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(54) **METHOD OF SCREENING FOR CANCER AND ADENOMA**

SCREENING-VERFAHREN FÜR KREBS UND ADENOM

PROCÉDÉ DE DÉPISTAGE DU CANCER ET D'UN ADÉNOME

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- **BRUEHL R E ET AL: "Minimal sulfated carbohydrates for recognition by L-selectin and the MECA-79 antibody" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOCHEMICAL BIOLOGISTS, BIRMINGHAM,; US, vol. 275, no. 42, 20 October 2000 (2000-10-20), pages 32642-32648, XP002475643 ISSN: 0021-9258**
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- **BISTRUP A. ET AL.: 'Detection of a sulfotransferase (HEC-GlcNAc6ST) in high endothelial venules of lymph nodes and in high endothelial venule-like vessels with ectopic lymphoid aggregates: relationship to the MECA-79 epitope' AM. J. PATHOL. vol. 164, no. 5, May 2004, pages 1635 - 1644, XP002996352**

Description

Field of the Invention

[0001] The present invention relates to examining methods of human carcinomas and adenomas except colorectal carcinomas and colorectal adenomas and to examination reagents thereof.

Prior Art

[0002] Since the number of carcinoma patients is increasing year by year, a method for early detection is necessary. Although, immunological fecal occult blood test and various tumor markers are used at present for the examination of carcinomas, these methods do not have satisfactory positive rates. Namely, the positive rate of immunological fecal occult blood test used for the examination of carcinomas and adenomas is 50 to 60%. As to the tumor markers of colorectal carcinomas, carcino-embryonic antigen (CEA), CA19-9, STX, which are used for examining the therapeutic effect and for monitoring recurrence, are not satisfactory as tumor markers for early detection of colorectal carcinomas.

[0003] Although sulfation of sugar residues is active in a normal large bowel, it is known that the sulfation of sugar residues is remarkably reduced in colorectal carcinomas. Namely, both 3'-sulfation of galactose and 6-sulfation of N-acetylglucosamine (hereinafter referred to as [GlcNAc]), which are abundant in colorectum, are reduced (Reference 1). A number of GlcNAc-6-sulfotransferase isozymes have been known in colorectal carcinoma tissues and in non-carcinoma colorectal tissues of patients, and I-GlcNAc6ST is significantly decreased in course of carcinogenesis, which leads to the reduced sulfation of sugar residues in colorectal carcinoma (Reference 2). While, GlcNAc6ST-1, one of the isozymes in a normal colorectum, does not show significant changes in the level in course of carcinogenesis. Furthermore, HEC-GlcNAc6ST, another isozyme, increases significantly in carcinoma (Reference 3).

[0004] HEC-GlcNAc6ST, which increases in carcinomas, synthesizes 6-sulfated GlcNAc and carries out sulfation of GlcNAc in various sugar residues. Therefore, there are a huge variety of the structures of intra-cellularly synthesized sugar residues and their antigenicity. Since GlcNAc6ST-1 and I-GlcNAc6ST also synthesize 6-sulfatedGlcNAc, only the fact that 6-sulfatedGlcNAc is synthesized from HEC-GlcNAc6ST cannot be used as a specific method for diagnosis of carcinomas and adenomas. However, it is known that the substrate selectivity of GlcNAc6ST-1 and I-GlcNAc6ST is more specific than that of HEC-GlcNAc6ST (References 3, 4). Therefore, certain 6-sulfated sugar residues might be produced by HEC-GlcNAc6ST, but not by GlcNAc6ST nor by I-GlcNAc6ST (Reference 5). However, an actual system for diagnosis of carcinomas and adenomas has not been established.

[0005] On the other hand, the monoclonal antibody (MECA-79 antibody, Reference 6), commercially available as an antibody against an immunological homing receptor of lymphocytes, is known to react with chemically synthesized GlcNAc6-sulfated sugar residues (Reference 7). Moreover, the antigens recognizable by the antibody (MECA-79) are reported to emerge on the cell surface, when a mouse gene encoding HEC-GlcNAc6ST enzyme is transduced into CHO cells (hamster ovary cells) (Reference 8).

[0006] The present inventors found that human colorectal carcinoma cells has the antigen recognizing the MECA-79 antibody, which could be applied for the examination of colorectal carcinomas and colorectal adenomas (Patent publication no. JP2005062125).

Reference 1: Izawa, M. et al., Cancer Res., 60: 1410-1416, 2000.
Reference 2:Abstract of the 22nd Research Meeting of Japan Molecular Tumor Maker pp42-43, 2002.
Reference 3: Seko, A. et al., Glycobiology, 10:919-929, 2000
Reference 4: Seko, A. et al., Glycobiology, 12:379-388, 2002
Reference 5: The Journal of Biological chemistry, vol. 277, No. 6, 3979-3984 (2002)
Reference 6: Streeter, P.R. et al., J. Cell Biol. 107: 1853-1862, 1988.
Reference 7: Bruehl, R.E. et al., J. Biol. Chem. 275: 32642-32648, 2000
Reference 8: Yeh, J.C. et al., Cell 105: 957-969, 2001.

Problems to be solved by the Invention

[0007] The present invention provides examination methods and reagents of carcinomas and adenomas, wherein the method enables to detect efficiently carcinoma and adenoma patients and patients (except colorectal carcinomas and colorectal adenomas) at high risk of carcinomas and is useful for diagnosis of carcinomas and adenomas.

Means to solve the Problems

[0008] The present inventors discovered that there are significant differences in the distribution of GlcNAc-6-sulfotrans-

ferase isozymes, sulfation enzymes of sugar residues, between non-carcinoma tissues and carcinoma tissues or adenoma tissues, during investigations. Then the inventors found that carcinomas and adenomas (except colorectal carcinomas and colorectal adenomas) could be detected specifically by assaying 6-sulfated sugar residues, which are synthesized only by HEC-GlcNAc6ST, but not by GlcNAc6ST 1 nor by I-GlcNAc6ST, in tissues of patients and in fecal samples.

