experiments.

**Sialyltransferase Activities in Sato Lung Cancer** We have thus far demonstrated that upon hepatocarcinogenesis, the sialyltransferase activity toward the *N*-linked sugar chains of glycoproteins is increased, or at least not decreased, while the activity toward the *O*-linked sugar chains is markedly decreased (Table I). To learn if this pattern of sialyltransferase alteration also occurs in other types of cancer, the activities of sialyltransferases responsible for the formation of glycoproteins were compared between rat lung and Sato lung cancer. Table IV shows that in the lung cancer too, the pattern of deviation is similar so that sialyl transfer to asialo-orosomucoid is slightly higher but that to asialo-submaxillary mucins is markedly lower. A preliminary study made on Sato lung cancer has also suggested that with *N*-acetyllactosamine as the acceptor, only the formation of ($\alpha$2→6) sialyl linkage is stimulated.

## Discussion

As far as the sialyltransferases responsible for the formation of gangliosides are concerned, MeDAB-induced hepatoma is distinguished from control liver (Wistar) primarily by elevated lactosylceramide sialyltransferase activity (Table III). Although this elevation seems to be associated with cell proliferation (see above), it also correlates with hepatocarcinogenesis, since the same elevation has been observed by Merritt *et al.*[28] in hyperplastic nodules following the administration of *N*-$\alpha$-fluorenylacetamide. Lactosylceramide sialyltransferase, however, is markedly decreased in AH-109A as compared with control liver (Donryu). This discrepancy between the two hepatomas may have arisen from the fact that AH-109A is more highly dedifferentiated than MeDAB-induced hepatoma: Merritt *et al.*[28] have reported that hepatomas tend to lose the lactosylceramide sialyltransferase activity with the progress of dedifferentiation. However, the fact that the two hepatomas have been induced/transplanted in rats of different strains (Wistar vs. Donryu) should also be considered since even Wistar and Donryu livers are not coincident in relative activities of glycolipid sialyltransferases including that toward lactosylceramide (Table III). These differences due to animal strain are probably related to the general observation that cellular ganglioside expression differs according to cell type and is easily affected by environmental changes.[29]

In contrast to these sialyltransferases, the hepatic levels of sialyltransferases responsible for the formation of glycoproteins are rather stable so that Wistar and Donryu livers exhibit activity patterns grossly similar to each other (Table I). As compared with control liver, MeDAB-induced hepatoma is more active in sialylating the *N*-linked sugar chains of glycoproteins but is much lower in the activities toward the *O*-linked sugar chains (Table I). This pattern of sialyltransferase alterations has been found in other tumors such as AH-109A (Table I) and Sato lung cancer (Table IV), thereby suggesting its close association with the cancerous state. We have further demonstrated that the increase in *N*-linked chain sialyltransferase activity described above is due primarily to the elevated level of Gal($\beta$1→4)GlcNAc ($\alpha$2→6) sialyltransferase (Table II). In contrast to this sialyltransferase, the hepatic level of Gal($\beta$1→3,4)GlcNAc ($\alpha$2→3) sialyltransferase is low and further lowered upon hepatocarcinogenesis.

Malignant transformation induces hypersialylation in cell surface glycoproteins.[3, 30, 31] The hypersialylation could arise from increased activity of *N*-acetylglucosaminyltransferase V[32] since this alteration should result in increased ($\beta$1→6) branching of *N*-linked sugar chains[33, 34] thereby creating new sites for sialylation. In this connection, it is

worthnoting that the increase in ($\beta$1→6) branching appears to be related to the metastatic potential of the cells.[35] The present quantitative study, however, suggests that the increased extent of sialic acid substitution due to increased activity of Gal($\beta$1→4)GlcNAc ($\alpha$2→6) sialyltransferase is also important as a cause of the neoplastic hypersialylation. This view is further strengthened by our previous observation that all the Gal($\beta$1→4)-GlcNAc ($\alpha$2→6) sialyltransferase molecules in MeDAB-induced hepatoma are in an active state while a considerable portion of the liver transferase remains inactive.[7,9] That the increased extent of sialic acid substitution increases the metastatic ability of the cells has already been reported.[36] Finally, it should be pointed out that in MeDAB-induced hepatoma, the rise of Gal($\beta$1→4)GlcNAc ($\alpha$2→6) sialyltransferase is accompanied by a fall of Gal($\beta$1→3,4)GlcNAc ($\alpha$2→3) sialyltransferase. This pattern of alteration may provide a further mechanism for the neoplastic hypersialylation, since the ($\alpha$2→6) sialyl linkage is much more resistant than the ($\alpha$2→3) linkage to mammalian sialidases.[37,38]

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