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(71) Applicant *(for all designated States except US)*: **ACADEMIA SINICA**; 128, Sec. 2, Academia Sinica Road, Nan-Kang, Taipei, 115 (TW).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **YU, Alice, L.** [US/—]; 1F., No. 8, Alley 2, Lane 61, Sec. 2, Academia Road, Nan-Kan, 115 Taipei (TW). **CHANG, Wen-Wei**; No. 9, Lane 159, Huatai Street, Kaohsiung County, 830 Fongshan City (TW). **YU, John** [US/—]; 1F., No. 8, Alley 2, Lane 61, Sec. 2, Academia Road Nan-Kang District, 115 Taipei City (TW). **WONG, Chi-Huey** [US/—]; 5F, No. 11, Alley 3, Lane 61, Sec 2, Academia Road, Nan-Kang, 115 Taipei (TW). **CHEN, I-Ju**; 2F., No. 7, Lane 20, Sec. 3, Academia, Road Nan-Kang District, 115 Taipei (TW). **LEE, Chien, Hsin**; No. 10, Lane 30, Hua-an Street, Taipei County, 235 Jhonghe City (TW).

(74) Agents: **TSAO, Rocky, Y.** et al.; Occhiuti Rohlicek & Tsao LLP, 10 Fawcett Street, Cambridge, MA 02138 (US).

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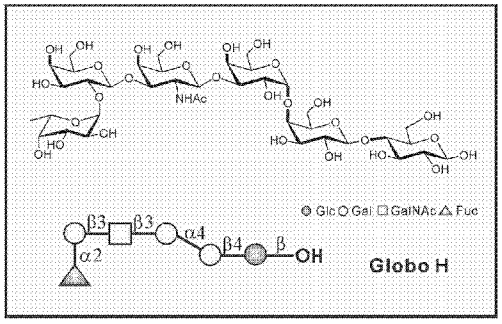
[Continued on next page]

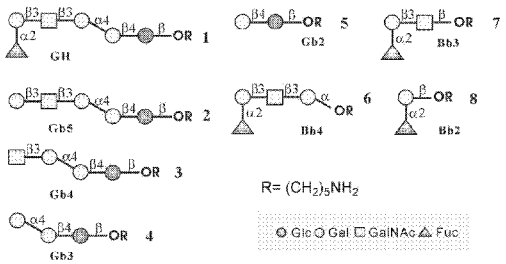
(54) Title: COMPOSITIONS FOR INDUCING IMMUNE RESPONSES SPECIFIC TO GLOBO H AND SSEA3 AND USES THEREOF IN CANCER TREATMENT

Fig. 1

A.



B.



(57) Abstract: An immune composition containing Globo H or its fragment (e.g., SSEA3) and an adjuvant, e.g., α -GalCer, for eliciting immune responses against Globo H or its fragment and uses thereof in cancer treatment. Also disclosed is a method of treating cancer by inhibiting the activity of one of *FUT1* and *FUT2*, both of which involve in Globo H biosynthesis.

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COMPOSITIONS FOR INDUCING IMMUNE RESPONSES SPECIFIC TO GLOBO H AND SSEA3 AND USES THEREOF IN CANCER TREATMENT

RELATED APPLICATION

This application claims priority to U.S. Provisional Application No. 61/061,968, filed on June 16, 2008, the content of which is hereby incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

Globo H is a cancer antigen overly expressed in various epithelial cancers. It has been suggested that this antigen can serve as a target in cancer immunotherapy. While vaccines have been developed to elicit antibody responses against Globo H, their anti-cancer efficacies are unsatisfactory due to low antigenecity of Globo H. There is a need for a new vaccine capable of eliciting high levels of immune responses targeting Globo H.

SUMMARY OF THE INVENTION

The present invention is based on an unexpected discoveries that (1) SSEA3, the immediate precursor of Globo H, is expressed at a high level in breast cancer stem cells and therefore can serve as a suitable target for breast cancer treatment, and (2) α-galactosyl-ceramide (α-GalCer) is an effective adjuvant that promotes production of anti-Globo H and anti-SSEA3 antibodies.

Accordingly, one aspect of this invention features an immune composition containing Globo H or its fragment (e.g., SSEA3) and an adjuvant (e.g., α-GalCer). Globo H or its fragment can be conjugated with Keyhole Limpet Hemocyanin (KLH). When administered into a subject (e.g., a human), this immune composition elicits immune responses (e.g., antibody production) targeting Globo H or its fragment and, therefore, is effective in treating cancer (e.g., breast cancer, prostate cancer, ovarian cancer, and lung cancer).

Another aspect of this invention relates to a method of producing antibody specific to Globo H or its fragment by administering to a non-human mammal (e.g., mouse, rabbit, goat, sheep, or horse) the immune composition described above and

isolating from the mammal antibody that binds to Globo H or its fragment.

In yet another aspect, this invention features a method of treating cancer with a first agent that inhibits the activity of 2-fucosyltransferase 1 (*FUT1*) or 2-fucosyltransferase 2 (*FUT2*). Both *FUT1* and *FUT2* are involved in Globo H biosynthesis. This agent can be an antibody that blocks the interaction between *FUT1*/*FUT2* and its substrate or an interfering RNA (e.g., si*FUT1* or si*FUT2)* that suppresses expression of *FUT1* or *FUT2*. Optionally, the first agent, targeting *FUT1*, can be combined with a second agent that inhibits the activity of *FUT2*. In one example, the first agent is si*FUT1* and the second agent is si*FUT2*.

Also within the scope of this invention is use of the immune composition or the first and second agents in treating cancer and in manufacturing a medicament for the treatment of cancer.

The details of one or more embodiments of the invention are set forth in the description below. Other features or advantages of the present invention will be apparent from the following drawings and detailed description of several examples, and also from the appended claims.

BREIF DESCRIPTION OF THE DARAWINGS

The drawings are first described.

Fig.1 is a diagram depicting the structures of the hexasaccharide epitope in Glogo H (GH) and fragments of this epitope. Panel A: the structure of the hexasaccharide epitope. Panel B: the structures of the hexasaccharide epitopes and its seven fragments.

Fig. 2 is a chart showing the levels of anti-Globo H and anti-SSEA3 antibodies in mice immunized with KLH-conjugated Globo H alone and with KLH-conjugated Globo H together with α-GalCer.

Fig. 3 is a diagram showing the effects of si*FUT1* and si*FUT2* on *FUT1* and *FUT2* expression in breast cancer cells. Panel A: suppression of *FUT1* expression by si*FUT1* in MB157 cells. Panel B: suppression of *FUT1* and *FUT2* expression by si*FUT1* and si*FUT2,* respectively, in T-47D cells.

