Quantitative and Qualitative Characterization of Human Cancer-associated Serum Glycoprotein Antigens Expressing Fucosyl or Sialyl-Fucosyl Type 2 Chain Poly lactosamine

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ABSTRACT

The quantity of tumor-associated antigens carrying type 2 chain glycolipid derivatives, Le" (X-hapten), poly-Le", sialyl Le", and Le" (Y-hapten), present in sera of patients with various malignant and non-malignant disorders, as well as the qualitative chemical properties of the carrier molecules in sera, have been investigated using four monoclonal antibodies, each of which defines one of these determinants. The following findings are of particular importance: (a) the serum levels of Le" defined by antibody FH2 and poly-Le" defined by ACFH18 in patients with cancer were occasionally high (incidence about 10%); however, the majority of patients did not show elevated levels; (b) the serum level of the antigen, defined by monoclonal antibody FH6 (termed sialyl Le"-i since this determinant is carried by i antigen), was significantly high in patients with cancers originating from organs from which adenocarcinomas often develop. For example, among various types of lung cancer, only adenocarcinoma but not squamous cell carcinoma, small cell carcinoma, or large cell carcinoma showed a high level of sialyl Le"-i antigen in sera. The incidence of high antigen levels in sera of patients with adenocarcinomas of lung was as high as 76% of the observed cases; (c) the serum level of Le" (Y-hapten) was frequently high in patients with hepatoma (incidence, 34%); (d) sialyl Le"-i antigen was separated on gel filtration as a glycoprotein with an average molecular weight greater than 10^6. It was characterized by its susceptibility to base-hydrolysis, Pronase digestion, sialidase and endo-β-galactosidase treatment and is assumed to be a high molecular weight mucin-type glycoprotein; (e) sialyl Le"-i antigen expressed in sera of patients with cancer was soluble in perchloric acid, while the same antigen in sera of patients with noncancerous diseases and normal subjects was mostly insoluble in perchloric acid. Le", a poly-Le", and essentially all Le" antigens in sera of patients with cancer were perichloric acid-insoluble.

INTRODUCTION

Structural analysis of glycolipids in tumor tissues combined with the monoclonal antibody approach have revealed that the most common human cancers, particularly adenocarcinomas of colon, stomach, breast, and lung, are characterized by the accumulation of fucosylated type 2 chain with Le" (X-hapten), Le" (Y-hapten), and sialyl Le" determinants, each of which is defined by specific monoclonal antibodies (for review, see Ref. 1). In contrast to type 2 chain glycolipids, changes in type 1 chain glycolipids lead to formation of sialyl Le" antigen defined by monoclonal antibody N-19-9 (2, 3) and CSLEA-1 (4). The levels of sialyl Le" and sialyl Le" antigens have been found to be complementarily elevated in sera of patients with various types of human cancer (4, 5). Sialyl Le" antigen present in sera of patients with cancer has been found to be a high molecular weight glycoprotein rather than a glycolipid (6).

Accumulation of lactofucopentaosyl(III)ceramide was initially observed in human adenocarcinoma (7), and more recently a series of unbranched type 2 chain poly lactosamine glycolipids with Le" structure was isolated and characterized from human adenocarcinoma (8). These were initially defined by antibodies directed to stage-specific embryonic antigen 1 (9–11) and many other antibodies with similar specificity (12–16), but some antibodies react preferentially with a polymeric Le" structure having α1→3 fucosyl substitutions at the internal GlcNAc (17–19). A series of glycolipids with Le" (Y-hapten), defined by monoclonal antibodies (20–22), was also found to accumulate in various types of human adenocarcinomas. Glycolipids with sialyl 2→3 substitution of Le" structure have also been found to accumulate in human adenocarcinoma, and a monoclonal antibody, FH6, directed to this structure carried by a repeating lactosaminyl chain has been established (23). This structure is hereby designated sialyl Le"-i in order to distinguish it from the antigen defined by antibody CSLEX-1, which reacts with all types of sialyl Le" irrespective of the carrier carbohydrate (24). In this study, we investigated the serum level of these four types of antigens (Le", polymeric Le", Le", and sialyl Le"-i) defined by monoclonal antibodies FH2, ACFH18, AH6, and FH6, respectively, and made a preliminary study of the properties of each type of antigen in the sera of patients with various cancers.

