Method for Determining Susceptibility of Tumor to Treatment with Anti-Neoplastic Agent

CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

[0001] This application claims benefit of U.S. provisional application No. 60/453,083, filed on March 7, 2003, the entire contents of which are incorporated herein by reference.

FIELD OF INVENTION

[0002] The invention relates to methods of determining the susceptibility of a tumor to treatment with anti-neoplastic agents, and finds application in biology and medicine.

BACKGROUND OF THE INVENTION

[0003] The term "cancer" generally refers to one of a group of more than 100 diseases caused by the uncontrolled growth and spread of abnormal cells that can take the form of solid tumors, lymphomas, and non-solid cancers such as leukemia. Physicians treating patients with cancer have a diverse arsenal of drug and non-drug therapies available. Identification of the appropriate therapy for a particular patient can be problematic, however, because although certain therapies can be matched to certain cancer types, patients' responses to a given therapy are not uniform.

[0004] A number of promising anti-cancer agents contain a glucose or glucose-like moiety and so may be a substrate for glucose transporters, membrane proteins that facilitate cell uptake of glucose. Examples include the anti-cancer drugs streptozotocin (STZ), glucofosfamide, and 2gluSNAP, and additional glucose or glucose-analog-containing drugs are being developed. The glucose-like moieties of these drugs mediate transport into cancer cells and thereby take advantage of the fact that many cancer cells exhibit, relative to normal cells, enhanced glucose metabolism thought to be due, in part, to an increase in the numbers of glucose transporters (see Medina *et al.*, 2002, *Biol Res.* 35:9-26). However, not all patients (or cancers) respond to treatment with drugs such as streptozotocin, glucofosfamide,

and 2gluSNAP. Methods to help predict which patients are most likely to benefit from specific treatment with specific anti-cancer agents and classes of agents would allow the physician to treat cancer more effectively and provide considerable benefit to patients. The present invention provides such methods.

SUMMARY OF THE INVENTION

[0005] The present invention has multiple aspects but generally provides methods and reagents for screening cancer patients to determine whether the cancer from which they suffer is susceptible to treatment with a glucose or glucose analog-containing anti-cancer agent.

[0006] In one aspect, the present invention provides a means to correlate the level of one or more glucose transporters in cancer cells with the susceptibility of said cells to an anti-cancer agent that is transported by said transporters, such as an anti-neoplastic agent containing glucose or a glucose analog. In one embodiment, the agent comprises a glucose moiety; in other embodiments, the agent contains a 2-deoxyglucose or a fructose moiety.

[0007] In one embodiment, the invention provides a method for determining whether a cancer is susceptible to treatment with an anti-neoplastic agent by (a) obtaining a sample of the cancer; (b) measuring the level of at least one glucose transporter in the sample; (c) comparing the level with a predetermined value, and (d) determining that, if the measured level is larger than the predetermined value, the cancer is susceptible to treatment with the anti-neoplastic agent. In a related aspect, the present invention provides a method for determining whether a human cancer is susceptible to treatment with an anti-cancer agent that contains glucose or a glucose analog and is transported by a glucose transporter, said method comprising: obtaining a sample of said cancer; measuring an amount of glucose transporter in said sample; comparing said amount in said sample to a predetermined value; and determining that, if said amount is larger than the predetermined value, then said cancer is susceptible to said anti-cancer agent and can be treated therewith, or, if said amount is smaller than said predetermined value, then said cancer is not susceptible or has a reduced susceptibility to said anti-cancer agent.

[0008] In one embodiment, the level in the cancer sample of more than one glucose transporter is measured. In an embodiment, the level of class I and/or a class II and/or a class III GLUT glucose transporter is measured. A variety of methods may be used to measure the

level of glucose transporter in the sample. One method is an immunological assay. Another suitable method involves amplification of an RNA or cDNA. In one embodiment, said human cancer sample, which can be, for example, a sample of fresh, frozen, fixed, or fixed and paraffin-embedded tumor cells, is treated to isolate total protein from said sample, and the isolate is contacted with antibodies specific for one or more of said glucose transporters, the levels of which in said isolate are determined by detecting the complex formed between the antibody and said glucose transporter. In other embodiments, the level or amount of glucose transporter in the sample is determined by detecting glucose transporter protein with a reagent other than an antibody, for example by measuring glucose uptake in a fresh tissue sample, or by detecting messenger RNA from one or more of the glucose transporter genes, or by otherwise measuring the activity of a glucose transporter or its corresponding gene.

[0009] In one embodiment of this method, the GLUT2 level in a cancer sample is measured. In another embodiment of this method, the anti-cancer agent is streptozotocin treatment. In another embodiment, the cancer is a pancreatic cancer or islet cell carcinoma. In a preferred embodiment, the GLUT2 level is measured, the anti-cancer agent is streptozotocin, glucofosfamide, or a gluSNAP compound, and the cancer is a pancreatic cancer or islet cell carcinoma.

[0010] In another aspect, the present invention provides kits useful in the practice of the invention. In one embodiment, the kits comprise diagnostic screening reagents and instructions for the use thereof in the present method. In another embodiment, the kits further comprise an anti-neoplastic therapeutic agent that comprises glucose or glucose analog.

[0011] These and other aspects and embodiments of the invention are described in more detail in the detailed description, example, and claims that follow.

DETAILED DESCRIPTION OF THE INVENTION

1. INTRODUCTON

[0012] The present invention provides methods, reagents and devices useful in assessing whether a cancer is susceptible to treatment with an anti-cancer agent or the degree of susceptibility of a cancer to an anti-cancer agent. In one aspect the anti-cancer agent is a drug that is transported by a glucose transporter, such as a drug comprising a glucose or glucose analog moiety that is a substrate for the glucose transporter. As used herein, a determination

that a cancer is "susceptible" to treatment with a drug means that there is a greater likelihood that the drug will be therapeutically efficacious against the tumor compared to the likelihood of efficacy for a tumor determined to be not "susceptible" to the agent. Determining that a cancer is susceptible to treatment with a drug or drug class therefore provides a method for determining a chemotherapeutic regimen for treatment of the patient.

[0013] In some methods of the invention, a cancer sample is obtained from a patient and tested to determine the level(s) of one or more glucose transporters; cancers expressing high levels of glucose transporters are determined to be susceptible to chemotherapy with anti-cancer agents that contain a glucose or glucose moiety and that are transported by a glucose transporter. Conversely, cancers expressing low levels of glucose transporters are determined not to be susceptible (or to be less susceptible) to such chemotherapy. The pre-therapy screening in accordance with the methods of the present invention allows the physician to treat cancer more effectively, as the physician is provided with a means for determining whether treatment with an anti-cancer agent will be effective, and if not, then to treat the cancer with a compound that may be more effective, and if so, then treating with that agent.

[0014] The term "glucose transporter" is used in the medical literature in two related, but slightly different, senses. In the first sense, glucose transporters are proteins that transport compounds (whether glucose, glucose analogs, other sugars such as fructose or inositol, or non-sugars such as ascorbic acids) across a cell membrane and are members of the glucose transporter "family" based on structural similarity (e.g., homology to other glucose transport proteins). However, some "glucose transporters" are believed to have a primary substrate other than glucose. For example, the glucose transporter GLUT5 is primarily a transporter of fructose, and is reported to transport glucose itself with low affinity. Similarly, the primary substrate for the glucose transporter HMIT is myo-inositol (a sugar alcohol). As used herein, the term "glucose transporter," unless otherwise specified, includes transporters of fructose and inositol. In one aspect, the invention contemplates measuring the level of any glucose transporter to assess the susceptibility of a tumor to an anti-cancer agent transported into a tumor cell by a glucose transporter (including, but not limited to, a glucose transporter selected from the groups of GLUT1-12, HMIT and SGLT1-6 transporters). In a preferred embodiment, the invention contemplates measuring the level of a glucose transporter that transports glucose with a higher affinity than fructose, inositol, H+-myoinositol or non-sugars such as ascorbic acids to assess the susceptibility of a tumor to an anti-cancer agent

comprising a glucose or glucose analog moiety transported into a tumor cell by a glucose transporter.

[0015] In a preferred embodiment of the invention, levels in the cancer sample of one or more glucose transporters are determined and compared to a reference value(s). A high transporter level in the cancer sample, relative to the reference value, indicates the tumor is susceptible to treatment with members of the class of anti-neoplastic agents that contain a glucose or glucose analog moiety.

[0016] Accordingly, in one embodiment, the present invention encompasses a method for determining whether a cancer is susceptible to treatment with an anti-neoplastic agent comprising glucose or a glucose analog that is transported into a cancer cell by a glucose transporter, said method comprising the steps of: (a) obtaining a sample of said cancer; (b) measuring an amount of or the activity of a glucose transporter in said sample; (c) comparing said amount or activity measured in step (b) with a predetermined amount or activity; and (d) determining that, if said measured amount or activity is larger than said predetermined amount or activity, then said cancer is susceptible to said treatment, and if said measured amount is less than said predetermined amount or activity, then said cancer is not or is less susceptible to said treatment.

[0017] In one embodiment, the present invention encompasses a method for determining whether a patient is susceptible to treatment with an anti-neoplastic agent comprising glucose or a glucose analog that is transported into a cancer cell by a glucose transporter, said method comprising the steps of: (a) diagnosing the patient as having a cancer; (b) obtaining a sample of said cancer from said patient; (c) measuring an amount or activity of glucose transporter in said sample; (d) comparing said amount or activity measured in step (c) with a predetermined amount or activity; and (e) determining that, if said measured amount or activity is larger than said predetermined amount or activity, then said patient is susceptible to said treatment, and if said measured amount or activity is less than said predetermined amount or activity, then said patient is not susceptible to said treatment.

[0018] In one embodiment, the present invention encompasses a method for treating a cancer patient with an anti-neoplastic agent comprising glucose or a glucose analog that is transported into a cancer cell by a glucose transporter comprising the steps of: (a) obtaining a sample of said cancer from said patient; (b) measuring an amount or activity of glucose transporter in said sample; (c) comparing said amount or activity measured in step (b) with a

predetermined amount or activity; (d) determining that, if said measured amount or activity is larger than said predetermined amount or activity, then said cancer is susceptible to said treatment, and if said measured amount or activity is less than said predetermined amount, then said cancer is not susceptible to said treatment; and if said measured amount or activity is larger than said predetermined amount or activity, (e) treating the cancer patient with the anti-neoplastic agent.

[0019] In one embodiment, the present invention encompasses a method for treating a patient with an anti-neoplastic agent comprising glucose or a glucose analog that is transported into a cancer cell by a glucose transporter comprising the steps of: (a) diagnosing the patient as having a cancer; (b) obtaining a sample of said cancer from said patient; (c) measuring an amount or activity of glucose transporter in said sample; (d) comparing said amount or activity measured in step (c) with a predetermined amount or activity; (e) determining that, if said measured amount or activity is larger than said predetermined amount or activity, then said cancer is susceptible to said treatment, and if said measured amount or activity is less than said predetermined amount or activity, then said cancer is not susceptible to said treatment; and if said measured amount or activity is larger than said predetermined amount or activity, (f) treating the patient with the anti-neoplastic agent.

2. "ANTI-CANCER AGENTS" AND "ANTI-NEOPLASTIC AGENTS"

[0020] Anti-cancer agents are compounds that prevent or impede the growth and/or spread of cancer cells in a patient. Administration of anti-cancer agents may result in delay or slowing of disease progression, amelioration or palliation of the disease state, remission (whether partial or total), and/or prolong survival (compared to expected survival if not receiving treatment). Anti-cancer agents presently in use or under development include, but are not limited to, alkylators, anthracyclines, antibiotics, metabolic poisons, radionucleotides, metal poisons, enzyme inhibitors, aromatase inhibitors, biphosphonates, cyclo-oxygenase inhibitors, estrogen receptor modulators, folate antagonists, inorganic arsenates, microtubule inhibitors, modifiers, nitrosoureas, nucleoside analogs, orthoclase inhibitors, platinum-containing compounds, retinoid, topoisomerase 1 inhibitors, topoisomerase 2 inhibitors, and tyrosine kinase inhibitors.

[0021] Of primary interest in the context of the present invention are anti-cancer agents transported into the cancer cell by a glucose transporter. In this context, the term "anti-

neoplastic agent" is used to refer to such anti-cancer agents and thus distinguish them from anti-cancer agents not transported into the cancer cell by a glucose transporter (such as, for example, cisplatin, Bevacizumab, Pemetrexed, and the like). Anti-neoplastic agents include molecules that comprise a glucose or glucose analog moiety that facilitates or otherwise allows transport into the cell by a glucose transporter. In one embodiment, the glucose analog is itself the anti-neoplastic agent. An example of such an agent is 2-deoxyglucose (2-DG). Other examples include 2-fluoro-2-deoxyglucose (2-FDG), and radioactively labeled 2-FDG compounds such as 2-^{18}FDG. In another embodiment, a glucose or glucose-like moiety is conjugated to another moiety, such as a cytotoxic compound, which may be an anti-cancer agent in its own right (i.e., when not conjugated to the glucose or glucose-like moiety) such as those listed above. For example, the glucose or glucose analog moiety can serve merely as a targeting molecule that brings the cytotoxic agent attached thereon into the cell. Anti-neoplastic agents that comprise a glucose or glucose analog conjugated to a toxic moiety are described in PCT publication WO 03/082301. In one embodiment, neither the glucose-like moiety nor the other moiety is toxic when administered in a non-conjugated form. In an alternative embodiment, both the glucose-like moiety and the other moiety are toxic when administered independently.

[0022] For anti-neoplastic agents comprising a moiety conjugated to a glucose or glucose analog, any linkage that is compatible with both the glucose or glucose analog portion and the non-glucose portion may be used; for example, any of the linkages described in WO 03/082301 or in U.S. Patent No. 5,622,936. The anti-neoplastic agent can be attached to the glucose or glucose analog at any functional site, for example, on the 1 position, 2 position, 3 position, or 4 position of glucose or glucose analogs.

[0023] Glucose analogs that are, or are a portion of, an anti-neoplastic agent for which the screening methods of the invention are useful are known in the art and include, but are not limited to, glucose derivatives such as D-(+)-2-deoxyglucose, D-(+)-2-amino-2-deoxy-glucose or N-acetyl D-(+)-2-amino-2-deoxyglucose; D-mannose and mannose derivatives; D-glucose and D-glucose derivatives, including but not limited to D-3-amino-3-deoxy-glucose and D-2-amino-2-deoxy-glucose; and D-galactose and galactose derivatives including but not limited to D-2-deoxy-D-galasctose, D-4-amino-4-deoxy-galactose and D-2-amino-2-deoxy-galactose. The glucose or glucose moiety thus can differ from D-glucose or a derivative such as 2-DG and 2-glucosamine in that it is an epimer thereof. In addition, the glucose or glucose analog moiety can be a fluorinated derivative of any of the foregoing compounds. Moreover,

the oxygen in the ring of any of the foregoing compounds can be substituted with an isostere selected from the group consisting of S, sulfone, and the like. For example, the glucose analog can be 5-thio-D-glucose or a derivative thereof. The term "glucose analog" also includes glucose derivatives, including, but not limited to, derivatives having (C1-C12)acyl groups or (C1-C12)akyl groups attached via –O- or –NH- groups at the 3- and 4- positions of the glucose molecules. Additionally, the glucose derivative may have a solubility or partitioning effector or component attached at the 1-, 3-, or 4- positions.

[0024] In some embodiments, the glucose analog is fructose, psicose, sorbose, tagatose, allose, altrose, gulose, idose, talose, inositol (e.g., myo-, scillo-, muco-, and chiro inositol) or other sugar alcohol that is a substrate for a glucose transporter, or a derivative thereof that is a substrate for a glucose transporter, such as those listed in Table 1.

[0025] A number of anti-neoplastic agents that comprise a glucose or glucose analog are known, and others are being tested or developed for future clinical use. For illustration and not limitation, examples of such anti-neoplastic agents include streptozotocin, glucofosfamide, glycol-S-nitrosothiols (including 2gluSNAP), single proton-emitting radio tracers (including 2-O-(3'-iodobenzyl-D-glucose and N-(4'-iodionbenzyl)-D-glucosamine, 2-deoxy-D-glucose (2-DG) and 2-DG conjugates described in WO 03/082301, conjugates described in WO 02/058741, conjugates described in WO 99/20316, conjugates described in U.S. Patent No. 6,489,302, and conjugates described in U.S. Patent No. 5,622,396, and their derivatives and analogs.