Fig. 4 is a diagram showing the effects of si*FUT1* and si*FUT2* on inhibiting growth of breast cancer xenografts. Panel a: a chart showing that si*FUT1* and si*FUT2* inhibited breast tumor growth. Panel b: a chart showing that si*FUT1* and si*FUT2* reduced weights of tumor mass.

DETAILED DEESCRIPTION OF THE INVENTION

We have discovered that Globo H and its immediate precursor SSEA3 both can serve as targets in cancer treatment.

Accordingly, one embodiment of this invention is a method of treating cancer by administering to a subject in need thereof an effective amount of an immune composition containing either Globo H or a fragment thereof (e.g., SSEA3, also known as Gb5) and an adjuvant. The types of target cancer include, but are not limited to, breast cancer (including stages 1-4), lung cancer (e.g., small cell lung cancer), liver cancer (e.g., hepatocellular carcinoma and cohlagiocarcinoma), oral cancer, stomach cancer (including T1-T4), colon cancer, nasopharynx cancer, skin cancer, kidney cancer, brain tumor (e.g., astrocytoma, glioblastoma multiforme, and meningioma), prostate cancer, ovarian cancer, cervical cancer, bladder cancer, and endometrium, rhabdomyosarcoma, osteosarcoma, leiomyosarcoma, and gastrointestinal stromal tumor. The term "treating" as used herein refers to the application or administration of a composition including one or more active agents to a subject, who has cancer, a symptom of cancer, or a predisposition toward cancer, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the cancer, the symptoms of the cancer, or the predisposition toward the cancer. "An effective amount" as used herein refers to the amount of each active agent required to confer therapeutic effect on the subject, either alone or in combination with one or more other active agents. Effective amounts vary, as recognized by those skilled in the art, depending on route of administration, excipient usage, and co-usage with other active agents.

The immune composition used in the above-described method can contain a glycan (i.e., a molecule containing a sugar moiety) that is Globo H or a fragment thereof and an adjuvant. Globo H is a glycan containing the hexasaccharide epitope

shown in Fig. 1, Panel A, and optionally, a non-sugar moiety. Its fragment is a glycan containing a fragment of the hexasaccharide epitope and, if applicable, the non-sugar moiety. Fragments of the hexasaccharide epitope are shown in Fig. 1, Panel B. These oligosaccharides can be prepared by routine methods. See, e.g., Huang et al., *Proc. Natl. Acad. Sci. USA* 103:15-20 (2006). If desired, they can be linked to a non-sugar moiety.

Any of the glycans described above can be conjugated to a protein carrier, such as KLH.

They can then be mixed with an adjuvant and optionally a pharmaceutically acceptable carrier (e.g., a phosphate buffered saline, or a bicarbonate solution) to form an immune composition (e.g., a vaccine) via conventional methods. See, e.g., U.S. Patents. 4,601,903; 4,599,231; 4,599,230; and 4,596,792. The composition may be prepared as injectables, as liquid solutions, or emulsions and the carrier is selected on the basis of the mode and route of administration, as well as on the basis of standard pharmaceutical practice. Suitable pharmaceutical carriers and diluents, and pharmaceutical necessities for their use, are described in Remington's Pharmaceutical Sciences. The immune composition preferably contains α-GalCer as an adjuvant. Other examples of adjuvant include, but are not limited to, a cholera toxin, Escherichia coli heat-labile enterotoxin (LT), liposome, immune-stimulating complex (ISCOM), or immunostimulatory sequences oligodeoxynucleotides (ISS-ODN). The composition can also include a polymer that facilitates in vivo delivery. See Audran R. et al. Vaccine 21:1250-5, 2003; and Denis-Mize et al. Cell Immunol., 225:12-20, 2003. When necessary, it can further contain minor amounts of auxiliary substances such as wetting or emulsifying agents, or pH buffering agents to enhance the ability of the composition to elicit immune responses against the sugar moiety in Globo H or its fragment.

The immune composition described herein can be administered parenterally (e.g., intravenous injection, subcutaneous injection or intramuscular injection). Alternatively, other modes of administration including suppositories and oral formulations may be desirable. For suppositories, binders and carriers may include, for example, polyalkalene glycols or triglycerides. Oral formulations may include

normally employed incipients such as, for example, pharmaceutical grades of saccharine, cellulose, magnesium carbonate and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain 10-95% of the immune composition described herein.

The immune composition is administered in a manner compatible with the dosage formulation, and in an amount that is therapeutically effective, protective and immunogenic. The quantity to be administered depends on the subject to be treated, including, for example, the capacity of the individual's immune system to synthesize antibodies, and if needed, to produce a cell-mediated immune response. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner. However, suitable dosage ranges are readily determinable by one skilled in the art. Suitable regimes for initial administration and booster doses are also variable, but may include an initial administration followed by subsequent administrations. The dosage of the vaccine may also depend on the route of administration and varies according to the size of the host.

The immune composition of this invention can also be used to generate antibodies in animals for production of antibodies, which can be used in both cancer treatment and diagnosis. Methods of making monoclonal and polyclonal antibodies and fragments thereof in animals (e.g., mouse, rabbit, goat, sheept, or horse) are well known in the art. See, for example, Harlow and Lane, (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York. The term "antibody" includes intact immunoglobulin molecules as well as fragments thereof, such as Fab, $F(ab')_2$, Fv, scFv (single chain antibody), and dAb (domain antibody; Ward, et. al. (1989) Nature, 341, 544).

Another embodiment of this invention is a method of treating cancer by inhibiting the activity of *FUT1* and/or *FUT2*, both being responsible for Globo H biosynthesis. *FUT1* and *FUT2* are well-known 2-fucosyltransferases that transfer a fucose unit to the reducing end of an oligosaccharide substrate via an α1,2 linkage. See, e.g., NCBI Gene ID:2523 and NCBI Gene ID:2524.

In one example, the just-described method is performed by administering to a subject in need thereof an effective amount of an antibody that interferes with the interaction between *FUT1/FUT2* and their substrate, i.e., an antibody specific to *FUT1/FUT2* or their substrate.

In general, to produce such an antibody, *FUT1/FUT2*, a fragment thereof, or a substrate thereof can be coupled to a carrier protein (e.g., KLH), if necessary, mixed with an adjuvant, and then injected into a host animal. Antibodies produced in the animal can then be purified by conventional methods, e.g., affinity chromatography. Commonly employed host animals include rabbits, mice, guinea pigs, and rats. Various adjuvants that can be used to increase the immunological response depend on the host species and include Freund's adjuvant (complete and incomplete), mineral gels such as aluminum hydroxide, CpG, surface-active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. Useful human adjuvants include BCG (bacille Calmette-Guerin) and Corynebacterium parvum.