MATERIALS AND METHODS

Monoclonal Antibodies Used and the Antigens Defined by these Antibodies. Monoclonal antibodies directed to various fucosyl type 2 chain poly lactosamines (FH2, FH6, ACFH18, and AH6; all murine IgM) were prepared as previously described (17, 20, 23, 24), and the structures of the antigens defined by these antibodies are illustrated in Fig. 1. Antibodies were purified from the culture supernatant of each hybridoma by gel filtration on a Sepharose 6B column. FH6 reacts maximally with the original immunogen, i.e., sialyl Le" carried by poly lactosamine with α1→3 internal fucosyl substitution (23), but it also reacts with sialyl Le" carried by a poly lactosamine with at least two repeating N-acetyllactosamine units, which was identified as i-antigen (23). FH6 does not react with short chain sialyl Le" hapten. The FH6 antigen is hereby designated sialyl Le"-i, which is distinguished from a simple sialyl Le" determinant defined by antibody CSLEX-1 (25).

Blood Samples. Patient serum was obtained from the Kyoto University Hospital, Kyoto, Japan. Some serum samples were donated by Shimane Hospital, Kyoto, Japan through the courtesy of Drs. Jiro Endo and Yoshinobu Yoshida. Serum samples from 310 randomly selected healthy individuals (12–65 years of age; 166 male, 144 female)
SERUM POLYLACTOSAMINE ANTIGENS

were supplied by the Otsuka Assay Institute, Tokushima, Japan.

The basic structure of Le" is formed by the attachment of α-fucose to the pentultimate N-acetylglucosamine in the long straight chain of polylactosamine. This structure is recognized by monoclonal antibodies directed to Le", such as FH2 (9). Monoclonal anti-stage-specific embryonic antigen 1 antibody requires at least two repeating N-acetyllactosamine units (i structure) in the core chain (6), and is hereby designated anti-Le"-i antibody. As the core polylactosamine chain is elongated, more α-fucose residues are sometimes attached to the internal N-acetylglucosamines, forming so-called poly-Le"-i antigen, as indicated in "2." This structure is specifically recognized by monoclonal antibodies such as FH4 and ACFH18 (9). If the terminal β-galactose is further fucosylated, the Le"(Y-hapten) structure is formed, as shown in "3," and is recognized by anti-Le"-i antibody such as AH6 (8). When the terminal β-galactose is modified by sialic acid, as illustrated in "4," sialyl Le" antigen is formed, which is recognized by antibodies such as CSLEX-1 and FH6 (10, 11, 13). FH6 needs two repeating N-acetylglactosamine structures present in the core chain besides the sialyl Le" structure at the terminus (11); hence it can be designated anti-sialyl Le"-i antibody.

Fig. 1. Carbohydrate structures of four antigens carried by fucosylated type 2 chain polylactosamines, Le", poly-Le", Le"-i, and sialyl Le"-i.
The abbreviation used is: PCA, perchloric acid.

Results

Standard Conditions Established for the Assay of Each Determinant by Its Respective Monoclonal Antibody. By using the standard material described in "Materials and Methods," a standard assay system was established for each monoclonal antibody. The assay condition was linear up to 224 units/ml for FH6 and ACFH18 and up to 112 units/ml for FH2 and AH6 antibodies. When 100 samples of normal sera were tested, the normal value of 8.3 ± 7.0 (mean ± SD) was obtained for Le" detected by FH2 antibody, 20.5 ± 6.1 for poly-Le" detected by ACFH18 antibody, 14.6 ± 6.1 for sialyl Le"-i detected by FH-6 antibody, and 4.8 ± 3.6 for Le" detected by AH6 antibody. Any values obtained from sera of patients that were greater than the mean + 2 SD of the normal population were designated as abnormally high values in this study.