[0026] Streptozotocin [2-deoxy-2-(3-methyl-3-nitroso-ureido)-D-glucose; N-methylnitrosocarbamoyl-D-glucosamine; STZ; Zanosar™] is an anti-mitotic alkylating agent in which a cytotoxic N-nitrosourea group is attached to the 2 position of glucosamine. Streptozotocin has been approved by the FDA to treat pancreatic islet cell cancer and carcinoid tumors. In contrast to other nitrosourea analogs, streptozotocin selectively targets pancreatic β-cells, due to the presence of a glucose moiety on the compound, which appears to mediate uptake into cells expressing GLUT2 (see, Schnedl *et al.*, Nov. 1994, *Diabetes 43*:1326-33; Elsner *et al.*, Dec. 2000, *Diabetologia* 43:1528-33; Hosokawa *et al.*, Dec. 2001, *Biochem. Biophys. Res. Commun. 289*:1114-17; Wang *et al.*, Jan. 1998, *Diabetes 47*:50-56; and Wang *et al.*, 1995, *Exp. Clin. Endocrinol. Diabetes 103 Suppl. 2*:83-97). See, e.g., U.S. Patent Nos. 3,694,428 and 3,940,383.

[0027] The anti-neoplastic agent glucofosfamide (beta-D-glucosyl-ifosfamide mustard; glc-IPM) contains a cytotoxic agent ifosfamide coupled to glucose via an ester linkage at the oxygen atom at the 1-position of glucose (see U.S. Patent No. 5,622,936 and U.S. Patent No. 6,489,302). Glucofosfamide has been tested for the treatment of patients with pancreatic cancer receiving first line treatment and in patients with non-small cell lung cancer receiving second line chemotherapy, as well as glioblastoma, breast cancer, and colon caner patients. See Niculescu-Duvaz, 2002, *Curr. Opin. Investig. Drug* 3:1527-32. Briasoulis *et al.*, 2000, *J. Clin. Oncol.* 18:3535-44, reports that cellular uptake of glucofosfamide is mediated by a Na+-dependent glucose transporter.

[0028] A glycol-S-nitrosothiol compound can be described as a cytotoxic agent linked to a sugar, and has been reported as targeting tumor cells that over-express GLUT1 preferentially (see Ramirez *et al.*, 1996, *Bioorg. Med. Chem. Lett.* 6:2575-80; and Cantuaria *et al.*, 2000, *Cancer* 88:381-88). An exemplary glycol-S-nitrosothiol, 2gluSNAP, has a structure in which a nitric oxide donating cytotoxic moiety (S-nitroso-N-acetyl-penicillamine) is linked to 2-deoxyglucoamine at the 2 position via an amide bond. These compounds have been reported to target tumor cells that overexpress GLUT1 (see Ramirez *et al.*, *supra*, and Cantuaria *et al.*, *supra*).

[0029] Other anti-neoplastic agents to which the screening methods of the present invention may be applied are compounds that contain a glucose moiety linked to a single photon-emitting moiety via a heterocyclic, hydrocarbon, or aromatic groups as described in PCT publication WO 99/20316. These compounds include, for example, 2-O-(3'-iodobenzyl)-D-glucose and N-(4'-iodobenzyl)-D-glucosamine. Other examples of anti-neoplastic agents that can be attached to glucose or glucose analogs described above include, for example, Yttrium-90, Iodine-125, Iodine-131, phosphate-32, hydoxyurea, triapine, 5-HP, camptothecin and analogs thereof, carboplat and analogs thereof, DOTA and other radiomethal ion chelators, methotrexate and analogs, mitoxantrone and related anthraquinone structures, small kinase inhibitors, dacarazine or procarbazine, and mitomycin.

[0030] Compounds other than glucose and glucose analogs are transported by one or more glucose transporters. For example, ascorbic acids have also been reported to be transported into the cell by glucose transporters in the form of dehydroascorbic acids. See Vera *et al.*, 1004, *Blood* 84:1628-34 and Agus *et al.*, 1997, *J. Clin. Invest.* 100:2842-48. Proton/myoinositol co-transporter (HMIT), a member of the facilitative glucose transporter

class, was found to be selective for myo-, scillo-, muco-, and chiro-inositol. Transport was with high affinity (Km = 100 uM) and was increased by low pH with a maximal rate reached at pH = 5.0.

[0031] The anti-neoplastic agents described herein are used for the treatment of cancer as primary, single-agent treatment, as well as in treatment in combination with radiation, surgery, or other anti-cancer agents, and combinations of such therapies.

3. GLUCOSE TRANSPORTERS

[0032] As noted above, in one aspect of the invention, levels of one or more glucose transporters are determined in a cancer sample. Accordingly, an appreciation of the nature and types of glucose transporters will provide the practitioner with guidance concerning the invention.

[0033] Glucose transporters include members of the facilitative glucose transporter protein family (GLUT/SLC2A), sodium-dependent glucose co-transporters (SGLT/SLC5A), the H+/myo-inositol co-transporter (HMIT1), and human or mammalian homologs and orthologs of the aforementioned. Exemplary glucose transporters, for illustration and not limitation, include those listed in Table 1.

TABLE 1

Glucose Transporter	Nucleic Acid Sequence Accession No. *	Protein Sequence Accession No. *	Class	Selected Tissues Reported to Express Transporter	Primary References
GLUT1	NM_006516	P11166	I	All tissues (abundant in erythrocytes and brain)	1, 2
GLUT2	NM_000340	P11168	I	Liver, pancreas, pancreatic islet cells, retina, intestine, kidney	2, 3
GLUT3	NM_006931	P11169	I	Brain	2, 4
GLUT4	NM_001042	P14672	I	Heart, muscle, fat, brain	5, 6
GLUT5	BC001692	P22732	II	Intestine, testes, kidney, erythrocytes	7, 8
GLUT6	NM_017585	Q9UGQ3	III	Brain, spleen, leucocytes	9, 10
GLUT7			II	Unknown	11
GLUT8	NM_014580	Q9NY64	III	Testis, brain, blastocyst	10, 12, 13, 14, 29
GLUT9	BC018897	Q9NRM0	II	Liver, kidney	15
GLUT10	NM_030777	Q95528	III	Liver, pancreas	16, 17
GLUT11	NM_030807	Q9BYW1	II	Heart, muscle	18, 19, 20
GLUT12	NM_145176	NP_660159	III	Heart, prostate, muscle, small intestine, fat	21
HMIT	NM_052885	NP_443117	III	Brain	22
GLUT14	NM_153449 AF481879	AAL89709 AAL89710	I	Testis	28
SGLT1	NM_000343	P13866		Small intestine, kidney, heart,	23

Glucose Transporter	Nucleic Acid Sequence Accession No. *	Protein Sequence Accession No. *	Class	Selected Tissues Reported to Express Transporter	Primary References
				liver, lung	
SGLT2	NM_003041.1	P31639		Ubiquitous (primarily kidney)	24, 25
SGLT3	AJ133127**	Q9NY91		Small intestine, skeletal muscle (based on pig SGLT3)	26
SGLT5	NM_152351				27

* Reference to specific sequences is provided for illustration and is not intended to limit the invention in any fashion. For example, there may be additional or alternative sequences corresponding to the transporters listed in column 1, including, but not limited to, polymorphic sequences and other variants.
** EMBL sequence accession number.

1. Mueckler et al. 1985, *Science* 229:941-5; 2. Gould et al. 1991, *Biochem.* 30:5139-45; 3. Fukumoto et al. 1988, *PNAS* 85:5434-8; 4. Kayano et al. 1988, *J. of Biol. Chem.* 263:15245-8; 5. Fukumoto et al. 1989, *J. of Biol. Chem.* 264:7776-9; 6. James et al. 1989, *Nature* 338:83-7; 7. Kayano et al. 1990, *J. of Biol. Chem.* 265:13276-82; 8. Davidson et al. 1992, *Am. J. of Physiol.* 262:C795-C800; 9. Doege et al. 2000a, *Biochem. J.* 350:771-6; 10. Lisinski et al., 2001, *Biochem. J.* 358:517-22; 11. Joost & Thorens, 2001, *Mol. Membrane Biol.* 18:247-56; 12. Carayannopoulos et al. 2000, *PNAS* 97:7313-8; 13. Doege et al. 2000b, *J. of Biol. Chem.* 275:16275-80; 14. Ibberson et al. 2000, *J. of Biol. Chem.* 275:4607-12; 15. Phay *et al.* 2000, *Surgery* 128:946-51; 16. Dawson et al. 2001, *Mol.Genetics and Metabol.* 74:186-99; 17. McVie-Wylie et al. 2001, *Genomics* 72:113-7; 18. Doege et al. 2001, *Biochem.l J.* 359:443-9; 19. Wu *et al.* 2002, *Mol. Genetics and Metabol.* 76:37-45; 20. Sasaki et al. 2001, *Biochem. and Biophys. Res. Comm.* 289:1218-24; 21. Rogers et al. 2002, *Am. J. of Physiol.* 282:E733-8; 22. Udry *et al.* 2001, *EMBO J.* 20:4467-7; 23. Hediger et al. 1989, *PNAS*, 86(5):5748-52; 24. Wells et al., 1992, *Am. J. Physiol.* 263(3):F459-65; 25. Kanai et al., 1994, *J Clin Invest.* 93(1):397-404; 26. Diez-Sampedro, 2003, *PNAS*, 100(20):11753-8; 27. Wood & Trayhurn, 2003, *British J. Nutr.* 89:3-9; 28. Wu et al., 2002, *Genomics* 80:553-7; 29. U.S. Patent Application No. 20030228592.

3.1 GLUTs

[0034] In some embodiments, the glucose transporter is a facilitative Na^+ independent sugar transporter or facilitative glucose transporter. "Facilitative glucose transporter" or "facilitative Na^+ independent sugar transporter" or "GLUT" refers to glucose transporters that utilize the diffusion gradient of their substrates such as glucose across plasma membranes to transport the substrates. Facilitative glucose transporters include, but are not limited to, GLUT1, GLUT2, GLUT3, GLUT4, GLUT6, GLUT7, GLUT8, GLUT9, GLUT10, GLUT11, GLUT12, and H+-coupled myo-inositol transporter HMIT1. GLUT14 is 94.5% identical to GLUT3 and is also included in the GLUT family.

[0035] Facilitative glucose transporters generally possess 12 membrane-spanning helices. The transmembrane domain of the protein may contain a water-filled pathway through which the substrate moves and, in some cases, the exoplasmic domain of the protein can contain a large loop bearing an N-linked oliogosaccharide moiety. The loop can be formed between any of two consecutive transmembrane helices, for example, between the first and the second transmembrane helices, or between the ninth and the tenth transmembrane helices.

[0036] In some embodiments, the glucose transporter comprises one or more "glucose transporter signatures." "Glucose transporter signature" as used herein refers to conserved residues on two or more glucose transporters that have been implicated in the function of the protein as transporters. In some embodiments, the glucose transporter signature is the presence of seven glycine residues in transmembrane helices 1, 2, 4, 5, 7, 8, and 10. In some embodiments, the glucose transporter signature is one or more charged residues on the cytoplasmic surface of the protein that are conserved among the known GLUTs. See Joost *et al.*, 2001, *Mol. Membrane Biol.* 18:247-256. In some embodiments, the glucose transporter signature is one or more of the following: tryptophan at helix 6, tryptophan at helix 11, tyrosine at helix 4, and tyrosine in helix 7. In some embodiments, the glucose transporter is a PXXPR motif after helix 6. In other embodiments, the glucose transporter does not comprise such a PXXPR motif.

[0037] In some embodiments, the glucose transporter signature is a tryptophan residue immediately following a conserved GXXPXP motif at helix 10 (corresponding to tryptophan 388 in GLUT1 when the sequence is aligned with GLUT1). In some embodiments, the glucose transporter signature is a tryptophan residue in helix 10 (corresponding to tryptophan 412 in GLUT1 when the sequence is aligned with GLUT1). These tryptophan residues have been shown to be important for binding of the ligands cytochalasin B and forskolin, and confer cytochalasin B or forskolin sensitivity to the glucose transporters. Garcia *et al.*, 1992, *J. Biol. Chem.* 267:7770-76; Schurmann *et al.*, 1993, *Biochem. J.* 290:497-501. Thus, some embodiments of the present invention encompass the assay of glucose transporters that are sensitive to cytochalasin B or forskolin treatment. In some embodiments, the glucose transporter has low binding affinity to cytochalasin B or forskolin. In some embodiments, the glucose transporter has high binding affinity to cytochalasin B or forskolin.

[0038] Glucose transporters assayed in the practice of the present invention have different affinities to glucose or glucose analogs. Accordingly, in some embodiments, the glucose transporter displays high affinity for glucose. As used in this context, for GLUTs, "high affinity" means a K_M less than 5 mM using the *Xenopus* oocytes assay described in Burant *et al.*, 1992, *J. Biol. Chem.* 267:14523-26; and for SGLTs "high affinity" means a $K_{0.5}$ less than 1 mM, preferably less than 0.5 mM, measured using the two-microelectrode clamp assay described in Panayotova-Heiermann *et al.*, 1996, *J. Biol. Chem.* 271:10029-34. In other embodiments, the glucose transporter displays a low affinity for glucose . In some embodiments, the glucose/glucose analog-binding affinity of the glucose transporter varies in

different tissues or under different physiological conditions, for example in a diseased state such as cancer.

[0039] Based on sequence similarities and characteristic elements, the set of known GLUT proteins can be divided into three smaller subsets, namely class I (GLUT1-4), class II (GLUT5, GLUT7, GLUT9, and GLUT11), and class III (GLUT6, 8, 10, 12, and HMIT1). See Joost *et al.*, 2001, *Mol. Membr. Biol.* 18:247-56. Abnormal and/or over-expression of GLUTs is observed in some tumor tissues (see the review Smith, 1999, *Br. J. Biomed. Sci.* 564:285-92; Bell *et al.*, 1990, Diabetes Care 13:198-206).

3.1.1 CLASS I GLUTs

[0040] Class I glucose transporters may comprise sequence motifs (a glucose transporter signature) such as a glutamine at helix 5 (corresponding to Q161 in GLUT1 when the sequence is aligned with GLUT1). In some embodiments, the glucose transporter comprises a STSIF motif in extracellular loop 7 (i.e., the loop between transmembrane helix 7 and transmembrane helix 8). In some embodiments, the glucose transporter comprises a QLS-motif at helix 7. Class I glucose transporters are reported to be expressed in various solid tumors such as breast cancer, renal cell carcinoma, brain tumors, gastrointestinal malignomas, and cervical carcinomas.

[0041] In some embodiments of the invention, levels of a Class I glucose transporter are measured.

[0042] In some embodiments of the invention, GLUT1 levels are measured. GLUT1 has been reported to be over-expressed in a variety of malignant tissues, including tumors of the bladder, breast, cervical, colorectal, gastric, esophageal, head and neck, leiomyosarcomas, lung, ovarian, pancreatic, penile, thyroid, uterous, vascular, and juvenile hemangiomas. Medina *et al.*, 2002, *Biol. Res.* 35:9-26. Expression of GLUT1 has also been shown to correlate with tumor hypoxia. Airley *et al.*, 2001, *Clin. Cancer Res.* 7:928-34; Chen et al. 2001, *J. Biol. Chem.* 12:9519-25.

[0043] In some embodiments of the invention, GLUT2 levels are measured. GLUT2 has been reported to be over-expressed in gastric cancer.

[0044] In some embodiments of the invention, GLUT3 levels are measured. GLUT3 has been reported to be over-expressed in brain cancer, lung cancer, breast cancer, gastric cancer,

head and neck cancer, meningiomas, and ovarian cancer. GLUT3 has high affinity for glucose and is thought to be responsible for transport of glucose in tissues where the demand for glucose as a fuel is considerable, such as in the brain.

[0045] In some embodiments, GLUT4 levels are measured. GLUT4 has been reported to be over-expressed in breast cancer, gastric cancer, lung cancer, and pancreatic cancer.

[0046] In some embodiments, GLUT14 levels are measured. GLUT14 has two alternatively spliced forms: the shorter form of GLUT14 is a 497-amino-acid protein that is 94.5% identical to GLUT3. The long form is a 520 amino acid protein that differs from the short form only at the N-terminus. Both isoforms are reported to be specifically expressed in testis.