Polyclonal antibodies, heterogeneous populations of antibody molecules, are present in the sera of the immunized subjects. Monoclonal antibodies, homogeneous populations of antibodies to *FUT1/FUT2* or their substrate, can be prepared using standard hybridoma technology (see, for example, Kohler et al. (1975) Nature 256, 495; Kohler et al. (1976) Eur. J. Immunol. 6, 511; Kohler et al. (1976) Eur J Immunol 6, 292; and Hammerling et al. (1981) Monoclonal Antibodies and T Cell Hybridomas, Elsevier, N.Y.). In particular, monoclonal antibodies can be obtained by any technique that provides for the production of antibody molecules by continuous cell lines in culture such as described in Kohler et al. (1975) Nature 256, 495 and U.S. Patent No. 4,376,110; the human B-cell hybridoma technique (Kosbor et al. (1983) Immunol Today 4, 72; Cole et al. (1983) Proc. Natl. Acad. Sci. USA 80, 2026, and the EBV-hybridoma technique (Cole et al. (1983) Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Such antibodies can be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD, and any subclass thereof. The hybridoma producing the monoclonal antibodies of the invention may be cultivated in vitro or in vivo. The ability to produce high titers of monoclonal

antibodies in vivo makes it a particularly useful method of production.

In addition, techniques developed for the production of "chimeric antibodies" can be used. See, e.g., Morrison et al. (1984) Proc. Natl. Acad. Sci. USA 81, 6851; Neuberger et al. (1984) Nature 312, 604; and Takeda et al. (1984) Nature 314:452. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Alternatively, techniques described for the production of single chain antibodies (U.S. Patent Nos. 4,946,778 and 4,704,692) can be adapted to produce a phage library of single chain Fv antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge. Moreover, antibody fragments can be generated by known techniques. For example, such fragments include, but are not limited to, $F(ab')_2$ fragments that can be produced by pepsin digestion of an antibody molecule, and Fab fragments that can be generated by reducing the disulfide bridges of $F(ab')_2$ fragments. Antibodies can also be humanized by methods known in the art. For example, monoclonal antibodies with a desired binding specificity can be commercially humanized (Scotgene, Scotland; and Oxford Molecular, Palo Alto, Calif.). Fully human antibodies, such as those expressed in transgenic animals are also features of the invention. See, e.g., Green et al. (1994) Nature Genetics 7, 13; and U.S. Patent Nos. 5,545,806 and 5,569,825.

In another example, the above-described method can be performed by administering to a subject in need of cancer treatment an effective amount of one or more double-strand RNAs (dsRNAs) that inhibit the expression of *FUT1* and/or *FUT2* via RNA interference, thereby reducing the level of Globo H. RNA interference (RNAi) is a process in which a dsRNA directs homologous sequence-specific degradation of messenger RNA. In mammalian cells, RNAi can be triggered by 21-nucleotide duplexes of small interfering RNA (siRNA) without activating the host interferon response.

A dsRNA can be synthesized by methods known in the art. See, e.g., Caruthers et al., 1992, Methods in Enzymology 211, 3-19, Wincott et al., 1995, Nucleic Acids Res. 23, 2677-2684, Wincott et al., 1997, Methods Mol. Bio. 74, 59,

Brennan et al., 1998, Biotechnol Bioeng., 61, 33-45, and Brennan, U.S. Pat. No. 6,001,311. It can also be transcribed from an expression vector and isolated using standard techniques.

The dsRNA or vector as described above can be delivered to cancer cells by methods, such as that described in Akhtar et al., 1992, Trends Cell Bio. 2, 139. For example, it can be introduced into cells using liposomes, hydrogels, cyclodextrins, biodegradable nanocapsules, or bioadhesive microspheres. Alternatively, the dsRNA or vector can be locally delivered by direct injection or by use of an infusion pump. Other approaches include employing various transport and carrier systems, for example through the use of conjugates and biodegradable polymers

As an example, the above-described dsRNA contains a first strand that is complementary to CGCGGACTTGAGAGATCCTTT, or the complement thereof (e.g., si*FUT1* described in Example 2 below). In another example, the dsRNA contains a first strand that is complementary to CTATGTCCATGTCATGCCAAA, or the complement thereof (e.g., si*FUT2* described in Example 2 below).

To facilitate delivery, the dsRNA mentioned above or a DNA plasmid expressing it can be conjugated with a chaperone agent. As used herein, "conjugated" means two entities are associated, preferably with sufficient affinity that the therapeutic benefit of the association between the two entities is realized. Conjugated includes covalent or noncovalent bonding as well as other forms of association, such as entrapment of one entitity on or within the other, or of either or both entities on or within a third entity (e.g., a micelle).

The chaperone agent can be a naturally occurring substance, such as a protein (e.g., human serum albumin, low-density lipoprotein, or globulin), carbohydrate (e.g., a dextran, pullulan, chitin, chitosan, inulin, cyclodextrin or hyaluronic acid), or lipid. It can also be a recombinant or synthetic molecule, such as a synthetic polyamino acid polymer (e.g., polylysine, poly L-aspartic acid, poly L-glutamic acid, styrene-maleic acid anhydride copolymer, poly(L-lactide-co-glycolied) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer, polyethylene glycol, polyvinyl alcohol, polyurethane, poly(2-ethylacryllic acid), N-isopropylacrylamide polymers, and polyphosphazine). Alternatively, the

chaperone agent is a micelle, liposome, nanoparticle, or microsphere, in which the dsRNA or the DNA plasmid is encapsulated.

In one instance, a chaperone agent serves as a substrate for attachment of one or more of a fusogenic agent, a condensing agent, or a targeting agent.

A fusogenic agent is responsive to the local pH. For instance, upon encountering the pH within an endosome, it can cause a physical change in its immediate environment (e.g., a change in osmotic properties, which disrupts or increases the permeability of the endosome membrane), thereby facilitating release of a dsRNA or DNA plasmid into host cell's cytoplasm. A preferred fusogenic agent changes charge, for example, becoming protonated at a pH lower than a physiological range (e.g., at pH 4.5-6.5). Fusogenic agents can be molecules containing an amino group capable of undergoing a change of charge (e.g., protonation) when exposed to a specific pH range. Such fusogenic agents include polymers that contain polyamino chains (e.g., polyethyleneimine) and membrane disruptive agents (e.g., mellittin). Other examples include polyhistidine, polyimidazole, polypyridine, polypropyleneimine, mellitin, and a polyacetal substance (e.g., a cationic polyacetal).