Sialyl Le"-i Antigen Levels Defined by FH6 in the Sera of Patients with Malignant and Non-Malignant Diseases. As shown in Fig. 2a, the level of sialyl Le"-i antigen defined by FH6 antibody was high in a significant number of sera from patients with malignant diseases. Among patients with various cancers, the highest incidence of abnormally high levels was obtained from patients with lung carcinoma (44%), followed by patients with cancers of the digestive system, breast cancer, and ovarian cancer. Collectively, sialyl Le"-i antigen levels were high in patients with cancer originating from organs which adenocarcinomas frequently develop. The incidence of abnormally high levels in sera of patients with other cancers, such as nasopharyngeal cancers, leukemia, or malignant lymphoma, was significantly lower than in patients with adenocarcinoma. In sera of patients with non-malignant diseases, the incidence of high levels was very low.

Le", poly-Le", and Le" Antigen Levels in the Sera of Patients with Malignant and Non-Malignant Diseases. As shown in Fig. 2, b–d, the serum level of Le", poly-Le", and Le" antigens was significantly high in a few patients with various cancers. The spectrum of cancers showing abnormally high levels resembles that of sialyl Le"-i antigen, i.e., abnormal values were frequently detected in lung cancer, cancers of the digestive system, breast cancer, and ovarian cancer. However, the frequencies of abnormally high levels were generally less than that of sialyl Le"-i antigen in every type of cancer, as summarized in Table 1. The only exceptions were the samples from patients with hepatoma, which showed a higher incidence of elevated levels of Le" than of Le"-i (see below).

Sialyl Le"-i Antigen Levels in Sera of Patients with Lung Cancer. Since the incidence of high levels of sialyl Le"-i antigen was highest in sera of patients with lung cancer, lung cancer cases were classified according to their histology, and their serum antigen levels were studied in detail. As shown in Fig. 3a, sialyl Le"-i antigen levels were remarkably high in sera of patients with adenocarcinomas, and the levels were much lower in sera of patients with squamous cell carcinoma, small cell carcinoma, and large cell carcinoma of the lung. The difference in sialyl Le"-i antigen levels between sera with adenocarcinoma and squamous cell carcinoma or between adenocarcinoma and small cell carcinoma was statistically significant at P < 0.001 by the χ²-test. The antigen level and the positive incidence were particularly high in patients with stage III or IV cancers (Fig. 3b). The difference between stage I and II patients and stage III and IV patients was significant at P < 0.005 by the Wilcoxon paired rank sum test.
Fig. 2. Levels of four antigens carried by fucosylated type 2 chain polylactosamines in sera of patients with various disorders, a. Serum levels of sialyl Le\(^{-}\)i antigen as determined by FH6 antibody; b. serum levels of Le\(^{a}\) as determined by FH2 antibody; c. serum levels of poly-Le\(^{*}\) as determined by ACFH18 antibody; d. serum levels of Le\(^{b}\) as determined by AH6 antibody. The four antigens were assayed on the same panel of sera (approximately 600 samples) collected from patients with various disorders. ca., carcinoma.

Le\(^{a}\) Antigen Levels in the Sera of Patients with Hepatoma. Since the incidence of abnormally high levels of Le\(^{a}\) antigen was highest in patients with hepatoma, the serum level of the antigen was further studied in patients with malignant and non-malignant liver diseases. As shown in Fig. 3, abnormally high Le\(^{a}\) antigen levels were observed only in patients with hepatoma and not in patients with non-malignant liver diseases, including liver cirrhosis.

