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(54) Title: PRMT1 AND PRMT6 FOR TARGET GENES OF CANCER THERAPY AND DIAGNOSIS

(57) Abstract: Objective methods for diagnosing a predisposition to developing cancer, particularly bladder cancer, gastric cancer, colorectal cancer, breast cancer, esophageal cancer, lung cancer, lymphoma, pancreatic cancer and testicular cancer, are described herein. In one embodiment, the diagnostic method involves determining an expression level of PRMT1 or PRMT6 gene. The present invention further provides methods of screening for therapeutic agents useful in the treatment of PRMT1 or PRMT6 associated diseases, such as a cancer, e.g., bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML. The present invention further provides methods of inhibiting the cell growth and treating or alleviating symptoms of PRMT1 or PRMT6 associated diseases. The present invention also features products, including double-stranded molecules and vectors encoding thereof as well as to compositions containing them.

Description

Title of Invention: PRMT1 AND PRMT6 FOR TARGET GENES OF CANCER THERAPY AND DIAGNOSIS

Technical Field

[0001] The present invention relates to methods of detecting and diagnosing a predisposition to developing cancer, particularly bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML. The present invention also relates to methods of screening for a candidate substance for treating and preventing cancer with over-expression of PRMT1 or PRMT6, particularly bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML. Moreover, the present invention relates to a double-stranded molecule which reduces PRMT6 gene expression and uses thereof.

[0002] Priority

The present application claims priority to US Serial No. 61/301,020, filed February 3, 2010, the disclosures of which are incorporated herein by reference in their entireties.

Background Art

[0003] SMYD3, a histone lysine methyltransferase, stimulates proliferation of cells and plays an important role in human carcinogenesis through its methyltransferase activity [PTL1, NPLs 1-5]. Arginine methyltransferases have also been characterized as transcriptional regulators, similar to lysine methyltransferases. In mammalian cells, protein arginine methyltransferases (PRMTs) have been classified into type I (PRMT1, 3, 4, 6 and 8) and type II (PRMT5, 7 and FBXO11), depending on their specific catalytic activity [NPLs 6, 7]. Type I PRMT activity is defined by the formation of asymmetric omega-N^G, N^G-dimethylarginine residues, whereas type II activity is defined by the formation of symmetric omega-N^G, N^G-dimethylarginine residues [NPLs 8, 9]. Despite a large body of information for the prominent role of PRMTs in transcriptional regulation, their physiological function and involvement in human disease is still not well understood.

[0004] PRMT1 (NM_001536.3, NM_198318.2, NM_198319.2) (for example SEQ ID NO: 1 encoded by SEQ ID NO: 2) is known to possess type I activity and catalyze methylation of the third arginine of histone H4 [NPLs 10, 11]. PRMT1 was originally identified as an interacting protein for both the BTG1 and BTG2 proteins, as well as the interferon-alpha/beta receptor [NPLs 12, 13]. PRMT1 has served as the pro-

totypical PRMT because it was the first eukaryotic PRMT to be cloned and has been shown to act as a coactivator of nuclear receptor-mediated gene transcription together with p300/CBP, a histone acethyltransferase, and PRMT4/CARM1 (coactivator-associated arginine methyltransferase 1) [NPLs 11, 14]. PRMT6 (NM_018137.2) (SEQ ID NO: 3 encoded by SEQ ID NO: 4) is also a type I enzyme and is the major protein arginine methyltransferase responsible for the methylation of the second arginine of histone H3 [NPLs 15, 16]. PRMT6 was shown to antagonize the MLL-complex-dependent methylation of the Lys-4 residue [NPL 16]. The biological role of PRMT6 is not clarified, but it has been suggested that its activity may affect gene regulation primarily through modifying protein-nucleic acid interactions. PRMT6 localizes exclusively in the nucleus, and methylates glycine- and arginine-rich (GAR) sequences in proteins [NPL 17]. Although PRMT1 and PRMT6 share substrates, some PRMT6-specific cellular targets do not contain the GAR consensus sequence, including high mobility group proteins (HMGA1a and HMGA1b) [NPL 18], DNA polymerase beta [NPL 19] and HIV-1 trans-activator of transcription (Tat) protein [NPL 20].

Citation List

Patent Literature

[0005] [PTL1] WO2005/071102

Non-Patent Literature

[0006] [NPL 1] Hamamoto R et al. Nat Cell Biol 2004;6:731-40
[NPL 2] Hamamoto R et al. Cancer Sci 2006;97:113-8
[NPL 3] Kunizaki M et al. Cancer Res 2007;67:10759-65
[NPL 4] Silva FP et al. Oncogene 2008;27:2686-92
[NPL 5] Tsuge M et al. Nat Genet 2005;37:1104-7
[NPL 6] Bedford MT et al., Mol Cell 2009;33:1-13
[NPL 7] Pahlich S et al. Biochim Biophys Acta 2006;1764:1890-903
[NPL 8] Gary JD et al. Prog Nucleic Acid Res Mol Biol 1998;61:65-131
[NPL 9] Scott HS et al. Genomics 1998;48:330-40
[NPL 10] Huang S et al. Genes Dev 2005;19:1885-93
[NPL 11] Strahl BD et al. Curr Biol 2001;11:996-1000
[NPL 12] Lin WJ et al. J Biol Chem 1996;271:15034-44
[NPL 13] Abramovich C et al., EMBO J 1997;16:260-6
[NPL 14] Koh SS et al. J Biol Chem 2001;276:1089-98.
[NPL 15] Guccione E et al. Nature 2007;449:933-7
[NPL 16] Hyllus D et al. Genes Dev 2007;21:3369-80
[NPL 17] Frankel A et al. J Biol Chem 2002;277:3537-43

[NPL 18] Sgarra R et al. J Biol Chem 2006;281:3764-72

[NPL 19] El-Andaloussi N et al. Mol Cell 2006;22:51-62

Summary of Invention

[0007] In order to investigate possible roles of PRMTs in human carcinogenesis, the expression profiles of all human PRMTs in clinical tissues were examined to identify a methyltransferase that can contribute to human carcinogenesis. We found that expression levels of PRMT1 and PRMT6 were significantly up-regulated in various types of cancer, compared with the levels in corresponding normal (non-cancer) tissues. We identified two Type I PRMTs (PRMT1 and PRMT6) overexpressed in various types of human cancer. Since the genes are scarcely expressed in adult normal organs, PRMT1 and 6 are appropriate and promising molecular targets for novel therapeutic approaches with minimal adverse effect. Functionally, knockdown of endogenous PRMT1 or 6 by siRNA in cancer cell lines results in drastic suppression of cancer cell growth, demonstrating the essential role of these genes in maintaining viability of cancer cells.

[0008] Accordingly, the present invention features a method of diagnosing or determining a predisposition to cancer, particularly bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML, in a subject by determining an expression level of PRMT1 or 6 in a subject derived biological sample, such as biopsy. An increase of the level of expression of either or both of PRMT1 and PRMT6 compared to a normal control level indicates that the subject suffers from or is at risk of developing cancer, particularly bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML. In such methods, PRMT1 or 6 gene can be detected by appropriate probes or the PRMT1 or 6 protein can be detected by each antibody.

[0009] The present invention further provides methods of identifying a substance that inhibits the expression of a PRMT1 or 6 gene or the activity of its gene product. Furthermore the present invention provides methods of identifying a candidate substance for treating or preventing PRMT1 or 6 associated-disease, such as cancer, e.g., bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML, or a candidate substance that inhibits growth of cells over-expressing the PRMT1 or 6 gene. The method can be carried out in vitro or in vivo. A decrease in the expression level of the PRMT1 or 6 gene and/or biological activity of its gene product as compared to that in the absence of the candidate substance indicates that the candidate substance is an inhibitor of the PRMT1 or 6 and may be used to inhibit the

growth of cells over-expressing the PRMT1 or 6 gene, such as cancerous cell, e.g., cells of bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML.

[0010] In another aspect, the present invention provides a method for inhibiting the growth of a cancerous cell over-expressing PRMT6 by administering a substance that inhibits expression of PRMT6 and/or the function of the PRMT6 protein. Preferably the substance is an inhibitory nucleic acid (e.g., an antisense, ribozyme, double stranded molecule). The substance may be a nucleic acid molecule or vector for providing double stranded molecule. Expression of the gene may be inhibited by introduction of a double stranded molecule into the target cell in an amount sufficient to inhibit expression of the PRMT6 gene. The present invention also provides methods for inhibiting the growth of cancerous cells over-expressing PRMT6 in a subject. The methods are useful for treating or preventing cancer, particularly bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML. In another aspect, the present invention relates to a pharmaceutical composition for treating or preventing cancer that includes double-stranded molecules or vectors encoding said double-stranded molecules as an active ingredient and pharmaceutically acceptable carrier. The double-stranded molecules provided in the present invention inhibit expression of the PRMT1 or PRMT6 gene and inhibit the growth of cancerous cells over-expressing PRMT1 or PRMT6 when introduced into cells. For example, such molecules target the sequence corresponding to SEQ ID NO: 29, 32, 35 or 38. The molecules of the present invention include a sense strand and an antisense strand, wherein the sense strand includes a sequence including the target sequence, and wherein the antisense strand includes a sequence which is complementary to the sense strand. The sense and the antisense strands of the molecule hybridize to each other to form a double-stranded molecule.

[0011] Furthermore, the present invention provides the method of diagnosing cancer in a subject by determining an asymmetric dimethyl arginine (ADMA) level in a subject derived biological sample, such as blood sample collected from a subject to be diagnosed or screened. In prefered embodiments, blood sample is whole blood, plasma or serum. An increase of the ADMA level compared to a normal control level indicates that the subject suffers from or is at risk of developing cancer. In the methods, ADMA can be detected by anti-ADMA antibody.

Brief Description of Drawings

[0012] [fig.1]Figure 1 depicts elevated PRMT1 and PRMT6 expressions in bladder cancer. (a)

Expression levels of PRMT1 and PRMT6 were analyzed by quantitative real-time PCR, and the result is shown by box-whisker plot (median 50% boxed). Relative mRNA expression shows the value normalized by GAPDH and SDH expressions. Mann-Whitney U test was used for statistical analysis. (b) Immunohistochemical staining of PRMT1 and PRMT6 in bladder tissues. Counterstaining was done with hematoxylin and eosin. Original magnification, x40 and x400.

[0013] [fig.2]Figure 2 depicts expression of PRMT1 and PRMT6 in 14 bladder cancer cell lines, four non-small cell lung cancer cells and one small cell lung cancer cell line.

[0014] [fig.3]Figure 3 depicts involvement of PRMT1 and PRMT6 in the growth of bladder and lung cancer cells. (a) Expression of PRMT1 and PRMT6 in LC319 cells treated with two independent specific siRNAs against PRMT1 and PRMT6 (siPRMT1#1, #2 and siPRMT6#1, #2) was analyzed by quantitative real-time PCR. siRNAs targeting EGFP (siEGFP) and siNegative control (siNC) were used as controls. mRNA expression levels were normalized by GAPDH and SDH expressions, and values are relative to siEGFP (siEGFP = 1). Results are the mean +/- SD of three independent experiments. P values were calculated using Student's t-test (**, $P < 0.01$; ***, $P < 0.001$). (b and c) Effects of PRMT1 (b) and PRMT6 (c) siRNA knockdown on the viability of two bladder cancer cell lines (SW780, RT4) and three lung cancer cell lines (A549, LC319 and SBC5). Relative cell number shows the value normalized to siEGFP-treated cells (siEGFP = 1). Results are the mean +/- SD in three independent experiments. P values were calculated using Student's t-test (*, $P < 0.05$; **, $P < 0.01$). (d) DNA content of SW780 and A549 cells was analyzed by FACS 72 h after the treatment with control siRNAs (siEGFP, siNC) and siPRMT1s (siPRMT1#1, #2). Results are the mean +/- SD in three independent experiments. P values were calculated using Student's t-test (*, $P < 0.05$; **, $P < 0.01$).

[0015] [fig.4]Figure 4 depicts two-dimensional, unsupervised hierarchical cluster analysis of SW780 and A549 mRNA expression profiles after knockdown of PRMT1 expression. Differentially expressed genes were selected for this analysis. Red, Up-regulated; Green, Down-regulated.

[0016] [fig.5]Figure 5 depicts two-dimensional, unsupervised hierarchical cluster analysis of SW780 and A549 mRNA expression profiles after knockdown of PRMT6 expression. Differentially expressed genes were selected for this analysis. Red, Up-regulated; Green, Down-regulated.

[0017] [fig.6]Figure 6 depicts confirmation of microarray data using quantitative real-time PCR. The present inventors randomly selected five downstream candidates (MAPK1, RRAS, NRAS, GALNT1 and RTN4) and evaluated expression of those genes based on three independent experiments. P values were calculated using Student's t-test.

[0018] [fig.7]Figure 7 depicts silver staining pattern of interacting proteins with PRMT1 and

PRMT6. The present inventors amplified the coding region of PRMT1 and PRMT6 by RT-PCR, and cloned the PCR products into p3xFLAG-CMV10 (SIGMA-ALDRICH). 293T cells transfected with p3xFLAG-CMV, p3xFLAG-CMV-PRMT1 and p3xFLAG-CMV-PRMT6 were washed with PBS and lysed in CelLytic-M lysis reagent (SIGMA-ALDRICH) containing 1X complete protease inhibitor cocktail (Roche). In an immunoprecipitation reaction, 500 micro g of whole-cell extract was incubated with optimum concentration of mouse anti-FLAG M2-agarose at 4 degree C for 1-2 h. After the beads were washed, proteins that bound to the beads were eluted by 3xFLAG peptide (SIGMA-ALDRICH). Purified complex was resolved on 5%-20% linear gradient SDS-PAGE, and proteins were visualized by silver staining. Bands were excised, digested with trypsin, and analyzed by mass spectrometry.

[0019] [fig.8]Figure 8 depicts measurement of serum ADMA levels in cancer patients. The serum ADMA levels were determined using the enzyme-linked immunosorbent assay method. P values were calculated using Student's t-test.

[0020] [fig.9]Figure 9 depicts expression levels of PRMT1 in various normal tissues and bladder tumor tissues were analyzed by quantitative real-time PCR. Relative mRNA expression shows the value normalized by GAPDH and SDH.

Description of Embodiments

[0021] Definition:

The words "a", "an", and "the" as used herein mean "at least one" unless otherwise specifically indicated.

An "isolated" or "purified" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. In a preferred embodiment, nucleic acid molecules encoding antibodies of the present invention are isolated or purified.

The terms "polypeptide", "peptide", and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is a modified residue, or a non-naturally occurring residue, such as an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers.

[0022] The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that similarly functions to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those modified after translation in cells (e.g., hydroxyproline, gamma-carboxyglutamate, and O-phosphoserine). The phrase "amino acid analog" refers to compounds that have the same basic chemical structure (an alpha

carbon bound to a hydrogen, a carboxy group, an amino group, and an R group) as a naturally occurring amino acid but have a modified R group or modified backbones (e.g., homoserine, norleucine, methionine, sulfoxide, methionine methyl sulfonium). The phrase "amino acid mimetic" refers to chemical compounds that have different structures but similar functions to general amino acids.

Amino acids may be referred to herein by their commonly known three letter symbols or the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission.

[0023] The terms "polynucleotides", "oligonucleotide", "nucleotides", "nucleic acids", and "nucleic acid molecules" are used interchangeably unless otherwise specifically indicated and, similarly to the amino acids, are referred to by their commonly accepted single-letter codes. Similar to the amino acids, they encompass both naturally-occurring and non-naturally occurring nucleic acid polymers. The polynucleotide, oligonucleotide, nucleotides, nucleic acids, or nucleic acid molecules may be composed of DNA, RNA or a combination thereof.

Unless otherwise defined, the term "cancer" refers to cancers over-expressing the PRMT1 or PRMT6. Examples of cancers over-expressing PRMT1 or PRMT6 include, but are not limited to, bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML.

[0024] Gene or protein of PRMT1 and PRMT6:

The present invention is based, at least in part, on the discovery that the genes encoding PRMT1 and PRMT6 are over-expressed in several cancers compared to non-cancerous tissue. The nucleic acid and polypeptide sequences of PRMT1 or 6 are not to be considered limited to what is shown in SEQ ID NOs: 1 and 2 or 3 and 4, respectively. The sequence data are also available via following accession numbers:

PRMT1: BC109283, NM_001536, NM_198319 or NM_198318,

PRMT6: NM_018137.2 (the entire disclosures of which are herein incorporated by reference).

[0025] According to an aspect of the present invention, functional equivalents of the protein (polypeptide) are also considered as "PRMT1 polypeptides" or "PRMT6 polypeptides". Herein, a "functional equivalent" of a protein (e.g., a PRMT1 polypeptide) is a polypeptide that has a biological activity equivalent to the protein. Namely, any polypeptide that retains the biological ability of the PRMT1 protein or the PRMT6 protein may be used as such a functional equivalent in the present invention. Such functional equivalents include those wherein one or more amino acids are substituted, deleted, added, or inserted to the natural occurring amino acid sequence of the PRMT1 protein or the PRMT6 protein. Alternatively, the polypeptide may be

composed an amino acid sequence having at least about 80% homology (also referred to as sequence identity) to the sequence of the respective protein, more preferably at least about 90% to 95% homology, often about 96%, 97%, 98% or 99% homology. In other embodiments, the polypeptide can be encoded by a polynucleotide that hybridizes under stringent conditions to the natural occurring nucleotide sequence of the PRMT1 gene or the PRMT6 gene.

A polypeptide of the present invention may have variations in amino acid sequence, molecular weight, isoelectric point, the presence or absence of sugar chains, or form, depending on the cell or host used to produce it or the purification method utilized. Nevertheless, so long as it has a function equivalent to that of the human PRMT1 protein or PRMT6 protein of the present invention, it is within the scope of the present invention.

[0026] The phrase "stringent (hybridization) conditions" refers to conditions under which a nucleic acid molecule will hybridize to its target sequence, typically in a complex mixture of nucleic acids, but not detectably to other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, Techniques in Biochemistry and Molecular Biology-Hybridization with Nucleic Probes, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, stringent conditions are selected to be about 5-10 degrees C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength pH. The Tm is the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at Tm, 50% of the probes are occupied at equilibrium). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal is at least two times of background, preferably 10 times of background hybridization. Exemplary stringent hybridization conditions include the following: 50% formamide, 5x SSC, and 1% SDS, incubating at 42 degrees C, or, 5x SSC, 1% SDS, incubating at 65 degrees C, with wash in 0.2x SSC, and 0.1% SDS at 50 degrees C.

[0027] In the context of the present invention, a condition of hybridization for isolating a DNA encoding a polypeptide functionally equivalent to the human PRMT1 or PRMT6 protein can be routinely selected by a person skilled in the art. For example, hybridization may be performed by conducting pre-hybridization at 68 degrees C for 30 min or longer using "Rapid-hyb buffer" (Amersham LIFE SCIENCE), adding a labeled probe, and warming at 68 degrees C for 1 hour or longer. The following washing step can be conducted, for example, in a low stringent condition. An exemplary low

stringent condition may include 42 degrees C, 2x SSC, 0.1% SDS, preferably 50 degrees C, 2x SSC, 0.1% SDS. High stringency conditions are often preferably used. An exemplary high stringency condition may include washing 3 times in 2x SSC, 0.01% SDS at room temperature for 20 min, then washing 3 times in 1x SSC, 0.1% SDS at 37 degrees C for 20 min, and washing twice in 1x SSC, 0.1% SDS at 50 degrees C for 20 min. However, several factors, such as temperature and salt concentration, can influence the stringency of hybridization and one skilled in the art can suitably select the factors to achieve the requisite stringency.

[0028] Generally, it is known that modifications of one or more amino acids in a protein do not influence the function of the protein. In fact, mutated or modified proteins, proteins having amino acid sequences modified by substituting, deleting, inserting, and/or adding one or more amino acid residues of a certain amino acid sequence, can retain the original biological activity (Mark et al., Proc Natl Acad Sci USA 81: 5662-6 (1984); Zoller and Smith, Nucleic Acids Res 10:6487-500 (1982); Dalbadie-McFarland et al., Proc Natl Acad Sci USA 79: 6409-13 (1982)). Accordingly, one of skill in the art will recognize that at least one mutation or alteration selected from the group consisting of individual additions, deletions, insertions, and substitutions to an amino acid sequence which alter a single amino acid or a small percentage of amino acids or those considered to be a "conservative modifications", wherein the alteration of a protein results in a protein with similar functions, are acceptable in the context of the instant invention.

[0029] So long as the activity of the protein is maintained, the number of amino acid mutations is not particularly limited. However, it is generally preferred to alter 5% or less of the amino acid sequence. Accordingly, in a preferred embodiment, the number of amino acids to be mutated in such a mutant is generally 30 amino acids or fewer, preferably 20 amino acids or fewer, more preferably 10 amino acids or fewer, more preferably 6 amino acids or fewer, and even more preferably 3 amino acids or fewer.

[0030] An amino acid residue to be mutated is preferably mutated into a different amino acid in which the properties of the amino acid side-chain are conserved (a process known as conservative amino acid substitution). Examples of properties of amino acid side chains are hydrophobic amino acids (A, I, L, M, F, P, W, Y, V), hydrophilic amino acids (R, D, N, C, E, Q, G, H, K, S, T), and side chains having the following functional groups or characteristics in common: an aliphatic side-chain (G, A, V, L, I, P); a hydroxyl group containing side-chain (S, T, Y); a sulfur atom containing side-chain (C, M); a carboxylic acid and amide containing side-chain (D, N, E, Q); a base containing side-chain (R, K, H); and an aromatic containing side-chain (H, F, Y, W). Conservative substitution tables providing functionally similar amino acids are well known in the art. For example, the following eight groups each contain amino acids

that are conservative substitutions for one another:

1) Alanine (A), Glycine (G);

2) Aspartic acid (D), Glutamic acid (E);

3) Aspargine (N), Glutamine (Q);

4) Arginine (R), Lysine (K);

5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V);

6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W);

7) Serine (S), Threonine (T); and

8) Cystein (C), Methionine (M) (see, e.g., Creighton, Proteins 1984).

[0031] Such conservatively modified polypeptides are included in the present PRMT1 protein or PRMT6 protein. However, the present invention is not restricted thereto and the PRMT1 protein or PRMT6 protein includes non-conservative modifications, so long as at least one biological activity of the PRMT1 protein or PRMT6 protein is retained. Furthermore, the modified proteins do not exclude polymorphic variants, interspecies homologues, and those encoded by alleles of these proteins.

[0032] Moreover, the PRMT1 gene or PRMT6 gene of the present invention encompasses polynucleotides that encode such functional equivalents of the PRMT1 protein or PRMT6 protein, respectively. In addition to hybridization, a gene amplification method, for example, the polymerase chain reaction (PCR) method, can be utilized to isolate a polynucleotide encoding a polypeptide functionally equivalent to the PRMT1 protein or PRMT6 protein, using a primer synthesized based on the sequence information of the protein encoding DNA (SEQ ID NO: 2 or SEQ ID NO:4). Polynucleotides and polypeptides that are functionally equivalent to the human PRMT1 and PRMT6 gene and protein, respectively, normally have a high homology to the originating nucleotide or amino acid sequence thereof . "High homology" typically refers to a homology of 40% or higher, preferably 60% or higher, more preferably 80% or higher, even more preferably 90% to 95% or higher. The homology of a particular polynucleotide or polypeptide can be determined by following the algorithm in "Wilbur and Lipman, Proc Natl Acad Sci USA 80: 726-30 (1983)".

[0033] A method for diagnosing cancer:

The expression of PRMT1 was found to be specifically elevated in diffuse-type gastric cancer, non-small cell lung cancer, small cell lung cancer, testicular cancer, bladder cancer, pancreatic cancer, lymphoma, esophageal cancer and breast cancer, and of PRMT6 was found to be specifically elevated in lymphoma, small cell lung cancer, cervical cancer, osteosarcoma, bladder cancer, prostate cancer, CML, breast cancer and non-small cell lung cancer (Figs. 1, 2 and table 5). Therefore, the PRMT1 and PRMT6 genes identified herein as well as their transcription and translation products find diagnostic utility as a marker for cancers as above, and by measuring the

expression of either or both of PRMT1 and PRMT6 in a sample. Those cancers can be diagnosed or detected by comparing the expression level of PRMT1 and/or PRMT6 between a subject-derived sample with a normal sample. Specifically, the present invention provides a method for diagnosing or detecting cancers by determining the expression level of PRMT1 and/or PRMT6 in the subject. In the present invention, cancer indicates bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, lung cancer, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML, and lung cancers that can be diagnosed by the present method include NSCLC and SCLC. Furthermore, NSCLC, including lung adenocarcinoma and lung squamous cell carcinoma (SCC), can also be diagnosed or detected by the present invention.

[0034] Alternatively, the present invention provides a method for detecting or identifying cancer cells in a subject-derived tissue sample of a patient, the method including the step of determining the expression level of the either or both of PRMT1 and PRMT6 gene in a subject-derived biological sample, wherein an increase in the expression level as compared to a normal control level of the gene indicates the presence or suspicion of cancer cells in the tissue.

According to the present invention, an intermediate result for examining the condition of a subject may be provided, i.e., a method of monitoring a subject. Such intermediate result may be combined with additional information to assist a doctor, nurse, or other practitioner to diagnose that a subject suffers from the disease. Alternatively, the present invention may be used to detect cancerous cells in a subject-derived tissue, and provide a doctor with useful information to diagnose that the subject suffers from the disease.

[0035] For example, according to the present invention, the expression level of the either or both of PRMT1 gene and PRMT6 gene can be used in combination with another diagnostic indicator, including tissue pathology, levels of known tumor marker(s) in blood, and clinical course of the subject, etc. For example, some well-known diagnostic pancreatic cancer markers in blood include ACT, AFP, BCA225, BFP, CA15-3, CA19-9, CA50, CA72-4, CA125, CA130, CA602, CEA, DUPAN-2, IAP, KMO-1, alpha-macrogloblin, NCC-ST-439, NSE, PIVKA-II, SCC, sICAM-1, SLX, SP1, SOD, Span-1, STN, TK activity, TPA, YH-206, elastase I, cytokeratin-19 fragment, and CYFRA21-1. Namely, in this particular embodiment of the present invention, the outcome of the gene expression analysis serves as an intermediate result for further diagnosis of a subject's disease state.

[0036] Specifically, the present invention provides the following methods [1] to [10]:

[1] A method of detecting or diagnosing cancer in a subject, including determining an expression level of PRMT1 and/or PRMT6 in a subject-derived biological sample,

wherein an increase of the level compared to a normal control level of the gene indicates that the subject suffers from or is at risk of developing cancer.[2] The method of [1], wherein the expression level is at least 10% greater than the normal control level.

[3] The method of [1], wherein the expression level is detected by a method selected from among:

(a) detecting an mRNA including the sequence of PRMT1 and/or PRMT6,

(b) detecting a protein including the amino acid sequence of PRMT1 and/or PRMT6, and

(c) detecting a biological activity of a protein including the amino acid sequence of PRMT1 and/or PRMT6.

[4] The method of [1], wherein the cancer is bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and/or CML (Alternatively, The method of [1], wherein the cancer is selected from the group consisting of bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML).

[5] The method of [3], wherein the expression level is determined by detecting hybridization of a probe to a gene transcript of the gene.

[6] The method of [3], wherein the expression level is determined by detecting the binding of an antibody against the protein encoded by a gene as the expression level of the gene.

[7] The method of [1], wherein the subject-derived biological sample includes biopsy, sputum or blood.

[8] The method of [1], wherein the subject-derived biological sample includes an epithelial cell.

[9] The method of [1], wherein the subject-derived biological sample includes a cancer cell.

[10] The method of [1], wherein the subject-derived biological sample includes a cancerous epithelial cell.

[0037] In another embodiments, [1] method of detecting or diagnosing cancer in a subject, including determining an expression level of either or both of PRMT1 and PRMT6 in a subject-derived biological sample, wherein an increase of the level compared to a normal control level of the gene indicates that the subject suffers from or is at risk of developing cancer is provided. In addition, [3] the method of [1], wherein the expression level is detected by a method selected from among:

(a) detecting an mRNA including the sequence of either or both of PRMT1 and

PRMT6,

(b) detecting a protein including the amino acid sequence of either or both of PRMT1 and PRMT6, and

(c) detecting a biological activity of a protein including the amino acid sequence of either or both of PRMT1 and PRMT6 is also provided.

[0038] The method of diagnosing cancer will be described in more detail below.

A subject to be diagnosed by the present method is preferably a mammal. Exemplary mammals include, but are not limited to, e.g., human, non-human primate, mouse, rat, dog, cat, horse, and cow.

A subject derived biological sample is a biological sample obtained from the subject to be diagnosed. Any biological material can be used as the biological sample for the determination so long as it includes the objective transcription or translation product of PRMT1 and/or PRMT6. The biological samples include, but are not limited to, bodily tissues which are desired for diagnosing or are suspicion of suffering from cancer, and fluids, such as biopsy, blood, sputum and urine. Preferably, the biological sample contains a cell population including an epithelial cell, more preferably a cancerous epithelial cell or an epithelial cell derived from tissue suspected to be cancerous. Further, if necessary, the cell may be purified from the obtained bodily tissues and fluids, and then used as the biological sample.

[0039] According to the present invention, the expression level of PRMT1 and/or PRMT6 in the subject-derived biological sample is determined. The expression level can be determined at the transcription product level, using methods known in the art. For example, the mRNA of PRMT1 and/or PRMT6 may be quantified using probes by hybridization methods (e.g., Northern hybridization). The detection may be carried out on a chip or an array. The use of an array is preferable for detecting the expression level of a plurality of genes (e.g., various cancer specific genes) including PRMT1 and/or PRMT6. Those skilled in the art can prepare such probes utilizing the sequence information of PRMT1 and/or PRMT6. For example, the cDNA of PRMT1 and/or PRMT6 may be used as the probes. If necessary, the probe may be labeled with a suitable label, such as dyes, fluorescent and isotopes, and the expression level of the gene may be detected as the intensity of the hybridized labels.

[0040] Furthermore, the transcription product of PRMT1 and/or PRMT6 may be quantified using primers by amplification-based detection methods (e.g., RT-PCR). Such primers can also be prepared based on the available sequence information of the gene. For example, the primers (SEQ ID NOs: 9 and 10 or 11 and 12 for PRMT1, and 13 and 14 or 15 and 16 for PRMT6) used in the Example may be employed for the detection by RT-PCR or Northern blot, but the present invention is not restricted thereto.

[0041] Specifically, a probe or primer used for the present method hybridizes under

stringent, moderately stringent, or low stringency conditions to the mRNA of PRMT1 and/or PRMT6. As used herein, the phrase "stringent (hybridization) conditions" refers to conditions under which a probe or primer will hybridize to its target sequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different under different circumstances. Specific hybridization of longer sequences is observed at higher temperatures than shorter sequences. Generally, the temperature of a stringent condition is selected to be about 5 degree Centigrade lower than the thermal melting point (Tm) for a specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Since the target sequences are generally present at excess, at Tm, 50% of the probes are occupied at equilibrium. Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30 degree Centigrade for short probes or primers (e.g., 10 to 50 nucleotides) and at least about 60 degree Centigrade for longer probes or primers. Stringent conditions may also be achieved with the addition of destabilizing agents, such as formamide.

[0042] Alternatively, the translation product may be detected for the diagnosis of the present invention. For example, the quantity of PRMT1 and/or PRMT6 protein may be determined. A method for determining the quantity of the protein as the translation product includes immunoassay methods that use an antibody specifically recognizing the protein. The antibody may be monoclonal or polyclonal. Furthermore, any fragment or modification (e.g., chimeric antibody, scFv, Fab, F(ab')2, Fv, etc.) of the antibody may be used for the detection, so long as the fragment retains the binding ability to PRMT1 and/or PRMT6 protein. Methods to prepare these kinds of antibodies for the detection of proteins are well known in the art, and any method may be employed in the present invention to prepare such antibodies and equivalents thereof.

[0043] As another method to detect the expression level of PRMT1 and/or PRMT6 gene based on its translation product, the intensity of staining may be observed via immunohistochemical analysis using an antibody against PRMT1 and/or PRMT6 protein. Namely, the observation of strong staining indicates increased presence of the protein and at the same time high expression level of PRMT1 and/or PRMT6 gene.

Moreover, in addition to the expression level of the PRMT1 and/or PRMT6 gene, the expression level of other cancer-associated genes, for example, genes known to be differentially expressed in cancer may also be determined to confirm the diagnosis.

[0044] The expression level of cancer marker gene including the PRMT1 and/or PRMT6 gene in a biological sample can be considered to be increased if it increases from the

control level of the cancer marker gene in a corresponding non-cancer (normal) sample by, for example, 10%, 25%, or 50%; or increases to more than 1.1 fold, more than 1.5 fold, more than 2.0 fold, more than 5.0 fold, more than 10.0 fold, or more.

[0045] The control level may be determined at the same time with the test biological sample by using a sample(s) previously collected and stored from a subject/subjects whose disease state (cancerous or non-cancerous) is/are known. Alternatively, the control level may be determined by a statistical method based on the results obtained by analyzing previously determined expression level(s) of PRMT1 and/or PRMT6 gene in samples from subjects whose disease state are known. Furthermore, the control level can be a database of expression patterns from previously tested cells. Moreover, according to an aspect of the present invention, the expression level of PRMT1 and/or PRMT6 gene in a biological sample may be compared to multiple control levels, which control levels are determined from multiple reference samples. It is preferred to use a control level determined from a reference sample derived from a tissue type similar to that of the subject-derived biological sample. Moreover, it is preferred to use the standard value of the expression levels of PRMT1 and/or PRMT6 gene in a population with a known disease state. The standard value may be obtained by any method known in the art. For example, a range of mean +/- 2 S.D. or mean +/- 3 S.D. may be used as standard value.

[0046] In the context of the present invention, a control level determined from a biological sample that is known not to be cancerous is referred to as a "normal control level". On the other hand, if the control level is determined from a cancerous biological sample, it is referred to as a "cancerous control level".

When the expression level of PRMT1 and/or PRMT6 gene is increased as compared to the normal control level or is similar to the cancerous control level, the subject may be diagnosed as suffering from or at a risk of developing cancer. Furthermore, in the case where the expression levels of multiple cancer-related genes are compared, a similarity in the gene expression pattern between the sample and the reference which is cancerous indicates that the subject is suffering from or at a risk of developing cancer.

Differences between the expression levels of a test biological sample and the control level can be normalized to the expression level of control nucleic acids, e.g., housekeeping genes, whose expression levels are known not to differ depending on the cancerous or non-cancerous state of the cell. Exemplary control genes include, but are not limited to, beta-actin, glyceraldehyde 3 phosphate dehydrogenase, and ribosomal protein P1.

[0047] A kit for diagnosing cancer:

The present invention provides a kit for diagnosing cancer. Preferably, the cancer may be selected from the group consisting of bladder cancer, diffuse-type gastric

cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML. Specifically, the kit includes at least one reagent for detecting the expression of the PRMT1 and/or PRMT6 gene in a subject-derived biological sample, which reagent may be selected from the group of:

(a) a reagent for detecting mRNA of either of the PRMT1 or PRMT6 gene or both;

(b) a reagent for detecting either of the PRMT1 or PRMT6 protein or both; and

(c) a reagent for detecting the biological activity of either of the PRMT1 or PRMT6 protein or both.

[0048] Suitable reagents for detecting mRNA of the PRMT1 and/or PRMT6 gene include nucleic acids that specifically bind to or identify the PRMT1 and/or PRMT6 mRNA, such as oligonucleotides which have a complementary sequence to a part of the PRMT1 and/or PRMT6 mRNA. These kinds of oligonucleotides are exemplified by primers and probes that are specific to the PRMT1 and/or PRMT6 mRNA. These kinds of oligonucleotides may be prepared based on methods well known in the art. If needed, the reagent for detecting the PRMT1 and/or PRMT6 mRNA may be immobilized on a solid matrix. Moreover, more than one reagent for detecting the PRMT1 and/or PRMT6 mRNA may be included in the kit.

[0049] A probe or primer of the present invention typically comprises a substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 2000, 1000, 500, 400, 350, 300, 250, 200, 150, 100, 50, or 25, consecutive sense strand nucleotide sequence of a nucleic acid comprising a PRMT1 or PRMT6 sequence, or an antisense strand nucleotide sequence of a nucleic acid comprising a PRMT1 or PRMT6 sequence, or of a naturally occurring mutant of these sequences. In particular, for example, in a preferred embodiment, an oligonucleotide having 5-50 in length can be used as a primer for amplifying the genes, to be detected. More preferably, mRNA or cDNA of PRMT1 or PRMT6 gene can be detected with oligonucleotide probe or primer having 15- 30b in length. In preferred embodiments, length of the oligonucleotide probe or primer can be selected from 15-25. Assay procedures, devices, or reagents for the detection of gene by using such oligonucleotide probe or primer are well known (e.g. oligonucleotide microarray or PCR). In these assays, probes or primers can also comprise tag or linker sequences. Further, probes or primers can be modified with detectable label or affinity ligand to be captured. Alternatively, in hybridization based detection procedures, a polynucleotide having a few hundreds (e.g., about 100-200) bases to a few kilo (e.g., about 1000-2000) bases in length can also be used for a probe (e.g., northern blotting assay or cDNA microarray analysis).

[0050] On the other hand, suitable reagents for detecting the PRMT1 and/or PRMT6 protein

include antibodies to the PRMT1 and/or PRMT6 protein. The antibody may be monoclonal or polyclonal. Furthermore, any fragment or modification (e.g., chimeric antibody, scFv, Fab, F(ab')2, Fv, etc.) of the antibody may be used as the reagent, so long as the fragment retains the ability to bind the PRMT1 and/or PRMT6 protein. Methods to prepare these kinds of antibodies for the detection of proteins are well known in the art, and any method may be employed in the present invention to prepare such antibodies and equivalents thereof. Furthermore, the antibody may be labeled with signal generating molecules via direct linkage or an indirect labeling technique. Labels and methods for labeling antibodies and detecting the binding of antibodies to their targets are well known in the art and any labels and methods may be employed for the present invention. Moreover, more than one reagent for detecting the PRMT1 and/or PRMT6 protein may be included in the kit.

[0051] Furthermore, the biological activity can be determined by, for example, measuring the cell proliferating activity due to the expressed PRMT1 and/or PRMT6 protein in the biological sample. For example, the cell proliferating activity of a subject derived biological sample can be determined by culturing a cell in the presence of the subject-derived biological sample, and detecting the speed of proliferation, measuring the cell cycle, or measuring the colony forming ability. If needed, the reagent for detecting the PRMT1 and/or PRMT6 mRNA may be immobilized on a solid matrix. Moreover, more than one reagent for detecting the biological activity of the PRMT1 and/or PRMT6 protein may be included in the kit.

[0052] The kit may contain more than one of the aforementioned reagents. Furthermore, the kit may include a solid matrix and reagent for binding a probe against the PRMT1 and/or PRMT6 gene or antibody against the proteins, a medium and container for culturing cells, positive and negative control reagents, and a secondary antibody for detecting an antibody against the PRMT1 and/or PRMT6 protein. For example, tissue samples obtained from subject suffering from cancer or not may serve as useful control reagents. A kit of the present invention may further include other materials desirable from a commercial and user standpoint, including buffers, diluents, filters, needles, syringes, and package inserts (e.g., written, tape, CD-ROM, etc.) with instructions for use. These reagents and such may be contained in a container with a label. Suitable containers include bottles, vials, and test tubes. The containers may be formed from a variety of materials, such as glass or plastic.