3.1.2 CLASS II GLUTs

[0047] In some embodiments, the glucose transporter is a class II GLUT. In some embodiments, the glucose transporter lacks a tryptophan residue following the conserved GXXPXP motif in helix 10 (corresponding to tryptophan 388 in GLUT1 when the sequence is aligned with GLUT1) that has been shown to confer cytochalasin B sensitivity to the glucose transporters. Class II glucose transporters are not sensitive to inhibition by cytochalasin B (see Joost *et al.*, 2001, *Mol. Mem. Biol.* 18:247-56).

[0048] In some embodiments of the invention, levels of a Class II glucose transporter are measured.

[0049] In one embodiment of the invention, GLUT5 levels are determined. GLUT5 has been reported to be over-expressed in lung cancer and breast cancer.

[0050] In one embodiment of the invention, GLUT7 levels are determined.

[0051] In one embodiment of the invention, GLUT9 levels are determined. An alternatively spliced form of GLUT9 was recently identified that differs from GLUT9 only at the N-terminus. Auguatin *et al.*, 2004, January 22, *J. Biol. Chem.*

[0052] In some embodiment of the invention, GLUT11 levels are determined. Two splice variants have been described for GLUT11, a long form (503 amino acids) and a short form (493 amino acids). Doege *et al.*, 2001, *Biol. J.* 359:443-49; Sasaki *et al.*, 2001, *Biochem. Biophys. Res. Comm.* 289:1218-24. The short form GLUT11 has been reported to mediate

low affinity glucose transport, and has been shown to be expressed predominantly in heart and skeletal muscle. The long form of GLUT11 has been reported to be expressed in liver, lung, trachea and brain, and was shown to increase fructose transport. Thus, in some embodiments of the present invention, the glucose transporter is the long form of GLUT11. In other embodiments, the glucose transporter is the short form of GLUT11. In yet other embodiments, the glucose transporter is a derivative, variant, or close homologue of GLUT11.

3.1.3 CLASS III GLUTs

[0053] In some embodiments, the glucose transporter is a class III GLUT. Motifs found in some Class III GLUTS include a glycosylation site on loop 9 (i.e., the loop between transmembrane helix 9 and transmembrane helix 10) and targeting motifs.

[0054] In some embodiments of the invention, levels of a Class III glucose transporter are determined.

[0055] In one embodiment of the invention, GLUT6 levels are determined. GLUT6 is reported to be predominantly expressed in brain, spleen and peripheral leukocytes.

[0056] In one embodiment of the invention, GLUT8 levels are determined. GLUT 8 is expressed in breast cancer cells.

[0057] In one embodiment of the invention, GLUT10 levels are determined.

[0058] In one embodiment of the invention, GLUT12 levels are determined. GLUT12 has been found to be expressed in breast tumors. See Rogers *et al.*, 2003, *Cancer Letters*, 93:225-33; Rogers et al., 2002, *Am. J. Physiol. Endocrinol. Metab.* 282(3):E733-8.

[0059] In one embodiment of the invention, HMIT1 levels are determined.

3.2 SGLTs

[0060] In some embodiments, the glucose transporter is a Na^+-dependent glucose co-transporter. "Na+-dependent glucose transporter" or "Na^+/glucose co-transporter" refers to a glucose transporter that actively transports glucose or glucose analogs in an energy-dependent manner. Some Na^+-dependent glucose transporters utilize the movement of Na^+

down an electrochemical gradient to drive the uptake of glucose or glucose analogs. Na^+-dependent glucose transporters include, but are not limited to, SGLT1, SGLT2, SGLT3, SGLT4, SGLT5, and SGLT6. See Table 1; also see Coady et al., 2002, "Identification of a novel Na+/myo-inositol cotransporter." *J Biol Chem.* 277:35219-24. In some embodiments, the glucose transporter is a high affinity, low capacity Na^+-dependent glucose transporter. In some embodiments, the glucose transporter is a low affinity, high capacity Na+-dependent glucose transporter.

[0061] In some embodiments, the Na+-dependent glucose transporter encompasses 14 transmembrane helices. In some embodiments, the glucose transporter further comprises an N-linked glycosylation site between helix 6 and 7 that has been shown to be conserved among several known SGLTs. See Wright, *Am. J. Physiol. Renal Physiol.* 2001, 280(1):F10-8.

[0062] In one embodiment of the invention, SGLT1 levels are determined. SGLT1 has a high glucose affinity with a reported Na^+/glucose binding ratio of 2:1. SGLT1 has been reported to be expressed in several intestinal tumor cell lines and primary lung cancers. Bissonette *et al.*, 1996, *Am. J. Physiol.*, 270:G833-G843; Delezay *et al.*, 1995, *J. Cell Physiol.* 163:120-128; Ishikawa *et al.*, 2001, *Jpn. J. Cancer Res.* 92:874-79.

[0063] In one embodiment of the invention, SGLT2 levels are determined. SGLT2 is a low affinity, high capacity transporter with a reported Na^+/glucose binding rate of 1:1.

[0064] In one embodiment of the invention, SGLT3 levels are determined. SGLT3 (formerly named SAAT1) has been reported to mediate transport of the chemotherapeutic agent β-D-glucosyllisophosphoramide mustard (D-19575) into tumor cells. Vehyl *et al.*, 1998, *Proc. Natl. Acad. Sci. USA*, 95:2914-19. Recently, it has been proposed that SGLT3 is not a Na+/glucose cotransporter but instead a glucose sensor in the plasma membrane of cholinergic neurons, skeletal muscle, and other tissues (Diez-Sampedro *et al.*, 2003, "A glucose sensor hiding in a family of transporters" *Proc. Natl. Acad. Sci. U S A* 100:11753-8). Accordingly, in some embodiments of this invention, the glucose transporter for which a level is determined is not, or does not include, SGLT3.

[0065] In one embodiment of the invention, SGLT4 levels are determined.

[0066] In one embodiment of the invention, SGLT5 levels are determined.

[0067] In one embodiment of the invention, SGLT6 levels are determined.

[0068] It will be apparent that the level of a glucose transporter other than a transporter described in Table 1 may be measured in the practice of the invention. For example, derivatives, isoforms, variants, mutants, and homologs of any of the glucose transporters listed in Table 1, including homologs in other mammals, may be measured or their levels or activities measured. Further, the methods of the invention are applicable to glucose transporters that may be discovered in the future, such as a transporter with structure or sequence characteristic of known transporters (including, for example, a glucose transporter with at least 28%, at least 30%, at least 50%, at least 60%, at least 80%, at least 90% amino acid sequence similarity to a glucose transporter listed in Table 1).

4. DETERMINING SUSCEPTIBILITY OF A CANCER TO TREATMENT WITH AN ANTI-NEOPLASTIC AGENT

[0069] In one aspect, the methods of the present invention involve measuring the level of glucose transporter in a cancer sample from a patient. The level is compared to a reference value to determine whether the cancer, by virtue of expressing a high level of one or more transporters, is susceptible to treatment with an anti-neoplastic agent. As is explained in more detail below, in various aspects of the invention, levels of a single transporter in the cancer sample are measured, levels of multiple different transporters in the cancer sample are independently measured (i.e., a distinct value is determined for the level of each transporter) and/or two or more different transporters in the cancer sample are measured in a combined measurement (e.g., by assaying with a probe that recognizes more than one transporter or by assaying with multiple probes).

[0070] The word "level," as used herein, is intended to encompass any measurement reflective of the number of glucose transporters in the cancer sample and, in various embodiments, may be quantitative, semi-quantitative or relative (e.g., merely determined to be "greater than" or "less than" another similarly measured level). This number can be inferred from a wide variety of different measurements. For example, the number can be inferred from the amount of glucose transporter protein, the abundance of messenger RNA, or from glucose transporter activity in the sample. A number of assays that can be employed in the methods of the invention are described below and others will be apparent to the practitioner guided by this disclosure.

[0071] In some embodiments, the measurement of glucose transporters in the sample is normalized as an amount (or number) per cell, per gram tissue, per mm^3 tissue, or the like. As is known in the art, the transporter level in a cancer sample also can be normalized by reference to the abundance of a second protein in the sample, and the level of transporters expressed as a ratio of the amount of transporter protein to second protein. An advantage of such a method is that by selecting a protein that is expressed at a relatively constant level under different conditions, it is possible to minimize any effects of general inhibition of protein synthesis, cell apoptosis, or loss of materials during the assay process. Suitable "second proteins" include, without limitation, structural proteins, such as actin, tubulin, and glyceraldehyde-3-phosphate dehydrogenase. Comparable normalization can be used when transporter RNA levels are measured (e.g., by comparing transporter RNA levels with ribosomal RNAs or mRNA encoding a structural protein). In one aspect, glucose-6-phosphatase activity in the tumor is also measured and the glucose transporter level is expressed as a ratio of transporter (protein, RNA or activity) to glucose-6-phosphatase (G6Pase) protein, RNA or activity. A high ratio is indicative of increased susceptibility to treatment with an anti- neoplastic agent. Glucose and some glucose analogs are phosphorylated upon entry into a cell, resulting in accumulation and, in the case of certain analogs, increased toxicity due to accumulation of the phosphorylated forms in the cell. Expression and activity assays for glucose-6-phosphatase are known (see, e.g., Schmoll *et al.*, 2001, *Cancer Letters*, 167:85-90; Taketa *et al.*, 1998, *Cancer Res.* 48:467-74) In some embodiments, the ratio of the glucose transporter level over the glucose-6-phosphatase level is determined. The higher the ratio, the more susceptible the cancer of the patient to the treatment with an anti-neoplastic agent comprising glucose or a glucose analog.

[0072] In another embodiment, the transporter level in the cancer sample is expressed as a ratio of transporter level in the cancer sample to the transporter level in a matching non-tumor tissue from the same patient. A "matching non-tumor tissue" refers to a sample of non-cancerous tissue, preferably, a matching non-tumor or non-malignant sample is derived from the same organ as the organ from which the tumor sample is derived. Most preferably, the matching non-tumor sample is derived from the same organ tissue layer from which the tumor sample is derived. Also, it is preferable to take a matching non-tumor tissue sample at the same time a tumor sample is biopsied. For example, when obtaining tissues from a pancreatic tumor for purposes of the present invention, the practitioner can also obtain non-malignant pancreatic tissue from a location removed from the tumor. Then, glucose

transporter levels are measured in each sample, and if the measured level in the tumor sample exceeds the level in the normal sample, then the tumor is determined to be susceptible to treatment with a glucose transporter anti-cancer agent.

[0073] In a related embodiment, the transporter level in the cancer sample is expressed as a ratio of transporter level in cancerous (transformed) cells in a cancer sample to the transporter level in non-cancerous cells from the same sample. This ratio can be conveniently determined, because when a cancer sample is obtained from a subject (e.g., by biopsy), the sample frequently also contains non-cancerous cells which can be identified by observation or using histological methods, or by detection of the absence of a cancer-specific molecular marker.

[0074] Although transporter levels are most often determined in a single cancer sample from a subject (and optionally a single non-cancer sample); in some embodiments, several biological samples of the cancerous and/or normal tissue are obtained from a single subject to obtain a mean value for the subject.

4.1. REFERENCE VALUES

[0075] The level of expression of glucose transporters in the cancer sample is compared to a reference value to determine relative susceptibility to treatment with an anti-neoplastic agent. For a given transporter, it will be appreciated that the reference value for determining susceptibility to treatment may differ for different cancers. A variety of methods can be employed to determine the reference value for a particular transporter.

[0076] In one embodiment of the method, a reference value for a cancer type is determined by assessing transporter level(s) in cancer samples from a number of different patients, herein referred to as the "survey population." Generally, the patients in the survey population and the subject from whom the cancer sample is obtained all have the same type of cancer. For example, in one embodiment, pancreatic cancer samples from 10, 50, 100, 200, 500, or 1000 or more patients are analyzed for GLUT2 levels. Classification of cancer by "type" will be within the skill of, and at the discretion of, the practitioner. Usually the cancer "type" is based on the tissue of origin (e.g., pancreatic cancer, breast cancer, for example) but can also be based on stage, metastasis, degree of hypoxia, presence of prognostic markers, and the like. Another parameter for classifying cancer by type is the demonstrated effect of an anti-

cancer agent or class of anti-cancer agents (including anti-neoplastic agents or a specific anti-neoplastic agent) in patients with the cancer "type". As used in this context, "effect" includes response (or lack thereof) to treatment with anti-cancer agent(s), length of increased survival of treated patients, and the like. In addition to being matched for cancer type, the survey population and subject also can be matched according to patient characteristics such as sex, age and ethnicity, and other criteria.

[0077] Transporter levels in the survey population (i.e., herein referred to as the "survey values") will form a distribution, which may be unimodal, bimodal or multimodal. In one embodiment of the invention, this distribution is used to determine the appropriate reference value, with, as previously discussed, cancers that express a level of transporter higher than the reference value being identified as susceptible to treatment with a neoplastic agent. The reference value can be a single cut-off value, such as a median or mean of the distribution of survey values in which patient samples with transporter values above the mean or median are identified as susceptible to treatment with an anti-neoplastic agent. In a related embodiment, the reference value is a percentile of the distribution of survey values, such as the 60^{th} percentile, the 75^{th} percentile, the 90^{th} percentile and the like. Alternatively, the reference value is a range, for example, where the distribution of survey values is divided into equal (or unequal) portions, such as quadrants, and each quadrant is correlated with a level of susceptibility to treatment (e.g., with the lowest quadrant correlated with least susceptibility and the highest quadrant correlated with the highest susceptibility). In some cases, the distribution of survey values is bimodal (with a low range and a high range). In such cases, cancer samples that have a glucose transporter level within or greater than the higher range are identified as originating from cancers susceptible to treatment with an anti-neoplastic agent. In some cases the distribution of survey values is multimodal (with a low range and more than one higher range). In such cases, cancer samples that have a glucose transporter level greater than the low range, and preferably within the higher of the higher ranges are identified as originating from cancers susceptible to treatment with an anti-neoplastic agent.

[0078] In some cases, the reference value may be zero or near zero, such as when a cancer type (for purposes of discussion, referred to herein as cancers of "tissue x") includes some tumors that do not express the specified glucose transporter or transporters and other tumors that do express the transporter or transporters. In such a case, any detectable expression of the transporter(s) is sufficient to indicate the patient is a candidate for treatment with an anti-neoplastic agent.

[0079] It will be appreciated that, so long as the units of the survey values (and thus the reference value) are the same as or similar to the units used to express transporter levels in the cancer sample (or, alternatively, the survey values and cancer sample levels can be otherwise compared), the particular method of describing transporter levels is not critical, but will depend on the nature of the cancer, the assay methods used, and the preference of the practitioner.

[0080] In another embodiment, the survey population is composed of subjects not having a cancer (e.g., healthy individuals), and a reference value for a cancer type is determined by assessing transporter level(s) in normal (non-malignant) samples from a matching tissue of subjects not diagnosed as having cancer (e.g., healthy subjects) who constitute a "survey population." The normal samples are matched to the cancer type in that they are derived from the same organ as the organ from which the tumor sample is derived, and preferably from the same tissue layer. The reference value is a value greater than the median or average of the normal values, and preferably is a value that is greater than that of 75% of the normal samples, often greater than that of 90% of the normal samples, sometimes greater than 95% or even greater than 99%.

[0081] Accordingly, the reference value may be determined using routine methods, e.g., collecting cancer samples (or normal samples) and determining transporter levels. Determination of particular threshold levels for assessing susceptibility of a tumor to treatment, selection of appropriate ranges, patient categories, cancer types, and the like are well within the skill of medical professionals guided by this disclosure. It will be understood that standard statistical methods may be employed by the practitioner in making such determinations. See, e.g., Principles of Biostatistics by Marcello Pagano *et al.* (Brook Cole; 2000); Fundamentals of Biostatistics by Bernard Rosner (Duxbury Press, 5th Ed, 1999); Biostatistics: a Foundation for Analysis in the Health Science by Wayne W. Daniels (John Wiley & Sons, 3rd Ed.; 1983); and Clinical Epidemiology and Biostatistics by Knapp and Miller (William and Wilkins, Harual Publishing Co. Malvern, PA 1992).