[0009] Previously, many antibodies such as AG223 (Biochem. (Tokyo), 124:670-678, 1998), G152, G72, AG97, AG107; AG273, G2706, G27011, G27039 (the above: J.Biol.Chem., 273: 11225-11233, 1998) and the like have been known to react with GlcNAc-6-sulfated sugar residues. Meanwhile, MECA-79 antibody (Pharmingen, Catalog No. 09961D; Distributor, Becton, Dickinson and Company), which is available commercially as an antibody against lymphocyte immunological homing receptor, has been known to react with some kinds of GlcNAc-6-sulfated sugar residues (Reference 6). The present inventors screened to look for antibodies, which are weakly or little reactive to cells expressing GlcNAc-6-sulfated sugar residues found in normal cells, but are reactive with cells expressing GlcNAc-6-sulfated sugar residues increased in carcinoma cells, examined the reactivity of the antibodies to samples from patients, found that these antibodies are highly positive to various carcinoma cells and completed the present invention.

[0010] In other words, the present invention is a method for examining carcinomas and adenomas, except colorectal carcinomas and colorectal adenomas, comprising assaying presence or absence, or intensity of the reactivity of an antibody to tissues, body fluid or feces of patients, or extracts thereof, wherein said antibodies react with such antigen that is present in cells expressing HEC-GlcNAc6ST gene encoding GlcNAc-6-sulfotransferase and that is absent or almost absent in cells expressing GlcNAc6ST-1 or I-GlcNAc6ST gene.

[0011] The antigen may be an antigen that is present in cells transduced with HEC-GlcNAc6ST gene and is absent or almost absent in cells transduced with GlcNAc6ST-1 gene or I-GlcNAc6ST gene.

[0012] The antigen comprises the sugar residues expressed by the following formula:

R1-Gal B1-3/4(SO_3-6) GlcNAc B1-R2

where, R1 represents sugar residues added by other enzymes and is not limited in structure, Gal β represents β galactose, GlcNAc β represents β N-acetylglucosamine, Gal β1-3/4 represents binding of 1 position of Gal β and 3 position and/or 4 position of GlcNAc β, (SO_3-6) represents addition of a sulfate group to 6 position of GlcNAc β, R2 represents -3GalNAc α, -3Ga1 β or -2Manα and binds to 1 position of GlcNAc β.

[0013] Furthermore, the present invention is a method for examining carcinomas and adenomas, except colorectal carcinomas and colorectal adenomas, comprising assaying the reactivity of MECA-79 antibody (Pharmingen, catalog No. 09961D) or its equivalent with tissues, body fluid, feces or extract thereof of test subjects.

[0014] Moreover, the present invention is any one of the above methods, further comprising reacting a labeled probe to said antibody and assaying the label qualitatively or quantitatively.

[0015] The preferable examination method comprises fixing the antigens present in tissues, body fluid or feces or extracts thereof of patients to a membrane, reacting with the antibody, reacting with a labeled probe and detecting the label. It is preferable to insert washing procedures appropriately between the above processes. The above probe includes anti-human-IgG antibody, protein G, protein A, and protein L. These probes are usually labeled. The labels include a radioactive isotope (^{125}I) and enzymes (peroxidase, alkaline phosphatase). An antibody with enzyme may involve observation of a change (i.e. color change) by the reaction between the enzyme and the substrate.

[0016] Still furthermore, we describe the use of an examination reagent for examining carcinomas and adenomas, except colorectal carcinomas and colorectal adenomas, comprising, as a major component, an antibody reacting specifically with an antigen carrying sugar residues, which is present in cells expressing HEC-GlcNAc6ST gene and is absent or almost absent in cells expressing GlcNAc6ST-1 or GlcNAc6ST gene. (see claims). Still moreover, the present invention is the use of an examination reagent for examining carcinomas and adenomas, except colorectal carcinomas and colorectal adenomas, comprising, as a major component, an antibody reacting specifically with an antigen carrying sugar residues, which is present in cells transduced with HEC-GlcNAc6ST gene and is absent or almost absent in cells transduced with GlcNAcGST-1 or GlcNAc6ST gene.

[0017] Also, the present invention is the use of an examination reagent for examining carcinomas and adenomas, except colorectal carcinomas and colorectal adenomas, comprising, as a major component, an antibody reacting specifically with an antigen carrying sugar residues, which are present in tissues, body fluid or feces of patients with colorectal cancer and colorectal adenoma and expressed by the following general formula:

R1-Gal β1-3/4 (SO_3-6) GlcNAc β1-R2

where, R1 represents sugar residues added by other enzymes and is not limited in structure, Gal β represents β galactose, GlcNAc β represents β N-acetylglucosamine, Gal β1-3/4 represents binding of 1 position of Gal β and 3 position and/or 4 position of GlcNAc β, (SO_3-6) represents addition of a sulfate group to 6 position of GlcNAc β, R2 represents -3GalNAc

α, -3Gal β or -2Manα and binds to 1 position of GlcNAc β.

Brief Description of the Drawings

[0018]

Figure 1 shows the results of flowcytometric analysis using MECA-79 antibody on colorectal cancer cells (COL0201 cells) and on normal colorectal epithelial cells (SW480 cells treated with Tricostatine A).
Figure 2 shows the result of the flowcytometric analysis on the reactivity of various antibodies with cells. The ordinate shows the cell frequency (the number of cells) and the abscissa axis shows the fluorescence (Arbitrary Unit). Transfectants show transgenic cells.
Figure 3 shows the result of determination of the amount of sugar residues in serum samples of various carcinoma patients by sandwich ELISA method by the use of 7A4 antibody of the present invention.
Figure 4 shows a catalog of MECA-79 antibody (Pharmingen, catalog No. 09961D).
Figure 5 shows a catalog of MECA-79 antibody (Pharmingen, catalog No. 09961D).

Detailed Description of the Invention

[0019] The structure of 6-sulfated sugar residues, which are rarely synthesized by GlcNAc6ST-1 or by I-GlcNAc6ST, but are synthesized only by HEC-GlcNAc6ST, is represented by the following general formula:

R1-Galβ-3/4(SO_3-6)GlcNAcβ1-R2.