[0053] As an embodiment of the present invention, when the reagent is a probe against the PRMT1 and/or PRMT6 mRNA, the reagent may be immobilized on a solid matrix, such as a porous strip, to form at least one detection site. The measurement or detection region of the porous strip may include a plurality of sites, each containing a nucleic acid (probe). A test strip may also contain sites for negative and/or positive

controls. Alternatively, control sites may be located on a strip separated from the test strip. Optionally, the different detection sites may contain different amounts of immobilized nucleic acids, i.e., a higher amount in the first detection site and lesser amounts in subsequent sites. Upon the addition of test sample, the number of sites displaying a detectable signal provides a quantitative indication of the amount of PRMT1 and/or PRMT6 mRNA present in the sample. The detection sites may be configured in any suitably detectable shape and are typically in the shape of a bar or dot spanning the width of a test strip.

[0054] The kit of the present invention may further include a positive control sample or PRMT1 and/or PRMT6 standard sample. The positive control sample of the present invention may be prepared by collecting PRMT1 and/or PRMT6 positive samples and then those PRMT1 and/or PRMT6 level are assayed. In one embodiment, either or both of the PRMT1 and PRMT6 positive tissue samples may be composed of cancer cells expressing either of PRMT1 or PRMT6 or both. Such cancer cells include, but are not limited to, cancer selected from the group consisting of bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML. The PRMT1 and/or PRMT6 level of the positive control sample is, for example, more than cut off value.

[0055] Serological diagnosis of cancer:

The present invention also provides asymmetric dimethylarginine (ADMA) as a novel serological cancer marker. Namely, by measuring the level of ADMA in subject-derived blood samples, the occurrence of, or a predisposition to, a cancer expressing PRMT1 and/or PRMT6 in a subject can be determined.

In the context of the present invention, any cancer related to PRMT1 and/or PRMT6 overexpression can be diagnosed, for example, bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML, more preferably cancer, hematopoietic tumor, gastric cancer and breast cancer.

[0056] Accordingly, the present invention involves determining (e.g., measuring) the level of ADMA in blood samples. In the present invention, a method for diagnosing cancer also includes a method for testing or detecting cancer. Alternatively, in the present invention, diagnosing cancer also refers to showing a suspicion, risk, or possibility of cancer in a subject, or using ADMA as a cancer marker.

[0057] Alternatively, by measuring the level of ADMA in subject-derived blood samples, the occurrence of, or a predisposition to, a cancer expressing PRMT1 and/or PRMT6 in a subject can be determined. Accordingly, the present invention involves determining (e.g., measuring) the level of ADMA in blood samples. In the present

invention, a method for diagnosing cancer also includes a method for testing or detecting cancer. Alternatively, in the present invention, diagnosing cancer also refers to showing a suspicion, risk, or possibility of cancer in a subject.

Any blood samples may be used for determining the level of ADMA so long as ADMA can be detected in the samples. Preferably, the blood samples include whole blood, serum, and plasma, more preferably serum.

[0058] In the present invention, the "level of ADMA in blood samples" refers to the concentration of ADMA present in the blood after correcting the corpuscular volume in the whole blood. One of skilled in the art will recognize that the percentage of corpuscular volume in the blood varies greatly between individuals. For example, the percentage of erythrocytes in the whole blood is very different between men and women. Furthermore, differences between individuals cannot be ignored. Therefore, the apparent concentration of a substance in the whole blood which includes corpuscular components varies greatly depending on the percentage of corpuscular volume. For example, even if the concentration in the serum is the same, the measured value for a sample with a large amount of corpuscular component will be lower than the value for a sample with a small amount of corpuscular component. Therefore, to compare the measured values of components in the blood, values for which the corpuscular volume has been corrected are usually used.

[0059] For example, by measuring components in the blood using, as samples, serum or plasma obtained by separating blood cells from the whole blood, measured values from which the effect from the corpuscular volume has been removed can be obtained. Therefore, the level of ADMA in the present invention can usually be determined as a concentration in the serum or plasma. Alternatively, it may first be measured as a concentration in the whole blood, and then the effect from the corpuscular volume may be corrected. Methods for measuring a corpuscular volume in a whole blood sample are known.

[0060] Subjects diagnosed for cancer according to the present methods are preferably mammals and include humans, non-human primates, mice, rats, dogs, cats, horses and cows. A preferable subject of the present invention is a human.

In the present invention, a subject may be a patient suspected of having cancer or a healthy individual. The patient may be diagnosed by the present invention to facilitate clinical decision-making. In another embodiment, the present invention may also be applied to healthy individuals for screening of cancer.

[0061] Furthermore, an intermediate result for examining the condition of a subject may be provided. Such intermediate result may be combined with additional information to assist a doctor, nurse, or other practitioner to diagnose that a subject suffers from the disease. Alternatively, the present invention may be used to detect ADMA as a cancer

marker in a blood sample, and provide a doctor with useful information to diagnose that the subject, from which the blood sample is derived, suffers from the disease. In other word, the present invention may provide a serological cancer marker for determined a blood sample derived from a subject who has cancerous cells.

[0062] In one embodiment of the present invention, the level of ADMA is determined by measuring the quantity or concentration of ADMA protein in blood samples. Methods for determining the quantity of the ADMA protein in blood samples include immunoassay methods. The immunoassay methods may be preferably ELISA, and antibodies to be used for the immunoassay methods may be preferably antibodies raised against the ADMA.

In the methods of diagnosis of the present invention, the blood concentration of other markers may be determined, in addition to the blood concentration of ADMA, to detect cancer. Therefore, the present invention provides methods for diagnosing cancer, in which cancer is detected when either the blood concentration of ADMA and the blood concentration of other markers, are higher as compared with healthy individuals.

[0063] In the present invention, a novel serological marker for cancer, ADMA, is provided. As shown in Examples discussed bellow, ADMA shows higher sensitivity, and therefore, improvement in the sensitivity of diagnostic or detection methods for cancer may be achieved by the present invention. Namely, the present invention provides a method for diagnosing cancer in a subject, including the steps of:

(a) collecting a blood sample from a subject to be diagnosed;

(b) determining a level of ADMA in the blood sample; and

(c) comparing the ADMA level determined in step (b) with that of a normal control, wherein a high ADMA level in the blood sample, as compared to the normal control, indicates that the subject suffers from or is at a risk of developing cancer.

[0064] In another embodiment, the method of the present invention may further include the steps of:

(d) determining a level of other markers in the blood sample;

(e) comparing the level of other markers determined in step (d) with that of a normal control; and

(f) judging that the subject suffers from or is at a risk of developing cancer, when the level of ADMA and/or the level of the other markers are higher than the control levels (Alternatively, judging that the subject suffers from or is at a risk of developing cancer, when either of the level of ADMA or the level of the other markers or both are higher than the control levels).

[0065] In the present invention, the standard value of the blood concentration of ADMA can be determined statistically. For example, the blood concentration of ADMA in healthy individuals can be measured to determine the standard blood concentration of ADMA

statistically. When a statistically sufficient population is gathered, a value in the range of twice or three times the standard deviation (S.D.) from the mean value is often used as the standard value. Therefore, values corresponding to the mean value + 2 x S.D. or mean value + 3 x S.D. may be used as standard values. The standard values set as described theoretically include 90% and 99.7% of healthy individuals, respectively.

[0066] Alternatively, standard values can also be set based on the actual blood concentration of ADMA in cancer patients. Generally, standard values set this way minimize the percentage of false positives, and are selected from a range of values satisfying conditions that can maximize detection sensitivity. In this case, the standard values are usually referred to as "cut off value". Herein, the percentage of false positives refers to a percentage, among healthy individuals, of patients whose blood concentration of ADMA is judged to be higher than a standard value (cut off value). On the contrary, the percentage, among healthy individuals, of patients whose blood concentration of ADMA is judged to be lower than a standard value (cut off value) indicates specificity. That is, the sum of the false positive percentage and the specificity is always 1. The detection sensitivity refers to the percentage of patients whose blood concentration of ADMA is judged to be higher than a standard value (cut off value), among all cancer patients within a population of individuals for whom the presence of cancer has been determined.

[0067] Furthermore, in the present invention, the percentage of cancer patients among patients whose ADMA concentration was judged to be higher than a standard value (cut off value) represents the positive predictive value. On the other hand, the percentage of healthy individuals among patients whose ADMA concentration was judged to be lower than a standard value (cut off value) represents the negative predictive value. The relationship between these values is summarized in Table 1. As the relationship shown below indicates, each of the values for sensitivity, specificity, positive predictive value, and negative predictive value, which are indexes for evaluating the diagnostic accuracy for cancer, varies depending on the standard value (cut off value) for judging the level of the blood concentration of ADMA.

[0068] [Table 1]

Blood concentration of ADMA	Cancer patients	Healthy individuals	
High	a: True positive	b: False positive	Positive predictive value a/(a+b)
Low	c: False negative	d: True negative	Negative predictive value d/(c+d)
	Sensitivity a/(a+c)	Specificity d/(b+d)	

As mentioned previously, a standard value (cut off value) is usually set such that the

false positive ratio is low and the sensitivity is high. However, as also apparent from the relationship shown above, there is a trade-off between the false positive ratio and sensitivity. That is, if the standard value (cut off value) is decreased, the detection sensitivity increases. However, since the false positive ratio also increases, it is difficult to satisfy the conditions to have a "low false positive ratio". Considering this situation, for example, values that give the following predicted results may be selected as the preferable standard values (cut off values) in the present invention.

Standard values (cut off values) for which the false positive ratio is 50% or less (that is, standard values (cut off values) for which the specificity is not less than 50%).

Standard values (cut off values) for which the sensitivity is not less than 20%.

[0069] In the present invention, the standard values (cut off values) can be set using a receiver operating characteristic (ROC) curve. An ROC curve is a graph that shows the detection sensitivity on the vertical axis and the false positive ratio (that is, "1 - specificity") on the horizontal axis. In the present invention, an ROC curve can be obtained by plotting the changes in the sensitivity and the false positive ratio, which were obtained after continuously varying the standard value (cut off value) for determining the high/low degree of the blood concentration of ADMA.

The "standard value (cut off value)" for obtaining the ROC curve is a value temporarily used for the statistical analyses. The "standard value (cut off value)" for obtaining the ROC curve can generally be continuously varied within a range that is allowed to cover all selectable standard values (cut off value). For example, the standard value (cut off value) can be varied between the smallest and largest measured ADMA values in an analyzed population.

[0070] Based on the obtained ROC curve, a preferable standard value (cut off value) to be used in the present invention can be selected from a range that satisfies the above-mentioned conditions. Alternatively, a standard value (cut off value) can be selected based on an ROC curve produced by varying the standard values (cut off values) from a range that includes most of the measured ADMA values. In the present invention, the standard value (cut off value) of the ADMA blood concentration may be set at, for example, 0.6 to 2.0 ng/ml, preferably 0.7 to 1.8 ng/ml, more preferably 0.8 to 1.5 ng/ml, more preferably 0.9 to 1.2 ng/ml, more preferably 1.0 ng/ml.

[0071] ADMA in the blood can be measured by any method that can quantitate proteins. For example, immunoassay, liquid chromatography, surface plasmon resonance (SPR), mass spectrometry, or the like can be used in the present invention. In mass spectrometry, proteins can be quantitated by using a suitable internal standard. For example, isotope-labeled ADMA can be used as the internal standard. The concentration of ADMA in the blood can be determined from the peak intensity of ADMA in the blood and that of the internal standard. Generally, the matrix-assisted laser

desorption/ionization (MALDI) method is used for mass spectrometry of proteins. With an analysis method that uses mass spectrometry or liquid chromatography, ADMA can also be analyzed simultaneously with other tumor markers (e.g., CEA or CYFRA).

[0072] A preferable method for measuring ADMA in the present invention is the immunoassay. Those skilled in the art can prepare antibodies by synthesizing necessary immunogens based on the amino acid sequence of ADMA. The peptide used as immunogen can be easily synthesized using a peptide synthesizer. The synthetic peptide can be used as an immunogen by linking it to a carrier protein.

Keyhole limpet hemocyanin, myoglobin, albumin, and the like can be used as the carrier protein. Preferable carrier proteins are KLH, bovine serum albumin, and such. The maleimidobenzoyl-N-hydrosuccinimide ester method (hereinafter abbreviated as the MBS method) and the like are generally used to link synthetic peptides to carrier proteins.

[0073] Specifically, a cysteine is introduced into the synthetic peptide and the peptide is crosslinked to KLH by MBS using the cysteine's SH group. The cysteine residue may be introduced at the N-terminus or C-terminus of the synthesized peptide.

Immunogens obtained in this manner are mixed with a suitable adjuvant and used to immunize animals. Known adjuvants include Freund's complete adjuvant (FCA) and incomplete adjuvant. The immunization procedure is repeated at appropriate intervals until an increase in the antibody titer is confirmed. There are no particular limitations on the immunized animals in the present invention. Specifically, animals commonly used for immunization such as mice, rats, or rabbits can be used.

[0074] When obtaining the antibodies as monoclonal antibodies, animals that are advantageous for their production may be used. For example in mice, many myeloma cell lines for cell fusion are known, and techniques for establishing hybridomas with a high probability are already well known. Therefore, mice are a desirable immunized animal to obtain monoclonal antibodies.

[0075] Furthermore, the immunization treatments are not limited to in vitro treatments. Methods for immunologically sensitizing cultured immunocompetent cells in vitro can also be employed. Antibody-producing cells obtained by these methods are transformed and cloned. Methods for transforming antibody-producing cells to obtain monoclonal antibodies are not limited to cell fusion. For example, methods for obtaining cloneable transformants by virus infection are known.

[0076] Hybridomas that produce the monoclonal antibodies used in the present invention can be screened based on their reactivity to ADMA. Specifically, antibody-producing cells are first selected by using as an index the binding activity toward ADMA, or a domain peptide thereof, that was used as the immunogen. Positive clones that are

selected by this screening are subcloned as necessary.

The monoclonal antibodies to be used in the present invention can be obtained by culturing the established hybridomas under suitable conditions and collecting the produced antibodies. When the hybridomas are homohybridomas, they can be cultured in vivo by inoculating them intraperitoneally in syngeneic animals. In this case, monoclonal antibodies are collected as ascites fluid. When heterohybridomas are used, they can be cultured in vivo using nude mice as a host.

[0077] In addition to in vivo cultures, hybridomas are also commonly cultured ex vivo, in a suitable culture environment. For example, basal media such as RPMI 1640 and DMEM are generally used as the medium for hybridomas. Additives such as animal sera can be added to these media to maintain the antibody-producing ability to a high level. When hybridomas are cultured ex vivo, the monoclonal antibodies can be collected as a culture supernatant. Culture supernatants can be collected by separating from cells after culturing, or by continuously collecting while culturing using a culture apparatus that uses a hollow fiber.

[0078] Monoclonal antibodies used in the present invention are prepared from monoclonal antibodies collected as ascites fluid or culture supernatants, by separating immunoglobulin fractions by saturated ammonium sulfate precipitation and further purifying by gel filtration, ion exchange chromatography, or such. In addition, if the monoclonal antibodies are IgGs, purification methods based on affinity chromatography with a protein A or protein G column are effective.

[0079] On the other hand, to obtain antibodies used in the present invention as polyclonal antibodies, blood is drawn from animals whose antibody titer increased after immunization, and the serum is separated to obtain an anti-serum. Immunoglobulins are purified from anti-sera by known methods to prepare the antibodies used in the present invention. ADMA-specific antibodies can be prepared by combining immunoaffinity chromatography which uses ADMA as a ligand with immunoglobulin purification.

[0080] The binding of antibodies to antigens can be detected by various immunoassay principles. Immunoassays can be broadly categorized into heterogeneous analysis methods and homogeneous analysis methods. To maintain the sensitivity and specificity of immunoassays to a high level, the use of monoclonal antibodies is desirable. Methods of the present invention for measuring ADMA by various immunoassay formats are explained in further detail herein.

First, exemplary methods for measuring substance (ADMA) using a heterogeneous immunoassay are described. In heterogeneous immunoassays, a mechanism for detecting antibodies that bind to the substance after separating them from those that do not bind to the substance is required.

[0081] To facilitate the separation, immobilized reagents are generally used. For example, a

solid phase onto which antibodies recognizing the substance have been immobilized is first prepared (immobilized antibodies). The substance is made to bind to these, and secondary antibodies are further reacted thereto.

When the solid phase is separated from the liquid phase and further washed, as necessary, secondary antibodies remain on the solid phase in proportion to the concentration of the substance. By labeling the secondary antibodies, the substance can be quantitated by measuring the signal derived from the label.

[0082] Any method may be used to bind the antibodies to the solid phase. For example, antibodies can be physically adsorbed to hydrophobic materials such as polystyrene. Alternatively, antibodies can be chemically bound to a variety of materials having functional groups on their surfaces. Furthermore, antibodies labeled with a binding ligand can be bound to a solid phase by trapping them using a binding partner of the ligand. Combinations of a binding ligand and its binding partner include avidin-biotin and such. The solid phase and antibodies can be conjugated at the same time or before the reaction between the primary antibodies and the substance.

Similarly, the secondary antibodies do not need to be directly labeled. That is, they can be indirectly labeled using antibodies against antibodies or using binding reactions such as that of avidin-biotin.

[0083] The concentration of the substance in a sample is determined based on the signal intensities obtained using standard samples with known concentrations of the substance.

Any antibody can be used as the immobilized antibody and secondary antibody for the heterogeneous immunoassays mentioned above, so long as it is an antibody, or a fragment including an antigen-binding site thereof, that recognizes the substance. Therefore, it may be a monoclonal antibody, a polyclonal antibody, or a mixture or combination of both. For example, a combination of monoclonal antibodies and polyclonal antibodies is a preferable combination in the present invention. Alternatively, when both antibodies are monoclonal antibodies, combining monoclonal antibodies recognizing different epitopes is preferable.

[0084] Since the antigens to be measured are sandwiched by antibodies, such heterogeneous immunoassays are called sandwich methods. Since sandwich methods excel in the measurement sensitivity and the reproducibility, they are a preferable measurement principle in the present invention.

The principle of competitive inhibition reactions can also be applied to the heterogeneous immunoassays. For example, immunoassays based on the phenomenon of competitive inhibition of the binding between the substance with a known concentration and an antibody can be used. The concentration of the substance in the sample can be determined by labeling substance with a known concentration and measuring the amount of substance that reacted (or did not react) with the antibody.

[0085] A competitive reaction system is established when antigens with a known concentration and antigens in a sample are simultaneously reacted to an antibody. Furthermore, analyses by an inhibitory reaction system are possible when antibodies are reacted with antigens in a sample, and antigens with a known concentration are reacted thereafter. In both types of reaction systems, reaction systems that excel in the operability can be constructed by setting either one of the antigens with a known concentration used as a reagent component or the antibody as the labeled component, and the other one as the immobilized reagent.

[0086] Radioisotopes, fluorescent substances, luminescent substances, substances having an enzymatic activity, macroscopically observable substances, magnetically observable substances, and such are used in these heterogeneous immunoassays. Specific examples of these labeling substances are shown below.

Substances having an enzymatic activity:
peroxidase,
alkaline phosphatase,
urease, catalase,
glucose oxidase,
lactate dehydrogenase, or
amylase, etc.
Fluorescent substances:
fluorescein isothiocyanate,
tetramethylrhodamine isothiocyanate,
substituted rhodamine isothiocyanate, or
dichlorotriazine isothiocyanate, etc.
Radioisotopes:
tritium,
^{125}I, or
^{131}I, etc.

[0087] Among these, non-radioactive labels such as enzymes are an advantageous labels in terms of safety, operability, sensitivity, and such. Enzymatic labels can be linked to antibodies or to ADMA by known methods such as the periodic acid method or maleimide method.

As the solid phase, beads, inner walls of a container, fine particles, porous carriers, magnetic particles, or such are used. Solid phases formed using materials such as polystyrene, polycarbonate, polyvinyl toluene, polypropylene, polyethylene, polyvinyl chloride, nylon, polymethacrylate, latex, gelatin, agarose, glass, metal, ceramic, or such can be used. Solid materials in which functional groups to chemically bind antibodies and such have been introduced onto the surface of the above solid materials

are also known. Known binding methods, including chemical binding such as poly-L-lysine or glutaraldehyde treatment and physical adsorption, can be applied for solid phases and antibodies (or antigens).

[0088] Although the steps of separating the solid phase from the liquid phase and the washing steps are required in all heterogeneous immunoassays exemplified herein, these steps can easily be performed using the immunochromatography method, which is a variation of the sandwich method.

Specifically, antibodies to be immobilized are immobilized onto porous carriers capable of transporting a sample solution by the capillary phenomenon, then a mixture of a sample including the substance (ADMA) and labeled antibodies is deployed therein by this capillary phenomenon. During deployment, the substance reacts with the labeled antibodies, and when it further contacts the immobilized antibodies, it is trapped at that location. The labeled antibodies that do not react with the substance pass through, without being trapped by the immobilized antibodies.

[0089] As a result, the presence of the substance can be detected using, as an index, the signals of the labeled antibodies that remain at the location of the immobilized antibodies. If the labeled antibodies are maintained upstream in the porous carrier in advance, all reactions can be initiated and completed by just dripping in the sample solutions, and an extremely simple reaction system can be constructed. In the immunochromatography method, labeled components that can be distinguished macroscopically, such as colored particles, can be combined to construct an analytical device that does not even require a special reader.

[0090] Furthermore, in the immunochromatography method, the detection sensitivity for the substance can be adjusted. For example, by adjusting the detection sensitivity near the cutoff value described below, the aforementioned labeled components can be detected when the cutoff value is exceeded. By using such a device, whether a subject is positive or negative can be judged very simply. By adopting a constitution that allows a macroscopic distinction of the labels, necessary examination results can be obtained by simply applying blood samples to the device for immunochromatography.

[0091] Various methods for adjusting the detection sensitivity of the immunochromatography method are known in the art. For example, a second immobilized antibody for adjusting the detection sensitivity can be placed between the position where samples are applied and the immobilized antibodies (Japanese Patent Application Kokai Publication No. (JP-A) H06-341989 (unexamined, published Japanese patent application)). The substance in the sample is trapped by the second immobilized antibody while deploying from the position where the sample was applied to the position of the first immobilized antibody for label detection. After the second immobilized antibody is saturated, the substance can reach the position of the first im-

mobilized antibody located downstream. As a result, when the concentration of the substance in the sample exceeds a predetermined concentration, the substance bound to the labeled antibody is detected at the position of the first immobilized antibody.

[0092] Next, exemplary homogeneous immunoassays are described. As opposed to heterogeneous immunological assay methods that require a separation of the reaction solutions as described above, the substance (ADMA) can also be measured using homogeneous analysis methods. Homogeneous analysis methods allow the detection of antigen-antibody reaction products without their separation from the reaction solutions.

A representative homogeneous analysis method is the immunoprecipitation reaction, in which antigenic substances are quantitatively analyzed by examining precipitates produced following an antigen-antibody reaction. Polyclonal antibodies are generally used for the immunoprecipitation reactions. When monoclonal antibodies are applied, multiple types of monoclonal antibodies that bind to different epitopes of the substance are preferably used. The products of precipitation reactions that follow the immunological reactions can be macroscopically observed or can be optically measured for conversion into numerical data.

[0093] The immunological particle agglutination reaction, which uses as an index the agglutination by antigens of antibody-sensitized fine particles, is a common homogeneous analysis method. As in the aforementioned immunoprecipitation reaction, polyclonal antibodies or a combination of multiple types of monoclonal antibodies can be used in this method as well. Fine particles can be sensitized with antibodies through sensitization with a mixture of antibodies, or they can be prepared by mixing particles sensitized separately with each antibody. Fine particles obtained in this manner gives matrix-like reaction products upon contact with the substance. The reaction products can be detected as particle aggregation. Particle aggregation may be macroscopically observed or can be optically measured for conversion into numerical data.

[0094] Immunological analysis methods based on energy transfer and enzyme channeling are known as homogeneous immunoassays. In methods utilizing energy transfer, different optical labels having a donor/acceptor relationship are linked to multiple antibodies that recognize adjacent epitopes on an antigen. When an immunological reaction takes place, the two parts approach and an energy transfer phenomenon occurs, resulting in a signal such as quenching or a change in the fluorescence wavelength. On the other hand, enzyme channeling utilizes labels for multiple antibodies that bind to adjacent epitopes, in which the labels are a combination of enzymes having a relationship such that the reaction product of one enzyme is the substrate of another. When utilizing the two parts approach due to an immunological reaction, the enzyme reactions are promoted; therefore, their binding can be detected as a change in the enzyme reaction rate.

[0095] In the present invention, blood for measuring ADMA can be prepared from blood drawn from patients. Preferable blood samples are the serum or plasma. Serum or plasma samples can be diluted before the measurements. Alternatively, the whole blood can be measured as a sample and the obtained measured value can be corrected to determine the serum concentration. For example, concentration in whole blood can be corrected to the serum concentration by determining the percentage of corpuscular volume in the same blood sample.

In a preferred embodiment, the immunoassay includes an ELISA. The present inventors established sandwich ELISA to detect serum ADMA in patients with cancer.

[0096] The ADMA level in the blood samples is then compared with an ADMA level associated with a reference sample such as a normal control sample. The phrase "normal control level" refers to the level of ADMA typically found in a blood sample of a population not suffering from cancer, respectively. The reference sample is preferably of a similar nature to that of the test sample. For example, if the test samples include patient serum, the reference sample should also be serum. The ADMA level in the blood samples from control and test subjects may be determined at the same time or, alternatively, the normal control level may be determined by a statistical method based on the results obtained by analyzing the level of ADMA in samples previously collected from a control group.

[0097] The ADMA level may also be used to monitor the course of treatment of cancer. In this method, a test blood sample is provided from a subject undergoing treatment for cancer. Preferably, multiple test blood samples are obtained from the subject at various time points, including before, during, and/or after the treatment. The level of ADMA in the post-treatment sample may then be compared with the level of ADMA in the pre-treatment sample or, alternatively, with a reference sample (e.g., a normal control level). For example, if the post-treatment ADMA level is lower than the pre-treatment ADMA level, one can conclude that the treatment was efficacious. Likewise, if the post-treatment ADMA level is similar to the normal control ADMA level, one can also conclude that the treatment was efficacious.

[0098] An "efficacious" treatment is one that leads to a reduction in the level of ADMA or a decrease in size, prevalence, or metastatic potential of cancer in a subject. When a treatment is applied prophylactically, "efficacious" means that the treatment retards or prevents occurrence of cancer or alleviates a clinical symptom of cancer. The assessment of cancer can be made using standard clinical protocols. Furthermore, the efficaciousness of a treatment can be determined in association with any known method for diagnosing or treating cancer. For example, cancer is routinely diagnosed histopathologically or by identifying symptomatic anomalies.

[0099] Kit for the serological diagnosis of cancer:

Components used to carry out the diagnosis of cancer according to the present invention can be combined in advance and supplied as a testing kit. Accordingly, the present invention provides a kit for detecting cancer, which relates to either of PRMT1 or PRMT6 or both overexpression, including:

(i) an immunoassay reagent for determining a level of ADMA in a blood sample.

In the preferable embodiments, the kit of the present invention may further include:

(ii) a positive control sample for ADMA.

The kit of the present invention may be preferably applicable to bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML.

[0100] The reagents for the immunoassays which constitute a kit of the present invention may include reagents necessary for the various immunoassays described above. Specifically, the reagents for the immunoassays include an antibody that recognizes the substance to be measured. The antibody can be modified depending on the assay format of the immunoassay. ELISA can be used as a preferable assay format of the present invention. In ELISA, for example, a first antibody immobilized onto a solid phase and a second antibody having a label are generally used.

[0101] Therefore, the immunoassay reagents for ELISA can include a first antibody immobilized onto a solid phase carrier. Fine particles or the inner walls of a reaction container can be used as the solid phase carrier. Magnetic particles can be used as the fine particles. Alternatively, multi-well plates such as 96-well microplates are often used as the reaction containers. Containers for processing a large number of samples, which are equipped with wells having a smaller volume than in 96-well microplates at a high density, are also known. In the present invention, the inner walls of these reaction containers can be used as the solid phase carriers.

[0102] The immunoassay reagents for ELISA may further include a second antibody having a label. The second antibody for ELISA may be an antibody onto which an enzyme is directly or indirectly linked. Methods for chemically linking an enzyme to an antibody are known. For example, immunoglobulins can be enzymatically cleaved to obtain fragments including the variable regions. By reducing the -SS- bonds included in these fragments to -SH groups, bifunctional linkers can be attached. By linking an enzyme to the bifunctional linkers in advance, enzymes can be linked to the antibody fragments.

[0103] Alternatively, to indirectly link an enzyme, for example, the avidin-biotin binding can be used. That is, an enzyme can be indirectly linked to an antibody by contacting a biotinylated antibody with an enzyme to which avidin has been attached. In addition, an enzyme can be indirectly linked to a second antibody using a third antibody which is an enzyme-labeled antibody recognizing the second antibody. For example, enzymes

such as those exemplified above can be used as the enzymes to label the antibodies.

[0104] Kits of the present invention include a positive control for ADMA. A positive control for ADMA includes ADMA whose concentration has been determined in advance. Preferable concentrations are, for example, a concentration set as the standard value (e.g., 1.0 ng/ml as the cut off value) in a testing method of the present invention. Alternatively, a positive control having a higher concentration can also be combined. The positive control for ADMA in the present invention can additionally include other markers whose concentration has been determined in advance.

[0105] The positive controls in the present invention are preferably in a liquid form. In the present invention, blood samples are used as samples. Therefore, samples used as controls also need to be in a liquid form. Alternatively, by dissolving a dried positive control with a predefined amount of liquid at the time of use, a control that gives the tested concentration can be prepared. By packaging, together with a dried positive control, an amount of liquid necessary to dissolve it, the user can obtain the necessary positive control by just mixing them. ADMA used as the positive control can be a naturally-derived protein or it may be a recombinant protein. Not only positive controls, but also negative controls can be combined in the kits of the present invention. The positive controls or negative controls are used to verify that the results indicated by the immunoassays are correct.

[0106] Screening for an anti-cancer substance:

In the context of the present invention, agents to be identified through the present screening methods may be any substance or composition including several substances. Furthermore, the test substance exposed to a cell or protein according to the screening methods of the present invention may be a single substance or a combination of substances. When a combination of substances is used in the methods, the substances may be contacted sequentially or simultaneously.

[0107] Any test substance, for example, cell extracts, cell culture supernatant, products of fermenting microorganism, extracts from marine organism, plant extracts, purified or crude proteins, peptides, non-peptide substances, synthetic micromolecular substances (including nucleic acid constructs, such as antisense RNA, siRNA, Ribozymes, and aptamer, etc.) and natural substances can be used in the screening methods of the present invention. The test substance of the present invention can be also obtained using any of the numerous approaches in combinatorial library methods known in the art, including (1) biological libraries, (2) spatially addressable parallel solid phase or solution phase libraries, (3) synthetic library methods requiring deconvolution, (4) the "one-bead one-compound" library method and (5) synthetic library methods using affinity chromatography selection. The biological library methods using affinity chromatography selection is limited to peptide libraries, while the other four approaches are

applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, Anticancer Drug Des 1997, 12: 145-67). Examples of methods for the synthesis of molecular libraries can be found in the art (DeWitt et al., Proc Natl Acad Sci USA 1993, 90: 6909-13; Erb et al., Proc Natl Acad Sci USA 1994, 91: 11422-6; Zuckermann et al., J Med Chem 37: 2678-85, 1994; Cho et al., Science 1993, 261: 1303-5; Carell et al., Angew Chem Int Ed Engl 1994, 33: 2059; Carell et al., Angew Chem Int Ed Engl 1994, 33: 2061; Gallop et al., J Med Chem 1994, 37: 1233-51). Libraries of compounds may be presented in solution (see Houghten, Bio/Techniques 1992, 13: 412-21) or on beads (Lam, Nature 1991, 354: 82-4), chips (Fodor, Nature 1993, 364: 555-6), bacteria (US Pat. No. 5,223,409), spores (US Pat. No. 5,571,698; 5,403,484, and 5,223,409), plasmids (Cull et al., Proc Natl Acad Sci USA 1992, 89: 1865-9) or phage (Scott and Smith, Science 1990, 249: 386-90; Devlin, Science 1990, 249: 404-6; Cwirla et al., Proc Natl Acad Sci USA 1990, 87: 6378-82; Felici, J Mol Biol 1991, 222: 301-10; US Pat. Application 2002103360).

[0108] A substance in which a part of the structure of the substance screened by any of the present screening methods is converted by addition, deletion and/or replacement, is included in the agents obtained by the screening methods of the present invention.

Furthermore, when the screened test substance is a protein, for obtaining a DNA encoding the protein, either the whole amino acid sequence of the protein may be determined to deduce the nucleic acid sequence coding for the protein, or partial amino acid sequence of the obtained protein may be analyzed to prepare an oligo DNA as a probe based on the sequence, and screen cDNA libraries with the probe to obtain a DNA encoding the protein. The obtained DNA is confirmed it's usefulness in preparing the test substance which is a candidate for treating or preventing cancer.

[0109] Test substances useful in the screenings described herein can also be antibodies that specifically bind to PRMT1 and/or PRMT6 protein or partial peptides thereof that lack the biological activity of the original proteins in vivo.

Although the construction of test substance libraries is well known in the art, herein below, additional guidance in identifying test substances and construction libraries of such agents for the present screening methods are provided.

[0110] It is herein revealed that suppression of either of the expression level or biological activity of PRMT1 or PRMT6, or both leads to suppression of the growth of cancer cells. Therefore, when a substance suppresses the expression and/or activity of PRMT1 or PRMT6, such suppression is indicative of a potential therapeutic effect in a subject. In the context of the present invention, a potential therapeutic effect refers to a clinical benefit with a reasonable expectation. Examples of such clinical benefit include but are not limited to;

(a) reduction in expression of the either of PRMT1 or PRMT6 gene or both,

(b) a decrease in size, prevalence, or metastatic potential of the cancer in the subject,

(c) preventing cancers from forming, or

(d) preventing or alleviating a clinical symptom of cancer.

[0111] (i) Molecular modeling:

Construction of test substance libraries is facilitated by knowledge of the molecular structure of compounds known to have the properties sought, and/or the molecular structure of PRMT1 and/or PRMT6. One approach to preliminary screening of test substances suitable for further evaluation is computer modeling of the interaction between the test substance and its target.

[0112] Computer modeling technology allows the visualization of the three-dimensional atomic structure of a selected molecule and the rational design of new compounds that will interact with the molecule. The three-dimensional construct typically depends on data from x-ray crystallographic analysis or NMR imaging of the selected molecule. The molecular dynamics require force field data. The computer graphics systems enable prediction of how a new compound will link to the target molecule and allow experimental manipulation of the structures of the compound and target molecule to perfect binding specificity. Prediction of what the molecule-compound interaction will be when small changes are made in one or both requires molecular mechanics software and computationally intensive computers, usually coupled with user-friendly, menu-driven interfaces between the molecular design program and the user.

[0113] An example of the molecular modeling system described generally above includes the CHARMm and QUANTA programs, Polygen Corporation, Waltham, Mass. CHARMm performs the energy minimization and molecular dynamics functions. QUANTA performs the construction, graphic modeling and analysis of molecular structure. QUANTA allows interactive construction, modification, visualization, and analysis of the behavior of molecules with each other.

[0114] A number of articles review computer modeling of drugs interactive with specific proteins, such as Rotivinen et al. Acta Pharmaceutica Fennica 1988, 97: 159-66; Ripka, New Scientist 1988, 54-8; McKinlay & Rossmann, Annu Rev Pharmacol Toxiciol 1989, 29: 111-22; Perry & Davies, Prog Clin Biol Res 1989, 291: 189-93; Lewis & Dean, Proc R Soc Lond 1989, 236: 125-40, 141-62; and, with respect to a model receptor for nucleic acid components, Askew et al., J Am Chem Soc 1989, 111: 1082-90.

Other computer programs that screen and graphically depict chemicals are available from companies such as BioDesign, Inc., Pasadena, Calif., Allelix, Inc, Mississauga, Ontario, Canada, and Hypercube, Inc., Cambridge, Ontario. See, e.g., DesJarlais et al., J Med Chem 1988, 31: 722-9; Meng et al., J Computer Chem 1992, 13: 505-24; Meng et al., Proteins 1993, 17: 266-78; Shoichet et al., Science 1993, 259: 1445-50.

[0115] Once a putative inhibitor has been identified, combinatorial chemistry techniques can be employed to construct any number of variants based on the chemical structure of the identified putative inhibitor, as detailed below. The resulting library of putative inhibitors, or "test substances" may be screened using the methods of the present invention to identify test substances treating or preventing a cancer, such as bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML.

[0116] (ii) Combinatorial chemical synthesis:

Combinatorial libraries of test substances may be produced as part of a rational drug design program involving knowledge of core structures existing in known inhibitors. This approach allows the library to be maintained at a reasonable size, facilitating high throughput screening. Alternatively, simple, particularly short, polymeric molecular libraries may be constructed by simply synthesizing all permutations of the molecular family making up the library. An example of this latter approach would be a library of all peptides of six amino acids in length. Such a peptide library could include every 6 amino acid sequence permutation. This type of library is termed a linear combinatorial chemical library.

[0117] Preparation of combinatorial chemical libraries is well known to those of skill in the art, and may be generated by either chemical or biological synthesis. Combinatorial chemical libraries include, but are not limited to, peptide libraries (see, e.g., US Patent 5,010,175; Furka, Int J Pept Prot Res 1991, 37: 487-93; Houghten et al., Nature 1991, 354: 84-6). Other chemistries for generating chemical diversity libraries can also be used. Such chemistries include, but are not limited to: peptides (e.g., PCT Publication No. WO 91/19735), encoded peptides (e.g., WO 93/20242), random bio-oligomers (e.g., WO 92/00091), benzodiazepines (e.g., US Patent 5,288,514), diversomers such as hydantoins, benzodiazepines and dipeptides (DeWitt et al., Proc Natl Acad Sci USA 1993, 90:6909-13), vinylogous polypeptides (Hagihara et al., J Amer Chem Soc 1992, 114: 6568), nonpeptidal peptidomimetics with glucose scaffolding (Hirschmann et al., J Amer Chem Soc 1992, 114: 9217-8), analogous organic syntheses of small compound libraries (Chen et al., J. Amer Chem Soc 1994, 116: 2661), oligocarbamates (Cho et al., Science 1993, 261: 1303), and/or peptidylphosphonates (Campbell et al., J Org Chem 1994, 59: 658), nucleic acid libraries (see Ausubel, Current Protocols in Molecular Biology 1995 supplement; Sambrook et al., Molecular Cloning: A Laboratory Manual, 1989, Cold Spring Harbor Laboratory, New York, USA), peptide nucleic acid libraries (see, e.g., US Patent 5,539,083), antibody libraries (see, e.g., Vaughan et al., Nature Biotechnology 1996, 14(3):309-14 and PCT/US96/10287), carbohydrate libraries (see, e.g., Liang et al., Science 1996, 274: 1520-22; US Patent

5,593,853), and small organic molecule libraries (see, e.g., benzodiazepines, Gordon EM. Curr Opin Biotechnol. 1995 Dec 1;6(6):624-31.; isoprenoids, US Patent 5,569,588; thiazolidinones and metathiazanones, US Patent 5,549,974; pyrrolidines, US Patents 5,525,735 and 5,519,134; morpholino compounds, US Patent 5,506,337; benzodiazepines, 5,288,514, and the like).