A condensing agent interacts with (e.g., attracts, holds, or binds to) the dsRNA or the DNA plasmid and causes it to condense (e.g., reducing the size of the dsRNA/plasmid), thus protecting the dsRNA/plasmid against degradation. Preferably, the condensing agent includes a moiety (e.g., a charged moiety) that interacts with the dsRNA or the DNA plasmid via, e.g., ionic interactions. Examples of the condensing agent include a polylysine, spermine, spermidine, polyamine or quarternary salt thereof, pseudopeptide-polyamine, peptidomimetic polyamine, dendrimer polyamine, arginine, amidine, protamine, cationic lipid, cationic porphyrin, and an alpha helical peptide.

Without further elaboration, it is believed that one skilled in the art can, based on the above description, utilize the present invention to its fullest extent. The following specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. All publications cited herein are incorporated by reference.

Example 1: Induction of Antibodies Specific to Globo H and SSEA3 with

KLH-conjugated Globo H and α-Galcer.

Globo H-KLH was purchased from Optimer Pharmaceuticals. Three groups of 6-week-old female BALB/b mice (BioLASCO), two in each group, were injected (s.c.) with PBS ("control mice"), 0.6 μg KLH-Globo H ("Globo H mice"), and 0.6 μg KLH-Globo H in combination with 2 μg α-GalCer ("Globo H-GalCer mice"), respectively, once every week for three weeks. Sera were collected from the mice of each group 10 days after the last injection and antibodies specific to Globo H and SSEA3 were detected following the method described in Huang et al., *Proc, Natl. Acad. Sci. USA* 103:15-20 (2006). Briefly, the sera were diluted 1:25 with 3% BSA/PBS buffer and 50 ml of each diluted serum were incubated with a slide, to which Globo H and SSEA3 were attached, in a humidifying chamber for 1 hour. The slide was washed three times with 0.05% PBS/Tween 20 (PBST) and subsequently incubated with 100 μl Cy5-conjugated goat anti-mouse IgG antibody(1:200) in the same chamber. After being air-dried, the slide was washed with PBST and water, each for three times, and then measured for the levels of fluorescence released thereby with a microarray scanner (GenePix 4000B; Molecular Devices). The results thus obtained were analyzed with the GenePix Pro software.

In the Globo H mice, only a low level of anti-Globo H IgG antibody was detected and the level of anti-SSEA IgG antibody was undetectable. See Fig. 2. Differently, the Globo H-GalCer mice showed high levels of both anti-Globo H and anti-SSEA3 IgG antibodies. See also Fig. 2. These findings indicated that Globo H-KLH in combination with α-GalCer is an effective vaccine for inducing both anti-Globo H and anti-SSEA3 antibodies.

Example 2: Inhibition of *FUT1* and *FUT2* via RNA Interference Reduced Levels of Globo H

The levels of *FUT1* and *FUT2* mRNAs in three breast cancer cell lines, MCF-7, MB157, and T-47D, were determined by quantitative RT-PCR as follows. Total RNAs were extracted from these cancer cells and cDNAs were produced via reverse transcription using the RNAs as the template and oligo(dT) as the primer. Fifty nanograms of the cDNAs were subjected to RT-PCR using the following

primers: L-fut1: CCTGCCAGACTCTGAGTTCC and AGGCTTAGCCAATGTCCAGA as well as L-fut2: GGGAGTTACCGGTGCAGATA and R-fut2: GTCCCAGTGCCTTTGATGTT. The RT-PCR reaction was carried out under the following conditions: 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 10 sec and 60 °C for 1 min, using an ABI Prism 7000 Sequence Detection System and the results thus obtained were analyzed using the ABI Prism 7000 SDS software (Applied Biosystems) to obtain a threshold cycle number (Ct value) for the mRNA levels of *FUT1* and *FUT2* in each cell line. The Ct value was normalized against the mRNA level of HPRT1 in the same cell line to obtain a ΔCt value. The ΔCt value of *FUT1* in MCF-7 was used to normalize the ΔCt value of either *FUT1* or *FUT2* in each cell-line. The fold-change of a mRNA level was calculated based on the following formula: $2^{-[\Delta Ct(\text{target gene})-\Delta Ct(FUT1 \text{ in MCF-7})]}$. The mRNA levels of HPRT1 and GAGDH were used as internal controls.

No significant difference in the mRNA levels of *FUT1* was found among the three breast cancer cell lines. On the other hand, *FUT2* mRNAs were barely detectable in MCF-7 and MB157 cells, while in the T47D cells, the level of *FUT2* mRNA was 6000-fold greater than that in the other two cell lines.

The levels of *FUT1* or *FUT2* were reduced via RNA interference as follows. Nucleotide sequences encoding si*FUT1* (containing a sequence complementary to CGCGGACTTGAGAGATCCTTT) and si*FUT2* (containing a sequence complementary to CTA TGTCCATGTCATGCCAAA) were cloned into a VSV-G-pseudotype lentiviral vector and introduced into 293T cells together with packaging plasmids pMD.G and pCMVΔR8.91. Lentiviral particles thus produced were harvested at 48 and 72 hours after transfection and concentrated by ultracentrifugation (25,000 rpm, 90 minutes). These virus particles, capable of expressing si*FUT1* or si*FUT2*, were incubated with ThT-47D or MB157 (plated at $2x10^5$ cells /well in 6-well plates) in the presence of 8 μg/mL polybrene (Sigma-Aldrich Corp.). The cells were harvested 96 hours later and the mRNA levels of *FUT1* and *FUT2* were determined by quantitative RT-PCR as described above.

As shown in Fig. 3, si*FUT1* successfully reduced the level of *FUT1* mRNA in MB157 cells (see panel A). Similarly, si*FUT1* and si*FUT2* reduced the levels of *FUT1* and *FUT2* mRNAs, respectively, in T-47D cells. See Fig. 3, panel B.

The levels of Globo H in both the MB157 and the T-47D cells were determined via flow cytometry using the AlexaFluor488-VK-9 antibody as follows. Aliquots of cells, each containing $1x10^5$ cells, were incubated first with anti-GloboH-Alexa488 (Vk9; see Chang et al., *Proc. Natl. Acad. Sci. USA* 105:11667-11672 (2008) for 1 hour on ice, then with biotinylated-UEA1 (Vector Labotories) for one hour on ice, and finally with FITC-conjugated streptavidin (Jackson ImmunoResearch) for I hour on ice. The cells were then subjected to flow cytometry using a FACSCanto flow cytometer and the data thus obtained were analyzed by the CellQuest program (BD Biosciences). Results obtained from this study show that suppression of *FUT1* expression via RNA interference in MB157 cells resulted in a decreased level of Globo H and suppression of *FUT2* expression resulted in a decreased level of Globo H in T-47D cells.