Incidence of Sialyl Le\(^{-}\)i, Le\(^{a}\), poly-Le\(^{*}\), and Le\(^{b}\) Antigens in Sera and the Overlap of Positive Incidence. In most of the cancers tested, the sialyl Le\(^{-}\)i antigen showed the highest incidence of abnormally high levels. This was typified by lung cancer, in which the incidence of high levels of sialyl Le\(^{-}\)i antigen far exceeded that of the other three antigens. This
**Table 1** Serum levels of sialylated type 2 chain polylactosamine antigens in patients with various disorders

<table>
<thead>
<tr>
<th>Patients</th>
<th>Cancers</th>
<th>Nonmalignant diseases</th>
<th>Other malignant diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stomach CA*</td>
<td>Liver</td>
<td>Normal</td>
</tr>
<tr>
<td>Le* (FH2)</td>
<td>8/89(9)</td>
<td>3/36(8)</td>
<td>0/34(0)</td>
</tr>
<tr>
<td>Poly-Le* (ACFH18)</td>
<td>6/77(8)</td>
<td>1/21(5)</td>
<td>1/34(9)</td>
</tr>
<tr>
<td>Sialyl Le*-i (FH6)</td>
<td>21/94(22)</td>
<td>1/47(2)</td>
<td>3/37(8)</td>
</tr>
<tr>
<td>Le* (AH6)</td>
<td>13/72(18)</td>
<td>4/27(17)</td>
<td>0/36(0)</td>
</tr>
<tr>
<td>Total</td>
<td>25/69(36)</td>
<td>4/21(19)</td>
<td>3/34(9)</td>
</tr>
</tbody>
</table>

* Difference from normal population is significant at P < 0.01 by the chi² test.
* Numbers in parentheses, percentage.
* Difference from normal population is significant at P < 0.001 by the chi² test.
* Number of sera where one or more of the four antigens is positive/number of sera tested for all four antigens.
* Difference from normal population is significant at P < 0.05 by the chi² test.
* Sum of stomach, colorectal, liver, pancreas, biliary tract, breast, lung, and ovarian carcinomas.
* Include leukemias, myelomas, malignant lymphomas, and other sarcomas.

Fig. 3. Levels of sialyl Le*-i antigen in sera of patients with lung cancer. In a, the antigen levels in sera of patients with lung cancer of different histological types at stages III and IV are shown. In b, the antigen levels in sera of patients with lung adenocarcinomas at various stages (stages I–IV) are shown, ca., carcinoma.

Fig. 4. Levels of Le* antigen in sera of patients with benign and malignant liver diseases.

Fig. 5. Coincidence of presence of the four antigens in sera of patients with various cancers. Antigen profiles of sera that showed high levels of at least one of the four antigens are shown, ca., carcinoma.

indicates that sialyl Le*-i antigen is the best marker for lung cancer among these four antigens.

In other types of cancer, however, significant numbers of patients had high serum levels of Le*, poly-Le*, or Le* antigens but not of sialyl Le*-i antigen. This was typical for patients with hepatoma, in which the incidence of high levels of Le* antigen far exceeded that of sialyl Le*-i, indicating that Le* antigen is the best marker for hepatoma among these four antigens (Fig. 5).

In stomach, colorectal, and breast cancers, a significant number of patients had high levels of Le*, poly-Le*, and Le* antigens but not sialyl Le*-i antigen. Thus, combining the determinations indicates that sialyl Le*-i antigen is the best marker for lung cancer among these four antigens.
tions of these four antigens in sera greatly enhanced the detection rate of these cancers in patients, as shown in Fig. 5 and Table 1.

Partial Characterization of Sialyl Le\textsuperscript{i} Antigen. The sialyl Le\textsuperscript{i} antigen was sialidase-sensitive, protease-sensitive, labile in alkaline, and decreased upon endo-\(\beta\)-galactosidase treatment, as shown in Fig. 6. When the sera of patients with cancers showing high levels of sialyl Le\textsuperscript{i} antigen were pooled and analyzed on Sepharose 6B column chromatography, the antigen was eluted at the void volume, indicating that the antigen has a molecular weight of more than 1,000,000 (Fig. 7a). Essentially the same results were obtained when the pooled sera from patients with non-malignant diseases showing relatively high levels of the antigen were tested (Fig. 7b). When normal sera showing relatively high levels of the antigen were pooled and analyzed in the same way, similar results were obtained (Fig. 7c).