[0118] Devices for the preparation of combinatorial libraries are commercially available (see, e.g., 357 MPS, 390 MPS, Advanced Chem Tech, Louisville KY, Symphony, Rainin, Woburn, MA, 433A Applied Biosystems, Foster City, CA, 9050 Plus, Millipore, Bedford, MA). In addition, numerous combinatorial libraries are themselves commercially available (see, e.g., ComGenex, Princeton, N.J., Tripos, Inc., St. Louis, MO, 3D Pharmaceuticals, Exton, PA, Martek Biosciences, Columbia, MD, etc.).

[0119] (iii) Other candidates:

Another approach uses recombinant bacteriophage to produce libraries. Using the "phage method" (Scott & Smith, Science 1990, 249: 386-90; Cwirla et al., Proc Natl Acad Sci USA 1990, 87: 6378-82; Devlin et al., Science 1990, 249: 404-6), very large libraries can be constructed (e.g., 106 -108 chemical entities). A second approach uses primarily chemical methods, of which the Geysen method (Geysen et al., Molecular Immunology 1986, 23: 709-15; Geysen et al., J Immunologic Method 1987, 102: 259-74); and the method of Fodor et al. (Science 1991, 251: 767-73) are examples. Furka et al. (14th International Congress of Biochemistry 1988, Volume #5, Abstract FR:013; Furka, Int J Peptide Protein Res 1991, 37: 487-93), Houghten (US Patent 4,631,211) and Rutter et al. (US Patent 5,010,175) describe methods to produce a mixture of peptides that can be tested as agonists or antagonists.

[0120] Aptamers are macromolecules composed of nucleic acid that bind tightly to a specific molecular target. Tuerk and Gold (Science. 249:505-510 (1990)) discloses SELEX (Systematic Evolution of Ligands by Exponential Enrichment) method for selection of aptamers. In the SELEX method, a large library of nucleic acid molecules (e.g., 10^{15} different molecules) can be used for screening.

[0121] Screening for a PRMT1 and/or PRMT6 binding substance:

In present invention, over-expression of either of PRMT1 or PRMT6 or both was detected in at least one of cancer selected from the group consisting of bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML, in spite of no expression in normal organs (Figs. 1, 2 and Table 5). Therefore, using the PRMT1 and/or PRMT6 genes and proteins encoded by the genes, the present invention provides a method of screening for a substance that binds to PRMT1 and/or PRMT6. Due to the expression of PRMT1 and/or PRMT6 in cancer, a substance that binds to PRMT1 and/or PRMT6 is expected to suppress the proliferation

of cancer cells, and thus be useful for treating or preventing cancer. Therefore, the present invention also provides a method for screening a substance that suppresses the proliferation of cancer cells, and a method for screening a substance for treating or preventing cancer using the PRMT1 and/or PRMT6 polypeptide. Specially, an embodiment of this screening method includes the steps of:

(a) contacting a test substancetest substance with a polypeptide encoded by a polynucleotide of PRMT1 or PRMT6;

(b) detecting the binding activity between the polypeptide and the test substance; and

(c) selecting the test substancetest substance that binds to the polypeptide.

[0122] According to the present invention, the therapeutic effect of the test agent or compound on inhibiting cell growth and treating or preventing PRMT1 or PRMT6 associating disease may be evaluated. Therefore, the present invention also provides a method of screening for an agent or compound for inhibiting cell growth and treating or preventing PRMT1 or PRMT6 associating disease, which includes the steps of:

a) contacting a test agent or compound with the PRMT1 or PRMT6 polypeptide or a functional fragment thereof;

b) detecting the binding between the polypeptide (or fragment) and the test agent or compound; and

c) correlating the binding of b) with the therapeutic effect of the test agent or compound.

[0123] In the present invention, the therapeutic effect may be correlated with the binding properties of the test agent or compound For example, when the test agent or compound binds to the polypeptide (or fragment), the test agent or compound may identified or selected as the candidate agent or compound having the therapeutic effect. Alternatively, when the test agent or compound does not bind to the polypeptide (or fragment), the test agent or compound may identified as the agent or compound having no significant therapeutic effect.

[0124] Alternatively, according to the present invention, the potential therapeutic effect of a test substance or compound on treating or preventing cancer can also be evaluated or estimated. In some embodiments, the present invention provides a method for evaluating or estimating a therapeutic effect of a test substance on treating or preventing cancer or inhibiting cancer associated with over-expression of PRMT1 or PRMT6, the method including steps of:

(a) contacting a test substance with a polypeptide encoded by a polynucleotide of PRMT1 or PRMT6;

(b) detecting the binding activity between the polypeptide and the test substance; and

(c) correlating the potential therapeutic effect and the test substance, wherein the potential therapeutic effect is shown, when a substance binds to the polypeptide.

[0125] The method of the present invention will be described in more detail below.

The PRMT1 and/or PRMT6 polypeptide to be used for screening may be a recombinant polypeptide or a protein derived from the nature or a partial peptide thereof. The polypeptide to be contacted with a test substance can be, for example, a purified polypeptide, a soluble protein, a form bound to a carrier or a fusion protein fused with other polypeptides.

[0126] As a method of screening for proteins, for example, that bind to the PRMT1 or PRMT6 polypeptide using the PRMT1 or PRMT6 polypeptide, many methods well known by a person skilled in the art can be used. Such a screening can be conducted by, for example, immunoprecipitation method, specifically, in the following manner. The gene encoding the PRMT1 or PRMT6 polypeptide is expressed in host (e.g., animal) cells and so on by inserting the gene to an expression vector for foreign genes, such as pSV2neo, pcDNA I, pcDNA3.1, pCAGGS and pCD8.

[0127] The promoter to be used for the expression may be any promoter that can be used commonly and include, for example, the SV40 early promoter (Rigby in Williamson (ed.), Genetic Engineering, vol. 3. Academic Press, London, 83-141 (1982)), the EF-alpha promoter (Kim et al., Gene 91: 217-23 (1990)), the CAG promoter (Niwa et al., Gene 108: 193 (1991)), the RSV LTR promoter (Cullen, Methods in Enzymology 152: 684-704 (1987)) the SR alpha promoter (Takebe et al., Mol Cell Biol 8: 466 (1988)), the CMV immediate early promoter (Seed and Aruffo, Proc Natl Acad Sci USA 84: 3365-9 (1987)), the SV40 late promoter (Gheysen and Fiers, J Mol Appl Genet 1: 385-94 (1982)), the Adenovirus late promoter (Kaufman et al., Mol Cell Biol 9: 946 (1989)), the HSV TK promoter and so on.

[0128] The introduction of the gene into host cells to express a foreign gene can be performed according to any methods, for example, the electroporation method (Chu et al., Nucleic Acids Res 15: 1311-26 (1987)), the calcium phosphate method (Chen and Okayama, Mol Cell Biol 7: 2745-52 (1987)), the DEAE dextran method (Lopata et al., Nucleic Acids Res 12: 5707-17 (1984); Sussman and Milman, Mol Cell Biol 4: 1641-3 (1984)), the Lipofectin method (Derijard B., Cell 76: 1025-37 (1994); Lamb et al., Nature Genetics 5: 22-30 (1993): Rabindran et al., Science 259: 230-4 (1993)) and so on.

[0129] The polypeptide encoded by the PRMT1 or PRMT6 gene can be expressed as a fusion protein including a recognition site (epitope) of a monoclonal antibody by introducing the epitope of the monoclonal antibody, whose specificity has been revealed, to the N- or C- terminus of the polypeptide. A commercially available epitope-antibody system can be used (Experimental Medicine 13: 85-90 (1995)). Vectors which can express a fusion protein with, for example, beta-galactosidase, maltose binding protein, glutathione S-transferase, green fluorescence protein (GFP) and so on

by the use of its multiple cloning sites are commercially available. Also, a fusion protein prepared by introducing only small epitopes consisting of several to a dozen amino acids so as not to change the property of the PRMT1 or PRMT6 polypeptide by the fusion is also reported. Epitopes, such as polyhistidine (His-tag), influenza aggregate HA, human c-myc, FLAG, Vesicular stomatitis virus glycoprotein (VSV-GP), T7 gene 10 protein (T7-tag), human simple herpes virus glycoprotein (HSV-tag), E-tag (an epitope on monoclonal phage) and such, and monoclonal antibodies recognizing them can be used as the epitope-antibody system for screening proteins binding to the PRMT1 or PRMT6 polypeptide (Experimental Medicine 13: 85-90 (1995)).

[0130] In immunoprecipitation, an immune complex is formed by adding these antibodies to cell lysate prepared using an appropriate detergent. The immune complex consists of the PRMT1 or PRMT6 polypeptide, a polypeptide including the binding ability with the polypeptide, and an antibody. Immunoprecipitation can be also conducted using antibodies against the PRMT1 or PRMT6 polypeptide, besides using antibodies against the above epitopes, which antibodies can be prepared as described above. An immune complex can be precipitated, for example, by Protein A sepharose or Protein G sepharose when the antibody is a mouse IgG antibody. If the polypeptide encoded by PRMT1 or PRMT6 gene is prepared as a fusion protein with an epitope, such as GST, an immune complex can be formed in the same manner as in the use of the antibody against the PRMT1 or PRMT6 polypeptide, using a substance specifically binding to these epitopes, such as glutathione-Sepharose 4B.

Immunoprecipitation can be performed by following or according to, for example, the methods in the literature (Harlow and Lane, Antibodies, 511-52, Cold Spring Harbor Laboratory publications, New York (1988)).

[0131] SDS-PAGE is commonly used for analysis of immunoprecipitated proteins and the bound protein can be analyzed by the molecular weight of the protein using gels with an appropriate concentration. Since the protein bound to the PRMT1 or PRMT6 polypeptide is difficult to detect by a common staining method, such as Coomassie staining or silver staining, the detection sensitivity for the protein can be improved by culturing cells in culture medium containing radioactive isotope, ^{35}S-methionine or ^{35}S-cysteine, labeling proteins in the cells, and detecting the proteins. The target protein can be purified directly from the SDS-polyacrylamide gel and its sequence can be determined, when the molecular weight of a protein has been revealed.

[0132] As a method of screening for proteins binding to the PRMT1 or PRMT6 polypeptide using the polypeptide, for example, West-Western blotting analysis (Skolnik et al., Cell 65: 83-90 (1991)) can be used. Specifically, a protein binding to the PRMT1 or PRMT6 polypeptide can be obtained by preparing a cDNA library from cultured cells

expected to express a protein binding to the PRMT1 or PRMT6 polypeptide using a phage vector (e.g., ZAP), expressing the protein on LB-agarose, fixing the protein expressed on a filter, reacting the purified and labeled PRMT1 or PRMT6 polypeptide with the above filter, and detecting the plaques expressing proteins bound to the PRMT1 or PRMT6 polypeptide according to the label. The PRMT1 or PRMT6 polypeptide may be labeled by utilizing the binding between biotin and avidin, or by utilizing an antibody that specifically binds to the PRMT1 or PRMT6 polypeptide, or a peptide or polypeptide (for example, GST) that is fused to the PRMT1 or PRMT6 polypeptide. Methods using radioisotope or fluorescence and such may be also used.

[0133] Alternatively, in another embodiment of the screening method of the present invention, a two-hybrid system utilizing cells may be used ("MATCHMAKER Two-Hybrid system", "Mammalian MATCHMAKER Two-Hybrid Assay Kit", "MATCHMAKER one-Hybrid system" (Clontech); "HybriZAP Two-Hybrid Vector System" (Stratagene); the references "Dalton and Treisman, Cell 68: 597-612 (1992)", "Fields and Sternglanz, Trends Genet 10: 286-92 (1994)").

[0134] In the two-hybrid system, the PRMT1 or PRMT6 polypeptide is fused to the SRF-binding region or GAL4-binding region and expressed in yeast cells. A cDNA library is prepared from cells expected to express a protein binding to the PRMT1 or PRMT6 polypeptide, such that the library, when expressed, is fused to the VP16 or GAL4 transcriptional activation region. The cDNA library is then introduced into the above yeast cells and the cDNA derived from the library is isolated from the positive clones detected (when a protein binding to the polypeptide of the present invention is expressed in yeast cells, the binding of the two activates a reporter gene, making positive clones detectable). A protein encoded by the cDNA can be prepared by introducing the cDNA isolated above to E. coli and expressing the protein. As a reporter gene, for example, Ade2 gene, lacZ gene, CAT gene, luciferase gene and such can be used in addition to the HIS3 gene.

[0135] A substance binding to the polypeptide encoded by PRMT1 or PRMT6 gene can also be screened using affinity chromatography. For example, the PRMT1 or PRMT6 polypeptide may be immobilized on a carrier of an affinity column, and a test substance, containing a protein capable of binding to the polypeptide of the present invention, is applied to the column. A test substance herein may be, for example, cell extracts, cell lysates, etc. After loading the test substance, the column is washed, and substances bound to the polypeptide of the present invention can be prepared. When the test substance is a protein, the amino acid sequence of the obtained protein is analyzed, an oligo DNA is synthesized based on the sequence, and cDNA libraries are screened using the oligo DNA as a probe to obtain a DNA encoding the protein.

[0136] A biosensor using the surface plasmon resonance phenomenon may be used as a

mean for detecting or quantifying the bound substance in the present invention. When such a biosensor is used, the interaction between the PRMT1 or PRMT6 polypeptide and a test substance can be observed real-time as a surface plasmon resonance signal, using only a minute amount of polypeptide and without labeling (for example, BIAcore, Pharmacia). Therefore, it is possible to evaluate the binding between the polypeptide of the present invention and a test substance using a biosensor such as BIAcore.

The methods of screening for molecules that bind when the immobilized PRMT1 or PRMT6 polypeptide is exposed to synthetic chemical compounds, or natural substance banks or a random phage peptide display library, and the methods of screening using high-throughput based on combinatorial chemistry techniques (Wrighton et al., Science 273: 458-64 (1996); Verdine, Nature 384: 11-13 (1996); Hogan, Nature 384: 17-9 (1996)) to isolate not only proteins but chemical compounds that bind to the PRMT1 or PRMT6 protein (including agonist and antagonist) are well known to one skilled in the art.

[0137] Screening for a substance suppressing the biological activity of PRMT1 and/or PRMT6:

The PRMT1 and PRMT6 protein have the activity of promoting cell proliferation of cancer cells. Moreover, arginine methylation of histones and other nuclear proteins is performed by the family of PRMTs (protein arginine methyltransferases). PRMTs use S-adenosylmethionine (SAM)-dependent methylation to modify the guanidino nitrogens of the arginine side chain by adding one or two methyl groups (Bedford MT and Richard S, Mol Cell 2005;18:263-272). PRMT1 is one of the members of type I PRMT family, and also have been demonstrated to have the methyltransferase activity, in particular, an ability to methylate H4/H2A at arginine 3 (Wang H. et al, Science 2001; 293: 853-857). PRMT6 is also a type I PRMT enzyme and is the major protein arginine methyltransferase responsible for the methylation of the second arginine of histone H3 (Guccione E et al. Nature 2007;449:933-7, Hyllus D et al. Genes Dev 2007; 21: 3369-80). Using these biological activities, the present invention provides a method for screening a substance that suppresses the proliferation of cancer cells expressing PRMT1 and/or PRMT6, and a method for screening a candidate substance for treating or preventing the cancer. Exemplary cancers includebladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML. Thus, the present invention provides a method of screening for a candidate substance for treating or preventing cancer using the polypeptide encoded by PRMT1 or PRMT6 gene including the steps as follows:

(a) contacting a test substance with a polypeptide encoded by a polynucleotide of

PRMT1 or PRMT6;

(b) detecting a biological activity of the polypeptide of step (a); and

(c) selecting the test substance that suppresses the biological activity of the polypeptide encoded by the polynucleotide of PRMT1 or PRMT6 as compared to the biological activity of the polypeptide detected in the absence of the test substance.

[0138] According to the present invention, the therapeutic effect of the test substance on suppressing the biological activity (e.g., the cell-proliferating activity or the methyl-transferase activity) of PRMT1 or PRMT6, or a candidate substance for treating or preventing cancer may be evaluated. Therefore, the present invention also provides a method of screening for a candidate substance for suppressing the biological activity of PRMT1 and/or PRMT6, or a candidate substance for treating or preventing cancer, using the PRMT1 or PRMT6 polypeptide or fragments thereof, including the following steps:

a) contacting a test substance with the PRMT1 or PRMT6 polypeptide or a functional fragment thereof; and

b) detecting the biological activity of the polypeptide or fragment of step (a), and

c) correlating the biological activity of b) with the therapeutic effect of the test substance.

[0139] In the present invention, the therapeutic effect may be correlated with the biological activity of PRMT1 or PRMT6 polypeptide or a functional fragment thereof. For example, when the test agent or compound suppresses or inhibits the biological activity of PRMT1 or PRMT6 polypeptide or a functional fragment thereof as compared to a level detected in the absence of the test agent or compound, the test agent or compound may identified or selected as the candidate agent or compound having the therapeutic effect. Alternatively, when the test agent or compound does not suppress or inhibit the biological activity PRMT1 or PRMT6 polypeptide or a functional fragment thereof as compared to a level detected in the absence of the test agent or compound, the test agent or compound may be identified as the agent or compound having no significant therapeutic effect.

[0140] Alternatively, in some embodiments, the present invention provides a method for evaluating or estimating a therapeutic effect of a test substance on treating or preventing cancer or inhibiting cancer associated with over-expression of PRMT1 or PRMT6, the method including steps of:

(a) contacting a test substance with a polypeptide encoded by a polynucleotide of PRMT1 or PRMT6 gene;

(b) detecting the biological activity of the polypeptide of step (a); and

(c) correlating the potential therapeutic effect and the test substance, wherein the potential therapeutic effect is shown, when a substance suppresses the biological

activity of the polypeptide encoded by the polynucleotide of PRMT1 or PRMT6 gene as compared to the biological activity of said polypeptide detected in the absence of the test substance.

[0141] Such cancer includes bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML. In the present invention, the therapeutic effect may be correlated with the biological activity of the PRMT1 or PRMT6 polypeptide or a functional fragment thereof. For example, when the test substance suppresses or inhibits the biological activity of the PRMT1 or PRMT6 polypeptide or a functional fragment thereof as compared to a level detected in the absence of the test substance, the test substance may identified or selected as the candidate substance having the therapeutic effect. Alternatively, when the test substance does not suppress or inhibit the biological activity of the PRMT1 or PRMT6 polypeptide or a functional fragment thereof as compared to a level detected in the absence of the test substance, the test substance may identified as the substance having no significant therapeutic effect.

[0142] The method of the present invention will be described in more detail below.

Any polypeptides can be used for screening so long as they include the biological activity of the PRMT1 or PRMT6 protein. Such biological activity includes cell-proliferating activity or methyltransferase activity of the PRMT1 or PRMT6 protein. For example, PRMT1 or PRMT6 protein can be used and polypeptides functionally equivalent to these proteins can also be used. Such polypeptides may be expressed endogenously or exogenously by cells.

[0143] The substance isolated by this screening is a candidate antagonist (inhibitor) of the polypeptide encoded by the PRMT1 or PRMT6 gene. The term "antagonist" refers to molecules that inhibit the function of the polypeptide by binding thereto. The term also refers to molecules that reduce or inhibit expression of the gene encoding PRMT1 or PRMT6. Moreover, a substance isolated by this screening is a candidate for substances which inhibit the in vivo interaction of the PRMT1 or PRMT6 polypeptide with molecules (including DNAs and proteins).

When the biological activity to be detected in the present method is cell proliferation, it can be detected, for example, by preparing cells which express the PRMT1 or PRMT6 polypeptide, culturing the cells in the presence of a test substance, and determining the speed of cell proliferation, measuring the cell cycle and such, as well as by measuring cell survival or colony forming activity. The substances that reduce the speed of proliferation of the cells expressed PRMT1 and/or PRMT6 are selected as candidate substance for treating or preventing cancer.

[0144] More specifically, the method includes the steps of:

(a) contacting a test substance with cells overexpressing PRMT1 or PRMT6;

(b) measuring cell-proliferating activity; and

(c) selecting the test substance that reduces the cell-proliferating activity in the comparison with the cell-proliferating activity in the absence of the test substance.

[0145] In preferable embodiments, the method of the present invention may further include the steps of:

(d) selecting the test substance that have substantially no effect to the cells expressing little or no detectable PRMT1 or PRMT6.

When the biological activity to be detected in the present method is methyl-transferase activity, the methyltransferase activity can be determined by contacting a polypeptide with a substrate (e.g., histone H4/H2A, H3 or fragments thereof including Arginine 3) and a co-factor (e.g., S-adenosyl-L-methionine) under conditions suitable for methylation of the substrate and detecting the methylation level of the substrate.

[0146] More specifically, the method includes the step of:

[1] A method of measuring methyl transferase activity of PRMT1 or PRMT6, the method including the steps of:

(a) contacting a test substrate with a polypeptide encoded by a polynucleotide of PRMT1 or PRMT6;

(b) detecting the methylation level of the substrate; and

(c) measuring the methyl transferase activity by correlating the methylation level of the step (b) with the methyl transferase activity.

[2] The method of [1], wherein the substrate is a histone or a fragment thereof including at least one methylation region.

[3] The method of [2], wherein the substrate is a histone H4, H2A, H3 or a fragment thereof including at least one methylation region.

[4] The method of [3], wherein .the methylation region is arginine 3.

[4] The method of [1], wherein the cofactor is an S-adenosylmethionine.

[6] The method of [1], wherein the polypeptide is contacted with the substrate and cofactor in the presence of an enhancing agent for the methylation.

[7] The method of [1], wherein the enhancing agent for the methylation is S-adenosyl homocysteine hydrolase (SAHH).

[0147] In the present invention, methyltransferase activity of a PRMT1 or PRMT6 polypeptide can be determined by methods known in the art. For example, the PRMT1 or PRMT6 and a substrate can be incubated with a labeled methyl donor, under suitable assay conditions. A histone H4, H2A or H3 peptides, and S-adenosyl-[methyl-^{14}C]-L-methionine, or S-adenosyl-[methyl-^{3}H]-L-methionine preferably can be used as the substrate and methyl donor, respectively. Transfer of the radiolabel to the histone H4, H2A or H3 peptides can be detected, for example, by SDS-PAGE electrophoresis

and fluorography. Alternatively, following the reaction, the histone H4, H2A or H3 peptides can be separated from the methyl donor by filtration, and the amount of radiolabel retained on the filter quantitated by scintillation counting. Other suitable labels that can be attached to methyl donors, such as chromogenic and fluorescent labels, and methods of detecting transfer of these labels to histones and histone peptides, are known in the art.

[0148] Alternatively, the methyltransferase activity of PRMT1 or PRMT6 can be determined using an unlabeled methyl donor (e.g., S-adenosyl-L-methionine) and reagents that selectively recognize methylated histones or histone peptides. For example, after incubation of the PRMT1 or PRMT6, substrate to be methylated and methyl donor, under the condition capable of methylation of the substrate, methylated substrate can be detected by immunological method. Any immunological techniques using an antibody recognizing methylated substrate can be used for the detection. For example, an antibody against methylated histone is commercially available (abcam Ltd.). ELISA or Immunoblotting with antibodies recognizing methylated histone can be used for the present invention.

[0149] In the present invention, an agent enhancing the methylation of the substance can be used. SAHH or a functional equivalent thereof is one of the preferable enhancing agent for the methylation. The agent enhances the methylation of the substance, the methyltransferase activity can be determined with higher sensitivity thereby. PRMT1 or PRMT6 may be contacted with substrate and cofactor under the existence of the enhancing agent.

[0150] Furthermore, the present method detecting methyltransferase activity can be performed by preparing cells which express the PRMT1 or PRMT6 polypeptide, culturing the cells in the presence of a test substance, and determining methylation level of a histone, for example, by using the antibody specific binding to methylation region.

[0151] More specifically, the method includes the step of:

[1] contacting a test substance with cells expressing PRMT1 or PRMT6;

[2] detecting a methylation level of histone H3, H4 or H2A arginine 3; and

[3] selecting the test substance that reduces the methylation level in the comparison with the methylation level in the absence of the test substance.

[0152] "Suppress the biological activity" as defined herein are preferably at least 10% suppression of the biological activity of PRMT1 or PRMT6 in comparison with in absence of the substance, more preferably at least 25%, 50% or 75% suppression and most preferably at 90% suppression.

[0153] In the preferred embodiments, control cells which do not express PRMT1 or PRMT6 polypeptide are used. Accordingly, the present invention also provides a method of

screening for a candidate substance for inhibiting the cell growth or a candidate substance for treating or preventing PRMT1 or PRMT6 associating disease, using the PRMT1 or PRMT6 polypeptide or fragments thereof including the steps as follows:
a) culturing cells which express a PRMT1 or PRMT6 polypeptide or a functional fragment thereof, and control cells that do not express a PRMT1 or PRMT6 polypeptide or a functional fragment thereof in the presence of the test substance;
b) detecting the biological activity of the cells which express the protein and control cells; and
c) selecting the test compound that inhibits the biological activity in the cells which express the protein as compared to the proliferation detected in the control cells and in the absence of said test substance.

[0154] Screening for a substance altering the expression of genes:

In the present invention, the decrease of the expression of PRMT1 or PRMT6 by siRNA causes inhibiting cancer cell proliferation (Fig. 3). Therefore, the present invention provides a method of screening for a substance that inhibits the expression of PRMT1 or PRMT6. A substance that inhibits the expression of PRMT1 and/or PRMT6 is expected to suppress the proliferation of cancer cells, and thus is useful for treating or preventing cancer, e.g., bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML. Therefore, the present invention also provides a method for screening a substance that suppresses the proliferation of cancer cells, and a method for screening a substance for treating or preventing cancer.

[0155] In the context of the present invention, such screening may include, for example, the following steps:

(a) contacting a candidate substance with a cell expressing PRMT1 or PRMT6; and

(b) selecting the candidate substance that reduces the expression level of PRMT1 or PRMT6 as compared to a control (e.g., without the substance).

[0156] According to the present invention, the therapeutic effect of the test agent or compound on inhibiting the cell growth or a candidate agent or compound for treating or preventing a PRMT1 or PRMT6 associated disease may be evaluated. Therefore, the present invention also provides a method for screening a candidate agent or compound that suppresses the proliferation of cancer cells, and a method for screening a candidate agent or compound for treating or preventing a PRMT1 or PRMT6 associated disease.

[0157] In the context of the present invention, such screening may include, for example, the following steps:

a) contacting a test agent or compound with a cell expressing the PRMT1 or PRMT6 gene;

b) detecting the expression level of the PRMT1 or PRMT6 gene; and

c) correlating the expression level of b) with the therapeutic effect of the test agent or compound.

[0158] In the present invention, the therapeutic effect may be correlated with the expression level of the PRMT1 or PRMT6 gene. For example, when the test agent or compound reduces the expression level of the PRMT1 or PRMT6 gene as compared to a level detected in the absence of the test agent or compound, the test agent or compound may identified or selected as the candidate agent or compound having the therapeutic effect. Alternatively, when the test agent or compound does not reduce the expression level of the PRMT1 or PRMT6 gene as compared to a level detected in the absence of the test agent or compound, the test agent or compound may be identified as the agent or compound having no significant therapeutic effect.

[0159] Alternatively, in some embodiments, the present invention also provides a method for evaluating or estimating a therapeutic effect of a test substance on treating or preventing cancer or inhibiting cancer associated with over-expression of PRMT1 or PRMT6, the method including steps of:

(a) contacting a candidate substance with a cell expressing PRMT1 or PRMT6; and;

(b) correlating the potential therapeutic effect and the test substance, wherein the potential therapeutic effect is shown, when a substance reduces the expression level of PRMT1 or PRMT6 as compared to a control.

[0160] The method of the present invention will be described in more detail below.

[0161] Cells expressing the PRMT1 or PRMT6 include, for example, cell lines established from bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML; such cells can be used for the above screening of the present invention. The expression level can be estimated by methods well known to one skilled in the art, for example, RT-PCR, Northern blot assay, Western blot assay, immunostaining and flow cytometry analysis. "Reduce the expression level" as defined herein is preferably at least a 10% reduction of expression level of PRMT1 or PRMT6 in comparison to the expression level in absence of the substance, more preferably at least 25%, 50% or 75% reduced level and most preferably at least 95% reduced level. The substance herein includes chemical compounds, double-strand nucleotides, and so on. The preparation of the double-strand nucleotides is in aforementioned description. In the method of screening, a substance that reduces the expression level of PRMT1 or PRMT6 can be selected as candidate substances to be used for the treatment or prevention of cancer.

[0162] Alternatively, the screening method of the present invention may include the following steps:

(a) contacting a candidate substance with a cell into which a vector, including the transcriptional regulatory region of PRMT1 or PRMT6 and a reporter gene that is expressed under the control of the transcriptional regulatory region, has been introduced;

(b) measuring the expression or activity of the reporter gene; and

(c) selecting the candidate substance that reduces the expression or activity of the reporter gene.

[0163] According to the present invention, the therapeutic effect of the test agent or compound on inhibiting the cell growth or a candidate agent or compound for treating or preventing a PRMT1 or PRMT6 associated disease may be evaluated. Therefore, the present invention also provides a method for screening a candidate agent or compound that suppresses the proliferation of cancer cells, and a method for screening a candidate agent or compound for treating or preventing a PRMT1 or PRMT6 associated disease.

[0164] According to another aspect, the present invention provides a method which includes the following steps of:

a) contacting a test agent or compound with a cell into which a vector, composed of the transcriptional regulatory region of the PRMT1 or PRMT6 gene and a reporter gene that is expressed under the control of the transcriptional regulatory region, has been introduced;

b) detecting the expression level or activity of said reporter gene; and

c) correlating the expression level of b) with the therapeutic effect of the test agent or compound.

[0165] In the present invention, the therapeutic effect may be correlated with the expression level or activity of said reporter gene. For example, when the test agent or compound reduces the expression level or activity of said reporter gene as compared to a level detected in the absence of the test agent or compound, the test agent or compound may be identified or selected as the candidate agent or compound having the therapeutic effect. Alternatively, when the test agent or compound does not reduce the expression level or activity of said reporter gene as compared to a level detected in the absence of the test agent or compound, the test agent or compound may be identified as the agent or compound having no significant therapeutic effect.

[0166] Alternatively, in some embodiments, the present invention also provides a method for evaluating or estimating a therapeutic effect of a test substance on treating or preventing cancer or inhibiting cancer associated with over-expression of PRMT1 or PRMT6, the method including steps of:

(a) contacting a test substance with a cell into which a vector, including the transcriptional regulatory region of PRMT1 or PRMT6 and a reporter gene that is expressed under the control of the transcriptional regulatory region, has been in-

troduced;

(b) measuring the expression or activity of said reporter gene; and

(c) correlating the potential therapeutic effect and the test substance, wherein the potential therapeutic effect is shown, when a test substance reduces the expression or activity of said reporter gene.

[0167] Suitable reporter genes and host cells are well known in the art. For example, reporter genes are luciferase, green fluorescence protein (GFP), Discosoma sp. Red Fluorescent Protein (DsRed), Chrolamphenicol Acetyltransferase (CAT), lacZ and beta-glucuronidase (GUS), and host cell is COS7, HEK293, HeLa and so on. The reporter construct required for the screening can be prepared by connecting reporter gene sequence to the transcriptional regulatory region of PRMT1 or PRMT6. The transcriptional regulatory region of PRMT1 or PRMT6 herein is the region from transcription stat site to at least 500 bp upstream, preferably 1,000 bp, more preferably 5,000 or 10,000 bp upstream. A nucleotide segment containing the transcriptional regulatory region can be isolated from a genome library or can be propagated or amplified by PCR. The reporter construct required for the screening can be prepared by connecting reporter gene sequence to the transcriptional regulatory region of any one of these genes. Methods for identifying a transcriptional regulatory region, and also assay protocol are well known (Molecular Cloning third edition chapter 17, 2001, Cold Springs Harbor Laboratory Press).

[0168] The vector containing the the reporter construct is introduced into host cells and the expression or activity of the reporter gene is detected by methods well known in the art (e.g., using luminometer, absorption spectrometer, flow cytometer and so on). "Reduces the expression or activity" as defined herein are preferably at least 10% reduction of the expression or activity of the reporter gene in comparison with in absence of the substance, more preferably at least 25%, 50% or 75% reduction and most preferably at least 95% reduction.

[0169] By screening for candidate substances that (i) bind to the PRMT1 or PRMT6 polypeptide; (ii) suppress/reduce the biological activity (e.g., the cell-proliferating activity or the methyltransferase activity) of the PRMT1 or PRMT6 polypeptide; or (iii) reduce the expression level of PRMT1 or PRMT6, candidate substances that have the potential to treat or prevent cancers (e.g., bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML) can be identified. Potential of these candidate substances to treat or prevent cancers may be evaluated by second and/or further screening to identify therapeutic substance for cancers. For example, when a substance that binds to the PRMT1 or PRMT6 polypeptide inhibits the above-described activities of cancer, it may be concluded that

such a substance has the PRMT1 or PRMT6 specific therapeutic effect.

[0170] In the present invention, the downstream genes regulated by PRMT1 or PRMT6 were identified. Accordingly, a substance that binds to PRMT1 or PRMT6 and regulates the downstream genes is useful for treating or preventing cancer. Therefore, the present invention provides a method of screening for a substance for treating or preventing cancer, such as bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML. In one embodiment, the present invention provides a method of screening for a candidate substance for treating or preventing cancer, the method including the steps of:

1-(a) contacting a candidate substance with a cell expressing PRMT1 and a downstream gene of PRMT1; and

1-(b) selecting the substance that reduces expression level of a downstream gene of PRMT1 in comparison with the expression level detected in the absence of the candidate substance, or

2-(a) contacting a candidate substance with a cell expressing PRMT6 and a downstream gene of PRMT6; and

2-(b) selecting the substance that reduces expression level of a downstream gene of PRMT6 in comparison with the expression level detected in the absence of the candidate substance.

[0171] According to the present invention, the therapeutic effect of the test agent or compound on inhibiting the cell growth or a candidate agent or compound for treating or preventing a PRMT1 or PRMT6 associated disease may be evaluated. Therefore, the present invention also provides a method for screening a candidate agent or compound that suppresses the proliferation of cancer cells, and a method for screening a candidate agent or compound for treating or preventing a PRMT1 or PRMT6 associated disease.

[0172] In the context of the present invention, such screening may include, for example, the following steps:

1-a) contacting a candidate substance with a cell expressing PRMT1 and a downstream gene of PRMT1;

1-b) detecting the expression level of the downstream gene of PRMT1; and

1-c) correlating the expression level of 1-b) with the therapeutic effect of the test agent or compound, or

2-(a) contacting a candidate substance with a cell expressing PRMT6 and a downstream gene of PRMT6; and

2-b) detecting the expression level of the downstream gene of PRMT6; and

2-c) correlating the expression level of 2-b) with the therapeutic effect of the test agent or compound.

[0173] In the present invention, the therapeutic effect may be correlated with the expression level of the downstream genes regulated by PRMT1 or PRMT6. For example, when the test agent or compound reduces the expression level of the downstream genes regulated by PRMT1 or PRMT6 as compared to a level detected in the absence of the test agent or compound, the test agent or compound may identified or selected as the candidate agent or compound having the therapeutic effect. Alternatively, when the test agent or compound does not reduce the expression level of the downstream genes regulated by PRMT1 or PRMT6 as compared to a level detected in the absence of the test agent or compound, the test agent or compound may be identified as the agent or compound having no significant therapeutic effect.

[0174] Alternatively, in some embodiments, the present invention also provides a method for evaluating or estimating a therapeutic effect of a test substance on treating or preventing cancer or inhibiting cancer associated with over-expression of PRMT1 or PRMT6, the method including steps of:

1'-(a) contacting a candidate substance with a cell expressing PRMT1 and a downstream gene of PRMT1, and;

1'-(b) correlating the potential therapeutic effect and the test substance, wherein the potential therapeutic effect is shown, when a substance reduces the expression level of a downstream gene of PRMT1 in comparison with the expression level detected in the absence of the candidate substance, or

2'-(a) contacting a candidate substance with a cell expressing PRMT6 and a downstream gene of PRMT6; and

2'-(b) correlating the potential therapeutic effect and the test substance, wherein the potential therapeutic effect is shown, when a substance reduces the expression level of a downstream gene of PRMT6 in comparison with the expression level detected in the absence of the candidate substance.

[0175] Furthermore, in the present invention, the genes indicated in tables 9 and 10 (indicated in Figs. 4 and 5 as raw data) were identified as downstream genes regulated by PRMT1 and PRMT6, respectively. Therefore, the downstream gene of the methods can be at least one of the genes described in tables 9 and 10. Particularly, the expression of MAPK1, RRAS, NRAS, GALNT1 and RTN4 were regulated by the regulation of PRMT1 expression (Fig. 6). MAPK1 is indicated in Genbank Accession No.: NM_002745.4 and NM_138957.2 (for example, SEQ ID NO: 39 encoding SEQ ID NO:40). RRAS is indicated in Genbank Accession No.: NM_006270.3 (SEQ ID NO:41 encoding SEQ ID NO: 42). NRAS is indicated in Genbank Accession No.: NM_002524.3 (SEQ ID NO: 43 encoding SEQ ID NO: 44). GALNT1 is indicated in Genbank Accession No.: NM_020474.3 (SEQ ID NO: 45 encoding SEQ ID NO: 46). RTN4 is indicated in Genbank Accession No.: NM_007008.2, NM_020532.4,

NM_153828.2, NM_207520.1 and NM_207521.1 (for example SEQ ID NO: 47 encoding SEQ ID NO: 48).

[0176] Accordingly, the present invention provides the method of screening for a substance for treating or preventing cancer, the method including the further steps of:

1-(c) contacting a candidate substance that bind to PRMT1 with a cell expressing PRMT1 and MAPK1, RRAS, NRAS, GALNT1 and/or RTN4; and

1-(d) selecting the substance that reduces expression level of MAPK1, RRAS, NRAS, GALNT1 and/or RTN4 in comparison with the expression level detected in the absence of the candidate substance.

[0177] Alternatively, according to the present invention, the potential therapeutic effect of a test substance or compound on treating or preventing cancer can also be evaluated or estimated. In some embodiments, the present invention provides a method for evaluating or estimating a therapeutic effect of a test substance on treating or preventing cancer or inhibiting cancer associated with over-expression of PRMT1, the method including steps of:

1-(e) contacting a candidate substance that bind to PRMT1 with a cell expressing PRMT1 and at least one of gene selected from the group consisting of MAPK1, RRAS, NRAS, GALNT1 and RTN4; and

1-(f) correlating the potential therapeutic effect and the test substance, wherein the potential therapeutic effect is shown, when a substance reduces the expression level of at least one of gene selected from the group consisting of MAPK1, RRAS, NRAS, GALNT1 and RTN4 in comparison with the expression level detected in the absence of the candidate substance.