[0020] Various sugar residue carriers carry GlcNAcB, which is the substrate of GlcNAc-6-sulfotransferase in a body. R2 is a carrier.
HEC-GlcNAc-6ST has been known to transfer sulfate residues to all kinds of GlcNAcβ1-R2 previously tested according to both our research and other peoples research (References 4 and 7). In contrast, GlcNAc6ST-1 and I-GlcNAc6ST transfer sulfate residues only to a special kind of GlcNAcβ-R2 carrying a specific form of R2.

[0021] The case, in which HEC-GlcNAc6ST but not GlcNAc6ST-1 nor I-GlcNAc6ST can transfer a sulfate residue, is known as the case that R2 is -3GalNAca (the structure after sulfation is SO^-_3-6GlcNAcβ1-3GalNAcα), the case that R2 is -3Galβ (the structure after sulfation is SO_3-6GlcNAcβ1-3Galβ) and the case that R2 is -2Manα (the structure after sulfation is SO_3-6GlcNAcβ1-2Manα) (J. Biol. Chem., 277: 3979-3984, 2002 and Glycobiology, 12: 379-388, 2002). In the test method of the present invention, a specific antibody to any one of the three cases or antibodies cross reacting to all three sugar residues may be usable.

[0022] GleNAc-6-sulfotransferase adds sulfate group to terminal GlcNAc of sugar residues and synthesizes said 6-sulfated GlcNAc (i.e. SO_3-6GlcNAc) intra-cellularly. However, after synthesis of terminal 6-sulfated GlcNAc of sugar residues, the modified sugar residues are further added with sugar residues (R1) by other enzyme groups intra-cellularly, then a large variety of the structure and antigenicity of the sugar residues are finally synthesized and are generated from cells. Generally the structure added to 6-sulfated GlcNAc is Galβ1-4 and Galβ1-3 (referred to as Galβ1-3/4). Moreover, it is known that NeuAcα2-3/G, SO_3-3/6, and Fucal-2/3/4 are added to the 6-sulfated GlcNAc. The R1 part is added after the synthesis of 6-sulfated GlcNAc by GlcNAc-6-sulfotransferase. Therefore, R1 part is not related to the substrate specificity of such GlcNAc-6-sulfotransferases as HEC-GlcNAc6ST, GlcNAc6ST1 and I-GlcNAc6ST.

[0023] The antigens carrying the above sugar residues are present in carcinoma tissues obtained from colorectal carcinoma patients by biopsy or by surgical operation, and in such samples as serum, ascites and feces containing the antigens derived from the above tissues. Also, the antigen may be easily extracted from these samples using phosphate buffered saline. Also, the antibody against this sugar residue antigen could be obtained by known arts producing antibodies (e.g. Methods in Enzymology, 312: 160-179, 2000; Methods in Molecular Biology, 199: 203-218, 2002 et al.). An example of these antibodies may include MECA-79 antibody (Pharmingen, Catalog No. 09961D shown in Fig. 4 and 5).

[0024] The examination method and reagent using the antibodies of the present invention could be applied not only to colorectal carcinomas and colorectal adenomas, but also to adenomas causing universal carcinomas and precancerous states, i.e. to universal malignant tumors, i.e. to epithelial carcinomas and non-epithelial malignant tumors, preferably to epithelial carcinomas.

[0025] Malignant tumors are classified to epithelial carcinomas and non-epithelial malignant tumors. Epithelial carcinomas are classified to adenocarcinoma, squamous cell carcinoma and other epithelial carcinoma, wherein adenocarcinomas involve colorectal carcinoma, breast carcinoma, gallbladder carcinoma, gastric carcinoma, renal carcinoma, ovarian cancer, prostate carcinoma, pancreatic carcinoma, a part of pulmonary carcinoma, thyroid carcinoma, bronchial cancer, bile duct carcinoma, ovarian duct carcinoma, salivary gland cancer and testicular cancer; squamous cell carcinomas involve esophageal carcinoma, a part of pulmonary carcinoma, uterine cancer, oral carcinoma, carcinoma linguae,

laryngeal cancer, pharyngeal cancer, cutaneous carcinoma, vaginal carcinoma and penile cancer; other epithelial carcinomas include hepatic carcinoma, bladder carcinoma and the like; and non-epithelial malignant tumors involve osteosarcoma, malignant melanoma, fibrosarcoma and the like as well as leukemia, malignant lymphoma and cerebral tumor.

[0026] The following Examples illustrate the present invention, but are not intended to limit the scope thereof.

Reference Example 1

[0027] Gene expression of GlcNAc-6-sulfotransferase isozymes was examined by RT-PCR on human-derived colorectal carcinoma cells (Colo201 cells) and on TSA-SW480 cells obtained by the treatment of normal colorectal epithelial cells (SW480 cells, obtained from Tohoku University, Cell Resource Center for Biomedical Research) with Tricostatin A.

[0028] In the RT-PCR analysis, PCR primers for detection of the expression of HEC-GlcNAc6ST gene (Genebank, AF131235) are synthetic oligonucleotides of SEQ ID NO. 1 for upper strand side and those of SEQ ID NO. 2 for lower strand side (Tm=59°C), those for GlcNAc6ST1 gene (Genebank, AB011451) are synthetic oligonucleotides of SEQ ID NO. 3 for upper strand side and those of SEQ ID NO. 4 for lower strand side (Tm=62°C), and those for I-GlcNAc6ST gene (Genebank, AF176838) are synthetic oligonucleotides of SEQ ID NO. 5 for upper strand side and those of SEQ ID NO.6 for lower strand side (Tm=60°C).

[0029] The results are shown in Fig. 1. It is found that the colorectal carcinoma cells (Colo 201 cells) are typical colorectal carcinoma cells, which show the pattern of strong expression of HEC-GlcNAc6ST gene and little expression of GlcNAc6ST-1 and I-GlcNAc6ST genes. Furthermore, it is found that TSA-SW480 cells are typical normal epithelial cells, which show the pattern of little expression of HEC-GlcNAc6ST gene and strong expression of GlcNAc6ST-1 and I-GlcNAc6ST genes.