[0082] As is discussed in greater detail below, transporter levels may be determined for a single transporter. Alternatively, levels of several different transporters can be determined (e.g., simultaneously and/or in total without distinction as to the level of each). In a different embodiment, all of the transporters in the sample are assayed for the ability of the cell to

transport a compound (such as a specified anti-neoplastic agent or a compound that should be transported by the same transporter that transports the specified anti-neoplastic agent).

4.2. ASSAYS

[0083] Methods of determining glucose transporter levels in a biological sample are known in the art, and such assays are further described herein solely for illustration and convenience of the practitioner. Suitable methods include, for example, assays for the transporter proteins, assays for transporter RNAs and assays for transporter activity.

[0084] Assay methods are described below and in the Examples. The assays employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry, nucleic acid chemistry, and immunology, all of which are within the skill of the ordinarily skilled artisan. Such techniques are explained fully in the literature, such as, MOLECULAR CLONING: A LABORATORY MANUAL, second edition (Sambrook *et al.*, 1989) and MOLECULAR CLONING: A LABORATORY MANUAL, third edition (Sambrook and Russel, 2001) (the two previous citations being jointly referred to herein as "Sambrook"); CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (F.M. Ausubel *et al.*, eds., 1987, including supplements and revisions through 2003); PCR: THE POLYMERASE CHAIN REACTION (Mullis *et al.*, eds., 1994); Harlow and Lane, 1988, ANTIBODIES, A LABORATORY MANUAL, Cold Spring Harbor Publications, New York, *and* Harlow and Lane, 1999, USING ANTIBODIES: A LABORATORY MANUAL Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (jointly referred to herein as "Harlow and Lane"), METHODS IN CELL BIOLOGY VOLUME 37: ANTIBODIES IN CELL BIOLOGY, Asai, ed. Academic Press, Inc. New York (1993); BASIC AND CLINICAL IMMUNOLOGY 7th Edition, Stites & Terr, eds. (1991); Hames *et al.*, ed., NUCLEIC ACID HYBRIDIZATION, A PRACTICAL APPROACH IRL Press, (1985); and Beaucage *et al.*, eds., CURRENT PROTOCOLS IN NUCLEIC ACID CHEMISTRY, 2000, John Wiley & Sons, Inc., New York).

[0085] The selection of a particular assay method for a given patient is, in view of this disclosure, within the skill of the practitioner, and can depend on a number of factors, including the type and stage of the cancer, whether a resection has been performed, the availability of cancer samples, and their type, which can be, for example, tissue specimens or

extracts or cells or cell lysates or extracts or supernatants from the same, as well as the convenience of the practitioner and the availability and cost of reagents.

4.2.1 BIOLOGICAL SAMPLES

[0086] Levels of glucose transporters in a cancer sample or in non-cancerous tissue can be determined by obtaining a biological sample from a subject and detecting the presence or amount of glucose transporter protein, mRNA, transporter activity, and other markers for transporter expression. For convenience, the term "cancer sample" is used to refer to the material (either cellular or cell derived) in which glucose transporters are measured. Generally, the cancer sample is from a human. Transporter levels can be measured in tissue specimens, tissue extracts, cells, cell lysates, cell extracts, body fluids, supernatants from preneoplastic cell lysates, or supernatant from neoplastic lysates using methods known in the art.

[0087] The cancer sample can be obtained from, and the methods of the present invention applied to, any cancer, including but not limited to leukemia, breast cancer, skin cancer, bone cancer, prostate cancer, liver cancer, lung cancer, brain cancer; cancer of the larynx, gallbladder, rectum, parathyroid, thyroid, adrenal, neural tissue, head and neck, colon, stomach, bronchi, and kidneys; basal cell carcinoma, squamous cell carcinoma of both ulcerating and papillary type, metastatic skin carcinoma, osteo sarcoma, Ewing's sarcoma, veticulum cell sarcoma, myeloma, giant cell tumor, small-cell lung tumor, islet cell carcinoma, primary brain tumor, acute and chronic lymphocytic and granulocytic tumors, hairy-cell tumor, adenoma, hyperplasia, medullary carcinoma, pheochromocytoma, mucosal neuromas, intestinal ganglloneuromas, hyperplastic corneal nerve tumor, marfanoid habitus tumor, Wilm's tumor, seminoma, ovarian tumor, leiomyomater tumor, cervical dysplasia and *in situ* carcinoma, neuroblastoma, retinoblastoma, soft tissue sarcoma, malignant carcinoid, topical skin lesion, mycosis fungoide, rhabdomyosarcoma, Kaposi's sarcoma, osteogenic and other sarcoma, malignant hypercalcemia, renal cell tumor, polycythermia vera, adenocarcinoma, glioblastoma multiforma, leukemias, lymphomas, malignant melanomas, and epidermoid carcinomas.

[0088] Methods of obtaining tissue samples are well known in the art and will vary according to the type and location of a tumor and preferences of the physician. In one embodiment, the sample is obtained from surgically excised tissue. Tissue samples and

cellular samples can also be obtained without invasive surgery, for example by punctuating the chest wall or the abdominal wall or from masses of breast, thyroid or other sites with a fine needle and withdrawing cellular material (fine needle aspiration biopsy).

[0089] In another embodiment, the biological samples are bodily fluids that may contain cancer cells, which can be, for example, blood, breast exudate (e.g., nipple aspirate fluid), fecal suspensions, sputum, mucous, urine, lymph, cytosols, ascites, pleural effusions, amniotic fluid, or bladder washes.

[0090] The biological samples obtained can be used in fresh, frozen, or fixed (e.g., paraffin-embedded) form, depending on the nature of the sample, the assay used, and the convenience of the practitioner. Although fresh, frozen and fixed materials are suitable for various RNA and protein assays, generally, fresh tissues will be preferred for *ex vivo* measurements of activity.

[0091] Fixed tissue samples can also be employed. Tissue obtained by biopsy is often fixed, usually by formalin, formaldehyde, or gluteraldehyde, for example, or by alcohol immersion. Fixed biological samples are often dehydrated and embedded in paraffin or other solid supports, as is known in the art. See the reference Plenat *et al.*, 2001, *Ann. Pathol.* 21:29-47. Non-embedded, fixed tissue, as well as fixed and embedded tissue, can be used in the present methods. Solid supports for embedding fixed tissue can be removed with organic solvents to enable subsequent rehydration of preserved tissue.

[0092] In some cases, the assay for transporter levels includes a step of cell or tissue culture. Culture methods are well known in the art. For example, cells from a biopsy can be disaggregated using enzymes (such as collagenase and hyaluronidase) and or physical disruption (e.g., repeated passage through a 25-gauge needle) to dissociate the cells, collected by centrifugation, and resuspended in desired buffer or culture medium for culture, immediate analysis, or further processing.

4.2.2 PROTEIN-BASED DETECTION

[0093] In one aspect of the invention, the level of glucose transporter is determined by measuring transporter protein. Most conveniently, this is done using an immunoassay (such as, without limitation, Western analysis, flow cytometry, EIA, ELISA, RIA, competition

immunoassay, dual antibody sandwich assay, immunochemical, immunocytochemical and immunohistochemical methods, agglutination assay, and immunoprecipitation).

[0094] Antibodies for use in immunoassays are readily obtainable. Antibodies specific for glucose transporter proteins (including monoclonal and polyclonal antibodies, Fab and $F(ab')_2$ fragments, and recombinantly produced equivalents) can be prepared using routine methods. See, for example, Harlow and Lane, *supra*; Kohler and Milstein, 1975, *Nature* 256:495.

[0095] Antibodies can be generated by using a purified transporter protein, or portion thereof (whether purified from tissue or recombinantly expressed) as immunogen. Alternatively, a synthetic peptide or polypeptide sequence can be used to generate or select antibodies of interest. See, Huse *et al.*, 1989, *Science* 246:1275-81; and Ward *et al.*, 1989, *Nature* 341:544-46. For some assays it may be preferable to use an antibody that binds an extracellular region of a transporter(s), and such specificity can be obtained by using an extracellular epitope in generation or selection of the antibody. Likewise, antibodies can be selected that recognize an epitope found in more than one transporter (e.g., a conserved sequence) to allow simultaneous assays for multiple transporters. Table 1 provides accession numbers for DNA and protein sequences of selected transporter proteins and genes, which may be used to express or synthesize antigen.

[0096] Monoclonal and polyclonal antibodies for glucose transporters have been described in the scientific literature (see, Bukhard *et al.*, 2004, *Oral Oncology*, 40:28-35 [anti-SGLT1]; Hasper *et al.*, 1988, *J. Biol. Chem.* 263:398-403 [describing rabbit antiserum raised against a 13-amino acid peptide corresponding to the C-terminal of GLUT1]; Rogers *et al.*, 2003, *Am. J. Physiol. Endocrinol. Metab.*. 282:E733-738 [describing a rabbit polyclonal anti-GLUT12 antibody, R1396, raised to the unique 16 C-terminal amino acids of human GLUT12] and are widely available from commercial vendors [e.g. Research Diagnostics Inc., Alpha Diagnostics, Inc., East Acres Biologicals, DAKO, Hamburg, Germany, Chemicon International, Inc., Temecula, CA, (see Table 2).

TABLE 2

Characteristics of Exemplary Commercially Available Antibodies

Rabbit anti-SGLT-1 (Antigen: Synthetic peptide corresponding to amino acids 402-420 of the putative extracellular loop of SGLT-1 of rabbit small intestine)
Rabbit anti-GLUT-1 [MYH antibody, advantageous because it detects extracellular region] (Antigen: 15 amino acid synthetic peptide corresponding to the exofacial loop of the human GLUT-1 (Thr-Trp-Asn-His-Arg-Tyr-Gly-Glu-Ser-Ile-Pro-Ser-Thr-Thr-Leu))
Rabbit anti- GLUT-2 (Antigen: Synthetic peptide (40-55 amino acid) corresponding to the exoplasmic loop between helices 1 and 2 of GLUT2)
Rabbit anti-GLUT-3 (Antigen: A 12 amino acid synthetic peptide corresponding to the carboxy terminus of human GLUT 3 (SIEPAKETTTNV))
Rabbit anti-GLUT-4 (Antigen: Synthetic peptide corresponding to the C-terminus (amino acids 498-510) of mouse Glut-4)
Rabbit anti-GLUT-5 (Antigen: A 12 amino acid synthetic peptide specific for the carboxy terminus of the human GLUT 5 sequence (ELKELPPVTSEQ))
Rabbit anti-GLUT-8 (Antigen: An 11 amino acid sequence near the C-terminus of mouse Glut-8).

[0097] There are a variety of protein-based approaches for detecting glucose transporter levels. For example, transporter proteins from a sample can be purified (e.g., by chromatography or electrophoresis) or enriched (e.g., by cell fractionation) and measured by Western analysis, or competitive or noncompetitive immunoassays (such as ELISA or other sandwich assays) can be used, with or without such purification or enrichment of the transporter. Further guidance regarding the methodology and steps of a variety of antibody assays is provided, for example, in U.S. Patent No. 4,376,110 to Greene; "Immunometric Assays Using Monoclonal Antibodies," in ANTIBODIES: A LABORATORY MANUAL, COLD SPRING HARBOR LABORATORY, CHAP. 14 (1988); Bolton and Hunter, "Radioimmunoassay and Related Methods," in HANDBOOK OF EXPERIMENTAL IMMUNOLOGY (D.M. Weir, ed.), Vol. 1, chap. 26, Blackwell Scientific Publications, 1986; Nakamura, *et al.*, "Enzyme Immunoassays: Heterogeneous and Homogenous Systems," in HANDBOOK OF EXPERIMENTAL IMMUNOLOGY (D.M. Weir, ed.), Vol. 1, chap. 27, Blackwell Scientific Publications, 1986; and Coligan, *supra.*

[0098] For example, Western blot (immunoblot) analysis can be used to detect and quantify the presence of transporters in the sample. Western blots are useful for detecting glucose transporters in homogenized tissue samples and body fluid samples. Western blot techniques are routinely used in the art to determine protein levels in a sample. See Sambrook, *supra.* Generally, samples are homogenized and cells are lysed using a detergent such as Triton-X. The material is then separated by gel electrophoresis, transferred to a

suitable solid support (such as a nitrocellulose filter, a nylon filter, or derivatized nylon filter), and incubated with antibodies that specifically bind the transporter. These antibodies may be directly labeled or alternatively may be subsequently detected using labeled antibodies (*e.g.*, labeled sheep anti-mouse antibodies) that specifically bind to the anti-transporter antibodies.

[0099] In another approach, immunohistological or immunocytological methods are used for assay of glucose transporter levels. Tissue sample preparation procedures are known in the art (see, e.g., Harlow, *supra*) and described in the Examples. Typically, tissue obtained by biopsy is fixed and embedded in paraffin or other solid supports, or placed on a solid support without embedding. Frozen sections may also be used. See Plenat *et al.*, 2001, *Ann. Pathol.* 21(1):29-47. Further treatment of the tissue section prior to, during, or following the immunostaining may be carried out; for example, epitope retrieval methods, such as heating the tissue sample in citrate buffer may be carried out. See, e.g., Leong *et al.*, 1996, *Appl. Immunohistochem.* 4:201. Following the optional blocking step, the tissue section is exposed to primary antibody (such as those described above, preferably those that bind to extracellular epitopes) for a sufficient period of time and under suitable conditions such that the primary antibody binds to the glucose transporter in the tissue sample. Appropriate conditions for achieving this can be determined by routine experimentation. The extent of binding of antibody to the sample can be assayed either directly or indirectly. For example, in one embodiment, a primary or secondary antibody is fluorescently labeled, and immunofluorescence microscopy is used to detect binding.

[0100] In another approach, anti-glucose transporter antibody is allowed to bind directly to cells (e.g., cells expressing transporters) and the antibody determined directly (e.g., using a fluorescent tag or an enzyme-labeled primary antibody) or indirectly (e.g., using a labeled secondary antibody). An example of such a binding assay is flow cytometry such as fluorescence activated cell analysis (see, e.g., Salih *et al.*, 2000, *J. Immunology* 165:2903-10). Tumor tissue samples can first be dissociated prior to the FACS analysis. Specifically, tumors are exercised and minced in buffer. A tumor cell suspension is obtained by adding enzymes (such as collagenase and hyaluronidase) to dissociate the cells. Cells are then washed in washing buffer and passed several times through a 25-gauge needle. After centrifugation, cells are resuspended in a buffered solution. In an embodiment, the flow cytometry results are displayed as cell number vs. staining intensity (e.g,. number of transporters) and the distribution for the sample compared to those of a survey population.

[0101] Quantification of the expression of glucose transporters can be performed using any suitable method that is compatible with the assay. For example, for Western blot analysis, the intensity of the bands can be scanned and quantified. For immunohistochemistry analysis of tumor biopsy, the biopsy sections can be given a score according to the intensity of the glucose transporter staining, for example, 0 for no staining, 1 for light staining, 2 for medium staining, and 3 for heavy staining. In some embodiments, it is possible to distinguish membrane staining from cytoplasmic staining. In those embodiments, the membrane staining may be scored. For methods of quantitation, also see, for example, Raleigh *et al.*, 2001, "Semiquantitative immunohistochemical analysis for hypoxia in human tumors" *Int. J. Radiat. Oncol. Biol. Phys.*, 2001 Feb 1, 49(2):569-74 and Hatanaka *et al.*, 2001, "Quantitative immunohistochemical evaluation of HER2/neu expression with HercepTest™ in breast carcinoma by image analysis" *Pathol. Int.* 51:33-6.

[0102] As discussed above, in some embodiments, the level of a reference protein, one relatively indifferent to cancer status, is also measured. Suitable reference proteins include actin, tubulin, and glyceralaldehyde-3-phosphate dehydrogenase. When a reference protein is employed, the glucose transporter level is "normalized" to the reference protein level by dividing the former by the latter. The resulting ratio is compared to a predetermined ratio as described herein.