[0178] Screening for a substance by detecting the binding activity among the PRMT1 or PRMT6 polypeptide and each binding proteins:

According to the present invention, the PRMT1 polypeptide interacts with the SRRT polypeptide, EIF4B polypeptide, HNRNPK polypeptide and/or SERBP1 polypeptide, and the PRMT6 polypeptide interacts with the MSH2 polypeptide, EIF4B polypeptide, HNRNPK polypeptide, RUVBL1 polypeptide, EEF1A1 polypeptide and/or HNRNPD polypeptide and the interactions among theses polypeptides are considered to be important for cancer cell growth. Therefore, agents that inhibit the above interactions are expected to be useful for inhibiting cancer cell growth and/or survival, thus useful for treating or preventing cancer. Thus, the present invention provides methods of screening for candidate substances for treating or preventing cancer based on the binding activity among the PRMT1 polypeptide with the SRRT polypeptide, EIF4B polypeptide, HNRNPK polypeptide and/or SERBP1 polypeptide, and the PRMT6 polypeptide with the MSH2 polypeptide, EIF4B polypeptide, HNRNPK polypeptide, RUVBL1 polypeptide, EEF1A1 polypeptide and/or HNRNPD polypeptide. The

present screening methods are also useful for screening for a candidate substance for inhibiting cancer cell growth and/or survival. The present screening methods include the following steps:

1-(1) contacting at least one of binding protein selected from group consisting of SRRT polypeptide, an EIF4B polypeptide, an HNRNPK polypeptide a SERBP1 polypeptide and functional equivalent thereof with an PRMT1 polypeptide or functional equivalent thereof in the presence of a test substance;

1-(2) detecting the binding between the binding proteins and PRMT1 polypeptide of the step 1-(1); and

1-(3) selecting the test substance that inhibits the binding between the the binding proteins and PRMT1 polypeptides, or,

2-(1) contacting at least one of binding protein selected from the group consisting of a MSH2 polypeptide, an EIF4B polypeptide, an HNRNPK polypeptide, an RUVBL1 polypeptide, an EEF1A1 polypeptide, an HNRNPD polypeptide and functional equivalent thereof with an PRMT6 polypeptide or functional equivalent thereof in the presence of a test substance,

2-(2) detecting the binding between the binding proteins and PRMT6 polypeptides of the step 2-(1); and

2-(3) selecting the test substance that inhibits the binding between the binding proteins and PRMT6 polypeptides.

[0179] Alternatively, in some embodiments, the present invention also provides a method for evaluating or estimating a therapeutic effect of a test substance on treating or preventing cancer or inhibiting cancer, the method including steps of:

1'-(1) contacting at least one of binding protein selected from group consisting of a SRRT polypeptide, an EIF4B polypeptide, an HNRNPK polypeptide, a SERBP1 polypeptide and functional equivalent thereof with an PRMT1 polypeptide or functional equivalent thereof in the presence of a test substance;

1'-(2) detecting the binding between the binding proteins and PRMT1 polypeptides of the step 1'-(1);

1'-(3) comparing the binding level detected in the step 1'-(2) with those detected in the absence of the test substance;and

1'-(4) correlating the potential therapeutic effect and the test substance, wherein the potential therapeutic effect is shown, when a test substance reduce the binding level, or,

2'-(1) contacting at least one of binding protein selected from the group consisting of a MSH2 polypeptide, an EIF4B polypeptide, an HNRNPK polypeptide, an RUVBL1 polypeptide, an EEF1A1 polypeptide, an HNRNPD polypeptide and functional equivalent thereof with an PRMT6 polypeptide or functional equivalent thereof in the

presence of a test substance,

2'-(2) detecting the binding between the binding proteins and PRMT6 polypeptides of the step 2'-(1);

2'-(3) comparing the binding level detected in the step 2'-(2) with those detected in the absence of the test substance and

2'-(4) correlating the potential therapeutic effect and the test substance, wherein the potential therapeutic effect is shown, when a test substance reduce the binding level.

[0180] According to the present invention, the therapeutic effect of the test substance on inhibiting the cell growth or a candidate substance for treating or preventing PRMT1 and/or PRMT6 associating disease may be evaluated. Therefore, the present invention also provides a method of screening for a candidate substance for inhibiting the cell growth or a candidate substance for treating or preventing PRMT1 and/or PRMT6 associating disease, using the PRMT1 and/or PRMT6 polypeptide or fragments thereof including the following steps:

1-(a) contacting at least one of binding protein selected from the group consisting of a SRRT polypeptide, an EIF4B polypeptide, an HNRNPK polypeptide, a SERBP1 polypeptide and functional equivalent thereof with an PRMT1 polypeptide or functional equivalent thereof in the presence of a test substance;

1-(b) detecting the binding between the polypeptides of the step 1-(a); and

1-(c) correlating the binding of 1-(b) with the therapeutic effect of the test substance, or

2-(a) contacting at least one of binding protein selected from the group consisting of a MSH2 polypeptide, an EIF4B polypeptide, an HNRNPK polypeptide, an RUVBL1 polypeptide, an EEF1A1 polypeptide, an HNRNPD polypeptide and functional equivalent thereof with an PRMT6 polypeptide or functional equivalent thereof in the presence of a test substance;

2-(b) detecting the binding between the binding proteins and PRMT6 polypeptide of the step 2-(a); and

2-(c) correlating the binding of 2-(b) with the therapeutic effect of the test substance.

[0181] Alternatively, in some embodiments, the present invention also provides a method for evaluating or estimating a therapeutic effect of a test substance on treating or preventing cancer or inhibiting cancer, the method including steps of:

1'-(a) contacting at least one of binding protein selected from the group consisting of a SRRT polypeptide, an EIF4B polypeptide, an HNRNPK polypeptide, a SERBP1 polypeptide and functional equivalent thereof with an PRMT1 polypeptide or functional equivalent thereof in the presence of a test substance;

1'-(b) detecting the binding level between the binding preoteins and PRMT1 polypeptides of the step 1'-(a);

1'-(c) comparing the binding level detected in the step 1'-(b) with those detected in the absence of the test substance;and

1'-(d) correlating the potential therapeutic effect and the test substance, wherein the potential therapeutic effect is shown, when a test substance reduce the binding level, or

2'-(a) contacting at least one of binding protein selected from thegroup consisting of a MSH2 polypeptide, an EIF4B polypeptide, an HNRNPK polypeptide, an RUVBL1 polypeptide, an EEF1A1 polypeptide, an HNRNPD polypeptide or functional equivalent thereof with an PRMT6 polypeptide or functional equivalent thereof in the presence of a test substance;

2'-(b) detecting the binding level between the binding proteins and RPMT6 polypeptides of the step 2'-(a);

2'-(c) comparing the binding level detected in the step 2'-(b) with those detected in the absence of the test substance;and

2'-(d) correlating the potential therapeutic effect and the test substance, wherein the potential therapeutic effect is shown, when a test substance reduce the binding level.

[0182] In the present invention, the therapeutic effect may be correlated with the binding activity among the PRMT1 polypeptide with the SRRT polypeptide, EIF4B polypeptide, HNRNPK polypeptide and/or SERBP1 polypeptide, and the PRMT6 polypeptide with the MSH2 polypeptide, EIF4B polypeptide, HNRNPK polypeptide, RUVBL1 polypeptide, EEF1A1 polypeptide and/or HNRNPD polypeptide or a functional fragment thereof. For example, when the test substance suppresses or inhibits binding activity among them as compared to a level detected in the absence of the test substance, the test substance may identified or selected as the candidate substance having the therapeutic effect. Alternatively, when the test substance does not suppress or inhibit binding activity among above polypeptides as compared to a level detected in the absence of the test substance, the test substance may identified as the substance having no significant therapeutic effect.

[0183] SRRT is indicated in Genbank Accession No.: NM_015908, NM_001128852.1, NM_001128853.1 and NM_001128854.1 (for example, SEQ ID NO: 50 encoded by SEQ ID NO: 49). EIF4B is indicated in Genbank Accession No.: NM_001417 (SEQ ID NO:52 encoded by SEQ ID NO: 51). HNRNPK is indicated in Genbank Accession No.: NM_002140.3, NM_031262.2, and NM_031263.2 (for example SEQ ID NO: 54 encoded by SEQ ID NO: 53). SERBP1 is indicated in Genbank Accession No.: NM_001018067.1, NM_001018068.1, NM_001018069.1 and NM_015640.3 (for example SEQ ID NO: 56 encoded by SEQ ID NO: 55). MSH2 is indicated in Genbank Accession No.: NM_000251 (SEQ ID NO: 58 encoded by SEQ ID NO: 57). RUVBL1 is indicated in Genbank Accession No.: NM_003707.2 (SEQ ID NO: 60 encoded by SEQ ID NO: 59). EEF1A1 is indicated in Genbank Accession No.: NM_001402.5

(SEQ ID NO: 62 encoded by SEQ ID NO: 61). HNRNPD is indicated in Genbank Accession No.: NM_031370.2, NM_031369.2, NM_002138.3 and NM_001003810.1 (for example SEQ ID NO: 64 encoded by SEQ ID NO: 63).

[0184] In the present invention, it is shown that suppressing the binding activity among the PRMT1 polypeptide with the SRRT polypeptide, EIF4B polypeptide, HNRNPK polypeptide and/or SERBP1 polypeptide, and the PRMT6 polypeptide with the MSH2 polypeptide, EIF4B polypeptide, HNRNPK polypeptide, RUVBL1 polypeptide, EEF1A1 polypeptide and/or HNRNPD or a functional fragment thereof may reduces cancer cell growth. Thus, by screening for candidate substances that suppresses the binding activity, candidate substances that have the potential to treat or prevent cancers can be identified. The potential of these candidate substances to treat or prevent cancers may be evaluated by second and/or further screening to identify therapeutic substance for cancers.

[0185] As a method of screening for agents that inhibit the binding between the PRMT1 polypeptide and the SRRT polypeptide, EIF4B polypeptide, HNRNPK polypeptide and/or SERBP1 polypeptide, or between the PRMT6 polypeptide and the MSH2 polypeptide, EIF4B polypeptide, HNRNPK polypeptide, RUVBL1 polypeptide, EEF1A1 polypeptide and/or HNRNPD polypeptide many methods well known by one skilled in the art can be used. For example, screening can be carried out as an in vitro assay system, such as a cellular system. More specifically, first, either the PRMT1 polypeptide or the SRRT polypeptide, EIF4B polypeptide, HNRNPK polypeptide and/or SERBP1 polypeptide, or either the PRMT6 polypeptide or the MSH2 polypeptide, EIF4B polypeptide, HNRNPK polypeptide, RUVBL1 polypeptide, EEF1A1 polypeptide and/or HNRNPD polypeptide is bound to a support, and the other protein is added together with a test substance thereto. Next, the mixture is incubated, washed and the other protein bound to the support is detected and/or measured.

[0186] Examples of supports that may be used for binding proteins include, for example, insoluble polysaccharides, such as agarose, cellulose and dextran; and synthetic resins, such as polyacrylamide, polystyrene and silicon; preferably commercial available beads and plates (e.g., multi-well plates, biosensor chip, etc.) prepared from the above materials may be used. When using beads, they may be filled into a column. Alternatively, the use of magnetic beads is also known in the art, and enables one to readily isolate proteins bound on the beads via magnetism.

The binding of a protein to a support may be conducted according to routine methods, such as chemical bonding and physical adsorption, for example. Alternatively, a protein may be bound to a support via antibodies that specifically recognize the protein. Moreover, binding of a protein to a support can be also conducted by means of avidin and biotin.

The binding between proteins is preferably carried out in buffer, examples of which include, but are not limited to, phosphate buffer and Tris buffer. However, the selected buffer must not inhibit binding between the proteins.

[0187] In the context of the present invention, a biosensor using the surface plasmon resonance phenomenon may be used as a means for detecting or quantifying the bound protein. When such a biosensor is used, the interaction between the proteins can be observed in real-time as a surface plasmon resonance signal, using only a minute amount of polypeptide and without labeling (for example, BIAcore, Pharmacia). Therefore, it is possible to evaluate binding between the PRMT1 polypeptide with and SRRT polypeptide, EIF4B polypeptide, HNRNPK polypeptide and/or SERBP1 polypeptide, or the PRMT6 polypeptide and the MSH2 polypeptide, EIF4B polypeptide, HNRNPK polypeptide, RUVBL1 polypeptide, EEF1A1 polypeptide and/or HNRNPD polypeptide using a biosensor such as BIAcore.

[0188] Alternatively, either the PRMT1 polypeptide or the SRRT polypeptide, EIF4B polypeptide, HNRNPK polypeptide and/or SERBP1 polypeptide, and either the PRMT6 polypeptide or the MSH2 polypeptide, EIF4B polypeptide, HNRNPK polypeptide, RUVBL1 polypeptide, EEF1A1 polypeptide and/or HNRNPD polypeptide may be labeled, and the label of the bound protein may be used to detect or measure the bound protein. Specifically, after pre-labeling one of the proteins, the labeled protein is contacted with the other protein in the presence of a test substance, and then bound proteins are detected or measured according to the label after washing.

[0189] Labeling substances including, but not limited to, radioisotopes (e.g., ^{3}H, ^{14}C, ^{32}P, ^{33}P, ^{35}S, ^{125}I, ^{131}I), enzymes (e.g., alkaline phosphatase, horseradish peroxidase, beta-galactosidase, beta-glucosidase), fluorescent substances (e.g., fluorescein isothiocyanate (FITC), rhodamine) and biotin/avidin may be used for the labeling of a protein in the present method. When the protein is labeled with a radioisotope, the detection or measurement can be carried out by liquid scintillation. Alternatively, proteins labeled with enzymes can be detected or measured by adding a substrate of the enzyme to detect the enzymatic change of the substrate, such as generation of color, with absorptiometer. Further, in case where a fluorescent substance is used as the label, the bound protein may be detected or measured using fluorophotometer.

[0190] Furthermore, binding of the PRMT1 polypeptide and SRRT polypeptide, EIF4B polypeptide, HNRNPK polypeptide and/or SERBP1 polypeptide, or the PRMT6 polypeptide and the MSH2 polypeptide, EIF4B polypeptide, HNRNPK polypeptide, RUVBL1 polypeptide, EEF1A1 polypeptide and/or HNRNPD polypeptide can be also detected or measured using antibodies to the polypeptide thereof. For example, after contacting the PRMT1 polypeptide immobilized on a support with a test substance and the SRRT polypeptide, EIF4B polypeptide, HNRNPK polypeptide and/or SERBP1

polypeptide, the mixture is incubated and washed, and detection or measurement can be conducted using an antibody against the SRRT polypeptide, EIF4B polypeptide, HNRNPK polypeptide and/or SERBP1 polypeptide. Alternatively, the SRRT polypeptide, EIF4B polypeptide, HNRNPK polypeptide and/or SERBP1 polypeptide may be immobilized on a support, and an antibody against the PRMT1 polypeptide may be used as the antibody. In a similar way, after contacting the PRMT6 polypeptide immobilized on a support with a test substance and the MSH2 polypeptide, EIF4B polypeptide, HNRNPK polypeptide, RUVBL1 polypeptide, EEF1A1 polypeptide and/or HNRNPD polypeptide, the mixture is incubated and washed, and detection or measurement can be conducted using an antibody against the MSH2 polypeptide, EIF4B polypeptide, HNRNPK polypeptide, RUVBL1 polypeptide, EEF1A1 polypeptide and/or HNRNPD polypeptide. Alternatively, the MSH2 polypeptide, EIF4B polypeptide, HNRNPK polypeptide, RUVBL1 polypeptide, EEF1A1 polypeptide and/or HNRNPD polypeptide may be immobilized on a support, and an antibody against the PRMT6 polypeptide may be used as the antibody.

[0191] When using an antibody in the present screening, the antibody is preferably labeled with one of the labeling substances mentioned above, and detected or measured based on the labeling substance. Alternatively, an antibody against the the PRMT1 polypeptide, SRRT polypeptide, EIF4B polypeptide, HNRNPK polypeptide, SERBP1 polypeptide, the PRMT6 polypeptide, the MSH2 polypeptide, EIF4B polypeptide, HNRNPK polypeptide, RUVBL1 polypeptide, EEF1A1 polypeptide or HNRNPD polypeptide may be used as a primary antibody to be detected with a secondary antibody that is labeled with a labeling substance. Furthermore, an antibody bound to the protein in the screening of the present invention may be detected or measured using a protein G or protein A column.

[0192] The polypeptides to be used in the present screening methods may be recombinantly produced using standard procedures. For example, a gene encoding a polypeptide of interest may be expressed in animal cells by inserting the gene into an expression vector for foreign genes, such as pSV2neo, pcDNA I, pcDNA3.1, pCAGGS and pCD8. The promoter to be used for the expression may be any promoter that can be used commonly and include, for example, the SV40 early promoter (Rigby in Williamson (ed.), Genetic Engineering, vol. 3. Academic Press, London, 83-141 (1982)), the EF-alpha promoter (Kim et al., Gene 91: 217-23 (1990)), the CAG promoter (Niwa et al., Gene 108: 193 (1991)), the RSV LTR promoter (Cullen, Methods in Enzymology 152: 684-704 (1987)) the SR alpha promoter (Takebe et al., Mol Cell Biol 8: 466-72 (1988)), the CMV immediate early promoter (Seed and Aruffo, Proc Natl Acad Sci USA 84: 3365-9 (1987)), the SV40 late promoter (Gheysen and Fiers, J Mol Appl Genet 1: 385-94 (1982)), the Adenovirus late promoter (Kaufman et al., Mol Cell Biol

9: 946-58 (1989)), the HSV TK promoter and so on. The introduction of the gene into animal cells to express a foreign gene can be performed according to any conventional method, for example, the electroporation method (Chu et al., Nucleic Acids Res 15: 1311-26 (1987)), the calcium phosphate method (Chen and Okayama, Mol Cell Biol 7: 2745-52 (1987)), the DEAE dextran method (Lopata et al., Nucleic Acids Res 12: 5707-17 (1984); Sussman and Milman, Mol Cell Biol 4: 1641-3 (1984)), the Lipofectin method (Derijard B, Cell 76: 1025-37 (1994); Lamb et al., Nature Genetics 5: 22-30 (1993): Rabindran et al., Science 259: 230-4 (1993)), and so on. The polypeptides may be expressed as a fusion protein including a recognition site (epitope) of a monoclonal antibody by introducing the epitope of the monoclonal antibody, whose specificity has been revealed, to the N- or C- terminus of the polypeptide. Alternatively, a commercially available epitope-antibody system may be used (Experimental Medicine 13: 85-90 (1995)). Vectors which are capable of expressing a fusion protein with, for example, beta-galactosidase, maltose binding protein, glutathione S-transferase, green fluorescence protein (GFP), and so on, by the use of its multiple cloning sites are commercially available.

[0193] A fusion protein can be prepared by introducing a small epitope, e.g., composed of several to a dozen amino acids so as not to change the property of the original polypeptide. Epitopes, such as polyhistidine (His-tag), influenza aggregate HA, human c-myc, FLAG, Vesicular stomatitis virus glycoprotein (VSV-GP), T7 gene 10 protein (T7-tag), human simple herpes virus glycoprotein (HSV-tag), E-tag (an epitope on monoclonal phage) and such, and antibodies recognizing them may be used as the epitope-antibody system for detecting the binding activity between the polypeptides (Experimental Medicine 13: 85-90 (1995)).

[0194] Antibodies to be used in the present screening methods can be prepared using techniques well known in the art. Antigens to prepared antibodies may be derived from any animal species, but preferably is derived from a mammal such as a human, mouse, rabbit, or rat, more preferably from a human. The polypeptide used as the antigen can be recombinantly produced or isolated from natural sources. The polypeptides to be used as an immunization antigen may be a complete protein or a partial peptide derived from the complete protein.

[0195] Any mammalian animal may be immunized with the antigen; however, the compatibility with parental cells used for cell fusion is preferably taken into account. In general, animals of the order Rodentia, Lagomorpha or Primate are used. Animals of the Rodentia order include, for example, mice, rats and hamsters. Animals of Lagomorpha order include, for example, hares, pikas, and rabbits. Animals of Primate order include, for example, monkeys of Catarrhini (old world monkey) such as Macaca fascicularis, rhesus monkeys, sacred baboons and chimpanzees.

[0196] Methods for immunizing animals with antigens are well known in the art. Intraperitoneal injection or subcutaneous injection of antigens is a standard method for immunizing mammals. More specifically, antigens may be diluted and suspended in an appropriate amount of phosphate buffered saline (PBS), physiological saline, etc. If desired, the antigen suspension may be mixed with an appropriate amount of a standard adjuvant, such as Freund's complete adjuvant, made into emulsion, and then administered to mammalian animals. Preferably, it is followed by several administrations of the antigen mixed with an appropriately amount of Freund's incomplete adjuvant every 4 to 21 days. An appropriate carrier may also be used for immunization. After immunization as above, the serum is examined by a standard method for an increase in the amount of desired antibodies.

[0197] Polyclonal antibodies may be prepared by collecting blood from the immunized mammal examined for the increase of desired antibodies in the serum, and by separating serum from the blood by any conventional method. Polyclonal antibodies include serum containing the polyclonal antibodies, as well as the fraction containing the polyclonal antibodies isolated from the serum. Immunoglobulin G or M can be prepared from a fraction which recognizes only the objective polypeptide using, for example, an affinity column coupled with the polypeptide, and further purifying this fraction using protein A or protein G column.

[0198] To prepare monoclonal antibodies, immune cells are collected from the mammal immunized with the antigen and checked for the increased level of desired antibodies in the serum as described above, and are subjected to cell fusion. The immune cells used for cell fusion are preferably obtained from spleen. Other preferred parental cells to be fused with the above immunocyte include, for example, myeloma cells of mammalians, and more preferably myeloma cells having an acquired property for the selection of fused cells by drugs.

The above immunocyte and myeloma cells can be fused according to known methods, for example, the method of Milstein et al., (Galfre and Milstein, Methods Enzymol 73: 3-46 (1981)).

[0199] Resulting hybridomas obtained by the cell fusion may be selected by cultivating them in a standard selection medium, such as HAT medium (hypoxanthine, aminopterin, and thymidine containing medium). The cell culture is typically continued in the HAT medium for several days to several weeks, the time being sufficient to allow all the other cells, with the exception of the desired hybridoma (non-fused cells), to die. Then, the standard limiting dilution is performed to screen and clone a hybridoma cell producing the desired antibody.

[0200] In addition to the above method, in which a non-human animal is immunized with an antigen for preparing hybridoma, human lymphocytes, such as those infected by the

EB virus, may be immunized with an antigen, cells expressing such antigen, or their lysates in vitro. Then, the immunized lymphocytes are fused with human-derived myeloma cells that are capable of indefinitely dividing, such as U266, to yield a hybridoma producing a desired human antibody that is able to bind to the antigen (Unexamined Published Japanese Patent Application No. (JP-A) Sho 63-17688).

[0201] The obtained hybridomas may be subsequently transplanted into the abdominal cavity of a mouse and the ascites may be extracted. The obtained monoclonal antibodies can be purified by, for example, ammonium sulfate precipitation, a protein A or protein G column, DEAE ion exchange chromatography, or an affinity column carrying an objective antigen.

[0202] Antibodies against the PRMT1 polypeptide,SRRT polypeptide, EIF4B polypeptide, HNRNPK polypeptide, SERBP1 polypeptide, the PRMT6 polypeptide, the MSH2 polypeptide, EIF4B polypeptide, HNRNPK polypeptide, RUVBL1 polypeptide, EEF1A1 polypeptide or HNRNPD polypeptide can be used not only in the present screening method, but also for the detection of the polypeptides as cancer markers in biological samples as described in "a method for diagnosing cancer". They may further serve as candidates for agonists and antagonists of the polypeptides of interest. In addition, such antibodies, serving as candidates for antagonists, can be applied to the antibody treatment for diseases related to the PRMT1 and/or PRMT6 polypeptide, including bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML as described infra.

[0203] Monoclonal antibodies thus obtained can be also recombinantly prepared using genetic engineering techniques (see, for example, Borrebaeck and Larrick, Therapeutic Monoclonal Antibodies, published in the United Kingdom by MacMillan Publishers LTD (1990)). For example, a DNA encoding an antibody may be cloned from an immune cell, such as a hybridoma or an immunized lymphocyte producing the antibody, inserted into an appropriate vector, and introduced into host cells to prepare a recombinant antibody. Such recombinant antibody can also be used in the context of the present screening.

[0204] Furthermore, antibodies used in the screening and so on may be fragments of antibodies or modified antibodies, so long as they retain the original binding activity. For instance, the antibody fragment may be an Fab, $F(ab')_2$, Fv, or single chain Fv (scFv), in which Fv fragments from H and L chains are ligated by an appropriate linker (Huston et al., Proc Natl Acad Sci USA 85: 5879-83 (1988)). More specifically, an antibody fragment may be generated by treating an antibody with an enzyme, such as papain or pepsin. Alternatively, a gene encoding an antibody fragment may be constructed, inserted into an expression vector, and expressed in an appropriate host cell

(see, for example, Co et al., J Immunol 152: 2968-76 (1994); Better and Horwitz, Methods Enzymol 178: 476-96 (1989); Pluckthun and Skerra, Methods Enzymol 178: 497-515 (1989); Lamoyi, Methods Enzymol 121: 652-63 (1986); Rousseaux et al., Methods Enzymol 121: 663-9 (1986); Bird and Walker, Trends Biotechnol 9: 132-7 (1991)).

[0205] An antibody may be modified by conjugation with a variety of molecules, such as polyethylene glycol (PEG). Modified antibodies can be obtained through chemically modification of an antibody. These modification methods are conventional in the field.

Antibodies obtained as above may be purified to homogeneity. For example, the separation and purification of the antibody can be performed according to separation and purification methods used for general proteins. For example, the antibody may be separated and isolated by appropriately selected and combined column chromatographies, such as affinity chromatography, filter, ultrafiltration, salting-out, dialysis, SDS polyacrylamide gel electrophoresis, isoelectric focusing, and others (Antibodies: A Laboratory Manual. Ed Harlow and David Lane, Cold Spring Harbor Laboratory (1988)); however, the present invention is not limited thereto. A protein A column and protein G column can be used as the affinity column. Exemplary protein A columns to be used include, for example, Hyper D, POROS, and Sepharose F.F. (Pharmacia).

[0206] Exemplary chromatography, with the exception of affinity, includes, for example, ion-exchange chromatography, hydrophobic chromatography, gel filtration, reverse-phase chromatography, adsorption chromatography, and the like (Strategies for Protein Purification and Characterization: A Laboratory Course Manual. Ed Daniel R. Marshak et al., Cold Spring Harbor Laboratory Press (1996)). The chromatographic procedures can be carried out by liquid-phase chromatography, such as HPLC and FPLC.

[0207] Alternatively, a two-hybrid system utilizing cells may be used for detecting or measuring the binding activity among the polypeptides ("MATCHMAKER Two-Hybrid system", "Mammalian MATCHMAKER Two-Hybrid Assay Kit", "MATCHMAKER one-Hybrid system" (Clontech); "HybriZAP Two-Hybrid Vector System" (Stratagene); the references "Dalton and Treisman, Cell 68: 597-612 (1992)", "Fields and Sternglanz, Trends Genet 10: 286-92 (1994)").

[0208] In the two-hybrid system, for example, the PRMT1 or PRMT6 polypeptide are fused to the SRF-binding region or GAL4-binding region and expressed in yeast cells. The SRRT polypeptide, EIF4B polypeptide, HNRNPK polypeptide, SERBP1 polypeptide, MSH2 polypeptide, EIF4B polypeptide, HNRNPK polypeptide, RUVBL1 polypeptide, EEF1A1 polypeptide and/or HNRNPD polypeptide are fused to the VP16 or GAL4 transcriptional activation region and also expressed in the yeast cells in the existence of

a test substance. Alternatively, the SRRT polypeptide, EIF4B polypeptide, HNRNPK polypeptide, SERBP1 polypeptide, MSH2 polypeptide, EIF4B polypeptide, HNRNPK polypeptide, RUVBL1 polypeptide, EEF1A1 polypeptide or HNRNPD polypeptide may be fused to the SRF-binding region or GAL4-binding region, and the PRMT1 or PRMT6 polypeptide may be fused to the VP16 or GAL4 transcriptional activation region. The binding of the two polypeptides activates a reporter gene, making positive clones detectable. As a reporter gene, for example, Ade2 gene, lacZ gene, CAT gene, luciferase gene and such can be used besides HIS3 gene.

[0209] Double stranded molecule:

As used herein, the term "isolated double-stranded molecule" refers to a nucleic acid molecule that inhibits expression of a target gene and includes, for example, short interfering RNA (siRNA; e.g., double-stranded ribonucleic acid (dsRNA) or small hairpin RNA (shRNA)) and short interfering DNA/RNA (siD/R-NA; e.g., double-stranded chimera of DNA and RNA (dsD/R-NA) or small hairpin chimera of DNA and RNA (shD/R-NA)).

[0210] As used herein, a target sequence is a nucleotide sequence within mRNA or cDNA sequence of a target gene, which will result in suppression of translation of the whole mRNA of the target gene if the double-stranded molecule is introduced within a cell expressing the gene. A nucleotide sequence within the mRNA or cDNA sequence of a target gene can be determined to be a target sequence when a double-stranded molecule having a sequence corresponding to the target sequence inhibits expression of the gene in a cell expressing the gene. When a target sequence is shown by cDNA sequence, a sense strand sequence of a double-stranded cDNA, i.e., a sequence that mRNA sequence is converted into DNA sequence, is used for defining a target sequence. A double-stranded molecule is composed of a sense strand that has a sequence corresponding to a target sequence and an antisense strand that has a complementary sequence to the target sequence, and the antisense strand hybridizes with the sense strand at the complementary sequence to form a double-stranded molecule.

[0211] Herein, the phrase " corresponding to" means converting a target sequence according to the kind of nucleic acid that constitutes a sense strand of a double-stranded molecule. For example, when a target sequence is shown in DNA sequence and a sense strand of a double-stranded molecule has an RNA region, base "t"s within the RNA region are replaced with base "u"s. On the other hand, when a target sequence is shown in RNA sequence and a sense strand of a double-stranded molecule has a DNA region, base "u"s within the DNA region are replaced with "t"s. For example, when a target sequence is the DNA sequence shown in SEQ ID NO: 29, 32, 35 or 38 and the sense strand of the double-stranded molecule is composed of RNA, "a sequence corresponding to a target sequence" is "CGGUGUUCUA CAUGGAGGA"(for SEQ ID NO:

29), " GAGUUCACAC GCUGCCACA"(for SEQ ID NO: 32), "CCAUGCAUGG CUUUGCCAU" (for SEQ ID NO: 35) or "CGGAACAGGU GGAUGCCAU" (for SEQ ID NO: 38).

[0212] Also, a complementary sequence to a target sequence for an antisense strand of a double-stranded molecule can be defined according to the kind of nucleic acid that constitutes the antisense strand. For example, when a target sequence is the DNA sequence shown in SEQ ID NO: 35 or 38 and the antisense strand of the double-stranded molecule is composed of RNA, " a complementary sequence to a target sequence " is "UCCUCCAUG UAGAACACCG"(for SEQ ID NO: 29), "UGUGGCAGC GUGUGAACUC"(for SEQ ID NO: 32), "AUGGCAAAG CCAUGCAUGG" (for SEQ ID NO: 35) or "AUGGCAUCC ACCUGUUCCG" (for SEQ ID NO: 38). A double-stranded molecule may have either of one or two 3'overhangs having 2 to 5 nucleotides in length (e.g., uu) or a loop sequence that links a sense strand and an antisense strand to form hairpin structure, or both, in addition to a sequence corresponding to a target sequence and complementary sequence thereto.

[0213] As used herein, the term "siRNA" refers to a double-stranded RNA molecule which prevents translation of a target mRNA. Standard techniques of introducing siRNA into the cell are used, including those in which DNA is a template from which RNA is transcribed. Alternatively, siRNA may also be directly introduced in cells to be treated. Methods of introducing siRNA in a subject are well known in the art. For example, an administration of siRNA in conjunction with a delivery substance is preferable for the introductionod siRNA.

[0214] The siRNA includes a PRMT1 or PRMT6 sense nucleic acid sequence (also referred to as "sense strand"), a PRMT1 or PRMT6 antisense nucleic acid sequence (also referred to as "antisense strand") or both. The siRNA may be constructed such that a single transcript has both the sense and complementary antisense nucleic acid sequences of the target gene, e.g., a hairpin. The siRNA may either be a dsRNA or shRNA.

[0215] As used herein, the term "dsRNA" refers to a construct of two RNA molecules composed of complementary sequences to one another and that have annealed together via the complementary sequences to form a double-stranded RNA molecule. The nucleotide sequence of two strands may include not only the "sense" or "antisense" RNAs selected from a protein coding sequence of target gene sequence, but also RNA molecule having a nucleotide sequence selected from non-coding region of the target gene.

[0216] The term "shRNA", as used herein, refers to an siRNA having a stem-loop structure, composed of first and second regions complementary to one another, i.e., sense and antisense strands. The degree of complementarity and orientation of the regions are

sufficient such that base pairing occurs between the regions, the first and second regions are joined by a loop region, the loop results from a lack of base pairing between nucleotides (or nucleotide analogs) within the loop region. The loop region of an shRNA is a single-stranded region intervening between the sense and antisense strands and may also be referred to as "intervening single-strand".

[0217] As used herein, the term "siD/R-NA" refers to a double-stranded polynucleotide molecule which is composed of both RNA and DNA, and includes hybrids and chimeras of RNA and DNA and prevents translation of a target mRNA. Herein, a hybrid indicates a molecule wherein a polynucleotide composed of DNA and a polynucleotide composed of RNA hybridize to each other to form the double-stranded molecule; whereas a chimera indicates that one or both of the strands composing the double stranded molecule may contain RNA and DNA. Standard techniques of introducing siD/R-NA into the cell are used. The siD/R-NA includes a PRMT1 or PRMT6 sense nucleic acid sequence (also referred to as "sense strand"), a PRMT1 or PRMT6 antisense nucleic acid sequence (also referred to as "antisense strand") or both. The siD/R-NA may be constructed such that a single transcript has both the sense and complementary antisense nucleic acid sequences from the target gene, e.g., a hairpin. The siD/R-NA may either be a dsD/R-NA or shD/R-NA.

[0218] As used herein, the term "dsD/R-NA" refers to a construct of two molecules composed of complementary sequences to one another and that have annealed together via the complementary sequences to form a double-stranded polynucleotide molecule. The nucleotide sequence of two strands may include not only the "sense" or "antisense" polynucleotides sequence selected from a protein coding sequence of target gene sequence, but also polynucleotide having a nucleotide sequence selected from non-coding region of the target gene. One or both of the two molecules constructing the dsD/R-NA are composed of both RNA and DNA (chimeric molecule), or alternatively, one of the molecules is composed of RNA and the other is composed of DNA (hybrid double-strand).

[0219] The term "shD/R-NA", as used herein, refers to an siD/R-NA having a stem-loop structure, composed of a first and second regions complementary to one another, i.e., sense and antisense strands. The degree of complementarity and orientation of the regions are sufficient such that base pairing occurs between the regions, the first and second regions are joined by a loop region, the loop results from a lack of base pairing between nucleotides (or nucleotide analogs) within the loop region. The loop region of an shD/R-NA is a single-stranded region intervening between the sense and antisense strands and may also be referred to as "intervening single-strand".

As used herein, an "isolated nucleic acid" is a nucleic acid removed from its original environment (e.g., the natural environment if naturally occurring) and thus, syn-

thetically altered from its natural state. In the present invention, examples of isolated nucleic acid includes DNA, RNA, and derivatives thereof.

[0220] A double-stranded molecule against PRMT1 or PRMT6, which molecule hybridizes to target mRNA, decreases or inhibits production of PRMT1 or PRMT6 protein encoded by PRMT1 or PRMT6 gene by associating with the normally single-stranded mRNA transcript of the gene, thereby interfering with translation and thus, inhibiting expression of the protein. As demonstrated herein, the expression of PRMT1 or PRMT6 in several cancer cell lines was inhibited by dsRNA (Fig. 3). Therefore the present invention provides isolated double-stranded molecules that are capable of inhibiting the expression of PRMT1 or PRMT6 gene when introduced into a cell expressing the gene. The target sequence of double-stranded molecule may be designed by an siRNA design algorithm such as that mentioned below.

PRMT1 target sequence includes, for example, nucleotides SEQ ID NO: 29 or 32, and PRMT6 target sequence includes, for example, nucleotides SEQ ID NO: 35 or 38.

[0221] Specifically, the present invention provides the following double-stranded molecules [1] to [18]:

[1] An isolated double-stranded molecule that, when introduced into a cell, inhibits in vivo expression of PRMT1 or PRMT6 and cell proliferation, such molecules composed of a sense strand and an antisense strand complementary thereto, hybridized to each other to form the double-stranded molecule;

[2] The double-stranded molecule of [1], wherein the double-stranded molecule acts on mRNA, matching a target sequence of SEQ ID NO: 29, 32, 35 or 38

[3] The double-stranded molecule of [2], wherein the sense strand contains a sequence corresponding to a target sequence of SEQ ID NO: 29, 32, 35 or 38;

[4] The double-stranded molecule of [3], having a length of less than about 100 nucleotides;

[5] The double-stranded molecule of [4], having a length of less than about 75 nucleotides;

[6] The double-stranded molecule of [5], having a length of less than about 50 nucleotides;

[7] The double-stranded molecule of [6] having a length of less than about 25 nucleotides;

[8] The double-stranded molecule of [7], having a length of between about 19 and about 25 nucleotides;

[9] The double-stranded molecule of [1], composed of a single polynucleotide having both the sense and antisense strands linked by an intervening single-strand;

[10] The double-stranded molecule of [9], having the general formula 5'-[A]-[B]-[A']-3' or 5'-[A']-[B]-[A]-3', wherein [A] is the sense strand containing a

sequence corresponding to a target sequence of SEQ ID NO: 29, 32, 35 or 38; [B] is the intervening single-strand composed of 3 to 23 nucleotides, and [A'] is the antisense strand containing a sequence complementary to [A];

[11] The double-stranded molecule of [1], composed of RNA;

[12] The double-stranded molecule of [1], composed of both DNA and RNA;

[13] The double-stranded molecule of [12], wherein the molecule is a hybrid of a DNA polynucleotide and an RNA polynucleotide;

[14] The double-stranded molecule of [13] wherein the sense and the antisense strands are composed of DNA and RNA, respectively;

[15] The double-stranded molecule of [12], wherein the molecule is a chimera of DNA and RNA;

[16] The double-stranded molecule of [15], wherein a region flanking to the 3'-end of the antisense strand, or both of a region flanking to the 5'-end of sense strand and a region flanking to the 3'-end of antisense strand are RNA;

[17] The double-stranded molecule of [16], wherein the flanking region is composed of 9 to 13 nucleotides; and

[18] The double-stranded molecule of [2], wherein the molecule contains 3' overhang;

[0222] The double-stranded molecule of the present invention will be described in more detail below.