Solubility of Antigens in PCA. When the sera of patients with malignant diseases showing abnormally high levels of sialyl Le\textsuperscript{i} antigen were treated with 0.6 N PCA, the antigen was recovered in significant quantity in the PCA extract. A low activity for sialyl Le\textsuperscript{i} was often found in sera of normal individuals. However, when sera of normal individuals were treated with PCA, the sialyl Le\textsuperscript{i} antigen was mostly precipitated by the treatment and was not recovered in the extract. The results from randomly selected serum samples are shown in Fig. 8. These results suggest that the degree of glycosylation of sialyl Le\textsuperscript{i} antigen in sera of patients with cancer is significantly different from that of the same antigen present in sera of normal individuals or patients with non-malignant diseases. This is based on the assumption that perchloric acid solubility of glycoproteins is dependent on the degree of glycosylation.

When the sera of cancer patients showing abnormally high levels of Le\textsuperscript{x} or Le\textsuperscript{a} antigen were subjected to the same PCA treatment, both antigens were easily precipitated by PCA in nearly all sera (Fig. 9, b and c). Poly-Le\textsuperscript{a} antigen was significantly recovered in the PCA extract of some sera, as shown in Fig. 9a. The average recovery of sialyl Le\textsuperscript{i} antigen in 0.6 N PCA extract from randomly selected serum samples was 84.6 ± 32.1% (\(n = 17\)), while the recovery of poly-Le\textsuperscript{a} antigen in PCA extracts was 30.1 ± 20.4% (\(n = 10\)), and recoveries for Le\textsuperscript{x} and Le\textsuperscript{a} were 12.3 ± 16.5 (\(n = 17\)) and 3.4 ± 2.3 (\(n = 17\)), respectively. These results indicate that sialyl Le\textsuperscript{i} antigen in serum of patients with cancer is largely PCA-soluble, while Le\textsuperscript{a} antigen and Le\textsuperscript{a} antigen are essentially insoluble in PCA.

To further investigate this point, the sera having simultane-

![Fig. 6. Effect of enzymatic and chemical treatment on the serum level of sialyl Le\textsuperscript{i} antigen.](image)

![Fig. 7. Sepharose 6B column chromatography of serum sialyl Le\textsuperscript{i} antigen.](image)

**DISCUSSION**

Elevated levels of these four antigens, Le\textsuperscript{x}, poly-Le\textsuperscript{a}, Le\textsuperscript{a}, and sialyl Le\textsuperscript{i}, defined by their monoclonal antibodies, were frequently observed in sera of patients with various malignant diseases, and the levels of these antigens were consistently low in sera of patients with benign diseases and in healthy individuals. These results indicate that the four antigens are all associated with human cancer, which is compatible with the findings that all of the antigens are present in various types of human cancer in high quantity, particularly in human adenocarcinoma.

Fig. 6. Effect of enzymatic and chemical treatment on the serum level of sialyl Le\textsuperscript{i} antigen. •, control; △, treated with \(\alpha\)-fucosidase from beef kidney; ○, treated with endo-\(\beta\)-galactosidase from E. freundii; ◆, treated with Pronase E; ■, treated with sialidase; □, treated with 0.05 N NaOH at 50°C for 20 h. Bars, SD.

Fig. 7. Sepharose 6B column chromatography of serum sialyl Le\textsuperscript{i} antigen. a, pooled sera collected from normal individuals that showed relatively high values for the antigen; b, pooled sera collected from patients with benign diseases; c, pooled sera from patients with cancer that showed high levels of the antigen. • - •, sialyl Le\textsuperscript{i} antigen level; • - •, protein concentration; ○ - ○, carcinoembryonic antigen (CEA) level.