Example 1

[0030] cDNA of HEC-GlcNAc6ST (Genebank, NM_005769), GlcNAcGST-1 (Genebank,NM_004267) and I-GlcNAc6ST (Genebank, NM_012126) genes are transduced into human colorectal carcinoma cells (SW480 cells, obtained from Tohoku University, Cell Resource Center for Biomedical Research) together with drug resistant neo gene. After cloning by drug selection, said gene expression was confirmed by RT-PCR. The monitoring of gene expression was performed by regular detection of 6-sulfotransferase gene products during maintenance culturing and was used for examining stable gene expression.

[0031] A mouse was immunized by the use of said carcinoma cells by a conventional method. Then, monoclonal antibody, which reacts with those carcinoma cells transformed with GlcNAc-6-sulfotransferase gene, but does not react with those carcinoma cells transformed with other GlcNAc-6-sulfotransferase genes such as GlcNAc6ST-1 or I-GlcNAc6ST gene, was prepared. As the results, several antibodies such as KN173, KN101, KN439 and 7A4, which satisfy the above condition, were obtained.

[0032] Screening of reactivity between cells and antibodies was performed by flowcytometric analysis by the use of FACScan (Becton Dickinson) after staining cells with an indirect fluorescent antibody method (the first antibody 1.0 μg/ml, 4°C, 30 min; the second antibody: rabbit anti rat IgM antibody (Zymed Laboratories), 4°C, 30 min).

[0033] After the above-obtained monoclonal antibody was reacted at 4°C for 30 min as the first antibody, cells were stained conventionally by the use of FITC-labeled rabbit anti mouse immunogloblin antibody (Zymed Laboratories, 4°C, 30 min) as the second antibody and were analyzed by FACScan (Becton Dickinson). The results are shown in Fig.2.

[0034] All antibodies react only with carcinoma cells transformed with HEC-GlcNAc6ST gene and do not react with carcinoma cells transformed with GlcNAc6ST-1 or I-GlcNAc6ST gene. Furthermore, MECA-79 antibody (Pharmingen, Catalog No. 09961D) reacts slightly with GlcNAc6ST-1 gene-transformed carcinoma cells in addition to HEC-GlcNAc6ST gene-transformed carcinoma cells.

[0035] Moreover, as shown in Fig. 2, positive control antibody KN412 is a general 6-sulfation antibody, which reacts with gene products of all kinds of 6-sulfotransferase genes and is a control antibody detecting gene expression of 6-sulfotransferase in gene-transformed cells.

Example 2

[0036] 6-sulfated sugar residues in serum samples of various cancer patients were assayed by sandwich ELISA method by the use of antibody secreted from the clone 7A4 obtained in Example 1. First, the monoclonal antibody 7A4 secreted from the clone 7A4 is fixed in wells of a microplate. Second, serum samples of patients are reacted in the wells. Third, said biotin-labeled antibody is reacted in the wells. Forth, streptavidin-labeled Horse Radish Peroxidase is reacted in the wells. Fifth, the reactants are colored by the use of TMB substrate and finally the ratio of absorbance at 450 nm to control absorbance at 620 nm is measured to determine the amount of reactants after stopping the color development. Positive or negative is judged based on a cut-off line of average +2SD of normal people.

[0037] The results are shown in Fig.3. The amount of said sulfated sugar residues are increased in various carcinoma cases such as breast, pancreatic, gall bladder, esophageal, gastric, hepatocellular, kidney, prostate, lung ovarian, uterine and the like. Normal people are all negative.

SEQUENCE LISTING

[0038]

<110> Japan Science and Technology Agency et al.

<120> METHOD FOR EXAMINING CARCINOMA AND ADENOMA

<130> FS05-446PCT

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Claims

1. A method for examining carcinomas and adenomas, except colorectal carcinomas and colorectal adenomas, comprising assaying presence or absence, or intensity of the reactivity of an antibody to tissues, body fluid or feces of patients, or extracts thereof, wherein said antibodies react with such antigen that is present in cells expressing HEC-GlcNAc6ST gene encoding GlcNAc-6-sulfotransferase and that is absent or almost absent in cells expressing GlcNAc6ST-1 or I-GlcNAc6ST gene.

2. The method of claim 1, wherein said antigen is present in cells transduced with HEC-GlcNAc6ST gene and is absent or almost absent in cells transduced with GlcNAc6ST-1 gene or I-GlcNAc6ST gene.

3. The method of claim 1 or 2, wherein said antigen comprises the sugar residues expressed by the following formula:

R1-Gal 61-3/4(SO_3-6) GlcNAc β1-R2

where, R1 represents sugar residues added by other enzymes and is not limited in structure, Gal β represents β galactose, GlcNAc β represents β N-acetylglucosamine, Gal β1-3/4 represents binding of 1 position of Gal β and 3 position and/or 4 position of GlcNAc β, (SO_3-6) represents addition of a sulfate group to 6 position of GlcNAc β, R2 represents -3GalNAcα, -3Gal β or -2Manα and binds to 1 position of GlcNAc β.

4. A method for examining carcinomas and adenomas, except colorectal carcinomas and colorectal adenomas, comprising assaying the reactivity of MECA-79 antibody (Pharmingen, catalog No. 09961D) or its equivalent with tissues, body fluid, feces or extract thereof of test subjects.

5. The method of any one of claims 1 to4 further comprising reacting a labeled probe to said antibody and assaying the label qualitatively or quantitatively.

6. The method of any one of claims 1 to 5, wherein said carcinoma and adenoma are causative of epithelial carcinoma and precancerous adenoma, respectively.

7. Use of an examination reagent for examining carcinomas and adenomas, except colorectal carcinomas and colorectal adenomas, the examination reagent comprising, as a major component, an antibody reacting specifically with

an antigen carrying sugar residues, which is present in cells expressing HEC-GlcNAc6ST gene and is absent or almost absent in cells expressing GlcNAc6ST-1 or GlcNAc6ST gene.