Example 3: Anti-Cancer Effects of Si*FUT1* and Si*FUT2*

Inhibiting Cancer Cell Growth

Breast cancer cells MB157 and T-47D were seeded at 1 x 10^4 cells per well in a 96-well plate (Corning). They were then mixed with or without the virus particles described in Example 2 above that express si*FUT1* or si*FUT2* and centrifuged at 300g for 5 min for spin infection. 24 hours later, alamar blue (AbD Serotec) was added to the cells at a final concentration of 1:10 dilution and the cells were then cultured at 37 °C, 5%CO_2 for 3 hr. Subsequently, the absorbance at 544 nm and 590 nm was measured with a SpectraMax M2 Reader. The cells were cultured under the same conditions with fresh medium and the absorbance at 544 nm and 590 nm was again measured at 48, 72, and 96 hr after the initial seeding process. Results obtained from this study show that both the MB157 and T-47D cells infected with the virus particles decreased their growth rate as compared with the non-infected

cells. These data demonstrate that suppressing *FUT1* or *FUT2* expression by

siRNAs resulted in inhibition of cancer cell growth.

Inhibiting Mammosphere Formation

MB157 and T-47D cells, infected with virus particles expressing si*FUT1* or si*FUT2*, were suspended in DMEM/F12 medium supplemented with 0.4% BSA, 20ng/ml EGF, 20ng/ml bFGF, 5ug/ml insulin, 1μM hydrocortisone, 4μg/ml heparin, 1x B27 supplement, and 1% methyl cellulose (Sigma–Aldrich)at a density of 1,000 cells/ml. The suspended cells were then seeded on ultra low attachment plates (Costar) and cultured under suitable conditions to allow mammosphere formation. The primary mammosphere thus formed were Cultures were fed weekly. For secondary mammosphere culture, primary mammospheres were harvested, dispersed with trypsin (Gibco), pelleted, suspended in the culture medium described above at 1,000 cells/ml. The suspended cells were then cultured following the method described above to allow formation of secondary mammosphere. The numbers of mammopheres formed by both MB157 and T-47D cells expressing si*FUT1* were only 50% of that of the mammopheres formed by non-infected cells, indicating that si*FUT1* significantly reduced the mammophere formation capacity of the cancer cells. Similarly, the number of the mammopheres formed by the T-47D cells expressing si*FUT2* was only 17% of that of the mammopheres formed by non-infected cells. This result shows that, like si*FUT1*, si*FUT2* also significantly reduced the mammophere formation capacity of cancer cells.

Reducing Tumorigenecity

Six-week-old balb/c nude mice and NOD/SCID mice were injected with 17-β-estradiol (1.7mg/ml) subcutaneously at the lateral side of each mouse. 8-week-old, female balb/c nude mice were injected at their mammory fat pad with (i) MB157 cells ($1x10^7$) stably expressing siFUT, (ii) vehicle control MB157 cells ($1x10^7$) , (iii) T-47D cells ($5x10^6$) stably expressing si*FUT1*, (iv) T-7D cells ($5x10^6$) stably expressing si*FUT2*, and (v) vehicle control T-47D cells ($5x10^6$), all suspended in 0.1ml of 50% Matrigel (BD Biosciences) and 50% supplemented RPMI-1640 medium. The sizes of the tumors formed in these treated mice were regularly monitored at various time points by measuring the length (l) and width (w). Tumor volumes were calculated as follows: $V = \pi/6 \times l \times w \times [l + w]/2$. The animals were

finally sacrificed and the tumors were excised and weighed.

As shown in Fig. 4, Panel a, the tumors in the mice treated with the vehicle controls kept on growing over time, while in the mice treating with cells expressing si*FUT1* or si*FUT2*, the tumor growth rate was significantly reduced. The weights of the tumor mass in these mice were also significantly lower than those in the mice treated with the vehicle controls. See Fig. 4, Panel b.

Changing of Cell Morphology and Adhesion Ability

While regular T-47D cells were in square-like shape, T-47D cells expressing both si*FUT1* and si*FUT2* were small, round-shaped cells forming dense clusters. Similar morphology changes were observed in MB-157 cells expressing si*FUT1*.

Cell adhesion was determined using the RT-CES apparatus (Real Time Cell Electronic Sensing, ACEABIO). Briefly, ACEA's 96 microtiter plates were coated with fibronectin (25ug/ml, Sigma), type IV collagen (2ug/ml, BD biosciences), or laminin (5ug/ml, Sigma), all being diluted at appropriate folds in PBS, at 37 °C for 1 hr and then blocked with 1% BSA for 1 h at 37 °C. MB157 and T-47D cells were seeded at 2.5 x10^4 per 100μl of culture medium in the coated ACEA's 96 microtiter plates. Cell adhesion was monitored every 10 min in a period of 1 hour using the RT-CES. Globo-H ceramide (50μg/ml in serum-free medium) were added to certain cells to examine whether it counteracted the effects of si*FUT1* and si*FUT2*. Results indicate that si*FUT1* and si*FUT2* reduced cancer cell adhesion to polystyrene by 0.63 fold as compared with cells not expressing either siRNA and that Globo-H ceramide rescued the adhesion inhibition caused by the siRNAs.

Suppressing Cell Migration

The migration capacity of cells expressing si*FUT1* or si*FUT2* was first examined in a wound healing assay as follows. MB157 and T-47D cells were plated in a 12-well plate in a serum-containing medium until they reached 60% confluence. The cells were then infected with the virus particles described in Example 1 above or introduced with a control plasmid. These cells reached 100% confluence in a 2-day culture period. The cells were then starved overnight and confluent monolayers of the cells were wounded with a 20ul plastic pipette tip sharply. After being washed

with RPMI medium supplemented with serum, the cells were incubated in the RPMI medium and examined using a time-lapse microscope with temperature and CO_2 controls. Phase-contrast images of the cells were acquired every 4 h for 3 days or every 2h for 1 day. The rate of cell migration was determined using Metamorph software that measures the distance that the cells have traveled during a desired time period. Results indicate that si*FUT1* suppressed migration of T-47D cells and MB158 cells by 2.81 and 2.13, respectively, as compared with the cells transfected with the control plasmid. Exogenous addition of Globo H-ceramide rescued the reduced migration capacity of cells expressing si*FUT1* or si*FUT2*. This data indicates that the observed migration reduction was caused by the decreased level of Globo H resulting from suppression of *FUT1* and *FUT2* expression via RNA interference.

Taken together, the above results demonstrate that si*FUT1* and si*FUT2* are effective in treating cancer by suppressing *FUT1* and *FUT2* expression and consequently, reducing the level of Globo H.