Elevated levels of both sialyl Le\textsuperscript{i} and Le\textsuperscript{a} antigens were selected from the large number of sera of cancer patients and subjected to PCA treatment. The results showed that only sialyl Le\textsuperscript{i} antigen was significantly recovered in the extract, while Le\textsuperscript{a} antigen was hardly detectable in the extract, as shown in Table 2. These results clearly indicate that, for the most part, these two carbohydrate determinants are present on different molecules, even in the same serum.
with cancers were extracted with 0.6 N PCA, and the level of the antigens were measured before and after PCA extraction. A, Le"; B, poly-Le"; C, Le*. Individual sera from patients with adenocarcinoma; the levels of Le" and poly-Le" antigens tend to be high in the sera of patients with cancers originating in organs from which adenocarcinomas frequently develop, such as stomach, intestine, pancreas, gall bladder, ovary, mammary glands, and lung.

Among the four antigens described above, the level of sialyl Le"-i antigen was most frequently elevated in sera of patients with adenocarcinoma; the levels of Le* and poly-Le* antigens in sera were less frequently elevated. It is noteworthy that the Le* antigen was elevated in sera of patients with hepatoma. While Le* and poly-Le* antigens frequently accumulate in cancer tissues (19) and their level in cancer tissues is even higher than that of sialyl Le"-i and Le*, the incidence of cases showing a high level of Le* and poly-Le* in sera was much less than the incidence of high levels of sialyl Le"-i and Le* in patient sera. It is therefore possible that Le* and poly-Le* antigens could be eliminated from sera by reticuloendothelial systems, which are equipped with receptors for the terminal 3-galactosyl residue of carbohydrates (27).

Since these four antigens have related carbohydrate structures (Fig. 1) and have a common synthetic pathway (28), it was expected that serum positive for one antigen could also be positive for other related antigens. This expectation turned out to be partially true, and some patients had all four antigens present in detectable levels in their sera; nevertheless, some other cases, particularly patients with lung cancer and hepatoma, showed a peculiar distribution pattern of specific antigens in their sera; i.e., the level of sialyl Le"-i antigen was particularly high in the sera of patients with lung adenocarcinoma, and the level of Le* antigen was significantly high in the sera of patients with hepatoma. These findings suggest a few possibilities: (a) synthesis, chemical quantity, and release of these antigens are specifically high in some tumors but not in others. The expression of poly-Le* antigen defined by FH4 has been correlated with the degree of differentiation; i.e., it is highly expressed in adenocarcinoma but is less expressed or not expressed in undifferentiated cancers (19); (b) although all of these antigens have been found in high quantity in gastrointestinal cancers, particularly adenocarcinoma, by immunohistologic technique (19, 24, 25), their release from gastrointestinal tumors may be limited in some cases. For example, sialyl Le* antigen in colonic adenocarcinoma was highly concentrated at the luminal surface and in secretions (25), and it may be difficult for it to be released in the blood stream; and (c) another important factor affecting the level of tumor antigens in sera is the anatomical location of the tumor and its metastatic lesions; e.g., an antigen in a lung cancer can be disseminated more readily than an antigen in a tumor in gastrointestinal organs, since an antigen released from gastrointestinal organs may first be trapped in the liver before being disseminated by circulation. An antigen in metastatic deposits in liver and lymph nodes can be more readily disseminated than those located in gastrointestinal mucosae.

Serum sialyl Le*-i antigen seems to be essentially a high molecular weight glycoprotein with a molecular weight greater than 10^6, since it is eluted in the void volume through a Sepharose 6B gel filtration. The antigenic site could be attached to the protein core through an 3-glycosidic linkage, since FH6 reactivity can be abolished by alkaline degradation. These antigen properties are similar to those found in other cancer-associated mucin-like glycoproteins such as CA-1 (29), DU-PAN-2 (30), CA-19-9 (6), or OC-125 (31) antigens. Qualitative differences of FH6-defined serum antigen among patients with cancer and non-malignant diseases and normal healthy individuals are clearly suggested by its solubility in perchloric acid; i.e., the FH6-defined glycoprotein antigen in sera of patients is more soluble in perchloric acid than the same antigen in sera of patients with non-cancerous diseases and healthy individuals. This concept is illustrated in Fig. 10, a and b. Sialyl Le*-i mucin seems to be highly glycosylated, while Le* mucin seems to be much less glycosylated, since it precipitates in perchloric acid. The degree of glycosylation as judged from the recoveries in
Table 2  Effect of perchloric acid (PCA) treatment on the levels of sialylated and fucosylated species of Le" antigen (SSEA-1) in 15 sera from patients with cancer having elevated levels of both antigens