8. Use of an examination reagent for examining carcinomas and adenomas, except colorectal carcinomas and colorectal adenomas, the examination reagent comprising, as a major component, an antibody reacting specifically with an antigen carrying sugar residues, which is present in cells transduced with HEC-GlcNAc6ST gene and is absent or almost absent in cells transduced with GlcNAc6ST-1 or GlcNAc6ST gene.

9. Use of an examination reagent for examining carcinomas and adenomas, except colorectal carcinomas and colorectal adenomas, the examination reagent comprising, as a major component, an antibody reacting specifically with an antigen carrying sugar residues, which are present in tissues, body fluid or feces of patients with carcinomas and adenomas, except colorectal cancer and colorectal adenomas and expressed by the following general formula:

R1-Gal by-3/4 (SO_3.6) GlcNAc β1-R2

where, R1 represents sugar residues added by other enzymes and is not limited in structure, Gal β represents β galactose, GlcNAc β represents β N-acetylglucosamine, Gal β1-3/4 represents binding of 1 position of Gal β and 3 position and/or 4 position of GlcNAc β, (SO_3-6) represents addition of a sulfate group to 6 position of GlcNAc β, R2 represents -3GalNAc α, -3Gal β or -2Manα and binds to 1 position of GlcNAc β.

Patentansprüche

1. Verfahren zur Untersuchung von Karzinomen und Adenomen, mit Ausnahme von kolorektalen Karzinomen und kolorektalen Adenomen, welches das Überprüfen der Anwesenheit oder Abwesenheit oder der Intensität der Reaktion von einem Antikörper gegen Gewebe, Körperflüssigkeit oder Fäkalien von Patienten oder Extrakten davon umfasst, wobei die Antikörper mit einem Antigen reagieren, das in Zellen vorkommt, die das HEC-GlcNAc6St-Gen, welches für die GlcNAc-6-sulfotransferase kodiert, exprimieren, und das in Zellen, die das GlcNAc6ST-1- oder I-GlcNAc6ST-Gen exprimieren, fehlt oder nahezu nicht vorhanden ist.

2. Verfahren nach Anspruch 1, wobei das Antigen in Zellen vorhanden ist, die mit dem HEC-GlcNAc6ST-Gen transduziert sind, und in Zellen, die mit dem GlcNAc6ST-1-oder dem I-GlcNAc6ST-Gen transduziert sind, fehlt oder nahezu nicht vorhanden ist.

3. Verfahren nach Anspruch 1 oder 2, wobei das Antigen Zuckerreste umfasst, welche durch die folgende Formel:

Rl-Gal-βl-3/4(SO_3-6) GlcNAc-β1-R2

repräsentiert werden, wobei R1 für Zuckerreste steht, welche durch andere Enzyme hinzugefügt sind, und in der Struktur nicht eingeschränkt ist, Gal-β für β-Galaktose steht, GlcNAc β für β-N-Acetylglucosamin steht, Gal-β1-3/4 für die Bindung von Gal-β an Position 1 und GlcNAc-β an Position 3 und/oder Position 4 steht, (S03-6) für die Addition von einer Sulfatgruppe an Position 6 von GlcNAc-β steht, R2 für -3GalNAc-α, -3Gal-β oder -2Man-α steht und an die Position 1 von GlcNAc-β bindet.

4. Verfahren zur Untersuchung von Karzinomen und Adenomen, außer kolorektalen Karzinomen und koloektalen Adenomen, welches das Überprüfen der Reaktivität des MECA-79-Antikörpers (Pharmingen, Katalog-Nr.09961D) oder eines äquivalenten Antikörpers mit Gewebe, Körperflüssigkeit, Fäkalien oder Extrakten davon von Testpersonen umfasst.

5. Verfahren nach einem der Ansprüche 1 bis 4, das ferner das Umsetzen einer markierten Sonde mit dem Antikörper und das qualitative oder quantitative Überprüfen der Markierung umfasst.

6. Verfahren nach einem der Ansprüche 1 bis 5, wobei das Karzinom oder das Adenom ein Epithelkarzinom, beziehungsweise eine Adenomvorstufe verursachen.

7. Verwendung eines Untersuchungsreagenzes zur Untersuchung von Karzinomen und Adenomen, mit Ausnahme von kolorektalen Karzinomen und kolorektalen Adenomen, wobei das Untersuchungsreagenz als eine Hauptkomponente einen Antikörper umfasst, der spezifisch mit einem Antigen reagiert, welches Zuckerreste trägt, das in

Zellen vorhanden ist, die das HEC-GlcNAc6ST-Gen exprimieren, und in Zellen, die das GlcNAc6ST-1- oder das GlcNAc6ST-Gen exprimieren, fehlt oder nahezu nicht vorhanden ist.

8. Verwendung eines Untersuchungsreagenzes zur Untersuchung von Karzinomen und Adenomen, mit Ausnahme von kolorektalen Karzinomen und kolorektalen Adenomen, wobei das Untersuchungsreagenz als eine Hauptkomponente einen Antikörper umfasst, der spezifisch mit einem Antigen reagiert, welches Zuckerreste trägt, das in Zellen vorhanden ist, die mit dem HEC-GlcNAc6ST-Gen transduziert sind, und in Zellen, die mit dem GlcNAc6ST-1- oder dem GlcNAc6ST-Gen transduziert sind, fehlt oder nahezu nicht vorhanden ist.