Methods for designing double-stranded molecules having the ability to inhibit target gene expression in cells are known. (See, for example, US Patent No. 6,506,559, herein incorporated by reference in its entirety). For example, a computer program for designing siRNAs is available from the Ambion website (ambion.com/techlib/misc/siRNA_finder.html).

The computer program selects target nucleotide sequences for double-stranded molecules based on the following protocol.

[0223] Selection of Target Sites:

1. Beginning with the AUG start codon of the transcript, scan downstream for AA di-nucleotide sequences. Record the occurrence of each AA and the 3' adjacent 19 nucleotides as potential siRNA target sites. Tuschl et al. recommend to avoid designing siRNA to the 5' and 3' untranslated regions (UTRs) and regions near the start codon (within 75 bases) as these may be richer in regulatory protein binding sites, and UTR-binding proteins and/or translation initiation complexes may interfere with binding of the siRNA endonuclease complex.

2. Compare the potential target sites to the appropriate genome database (human, mouse, rat, etc.) and eliminate from consideration any target sequences with significant homology to other coding sequences. BLAST, which can be found on the NCBI server at: ncbi.nlm.nih.gov/BLAST/, is used (Altschul SF et al., Nucleic Acids Res 1997 Sep

1, 25(17): 3389-402).

3. Select qualifying target sequences for synthesis. Selecting several target sequences along the length of the gene to evaluate is typical.

[0224] Using the above protocol, the target sequence of the isolated double-stranded molecules of the present invention were designed as

SEQ ID NO: 29 or 32 for PRMT1 gene and SEQ ID NO: 35 or 38 for PRMT6 gene.

Double-stranded molecules targeting the above-mentioned target sequences were respectively examined for their ability to suppress the growth of cells expressing the target genes. Therefore, the present invention provides double-stranded molecule targeting the sequences of SEQ ID NO: 29 or 32 for PRMT1 gene and SEQ ID NO: 35 or 38 for PRMT6 gene.

[0225] Examples of double-stranded molecules of the present invention that target the above-mentioned target sequence of the PRMT1 or PRMT6 gene include isolated polynucleotides that contain the nucleic acid sequences corresponding to either of target sequences or complementary sequences to the target sequences, or both. Preferred examples of polynucleotides targeting the PRMT1 gene include those containing the sequence corresponding to SEQ ID NO: 29 or 32 and/or complementary sequences to these sequences. Preferred examples of polynucleotides targeting the PRMT6 gene include those containing the sequence corresponding to SEQ ID NO: 35 or 38 and/or complementary sequences to these sequences. In an embodiment, a double-stranded molecule is composed of two polynucleotides, one polynucleotide has a sequence corresponding to a target sequence, i.e., sense strand, and another polypeptide has a complementary sequence to the target sequence, i.e., antisense strand. The sense strand polynucleotide and the antisense strand polynucleotide hybridize to each other to form double-stranded molecule. Examples of such double-stranded molecules include dsRNA and dsD/R-NA .

[0226] In another embodiment, a double-stranded molecule is composed of a polynucleotide that has both a sequence corresponding to a target sequence, i.e., sense strand, and a complementary sequence to the target sequence, i.e., antisense strand. Generally, the sense strand and the antisense strand are linked by a intervening strand, and hybridize to each other to form a hairpin loop structure. Examples of such double-stranded molecule include shRNA and shD/R-NA.

[0227] In other words, a double-stranded molecule of the present invention is composed of a sense strand polynucleotide having a nucleotide sequence of the target sequence and anti-sense strand polynucleotide having a nucleotide sequence complementary to the target sequence, and both of polynucleotides hybridize to each other to form the double-stranded molecule. In the double-stranded molecule including the polynucleotides, a part of the polynucleotide of either or both of the strands may be RNA, and

when the target sequence is defined with a DNA sequence, the nucleotide "t" within the target sequence and complementary sequence thereto is replaced with "u".

[0228] In one embodiment of the present invention, such a double-stranded molecule of the present invention includes a stem-loop structure, composed of the sense and antisense strands. The sense and antisense strands may be joined by a loop. Accordingly, the present invention also provides the double-stranded molecule composed of a single polynucleotide containing both the sense strand and the antisense strand linked or flanked by an intervening single-strand.

The double-stranded molecule of the present invention may be directed to a single target PRMT1 or PRMT6 gene sequence or may be directed to a plurality of target PRMT1 or PRMT6 gene sequences.

[0229] A double-stranded molecule of the present invention targeting the above-mentioned targeting sequence of PRMT1 or PRMT6 gene include isolated polynucleotides that contain the nucleic acid sequences of target sequences and/or complementary sequences to the target sequence. Example of polynucleotide targeting PRMT6 gene includes that containing the sequence of SEQ ID NO: 35 or 38 and/or complementary sequences to these nucleotides. However, the present invention is not limited to this example, and minor modifications in the aforementioned nucleic acid sequences are acceptable so long as the modified molecule retains the ability to suppress the expression of PRMT6 gene. Herein, the phrase "minor modification" as used in connection with a nucleic acid sequence indicates one, two or several substitution, deletion, addition or insertion of nucleic acids to the sequence.

[0230] In the context of the present invention, the term "several" as applies to nucleic acid substitutions, deletions, additions and/or insertions may mean 3-7, preferably 3-5, more preferably 3-4, even more preferably 3 nucleic acid residues.

[0231] According to the present invention, a double-stranded molecule of the present invention can be tested for its ability to inhibit expression using the methods utilized in the Examples. In the Examples herein below, double-stranded molecules composed of sense strands of various portions of mRNA of PRMT1 or PRMT6 genes or antisense strands complementary thereto were tested in vitro for their ability to decrease production of PRMT1 or PRMT6 gene product in cancer cell lines according to standard methods. Furthermore, for example, reduction in PRMT1 or PRMT6 gene product in cells contacted with the candidate double-stranded molecule compared to cells cultured in the absence of the candidate molecule can be detected by, e.g., RT-PCR using primers for PRMT1 or PRMT6 mRNA mentioned under Example 1, item "Quantitative RT-PCR". Sequences which decrease the production of PRMT1 or PRMT6 gene product in in vitro cell-based assays can then be tested for there inhibitory effects on cell growth. Sequences which inhibit cell growth in in vitro cell-

based assay can then be tested for their in vivo ability using animals with cancer, e.g., nude mouse xenograft models, to confirm decreased production of PRMT1 or PRMT6 product and decreased cancer cell growth.

[0232] When the isolated polynucleotide is RNA or derivatives thereof, base "t" should be replaced with "u" in the nucleotide sequences. As used herein, the term "complementary" refers to Watson-Crick or Hoogsteen base pairing between nucleotides units of a polynucleotide, and the term "binding" means the physical or chemical interaction between two polynucleotides. When the polynucleotide includes modified nucleotides and/or non-phosphodiester linkages, these polynucleotides may also bind each other as same manner. Generally, complementary polynucleotide sequences hybridize under appropriate conditions to form stable duplexes containing few or no mismatches. Furthermore, the sense strand and antisense strand of the isolated polynucleotide of the present invention can form double-stranded molecule or hairpin loop structure by the hybridization. In a preferred embodiment, such duplexes contain no more than 1 mismatch for every 10 matches. In an especially preferred embodiment, where the strands of the duplex are fully complementary, such duplexes contain no mismatches.

[0233] The polynucleotide is preferably less than 1,000 nucleotides in length for PRMT1 or PRMT6. For example, the polynucleotide is less than 500, 200, 100, 75, 50, or 25 nucleotides in length for all of the genes. The isolated polynucleotides of the present invention are useful for forming double-stranded molecules against PRMT1 or PRMT6 gene or preparing template DNAs encoding the double-stranded molecules. When the polynucleotides are used for forming double-stranded molecules, the sense strand of polynucleotide may be longer than 19 nucleotides, preferably longer than 21 nucleotides, and more preferably has a length of between about 19 and 25 nucleotides. Accordingly, the present invention provides the double-stranded molecules comprising a sense strand and an antisense strand, wherein the sense strand comprises a nucleotide sequence corresponding to a target sequence. In preferable embodiments, the sense strand hybridizes with antisense strand at the target sequence to form the double-stranded molecule having between 19 and 25 nucleotide pair in length.

[0234] Alternatively, the double-stranded molecules of the present invention may be double-stranded molecules, wherein the sense strand is hybridize with antisense strand at the target sequence to form the double-stranded molecule having less than 500, 200, 100, 75, 50 or 25 nucleotides pair in length. Preferably, the double-stranded molecules have between about 19 and about 25 nucleotides pair in length. Further, the sense strand of the double-stranded molecule may preferably include less than 500, 200, 100, 75, 50, 30, 28, 27, 26, 25 nucleotides, more preferably, between about 19 and about 25 nucleotides.

[0235] The double-stranded molecules of the present invention may contain one or more modified nucleotides and/or non-phosphodiester linkages. Chemical modifications well known in the art are capable of increasing stability, availability, and/or cell uptake of the double-stranded molecule. The skilled person will be aware of other types of chemical modification which may be incorporated into the present molecules (WO03/070744; WO2005/045037). In one embodiment, modifications can be used to provide improved resistance to degradation or improved uptake. Examples of such modifications include, but are not limited to, phosphorothioate linkages, 2'-O-methyl ribonucleotides (especially on the sense strand of a double-stranded molecule), 2'-deoxy-fluoro ribonucleotides, 2'-deoxy ribonucleotides, "universal base" nucleotides, 5'-C- methyl nucleotides, and inverted deoxybasic residue incorporation (US20060122137).

[0236] In another embodiment, modifications can be used to enhance the stability or to increase targeting efficiency of the double-stranded molecule. Examples of such modifications include, but are not limited to, chemical cross linking between the two complementary strands of a double-stranded molecule, chemical modification of a 3' or 5' terminus of a strand of a double-stranded molecule, sugar modifications, nucleobase modifications and/or backbone modifications, 2 -fluoro modified ribonucleotides and 2'-deoxy ribonucleotides (WO2004/029212). In another embodiment, modifications can be used to increased or decreased affinity for the complementary nucleotides in the target mRNA and/or in the complementary double-stranded molecule strand (WO2005/044976). For example, an unmodified pyrimidine nucleotide can be substituted for a 2-thio, 5-alkynyl, 5-methyl, or 5-propynyl pyrimidine. Additionally, an unmodified purine can be substituted with a 7-deaza, 7-alkyl, or 7-alkenyl purine. In another embodiment, when the double-stranded molecule is a double-stranded molecule with a 3' overhang, the 3'- terminal nucleotide overhanging nucleotides may be replaced by deoxyribonucleotides (Elbashir SM et al., Genes Dev 2001 Jan 15, 15(2): 188-200). For further details, see, e.g., US20060234970. The present invention is not limited to these examples and any known chemical modifications may be employed for the double-stranded molecules of the present invention so long as the resulting molecule retains the ability to inhibit the expression of the target gene.

[0237] Furthermore, the double-stranded molecules of the present invention may include both DNA and RNA, e.g., dsD/R-NA or shD/R-NA. Specifically, a hybrid polynucleotide of a DNA strand and an RNA strand or a DNA-RNA chimera polynucleotide shows increased stability. Mixing of DNA and RNA, i.e., a hybrid type double-stranded molecule composed of a DNA strand (polynucleotide) and an RNA strand (polynucleotide), a chimera type double-stranded molecule containing both DNA and RNA on any or both of the single strands (polynucleotides), or the like may be formed

for enhancing stability of the double-stranded molecule.

[0238] The hybrid of a DNA strand and an RNA strand may be either where the sense strand is DNA and the antisense strand is RNA, or the opposite so long as it can inhibit expression of the target gene when introduced into a cell expressing the gene. Preferably, the sense strand polynucleotide is DNA and the antisense strand polynucleotide is RNA. Also, the chimera type double-stranded molecule may be either where both of the sense and antisense strands are composed of DNA and RNA, or where any one of the sense and antisense strands is composed of DNA and RNA so long as it has an activity to inhibit expression of the target gene when introduced into a cell expressing the gene. In order to enhance stability of the double-stranded molecule, the molecule preferably contains as much DNA as possible, whereas to induce inhibition of the target gene expression, the molecule is required to be RNA within a range to induce sufficient inhibition of the expression.

[0239] As a preferred example of the chimera type double-stranded molecule, an upstream partial region (i.e., a region flanking to the target sequence or complementary sequence thereof within the sense or antisense strands) of the double-stranded molecule is RNA. Preferably, the upstream partial region indicates the 5' side (5'-end) of the sense strand and the 3' side (3'-end) of the antisense strand. Alternatively, regions flanking to 5'-end of sense strand and/or 3'-end of antisense strand are referred to upstream partial region. That is, in preferable embodiments, a region flanking to the 3'-end of the antisense strand, or both of a region flanking to the 5'-end of sense strand and a region flanking to the 3'-end of antisense strand are composed of RNA. For instance, the chimera or hybrid type double-stranded molecule of the present invention include following combinations.

[0240] sense strand:
5'-[---DNA---]-3'
3'-(RNA)-[DNA]-5'
:antisense strand,
sense strand:
5'-(RNA)-[DNA]-3'
3'-(RNA)-[DNA]-5'
:antisense strand, and
sense strand:
5'-(RNA)-[DNA]-3'
3'-(---RNA---)-5'
:antisense strand.

[0241] The upstream partial region preferably is a domain composed of 9 to 13 nucleotides counted from the terminus of the target sequence or complementary sequence thereto

within the sense or antisense strands of the double-stranded molecules. Moreover, preferred examples of such chimera type double-stranded molecules include those having a strand length of 19 to 21 nucleotides in which at least the upstream half region (5' side region for the sense strand and 3' side region for the antisense strand) of the polynucleotide is RNA and the other half is DNA. In such a chimera type double-stranded molecule, the effect to inhibit expression of the target gene is much higher when the entire antisense strand is RNA (US20050004064).

[0242] In the present invention, the double-stranded molecule may form a hairpin, such as a short hairpin RNA (shRNA) and short hairpin consisting of DNA and RNA (shD/R-NA). The shRNA or shD/R-NA is a sequence of RNA or mixture of RNA and DNA making a tight hairpin turn that can be used to silence gene expression via RNA interference. The shRNA or shD/R-NA includes the sense target sequence and the antisense target sequence on a single strand wherein the sequences are separated by a loop sequence. Generally, the hairpin structure is cleaved by the cellular machinery into dsRNA or dsD/R-NA, which is then bound to the RNA-induced silencing complex (RISC). This complex binds to and cleaves mRNAs which match the target sequence of the dsRNA or dsD/R-NA.

[0243] A loop sequence composed of an arbitrary nucleotide sequence can be located between the sense and antisense sequence in order to form the hairpin loop structure. Such loop sequence may be joined to 5' or 3' end of a sense strands to form the hairpin loop structure. Thus, the present invention also provides a double-stranded molecule having the general formula 5'-[A]-[B]-[A']-3' or 5'-[A']-[B]-[A]-3', wherein [A] is the sense strand containing a sequence corresponding to a target sequence, [B] is an intervening single-strand and [A'] is the antisense strand containing a complementary sequence to [A]. The target sequence may be selected from among, for example, nucleotide of SEQ ID NO: 29 or 32 for PRMT1 or SEQ ID NO: 35 or 38 for PRMT6.

[0244] The present invention is not limited to these examples, and the target sequence in [A] may be modified sequences from these examples so long as the double-stranded molecule retains the ability to suppress the expression of the targeted PRMT1 and PRMT6 gene. The region [A] hybridizes to [A'] to form a loop composed of the region [B]. The intervening single-stranded portion [B], i.e., loop sequence may be preferably 3 to 23 nucleotides in length. The loop sequence, for example, can be selected from among the sequences found, e.g., at the Ambion website (ambion.com/techlib/tb/tb_506.html). Furthermore, loop sequence consisting of 23 nucleotides also provides active siRNA (Jacque JM et al., Nature 2002 Jul 25, 418(6896): 435-8, Epub 2002 Jun 26):

CCC, CCACC, or CCACACC: Jacque JM et al., Nature 2002 Jul 25, 418(6896): 435-8, Epub 2002 Jun 26;

UUCG: Lee NS et al., Nat Biotechnol 2002 May, 20(5): 500-5; Fruscoloni P et al., Proc Natl Acad Sci USA 2003 Feb 18, 100(4): 1639-44, Epub 2003 Feb 10; and UUCAAGAGA: Dykxhoorn DM et al., Nat Rev Mol Cell Biol 2003 Jun, 4(6): 457-67.

[0245] Examples of preferred double-stranded molecules of the present invention having hairpin loop structure are shown below. In the following structure, the loop sequence can be selected from among AUG, CCC, UUCG, CCACC, CTCGAG, AAGCUU, CCACACC, and UUCAAGAGA; however, the present invention is not limited thereto:

CGGUGUUCUACAUGGAGGA-[B]- UCCUCCAUGUAGAACACCG (for target sequence SEQ ID NO: 29),

GAGUUCACACGCUGCCACA-[B]- UGUGGCAGCGUGUGAACUC (for target sequence SEQ ID NO: 32),

CCAUGCAUGGCUUUGCCAU-[B]- AUGGCAAAGCCAUGCAUGG (for target sequence SEQ ID NO: 35), and

CGGAACAGGUGGAUGCCAU-[B]- AUGGCAUCCACCUGUUCCG (for target sequence SEQ ID NO: 38).

[0246] Furthermore, in order to enhance the inhibition activity of the double-stranded molecules, several nucleotides can be added to 3' end of the sense strand and/or antisense strand of the target sequence, as 3' overhangs. The preferred examples of nucleotides constituting a 3' overhang include "t" and "u", but are not limited thereto . The number of nucleotides to be added is at least 2, generally 2 to 10, preferably 2 to 5. The added nucleotides form single strand at the 3' end of the sense strand and/or antisense strand of the double-stranded molecule. In cases where double-stranded molecules consists of a single polynucleotide to form a hairpin loop structure, a 3' overhang sequence may be added to the 3' end of the single polynucleotide.

[0247] The method for preparing the double-stranded molecule is not particularly limited though it is preferable to use a chemical synthetic method known in the art. According to the chemical synthesis method, sense and antisense single-stranded polynucleotides are separately synthesized and then annealed together via an appropriate method to obtain a double-stranded molecule. According to a specific example for the annealing, the synthesized single-stranded polynucleotides are mixed in a molar ratio of preferably at least about 3:7, more preferably about 4:6, and most preferably substantially equimolar amount (i.e., a molar ratio of about 5:5). Next, the mixture is heated to a temperature at which double-stranded molecules dissociate and then is gradually cooled down. The annealed double-stranded polynucleotide can be purified by usually employed methods known in the art. Example of purification methods include methods utilizing agarose gel electrophoresis or wherein remaining single-stranded polynucleotides are optionally removed by, e.g., degradation with appropriate

enzyme.

The regulatory sequences flanking PRMT1 or PRMT6 sequences may be identical or different, such that their expression can be modulated independently, or in a temporal or spatial manner. The double-stranded molecules can be transcribed intracellularly by cloning PRMT1 or PRMT6 gene templates into a vector containing, e.g., an RNA pol III transcription unit from the small nuclear RNA (snRNA) U6 or the human H1 RNA promoter.

[0248] Vector containing a double-stranded molecule of the present invention:

Also included in the present invention are vectors containing one or more of the double-stranded molecules described herein, and a cell containing such a vector.

Specifically, the present invention provides the following vectors of [1] to [10].

[0249] [1] A vector, encoding a double-stranded molecule that, when introduced into a cell, inhibits in vivo expression of PRMT1 or PRMT6 and cell proliferation, such molecules composed of a sense strand and an antisense strand complementary thereto, hybridized to each other to form the double-stranded molecule.

[2] The vector of [1], encoding the double-stranded molecule acts on mRNA, matching a target sequence of SEQ ID NO: 29, 32, 35 or 38;

[3] The vector of [1], wherein the sense strand contains a sequence corresponding to a target sequence of SEQ ID NO: 29, 32, 35 or 38;

[4] The vector of [3], encoding the double-stranded molecule having a length of less than about 100 nucleotides;

[5] The vector of [4], encoding the double-stranded molecule having a length of less than about 75 nucleotides;

[6] The vector of [5], encoding the double-stranded molecule having a length of less than about 50 nucleotides;

[7] The vector of [6] encoding the double-stranded molecule having a length of less than about 25 nucleotides;

[8] The vector of [7], encoding the double-stranded molecule having a length of between about 19 and about 25 nucleotides;

[9] The vector of [1], wherein the double-stranded molecule is composed of a single polynucleotide having both the sense and antisense strands linked by an intervening single-strand;

[10] The vector of [9], encoding the double-stranded molecule having the general formula 5'-[A]-[B]-[A']-3' or 5'-[A']-[B]-[A]-3', wherein [A] is the sense strand containing a sequence corresponding to a target sequence of SEQ ID NO: 29, 32, 35 or 38, [B] is the intervening single-strand composed of 3 to 23 nucleotides, and [A'] is the antisense strand containing a sequence complementary to [A].

[0250] A vector of the present invention preferably encodes a double-stranded molecule of

the present invention in an expressible form. Herein, the phrase "in an expressible form" indicates that the vector, when introduced into a cell, will express the molecule. In a preferred embodiment, the vector includes regulatory elements necessary for expression of the double-stranded molecule. Accordingly, in one embodiment, the expression vector encodes the nucleic acid sequences of the present invention and is adapted for expression of said nucleic acid sequences. Such vectors of the present invention may be used for producing the present double-stranded molecules, or directly as an active ingredient for treating cancer.

[0251] Vectors of the present invention can be produced, for example, by cloning PRMT1 or PRMT6 sequence into an expression vector so that regulatory sequences are operatively-linked to PRMT1 or PRMT6 sequence in a manner to allow expression (by transcription of the DNA molecule) of both strands (Lee NS et al., Nat Biotechnol 2002 May, 20(5): 500-5). For example, the RNA molecule that is the antisense to mRNA is transcribed by a first promoter (e.g., a promoter sequence flanking to the 3' end of the cloned DNA) and the RNA molecule that is the sense strand to the mRNA is transcribed by a second promoter (e.g., a promoter sequence flanking to the 5' end of the cloned DNA). The sense and antisense strands hybridize in vivo to generate a double-stranded molecule constructs for silencing of the gene. Alternatively, two vector constructs respectively encoding the sense and antisense strands of the double-stranded molecule are utilized to respectively express the sense and anti-sense strands and then forming a double-stranded molecule construct. Furthermore, the cloned sequence may encode a construct having a secondary structure (e.g., hairpin); namely, a single transcript of a vector contains both the sense and complementary antisense sequences of the target gene.

[0252] The vectors of the present invention may also be equipped so to achieve stable insertion into the genome of the target cell (see, e.g., Thomas KR & Capecchi MR, Cell 1987, 51: 503-12 for a description of homologous recombination cassette vectors). See, e.g., Wolff et al., Science 1990, 247: 1465-8; US Patent Nos. 5,580,859; 5,589,466; 5,804,566; 5,739,118; 5,736,524; 5,679,647; and WO 98/04720. Examples of DNA-based delivery technologies include "naked DNA", facilitated (bupivacaine, polymers, peptide-mediated) delivery, cationic lipid complexes, and particle-mediated ("gene gun") or pressure-mediated delivery (see, e.g., US Patent No. 5,922,687).

[0253] The vectors of the present invention include, for example, viral or bacterial vectors. Examples of expression vectors include attenuated viral hosts, such as vaccinia or fowlpox (see, e.g., US Patent No. 4,722,848). This approach involves the use of vaccinia virus, e.g., as a vector to express nucleotide sequences that encode the double-stranded molecule. Upon introduction into a cell expressing the target gene, the recombinant vaccinia virus expresses the molecule and thereby suppresses the pro-

liferation of the cell. Another example of useable vector includes Bacille Calmette Guerin (BCG). BCG vectors are described in Stover et al., Nature 1991, 351: 456-60. A wide variety of other vectors are useful for therapeutic administration and production of the double-stranded molecules; examples include adeno and adeno-associated virus vectors, retroviral vectors, Salmonella typhi vectors, detoxified anthrax toxin vectors, and the like. See, e.g., Shata et al., Mol Med Today 2000, 6: 66-71; Shedlock et al., J Leukoc Biol 2000, 68: 793-806; and Hipp et al., In Vivo 2000, 14: 571-85.

[0254] Method of inhibiting or reducing growth of a cancer cell or treating cancer using a double-stranded molecule of the present invention:

In the present invention, dsRNAs for PRMT1 or PRMT6 were tested for their ability to inhibit cell growth. The dsRNA for PRMT1 or PRMT6 (Fig. 3), effectively knocked down the expression of the gene in several cancer cell lines coincided with suppression of cell proliferation.

Therefore, the present invention provides methods for inhibiting cell growth, i.e., a cancer cell, by inducing dysfunction of PRMT1 or PRMT6 gene via inhibiting the expression of PRMT1 or PRMT6. Exemplary cancer includes, but is not limited to, e.g., bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML. PRMT1 or PRMT6 gene expression can be inhibited by any of the aforementioned double-stranded molecules of the present invention which specifically target of PRMT1 or PRMT6 gene.

[0255] Such ability of the present double-stranded molecules and vectors to inhibit cell growth of cancerous cell indicates that they can be used for methods for treating cancer such as bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML. Thus, the present invention provides methods to treat patients with cancer by administering a double-stranded molecule against PRMT1 or PRMT6 gene or a vector expressing the molecule without adverse effect because PRMT1 or PRMT6 gene was minimally detected in normal organs (Fig. 1, and 9).

[0256] Specifically, the present invention provides the following methods [1] to [32]:

[1] A method for inhibiting a growth of cancer cell and treating a cancer, wherein the cancer cell or the cancer expresses a PRMT1 or PRMT6 gene, which method includes the step of administering at least one isolated double-stranded molecule inhibiting the expression of PRMT1 or PRMT6 in a cell over-expressing the gene and the cell proliferation, wherein the double-stranded molecule is composed of a sense strand and an antisense strand complementary thereto, hybridized to each other to form the double-stranded molecule;

[2] The method of [1], wherein the double-stranded molecule acts at mRNA which matches a target sequence of SEQ ID NO: 29, 32, 35 or 38;
[3] The method of [2], wherein the sense strand contains the sequence corresponding to a target sequence of SEQ ID NO: 29, 32, 35 or 38;
[4] The method of [1], wherein the cancer to be treated is selected from bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML;
[5] The method of [3], wherein the double-stranded molecule has a length of less than about 100 nucleotides;
[6] The method of [5], wherein the double-stranded molecule has a length of less than about 75 nucleotides;
[7] The method of [6], wherein the double-stranded molecule has a length of less than about 50 nucleotides;
[8] The method of [7], wherein the double-stranded molecule has a length of less than about 25 nucleotides;
[9] The method of [8], wherein the double-stranded molecule has a length of between about 19 and about 25 nucleotides in length;
[10] The method of [1], wherein the double-stranded molecule is composed of a single polynucleotide containing both the sense strand and the antisense strand linked by an intervening single-strand;
[11] The method of [10], wherein the double-stranded molecule has the general formula 5'-[A]-[B]-[A']-3' or 5'-[A']-[B]-[A]-3', wherein [A] is the sense strand containing a sequence corresponding to a target sequence of SEQ ID NO: 29, 32, 35 or 38, [B] is the intervening single strand composed of 3 to 23 nucleotides, and [A'] is the antisense strand containing a sequence complementary to [A];
[12] The method of [1], wherein the double-stranded molecule is an RNA;
[13] The method of [1], wherein the double-stranded molecule contains both DNA and RNA;
[14] The method of [13], wherein the double-stranded molecule is a hybrid of a DNA polynucleotide and an RNA polynucleotide;
[15] The method of [14] wherein the sense and antisense strand polynucleotides are composed of DNA and RNA, respectively;
[16] The method of [13], wherein the double-stranded molecule is a chimera of DNA and RNA;
[17] The method of [16], wherein a region flanking to the 3'-end of the antisense strand, or both of a region flanking to the 5'-end of sense strand and a region flanking to the 3'-end of antisense strand are composed of RNA;

[18] The method of [17], wherein the flanking region is composed of 9 to 13 nucleotides;

[19] The method of [1], wherein the double-stranded molecule contains 3' overhangs;

[20] The method of [1], wherein the double-stranded molecule is contained in a composition which includes, in addition to the molecule, a transfection-enhancing agent and pharmaceutically acceptable carrier;

[21] The method of [1], wherein the double-stranded molecule is encoded by a vector;

[22] The method of [21], wherein the double-stranded molecule encoded by the vector acts at mRNA which matches a target sequence of SEQ ID NO: 29, 32, 35 or 38;

[23] The method of [22], wherein the sense strand of the double-stranded molecule encoded by the vector contains the sequence corresponding to a target sequence selected from among SEQ ID NO: 29, 32, 35 or 38;

[24] The method of [21], wherein the cancer to be treated is bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML;

[25] The method of [23], wherein the double-stranded molecule encoded by the vector has a length of less than about 100 nucleotides;

[26] The method of [25], wherein the double-stranded molecule encoded by the vector has a length of less than about 75 nucleotides;

[27] The method of [26], wherein the double-stranded molecule encoded by the vector has a length of less than about 50 nucleotides;

[28] The method of [27], wherein the double-stranded molecule encoded by the vector has a length of less than about 25 nucleotides;

[29] The method of [28], wherein the double-stranded molecule encoded by the vector has a length of between about 19 and about 25 nucleotides in length;

[30] The method of [21], wherein the double-stranded molecule encoded by the vector is composed of a single polynucleotide containing both the sense strand and the antisense strand linked by an intervening single-strand;

[31] The method of [30], wherein the double-stranded molecule encoded by the vector has the general formula 5'-[A]-[B]-[A']-3' or 5'-[A']-[B]-[A]-3', wherein [A] is the sense strand containing a sequence corresponding to a target sequence of SEQ ID NO: 29, 32, 35 or 38, [B] is a intervening single-strand is composed of 3 to 23 nucleotides, and [A'] is the antisense strand containing a sequence complementary to [A]; and

[32] The method of [21], wherein the double-stranded molecule encoded by the vector is contained in a composition which includes, in addition to the molecule, a transfection-enhancing agent and pharmaceutically acceptable carrier.

[0257] The method of the present invention will be described in more detail below.

The growth of cells expressing PRMT1 or PRMT6 gene may be inhibited by contacting the cells with a double-stranded molecule against PRMT1 or PRMT6 gene, a vector expressing the molecule or a composition containing the same. The cell may be further contacted with a transfection agent. Suitable transfection agents are known in the art. The phrase "inhibition of cell growth" indicates that the cell proliferates at a lower rate or has decreased viability as compared to a cell not exposed to the molecule. Cell growth may be measured by methods known in the art, e.g., using the MTT cell proliferation assay.

[0258] The growth of any kind of cell may be suppressed according to the present method so long as the cell expresses or over-expresses the target gene of the double-stranded molecule of the present invention. Exemplary cells include bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML.

[0259] Thus, patients suffering from or at risk of developing disease related to PRMT1 or PRMT6 may be treated by administering the present double-stranded molecule, at least one vector expressing the molecule or composition containing the molecule. For example, cancer patients may be treated according to the present methods. The type of cancer may be identified by standard methods according to the particular type of tumor to be diagnosed. More preferably, patients treated by the methods of the present invention are selected by detecting the expression of PRMT1 or PRMT6 in a biopsy from the patient by RT-PCR or immunoassay. Preferably, before the treatment of the present invention, the biopsy specimen from the subject is confirmed for PRMT1 or PRMT6 gene over-expression by methods known in the art, for example, immunohistochemical analysis or RT-PCR.

[0260] For inhibiting cell growth, a double-stranded molecule of the present invention may be directly introduced into the cells in a form to achieve binding of the molecule with corresponding mRNA transcripts. Alternatively, as described above, a DNA encoding the double-stranded molecule may be introduced into cells as a vector. For introducing the double-stranded molecules and vectors into the cells, transfection-enhancing agent, such as FuGENE (Roche diagnostics), Lipofectamine 2000 (Invitrogen), Oligofectamine (Invitrogen), and Nucleofector (Wako pure Chemical), may be employed.

[0261] A treatment is deemed "efficacious" if it leads to clinical benefit such as, reduction in expression of PRMT1 or PRMT6 gene, or a decrease in size, prevalence, or metastatic potential of the cancer in the subject. When the treatment is applied prophylactically, "efficacious" means that it retards or prevents cancers from forming or prevents or alleviates a clinical symptom of cancer. Efficaciousness is determined in association with any known method for diagnosing or treating the particular tumor type.

[0262] It is understood that the double-stranded molecule of the present invention degrades the t PRMT1 or PRMT6 mRNA in substoichiometric amounts. Without wishing to be bound by any theory, it is believed that the double-stranded molecule of the present invention causes degradation of the target mRNA in a catalytic manner. Thus, compared to standard cancer therapies, significantly less a double-stranded molecule needs to be delivered at or near the site of cancer to exert therapeutic effect.

[0263] One skilled in the art can readily determine an effective amount of the double-stranded molecule of the present invention to be administered to a given subject, by taking into account factors such as body weight, age, sex, type of disease, symptoms and other conditions of the subject; the route of administration; and whether the administration is regional or systemic. Generally, an effective amount of the double-stranded molecule of the present invention is an intercellular concentration at or near the cancer site of from about 1 nanomolar (nM) to about 100 nM, preferably from about 2 nM to about 50 nM, more preferably from about 2.5 nM to about 10 nM. It is contemplated that greater or smaller amounts of the double-stranded molecule can be administered. The precise dosage required for a particular circumstance may be readily and routinely determined by one of skill in the art.

[0264] The present methods can be used to inhibit the growth or metastasis of cancer expressing PRMT1 or/and PRMT6; for example, bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML. In particular, a double-stranded molecule containing a target sequence of PRMT1 or PRMT6 (i.e., SEQ ID NO: 29, 32, 35 or 38) is particularly preferred for the treatment of cancer.

[0265] For treating cancer, the double-stranded molecule of the present invention can also be administered to a subject in combination with a pharmaceutical substance different from the double-stranded molecule. Alternatively, the double-stranded molecule of the present invention can be administered to a subject in combination with another therapeutic method designed to treat cancer. For example, the double-stranded molecule of the present invention can be administered in combination with therapeutic methods currently employed for treating cancer or preventing cancer metastasis (e.g., radiation therapy, surgery and treatment using chemotherapeutic agents, such as cisplatin, carboplatin, cyclophosphamide, 5-fluorouracil, adriamycin, daunorubicin or tamoxifen).

In the present methods, the double-stranded molecule can be administered to the subject either as a naked double-stranded molecule, in conjunction with a delivery reagent, or as a recombinant plasmid or viral vector which expresses the double-stranded molecule.

Suitable delivery reagents for administration in conjunction with the present a double-stranded molecule include the Mirus Transit TKO lipophilic reagent; lipofectin; lipofectamine; cellfectin; or polycations (e.g., polylysine), or liposomes. A preferred delivery reagent is a liposome.

[0266] Liposomes can aid in the delivery of the double-stranded molecule to a particular tissue, such as lung tumor tissue, and can also increase the blood half-life of the double-stranded molecule. Liposomes suitable for use in the present invention are formed from standard vesicle-forming lipids, which generally include neutral or negatively charged phospholipids and a sterol, such as cholesterol. The selection of lipids is generally guided by consideration of factors such as the desired liposome size and half-life of the liposomes in the blood stream. A variety of methods are known for preparing liposomes, for example, as described in Szoka et al., Ann Rev Biophys Bioeng 1980, 9: 467; and US Pat. Nos. 4,235,871; 4,501,728; 4,837,028; and 5,019,369, the entire disclosures of which are herein incorporated by reference.

[0267] Preferably, the liposomes encapsulating the present double-stranded molecule include a ligand molecule that can deliver the liposome to the cancer site. Ligands which bind to receptors prevalent in tumor or vascular endothelial cells, such as monoclonal antibodies that bind to tumor antigens or endothelial cell surface antigens, are preferred.

Particularly preferably, the liposomes encapsulating the present double-stranded molecule are modified so as to avoid clearance by the mononuclear macrophage and reticuloendothelial systems, for example, by having opsonization-inhibition moieties bound to the surface of the structure. In one embodiment, a liposome of the present invention can include both opsonization-inhibition moieties and a ligand.

[0268] Opsonization-inhibiting moieties for use in preparing the liposomes of the present invention are typically large hydrophilic polymers that are bound to the liposome membrane. As used herein, an opsonization inhibiting moiety is "bound" to a liposome membrane when it is chemically or physically attached to the membrane, e.g., by the intercalation of a lipid-soluble anchor into the membrane itself, or by binding directly to active groups of membrane lipids. These opsonization-inhibiting hydrophilic polymers form a protective surface layer which significantly decreases the uptake of the liposomes by the macrophage-monocyte system ("MMS") and reticuloendothelial system ("RES"); e.g., as described in US Pat. No. 4,920,016, the entire disclosure of which is herein incorporated by reference. Liposomes modified with opsonization-inhibition moieties thus remain in the circulation much longer than unmodified liposomes. For this reason, such liposomes are sometimes called "stealth" liposomes.

[0269] Stealth liposomes are known to accumulate in tissues fed by porous or "leaky" microvasculature. Thus, target tissue characterized by such microvasculature defects, for

example, solid tumors, will efficiently accumulate these liposomes; see Gabizon et al., Proc Natl Acad Sci USA 1988, 18: 6949-53. In addition, the reduced uptake by the RES lowers the toxicity of stealth liposomes by preventing significant accumulation in liver and spleen. Thus, liposomes of the present invention that are modified with opsonization-inhibition moieties can deliver the present double-stranded molecule to tumor cells.

[0270] Opsonization inhibiting moieties suitable for modifying liposomes are preferably water-soluble polymers with a molecular weight from about 500 to about 40,000 daltons, and more preferably from about 2,000 to about 20,000 daltons. Such polymers include polyethylene glycol (PEG) or polypropylene glycol (PPG) derivatives; e.g., methoxy PEG or PPG, and PEG or PPG stearate; synthetic polymers such as polyacrylamide or poly N-vinyl pyrrolidone; linear, branched, or dendrimeric polyamidoamines; polyacrylic acids; polyalcohols, e.g., polyvinylalcohol and polyxylitol to which carboxylic or amino groups are chemically linked, as well as gangliosides, such as ganglioside GM.sub.1. Copolymers of PEG, methoxy PEG, or methoxy PPG, or derivatives thereof, are also suitable. In addition, the opsonization inhibiting polymer can be a block copolymer of PEG and either a polyamino acid, polysaccharide, polyamidoamine, polyethyleneamine, or polynucleotide. The opsonization inhibiting polymers can also be natural polysaccharides containing amino acids or carboxylic acids, e.g., galacturonic acid, glucuronic acid, mannuronic acid, hyaluronic acid, pectic acid, neuraminic acid, alginic acid, carrageenan; aminated polysaccharides or oligosaccharides (linear or branched); or carboxylated polysaccharides or oligosaccharides, e.g., reacted with derivatives of carbonic acids with resultant linking of carboxylic groups.

Preferably, the opsonization-inhibiting moiety is a PEG, PPG, or derivatives thereof. Liposomes modified with PEG or PEG-derivatives are sometimes called "PEGylated liposomes".