Other Embodiments

All of the features disclosed in this specification may be combined in any combination. Each feature disclosed in this specification may be replaced by an alternative feature serving the same, equivalent, or similar purpose. Thus, unless expressly stated otherwise, each feature disclosed is only an example of a generic series of equivalent or similar features.

From the above description, one skilled in the art can easily ascertain the essential characteristics of the present invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, other embodiments are also within the claims.

What is claimed is:

1. An immune composition comprising a glycan and α-galactosyl-ceramide (α-GalCer), wherein the glycan is Globo H or a fragment thereof.

2. The immune composition of claim 1, wherein the glycan is Globo H.

3. The immune composition of claim 1, wherein the glycan is SSEA3.

4. The immune composition of claim 1, wherein the glycan or its fragment is conjugated with Keyhole Limpet Hemocyanin (KLH).

5. An immune composition comprising SSEA3 and an adjuvant.

6. A method of treating cancer, comprising administering to a subject in need thereof an effective amount of the immune composition of claim 1.

7. The method of claim 6, wherein the cancer is selected from the group consisting of breast cancer, prostate cancer, ovarian cancer, and lung cancer.

8. The method of claim 7, wherein the cancer is breast cancer.

9. The method of claim 6, wherein the glycan is Globo H and the immune composition elicits an immune response against Globo H.

10. The method of claim 6, wherein the glycan is SSEA3 and the immune composition elicits an immune response against SSEA3.

11. A method of treating cancer, comprising administering to a subject in need thereof an effective amount of the immune composition of claim 5.

12. The method of claim 11, wherein the cancer is selected from the group consisting of breast cancer, lung cancer, liver cancer, oral cancer, stomach cancer, colon cancer, nasopharynx cancer, skin cancer, kidney cancer, brain tumor, prostate cancer, ovarian cancer, cervical cancer, and bladder cancer.

13. The method of claim 12, wherein the cancer is breast cancer.

14. A method of producing an antibody specific to Globo H or its fragment, comprising

administering to a non-human mammal an effective amount of a composition containing Globo H or a fragment thereof and α-galactosyl-ceramide (α-GalCer) to induce production of an antibody that binds to Globo H or its fragment, and

isolating the antibody.

15. The method of claim 14, wherein the composition contains Globo H.

16. The method of claim 14, wherein the composition contains SSEA3.

17. A method of treating cancer, comprising administering to a subject in need thereof an effective amount of a first agent that inhibits the activity of 2-fucosyltransferase 1 (*FUT1*) or 2-fucosyltransferase 2 (*FUT2*), wherein the agent is an antibody that blocks interaction between *FUT1* or *FUT2* and its substrate or an interfering RNA that suppresses expression of *FUT1* or *FUT2*.

18. The method of claim 17, wherein the first agent is a small interfering RNA (siRNA).

19. The method of claim 18, wherein the siRNA is si*FUT1* or si*FUT2*.

20. The method of claim 17, wherein the first agent inhibits the activity of *FUT1* and the subject is further administered with an effective amount of a second

agent that inhibits the activity of *FUT2*, the second agent being an antibody that blocks interaction between *FUT2* and its substrate or an interfering RNA that suppresses expression of *FUT2*.

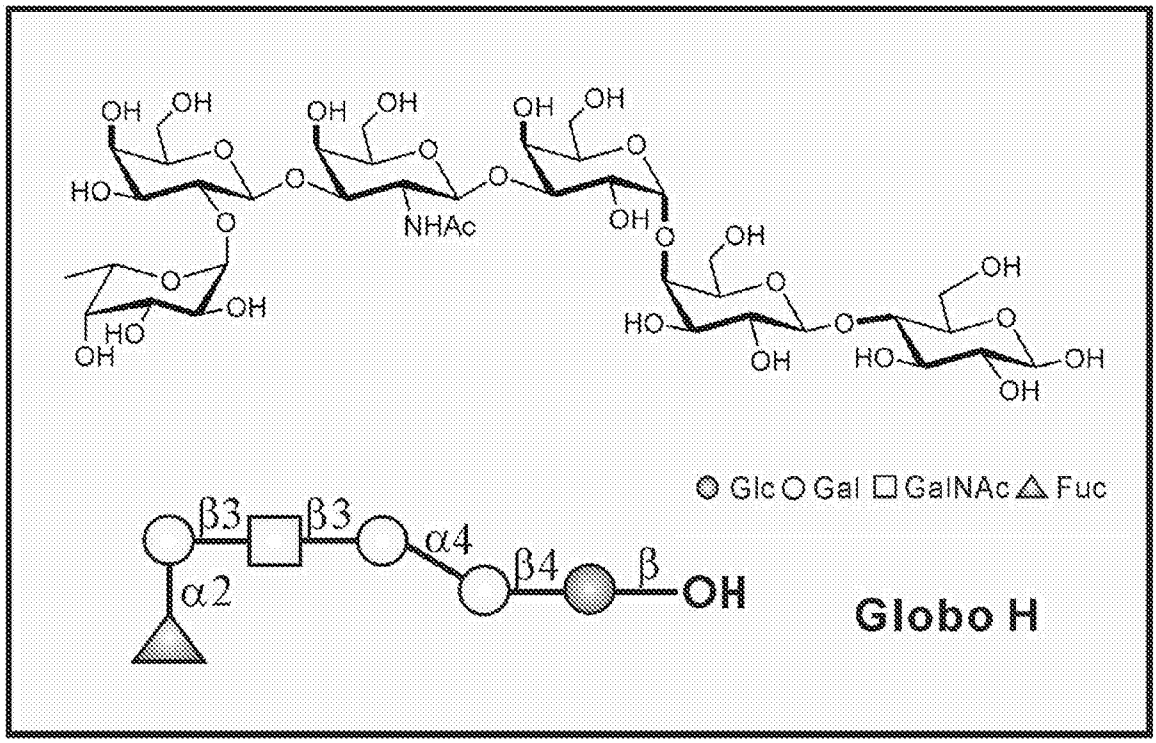
21. The method of claim 20, wherein the first and second agents are siRNAs.

22. The method of claim 21, wherein the first and second agents are si*FUT1* and si*FUT2*, respectively.

23. The method of claim 17, wherein the cancer is breast cancer.

Fig. 1

A.


Glc Gal GalNAc Fuc
Globo H

B.