9. Verwendung eines Untersuchungsreagenzes zur Untersuchung von Karzinomen und Adenomen, mit Ausnahme von kolorektalen Karzinomen und kolorektalen Adenomen, wobei das Untersuchungsreagenz als eine Hauptkomponente einen Antikörper umfasst, der spezifisch mit einem Antigen reagiert, welches Zuckerreste trägt, die in Geweben, Körperflüssigkeit oder Fäkalien von Patienten mit Karzinomen und Adenomen, mit Ausnahme von kolorektalen Karzinomen und kolorektalen Adenomen, vorhanden sind und durch die folgende allgemeine Formel:

Rl-Gal-βl-3/4(SO_3-6) GlcNAc-β1-R2

repräsentiert werden, wobei R1 für Zuckerreste steht, welche durch andere Enzyme hinzugefügt sind, und in seiner Struktur nicht eingeschränkt ist, Gal-β für β-Galaktose steht, GlcNAc β für β-N-Acetylglucosamin steht, Gal β1-3/4 für die Bindung von Gal-β an Position 1 und GlcNAc-β an Position 3 und/oder Position 4 steht, (SO_3-6) für die Addition von einer Sulfatgruppe an Position 6 von GlcNAc-β steht, R2 für -3GalNAc-α, -3Ga1-β oder -2Man-α steht und an die Position 1 von GlcNAc-β bindet.

Revendications

1. Procédé pour examiner des carcinomes et des adénomes, à l'exception des carcinomes colorectaux et des adénomes colorectaux, comprenant l'étape consistant à tester la présence ou l'absence, ou l'intensité de la réactivité d'un anticorps contre des tissus, un fluide corporel ou des matières fécales de patients, ou contre des extraits tirés de ceux-ci, dans lequel lesdits anticorps réagissent avec l'antigène qui est présent dans des cellules exprimant le gène HEC-GlcNAc6ST codant pour la GlcNAc-6-sulfotransférase et qui est absent, ou quasiment absent, de cellules exprimant le gène GlcNAc6ST-1 ou I-GlcNAc6ST.

2. Procédé selon la revendication 1, dans lequel ledit antigène est présent dans des cellules transduites avec le gène HEC-GlcNAc6ST et est absent, ou quasiment absent, de cellules transduites avec le gène GlcNAc6ST-1 ou avec le gène I-GlcNAc6ST.

3. Procédé selon la revendication 1 ou 2, dans lequel ledit antigène comprend les résidus de sucre exprimés par la formule suivante :

R1-Gal β1-3/4(SO_3-6) GlcNAc β1-R2

dans laquelle, le radical R1 représente des résidus de sucre ajoutés par d'autres enzymes et n'est pas limité en matière de structure, le résidu Gal β représente le β-galactose, le résidu GlcNAc β représente la β-N-acétylglucosamine, le terme Gal β1-3/4 représente la liaison entre la position 1 du résidu Gal β et la position 3 et/ou la position 4 du résidu GlcNAc β, le terme (SO_3-6) représente l'addition d'un groupement sulfate sur la position 6 du résidu GlcNAc β, le radical R2 représente un résidu -3GalNAc α, -3Gal β ou -2Mana et est lié à la position 1 du résidu GlcNAc β.

4. Procédé pour examiner des carcinomes et des adénomes, à l'exception des carcinomes colorectaux et des adénomes colorectaux, comprenant l'étape consistant à tester la réactivité de l'anticorps MECA-79 (Pharmingen, numéro de catalogue 09961D), ou son équivalent, contre des tissus, un fluide corporel, des matières fécales, ou un extrait tiré de ceux-ci, de patients à tester.

5. Procédé selon l'une quelconque des revendications 1 à 4, comprenant en outre l'étape consistant à faire réagir une sonde marquée avec ledit anticorps et à tester le marqueur au plan qualitatif ou quantitatif.

6. Procédé selon l'une quelconque des revendications 1 à 5, dans lequel ledit carcinome et ledit adénome peuvent

provoquer un carcinome épithélial et un adénome précancéreux, respectivement.

7. Utilisation d'un réactif d'examen pour examiner des carcinomes et des adénomes, à l'exception des carcinomes colorectaux et des adénomes colorectaux, le réactif d'examen comprenant, comme composant principal, un anticorps réagissant de manière spécifique avec un antigène portant des résidus de sucre, qui est présent dans des cellules exprimant le gène HEC-GlcNAc6ST et qui est absent, ou quasiment absent, de cellules exprimant le gène GlcNAc6ST-l ou GlcNAc6ST.

8. Utilisation d'un réactif d'examen pour examiner des carcinomes et des adénomes, à l'exception des carcinomes colorectaux et des adénomes colorectaux, le réactif d'examen comprenant, comme composant principal, un anticorps réagissant de manière spécifique avec un antigène portant des résidus de sucre, qui est présent dans des cellules transduites avec le gène HEC-GlcNAc6ST et qui est absent, ou quasiment absent, de cellules transduites avec le gène GlcNAc6ST-1 ou GlcNAc6ST.

9. Utilisation d'un réactif d'examen pour examiner des carcinomes et des adénomes, à l'exception des carcinomes colorectaux et des adénomes colorectaux, le réactif d'examen comprenant, comme composant principal, un anticorps réagissant de manière spécifique avec un antigène portant des résidus de sucre, qui sont présents dans des tissus, un fluide corporel ou des matière fécales de patients présentant des carcinomes et des adénomes, à l'exception du cancer colorectal et des adénomes colorectaux, et sont exprimés selon la formule générale suivante :

$$R1\text{-}Gal\ \beta 13/4\ (SO_3\text{-}6)\ GlcNAc\ \beta 1\text{-}R2$$

dans laquelle, le radical R1 représente des résidus de sucre ajoutés par d'autres enzymes et n'est pas limité en matière de structure, le résidu Gal β représente le β galactose, le résidu GlcNAc β représente la β N-acétylglucosamine, le terme Gal β1-3/4 représente la liaison entre la position 1 du résidu Gal β et la position 3 et/ou la position 4 du résidu GlcNAc β, le terme (SO_3-6) représente l'addition d'un groupement sulfate sur la position 6 du résidu GlcNAc β, le radical R2 représente un résidu -3GalNAc α, -3Gal β ou -2Manα et est lié sur la position 1 du résidu GlcNAc β.