4.2.3 RNA-BASED DETECTION

[0103] In another embodiment, levels of glucose transporters are determined by measuring transporter mRNA levels. Assays for the messenger RNA levels of one or more glucose transporters in a sample include Northern analysis, polymerase chain reaction (PCR), including quantitative PCR, ligase chain reaction (LCR), RNase protection, *in situ* hybridization, serial analysis of gene expression (SAGE), differential display (DD) analysis, RNA arbitrarily primed (RAP)-PCR, restriction endonucleolytic analysis of differentially expressed sequences (READS), amplified restriction fragment-length polymorphism (AFLP), total gene expression analysis (TOGA), and use of internal standard competitive template primers (CTs) in a quantitative multiplex RT-PCR method [StaRT-(PCR)], high density cDNA filter hybridization (HDFCA) analysis, suppression subtractive hybridization (SSH), differential screening (DS), high-density cDNA or oligonucleotide arrays. For review, see, Ahmed, 2002, "Molecular techniques for studying gene expression in carcinogenesis" *J. Environ. Sci. Health Part C Environ. Carcinog. Ecotoxicol. Rev.* 20:77-116. Also see:

Lipshutz *et al. Nat. Genet.* 1999, 21:20-4; U.S. Pat. Nos. 5,445,934; 5,578,832; 5,556,752; and 5,510,270; Schena *et al.*, 1995, *Science* 270:467-70 (high density cDNA arrays); Lynn *et al.*, 1983, *Proc. Natl. Acad. Sci.* 80:2656; Zinn *et al.*, 1983, *Cell* 34:865; and Sambrook and Ausubel, *supra* (ribonuclease protection assays).

[0104] Methods for isolating RNA from tissues are well known (see, e.g., Ausubel, *supra*; Rapley *et al.*, "RNA Isolation and Characterization Protocols" 1998. Humana Press, Inc. Totowa, New Jersey; Farrell *et al.*, "RNA Methodologies" 1993. Academic Press, Inc. San Diego, California). RNA for amplification also can be isolated from fixed and/or paraffin-embedded tissue sections. See Stanta *et al.*, "RNA Extracted from Paraffin-Embedded Human Tissues is Amenable to Analysis by PCR Amplification" *BioTechniques* 11 (3):304-308. 1991; Finke *et al.*, "An Improved Strategy and a Useful Housekeeping Gene for RNA Analysis from Formalin-Fixed, Paraffin-Embedded Tissues by PCR" *BioTechniques* 14 (3):448-453. 1993; De Andres *et al.*, "Improved Method for mRNA Extraction from Paraffin-Embedded Tissues. *BioTechniques* 18 (1):42-43. 1995"; Rupp *et al.*, "Purification and Analysis of RNA from Paraffin-Embedded Tissues". *BioTechniques* 6 (1):56-60. 1988; Sorg *et al.*, "Detection of Borna Disease Virus RNA in Formalin-Fixed, Paraffin-embedded Brain Tissues by Nested PCR". *Journal of Clinical Microbiology* 33 (4):821-823. 1995; Werner *et al.*, "Effect of formalin tissue fixation and processing on immunohistochemistry" *American Journal of Surgical Pathology* 24 (7):1016-1019. 2000. See also, U.S. Patent No 6,602,670, and publications cited in the specification and in the "references cited" section of that patent.

[0105] For illustration, in one embodiment, the level of glucose transporter is determined using quantative PCR. Quantative PCR refers to methods that are able to quantitate the amount of cDNA derived from reverse transcription of mRNA in cells from a tissue sample. The mRNA in the cells obtained from a tissue sample can be reverse transcribed by methods known in the art, including, for example, Sambrook, *supra*, and Ausubel, *supra*. Quantative PCR is carried out, for example, by amplifying RNA according to standard PCR methods using a primer which is modified to allow capture of the resulting product onto a solid surface. For example, the 5' or 3' primer can be biotinylated at the 5' terminus or 3' terminus, respectively, to allow capture of the resulting product onto avidin-coated microplates. The product attached to the solid surface is then quantitated, for example, by hybridizing to an oligonucleotide probe having a quantifiable label attached thereto. A "quantifiable label" can be, for example, a radioactive atom or group. The level of transporter mRNA expression is then assessed by determining the level of radioactivity in the

sample. Alternatively, a "quantifiable label" is a group or moiety such as digoxigenin. In that embodiment, the PCR product is quantitated by addition of 1) an anti-digoxigenin antibody that is coupled with alkaline phosphatase and 2) a colorigenic substrate for alkaline phosphatase, followed by absorptometry. Optionally, the result can be normalized with respect to a cDNA standard curve.

[0106] A variety of so-called "real time amplification" methods or "real time quantitative PCR" methods can also be utilized to determine the quantity of glucose transporter mRNA present in a sample. Such methods involve measuring the amount of amplification product formed during an amplification process. Fluorogenic nuclease assays are one specific example of a real time quantitation methodology that can be used to detect and quantitate glucose transporter transcripts. In general, such assays continuously measure PCR product accumulation using a dual-labeled fluorogenic oligonucleotide probe, an approach frequently referred to in the literature simply as the "TaqMan" method. The probe used in such assays is typically a short (ca. 20-25 bases) polynucleotide that is labeled with two different fluorescent dyes. The 5' terminus of the probe is typically attached to a reporter dye and the 3' terminus is attached to a quenching dye, although the dyes can be attached at other locations on the probe as well. For measuring a glucose transporter transcript, the probe is designed to have at least substantial sequence complementarity with a probe binding site on a glucose transporter transcript. Upstream and downstream PCR primers that bind to regions that flank glucose transporter coding sequences are also added to the reaction mixture for use in amplifying the glucose transporter polynucleotide. When the probe is intact, energy transfer between the two fluorophors occurs and the quencher quenches emission from the reporter. During the primer extension phase of PCR, the probe is cleaved by the 5' nuclease activity of a nucleic acid polymerase such as Taq polymerase, thereby releasing the reporter dye from the polynucleotide-quencher complex and resulting in an increase of reporter emission intensity that can be measured by an appropriate detection system.

[0107] In one embodiment, *in situ* hybridization is used to detect glucose transporter sequences in a sample. In situ hybridization assays are well known and are generally described in Angerer *et al.*, METHODS ENZYMOL. 152: 649-660 (1987). For example, *in situ* hybridization can be performed as described in Ruglowski *et al.*, 2003, *Am. J. Clin. Pathol.* 120:691-698. Paraffin embedded tissue samples can be used. Briefly, the slides are pretreated with proteinase K and acetylation agents, and incubated with ^{33}P-labeled sense and anti-sense cRNA riboprobes of glucose transporter RNAs. After incubation, the slides are washed in washing buffer, digested with RNase A, washed again, and subject to dehydration.

After dehydration, slides are coated with silver emulsion and exposed for a certain amount of time (for example 8-10 days). The intensity of the silver stain can be examined using methods known in the art, for example by darkfield microscopy. Digitized images can also be examined. *In situ* hybridization methods are also described in Harris, 1996, *Anal. Biochem.* 243:249-256; Singer *et al.*, 1986, *Biotechniques* 4:230-250; Haase *et al.*, 1984, METHODS IN VIROLOGY, vol. VII, pp. 189-226; and NUCLEIC ACID HYBRIDIZATION: A PRACTICAL APPROACH (Hames *et al.*, eds., 1987).

[0108] Quantitation of the expression of glucose transporters can be performed using any method that is compatible for the method of the assay.

[0109] Probes and primers useful for detection of glucose transporter protein RNAs are readily obtainable or can be prepared using routine methods based on the nucleotide sequences of glucose transporters. Primers useful for amplification-based detection can be readily designed based on knowledge of the target sequence (sequence to be detected). Particularly suitable primers for some assays have a T_M close to 60°C, are between 100 and 600 [this seems more like a probe length than a primer length] bp in length and are specific for the region to be amplified (which can be determined by BLAST analysis of GenBank and the prospective primers, for example using software such as Oligo 6 (Molecular Biology Insights, Inc.; http://www.oligo.net). Preferably primers span an intron/exon splice junction so that amplification of desired RNA/cDNA can be easily separated from that of contaminating genomic DNA. It is well known that primers should be selected that do not form duplexes within themselves or with the other primer of the pair (if present) used for amplification. Probes and primers are described in the literature; see for example, Helmke *et al.*, 2004, *Oral Oncology*, 40:28-35 (SGLT1, SGLT2); U.S. Patent Application No. 20030228592 (GLUT8); Rogers, 2003, *Cancer Letters* 193:225-33 (GLUT12, GLUT4); Brukhard *et al.*, 2004, *Oral Oncology* 40:28-35 (SGLT1 and SGLT2); and Auguatin *et al.*, Jan 22, 2004, *J. Biol. Chem.* (GLUT9). Many other primers and probes are described in the scientific literature. Other probes and primers, for illustration and not limitation, are shown in Table 3. Other probes can be made by routine methods, for example, they can be amplified using primers listed in Table 3.

TABLE 3

Gene	Primer Name	Primer Sequence	Primer Length (bp)	Tm (°C)	CG%	Product size (bp)
GLUT1	F_1_(1231->1252)	TGACCATCGCGCTAGCACTGC	21	61.7	61.9	759
GLUT1	R_2_(1969<=1990)	TCCACCCTCAGGCATGGAACC	21	61.2	61.9	
GLUT1	F_4_(1341->1362)	TGGTTCATCGTGGCTGAACTC	21	57	52.4	1017
GLUT1	R_1_(2337<=2358)	TGAGTTTGCAGGCTCCCACAG	21	59.5	57.1	
GLUT1	F_2_(1192->1213)	TCATAGGCCTCGCTGGCATGG	21	61.3	61.9	1171
GLUT1	R_3_(2342<=2363)	AGCAGTGAGTTTGCAGGCTCC	21	59.8	57.1	
GLUT2	F_3_(980->1001)	TGTCTGGTGTGCGAGCCATCC	21	61.5	61.9	2295
GLUT2	R_3_(3254<=3275)	GATCAGTGCTCCAGTTGGTGG	21	57.9	57.1	
GLUT2	F_2_(1232->1253)	TGATGCTGCATGTGGCTCAGC	21	60.3	57.1	2044
GLUT2	R_1_(3255<=3276)	TGATCAGTGCTCCAGTTGGTG	21	56.9	52.4	
GLUT2	F_3_(980->1001)	TGTCTGGTGTGCGAGCCATCC	21	61.5	61.9	868
GLUT2	R_2_(1827<=1848)	ACAGCAGCTTTTGGCCTGTGG	21	60.6	57.1	
GLUT3	F_1_(1451->1472)	AGTGGCCGGCTGCTCCAACTG	21	64.2	66.7	2254
GLUT3	R_3_(3684<=3705)	AGACGGAGTCTCGCCCTGTGG	21	62.5	66.7	
GLUT3	F_4_(1589->1610)	AGTCCCTGAGACCCGTGGCAG	21	62.7	66.7	700
GLUT3	R_1_(2268<=2289)	ACAAACCTGCACATTCGGCAC	21	58.6	52.4	
GLUT4	F_2_(762->783)	TGGGCCTCACAGTGCTACCTG	21	60.9	61.9	379
GLUT4	R_2_(1420<=1441)	AAGTTGCTCGTCCAGTTGGAG	21	57	52.4	
GLUT4	F_3_(720->741)	TGGAGTCCCTCCTGGGCACTG	21	62.7	66.7	664
GLUT4	R_3_(1363<=1384)	TGGCTGAAGAGCTCGGCCACG	21	63.6	66.7	
GLUT4	F_3_(720->741)	TGGAGTCCCTCCTGGGCACTG	21	62.7	66.7	721
GLUT4	R_2_(1420<=1441)	AAGTTGCTCGTCCAGTTGGAG	21	57	52.4	
GLUT5	F_1_(102->123)	TGCCCTGGCAACCCTGATAGC	21	61.7	61.9	1071
GLUT5	R_2_(1152<=1173)	TATGGCATCCAGGACACTGTG	21	56.5	52.4	
GLUT5	F_2_(619->640)	GATGGCTGGCCGATCCTGCTG	21	62.7	66.7	1229
GLUT5	R_1_(1827<=1848)	AGCCACGTTACCAGGAGCCAC	21	61.3	61.9	
GLUT5	F_3_(148->169)	GGGTACAACGTGGCTGCTGTC	21	60.5	61.9	1025
GLUT5	R_2_(1152<=1173)	TATGGCATCCAGGACACTGTG	21	56.5	52.4	
GLUT6	F_1_(482->503)	ACAGCTGCCTGCATCCCGGTG	21	64.2	66.7	991
GLUT6	R_7_(1452<=1473)	TGAACACCAGGCTCACCAAGC	21	59.7	57.1	
GLUT6	F_2_(762->783)	TCGATGTCCACTGGGAGTTCG	21	58.3	57.1	980
GLUT6	R_1_(1721<=1742)	AGCAGTGCTACCTGTCCCGAG	21	60.5	61.9	

GLUT6	F_3_(266->287)	ACCAAATCCCAGGCATCCTGG	21	59.5	57.1	1485
GLUT6	R_4_(1730<=1751)	TGGCTGGACAGCAGTGCTACC	21	61.3	61.9	
GLUT8	F_1_(956->977)	TCCAGGTGCTGTTCACAGCTG	21	59.4	57.1	775
GLUT8	R_4_(1710<=1731)	ACCGCAGGTCTGCAAAGCTCG	21	62	61.9	
GLUT8	F_2_(944->965)	TCGTGGGTGTCATCCAGGTGC	21	61.3	61.9	588
GLUT8	R_5_(1511<=1532)	AGCTTGGAGTCACAGGCTTGC	21	59.8	57.1	
GLUT8	F_1_(956->977)	TCCAGGTGCTGTTCACAGCTG	21	59.4	57.1	416
GLUT8	R_3_(1351<=1372)	TCCATAGGGCCTGAGGACCTC	21	59.7	61.9	
GLUT9	F_2_(3->24)	TGGCTCTAGGGCTGGCACCAG	21	63.2	66.7	668
GLUT9	R_2_(650<=671)	AGCCACGGATCTCCTTGGGTG	21	61	61.9	
GLUT9	F_1_(422->443)	TCGCCATCGGTGGACTTGTGG	21	61.4	61.9	612
GLUT9	R_1_(1013<=1034)	TCACGGTGACCACCTGCCAGC	21	63.8	66.7	
GLUT10	F_1_(1068->1089)	ACTCAGGCCCAAGCTGTCTGG	21	61.3	61.9	1256
GLUT10	R_1_(2303<=2324)	TGGTTGCATGCGCCTGTAGTC	21	59.9	57.1	
GLUT10	F_4_(1642->1663)	ACGGTTCACCCTGAGCTTTGG	21	59.5	57.1	1591
GLUT10	R_2_(3212<=3233)	TGGCAAAGCCAGCTCCAGCAC	21	62.7	61.9	
GLUT11	F_1_(701->722)	TCTTTACGGCTCTGGGGATCG	21	58.2	57.1	561
GLUT11	R_4_(1241<=1262)	AGCAGGTCATCAGGCTGTACC	21	58.6	57.1	
GLUT11	F_2_(211->232)	TCCTTACGGCCTCGGACGCAG	21	62.8	66.7	951
GLUT11	R_3_(1141<=1162)	AGTCCCGATGATCGCGTACTG	21	58.2	57.1	
GLUT11	F_3_(749->770)	AGCTCCTAGGTGGCCCTCAGG	21	62.4	66.7	657
GLUT11	R_6_(1385<=1406)	ACAGCTCTGTGGCCAGGATCC	21	61.1	61.9	
GLUT11	F_5_(450->471)	TGGAGCACTGCTTGCAGGTCC	21	61.9	61.9	615
GLUT11	R_7_(1044<=1065)	TCCATGGCACTGCCCAGAACC	21	61.9	61.9	
GLUT12	F_1_(541->562)	ACGCATTGCCATAGGGGTCTC	21	59.2	57.1	1061
GLUT12	R_2_(1581<=1602)	TCTCGCTGAGCACCAGCCAGG	21	63.3	66.7	
GLUT12	F_4_(1091->1112)	TCCACTGGGGTTGGAGTCGTC	21	60.5	61.9	1384
GLUT12	R_1_(2454<=2475)	TGGGCAGTTGTCCACACTGTG	21	59.7	57.1	
GLUT12	F_4_(1091->1112)	TCCACTGGGGTTGGAGTCGTC	21	60.5	61.9	511
GLUT12	R_2_(1581<=1602)	TCTCGCTGAGCACCAGCCAGG	21	63.3	66.7	
SGLT1	F_1_(1083->1104)	AGGTTGGCTGTACCAACATCG	21	57.1	52.4	477
SGLT1	R_1_(1539<=1560)	AGTTGCTGGGCTCCATGCAGC	21	62.5	61.9	
SGLT1	F_4_(209->230)	TGGTGGCCGATTGGAGCCTCC	21	63.7	66.7	1143
SGLT1	R_2_(1331<=1352)	TGCTGACTGCACAATGGGCAC	21	60.5	57.1	
SGLT1	F_3_(1122->1143)	TGGAGCTCATGCCCAATGGAC	21	59.6	57.1	964