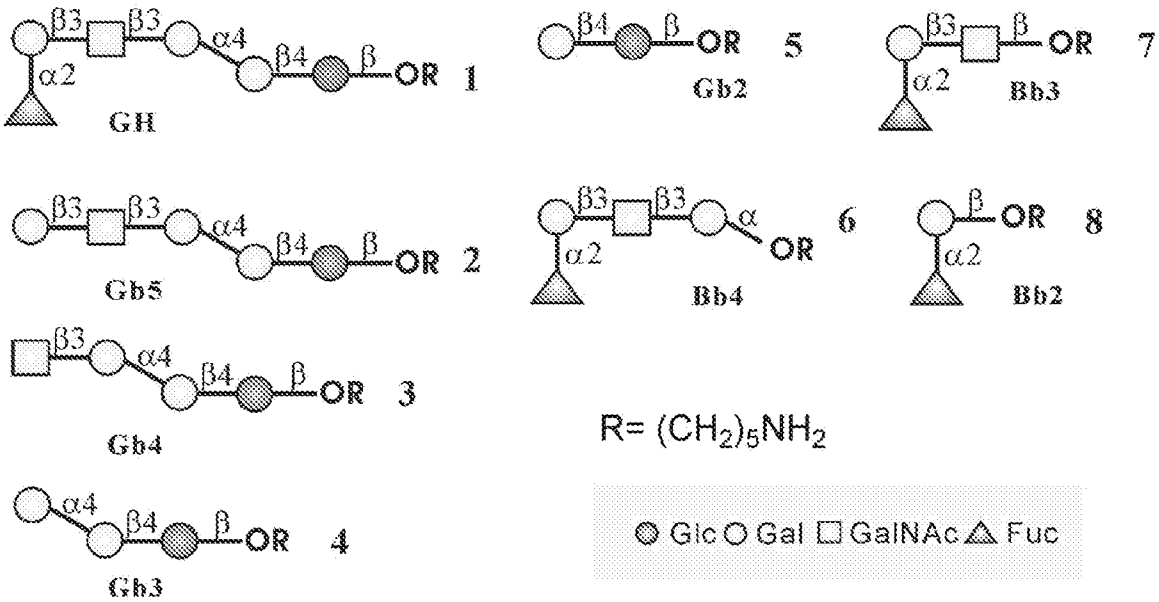

GH
1
Gb5
2
Gb4
3
Gb3
4
Gb2
5
Bb4
6
Bb3
7
Bb2
8
R= (CH2)5NH2
Glc Gal GalNAc Fuc

Fig. 2

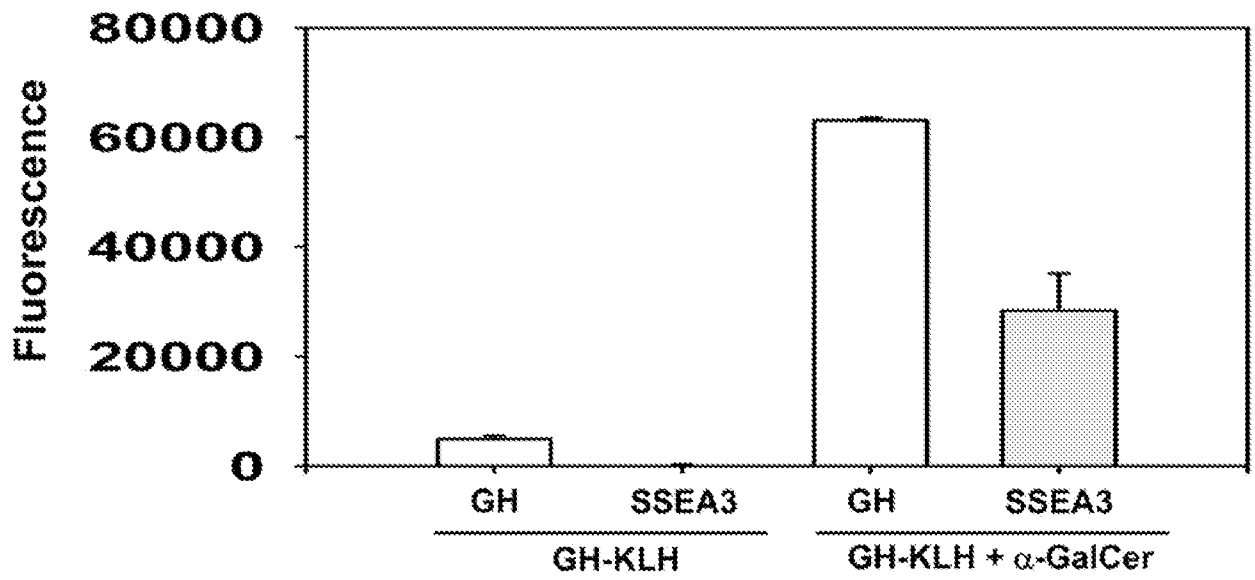
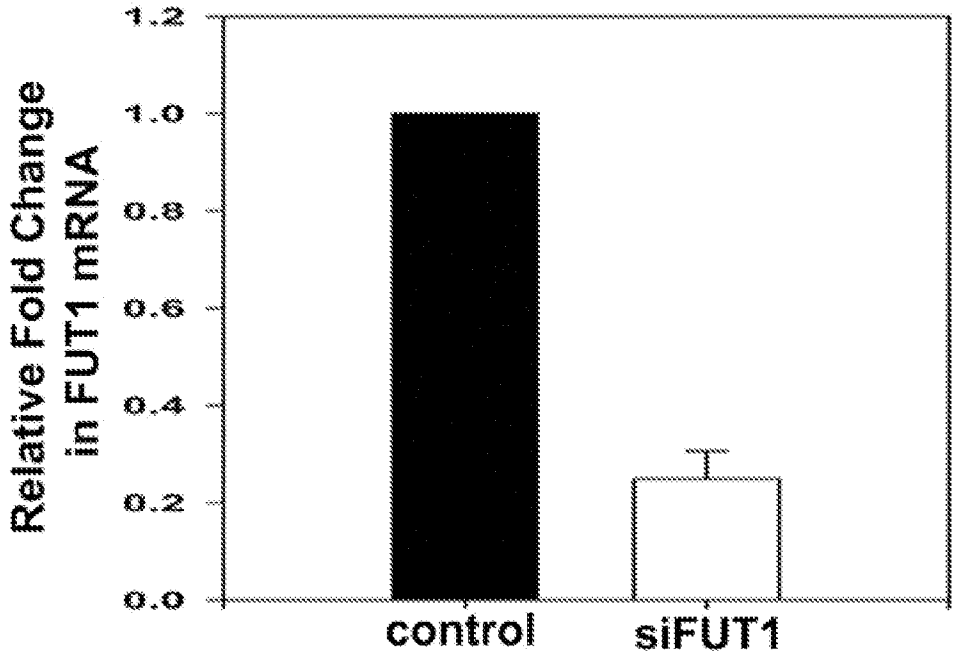

80000
60000
40000
20000
0
Fluorescence
GH
SSEA3
GH
SSEA3
GH-KLH
GH-KLH + α-GalCer

Fig. 3

A.