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Original Le&quot; antigen (units/ml)</th>
<th>0.6 n PCA extract (%)</th>
<th>% of recovery in the extract (%)</th>
<th>Original Le&quot; antigen (units/ml)</th>
<th>0.6 n PCA extract (%)</th>
<th>% of recovery in the extract (%)</th>
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<tr>
<td>51013</td>
<td>Lung cancer</td>
<td>43.5</td>
<td>39.0</td>
<td>90</td>
<td>396.6</td>
<td>12.7</td>
<td>3</td>
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<tr>
<td>51018</td>
<td>Lung cancer</td>
<td>80.3</td>
<td>41.3</td>
<td>51</td>
<td>60.8</td>
<td>2.5</td>
<td>4</td>
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<td>51021</td>
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<td>51.8</td>
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<td>7</td>
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<td>Ovarial cancer</td>
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<td>98</td>
<td>19.6</td>
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<td>100026</td>
<td>Pancreas cancer</td>
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<td>722.5</td>
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<tr>
<td>100035</td>
<td>Biliary tract</td>
<td>42.6</td>
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<td>68</td>
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<td>61</td>
<td>66.5</td>
<td>2.7</td>
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4) CA, carcinoma.

Fig. 10. Schematic model of the structures of serum mucins carrying fucosylated type 2 chain polylactosamine antigens. Most of the sialyl Le"-i in sera of patients with cancer is present on mucin molecules having a significantly high carbohydrate/protein ratio as in a, which are therefore extractable with PCA. On the other hand, most of the sialyl Le"-i in sera from normal individuals or patients with benign diseases is present on mucin molecules having a relatively low carbohydrate/protein ratio as in b and are therefore less extractable with PCA. Le" antigen must be present exclusively on mucin molecules that have low carbohydrate/protein ratios, as illustrated in e, since essentially none of the antigen is recovered in PCA extracts. The order of the average carbohydrate/protein ratio of these cancer-associated mucins carrying fucosylated type 2 chain polylactosamines must be sialyl Le"-i > poly-Le" > Le" > Le', as illustrated in a-e. Sialyl Le"-i and Le" are mostly present on different molecular species of mucins, even when they co-exist in the same serum.

Perchloric acid extract was sialyl Le"-i > poly-Le" > Le" > Le' as shown in Fig. 10, a and c-e. Even when sialyl Le"-i antigen and Le" antigen coexist in sera from the same patients, most of the sialyl Le"-i is carried by a highly glycosylated mucin-like glycoprotein, which is perchloric acid-soluble, while the glycoprotein antigen carrying Le" hapten is exclusively present in the less glycosylated perchloric acid-insoluble fraction. Therefore, these two types of glycoproteins can be readily separated by perchloric acid fractionation. These findings indicate that the cancer-associated mucin-type glycoproteins carrying fucosylated type 2 chain polylactosamines are highly heterogeneous and can be classified into several characteristic molecular species. Further studies on the biochemical characteristics of each species of cancer-associated mucin-type glycoprotein carrying fucosylated type 2 chain polylactosamines may deepen our knowledge of cancer markers that are useful for diagnosis of human cancer.


Quantitative and Qualitative Characterization of Human Cancer-associated Serum Glycoprotein Antigens Expressing Fucosyl or Sialyl-Fucosyl Type 2 Chain Polylactosamine

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