Figure 1

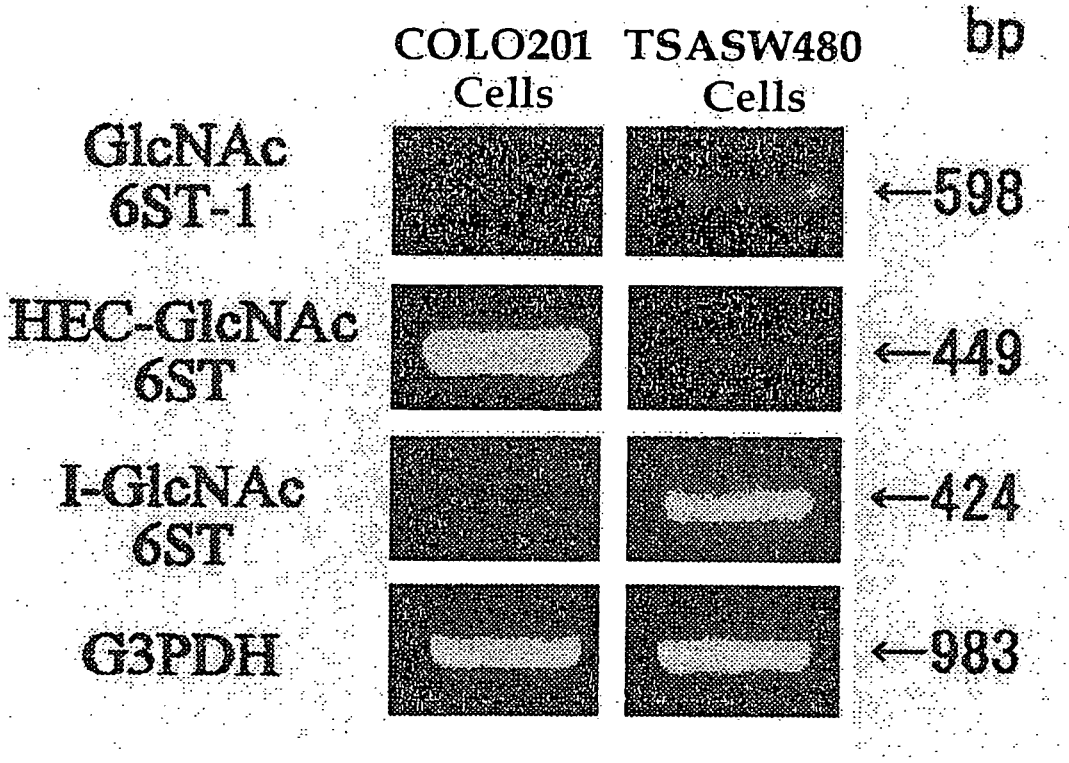
COLO201 Cells
TSASW480 Cells
bp
GlcNAc 6ST-1
←598
HEC-GlcNAc 6ST
←449
I-GlcNAc 6ST
←424
G3PDH
←983

Figure 2

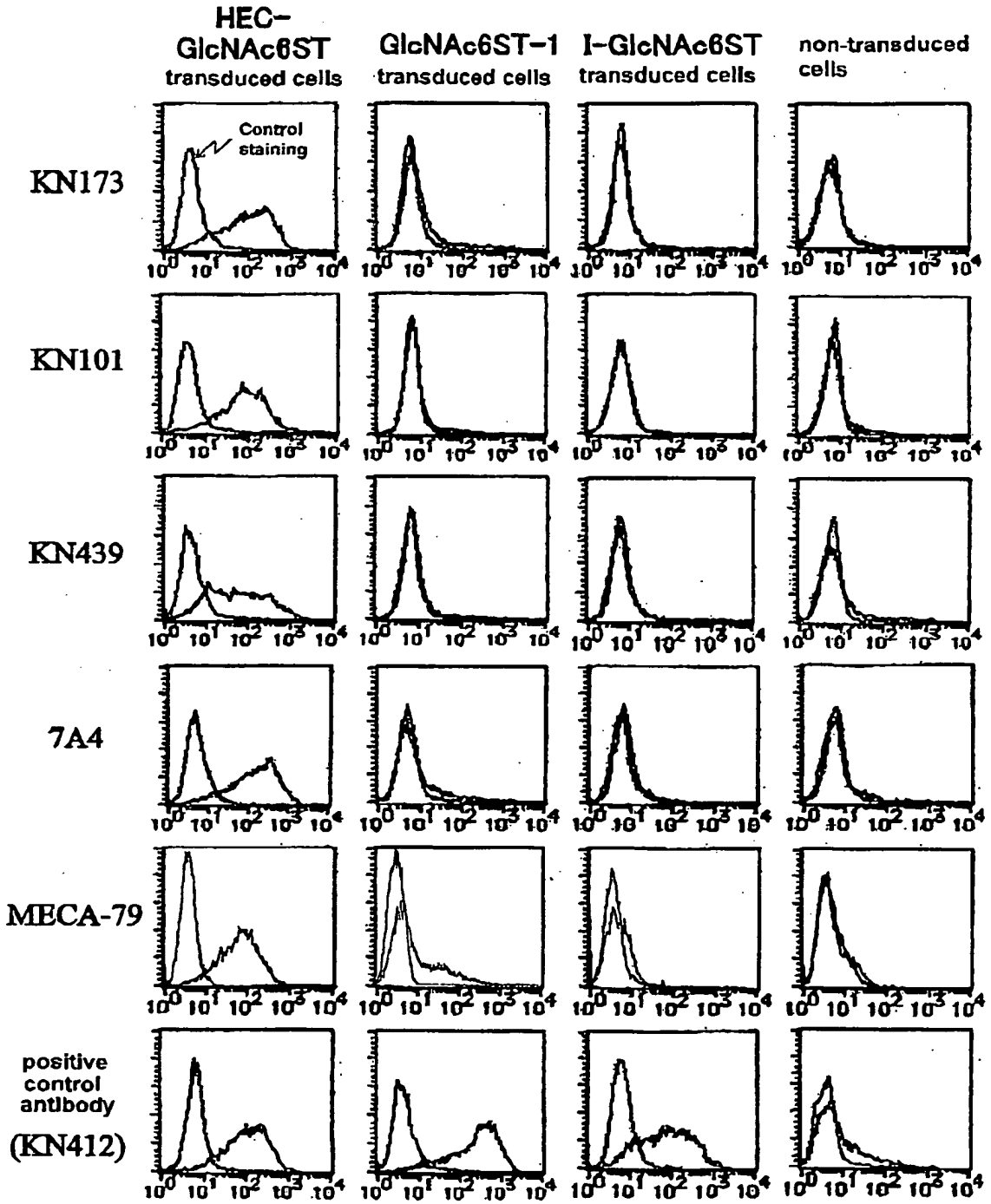
HEC-GlcNAc6ST transduced cells
GlcNAc6ST-1 transduced cells
I-GlcNAc6ST transduced cells
non-transduced cells
Control staining
KN173
KN101
KN439
7A4
MECA-79
positive control antibody (KN412)