SGLT1	R_3_(2065<=2086)	TCCCTTCAACACCACAGGACG	21	58.9	57.1	
SGLT2	F_2_(175->196)	TGGGCGGCTACTTCCTGGCAG	21	63.5	66.7	909
SGLT2	R_4_(1063<=1084)	ACACGCGCCTGCACACCTCAG	21	64.2	66.7	
SGLT2	F_3_(394->415)	TGCCACAGTACCTGCGCAAGC	21	62.3	61.9	758
SGLT2	R_1_(1131<=1152)	ACCGTTGGGCATGAGCTTCAC	21	60	57.1	
SGLT2	F_4_(894->915)	TGCAGCGACCAGGTCATCGTG	21	61.5	61.9	449
SGLT2	R_7_(1322<=1343)	AGCCAGGCCACCGACACTACC	21	63.2	66.7	
SGLT5	F_6_(643->664)	TCGCAGCTTTTGACCAGATCG	21	57.4	52.4	934
SGLT5	R_7_(1556<=1577)	TGCTCGTTGGCACGTCGCCAG	21	64.2	66.7	
SGLT5	F_1_(843->864)	TGCACCGACCAGGTCATCGTG	21	61.3	61.9	597
SGLT5	R_1_(1419<=1440)	ACTCACGCCGATGAGTGCCAC	21	61.5	61.9	
SGLT5	F_2_(739->760)	TGCCACGTACAGACGCCATGC	21	62	61.9	745
SGLT5	R_5_(1463<=1484)	AGTTGCCCGCTGTTGGAGTCC	21	61.8	61.9	

4.2.4 FUNCTIONAL ASSAYS

[0110] In one embodiment, the level of glucose transporters is determined by measuring glucose transporter activity, e.g., uptake of glucose or a glucose analog *in vitro* or *in vivo*. For example, the activity of a glucose transporter may be measured *in vitro* in a glucose uptake assay. Glucose uptake assays are known in the art (see e.g., Gnudi *et al.*, 1997, *Mol. Endocrinol.* 11:67-76; see also PCT Publication WO 03/082301). In one version, for illustration, and not limitation, a glucose uptake assay measures uptake of a labeled hexose. Cells are obtained by dissociation of tumor tissue samples, as described above. Optionally cells are cultured, and optionally subcultured to confluence, and then detached and resuspended. The cells are then incubated in the presence or absence of cytochalasin B (for GLUTs) or phlorizin (for SGLTs) with radio-labeled (for example, ^{14}C- or ^{3}H- labeled) glucose or glucose analogs (such as 2-deoxyglucose). After incubation, the cells are washed and analyzed for radioactivity. Cytochalasin B inhibits Class I and Class III glucose transporters and so is useful for assessing activity of such transporters. Phlorizin inhibits SGLTs and so is useful for assessing activity of such transporters.

[0111] In some embodiments, the levels of SGLTs are measured, and the glucose analog a-methyl-D-glucoside (a-MDG) is used. The a-MDG can be transported in a sodium-dependent manner with apparent affinity ($K_{0.5}$) of 0.4 (SGLT1) and 2 mM (SGLT2 and SGLT3). In some embodiments, the glucose analogs are transferred only by one or a small

number of specific glucose transporters, so that the uptake of glucose tracers directly correlates with the expression levels of those glucose transporters. For example, it has been reported that an increased glucose uptake of cervical cancer cells was related to an exclusive transmembranous over-expression of GLUT1. An increased glucose uptake in glucose uptake assays thus indicates an increased expression of GLUT1.

[0112] In some embodiments, the glucose analogs are transported by more than one glucose transporter. The amount of total glucose uptake will thus correlate with the overall level of glucose transporters expressed by the cells.

[0113] Another assay for assessing the expression level of glucose transporters is the cytochalasin B binding assay (see Ogura *et al.*, 1999, *J. Endocrinology*, 160:443-452; Ozaki *et al.*, 1996, *Mech Ageing Dev.* 88:149-158; and Gorga & Lienhard, 1981, *Biochem.* 20:5108-13). Briefly, membrane extracts are prepared from the tissue or cell sample and mixed with radio-labeled (such as ^{3}H-labeled) cytochalasin B. At the end of the reaction, the membrane-bound and free cytochalasin B are separated (for example by filtration or by centrifugation). The membrane portion is counted for radioactivity. The level of cytochalasin B binding is correlated with the level of GLUTs.

[0114] In another embodiment, the level of glucose transporters is determined by measuring glucose uptake *in vivo*. A correlation between glucose transporter expression and glucose uptake is made in this method. For example, Kato *et al.*, 2003, *Anti-Cancer Res.* 23:3263-72, reported a correlation of 18-F-fluorodeoxyglucose accumulation with GLUT1 expression in esophageal squamous cell carcinoma. Glucose uptake assays are known in the art. See, e.g., Reske *et al.*, 1997, *J. Nucl. Med.*, 38:1344-48. Generally, glucose uptake is assessed with nonmetabolizable glucose analogues, such as 2-fluorodeoxyglucose (2-FDG), 2-deoxyglucose (2-DG), and 3-O-methylglucose. Glucose transporter activity can be determined, for example, by positron emission tomography using a fluorescently labeled glucose analog.

5. SELECTION OF ANTI-NEOPLASTIC AGENTS

[0115] Certain anti-neoplastic agents are preferentially transported by certain glucose transporters. For example, the drug streptozotocin is transported into pancreatic cancer cells by the GLUT2 transporter. Detection of a high level of GLUT2 transporter in a cancer tissue provides, in accordance with the methods of the invention, a basis for predicting that the tumor is susceptible to treatment with streptozotocin. In general, the higher the level of a

particular transporter the more susceptible the tumor to treatment with an anti-neoplastic agent transported by the particular transporter.

[0116] Thus, in some embodiments, the amount of a glucose transporter is determined before an anti-neoplastic agent is selected for the treatment. Once the glucose transporter level is found to be larger than a predetermined amount, a determination of that the cancer is susceptible to the treatment of a particular anti-neoplastic agent is then made. The choice of the anti-neoplastic agent can be based on a number of factors, including, but not limited to, statistical analysis or substrate specificity of the glucose transporter.

[0117] In some embodiments, the glucose transporter is known to specifically transport a certain anti-neoplastic agent. A correlation is then immediately made between the larger amount of the glucose transporter and the susceptibility to that anti-neoplastic agent. For example, glucofosfamide, also known as glufosfamide, has been reported to be transported by SGLT1. A larger amount of SGLT1 than a predetermined amount is then indicative, in accordance with the methods of the invention, that the cancer is susceptible to treatment with glucofosfamide. In another embodiment, the glucose transporter is known to transport a certain class of anti-neoplastic agents. For example, GLUT2 is known to transport glucose analogs with a non-modified 2-position.

[0118] In some embodiments, the anti-neoplastic agent is preselected, and the level of a glucose transporter for which the agent is a substrate is determined to see whether a patient has a cancer of a type that is susceptible to such treatment. If the cancer sample from the patient has a level of a glucose transporter higher than a reference value, the patient is treated with the anti-neoplastic agent.

[0119] An agent can be identified as a substrate of a particular transporter using art-known methods (as described below). Alternatively, a correlation between levels of a transporter and susceptibility to an agent can be established by identifying a population of patients for whom the agent has proven effective, and determining what transporters are expressed. A level or expression profile for a particular patient can then be compared with the reference profile for the population for whom the treatment has proved effective.

[0120] A variety of substrate assays may be used. For example, hexose uptake and competition assays carried out in primary cell cultures or in *Xenopus* oocytes has been routinely used to determine whether a glucose transporter transports a particular substrate. See, e.g., Garcia *et al.*, 2003, *J. Neurochemistry*, 86:709; Burant *et al.*, *J. Biol. Chem.*, 1992, 267:14523-6; and Veyhl *et al.*, 1998, *Proc. Natl. Acad. Sci. USA* 95:2914-29. Sense cRNAs from the glucose transporter can be prepared as described in Veyhl *et al.* and injected into

defolliculated *Xenopus* oocytes. The oocytes are incubated in the presence or absence of cytochalasin B (for GLUTs) or phlorizin (for SGLTs) containing various radio-labeled (^{14}C, ^{3}H, etc) glucose or glucose analogs to be tested. After incubation, radioactivity in the oocytes can be analyzed. For SGLTs, the electric properties of the glucose or glucose analog uptake can be analyzed by employing the two-microelectrode voltage-clamp techniques.

[0121] Other assays are done in primary cells, such as human carcinoma cells, that are known to express a particular glucose transporter. Cells are grown and subcultured to confluence and then detached and resuspended. The cells are then incubated in the presence or absence of cytochalasin B (for GLUTs) or phlorizin (for SGLTs) with various radio-labeled (for example, ^{14}C- or ^{3}H- labeled) glucose or glucose analogs to be tested. After incubation, the cells are washed and analyzed for radioactivity.

[0122] In a related aspect of the invention, an *in vitro* activity assay is used to determine the susceptibility of a particular tumor (or tumor type) to a particular anti-neoplastic agent. According to this method, cells from the tumor are incubated with the anti-neoplastic agent at a variety of concentrations bracketing the estimated *in vivo* concentration when the agent is administered to a human patient. After one of more time intervals (e.g., 10 m, 30 m, 1 h, and 3 h) the effect of the agent on cell growth and viability is determined and compared to controls in which no agent was added and/or controls in which different anti-neoplastic agents or different anti-cancer agents were added. A reduction in cell growth compared to controls (e.g., as assessed by measuring cell number or a surrogate such as DNA content) or a reduction in viability (e.g., as assessed by monitoring apoptosis in the culture) is an indication that the tumor is susceptible to the agent. In one embodiment, a panel of several (e.g., at least 2, at least 3, at least 4, at least 5 or at least 10) different agents are screened according to this method to identify the most promising candidates for administration to the patient.

6. DEVICES AND METHODS FOR MEASURING LEVELS OF TRANSPORTERS, INCLUDING MULTIPLE TRANSPORTERS

[0123] A number of methods for determining levels of glucose transporters are described herein, and other methods will be apparent to one of skill in the relevant arts upon consideration of this disclosure. In some embodiments of the invention, only one of the assay methods is used for the determination of the glucose transporter levels. In other embodiments, two or more methods are used in combination to determine the glucose transporter levels. For example, samples showing high expression levels of glucose

transporters by immunohistochemistry assays may be further processed to quantify the protein level by Western blot assays, if such information is useful to the practitioner. Similarly, samples showing high glucose uptake may be further examined for the protein or mRNA levels of glucose transporters.

[0124] In some embodiments, the expression level of only one glucose transporter is measured. In other embodiments, the expression levels of two or more glucose transporters are measured. The measurement of two or more glucose transporters can be done sequentially or simultaneously.

[0125] In some embodiments, the expression of more than one glucose transporters is measured. This is particularly relevant when multiple glucose transporters are expressed in the same tissue. The practitioner may (1) determine which transporters are expressed at a level higher than the reference value for that tissue by assaying for several transporters; (2) select anti-neoplastic agents based on the knowledge of which drugs are transported by which transporters.

[0126] For example, multiple glucose transporters, including GLUT1, GLUT4, GLUT5, GLUT8, GLUT12, and HMIT have all been shown to be expressed in adipocytes. The detection of multiple glucose transporters can be done by methods that allow the detection or measurement of multiple glucose transporters, either sequentially or simultaneously.

[0127] Methods of simultaneous measurement of the expression levels of more than one protein is known in the art and the further description provided herein is for illustrative purposes only. For example, microarray analysis can be applied at both the RNA and protein levels, including for the determination of levels of more than one transporter protein or RNA.

[0128] Methods for measuring levels of multiple RNA species expressed in a cell or tissue are well known and include high-density polynucleotide or oligonucleotide arrays (Lipshutz *et al.*, *Nat. Genet.,* 1999, 21:20-4; U.S. Pat. Nos. 5,445,934; 5,578,832; 5,556,752; and 5,510,270), high density cDNA arrays (see, e.g., Schena *et al.*, 1995, *Science* 270:467-7), dot and slot blots, dip sticks, pins, chips, or beads. All of these techniques and devices are well known in the art and are the basis of many commercially available diagnostic kits. These techniques can be easily adapted, guided by the present disclosure, to measuring levels of glucose transporters.

[0129] In some embodiments, the levels of the glucose transporter(s) are determined using a protein array. "Protein arrays" contain different capture agents immobilized at different positions on a solid support that allow independent interaction between each capture agent and its respective target protein. The capture agents can be any molecules that

selectively bind to the target protein, such as antibodies, recombinant proteins, and small chemicals. Protein arrays may be used to determine quantities of specific proteins in a sample. See, Von Eggeling *et al.*, 2000, *BioTechniques* 29:1066-70; Haab, *Proteomics*, 3:2116-22; Wiesner, 2003, *J. Lab. Medicine* 27:85-91; Kodadek, 2002, *Trends Biochem. Sci.* 27:295-300.

[0130] In some embodiments of the invention, levels for only one, or a small number, of glucose transporters are measured. In some cases, for example, the patient's cancer is known to associate at some frequency with the over-expression of a particular glucose transporter. For example, GLUT1 over-expression has been reported to be associated with bladder cancer, breast cancer, cervical cancer, colorectal cancer, esophageal cancer, gastric cancer, head and neck cancer, leiomyosarcomas, ovarian cancer, and thyroid cancer. Accordingly, in one embodiment of the invention, the level of GLUT1 in a cancer sample from a subject with bladder cancer, breast cancer, cervical cancer, colorectal cancer, esophageal cancer, gastric cancer, head and neck cancer, leiomyosarcomas, ovarian cancer, or thyroid cancer is determined.

[0131] GLUT2 over-expression has been reported to be associated with pancreatic cancer or gastric cancer. Accordingly, in one embodiment of the invention, the level of GLUT2 in a cancer sample from a subject with pancreatic cancer or gastric cancer is determined. In one embodiment, the cancer is a pancreatic cancer, and the level of GLUT2 in the cancer sample is measured, and the anti-cancer agent is selected from the group consisting of streptozotocin, glucofosfamide, and a gluSNAP compound.

[0132] GLUT3 over-expression has been reported to be associated with brain cancer or lung cancer. Accordingly, in one embodiment of the invention, the level of GLUT3 in a cancer sample from a subject with brain cancer or lung cancer is determined.

[0133] In some embodiments, the correlation between the cancer and the glucose transporter over-expression will be unknown. In these embodiments, a correlation between the glucose transporter and different types of cancer is first established by methods such as expression profiling before the levels of a selected glucose transporter is determined in a patient. Alternatively, one may simply measure the total level of transporter in a cancer sample and administer the drug if the total level exceeds the reference level by the requisite amount selected for administration of the drug.

7. KITS AND DEVICES

[0134] In one aspect, the invention provides kits and devices for screening patient tumors to determine susceptibility to particular anti-neoplastic agents.

[0135] Kits of the invention comprise reagents for assessing expression of one or more glucose transporter genes, such as probes and/or primers for detection or amplification of glucose transporter gene products. In one embodiment, the probes are nucleic acid probes or primers that specifically bind to one or more polynucleotides transcribed from a glucose transporter gene. In one embodiment, the kit contains antibodies specific for one or a plurality (at least 2, preferably 3, often 4, sometime 5 or more) of different human glucose transporters. The kit of the invention may optionally comprise additional components useful for performing the methods of the invention, such as devices for use in dissociating cells or isolating proteins or nucleic acids from cells. In addition, the kits may contain calibration curves, a reference sample (or protein or nucleic acid) for comparison to a predetermined value, and/or reference values (e.g., in table format) as described herein.

[0136] The present invention also provides kits for determining the level or amount of glucose transporters in human tissue or body fluid samples. In one embodiment, the kits comprise diagnostic screening reagents and instructions for the use thereof in the present method.

[0137] The invention also provides devices useful for the screening methods of the invention. In one aspect, a device comprising immobilized probe(s) specific for one or more glucose transporter gene products (polynucleotides or proteins) is provided. The probes can bind polynucleotides (e.g., based on hybridization), polypeptides or cells expressing polypeptides.