[0271] The opsonization inhibiting moiety can be bound to the liposome membrane by any one of numerous well-known techniques. For example, an N-hydroxysuccinimide ester of PEG can be bound to a phosphatidyl-ethanolamine lipid-soluble anchor, and then bound to a membrane. Similarly, a dextran polymer can be derivatized with a stearylamine lipid-soluble anchor via reductive amination using $Na(CN)BH_3$ and a solvent mixture such as tetrahydrofuran and water in a 30:12 ratio at 60 degrees C.

[0272] Vectors expressing a double-stranded molecule of the present invention are discussed above. Such vectors expressing at least one double-stranded molecule of the present invention can also be administered directly or in conjunction with a suitable delivery reagent, including the Mirus Transit LT1 lipophilic reagent; lipofectin; lipofectamine; cellfectin; polycations (e.g., polylysine) or liposomes. Methods for delivering re-

combinant viral vectors, which express a double-stranded molecule of the present invention, to an area of cancer in a patient are within the skill of the art.

[0273] The double-stranded molecule of the present invention can be administered to the subject by any means suitable for delivering the double-stranded molecule into cancer sites. For example, the double-stranded molecule can be administered by gene gun, electroporation, or by other suitable parenteral or enteral administration routes.

Suitable enteral administration routes include oral, rectal, or intranasal delivery.

[0274] Suitable parenteral administration routes include intravascular administration (e.g., intravenous bolus injection, intravenous infusion, intra-arterial bolus injection, intra-arterial infusion and catheter instillation into the vasculature); peri- and intra-tissue injection (e.g., peri-tumoral and intra-tumoral injection); subcutaneous injection or deposition including subcutaneous infusion (such as by osmotic pumps); direct application to the area at or near the site of cancer, for example, by a catheter or other placement device (e.g., a suppository or an implant including a porous, non-porous, or gelatinous material); and inhalation. It is preferred that injections or infusions of the double-stranded molecule or vector be given at or near the site of cancer.

[0275] The double-stranded molecule of the present invention can be administered in a single dose or in multiple doses. Where the administration of the double-stranded molecule of the present invention is by infusion, the infusion can be a single sustained dose or can be delivered by multiple infusions. Injection of the substance directly into the tissue is at or near the site of cancer preferred. Multiple injections of the substance into the tissue at or near the site of cancer are particularly preferred.

[0276] One skilled in the art can also readily determine an appropriate dosage regimen for administering the double-stranded molecule of the present invention to a given subject. For example, the double-stranded molecule can be administered to the subject once, for example, as a single injection or deposition at or near the cancer site. Alternatively, the double-stranded molecule can be administered once or twice daily to a subject for a period of from about three to about twenty-eight days, more preferably from about seven to about ten days. In a preferred dosage regimen, the double-stranded molecule is injected at or near the site of cancer once a day for seven days. Where a dosage regimen includes multiple administrations, it is understood that the effective amount of a double-stranded molecule administered to the subject can include the total amount of a double-stranded molecule administered over the entire dosage regimen.

[0277] In the present invention, a cancer overexpressing PRMT1 or PRMT6 can be treated with at least one active ingredient selected from the group consisting of:

(a) a double-stranded molecule of the present invention,

(b) DNA encoding thereof, and

(c) a vector encoding thereof.

[0278] The cancers to be treated include, but are not limited to, bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML. Accordingly, prior to the administration of the double-stranded molecule of the present invention as active ingredient, it is preferable to confirm whether the expression level of PRMT1 or PRMT6 in the cancer cells or tissues to be treated is enhanced as compared with normal cells of the same organ. Thus, in one embodiment, the present invention provides a method for treating a cancer (over)expressing PRMT1 or PRMT6, which method may include the steps of:

i) determining the expression level of PRMT1 or PRMT6 in cancer cells or tissue(s) obtained from a subject with the cancer to be treated;

ii) comparing the expression level of PRMT1 or PRMT6 with normal control; and

iii) administrating at least one component selected from the group consisting of

(a) a double-stranded molecule of the present invention,

(b) DNA encoding said double-stranded molecule, and

(c) a vector encoding said double-stranded molecule,

to a subject with a cancer overexpressing PRMT1 or PRMT6 compared with normal control. Alternatively, the present invention also provides a pharmaceutical composition comprising at least one component selected from the group consisting of:

(a) a double-stranded molecule of the present invention,

(b) DNA encoding said double-stranded molecule, and

(c) a vector encoding said double-stranded molecule,

for use in administrating to a subject having a cancer overexpressing PRMT1 or PRMT6. In other words, the present invention further provides a method for identifying a subject to be treated with:

(a) a double-stranded molecule of the present invention,

(b) DNA encoding said double-stranded molecule, or

(c) a vector encoding said double-stranded molecule,

, which method may include the step of determining an expression level of PRMT1 or PRMT6 in subject-derived cancer cells or tissue(s), wherein an increase of the level compared to a normal control level of the gene indicates that the subject has cancer which may be treated with a double-stranded molecule of the present invention.

[0279] The method of treating a cancer of the present invention will be described in more detail below.

A subject to be treated by the present method is preferably a mammal. Exemplary mammals include, but are not limited to, e.g., human, non-human primate, mouse, rat, dog, cat, horse, and cow.

According to the present invention, the expression level of PRMT1 or PRMT6 in

cancer cells or tissues obtained from a subject is determined. The expression level can be determined at the transcription (nucleic acid) product level, using methods known in the art. For example, hybridization methods (e.g., Northern hybridization), a chip or an array, probes, RT-PCR can be used to determine the transcription product level of PRMT1 or PRMT6.

[0280] Alternatively, the translation product may be detected for the treatment of the present invention. For example, the quantity of observed protein (SEQ ID NOs: 1-4) may be determined.

As another method to detect the expression level of PRMT1 or PRMT6 gene based on its translation product, the intensity of staining may be measured via immunohistochemical analysis using an antibody against the PRMT1 or PRMT6 protein. Namely, in this measurement, strong staining indicates increased presence/level of the protein and, at the same time, high expression level of PRMT1 or PRMT6 gene.

[0281] Methods for detecting or measuring either of the PRMT1 or PRMT6 polypeptide or polynucleotide encoding thereof, or both can be exemplified as described above (A method for diagnosing cancer).

[0282] Compositions containing a double-stranded molecule of the present invention:

In addition to the above, the present invention also provides pharmaceutical composition that include the present double-stranded molecule or the vector coding for the molecules. Specifically, the present invention provides the following compositions [1] to [32]:

[1] A composition for inhibiting a growth of cancer cell and treating a cancer, wherein the cancer cell and the cancer expresses a PRMT1 or PRMT6 gene, including isolated double-stranded molecule inhibiting the expression of PRMT1 or PRMT6 and the cell proliferation, which molecule is composed of a sense strand and an antisense strand complementary thereto, hybridized to each other to form the double-stranded molecule;

[2] The composition of [1], wherein the double-stranded molecule acts on mRNA which matches a target sequence of SEQ ID NO: 29, 32, 35 or 38;

[3] The composition of [2], wherein the double-stranded molecule, wherein the sense strand contains a sequence corresponding to a target sequence of SEQ ID NO: 29, 32, 35 or 38;

[4] The composition of [1], wherein the cancer to be treated is selected from bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML;

[5] The composition of [3], wherein the double-stranded molecule has a length of less than about 100 nucleotides;

[6] The composition of [5], wherein the double-stranded molecule has a length of less than about 75 nucleotides;

[7] The composition of [6], wherein the double-stranded molecule has a length of less than about 50 nucleotides;

[8] The composition of [7], wherein the double-stranded molecule has a length of less than about 25 nucleotides;

[9] The composition of [8], wherein the double-stranded molecule has a length of between about 19 and about 25 nucleotides;

[10] The composition of [1], wherein the double-stranded molecule is composed of a single polynucleotide containing the sense strand and the antisense strand linked by an intervening single-strand;

[11] The composition of [10], wherein the double-stranded molecule has the general formula 5'-[A]-[B]-[A']-3' or 5'-[A']-[B]-[A]-3', wherein [A] is the sense strand sequence contains a sequence corresponding to a target sequence of SEQ ID NO: 29, 32, 35 or 38, [B] is the intervening single-strand consisting of 3 to 23 nucleotides, and [A'] is the antisense strand contains a sequence complementary to [A];

[12] The composition of [1], wherein the double-stranded molecule is an RNA;

[13] The composition of [1], wherein the double-stranded molecule is DNA and/or RNA (alternatively, The composition of [1], wherein the double-stranded molecule is either of DNA or RNA, or both);

[14] The composition of [13], wherein the double-stranded molecule is a hybrid of a DNA polynucleotide and an RNA polynucleotide;

[15] The composition of [14], wherein the sense and antisense strand polynucleotides are composed of DNA and RNA, respectively;

[16] The composition of [13], wherein the double-stranded molecule is a chimera of DNA and RNA;

[17] The composition of [14], wherein a region flanking to the 3'-end of the antisense strand, or both of a region flanking to the 5'-end of sense strand and a region flanking to the 3'-end of antisense strand are composed of RNA;

[18] The composition of [17], wherein the flanking region is composed of 9 to 13 nucleotides;

[19] The composition of [1], wherein the double-stranded molecule contains 3' overhangs;

[20] The composition of [1], wherein the composition includes a transfection-enhancing agent and pharmaceutically acceptable carrier;

[21] The composition of [1], wherein the double-stranded molecule is encoded by a vector and contained in the composition;

[22] The composition of [21], wherein the double-stranded molecule encoded by the

vector acts at mRNA which matches a target sequence of SEQ ID NO: 29, 32, 35 or 38;

[23] The composition of [22], wherein the sense strand of the double-stranded molecule encoded by the vector contains the sequence corresponding to a target sequence of SEQ ID NO: 29, 32, 35 or 38;

[24] The composition of [21], wherein the cancer to be treated is selected from bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer and CML;

[25] The composition of [23], wherein the double-stranded molecule encoded by the vector has a length of less than about 100 nucleotides;

[26] The composition of [25], wherein the double-stranded molecule encoded by the vector has a length of less than about 75 nucleotides;

[27] The composition of [26], wherein the double-stranded molecule encoded by the vector has a length of less than about 50 nucleotides;

[28] The composition of [27], wherein the double-stranded molecule encoded by the vector has a length of less than about 25 nucleotides;

[29] The composition of [28], wherein the double-stranded molecule encoded by the vector has a length of between about 19 and about 25 nucleotides in length;

[30] The composition of [21], wherein the double-stranded molecule encoded by the vector is composed of a single polynucleotide containing both the sense strand and the antisense strand linked by an intervening single-strand;

[31] The composition of [30], wherein the double-stranded molecule has the general formula 5'-[A]-[B]-[A']-3' or 5'-[A']-[B]-[A]-3', wherein [A] is the sense strand containing a sequence corresponding to a target sequence of SEQ ID NO: 29, 32, 35 or 38, [B] is a intervening single-strand composed of 3 to 23 nucleotides, and [A'] is the antisense strand containing a sequence complementary to [A]; and

[32] The composition of [21], wherein the composition includes a transfection-enhancing agent and pharmaceutically acceptable carrier.

[0283] Suitable compositions of the present invention are described in additional detail below.

The double-stranded molecule of the present invention is preferably formulated as pharmaceutical compositions prior to administering to a subject, according to techniques known in the art. Pharmaceutical composition of the present invention is characterized as being at least sterile and pyrogen-free. As used herein, "pharmaceutical composition" includes formulations for human and veterinary use. Methods for preparing pharmaceutical compositions of the present invention are within the skill known in the art, for example, as described in Remington's Pharmaceutical Science,

17th ed., Mack Publishing Company, Easton, Pa. (1985), the entire disclosure of which is herein incorporated by reference.

[0284] The present pharmaceutical composition contains the double-stranded molecule or vector encoding that of the present invention (e.g., 0.1 to 90% by weight), or a physiologically acceptable salt of the molecule, mixed with a physiologically acceptable carrier medium. Preferred physiologically acceptable carrier media are water, buffered water, normal saline, 0.4% saline, 0.3% glycine, hyaluronic acid and the like.

Moreover, the present double-stranded molecule may be contained as liposomes in the present composition. See under the item of "Methods of treating cancer using the double-stranded molecule" for details of liposomes.

[0285] Pharmaceutical compositions of the present invention can also include conventional pharmaceutical excipients and/or additives. Suitable pharmaceutical excipients include stabilizers, antioxidants, osmolality adjusting agents, buffers, and pH adjusting agents. Suitable additives include physiologically biocompatible buffers (e.g., tromethamine hydrochloride), additions of chelants (such as, for example, DTPA or DTPA-bisamide) or calcium chelate complexes (for example, calcium DTPA, CaNaDTPA-bisamide), or, optionally, additions of calcium or sodium salts (for example, calcium chloride, calcium ascorbate, calcium gluconate or calcium lactate). Pharmaceutical compositions of the present invention can be packaged for use in liquid form, or can be lyophilized.

For solid compositions, conventional nontoxic solid carriers can be used; for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like.

[0286] For example, a solid pharmaceutical composition for oral administration can include any of the carriers and excipients listed above and 10-95%, preferably 25-75%, of one or more double-stranded molecules of the present invention. A pharmaceutical composition for aerosol (inhalational) administration can include 0.01-20% by weight, preferably 1-10% by weight, of one or more double-stranded molecules of the present invention encapsulated in a liposome as described above, and propellant. A carrier can also be included as desired; e.g., lecithin for intranasal delivery.

[0287] In addition to the above, the present composition may contain other pharmaceutical active ingredients so long as they do not inhibit the in vivo function of the present double-stranded molecules. For example, the composition may contain chemotherapeutic agents conventionally used for treating cancers.

[0288] In another embodiment, the present invention also provides the use of the double-stranded nucleic acid molecule of the present invention in manufacturing a pharmaceutical composition for treating a cancer characterized by the expression of PRMT1 or PRMT6. For example, the present invention relates to a use of double-stranded

nucleic acid molecule inhibiting the expression of PRMT1 or PRMT6 gene in a cell, which molecule includes a sense strand and an antisense strand complementary thereto, hybridized to each other to form the double-stranded nucleic acid molecule and target to a sequence of SEQ ID NO: 29, 32, 35 or 38, for manufacturing a pharmaceutical composition for treating cancer expressing PRMT1 or PRMT6.

Alternatively, the present invention further provides the double-stranded nucleic acid molecules of the present invention for use in treating a cancer expressing the PRMT1 or PRMT6 gene.

[0289] Alternatively, the present invention further provides a method or process for manufacturing a pharmaceutical composition for treating a cancer characterized by the expression of PRMT1 or PRMT6, wherein the method or process includes a step for formulating a pharmaceutically or physiologically acceptable carrier with a double-stranded nucleic acid molecule inhibiting the expression of PRMT1 or PRMT6 in a cell, which over-expresses the gene, which molecule includes a sense strand and an antisense strand complementary thereto, hybridized to each other to form the double-stranded nucleic acid molecule and target to a sequence of SEQ ID NO: 29, 32, 35 or 38 as active ingredients.

[0290] In another embodiment, the present invention also provides a method or process for manufacturing a pharmaceutical composition for treating a cancer characterized by the expression of PRMT1 or PRMT6, wherein the method or process includes a step for admixing an active ingredient with a pharmaceutically or physiologically acceptable carrier, wherein the active ingredient is a double-stranded nucleic acid molecule inhibiting the expression of PRMT1 or PRMT6 in a cell, which over-expresses the gene, which molecule includes a sense strand and an antisense strand complementary thereto, hybridized to each other to form the double-stranded nucleic acid molecule and targets to a sequence of SEQ ID NO: 29, 32, 35 or 38.

[0291] Hereinafter, the present invention is described in more detail with reference to the Examples. However, the following materials, methods and examples only illustrate aspects of the present invention and in no way are intended to limit the scope of the present invention. As such, methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention.

[0292] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will

control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Examples

[0293] The present invention will be further described in the following examples, which do not limit the scope of the present invention described in the claims.

[0294] Example 1: General Methods

Tissue samples and RNA preparation

121 surgical specimens of primary urothelial carcinoma were collected, either at cystectomy or transurethral resection of bladder tumor (TURBT), and snap frozen in liquid nitrogen. 24 specimens of normal bladder urothelial tissue were collected from areas of macroscopically normal bladder urothelium in patients with no evidence of malignancy. Five sequential sections of 7 micro-m thickness were cut from each tissue and stained using Histogene™ staining solution (Arcturus, California, USA) following the manufacturer's protocol, and assessed for cellularity and tumor grade by an independent consultant urohistopathologist. Slides were then transferred for microdissection using a Pix Cell II laser capture microscope (Arcturus, CA, USA). This technique employs a low-power infrared laser to melt a thermoplastic film over the cells of interest, to which the cells become attached. Additionally, the sections were graded according to the degree of inflammatory cell infiltration (low, moderate and severe). Samples showing significant inflammatory cell infiltration were excluded (Wallard MJ, et al. Br J Cancer 2006;94:569-77).

[0295] Approximately 10,000 cells were microdissected from both stromal and epithelial/tumor compartments in each tissue. RNA was extracted using an RNeasy Micro Kit (QIAGEN, Crawley, UK). Areas of cancer or stroma containing significant inflammatory areas of tumor or stroma containing significant inflammatory cell infiltration were avoided to prevent contamination (Wallard MJ, et al. Br J Cancer 2006;94:569-77). Total RNA was reverse transcribed, and qRT-PCR was performed as described below. Given the low yield of RNA from such small samples, NanoDrop™ quantification was not performed, but correction for the endogenous 18S CT value was used as an accurate measure of the amount of intact starting RNA. To validate the accuracy of microdissection, primers and probes for Vimentin and Uroplakin were sourced and qRT-PCR performed according to the manufacturer's instructions (Assays on demand, Applied Biosystems, Warrington, UK). Vimentin is primarily expressed in messenchymally derived cells, and was used as a stromal marker. Uroplakin is a marker of urothelial differentiation and is preserved in up to 90% of epithelially derived tumors (Olsburgh Jet al. The Journal of pathology 2003;199:41-9.). Use of tissues for this study was approved by Cambridge shire Local Research Ethics

Committee (Ref 03/018).

[0296] Cell culture

All cell lines were grown in monolayers in appropriate media: Eagle's minimal essential medium (EMEM) for 253J, 253J B-V, HT-1197, HT-1376, J82, SCaBER, UMUC3 bladder cancer cells and SBC5 small cell lung cancer cells; RPMI1640 medium for 5637 bladder cancer cells and A549, NCI-H2170 and LC319 non-small cell lung cancer cells; Dulbecco's modified Eagle's medium (DMEM) for EJ28 bladder cancer cells and RERF-LC-AI non-small cell lung cancer cells; McCoy's 5A medium for RT4 and T24 bladder cancer cells; Leibovitz's L-15 for SW780 cells supplemented with 10% fetal bovine serum and 1% antibiotic/antimycotic solution (Sigma). All cells were maintained at 37°C in humid air with 5% CO_2, (253J, 253J B-V, HT-1197, HT-1376, J82, SCaBER, UMUC3, SBC-5, 5637, A549 NCI-H2170, LC319, EJ28, RERF-LC-AI, RT4 and T24) or without CO_2 (SW780). Cells were transfected with FuGENE6 (ROCHE, Basel, Switzerland) according to manufacturer's protocols.

[0297] Expression profiling in cancer using cDNA microarrays

The genome-wide cDNA microarray was established with 36,864 cDNAs selected from the UniGene database of the National Center for Biotechnology Information (NCBI). This microarray system was constructed essentially as described previously (Kikuchi T, et al. Oncogene 2003;22:2192-205., Kitahara O, et al. Cancer Res 2001;61:3544-9., Nakamura T, et al. G Oncogene 2004;23:2385-400.). Briefly, the cDNAs were amplified by RT-PCR using poly $(A)^+$ RNAs isolated from various human organs as templates; the lengths of the amplicons ranged from 200 to 1,100 bp, without any repetitive or poly (A) sequences. Many types of tumor and corresponding non-neoplastic tissues were prepared in 8 micro-m, as described previously (Kitahara O, et al. Cancer Res 2001;61:3544-9.). A total of 30,000-40,000 cancer or non-cancerous cells were collected selectively using the EZ cut system (SL Microtest GmbH, Germany) according to the manufacturer's protocol. Extraction of total RNA, T7-based amplification, and labeling of probes were performed as described previously (Kitahara O, et al. Cancer Res 2001;61:3544-9.). A measure of 2.5-micro-g aliquots of twice-amplified RNA (aRNA) from each cancerous and non cancerous tissue was then labeled, respectively, with Cy3-dCTP or Cy5-dCTP.

[0298] Quantitative real-time PCR

As described previously, 121 bladder cancer and 24 normal bladder tissues in Cambridge Addenbrooke's Hospital were prepared. For quantitative RT-PCR reactions, specific primers for all human GAPDH (housekeeping gene), SDH (housekeeping gene), PRMT1 and PRMT6 were designed (primer sequences in Table 2). PCR reactions were performed using the ABI prism 7700 Sequence Detection System (Applied Biosystems, Warrington, UK) following the manufacture's protocol. 50%

SYBR GREEN universal PCR Master Mix without UNG (Applied Biosystems, Warrington, UK), 50 nM each of the forward and reverse primers and 2 micro-l of reverse transcriptional cDNA were applied. Amplification conditions were firstly 5 min at 95 degree C and then 45 cycles each consisting of 10 sec at 95 degree C, 1 min at 55 degree C and 10 sec at 72 degree C. After this, samples were incubated for 15 sec at 95 degree C, 1 min at 65 degree C to draw the melting curve, and cooled to 50 degree C for 10 sec. Reaction conditions for target gene amplification were as described above and 5 ng of reverse transcribed RNA was used in each reaction.

[0299] [Table 2]

Primer sequences for quantitative RT-PCR

Gene name	Primer sequence	SEQ ID NO.
GAPDH (housekeeping gene) - f	5' GCAAATTCCATGGCACCGTC 3'	5
GAPDH (housekeeping gene) - r	5' TCGCCCCACTTGATTTTGG 3'	6
SDH (housekeeping gene) - f	5' TGGGAACAAGAGGGCATCTG 3'	7
SDH (housekeeping gene) - r	5' CCACCACTGCATCAAATTCATG 3'	8
PRMT1 – f1	5' GGGCTACTGCCTCTTCTACGAGTC 3'	9
PRMT1 – r1	5' GTCTTTGTACTGCCGGTCCTCGATG 3'	10
PRMT1 – f2	5' GGTGGACATCATCATCAGCGAGTGG 3'	11
PRMT1 – r2	5' TCACATACAGCGTGGCCCGGTCTGG 3'	12
PRMT6 – f1	5' GTTCCAGGTGACCTTCCCTGGAG 3'	13
PRMT6 – r1	5' CCGGCTCGTTCAGGTAGAGGAGC 3'	14
PRMT6 – f2	5' GAGTCGGAGAAACCCCTGGTGCTG 3'	15
PRMT6 – r2	5' AACGTCCGTGTCTTGCTCCACTTG 3'	16

Immunohistochemical staining

Sections of human bladder tissues were stained by VECTASTAIN (R) ABC KIT (VECTOR LABORATORIES, CA, USA). Briefly, endogenous peroxidase activity of xylene-deparaffinized and dehydrated sections was inhibited by treatment with 0.3% H_2O_2/methanol. Nonspecific binding was blocked by incubating sections with 3% BSA in a humidified chamber for 30 min at ambient temperature followed by overnight incubation at 4 degree C with a 1:500 dilution of rabbit polyclonal anti-PRMT1 (NQ15, SIGMA) antibody and a 1:1,000 dilution of mouse monoclonal anti-PRMT6 (ab50336, abcam, Cambridge, UK). The sections were washed twice with PBS (-), incubated with a 1:500 dilution of goat anti-rabbit biotinylated IgG and a 1:500 dilution of goat anti-mouse biotinylated IgG in PBS (s) containing 1% BSA for 30 min at ambient temperature, and then incubated with ABC reagent for 30 min. Specific immunostaining was visualized by 3,3'-diaminobenzidine. Slides were dehydrated through graded alcohol and xylene washing, and mounted on cover slips. Hematoxylin was used for nuclear counterstaining.

[0300] siRNA transfection

siRNA oligonucleotide duplexes were purchased from SIGMA Genosys for targeting the human PRMT1 and PRMT6 transcripts. siEGFP and siNegative control (siNC), which is a mixture of three different oligonucleotide duplexes, were used as control siRNAs. The siRNA sequences are described in Table 3. siRNA duplexes (100 nM final concentration) were transfected into bladder and lung cancer cell lines with Lipofectamine 2000 (Invitrogen) for 72 h, and cell viability was examined using the Cell Counting Kit-8 (Dojindo, Kumamoto, Japan).

[0301] [Table 3]

siRNA sequences

siRNA name		Sequence	SEQ ID NO.
siEGFP		Sense: 5' GCAGCACGACUUCUUCAAGTT 3'	17
		Antisense: 5' CUUGAAGAAGUCGUGCUGCTT 3'	18
siFFLuc		Sense: 5' GUGCGCUGCUGGUGCCAACTT 3'	19
		Antisense: 5' GUUGGCACCAGCAGCGCACTT 3'	20
siNegative control (Cocktail)	Target#1	Sense: 5' AUCCGCGCGAUAGUACGUA 3'	21
		Antisense: 5' UACGUACUAUCGCGCGGAU 3'	22
	Target#2	Sense: 5' UUACGCGUAGCGUAAUACG 3'	23
		Antisense: 5' CGUAUUACGCUACGCGUAA 3'	24
	Target#3	Sense: 5' UAUUCGCGCGUAUAGCGGU 3'	25
		Antisense: 5' ACCGCUAUACGCGCGAAUA 3'	26
siPRMT1#1		Sense: 5' CGGUGUUCUACAUGGAGGATT 3'	27
		Antisense: 5' UCCUCCAUGUAGAACACCGTT 3'	28
		Target: 5' CGGTGTTCTACATGGAGGA 3'	29
siPRMT1#2		Sense: 5' GAGUUCACACGCUGCCACATT 3'	30
		Antisense: 5' UGUGGCAGCGUGUGAACUCTT 3'	31
		Target: 5' GAGTTCACACGCTGCCACA 3'	32
siPRMT6#1		Sense : 5' CCAUGCAUGGCUUUGCCAUTT 3'	33
		Antisense: 5' AUGGCAAAGCCAUGCAUGGTT 3'	34
		Target: 5' CCATGCATGGCTTTGCCAT 3'	35
siPRMT6#2		Sense: 5' CGGAACAGGUGGAUGCCAUTT 3'	36
		Antisense: 5' AUGGCAUCCACCUGUUCCGTT 3'	37
		Target: 5' CGGAACAGGTGGATGCCAT 3'	38

[0302] Flow cytometry assays (FACS)

To examine the role of PRMT1 and PRMT6 in the cell cycle, SW780 and A549 cells were treated with siPRMT1s (siPRMT1#1, siPRMT1#2), siPRMT6s (siPRMT6#1, siPRMT6#2) or control siRNAs (siEGFP and siNC), and cultured in a CO_2 incubator at 37 degree C for 72 hours. Aliquots of 1 X 10^5 cells were collected by trypsinization, and stained with propidium iodide following the manufacturer's instructions (Cayman Chemical, Ann Arbor, MI). Cells were analyzed by FACScan (BECKMAN COULTER, Brea, CA) with MultiCycle for Windows software (BECKMAN COULTER) for detailed cell cycle status. The percentages of cells in G_0/G_1, S and G_2 / M phases of the cell cycle were determined from at least 20,000 ungated cells.

[0303] Microarray hybridization and statistical analysis for the clarification of down-stream genes

Purified total RNA was labeled and hybridized onto Affymetrix GeneChip U133 Plus 2.0 oligonucleotide arrays (Affymetrix, Santa Clara, CA) according to the manufacturer's instructions. Probe signal intensities were normalized by RMA and Quantile (using R and Bioconductor). Next, signal intensity fluctuation due to inter-experimental variation was estimated. Each experiment was replicated (1 and 2), and the standard deviation (stdev) of $\log_2(\text{intensity}_2/\text{intensity}_1)$ was calculated for each of a set of intensity ranges with the midpoints being at $\log_2((\text{intensity}_1+\text{intensity}_2) / 2) = 5, 7, 9, 11, 13$, and 15. The intensity variation was modeled using the formula $\text{stdev}(\log_2(\text{intensity}_2/\text{intensity}_1)) = a * (\log_2((\text{intensity}_1+\text{intensity}_2) / 2)) + b$ and estimated parameters a and b using the method of least squares. Using these values, the standard deviation of intensity fluctuation was calculated. The signal intensities of each probe were then compared between siPRMT1 or siPRMT6 (EXP) and controls (EGFP/FFLuc) (CONT) and tested for up/down-regulation by calculating the z-score: $\log_2(\text{intensity}_{EXP}/\text{intensity}_{CONT}) / (a * (\log_2((\text{intensity}_{EXP}+\text{intensity}_{CONT}) / 2)) + b)$. Resultant P values for the replication sets were multiplied to calculate the final P value of each probe. These procedures were applied to each comparison: siEGFP vs. siPRMT1 or siPRMT6, siFFLuc vs. siPRMT1 or siPRMT6, and siEGFP vs. siFFLuc, respectively. The up/down-regulated gene sets were determined as those that simultaneously satisfied the following criteria: (1) The Benjamini-Hochberg false discovery rate (FDR) <=0.05 for EGFP vs. siPRMT1 or siPRMT6, (2) FDR<=0.05 for FFLuc vs. siPRMT1 or siPRMT6 and the regulation direction is the same as (1), and (3) EGFP vs. FFLuc has the direction opposite to (1) and (2) or $P > 0.05$ for EGFP vs. FFLuc. Finally, a pathway analysis was performed using the hyper-geometric distribution test, which calculates the probability of overlap between the up/down-regulated gene set and each GO category compared against another gene list that is randomly sampled. The test was applied to the identified up/down-regulated genes to test whether or not they are significantly enriched (FDR<=0.05) in each category of "Biological processes" (857 categories) as defined by the Gene Ontology database.

[0304] Evaluation of serum ADMA levels

The serum ADMA levels were measured using the enzyme-linked immunosorbent assay (ELISA) method (Immunodiagnostik; Bensheim, Germany), following manufacturer's instructions (Ozgurtas T, et al. Atherosclerosis 2008;200:336-44.). The detection limit of the ADMA assay was 0.05 micro-M. All serum samples were stored in BioBank Japan (Nakamura Y. Clin Adv Hematol Oncol 2007;5:696-7.). Herein, the serum from 118 cancer patients were examined, including 33 lung cancer cases, 22 hematopoietic tumor cases, 33 gastric cancer cases and 30 breast cancer cases. 22

bronchial asthma cases and 19 periodontitis cases were used as controls. Differences of serum ADMA levels between cancer and non-cancerous cases were tested with two-tailed t-test. Relations between variables were investigated by Pearson's correlation test.

[0305] Example 2: Overexpression of PRMT1 and PRMT6 in clinical cancer tissues.

When first examined, expression levels of all PRMT genes in a small subset of British clinical bladder cancer samples, the significant overexpression of PRMT1 and PRMT6 was found in the cancer samples compared with non-cancerous samples. Subsequently, 121 bladder cancer samples and 24 normal control samples (British) were analyzed, and confirmed significant elevation of PRMT1 and PRMT6 expression levels in tumor cells compared with normal cells (both $P < 0.0001$, Mann-Whitney U test, Fig. 1a). Subclassification of tumors according to metastasis status, gender, recurrence status and smoking history identified no significant correlation with expression levels (Table 4). To evaluate protein expression levels of PRMT1 and PRMT6 in bladder tissues, immunohistochemical analysis was performed using anti-PRMT1 and PRMT6 antibodies, and it was observed strong PRMT1 and PRMT6 staining in the nucleus of malignant cells, but weak staining in non-neoplastic tissues. In addition, the previous microarray expression analysis of a large number of clinical samples (Kikuchi T, et al. Oncogene 2003;22:2192-205, Nakamura T, et al. G Oncogene 2004;23:2385-400, Nishidate T, et al. Int J Oncol 2004;25:797-819, Takata R, et al. Clin Cancer Res 2005;11:2625-36) indicated that both PRMT1 and PRMT6 expressions were significantly up-regulated in various types of cancer, including non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC) and breast cancer (Table 5).

[0306]

[Table 4]

Statistical analysis of PRMT1 and PRMT6 expression levels in clinical bladder tissues

		PRMT1				PRMT6		
Subjects	Case (n)	Mean	SD	95%CI	Case (n)	Mean	SD	95%CI
Normal (Control)	24	3.163	1.045	2.745 – 3.581	24	0.804	0.453	0.623 – 0.985
Tumor (Total)	121	9.421	12.712	6.864 – 10.842	121	6.750	7.604	5.395 – 8.105
Metastasis								
Negative or unknown	94	9.603	13.540	6.515 – 11.230	94	6.808	8.050	5.180 – 8.435
Positive	27	8.788	9.465	5.218 – 12.358	27	6.548	5.916	4.316 – 8.779
Gender								
Male	91	9.739	12.573	7.142 – 12.337	91	7.343	8.207	5.657 – 9.030
Female	30	6.196	3.508	4.941 – 7.451	30	4.949	5.077	3.132 – 6.765
Recurrence								
No	27	11.536	15.914	5.533 – 17.538	27	8.651	7.570	5.796 – 11.506
Yes	51	8.800	11.326	5.660 – 11.939	51	7.169	9.280	4.622 – 9.716
Died	8	5.909	4.170	3.019 – 8.798	8	3.670	4.145	0.798 – 6.542
Smoking								
No	27	12.322	18.752	5.249 – 19.395	27	8.383	10.286	4.504 – 12.263
Yes	49	8.711	8.863	6.229 – 11.193	49	6.082	7.067	4.104 – 8.061

[0307] [Table 5]

Expression of PRMT1 and PRMT6 in cancer tissues analyzed by cDNA microarray*

		Ratio (Tumor / Normal)		
Tissue type	Case (n)	Count >2 (T/N)	Count >3 (T/N)	Count >5 (T/N)
PRMT1				
Diffuse-type gastric cancer	17	13 (76.5%)	7 (41.1%)	3 (25%)
Non-small cell lung cancer	12	9 (75%)	5 (41.7%)	3 (25%)
Small cell lung cancer	15	11 (73.3%)	6 (40%)	0 (0%)
Testicular tumor	10	7 (70%)	7 (70%)	7 (70%)
Bladder cancer	29	13 (44.8%)	9 (31.0%)	3 (10.3%)
Pancreatic cancer	16	6 (37.5%)	2 (12.5%)	2 (12.5%)
Lymphoma	20	6 (30%)	1 (5%)	1 (5%)
Esophageal cancer	61	13 (21.3%)	5 (8.2%)	2 (3.3%)
Breast cancer	75	15 (21.1%)	12 (16.9%)	9 (12.7%)
PRMT6				
Lymphoma	15	13 (86.7%)	9 (60%)	0 (0%)
Small cell lung cancer	15	12 (80%)	8 (53.3%)	3 (20%)
Cervical cancer	17	11 (64.7%)	5 (29.4%)	3 (17.6%)
Osteosarcoma	25	12 (48%)	4 (16%)	1 (4%)
Bladder cancer	34	14 (41.2%)	8 (23.5%)	2 (5.9%)
Prostate cancer	56	16 (28.6%)	9 (16.1%)	4 (7.1%)
CML	44	11 (25%)	10 (22.7%)	7 (15.9%)
Breast cancer	80	19 (23.8%)	6 (7.5%)	3 (3.8%)
Non-small cell lung cancer	33	7 (21.2%)	2 (6.1%)	0 (0%)

* The signal intensity of PRMT1 and PRMT6 between tumor tissues and corresponding nonneoplatic tissues derived from the same patient were compared.

[0308] Example 3: PRMT1 and PRMT6 regulate the growth of cancer cells.

To examine whether elevated expression of PRMT1 and PRMT6 plays some critical

roles in the proliferation of cancer cells, it was prepared siRNA oligonucleotide duplexes, which specifically suppressed the expression of PRMT1 (siPRMT1#1, #2) or PRMT6 (siPRMT6#1, #2), and transfected either of them into lung and bladder cancer cell lines that expressed PRMT1 and PRMT6 abundantly (Fig 2). As shown in Fig. 3A, four siRNAs for PRMT1 and PRMT6 suppressed the expression of the corresponding genes, compared with siEGFP and siNC controls. The effect of siRNAs on the growth of cancer cells was examined by the cell counting kit system (Figs. 3B and C) and revealed that transfection of two independent siPRMT1s into two bladder cancer cell lines (SW780 and RT4) and three lung cancer cell lines (A549, LC319 and SBC5) significantly suppressed their growth, compared with those with siEGFP or siNC. When siRNAs for PRMT6 was used, a significant suppression of growth of two bladder cancer cell lines (SW780 and RT4) and three lung cancer cell lines (A549, LC319 and SBC5) was observed, while no effect was observed by the transfection of siEGFP or siNC.

Furthermore, cell cycle analysis using SW780 and A549 cells treated with two independent siPRMT1s showed that the cells in the S phase were significantly decreased by the reduction of PRMT1 expression and those in G_0 and G_1 phases were concomitantly increased (Fig. 3D). Similar results were observed with the reduction of PRMT6(data not shown). These data show that PRMT1 and PRMT6 play a role in the G_1-S transition of cancer cells.

[0309] Example 4: PRMT1 and PRMT6 can contribute to carcinogenesis through the regulation of RNA processing and DNA replication.

In order to find downstream genes of PRMT1 and PRMT6, expression profiles of cancer cells treated with siRNAs were analyzed, using the GeneChip Human Genome U133 Plus 2.0 microarrays (see Methods). Expression profiles of A549 and SW780 cells that were transfected with siPRMT1, siPRMT6, siEGFP or siFFLuc, were analyzed and revealed that expression of 521 genes was down-regulated and that of 110 genes was increased statistically after knockdown of PRMT1 (Fig. 4). When the cells were treated with siPRMT6, expression levels of 259 genes were decreased and those of 7 genes were increased (Fig. 5). The result of microarray analysis was confirmed by real-time quantitative RT-PCR of several candidate genes selected randomly from listed the Table 9. (Fig. 6). These down stream genes are listed in Table 9 (PRMT1) and Table 10 (PRMT6).

[0310] To further identify the functional networks of PRMT1 and PRMT6 in the whole cell, pathway analysis referring to the Gene Ontology (Methods; Table 6), was performed and revealed that PRMT1 could regulate RNA processing and DNA replication in cancer cells, and that PRMT6 regulates a similar pathway of PRMT1. These results indicate that PRMT1 and PRMT6 can promote the cell malignancy through the

regulation of RNA processing and DNA replication.