Figure 3

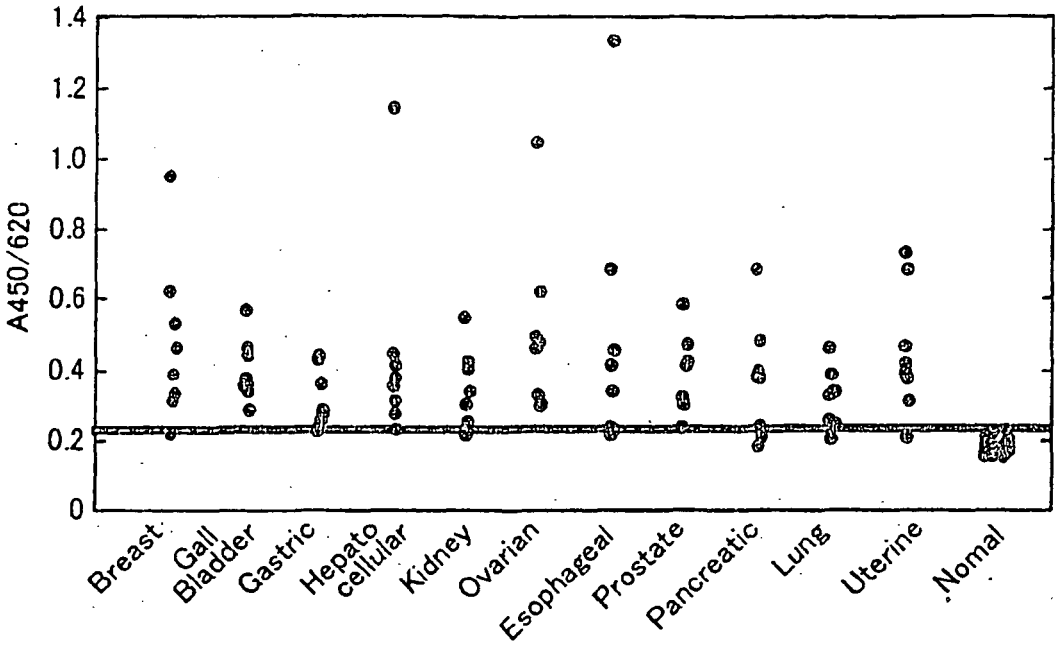
A450/620
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
Breast
Gall Bladder
Gastric
Hepato cellular
Kidney
Ovarian
Esophageal
Prostate
Pancreatic
Lung
Uterine
Nomal

Figure 4

BD PharMingen Technical Data Sheet

PURIFIED RAT ANTI-MOUSE PNAd CARBOHYDRATE EPITOPE (CD62L Ligand) MONOCLONAL ANTIBODY

PRODUCT INFORMATION

Catalog Number:	553863 (Was: 09961D), 0.5 mg
Description:	Purified anti-mouse PNAd Carbohydrate Epitope (CD62L Ligand)
Clone:	MECA-79
Immunogen:	Collagenase-dispersed BALB/c lymph node stroma[1]
Isotype:	Rat (Wistar) IgM, κ
Contents:	Purified immunoglobulin in 10 mM phosphate buffer, pH 7.2 with 500 mM NaCl and 0.09% (w/v) sodium azide.

SPECIFICITY

The MECA-79 antibody reacts with sulfate-dependent carbohydrate epitopes of peripheral lymph node addressin (PNAd).[2] The MECA-79–reactive antigen is closely associated with the carbohydrate ligands for L-selectin (*e.g.*, CD34, GlyCAM-1, MAdCAM-1), which are expressed on high endothelial venules (HEV) in lymphoid tissues and at sites of chronic inflammation.[1,2,3,4,5,6] Cross-reactivity with human,[3,4] ovine,[7] bovine,[7] primate,[7] and porcine[8] tissues has been observed. MECA-79 antibody inhibits L-selectin– dependent lymphocyte and platelet homing to lymph nodes *in vivo*[1,9] and *in vitro* adhesion to lymphoid tissue HEV[1,4] and immobilized PNAd.[3,9,10]

PREPARATION AND STORAGE

The antibody was purified from tissue culture supernatant by affinity chromatography. The antibody solution should be stored undiluted at 4°C.

USAGE

This antibody has been tested by immunohistochemical staining (IHC) of citrate-pretreated formalin-fixed paraffin-embedded sections (5 - 20 µg/ml) to assure specificity and reactivity. Other reported applications include IHC of acetone-fixed frozen sections,[1,4,5] immunoprecipitation[2,3] western blot analysis,[10] and *in vitro* and *in vivo* adhesion blocking.[1,3,4,9,10] Since applications vary, each investigator must determine dilutions appropriate for individual use.

Caution: Sodium azide is a reversible inhibitor of oxidative metabolism; therefore, antibody preparations containing this preservative agent must not be used in cell cultures nor injected into animals. Sodium azide may be removed by washing stained cells or plate-bound antibody or dialyzing soluble antibody in sodium azide-free buffer. Since endotoxin may also affect the results of functional studies, we recommend the NA/LE™ (No Azide/Low Endotoxin) antibody format for *in vitro* and *in vivo* use.

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Please see Page 2.

Figure 5

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