[0138] In some embodiments, a device comprising a single immobilized probe is used for screening. In one embodiment, an array format is used in which a plurality (at least 2, usually at least 4 or more) of different probes is immobilized. The term "array" is used in its usual sense and means that each of a plurality of probes, immobilized on a substrate, has a defined location (address) on the substrate. The number of probes on the array can vary depending on the nature and use of the device. For example, a dipstick format array for detecting glucose transporters can have as few as 1 probe, although usually at least 2, or more than 2, distinct probes are present.

[0139] A variety of binding and hybridization formats are known, including oligonucleotide arrays, cDNA arrays, dip sticks, pins, chips, or beads, Southern, northern, dot

and slot blots. Thus a device comprising a probe for a glucose transporter gene product immobilized on a solid substrate is provided by the invention. Any of a variety of solid supports can be used, which may be made from glass (e.g., glass slides), plastic (e.g., polypropylene, nylon), polyacrylamide, nitrocellulose, or other materials. One method for attaching the nucleic acids to a surface is by printing on glass plates, as is described generally by Schena *et al.*, 1995, *Science* 270:467-470; Shalon *et al.*, 1996, *Genome Res.* 6:639-645. Another method for making microarrays is by making high-density oligonucleotide arrays. See, Fodor *et al.*, 1991, *Science* 251:767-73; Lockhart *et al.*, 1996, *Nature Biotech* 14:1675; and U.S. Pat. Nos. 5,578,832; 5,556,752; and 5,510,270.

[0140] Although arrays are known that include probes for glucose transporters (e.g., an array comprising probes for a substantial fraction of a genome) the devices of the present invention are directed to measuring glucose transporters. Thus, in embodiments, at least about 10%, and sometimes at least about 25%, at least about 50% or at least about 75% of the immobilized probes on a device or array specifically bind (e.g., hybridize to) glucose transport gene products. In one embodiment, the substrate comprises fewer than about 100 distinct probes, fewer than about 50 distinct probes, fewer than about 10 distinct probes, fewer than about 5 distinct probes or fewer than about 3 distinct probes. As used in this context, a probe is "distinct" from a second probe if the two probes do not specifically bind the same polypeptide or polynucleotide (i.e., such as cDNA probes for different genes).

[0141] In one embodiment, the probes are selected from monoclonal antibodies or other specific binding proteins (e.g., antibody derivatives or fragments) that specifically bind a glucose transporter protein or a cell expressing such a protein. Probes for polypeptides can also be immobilized in an array format, for example, in an ELISA format in multi-well plates.

8. EXAMPLES

[0142] The present invention has been described in detail in the preceding sections. Aspects of the invention are illustrated in the following examples. In each case, the level of glucose transporter level determined in the cancer sample is compared to a reference value to determine whether the tumor is susceptible to treatment with an anti-neoplastic agent comprising a glucose moiety.

EXAMPLE 1

Western Assay for GLUT2 Levels in a Pancreatic Tumor

[0143] This example describes an antibody-based glucose transporter assay for determining the level of GLUT2 in a sample from a pancreatic cancer patient.

[0144] Human pancreatic islet cell cancer or carcinoid tumor cancer tissue is obtained from a patient undergoing surgery for treatment of cancer of the pancreas. In the operating room, the excised tissue is placed in a container, clearly marked and transported to the pathology department. After pathological evaluation to assess tumor pathological characteristics and to ensure that the sample contains tumor cells, a portion of the tumor is placed in a well-marked container and transported to the laboratory on dry ice, where it is stored at –70 degrees C until used. In some instances, a needle aspirate biopsy is used to assess tumor presence and characteristics. All steps below apply to either type of sample.

[0145] The frozen sample is weighed and pulverized in a thermovac tissue pulverizer on dry ice, and the tissue powder is placed in a clean tube. The tissue powder is homogenized in buffer (50 mM Tris-HCl-sodium ethylene diamie tetraacetic acid (EDTA), 1% Triton X-100, 10% glycereol, 10 mM sodium molybdate, 10 mM monothioglycerol, 10 ug/ml aprotinin, 10 ug/ml leupeptin, 0.5 mM phenylmethyl sulphonyl fluoride and 10 ug/ml pepstanin), using polytrone with the appropriate probe depending on the size of the tissue sample. The homogenization is carried out at 0-4 degrees C, with 30 second bursts and 1 minute cooling between bursts. The tissue homogenate is centrifuged at 10,000g for 10 minutes to separate the nuclear fraction and cell debris from the cytosolic/membrane soluble fraction. The resulting pellet is discarded, and the supernatant, which contains mostly cytosolic and membrane-bound proteins, including GLUT2, is removed and stored frozen until testing.

[0146] Resolving gels (10%) are made by mixing 20 ml of acrylamide-bis (30%-0.8% wt/vol), 7.5 ml of 3 M Tris-HCl, pH 8.8, 31.56 ml of ddH_2O, and 0.6 ml of 10% SDS; the resulting mixture is degassed for 30 minutes. TEMED (35 ul) is added, and the mixture is again degassed for 30 minutes. Ammonium persulfate (APS) (0.33 ml of 10%) is added, and the mixture is immediately poured into a gel apparatus using 60 cc syringes. 1-Butanol (1 ml) is overlaid on top of the resolving gel to prevent drying, and the gel is allowed to polymerize for 1 hour. The layer of butanol is removed by washing with water, just prior to pouring the stacking gel (see below).

[0147] The stacking gel consists of 3.75 ml of acrylamide-bis (30%-8% wt/vol), 1.875 ml of 2M Tris-HCl, pH 6.8, 24 ml of ddH_2O, and 0.3 ml of 10% SDS; this gel mixture is

prepared and degassed for 30 minutes. TEMED (25:l) is added to the mixture, and the mixture is again degassed for 30 minutes. Ammonium persulphate (0.3 ml; 10%) is added, and the stacking gel is poured into the gel apparatus and the combs placed appropriately. The stacking gel is allowed to polymerize for one hour. The combs are removed, and electrode buffer (25 mM Tris, 192 mM glycine, 4 mM sodium dodecylsulfate, pH 8.3) is added to the gel holder. Protein samples to be resolved are mixed with 5X sample buffer (2 g SDS, 1.0 ml of Triton X100, 3.126 ml of 2M Tris-HCl, 10.8 ml of ddH_2O; adjusted to a final volume of 20 ml and pH 6.8, 100 mg of bromophenol blue, 1.0 ml of mercaptoethanol, and 4 ml of glycerol) to give a final concentration of 1X and are layered into the wells in the gel. Molecular weight (MW) markers (BioRad) can be included in the gel (10 ul of MW markers to 0.150 ml of 1X sample buffer). The samples are layered onto the gel and electrophoresed for 12-17 hours at 17mA and 500V at room temperature.

[0148] The gels are removed from the apparatus and placed in a BioRad trans-blot apparatus, which is filled with transfer buffer (20% methanol, 192 mM glycine and 20 mM Tris, pH 8.3). The proteins in the gels are transferred to nitrocellulose membrane for three hours at 0-4 degrees C, with stirring, at 0.36A and 100V. Nitrocellulose membranes are either placed between two filter papers and stored at room temperature or used immediately in the Western blot analysis.

[0149] Nonspecific protein binding sites on the nitrocellulose membranes are blocked by incubation in buffer TBST/5% fat-free milk (10 mM Tris, 150 mM sodium chloride adjusted to pH 8.0; then 0.5% Tween 20 is added) for 1 hour on a shaker at room temperature. Subsequently, the membranes are washed with TBST, and the primary anti-GLUT2 antibodies in the desired dilutions, 1:1000 or 1:2000 in TBST/5% milk) are added. The antibody-antigen interaction is carried out with gentle shaking at room temperature for 1 hour. The membranes are then washed 3 times with TBST for 5 minutes each time to remove unbound primary antibodies.

[0150] Secondary antibodies (immuno pure goat anti-rabbit IgG conjugated to peroxidase) are added to the nitrocellulose membrane (1:50,000 in TBST/milk). This incubation is carried out at room temperature for 40 minutes with gentle shaking. The membranes are then washed 4 times for 5 minutes each, with TBST. The membranes are then incubated in one part stable peroxide solution to one part of luminol/enhancer solution for 3-5 minutes (Pierce), as suggested by the manufacturer. The membranes are wrapped in Saran wrap and placed in a cassette. In a dark room, X-ray film is placed on the membrane for 1-30 seconds. The film is developed, and the bands are visualized and quantitation is performed.

The level of GLUT2 protein in the sample is normalized to units per weight tumor and compared to a reference value to determine whether the tumor is susceptible to treatment with an anti-neoplastic agent comprising a glucose moiety.

EXAMPLE 2

Immunohistochemical Assays for GLUT2 Protein Level in Pancreatic Cancer

[0151] In the illustrative method described below, the following buffers and solutions are employed: Phosphate Buffered Saline (PBS): 5 mM $Na_2 PO_4$, 0.9 mM $KH_2 PO_4$, 72 mM NaCl, 1.6 mM KCl, pH 7.4; PBS/Triton X 100: PBS with Triton X 100 at a 1:500 dilution; and Citrate Buffer: 18 ml of 0.1M citric acid and 82 ml of 0.1M sodium citrate.

[0152] Pancreatic cancer tissue samples are obtained as described in Example 1. Five-micron sections are cut and mounted onto silane-coated slides. The slides are allowed to dry overnight. Slides are heated at 65 degrees C for 45 minutes to 1 hour, and then deparaffinized and rehydrated in xylene three times for 5 minutes each, 100% ethanol two times for 3 minutes each, and 95% ethanol two times for two minutes each. Endogenous peroxidases are blocked by incubating slides in 45 ml of methanol and 5 ml of 30% hydrogen peroxide for 20 minutes. Slides are rinsed with PBS/Triton X-100 two times for two minutes each.

[0153] Antigen retrieval is performed by adding Citrate Buffer to the glass holder in which the slides are submerged and heating in the microwave for 15 minutes on high; then 5 minutes on 50% power; and then another 5 minutes at 50% power. Slides are then cooled to room temperature for approximately 30 minutes and are then rinsed in PBS/Triton X-100 two times for two minutes each. Primary GLUT2 antibody is added at the appropriate dilution to each slide (diluted in PBS, 200 ul on each slide) and incubated for 1 hour at 37C in a humidity chamber. The slides are washed two times for two minutes each with PBS 5% Triton X-100. Biotinylated secondary antibody is applied to the slide for 30 minutes at room temperature. Slides are washed in PBS 5% Triton X-100.

[0154] Streptavidin is then applied for 30 minutes at room temperature in a humidity chamber, and slides are then rinsed in PBS 5% Triton X-100. Chromogen is added (2.5 ml PBS, 1/4 tablet 3,3' diaminobenzidine tetrahydrochloride (DAB) and two drops of 0.8% hydrogen peroxide), and the slides are incubated for 10 minutes at room temperature in a humidity chamber. The slides are then rinsed in ddH_2O for five minutes, counterstained in Gill's Hematoxylin for 1 minute, and washed in running water until clear. The cytoplasm is

cleared with 0.25% acid alcohol (three dips). The slides are then washed under a gentle stream of running water and differentiated in 1% ammonia water for 10 seconds, followed by a wash in running water. The slides are then dehydrated by incubating in 95% ethanol two times for 8-10 dips each; 100% ethanol two times for 8-10 dips each; and xylene three times for 10-15 dips each; coverslips are applied with Permount for visualization with a light microscope. The slides can be photographed with Kodak Ectochrome speed 100 film and the level of staining determined.

EXAMPLE 3

Immunohistochemical Assay of GLUT1

[0155] This example describes an antibody-based, immunohistochemical glucose transporter assay useful in determining the level of GLUT1 protein in a tumor specimen.

[0156] Tissue specimens are isolated by surgical resection or tumor biopsy using routine procedures in the course of treatment of cancer patients. Immediately upon isolation, the tissue specimens are placed in a clearly marked container and transported to the pathology department. After pathological evaluation to assess tumor pathological characteristics and to ensure that the sample contains tumor cells, a portion of the tissue specimen is placed in a well-marked container on dry ice and transported to the laboratory.

[0157] Specimens are immediately immersed in 10% formalin solution and processed according to the standard method as paraffin-embedded tissue blocks. At least three serial sections, each of four micron thickness, are cut from the paraffin-embedded fixed tissue blocks using a cryostat and mounted onto Vectabond coated slides (Vector Laboratories, Burlingame, CA). The slides are allowed to dry overnight before they are heated at 56 °C for 30 minutes, and are then deparaffinized and rehydrated in xylene three times for 5 minutes each, 100% ethanol two times for 3 minutes each, and 95% ethanol two times for two minutes each.

[0158] Endogenous peroxidases are blocked by incubating slides in 0.3% hydrogen peroxide in methanol for 20 minutes. Slides are rinsed with PBS (5 mM $Na_2 PO_4$, 0.9 mM $KH_2 PO_4$, 72 mM NaCl, 1.6 mM KCl, pH 7.4) two times for two minutes each.

[0159] Antigen retrieval is performed by microwaving the slides in 10 mM citrate buffer, pH 6.0, for three cycles of 5 minutes. Slides are subsequently cooled to RT (22°C) for approximately 30 minutes and are then rinsed in PBS two times for two minutes each. To

block non-specific protein binding, the slides are immersed 2% normal goat serum in 1% BSA in PBS for 30 minutes at RT.

[0160] Primary anti-human GLUT1 antibody (Chemicon International, Inc., Temecula, CA) is added at 1:300 dilution to each slide (diluted in 0.1% BSA in PBS, 200 ul on each slide) and incubated for two hours at RT in a humidity chamber. The anti-GLUT1 antibody is an affinity-purified rabbit polyclonal antibody generated against a 15-amino acid synthetic peptide corresponding to the exofacial loop of the human GLUT-1 sequence.

[0161] For negative controls, non-tumor tissues are also isolated from healthy portions of the affected organ or healthy organs of the same patient. In addition, negative controls can include staining without the primary antibody, substitution of anti-glucose transporter antibody by non-immune rabbit IgG (20 ug/ml), and staining with primary antibodies pre-absorbed with the target antigen peptide. A positive control for the assay is staining observed in the vascular tissues and erythrocytes that are present in the tissue sections.

[0162] After incubation with primary antibody, the slides are washed two times for two minutes each with PBS. A standard peroxidase-labelled streptavidin-biotin detection method can be used to detect the primary antibody. Biotinylated secondary antibody is applied to the slide for 30 minutes at RT. Slides are washed in PBS. Streptavidin is then applied for 30 minutes at RT in a humidity chamber, and slides are then rinsed in PBS. Chromogen (2.5 ml PBS, 1/4 tablet 3,3' diaminobenzidine tetrahydrochloride (DAB) and two drops of 0.8% hydrogen peroxide) is added, and the slides are incubated for 10 minutes at RT in a humidity chamber.

[0163] The slides are then rinsed in ddH_2O for five minutes, counterstained in Gill's Hematoxylin for 1 minute, and washed in running water until clear. The cytoplasm is cleared with 0.25% acid alcohol (three dips). The slides are then washed under a gentle stream of running water and differentiated in 1% ammonia water for 10 seconds, followed by a wash in running water.

[0164] The slides are dehydrated by incubating in 95% ethanol two times for 8-10 dips each; 100% ethanol two times for 8-10 dips each; and xylene three times for 10-15 dips each; coverslips are applied with Permount for visualization with a light microscope. The slides can be photographed with Kodak Ectochrome speed 100 film.

[0165] A semi-quantitative estimate of the proportion of cells with membrane staining and intensity of staining is recorded. The proportional area occupied by immunoreactive protein can be calculated by using a computer-assisted image analysis system KS-300 (Zeiss) connected to a BX60 microscope (Olympus) and a KY-F55B (JVC) color videocamera.

Staining results are scored from 0 to 4 according to the intensity and positive rate of staining. Experiments are done in duplicate and average values are calculated.

[0166] Depending on the source of tissue specimen and reagents, this assay method will require titration of optimal parameters. Parameters to adjust include, but are not limited to, adhesion of tissue sections on slides, dilution of primary antibody, time and temperature of incubation with primary antibody, and stringency of washes after incubation with the primary antibody.