[0311] [Table 6]

Gene Ontology pathway analysis based on the Affymetrix's microarray data

Entry ID	Name	Definition	P
PRMT1			
GO0007049	Cell cycle	The progression of biochemical and morphological phases and events that occur in a cell during successive cell replication or nuclear replication events. Canonically, the cell cycle comprises the replication and segregation of genetic material followed by the division of the cell, but in endocycles or syncytial cells nuclear replication or nuclear division may not be followed by cell division.	1.55×10^{-4}
GO0016071	mRNA metabolic process	The chemical reactions and pathways involving mRNA, which is responsible for carrying the coded genetic 'message', transcribed from DNA, to sites of protein assembly at the ribosomes.	1.49×10^{-3}
GO0006397	mRNA processing	Any process involved in the conversion of a primary mRNA transcript into one or more mature mRNA(s) prior to translation into polypeptide.	3.25×10^{-3}
GO0022402	Cell cycle process	A cellular process that is involved in the progression of biochemical and morphological phases and events that occur in a cell during successive cell replication or nuclear replication events.	6.36×10^{-3}
GO0051301	Cell division	The process resulting in the physical partitioning and separation of a cell into daughter cells.	7.49×10^{-3}
GO0000278	Mitotic cell cycle	Progression through the phases of the mitotic cell cycle, the most common eukaryotic cell cycle, which canonically comprises four successive phases called G_1, S, G_2, and M and includes replication of the genome and the subsequent segregation of chromosomes into daughter cells	9.46×10^{-3}
GO0022403	Cell cycle phase	A cell cycle process comprising the steps by which a cell progresses through one of the biochemical and morphological phases and events that occur during successive cell replication or nuclear replication events.	1.15×10^{-2}
GO0000915	Cytokinesis, contractile ring formation	The process of formation of a ring composed of actin, myosin, and associated proteins that will function in cytokinesis.	2.34×10^{-2}

GO0007108	Cytokinesis, initiation of separation	The process involved in starting cell separation.	2.34	$\times 10^{-2}$
GO0006260	DNA replication	The process whereby new strands of DNA are synthesized. The template for replication can either be an existing DNA molecule or RNA.	2.83	$\times 10^{-2}$
PRMT6				
GO0051726	Regulation of cell cycle	Any process that modulates the rate or extent of progression through the cell cycle.	6.17×10^{-4}	
GO0006275	Regulation of DNA replication	Any process that modulates the frequency, rate or extent of DNA replication.	6.48×10^{-3}	
GO0045893	Positive regulation of transcription, DNA-dependent	Any process that activates or increases the frequency, rate or extent of DNA-dependent transcription.	7.71×10^{-3}	
GO0051254	Positive regulation of RNA metabolic process	Any process that activates or increases the frequency, rate or extent of the chemical reactions and pathways involving RNA.	8.09×10^{-3}	
GO0046833	Positive regulation of RNA export from nucleus	Any process that activates or increases the frequency, rate or extent of directed movement of RNA from the nucleus into the cytoplasm.	1.20×10^{-2}	
GO0016568	Chromatin modification	The alteration of DNA or protein in chromatin, which may result in changing the chromatin structure.	2.62×10^{-2}	
GO0000075	Cell cycle checkpoint	A point in the eukaryotic cell cycle where progress through the cycle can be halted until conditions are suitable for the cell to proceed to the next stage.	3.08×10^{-2}	

[0312] To identify interacting proteins, immunoprecipitation-mass spectrometry (IP-MS) analysis was performed and showed several proteins interacting with PRMT1 and PRMT6. For PRMT1, SRRT, EIF4B, HNRNPK and SERBP1 were found as in-

teracting proteins (Fig. 7). These candidates all bind to RNA and are involved in RNA processing, RNA metabolism and DNA replication. PRMT6 interacted with MSH2, EIF4B, HNRNPK, RUVBL1, EEF1A1 and HNRNPD (Fig. 7). EEF1A1 and HNRNPD proteins can also bind to RNA and regulate RNA processing. Two interacting proteins, EIF4B and HNRNPK, bound both PRMT1 and PRMT6. MSH2 is a component of the post-replicative DNA mismatch repair system (MMR), and RUVBL1 possesses single-stranded DNA-stimulated ATPase and ATP-dependent DNA helicase (3' to 5') activity. These results indicate that PRMT1 and PRMT6 directly interact with proteins, involved in RNA processing and DNA replication, consistent with our microarray data.

[0313] Example 4: Elevation of serum ADMA levels in cancer patients

ADMA results from methylation of arginine residues in intracellular proteins with PRMTs and is likely to be released into the blood when such proteins are hydrolyzed. To evaluate the possibility that serum ADMA levels can be increased by the overexpression of PRMT1 and PRMT6 in cancer cells, the serum level of ADMA was measured using a number of serum samples stored in BioBank Japan (Nakamura Y. Clin Adv Hematol Oncol 2007;5:696-7). 118 cancer cases (33 lung cancer cases, 22 hematopoietic tumor cases, 33 gastric cancer cases and 30 breast cancer cases) were examined, as well as 41 non-cancer patient controls (22 bronchial asthma and 19 periodontitis cases). Individuals affected with cardiovascular disease (myocardial infarction, unstable angina pectoris, stable angina pectoris, arrhythmia, cardiac failure, and arteriosclerosis obliterans) and diabetes mellitus, for which the ADMA elevation has already been reported, were omitted. Detailed information for cases is shown in Table 7. Serum ADMA levels were validated by ELISA, and found that serum ADMA levels in cancer patients to be significantly higher than those in controls (Fig. 8; $P = 6.14 \times 10^{-5}$ [lung cancer], $P = 1.54 \times 10^{-4}$ [hematopoietic tumor], $P = 5.98 \times 10^{-4}$ [gastric cancer], and $P = 1.94 \times 10^{-2}$ [breast cancer], respectively). Other clinical variables such as gender, age and smoking were not significantly correlated with serum ADMA levels (Table 8). Additionally, ADMA levels were evaluated based on three independent experiments, and values were almost same in each experiment. These results reveal that serum ADMA levels can increase, following the elevation of PRMT1 and PRMT6 expression in various types of cancer.

[0314]

[Table 7]

Clinical information of BioBank Japan samples

Subjects	Non-tumor		Tumor			
	Bronchial Asthma	Periodontitis	Lung cancer	Hematopoietic tumor	Gastric cancer	Breast cancer
No. of patients	22	19	33	22	33	30
Serum ADMA level						
Mean (μM)	0.448	0.474	0.549	0.555	0.527	0.502
95%CI (μM)	0.417-0.479	0.442-0.506	0.502-0.595	0.508-0.602	0.497-0.556	0.476-0.528
Gender						
Male	10	10	26	13	20	0
Female	12	9	7	9	13	30
Age						
Mean	55.8	59.8	69.5	60.4	67.4	57.5
Range	35-86	35-81	39-87	26-79	32-86	32-83
Blood Pressure						
Mean SBP (mmHg)	119.5	126.2	123.3	119.7	121.4	126.9
Range (mmHg)	100-141	100-140	80-140	90-140	94-150	100-166
Mean DBP (mmHg)	68.7	75.5	74.2	73.7	70.4	76.4
Range (mmHg)	62-75	50-90	58-95	55-93	59-85	60-108
Smoking						
No	9	9	4	7	14	28
Yes	11	10	28	14	18	2
Drinking						
No	10	5	16	8	18	19
Yes	9	13	16	13	13	11

[0315] [Table 8]

Correlation between serum ADMA levels and clinical variables of non-tumor subjects

Variable	ADMA	Gender	Age	SBP	DBP	Smoking	Drinking
ADMA	1.00						
Gender	0.071	1.00					
Age	0.121	- 0.232	1.00				
SBP	0.378	- 0.071	0.421*	1.00			
DBP	0.292	0.149	0.289	0.520**	1.00		
Smoking	0.060	0.641***	- 0.167	- 0.088	0.196	1.00	
Drinking	0.029	0.583***	- 0.072	0.156	0.347	0.518**	1.00

Data are presented as the r value in Pearson's correlation test.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$
SBP: Systolic blood pressure DBP: Diastolic blood pressure

[0316] Discussion

Our results show that PRMT1 and PRMT6 are type I arginine methyltranserases that are upregulated in many cancer types. We also show that PRMT1 and PRMT6 have a role in the growth regulation of cancer cells, especially at the G_1-S transition. The pathway analysis indicated that these two type I protein arginine methyltransferases may mainly regulate RNA processing and DNA replication. Importantly, IP-MS analysis revealed that both PRMT1 and PRMT6 interact with several proteins that are

related to RNA processing and DNA replication. Interestingly, among interacting proteins, some were previously reported their association with human carcinogenesis. Multiple studies suggested that the redistribution of EEF1A1 from cytoplasm to the nucleus was related to cell proliferation and tumor development (Gangwani L, et al. J Cell Biol 1998;143:1471-84., Grassi G, et al. Biochimie 2007;89:1544-52.). A proportional increase of nuclear-localized EEF1A1 and TSPY (Testis-specific protein Y-encoded) was recently demonstrated, and such increasing nuclear localization of both EEF1A1 and TSPY was associated with a higher protein synthesis activity (Kido T, et al. Int J Cancer 2008;123:1573-85.). In ovarian cancer, SERBP1 mRNA was significantly overexpressed in tumor cells, compared to normal ovarian tissues. Furthermore, a significant correlation was found between SERBP1 expression and advanced disease stage (FIGO) (Koensgen D, et al. Gynecol Oncol 2007;107:266-73.). Because RNA processing and DNA replication must be a fundamental step for cell proliferation, it was suspected that the protein-protein interactions of PRMT1 and PRMT6 with these proteins must play an important role in modulating cancer cell growth.

[0317] Arginine residues are common targets for methylation in mammalian cells (Bedford MT, et al. Mol Cell 2009;33:1-13., Najbauer J, et al. J Biol Chem 1993;268:10501-9.). Arginine contains five potential hydrogen-bond donors located at a favorable position for interaction with biological hydrogen-bond acceptors. In protein-DNA complexes, arginine residues are the most easily-accessible hydrogen-bond donors. They bind DNA backbone phosphate groups, as well as thymine, adenine and guanine bases (Luscombe NM, et al. Nucleic Acids Res 2001;29:2860-74.). Specific networks of hydrogen bonds are formed by the interaction with one or more arginine residues (Mitchell JB, et al. J Mol Biol 1992;226:251-62.). Addition of a methyl group to an arginine residue not only influences the protein structure, but also results in the loss of a potential hydrogen-bond donor and reduction of the binding affinity to some binding partners. Methylation of arginine residues might also increase the affinity to aromatic rings, in cation-pi interactions (Hughes RM, et al. J Am Chem Soc 2006;128:12735-42.). Hence, modification of arginine residues in proteins is likely to affect the physiological function of a protein.

[0318] ADMA is produced by methylation of arginine residues in intracellular proteins by type I protein arginine N-methyltransferases (PRMTs) (Kielstein JT, et al. Clin Chem 2007;53:161-3.). When these proteins are hydrolyzed, ADMA is released, and the primary route of ADMA clearance is the enzymatic degradation by dimethylamine dimethylaminohydrolase (DDAH), which converts ADMA to L-citrulline and dimethylarigine. Several studies have shown elevated concentrations of serum ADMA in patients with conditions characterized by endothelial dysfunction, including pe-

ripheral arterial disease (Mittermayer F, et al.. Arterioscler Thromb Vasc Biol 2006;26:2536-40.) and diabetes mellitus (Abbasi F, et al. Am J Cardiol 2001;88:1201-3.). Herein, it is shown that PRMT1 and PRMT6 are significantly over-expressed in various types of cancer, and that free serum ADMA is significantly increased in cancer patients. This is the first correlation of serum levels of free ADMA in human carcinogenesis.

[0319] As expression levels of PRMT1 in bladder cancer tissues are significantly higher than those in any normal tissues, including heart, liver, lung and kidney (Fig. 9), PRMT1 is a promising target for cancer therapy. Furthermore, as knockdown of either PRMT1 or PRMT6 suppressed the growth of several cancer cells, these enzymes are shown to have a critical role in the growth regulation of cancer cells. Importantly, Northern blot analysis indicated that expression levels of PRMT6 in normal tissues are uniformly low, except in the testis (data not shown). Thus, an inhibitor(s) for PRMT1 and PRMT6 is an ideal candidate for molecular targeted therapy of cancer.

Industrial Applicability

[0320] The present inventors have shown that the cell growth is suppressed by a double-stranded nucleic acid molecule that specifically targets the PRMT1 or PRMT6 gene. Thus, the targeted double-stranded nucleic acid molecule is useful for the development of anti-cancer pharmaceuticals. For example, agents that block the expression of PRMT1 or PRMT6 protein or prevent its activity may find therapeutic utility as anti-cancer agents, particularly anti-cancer agents for the treatment of bladder cancer, diffuse-type gastric cancer, breast cancer, esophageal cancer, NSCLC, SCLC, lymphoma, pancreatic cancer, testicular cancer, cervical cancer, osteosarcoma, prostate cancer or CML.

[0321] While the present invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and scope of the present invention. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entireties. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

[0322]

[Table 9]

Down stream genes of PRMT1

Gene Symbol	Gene Title
Up-regulated	
ALS2CR13	amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 13
BID	BH3 interacting domain death agonist
BTBD3	BTB (POZ) domain containing 3
C14orf179	chromosome 14 open reading frame 179
C1orf106	chromosome 1 open reading frame 106
C20orf194	chromosome 20 open reading frame 194
C8orf4	chromosome 8 open reading frame 4
C9orf16	chromosome 9 open reading frame 16
CAB39L	calcium binding protein 39-like
CBX6	chromobox homolog 6
CCNG2	cyclin G2
CD164	CD164 molecule, sialomucin
CHMP4B	chromatin modifying protein 4B
COL1A1	collagen, type I, alpha 1
CPEB4	cytoplasmic polyadenylation element binding protein 4
CTSB	cathepsin B
CXCL1	chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)
CYP4F3	cytochrome P450, family 4, subfamily F, polypeptide 3
DDA1	DET1 and DDB1 associated 1
DDX52	DEAD (Asp-Glu-Ala-Asp) box polypeptide 52
DUSP16	dual specificity phosphatase 16
EGLN3	egl nine homolog 3 (C. elegans)
ENC1	ectodermal-neural cortex (with BTB-like domain)
EPS15L1	epidermal growth factor receptor pathway substrate 15-like 1
FADS3	fatty acid desaturase 3
FAM79A	family with sequence similarity 79, member A
FBXL18	F-box and leucine-rich repeat protein 18
FNDC3B	fibronectin type III domain containing 3B
FZD2	frizzled homolog 2 (Drosophila)
GABARAPL1	GABA(A) receptor-associated protein like 1
GADD45B	growth arrest and DNA-damage-inducible, beta
GLS	glutaminase
GPR157	G protein-coupled receptor 157
GRAMD3	GRAM domain containing 3
IGFBP3	insulin-like growth factor binding protein 3
IL32	interleukin 32
ITGB6	integrin, beta 6
ITGB8	integrin, beta 8
LIF	leukemia inhibitory factor (cholinergic differentiation factor)
LOC652968	hypothetical protein LOC652968
LOC653879	similar to Complement C3 precursor

LZTFL1	leucine zipper transcription factor-like 1
MAFF	v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian)
MAPRE3	microtubule-associated protein, RP/EB family, member 3
MARCKS	myristoylated alanine-rich protein kinase C substrate
MARK3	MAP/microtubule affinity-regulating kinase 3
MAST4	microtubule associated serine/threonine kinase family member 4
MORF4 /// MORF4L1	mortality factor 4 like 1 /// mortality factor 4
MORF4L1	mortality factor 4 like 1
MYO1B	myosin IB
MYOCD	myocardin
NANOS1	nanos homolog 1 (Drosophila)
NEDD9	neural precursor cell expressed, developmentally down-regulated 9
NINJ1	ninjurin 1
NRBP1	nuclear receptor binding protein 1
NUAK1	NUAK family, SNF1-like kinase, 1
PAIP2	poly(A) binding protein interacting protein 2
PDXK	pyridoxal (pyridoxine, vitamin B6) kinase
PIK3R3	phosphoinositide-3-kinase, regulatory subunit 3 (p55, gamma)
POLD4	polymerase (DNA-directed), delta 4
PRKAR1A	protein kinase, cAMP-dependent, regulatory, type I, alpha (tissue specific extinguisher 1)
PSCD2	pleckstrin homology, Sec7 and coiled-coil domains 2 (cytohesin-2)
PVRL2	poliovirus receptor-related 2 (herpesvirus entry mediator B)
RELB	v-rel reticuloendotheliosis viral oncogene homolog B, nuclear factor of kappa light polypeptide gene enhancer in B-cells 3 (avian)
RFX5	regulatory factor X, 5 (influences HLA class II expression)
RHPN2	rhophilin, Rho GTPase binding protein 2
RYK	RYK receptor-like tyrosine kinase
SEC14L2	SEC14-like 2 (S. cerevisiae)
SERINC2	serine incorporator 2
SGK1	serum/glucocorticoid regulated kinase 1
SH3BP4	SH3-domain binding protein 4
SLFN5	Schlafen family member 5
SNF1LK	SNF1-like kinase
SOX4	SRY (sex determining region Y)-box 4
SQRDL	sulfide quinone reductase-like (yeast)
SRGN	Serglycin
STK17A	serine/threonine kinase 17a
STX3	syntaxin 3
SYTL4	synaptotagmin-like 4 (granuphilin-a)
TBC1D9	TBC1 domain family, member 9 (with GRAM domain)
TCEA2	transcription elongation factor A (SII), 2
TGFBR2	transforming growth factor, beta receptor II (70/80kDa)
TGM2	transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase)
TRAPPC6A	trafficking protein particle complex 6A
TRIM31	tripartite motif-containing 31
UGCG	UDP-glucose ceramide glucosyltransferase

VAMP3	vesicle-associated membrane protein 3 (cellubrevin)
WIPI1	WD repeat domain, phosphoinositide interacting 1
ZFAND5	zinc finger, AN1-type domain 5
ZFHX3	zinc finger homeobox 3
ZNF697	zinc finger protein 697
AASS	aminoadipate-semialdehyde synthase
ADAM10	ADAM metallopeptidase domain 10
ANXA1	annexin A1
APLP2	amyloid beta (A4) precursor-like protein 2
B4GALT5	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 5
BAK1	BCL2-antagonist/killer 1
C20orf121	chromosome 20 open reading frame 121
CALM3	calmodulin 3 (phosphorylase kinase, delta)
CHMP1B	chromatin modifying protein 1B
DCP2	DCP2 decapping enzyme homolog (S. cerevisiae)
DUSP5	dual specificity phosphatase 5
EDG3	endothelial differentiation, sphingolipid G-protein-coupled receptor, 3
FAM3C	family with sequence similarity 3, member C
FRMD6	FERM domain containing 6
GFM1	G elongation factor, mitochondrial 1
JUNB	jun B proto-oncogene
KIAA0256	KIAA0256 gene product
LACTB	lactamase, beta
LATS2	LATS, large tumor suppressor, homolog 2 (Drosophila)
MBIP	MAP3K12 binding inhibitory protein 1
MIDN	Midnolin
MOBKL1B	MOB1, Mps One Binder kinase activator-like 1B (yeast)
MT1X	metallothionein 1X
MT2A	metallothionein 2A
MXD1	MAX dimerization protein 1
NCOA3	nuclear receptor coactivator 3
OAT	ornithine aminotransferase (gyrate atrophy)
PBXIP1	pre-B-cell leukemia homeobox interacting protein 1
PGK1	phosphoglycerate kinase 1
PGM2L1	phosphoglucomutase 2-like 1
PICALM	phosphatidylinositol binding clathrin assembly protein
PLAUR	plasminogen activator, urokinase receptor
PURB	purine-rich element binding protein B
RNASEK	ribonuclease, RNase K
SELS	selenoprotein S
SELT	selenoprotein T
SLC30A1	Solute carrier family 30 (zinc transporter), member 1
SLC6A6	solute carrier family 6 (neurotransmitter transporter, taurine), member 6
SRPR	signal recognition particle receptor ('docking protein')
UBE2K	ubiquitin-conjugating enzyme E2K (UBC1 homolog, yeast)
UBXD6	UBX domain containing 6
VPS25	vacuolar protein sorting 25 homolog (S. cerevisiae)

YOD1	YOD1 OTU deubiquinating enzyme 1 homolog (S. cerevisiae)
Down-regulated	
RAD51L1	RAD51-like 1 (S. cerevisiae)
38053	membrane-associated ring finger (C3HC4) 8
ACTR3B	ARP3 actin-related protein 3 homolog B (yeast)
ADD3	adducin 3 (gamma)
ADH5	alcohol dehydrogenase 5 (class III), chi polypeptide
ADI1	acireductone dioxygenase 1
ADIPOR2	adiponectin receptor 2
ADO	2-aminoethanethiol (cysteamine) dioxygenase
AK2	adenylate kinase 2
AKR1A1	aldo-keto reductase family 1, member A1 (aldehyde reductase)
ALDH5A1	aldehyde dehydrogenase 5 family, member A1 (succinate-semialdehyde dehydrogenase)
ANKFY1	ankyrin repeat and FYVE domain containing 1
ANP32A	Acidic (leucine-rich) nuclear phosphoprotein 32 family, member A
AP1S2	adaptor-related protein complex 1, sigma 2 subunit
AP2B1	adaptor-related protein complex 2, beta 1 subunit
APBA3	amyloid beta (A4) precursor protein-binding, family A, member 3 (X11-like 2)
APPBP2	amyloid beta precursor protein (cytoplasmic tail) binding protein 2
ARF6	ADP-ribosylation factor 6
ARHGDIB	Rho GDP dissociation inhibitor (GDI) beta
ARL13B	ADP-ribosylation factor-like 13B
ARL6IP2	ADP-ribosylation factor-like 6 interacting protein 2
ARMC1	armadillo repeat containing 1
ARPC5	actin related protein 2/3 complex, subunit 5, 16kDa
ARSB	arylsulfatase B
ASB3	ankyrin repeat and SOCS box-containing 3
ASNSD1	asparagine synthetase domain containing 1
ATP11A	ATPase, class VI, type 11A
B3GALTL	beta 1,3-galactosyltransferase-like
BARD1	BRCA1 associated RING domain 1
BCS1L	BCS1-like (yeast)
BMI1	BMI1 polycomb ring finger oncogene
BRIP1	BRCA1 interacting protein C-terminal helicase 1
BTBD12	BTB (POZ) domain containing 12
BXDC5	brix domain containing 5
C10orf56	chromosome 10 open reading frame 56
C12orf31	chromosome 12 open reading frame 31
C12orf32	chromosome 12 open reading frame 32
C13orf23	chromosome 13 open reading frame 23
C13orf3	chromosome 13 open reading frame 3
C14orf1	chromosome 14 open reading frame 1
C14orf108	chromosome 14 open reading frame 108
C14orf130	chromosome 14 open reading frame 130
C14orf133	chromosome 14 open reading frame 133
C14orf65	chromosome 14 open reading frame 65
C16orf5	chromosome 16 open reading frame 5

C16orf52	chromosome 16 open reading frame 52
C17orf37	chromosome 17 open reading frame 37
C17orf70	chromosome 17 open reading frame 70
C18orf19	chromosome 18 open reading frame 19
C18orf55	chromosome 18 open reading frame 55
C1orf19	chromosome 1 open reading frame 19
C1orf216	chromosome 1 open reading frame 216
C1orf27	chromosome 1 open reading frame 27
C20orf27	chromosome 20 open reading frame 27
C21orf33	chromosome 21 open reading frame 33
C21orf45	chromosome 21 open reading frame 45
C22orf13	chromosome 22 open reading frame 13
C22orf39	chromosome 22 open reading frame 39
C6orf153	chromosome 6 open reading frame 153
C8orf30A	chromosome 8 open reading frame 30A
C8orf37	chromosome 8 open reading frame 37
C9orf41	chromosome 9 open reading frame 41
C9orf69	chromosome 9 open reading frame 69
C9orf89	chromosome 9 open reading frame 89
CABYR	calcium binding tyrosine-(Y)-phosphorylation regulated
CAPZA1	capping protein (actin filament) muscle Z-line, alpha 1
CAPZB	capping protein (actin filament) muscle Z-line, beta
CASP6	caspase 6, apoptosis-related cysteine peptidase
CAST	Calpastatin
CBFB	core-binding factor, beta subunit
CCDC126	coiled-coil domain containing 126
CCDC72	coiled-coil domain containing 72
CCNYL1	cyclin Y-like 1
CD151	CD151 molecule (Raph blood group)
CD59	CD59 molecule, complement regulatory protein
CD68	CD68 molecule
CEP55	centrosomal protein 55kDa
CHCHD4	coiled-coil-helix-coiled-coil-helix domain containing 4
CHSY1	carbohydrate (chondroitin) synthase 1
CIZ1	CDKN1A interacting zinc finger protein 1
CNlH4	cornichon homolog 4 (Drosophila)
COMMD10	COMM domain containing 10
COQ9	coenzyme Q9 homolog (S. cerevisiae)
CRNKL1	crooked neck pre-mRNA splicing factor-like 1 (Drosophila)
CS	citrate synthase
CSRP1	cysteine and glycine-rich protein 1
CTB-1048E9.5	Similar to SRR1-like protein
CTDSPL	CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase-like
CUL4B	cullin 4B
CYCS	cytochrome c, somatic
CYP2S1	cytochrome P450, family 2, subfamily S, polypeptide 1
DARS	aspartyl-tRNA synthetase

DCTN4	dynactin 4 (p62)
DCUN1D4	DCN1, defective in cullin neddylation 1, domain containing 4 (S. cerevisiae)
DDHD2	DDHD domain containing 2
DDR1	discoidin domain receptor family, member 1
DEPDC1B	DEP domain containing 1B
DERL1	Der1-like domain family, member 1
DGKA	diacylglycerol kinase, alpha 80kDa
DGKZ	diacylglycerol kinase, zeta 104kDa
DIAPH1	diaphanous homolog 1 (Drosophila)
DKFZP564J0863	DKFZP564J0863 protein
DNAJC9	DnaJ (Hsp40) homolog, subfamily C, member 9
DNMT3B	DNA (cytosine-5-)-methyltransferase 3 beta
DPF2	D4, zinc and double PHD fingers family 2
DRAM	damage-regulated autophagy modulator
DTX3L	deltex 3-like (Drosophila)
ECE1	endothelin converting enzyme 1
EEF2K	eukaryotic elongation factor-2 kinase
EFTUD1	elongation factor Tu GTP binding domain containing 1
EHBP1	EH domain binding protein 1
EIF2S2	eukaryotic translation initiation factor 2, subunit 2 beta, 38kDa
EIF2S3	eukaryotic translation initiation factor 2, subunit 3 gamma, 52kDa
ENOPH1	enolase-phosphatase 1
EPS15L1	epidermal growth factor receptor pathway substrate 15-like 1
ERAP1	endoplasmic reticulum aminopeptidase 1
EZR	Ezrin
FAM102A	family with sequence similarity 102, member A
FAM108C1	family with sequence similarity 108, member C1
FAM122B	family with sequence similarity 122B
FAM127A	family with sequence similarity 127, member A
FAM127B	family with sequence similarity 127, member B
FAM33A	family with sequence similarity 33, member A
FAM54A	family with sequence similarity 54, member A
FAM65A	family with sequence similarity 65, member A
FAM86B1	family with sequence similarity 86, member B1
FAM86C	family with sequence similarity 86, member C
FANCI	Fanconi anemia, complementation group I
FBXO45	F-box protein 45
FBXO5	F-box protein 5
FERMT2	fermitin family homolog 2 (Drosophila)
FLII	flightless I homolog (Drosophila)
FLJ35348	FLJ35348
FLJ44896	FLJ44896 protein
FSCN1	fascin homolog 1, actin-bundling protein (Strongylocentrotus purpuratus)
FTO	fat mass and obesity associated
GALNT1	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 1 (GalNAc-T1)

GALNT11	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 11 (GalNAc-T11)
GALNT7	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 7 (GalNAc-T7)
GCA	grancalcin, EF-hand calcium binding protein
GCH1	GTP cyclohydrolase 1 (dopa-responsive dystonia)
GDA	guanine deaminase
GNB4	guanine nucleotide binding protein (G protein), beta polypeptide 4
GNPDA1	glucosamine-6-phosphate deaminase 1
GOLPH3L	golgi phosphoprotein 3-like
GORASP2	golgi reassembly stacking protein 2, 55kDa
GOSR1	golgi SNAP receptor complex member 1
GPD2	glycerol-3-phosphate dehydrogenase 2 (mitochondrial)
GPS2	G protein pathway suppressor 2
GRSF1	G-rich RNA sequence binding factor 1
GSG2	germ cell associated 2 (haspin)
HACE1	HECT domain and ankyrin repeat containing, E3 ubiquitin protein ligase 1
HECTD2	HECT domain containing 2
HECTD3	HECT domain containing 3
HIGD2A	HIG1 domain family, member 2A
HMG20A	high-mobility group 20A
HNRNPA0	heterogeneous nuclear ribonucleoprotein A0
HNRNPA2B1	heterogeneous nuclear ribonucleoprotein A2/B1
HNRPA3	heterogeneous nuclear ribonucleoprotein A3
HNRPA3 /// HNRPA3P1	heterogeneous nuclear ribonucleoprotein A3 pseudogene 1 /// heterogeneous nuclear ribonucleoprotein A3
HSPA1B	heat shock 70kDa protein 1B
IAH1	isoamyl acetate-hydrolyzing esterase 1 homolog (S. cerevisiae)
IARS	isoleucyl-tRNA synthetase
ICMT	isoprenylcysteine carboxyl methyltransferase
ID2	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein
ID4	inhibitor of DNA binding 4, dominant negative helix-loop-helix protein
IDE	insulin-degrading enzyme
ILK	integrin-linked kinase
IMPAD1	inositol monophosphatase domain containing 1
ING5 /// LOC727773	inhibitor of growth family, member 5 /// similar to inhibitor of growth family, member 5
IQGAP1	IQ motif containing GTPase activating protein 1
IQGAP3	IQ motif containing GTPase activating protein 3
IRX3	iroquois homeobox 3
ISCA2	iron-sulfur cluster assembly 2 homolog (S. cerevisiae)
ITGB5	integrin, beta 5
IVNS1ABP	influenza virus NS1A binding protein
KCTD20	potassium channel tetramerisation domain containing 20
KHSRP	KH-type splicing regulatory protein (FUSE binding protein 2)
KIAA0247	KIAA0247
KIAA0265	KIAA0265 protein
KIAA0494	KIAA0494
KIAA1430	KIAA1430

KIF11	kinesin family member 11
KLHL12	kelch-like 12 (Drosophila)
KLHL2	kelch-like 2, Mayven (Drosophila)
LAMC1	laminin, gamma 1 (formerly LAMB2)
LASS6	LAG1 homolog, ceramide synthase 6
LCMT1	leucine carboxyl methyltransferase 1
LMAN1	Lectin, mannose-binding, 1
LOC100101267 /// LOC729316 /// POM121	POM121 membrane glycoprotein (rat) /// POM121 membrane glycoprotein (rat) pseudogene /// POM121 membrane glycoprotein (rat)-like
LOC116236	hypothetical protein LOC116236
LOC134145	hypothetical protein LOC134145
LOC153346	hypothetical protein LOC153346
LOC158402	hypothetical protein LOC158402
LOC201725	hypothetical protein LOC201725
LOC203547	hypothetical protein LOC203547
LOC205251	LOC205251
LOC285636	hypothetical protein LOC285636
LOC339290	hypothetical protein LOC339290
LOC374443	CLR pseudogene
LOC400506	similar to TSG118.1
LOC645094 /// MRCL3	myosin regulatory light chain MRCL3 /// similar to myosin regulatory light chain-like
LOC647859	occludin pseudogene
LOC653381	similar to Sorbitol dehydrogenase (L-iditol 2-dehydrogenase)
LOC727922	similar to FUS-interacting serine-arginine-rich protein 1 (TLS-associated protein with Ser-Arg repeats) (TLS-associated protein with SR repeats) (TASR) (TLS-associated serine-arginine protein) (TLS-associated SR protein) (Neural-specific SR protein...
LOC728866 /// RP11-217H1.1	implantation-associated protein /// similar to implantation-associated protein
LOC93622	hypothetical protein BC006130
LOC96610	hypothetical gene LOC96610
LRRC58	leucine rich repeat containing 58
LRRC59	leucine rich repeat containing 59
MAF1	MAF1 homolog (S. cerevisiae)
MALL	mal, T-cell differentiation protein-like
MAOA	monoamine oxidase A
MAP2K4	mitogen-activated protein kinase kinase 4
MAPK1	mitogen-activated protein kinase 1
MAPK9	Mitogen-activated protein kinase 9
MARVELD2	MARVEL domain containing 2
MCFD2	multiple coagulation factor deficiency 2
MCM8	minichromosome maintenance complex component 8
MED14	mediator complex subunit 14
MESDC2	mesoderm development candidate 2
MEST	mesoderm specific transcript homolog (mouse)

METTL4	methyltransferase like 4
MGC16169	hypothetical protein MGC16169
MICB	MHC class I polypeptide-related sequence B
MIR16	membrane interacting protein of RGS16
MKI67	antigen identified by monoclonal antibody Ki-67
MLXIP	MLX interacting protein
MPHOSPH9	M-phase phosphoprotein 9
MRCL3 /// MRLC2	myosin regulatory light chain MRCL3 /// myosin regulatory light chain MRLC2
MRLC2	myosin regulatory light chain MRLC2
MRPL10	mitochondrial ribosomal protein L10
MRPS27	mitochondrial ribosomal protein S27
MTDH	Metadherin
MYADM	myeloid-associated differentiation marker
NAT10	N-acetyltransferase 10
NBPF11 /// NBPF20 /// XXyac-YX15 5B6.1	neuroblastoma breakpoint family, member 11 /// neuroblastoma breakpoint family, member 20 /// CLIP-190-like
NEDD4	neural precursor cell expressed, developmentally down-regulated 4
NEK3	NIMA (never in mitosis gene a)-related kinase 3
NEK4	NIMA (never in mitosis gene a)-related kinase 4
NFE2L1	nuclear factor (erythroid-derived 2)-like 1
NFYC	nuclear transcription factor Y, gamma
NLN	neurolysin (metallopeptidase M3 family)
NRAS	neuroblastoma RAS viral (v-ras) oncogene homolog
NUDCD1	NudC domain containing 1
NUDCD3	NudC domain containing 3
NUP214	nucleoporin 214kDa
NUP62	nucleoporin 62kDa
NUPL1	nucleoporin like 1
ORC2L	origin recognition complex, subunit 2-like (yeast)
P2RY2	purinergic receptor P2Y, G-protein coupled, 2
PAFAH1B1	platelet-activating factor acetylhydrolase, isoform Ib, alpha subunit 45kDa
PAICS	phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoimidazole succinocarboxamide synthetase
PAPSS2	3'-phosphoadenosine 5'-phosphosulfate synthase 2
PAQR3	progestin and adipoQ receptor family member III
PARD6G	par-6 partitioning defective 6 homolog gamma (C. elegans)
PATL1	protein associated with topoisomerase II homolog 1 (yeast)
PCBD2	pterin-4 alpha-carbinolamine dehydratase/dimerization cofactor of hepatocyte nuclear factor 1 alpha (TCF1) 2
PCTK1	PCTAIRE protein kinase 1
PCTP	phosphatidylcholine transfer protein
PDCD4	programmed cell death 4 (neoplastic transformation inhibitor)
PDS5A	PDS5, regulator of cohesion maintenance, homolog A (S. cerevisiae)
PDXDC1	pyridoxal-dependent decarboxylase domain containing 1
PDXK	pyridoxal (pyridoxine, vitamin B6) kinase
PERP	PERP, TP53 apoptosis effector

PHTF2	putative homeodomain transcription factor 2
PIK3R2	phosphoinositide-3-kinase, regulatory subunit 2 (p85 beta)
PLK4	polo-like kinase 4 (Drosophila)
PLS1	plastin 1 (I isoform)
POLR1B	polymerase (RNA) I polypeptide B, 128kDa
PPAPDC1B	phosphatidic acid phosphatase type 2 domain containing 1B
PPAT	phosphoribosyl pyrophosphate amidotransferase
PPFIBP1	PTPRF interacting protein, binding protein 1 (liprin beta 1)
PPIL1	peptidylprolyl isomerase (cyclophilin)-like 1
PPM1B	protein phosphatase 1B (formerly 2C), magnesium-dependent, beta isoforms
PPME1	protein phosphatase methylesterase 1
PPP1R11	protein phosphatase 1, regulatory (inhibitor) subunit 11
PPP1R7	protein phosphatase 1, regulatory (inhibitor) subunit 7
PPP3CB	protein phosphatase 3 (formerly 2B), catalytic subunit, beta isoforms
PREP	Prolyl endopeptidase
PRKACB	protein kinase, cAMP-dependent, catalytic, beta
PRMT1	protein arginine methyltransferase 1
PRMT3	protein arginine methyltransferase 3
PRPS2	phosphoribosyl pyrophosphate synthetase 2
PRSS16	protease, serine, 16 (thymus)
PSMF1	proteasome (prosome, macropain) inhibitor subunit 1 (PI31)
PTPRJ	protein tyrosine phosphatase, receptor type, J
PTRH1	peptidyl-tRNA hydrolase 1 homolog (S. cerevisiae)
PYCR1	pyrroline-5-carboxylate reductase 1
R3HDM1	R3H domain containing 1
RAB31	RAB31, member RAS oncogene family
RABEP1	rabaptin, RAB GTPase binding effector protein 1
RACGAP1	Rac GTPase activating protein 1
RAD51L3	RAD51-like 3 (S. cerevisiae)
RAP1GAP	RAP1 GTPase activating protein
RAP1GDS1	RAP1, GTP-GDP dissociation stimulator 1
RB1	retinoblastoma 1 (including osteosarcoma)
RBBP4	retinoblastoma binding protein 4
RBM12	RNA binding motif protein 12
RBM13	RNA binding motif protein 13
RBM23	RNA binding motif protein 23
RCHY1	ring finger and CHY zinc finger domain containing 1
RFC5	replication factor C (activator 1) 5, 36.5kDa
RIC8A	resistance to inhibitors of cholinesterase 8 homolog A (C. elegans)
RNF130	ring finger protein 130
RNF216	ring finger protein 216
RNF24	ring finger protein 24
RNF4	ring finger protein 4
RNPS1	RNA binding protein S1, serine-rich domain
RRAGC	Ras-related GTP binding C
RRN3	RRN3 RNA polymerase I transcription factor homolog (S. cerevisiae)
RTN4	reticulon 4
SAFB2	scaffold attachment factor B2