Relative Fold Change in FUT1 mRNA
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1.0
0.8
0.6
0.4
0.2
0.0
control
siFUT1

B.

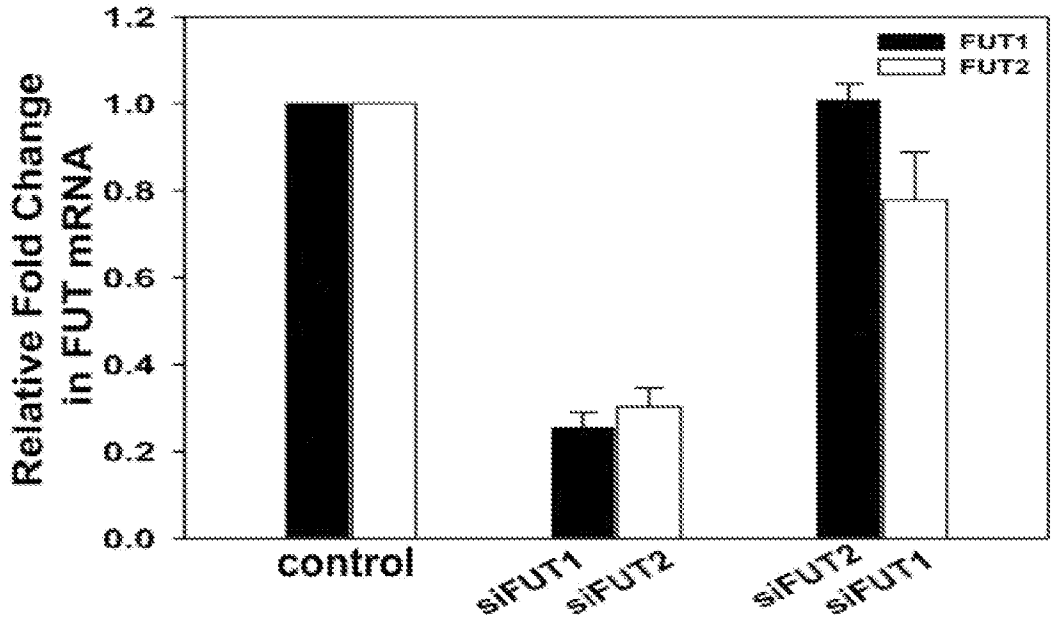
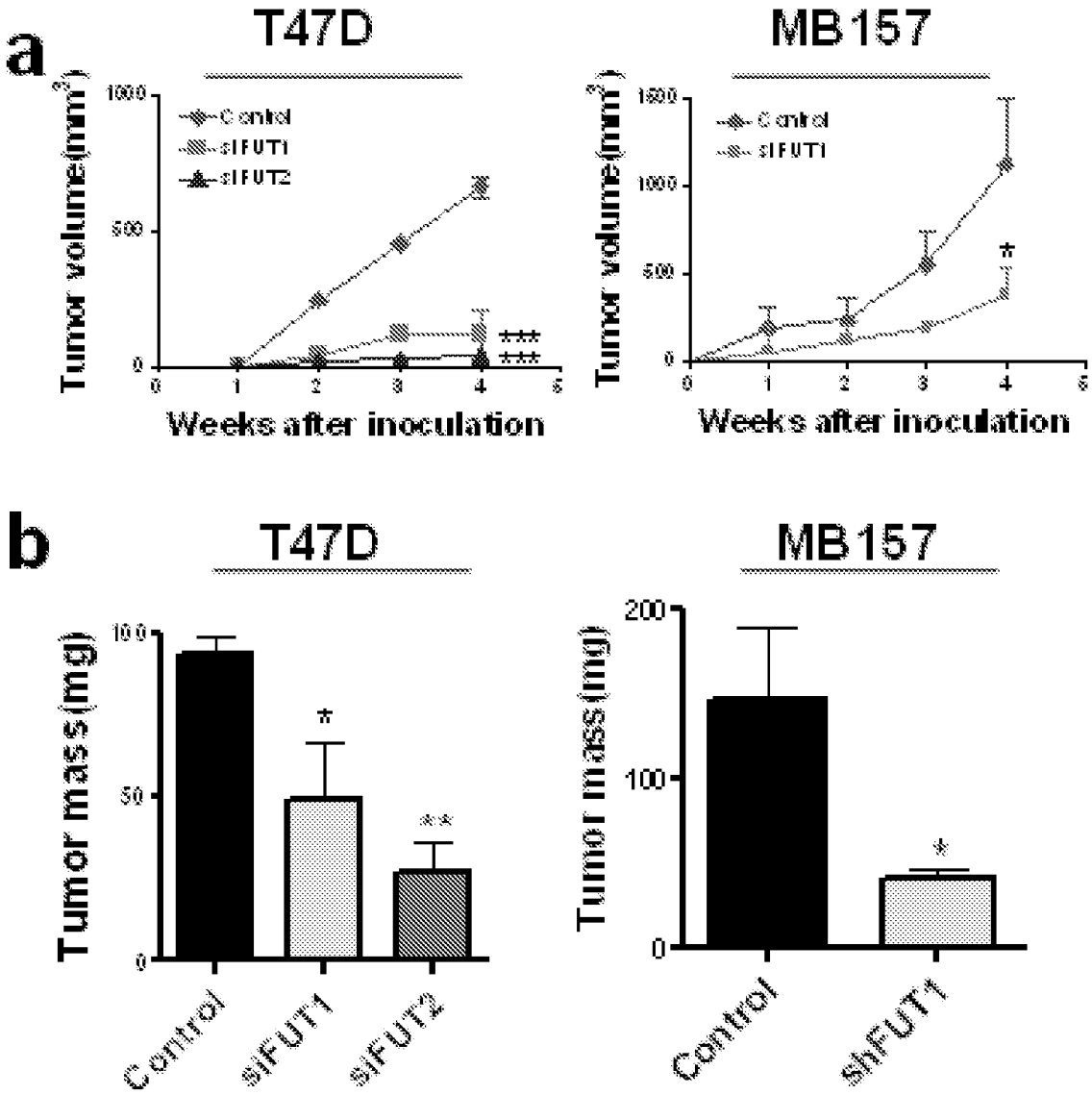

Relative Fold Change in FUT mRNA
1.2
1.0
0.8
0.6
0.4
0.2
0.0
FUT1
FUT2
control
siFUT1
siFUT2
siFUT2
siFUT1

Fig. 4


a
T47D
MB157
Tumor volume(mm³)
Control
siFUT1
siFUT2
Weeks after inoculation
b
T47D
MB157
Tumor mass (mg)
Control
siFUT1
siFUT2
shFUT1