EXAMPLE 4

Assay of GLUT8 mRNA Level

[0167] This example describes a glucose transporter assay useful in determining the level of GLUT8 mRNA in a tumor specimen.

[0168] Tissue specimens are isolated by surgical resection or tumor biopsy using routine procedures in the course of treatment of cancer patients. Immediately upon isolation, the tissue specimens are placed in a clearly marked container and transported to the pathology department. After pathological evaluation to assess tumor pathological characteristics and to ensure that the sample contains tumor cells, a portion of the tissue specimen is placed in a well-marked container and transported to the laboratory on dry ice.

[0169] Tissues specimens are homogenized in 4 M guanidine thiocyanate. Poly(A) RNAs are isolated using the Dynabeads mRNA purification kit (Dynal Biotech) according to the manufacturer's recommendations.

[0170] 1-5 ug of poly(A) RNA are separated by denaturing gel electrophoresis on 1% agarose gels containing 1% formaldehyde. The resolved RNA is blotted onto nylon membranes (Hybond N^+, Amersham Pharmacia Biotech, Braunschweig, Germany) by capillary action.

[0171] The GLUT8 cDNA is radioactively labeled with the Klenow fragment of DNA polymerase I and [α-^{32}P]dCTP by random oligonucleotide priming. The nylon membranes are hybridized at 42°C in ExpressHyb hybridization solution (Clontech Laboratories, Palo Alto, CA).

[0172] The membrane is subsequently washed two times at 55°C with 0.12 M NaCl, 0.012 M sodium citrate, 0.1% SDS before they are dried and exposed overnight on a phosphoimager plate (Fugi Photo Film). Experiments are done in duplicate and average values are calculated.

EXAMPLE 5

Immunohistochemical Assays for GLUT12 Protein Levels

[0173] This example describes an antibody-based, immunohistochemical glucose transporter assay useful in determining the level of GLUT12 protein in a tumor specimen.

[0174] Tumor tissue samples are obtained and processed as described in Example 2 prior to the step of antibody binding. Slides are then incubated overnight at 4°C in a 1:150 or 1:300 dilution of primary (R1396) anti-GLUT12 antibody diluted in 5% FBS/PBS (200 ul on each slide) in a humidity chamber. The rabbit polyclonal anti-GLUT12 antibody, R1396, was raised to the unique 16 C-terminal amino acids of human GLUT12 (Rogers *et al.*, 2002, "Identification of a novel glucose transporter-like protein-GLUT-12" *Am. J. Physiol. Endocrinol. Metab.* 282:E733–E738].

[0175] After incubation with primary antibody, the slides are washed with 0.1% Tween-20 in PBS and then incubated for 1 h with biotinylated swine anti-rabbit IgG (Dako, Carpinteria, USA). A standard peroxidase-labelled streptavidin-biotin detection method can be used to detect the primary antibody. Biotinylated secondary antibody is applied to the slide for 30 minutes at RT. Slides are washed in PBS. Streptavidin is then applied for 30 minutes at RT in a humidity chamber, and slides are then rinsed in PBS. Chromogen is added (2.5 ml PBS, 1/4 tablet 3,3' diaminobenzidine tetrahydrochloride (DAB) (Sigma, St. Louis, USA) and two drops of 0.8% hydrogen peroxide), and the slides are incubated for 10 minutes at RT in a humidity chamber.

[0176] The slides are then rinsed in ddH_2O for five minutes, counterstained in Gill's Hematoxylin for 1 minute, and washed in running water until clear. The cytoplasm is cleared with 0.25% acid alcohol (three dips). The slides are then washed under a gentle stream of running water and differentiated in 1% ammonia water for 10 seconds, followed by a wash in running water.

[0177] The slides are dehydrated by incubating in 95% ethanol two times for 8-10 dips each; 100% ethanol two times for 8-10 dips each; and xylene three times for 10-15 dips each; coverslips are applied with Permount for visualization with a light microscope. The slides can be photographed with Kodak Ectochrome speed 100 film.

[0178] A semi-quantitative estimate of the proportion of cells with membrane staining and intensity of staining is recorded. The criteria for assessing staining are based on those described by Southby *et al.* The proportional area occupied by immunoreactive protein can be calculated by using a computer-assisted image analysis system KS-300 (Zeiss) connected to a

BX60 microscope (Olympus) and a KY-F55B (JVC) color videocamera. Staining results are scored from 0 to 4 according to the intensity and positive rate of staining.

EXAMPLE 6

FDG-PET Scan

[0179] Positron emission tomography (PET) with 18-F-fluorodeoxyglucose (FDG) are used as described in Manda *et al., Anti-cancer Res.* (2003) 23(4):3263-72 to determine the level of glucose transport in patients with thoracic esophageal SCC. The study is performed on patients who underwent pre-operative FDG-PET imaging. FDG-PET studies are performed using a SET 2400 W PET scanner (Schimazu Corporation, Kyoto, Japan) with a 59.5-cm transaxial field of view and a 20-cm axial field of view, which produces 63 image planes, spaced 3.125 mm apart. Transaxial and coronal FDG PET images are interpreted visually by nuclear physicians in conjunction with CT or MRI. Regions of interest are used to evaluate the FDG uptake in segments, with a 4x4 pixel square, including the area of highest activity but not covering the entire tumor.

EXAMPLE 7

Immunohistochemical Assay

[0180] The following assay may be used to assay for glucose transporter expression. Sections of 3- to 4 mm thickness of the primary tumors are cut, deparaffinized in xylene, and rehydrated in descending grades (100-70%) of ethanol. Endogenous peroxidase activity is blocked with 3% hydrogen peroxide in methanol. After several washes in distilled water and phosphate-buffered saline, the sections are incubated with a 1:10 dilution of normal horse serum to minimize background staining. This is followed by incubation for 1 hr at room temperature with the primary antibody (Chemicon GLUT1 antibody). The peroxidase staining procedure utilizes ABC Elite Kits (Vector Laboratories, Burlingame, Calif.). The immunostaining reactions are visualized using 3-amino-9-ethylcarbazole as the chromogen. The sections and/or cytospin preparations are stained with toluidine blue and mounted in Permount. Positive and negative control immunostains are also prepared.

[0181] The sections are reviewed by the pathologist. Two features of the immunoreaction will be recorded using a semi quantitative scale: the relative number of positive cells (0%, <10%, 10-50%, and >50%) and the intensity of the reaction (0-3). The pattern of

immunostaining (membranous, cytoplasmic) is recorded separately. A tumor is considered to over-express glucose transporter if any neoplastic cells show cell membrane reactivity.

[0182] The quantitative measurement of glucose transporter immunostaining will be performed using computerized image analysis with the SAMBA 4000 Cell Image Analysis System (Image Products International, Inc., Chantilly, Va.) integrated with a Windows based software. A strong staining tumor tissue section will be used as positive control. The primary antibody will be replaced by an isotype-matched irrelevant antibody to set the negative control threshold, averaging the results from ten fields.

EXAMPLE 8

Activity-Based Glucose Transporter Assay for Cytochalasin B-Sensitive Glucose Transporters

[0183] This example describes an activity-based glucose transporter assay useful in determining the level and activity of cytochalasin B-sensitive glucose transporters in a tumor specimen.

[0184] Tissue specimens are isolated by surgical resection or tumor biopsy according to routine procedures in the course of treatment of cancer patients. Immediately upon isolation, the tissue specimens are placed in a clearly marked container and transported to the pathology department. After pathological evaluation to assess tumor pathological characteristics and to ensure that the sample contains tumor cells, a portion of the tissue specimen is placed in a well-marked container and transported to the laboratory on ice.

[0185] A tumor cell suspension is obtained by adding enzymes to a final concentration of 0.02% DNase, 0.3% collagenase, and 0.4% hyaluronidase, and incubated for 2 hours at 37 °C. Cells are washed three times in PBS and passed three times through a 25-gauge needle. After centrifugation, cells are resuspended in incubation buffer (15 mM HEPES, 135 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgCl_2$) and incubated in this medium for 30 minutes at RT. The uptake assay is then performed in 0.5 mL incubation buffer containing 0.5 mmol 2-deoxyglucose and 6 uL of 2-[1,2-^{3}H]deoxy-D-glucose (25-50 Ci/mmol; NEN Life Science Products, Boston, MA) at RT for 1 min. Glucose uptake is stopped by washing the cells with 10 mL of ice-cold PBS.

[0186] The cells are collected by centrifugation and washed twice with cold PBS. They are then lysed in 0.5 mL lysis buffer (10 mM Tris-HCl, pH 8.0, 0.2% SDS) and the incorporated radioactivity is assayed by liquid scintillation counting.

[0187] Experiments are done in duplicate and average values are calculated. 2-deoxyglucose uptake is calculated after subtraction of nonspecific uptake in parallel samples incubated in the presence of 10 μmol/L cytochalasin B, a potent inhibitor of glucose transporters. Results are normalized for cell number determined in parallel samples.

[0188] For negative controls, non-tumor tissues are also isolated from healthy organs of the same patient. An optional positive control can be glucose transport observed with tissues known to express high levels of GLUT4, such as human muscular and fat tissues, or cultured COS cells transfected with full-length GLUT4 cDNA.

EXAMPLE 9

Flow-Cytometry-Based Glucose Transporter Assay for the Level of GLUT3

[0189] This example describes an antibody-based, flow-cytometry-based glucose transporter assay useful in determining the level of GLUT3 protein in a tumor specimen.

[0190] Tissue specimens are isolated by surgical resection or tumor biopsy. Immediately upon isolation, the tissue specimens are placed in a clearly marked container and transported to the pathology department. After pathological evaluation to assess tumor pathological characteristics and to ensure that the sample contains tumor cells, a portion of the tissue specimen is placed in a well-marked container on dry ice and transported to the laboratory.

[0191] Specimens are dispersed into single-cell suspension using collagenase-digestion Ficoll gradient purification method. The cells are washed twice in Dulbecco's phosphate-buffered saline (PBS) (pH 7.6) by sedimentation at 500 x g for 30 seconds at room temperature (RT). The cells are then fixed for 15 minutes at RT in 4% paraformaldehyde in phosphate-buffered saline (PBS) buffered to pH 7.2. They are washed three times in PBS and resuspended in PBS with 3% bovine serum albumin (BSA) and divided into 1.5 ml microcentrifuge tubes at a density of approximately 10^5 cells per tube.

[0192] Primary anti-human GLUT3 antibody (Chemicon International, Inc., Temecula, CA) is added at 1:60 dilution to each tube and incubated overnight at 4°C. After incubation with primary antibody, the cells are washed twice by centrifugation at 500 x g for 30 seconds in PBS. The cell pellets are resuspended in R-phycoerythrin-labeled goat anti-rabbit IgG (Fisher Scientific) and incubated for 1 hour at 4°C with occasional shaking. After two washes by centrifugations at 500 x g for 30 seconds in PBS, the cells are resuspended in 500 μl of PBS.

[0193] IgG binding is determined by flow cytometry performed on a FACScan (Becton Dickinson) flow cytometer. Cell populations are gated to exclude dead cells. Depending on

the source of tissue specimen and reagents, this assay method will require titration of optimal parameters. Parameters that may be varied include the dilution of primary antibody, the time and temperature of incubation with primary antibody, and stringency of washes after incubation with the primary antibody. The proportion of cells staining positive for each specimen is determined and may be compared with negative control samples. In an embodiment, the flow cytometry results are displayed as cell number vs. staining intensity (e.g,. number of transporters) and the distribution for the sample compared to those of a survey population.

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[0194] Although the present invention has been described in detail with reference to specific embodiments, those of skill in the art will recognize that modifications and improvements are within the scope and spirit of the invention, as set forth in the claims which follow. All publications and patent documents (patents, published patent applications, and unpublished patent applications) cited herein are incorporated herein by reference as if each such publication or document was specifically and individually indicated to be incorporated herein by reference. Citation of publications and patent documents is not intended as an admission that any such document is pertinent prior art, nor does it constitute any admission as to the contents or date of the same. The invention having now been described by way of written description and example, those of skill in the art will recognize that the invention can be practiced in a variety of embodiments and that the foregoing description and examples are for purposes of illustration and not limitation of the following claims.

What is claimed is:

1. A method for determining whether a cancer is susceptible to treatment with an anti-neoplastic agent, comprising the steps of:

(a) obtaining a sample of the cancer,

(b) measuring the level of at least one glucose transporter in the sample,

(c) comparing the level with a predetermined value, and

(d) determining that, if the measured level is larger than the predetermined value, the cancer is susceptible to treatment with the anti-neoplastic agent.

2. The method of claim 1, wherein the anti-neoplastic agent comprises a glucose moiety.

3. The method of claim 2, wherein the anti-neoplastic agent comprises a glucose analog moiety.

4. The method of claim 3, wherein the glucose analog moiety is a fructose moiety.

5. The method of claim 1, wherein the glucose transporter is a Class I GLUT.

6. The method of claim 1, wherein the glucose transporter is a Class II GLUT.

7. The method of claim 1, wherein the glucose transporter is a Class III GLUT.

8. The method of any preceding claim wherein at least two different glucose transporters are measured.

9. The method of claim 8 wherein the levels of at least one Class I GLUT, at least one Class II GLUT, and at least one Class III GLUT are measured.

10. The method of claim 1, wherein the anti-neoplastic agent is streptozotocin.

11. The method of claim 10 wherein the glucose transporter is GLUT2.

12. The method of claim 1, wherein the anti-neoplastic agent is glucofosfamide.

13. The method of claim 12, wherein the glucose transporter is Na+-dependent glucose transporter.

14. The method of claim 1, wherein the anti-neoplastic agent is a glyco-S-nitrosothiol.

15. The method of claim 14, wherein the glucose transporter is GLUT1.

16. The method of claim 1, wherein the level of glucose transporter in the sample is measured by an immunological assay.

17. The method of claim 1, wherein the level of glucose transporter in the sample is measured by an amplification of an RNA or cDNA.

18. A method according to claim 1, wherein the cancer is selected from the group consisting of leukemia, breast cancer, skin cancer, bone cancer, prostate cancer, liver cancer, lung cancer, brain cancer, cancer of the larynx, gallbladder, pancreas, islet cells, rectum, parathyroid, thyroid, adrenal, neural tissue, head and neck, colon, stomach, bronchi, kidneys, basal cell carcinoma, squamous cell carcinoma of both ulcerating and papillary type, metastatic skin carcinoma, osteo sarcoma, Ewing's sarcoma, veticulum cell sarcoma, myeloma, giant cell tumor, small-cell lung tumor, islet cell carcinoma, primary brain tumor, acute and chronic lymphocytic and granulocytic tumors, hairy-cell tumor, adenoma, hyperplasia, medullary carcinoma, pheochromocytoma, mucosal neuronms, intestinal ganglloneuromas, hyperplastic corneal nerve tumor, marfanoid habitus tumor, Wilm's tumor, seminoma, ovarian tumor, leiomyomater tumor, cervical dysplasia and in situ carcinoma, neuroblastoma, retinoblastoma, soft tissue sarcoma, malignant carcinoid, topical skin lesion, mycosis fungoide, rhabdomyosarcoma, Kaposi's sarcoma, osteogenic and other sarcoma, malignant hypercalcemia, renal cell tumor, polycythermia vera, adenocarcinoma, glioblastoma multiforma, leukemias, lymphomas, malignant melanomas, and epidermoid carcinomas.

19. The method of claim 18, wherein the cancer is a pancreatic cancer.

20. The method of claim 19, wherein the cancer is a pancreatic islet cell carcinoma.

21. The method of claim 1 wherein the glucose transporter is GLUT2, the cancer is a pancreatic cancer or islet cell carcinoma, and the anti-neoplastic agent is streptozotocin, glucofosfamide, or a gluSNAP compound.

22. A method for determining a chemotherapeutic regimen for a patient, said method comprising the steps of: (a) diagnosing the patient as having cancer; (b) obtaining a cancer sample from the patient; (c) measuring the level of a glucose transporter in the sample; (d) comparing said amount measured in step (c) with a predetermined value; and (e) determining that if the amount measured in step (c) is larger than said predetermined amount, the patient is a candidate for treatment with an antineoplastic agent transported by the glucose transporter.

23. A kit for determining whether a cancer is susceptible to treatment with an anti-neoplastic agent, said kit comprising (a) reagents for determining the level of at least one, and optionally more than one, glucose transporter in a cancer sample, and (b) instructions including a reference value.