SCAMP3	secretory carrier membrane protein 3
SCAMP4	secretory carrier membrane protein 4
SCD	stearoyl-CoA desaturase (delta-9-desaturase)
SCFD2	sec1 family domain containing 2
SCMH1	sex comb on midleg homolog 1 (Drosophila)
SCOTIN	Scotin
SDC1	syndecan 1
SDCCAG8	serologically defined colon cancer antigen 8
SDHB	succinate dehydrogenase complex, subunit B, iron sulfur (Ip)
SERBP1	SERPINE1 mRNA binding protein 1
SET	SET translocation (myeloid leukemia-associated)
SFRS6	splicing factor, arginine/serine-rich 6
SFXN1	sideroflexin 1
SFXN2	sideroflexin 2
SHCBP1	SHC SH2-domain binding protein 1
SIAH2	seven in absentia homolog 2 (Drosophila)
SLC15A4	Solute carrier family 15, member 4
SLC1A4	solute carrier family 1 (glutamate/neutral amino acid transporter), member 4
SLC24A6	solute carrier family 24 (sodium/potassium/calcium exchanger), member 6
SLC25A17	solute carrier family 25 (mitochondrial carrier; peroxisomal membrane protein, 34kDa), member 17
SLC25A36	solute carrier family 25, member 36
SLC29A1	solute carrier family 29 (nucleoside transporters), member 1
SLC30A6	solute carrier family 30 (zinc transporter), member 6
SLC35E3	solute carrier family 35, member E3
SLC39A9	solute carrier family 39 (zinc transporter), member 9
SLK	STE20-like kinase (yeast)
SMARCAD1	SWI/SNF-related, matrix-associated actin-dependent regulator of chromatin, subfamily a, containing DEAD/H box 1
SNRPB2	small nuclear ribonucleoprotein polypeptide B"
SNX10	sorting nexin 10
SNX6	sorting nexin 6
SNX9	sorting nexin 9
SOCS4	suppressor of cytokine signaling 4
SPC24	SPC24, NDC80 kinetochore complex component, homolog (S. cerevisiae)
SPCS3	signal peptidase complex subunit 3 homolog (S. cerevisiae)
SPNS1	spinster homolog 1 (Drosophila)
SSR2	signal sequence receptor, beta (translocon-associated protein beta)
STK40	serine/threonine kinase 40
TARDBP	TAR DNA binding protein
TAX1BP3	Tax1 (human T-cell leukemia virus type I) binding protein 3
TBC1D16	TBC1 domain family, member 16
TBCEL	tubulin folding cofactor E-like
TBRG1	transforming growth factor beta regulator 1
TCFL5	transcription factor-like 5 (basic helix-loop-helix)
TDP1	tyrosyl-DNA phosphodiesterase 1
TGS1	trimethylguanosine synthase homolog (S. cerevisiae)

THAP2	THAP domain containing, apoptosis associated protein 2
THAP6	THAP domain containing 6
TICAM2	toll-like receptor adaptor molecule 2
TIMM8A	translocase of inner mitochondrial membrane 8 homolog A (yeast)
TIPRL	TIP41, TOR signaling pathway regulator-like (S. cerevisiae)
TK1	thymidine kinase 1, soluble
TMED8	transmembrane emp24 protein transport domain containing 8
TMEM127	transmembrane protein 127
TMEM184B	transmembrane protein 184B
TMEM185A	transmembrane protein 185A
TMEM201	transmembrane protein 201
TMEM65	transmembrane protein 65
TNPO1	transportin 1
TOM1L2	target of myb1-like 2 (chicken)
TOR2A	torsin family 2, member A
TRDMT1	tRNA aspartic acid methyltransferase 1
TRIM69	tripartite motif-containing 69
TRMT11	tRNA methyltransferase 11 homolog (S. cerevisiae)
TSGA14	testis specific, 14
TSHZ1	teashirt zinc finger homeobox 1
TTL	tubulin tyrosine ligase
TTLL12	tubulin tyrosine ligase-like family, member 12
TXN2	thioredoxin 2
TYMS	thymidylate synthetase
UBASH3B	ubiquitin associated and SH3 domain containing, B
UBE2B	ubiquitin-conjugating enzyme E2B (RAD6 homolog)
UBE2E1	ubiquitin-conjugating enzyme E2E 1 (UBC4/5 homolog, yeast)
UBE2G1	ubiquitin-conjugating enzyme E2G 1 (UBC7 homolog, yeast)
UBE2G2	ubiquitin-conjugating enzyme E2G 2 (UBC7 homolog, yeast)
UBE2N	ubiquitin-conjugating enzyme E2N (UBC13 homolog, yeast)
UBE2Q2	ubiquitin-conjugating enzyme E2Q (putative) 2
UBE3A	ubiquitin protein ligase E3A (human papilloma virus E6-associated protein, Angelman syndrome)
UCP2	uncoupling protein 2 (mitochondrial, proton carrier)
UNC13B	unc-13 homolog B (C. elegans)
USP46	ubiquitin specific peptidase 46
VCP	valosin-containing protein
VDAC3	voltage-dependent anion channel 3
VRK3	vaccinia related kinase 3
VTI1B	vesicle transport through interaction with t-SNAREs homolog 1B (yeast)
WDFY1	WD repeat and FYVE domain containing 1
WDR37	WD repeat domain 37
WDR5	WD repeat domain 5
WDR82	WD repeat domain 82
WIZ	widely interspaced zinc finger motifs
WSB2	WD repeat and SOCS box-containing 2
XPO6	exportin 6
YES1	v-yes-1 Yamaguchi sarcoma viral oncogene homolog 1

YWHAH	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta polypeptide
ZC3H13	zinc finger CCCH-type containing 13
ZDHHC16	zinc finger, DHHC-type containing 16
ZDHHC18	zinc finger, DHHC-type containing 18
ZDHHC23	zinc finger, DHHC-type containing 23
ZFP41	zinc finger protein 41 homolog (mouse)
ZNF286A	zinc finger protein 286A
ZNF586	zinc finger protein 586
ZWILCH	Zwilch, kinetochore associated, homolog (Drosophila)
ADD3	adducin 3 (gamma)
ADH5	alcohol dehydrogenase 5 (class III), chi polypeptide
ADORA2B	adenosine A2b receptor
ADRBK2	adrenergic, beta, receptor kinase 2
AK2	adenylate kinase 2
AKR1A1	aldo-keto reductase family 1, member A1 (aldehyde reductase)
ALDH5A1	aldehyde dehydrogenase 5 family, member A1 (succinate-semialdehyde dehydrogenase)
ANKRD6	ankyrin repeat domain 6
AP1S2	adaptor-related protein complex 1, sigma 2 subunit
APPBP2	amyloid beta precursor protein (cytoplasmic tail) binding protein 2
BARD1	BRCA1 associated RING domain 1
BCL11B	B-cell CLL/lymphoma 11B (zinc finger protein)
C11orf41	chromosome 11 open reading frame 41
C13orf23	chromosome 13 open reading frame 23
C14orf133	chromosome 14 open reading frame 133
C1orf19	chromosome 1 open reading frame 19
C1orf21	chromosome 1 open reading frame 21
C5orf25	chromosome 5 open reading frame 25
CAMK2D	Calcium/calmodulin-dependent protein kinase (CaM kinase) II delta
CCDC88C	coiled-coil domain containing 88C
CDCA7	cell division cycle associated 7
CDCA7L	cell division cycle associated 7-like
CDK6	cyclin-dependent kinase 6
CNIH4	cornichon homolog 4 (Drosophila)
COL4A2	collagen, type IV, alpha 2
COX7A2L	cytochrome c oxidase subunit VIIa polypeptide 2 like
CUL4B	cullin 4B
CYP2S1	cytochrome P450, family 2, subfamily S, polypeptide 1
DARS	aspartyl-tRNA synthetase
DDHD2	DDHD domain containing 2
DGKA	diacylglycerol kinase, alpha 80kDa
DRAM	damage-regulated autophagy modulator
EEF2K	eukaryotic elongation factor-2 kinase
EHBP1	EH domain binding protein 1
EIF2S2	eukaryotic translation initiation factor 2, subunit 2 beta, 38kDa
EIF2S3	eukaryotic translation initiation factor 2, subunit 3 gamma, 52kDa
ERAP1	endoplasmic reticulum aminopeptidase 1
ERLIN1	ER lipid raft associated 1

EXO1	exonuclease 1
FADS1	fatty acid desaturase 1
FAM149B1	Family with sequence similarity 149, member B1
FAM44B	family with sequence similarity 44, member B
FAM86B1	family with sequence similarity 86, member B1
FAM86C	family with sequence similarity 86, member C
FANCI	Fanconi anemia, complementation group I
FBXO45	F-box protein 45
FERMT2	fermitin family homolog 2 (Drosophila)
FOXF2	forkhead box F2
FTO	fat mass and obesity associated
GNPAT	glyceronephosphate O-acyltransferase
GRB14	growth factor receptor-bound protein 14
H1F0	H1 histone family, member 0
HACE1	HECT domain and ankyrin repeat containing, E3 ubiquitin protein ligase 1
HMG20A	high-mobility group 20A
HNRNPA2B1	heterogeneous nuclear ribonucleoprotein A2/B1
IAH1	isoamyl acetate-hydrolyzing esterase 1 homolog (S. cerevisiae)
IARS	isoleucyl-tRNA synthetase
ID4	inhibitor of DNA binding 4, dominant negative helix-loop-helix protein
IFRD1	interferon-related developmental regulator 1
IMPDH2	IMP (inosine monophosphate) dehydrogenase 2
IQGAP3	IQ motif containing GTPase activating protein 3
IRX3	iroquois homeobox 3
ISCA2	iron-sulfur cluster assembly 2 homolog (S. cerevisiae)
KATNB1	katanin p80 (WD repeat containing) subunit B 1
KIAA1430	KIAA1430
KIAA1804	mixed lineage kinase 4
KLHL12	kelch-like 12 (Drosophila)
LAMC1	laminin, gamma 1 (formerly LAMB2)
LOC286167	hypothetical protein LOC286167
LOC374443	CLR pseudogene
MAOA	monoamine oxidase A
MDC1	mediator of DNA damage checkpoint 1
MGC16169	hypothetical protein MGC16169
MICAL2	microtubule associated monoxygenase, calponin and LIM domain containing 2
MID1IP1	MID1 interacting protein 1 (gastrulation specific G12 homolog (zebrafish))
MRPL10	mitochondrial ribosomal protein L10
MRPS27	mitochondrial ribosomal protein S27
MYLK	myosin, light chain kinase
NCOR2	nuclear receptor co-repressor 2
PBK	PDZ binding kinase
PDCD4	programmed cell death 4 (neoplastic transformation inhibitor)
PODXL	podocalyxin-like
PRKACB	protein kinase, cAMP-dependent, catalytic, beta
PRKAR2B	protein kinase, cAMP-dependent, regulatory, type II, beta
PRPS2	Phosphoribosyl pyrophosphate synthetase 2

PRSS16	protease, serine, 16 (thymus)
PYCR1	pyrroline-5-carboxylate reductase 1
RAD51L1	RAD51-like 1 (S. cerevisiae)
RBBP4	retinoblastoma binding protein 4
RGS2	regulator of G-protein signaling 2, 24kDa
RHBDD1	rhomboid domain containing 1
RRAS	related RAS viral (r-ras) oncogene homolog
SCD	stearoyl-CoA desaturase (delta-9-desaturase)
SCMH1	sex comb on midleg homolog 1 (Drosophila)
SDC1	syndecan 1
SIAH2	seven in absentia homolog 2 (Drosophila)
SLC1A4	solute carrier family 1 (glutamate/neutral amino acid transporter), member 4
SLC25A36	solute carrier family 25, member 36
SNHG7	small nucleolar RNA host gene (non-protein coding) 7
SNX1	sorting nexin 1
ST6GALNAC 4	ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgalactosa minide alpha-2,6-sialyltransferase 4
SUCLG1	succinate-CoA ligase, alpha subunit
SUPT3H	suppressor of Ty 3 homolog (S. cerevisiae)
TAS2R14	taste receptor, type 2, member 14
TBC1D16	TBC1 domain family, member 16
TCFL5	transcription factor-like 5 (basic helix-loop-helix)
TDP1	tyrosyl-DNA phosphodiesterase 1
TIPRL	TIP41, TOR signaling pathway regulator-like (S. cerevisiae)
TMED8	transmembrane emp24 protein transport domain containing 8
TSGA14	testis specific, 14
TSHZ1	teashirt zinc finger homeobox 1
UBASH3B	ubiquitin associated and SH3 domain containing, B
UBE2T	ubiquitin-conjugating enzyme E2T (putative)
YARS	tyrosyl-tRNA synthetase
ZNF573	zinc finger protein 573

[0323]

[Table 10]

The down stream genes of PRMT6

Gene Symbol	Gene Title
UP-regulated	
ATF3	activating transcription factor 3
BAG2	BCL2-associated athanogene 2
BAK1	BCL2-antagonist/killer 1
ETS1	v-ets erythroblastosis virus E26 oncogene homolog 1 (avian)
JARID1B	jumonji, AT rich interactive domain 1B
LYN	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog
MAP3K7	mitogen-activated protein kinase kinase kinase 7
RAB5C	RAB5C, member RAS oncogene family
Down-regulated	
BNIP3L	BCL2/adenovirus E1B 19kDa interacting protein 3-like
CASC5	cancer susceptibility candidate 5
CASP9	caspase 9, apoptosis-related cysteine peptidase
CCNA2	cyclin A2
CCNC	cyclin C
CDC20	cell division cycle 20 homolog (S. cerevisiae)
CDC2L6	cell division cycle 2-like 6 (CDK8-like)
CDC37	cell division cycle 37 homolog (S. cerevisiae)
CDK2	cyclin-dependent kinase 2
CRK	v-crk sarcoma virus CT10 oncogene homolog (avian)
DEPDC1	DEP domain containing 1
H2AFV	H2A histone family, member V
HIF1A	hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)
HIG2	hypoxia-inducible protein 2
HIST1H4C	histone cluster 1, H4c
HMG20A	high-mobility group 20A
HMGB3	high-mobility group box 3
IQGAP1	IQ motif containing GTPase activating protein 1
JARID1C	jumonji, AT rich interactive domain 1C
MAPK14	mitogen-activated protein kinase 14
PBX2	pre-B-cell leukemia homeobox 2
PCNA	proliferating cell nuclear antigen
PRCC	papillary renal cell carcinoma (translocation-associated)
PRMT6	protein arginine methyltransferase 6
PTK2	PTK2 protein tyrosine kinase 2
RABIF	RAB interacting factor
RAP2B	RAP2B, member of RAS oncogene family
RBBP4	retinoblastoma binding protein 4
RHOU	ras homolog gene family, member U
SETD7	SET domain containing (lysine methyltransferase) 7
SMEK1	SMEK homolog 1, suppressor of mek1 (Dictyostelium)
SUV39H1	suppressor of variegation 3-9 homolog 1 (Drosophila)

TBC1D7	TBC1 domain family, member 7
TGFBR3	Transforming growth factor, beta receptor III
TIAM1	T-cell lymphoma invasion and metastasis 1
WHSC1	Wolf-Hirschhorn syndrome candidate 1

Claims

[Claim 1] A method of detecting or diagnosing cancer in a subject, comprising determining an expression level of a PRMT6 gene in a subject-derived biological sample, wherein an increase of said level compared to a normal control level of said gene indicates that said subject suffers from or is at risk of developing cancer, wherein the expression level is determined by a method selected from the group consisting of:
(a) detecting the mRNA of the PRMT6 gene;
(b) detecting the protein encoded by the PRMT6 gene; and
(c) detecting the biological activity of the protein encoded by the PRMT6 gene.

[Claim 2] The method of claim 1, wherein said increase is at least 10% greater than said normal control level.

[Claim 3] The method of claim 1, wherein the subject-derived biological sample is a biopsy.

[Claim 4] A kit for diagnosing cancer, which comprises a reagent selected from the group consisting of:
(a) a reagent for detecting mRNA of the PRMT6 gene;
(b) a reagent for detecting the protein encoded by the PRMT6 gene; and
(c) a reagent for detecting the biological activity of the protein encoded by the PRMT6 gene.

[Claim 5] The kit of claim 4, wherein the reagent is a probe to a gene transcript of PRMT6.

[Claim 6] The kit of claim 4, wherein the reagent is an antibody against the protein encoded by the PRMT6 gene.

[Claim 7] A method of detecting or diagnosing cancer in a subject, comprising determining an ADMA (asymmetric dimethylarginine) level in a subject-derived biological sample, wherein an increase of said level compared to a normal control level indicates that said subject suffers from or is at risk of developing cancer.

[Claim 8] The method of claim 7, wherein said increase is at least 10% greater than said normal control level.

[Claim 9] The method of claim 7, wherein the subject-derived biological sample is blood sample.

[Claim 10] The method of claim 9, wherein the blood sample is selected from the group consisting of whole blood, serum and plasma.

[Claim 11] The method of claim 10, wherein the ADMA is detected by immunoassay.

[Claim 12] The method of claim 11, wherein the immunoassay is an ELISA.

[Claim 13] A kit for diagnosing cancer, which comprises an ADMA detecting reagent.

[Claim 14] The kit of claim 13, wherein the reagent is an antibody against ADMA.

[Claim 15] A method of screening for a candidate substance for treating or preventing cancer or inhibiting cancer cell growth, said method comprising the steps of:
(a) contacting a test substance with a polypeptide encoded by the PRMT6 gene;
(b) detecting the binding activity between the polypeptide and the test substance; and
(c) selecting the test substance that binds to the polypeptide.

[Claim 16] A method of screening for a candidate substance for treating or preventing cancer or inhibiting cancer cell growth, said method comprising the steps of:
(a) contacting a test substance with a cell expressing the PRMT6 gene; and
(b) selecting the test substance that reduces the expression level of the PRMT6 gene in comparison with the expression level in the absence of the test compound.

[Claim 17] A method of screening for a candidate substance for treating or preventing cancer or inhibiting cancer cell growth, said method comprising the steps of:
(a) contacting a test substance with a polypeptide encoded by the PRMT6 gene;
(b) detecting the biological activity of the polypeptide of step (a); and
(c) selecting the test substance that suppresses the biological activity of the polypeptide in comparison with the biological activity detected in the absence of the test substance.

[Claim 18] The method of claim 17, wherein the biological activity is cell proliferative activity or methyltransferase activity.

[Claim 19] A method of screening for a candidate substance for treating or preventing cancer or inhibiting cancer cell growth, said method comprising the steps of:
a) contacting a test substance with a cell into which a vector comprising the transcriptional regulatory region of the PRMT6 gene and a reporter

gene that is expressed under the control of the transcriptional regulatory region has been introduced,

b) measuring the expression or activity of said reporter gene; and

c) selecting the test substance that reduces the expression or activity level of said reporter gene, as compared to a level in the absence of the test substance.

[Claim 20] A method of screening for a candidate substance for treating or preventing cancer, said method comprising steps of:

(a) contacting at least one of binding protein selected from group consisting of SRRT polypeptide, EIF4B polypeptide, HNRNPK polypeptide, SERBP1 polypeptide and functional equivalent thereof with a PRMT1 polypeptide or functional equivalent thereof in the presence of a test substance;

(b) detecting the binding between the polypeptides of step (a); and

(c) selecting the test substance that inhibits the binding between the binding protein and the PRMT1 polypeptide.

[Claim 21] A method of screening for a candidate substance for treating or preventing cancer, said method comprising steps of:

(a) contacting at least one of binding protein selected from the group consisting of MSH2 polypeptide, EIF4B polypeptide, HNRNPK polypeptide, RUVBL1 polypeptide, EEF1A1 polypeptide, HNRNPD polypeptide and functional equivalent thereof with a PRMT6 polypeptide or functional equivalent thereof in the presence of a test substance;

(b) detecting the binding between the polypeptides of step (a); and

(c) selecting the test substance that inhibits the binding between the the binding porotein and the PRMT6 polypeptide.

[Claim 22] A method of screening for a candidate substance for treating or preventing cancer, said method comprising the steps of:

(a) contacting a test substance with a cell expressing PRMT1 and a downstream gene of PRMT1; and

(b) selecting the substance that reduces the expression level of a downstream gene of PRMT1 in comparison with the expression level detected in the absence of the test substance.

[Claim 23] The method of claim 21, wherein the downstream gene of PRMT1 is selected from the group consisting of the genes described in Table 9.

[Claim 24] A method of screening for a candidate substance for treating or preventing cancer, said method comprising the steps of:

(a) contacting a test substance with a cell expressing PRMT6 and a downstream gene of PRMT6; and
(b) selecting the substance that reduces expression level of a downstream gene of PRMT6 in comparison with the expression level detected in the absence of the test substance.

[Claim 25] The method of claim 24, wherein the downstream gene of PRMT6 is selected from the group consisting of the genes described in Table 10.

[Claim 26] A double-stranded molecule comprising a sense strand and an antisense strand, wherein the sense strand comprises a nucleotide sequence corresponding to a target sequence consisting of SEQ ID NO: 29, and wherein the antisense strand comprises a nucleotide sequence which is complementary to said sense strand, wherein said sense strand and said antisense strand hybridize to each other to form said double-stranded molecule, and wherein said double-stranded molecule, when introduced into a cell expressing the PRMT1 gene, inhibits expression of said gene.

[Claim 27] A double-stranded molecule comprising a sense strand and an antisense strand, wherein the sense strand comprises a nucleotide sequence corresponding to a target sequence consisting of SEQ ID NO: 35 or 38, and wherein the antisense strand comprises a nucleotide sequence which is complementary to said sense strand, wherein said sense strand and said antisense strand hybridize to each other to form said double-stranded molecule, and wherein said double-stranded molecule, when introduced into a cell expressing the PRMT6 gene, inhibits expression of said gene.

[Claim 28] The double-stranded molecule of claim 26 or 27, wherein the double-stranded molecule is between about 19 and about 25 nucleotides in length.

[Claim 29] The double-stranded molecule of claim 26 or 27, wherein said double-stranded molecule is a single polynucleotide molecule comprising the sense strand and the antisense strand linked via a single-stranded nucleotide sequence.

[Claim 30] The double-stranded molecule of claim 29, wherein said polynucleotide has the general formula
5'-[A]-[B]-[A']-3'
wherein [A] is a sense strand comprising a nucleotide sequence corresponding to a target sequence of SEQ ID NO: 29, 35 or 38; [B] is a nucleotide sequence consisting of about 3 to about 23 nucleotides; and

[A'] is an antisense strand comprising a nucleotide sequence complementary to [A].

[Claim 31] A vector encoding the double-stranded molecule of any one of claims 26 to 30.

[Claim 32] A vector comprising a sense strand nucleic acid and an antisense strand nucleic acid, wherein said sense strand nucleic acid comprises nucleotide sequence selected from the group consisting of SEQ ID NO: 29, 35 or 38, and said antisense strand nucleic acid consists of a sequence complementary to the sense strand, wherein the transcripts of said sense strand and said antisense strand hybridize to each other to form a double-stranded molecule, and wherein said vector inhibits expression of target gene.

[Claim 33] A method of treating or preventing cancer in a subject comprising administering to said subject a pharmaceutically effective amount of a double-stranded molecule directed against a PRMT1 or PRMT6 gene or a vector encoding said double-stranded molecule, and a pharmaceutically acceptable carrier, wherein the double-stranded molecule inhibits cell proliferation as well as the expression of the PRMT1 or PRMT6 gene when introduced into a cell expressing the PRMT1 or PRMT6 gene.

[Claim 34] The method of claim 33, wherein the double-stranded molecule is that of claim 26.

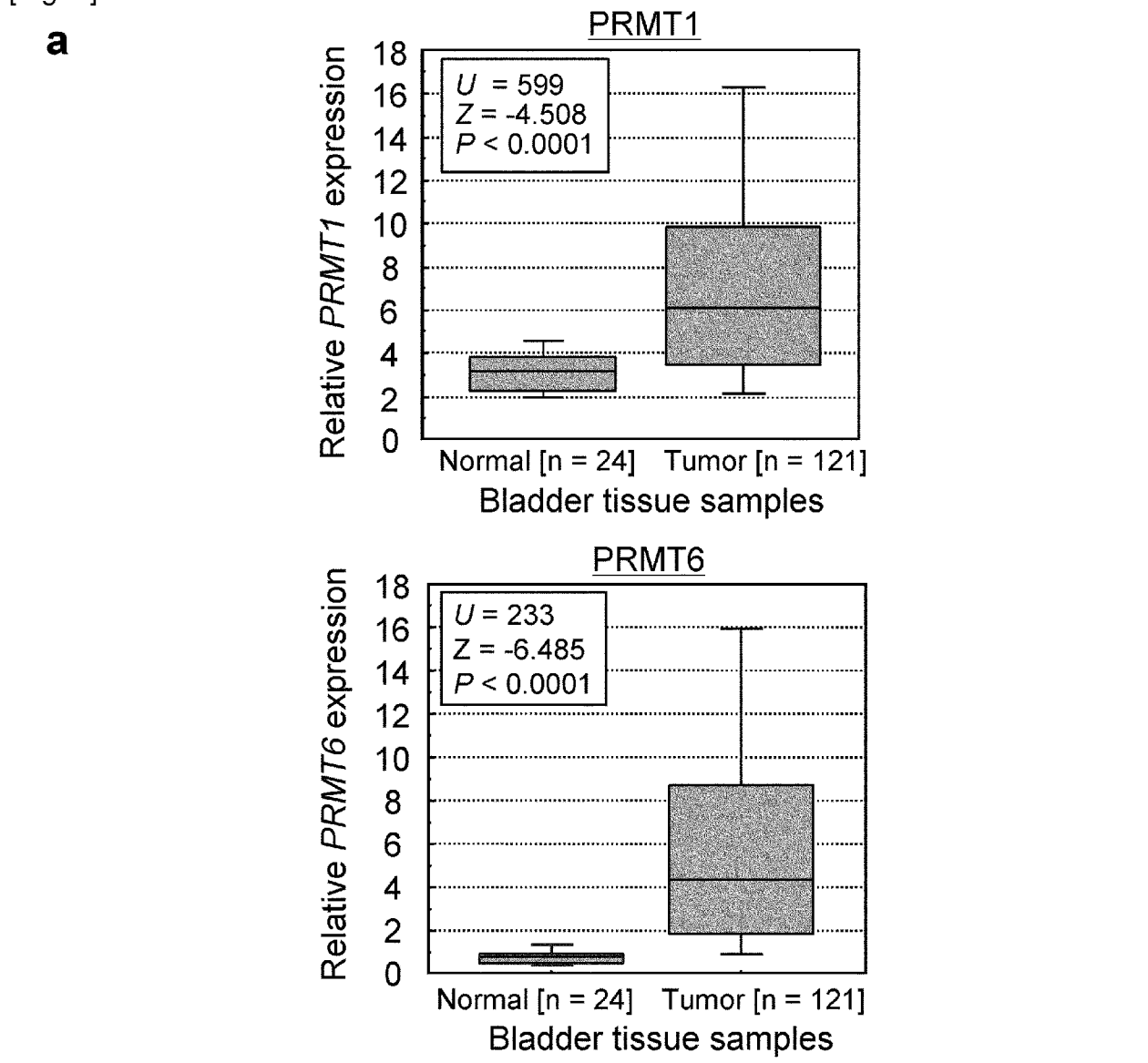
[Claim 35] The method of claim 33, wherein the vector is that of claim 31.

[Claim 36] A composition for treating or preventing cancer, which comprises a pharmaceutically effective amount of a double-stranded molecule directed against a PRMT1 or PRMT6 gene or a vector encoding said double-stranded molecule, and a pharmaceutically acceptable carrier, wherein the double-stranded molecule inhibits cell proliferation as well as the expression of the PRMT1 or PRMT6 gene when introduced into a cell expressing the PRMT1 or PRMT6 gene.

[Claim 37] The composition of claim 36, wherein the double-stranded molecule is that of claim 26.

[Claim 38] The composition of claim 36, wherein the vector is that of claim 31.

[Fig. 1]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PRMT1
Relative PRMT1 expression
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P < 0.0001
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Tumor [n = 121]
Bladder tissue samples
PRMT6
Relative PRMT6 expression
U = 233
Z = -6.485
P < 0.0001
Normal [n = 24]
Tumor [n = 121]
Bladder tissue samples
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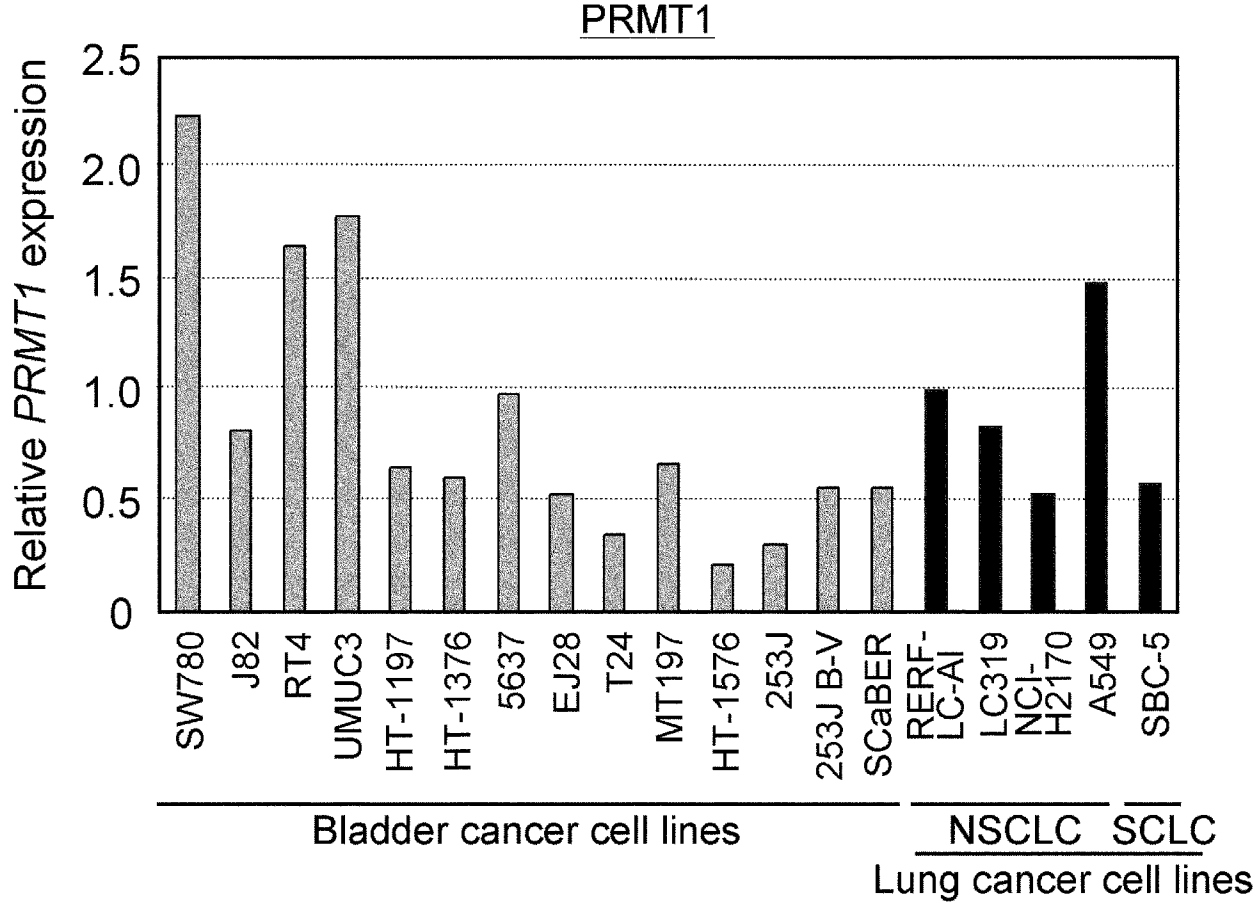
PRMT1
PRMT6
x40
x400
Normal bladder
Bladder tumor

[Fig. 2]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1.0
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HT-1376
5637
EJ28
T24
MT197
HT-1576
253J
253J B-V
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LC319
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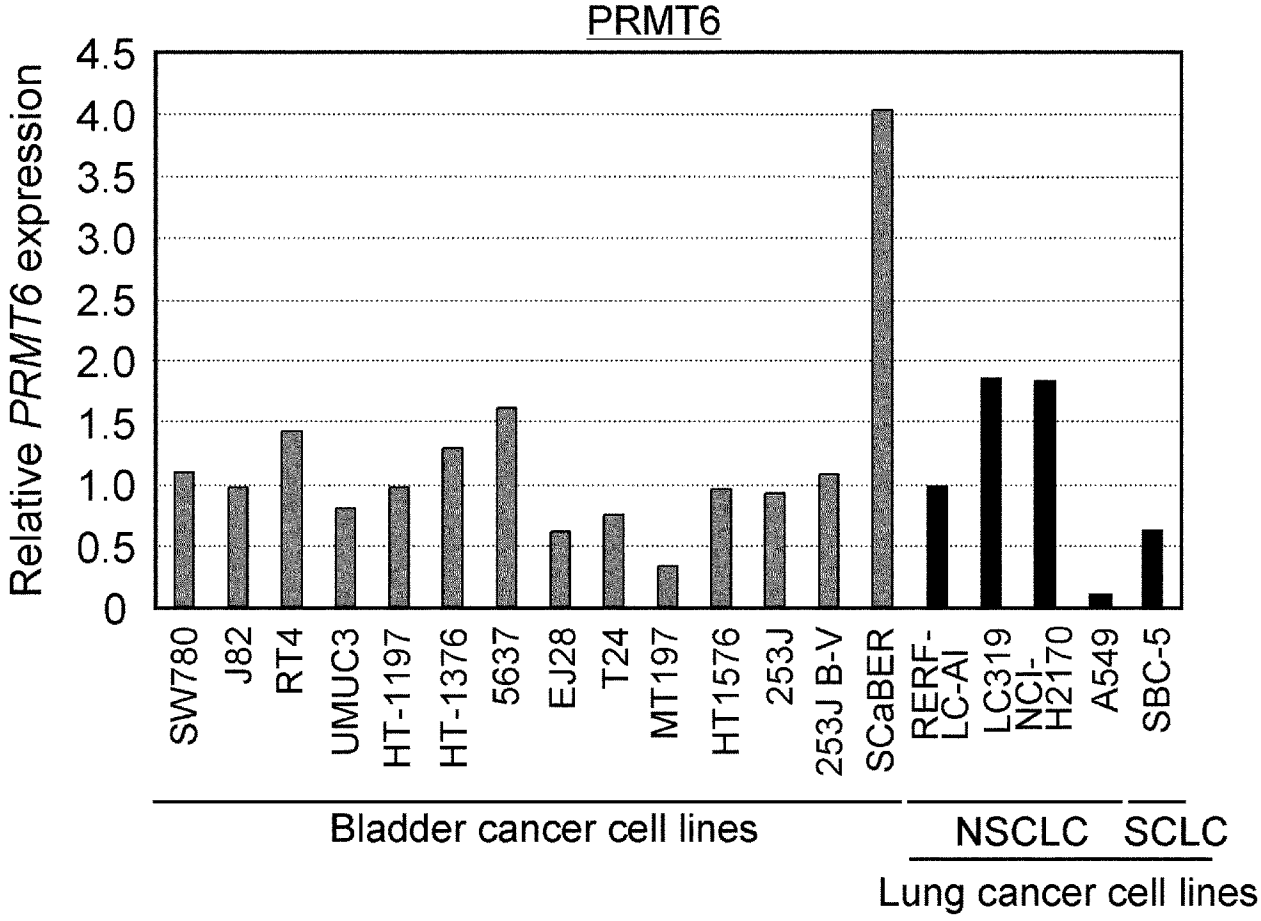
A549
SBC-5
Bladder cancer cell lines
NSCLC
SCLC
Lung cancer cell lines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RT4
UMUC3
HT-1197
HT-1376
5637
EJ28
T24
MT197
HT1576
253J
253J B-V
SCaBER
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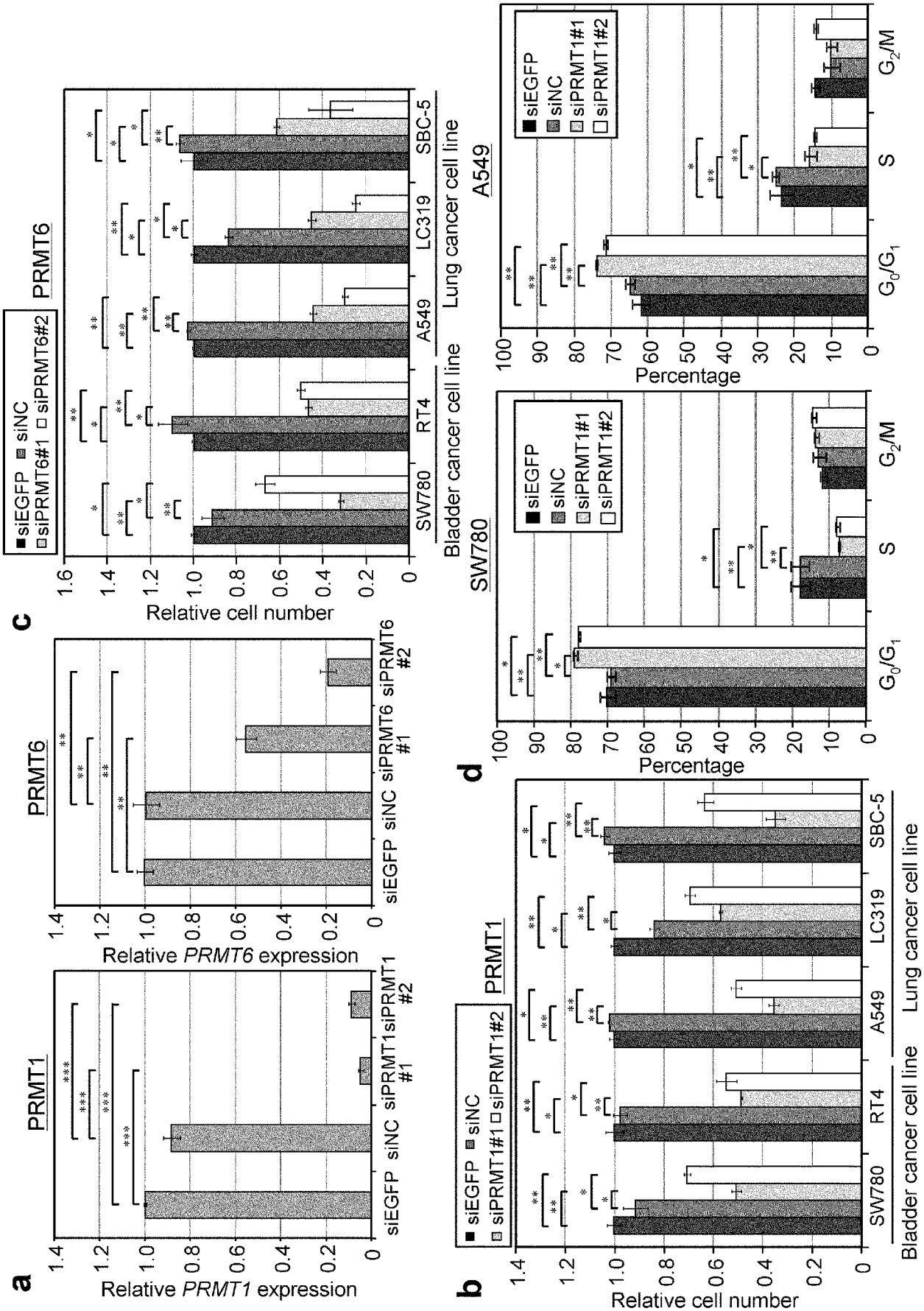
NCI-H2170
A549
SBC-5
Bladder cancer cell lines
NSCLC
SCLC
Lung cancer cell lines

[Fig. 3]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PRMT1
Relative PRMT1 expression
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siNC
siPRMT1 #1
siPRMT1 #2
b
PRMT1
siEGFP
siNC
siPRMT1#1
siPRMT1#2
Relative cell number
SW780
RT4
A549
LC319
SBC-5
Bladder cancer cell line
Lung cancer cell line
c
PRMT6
siPRMT6#1
siPRMT6#2
Relative cell number
d
SW780
A549
Percentage
G0/G1
S
G2/M

Fig. 4

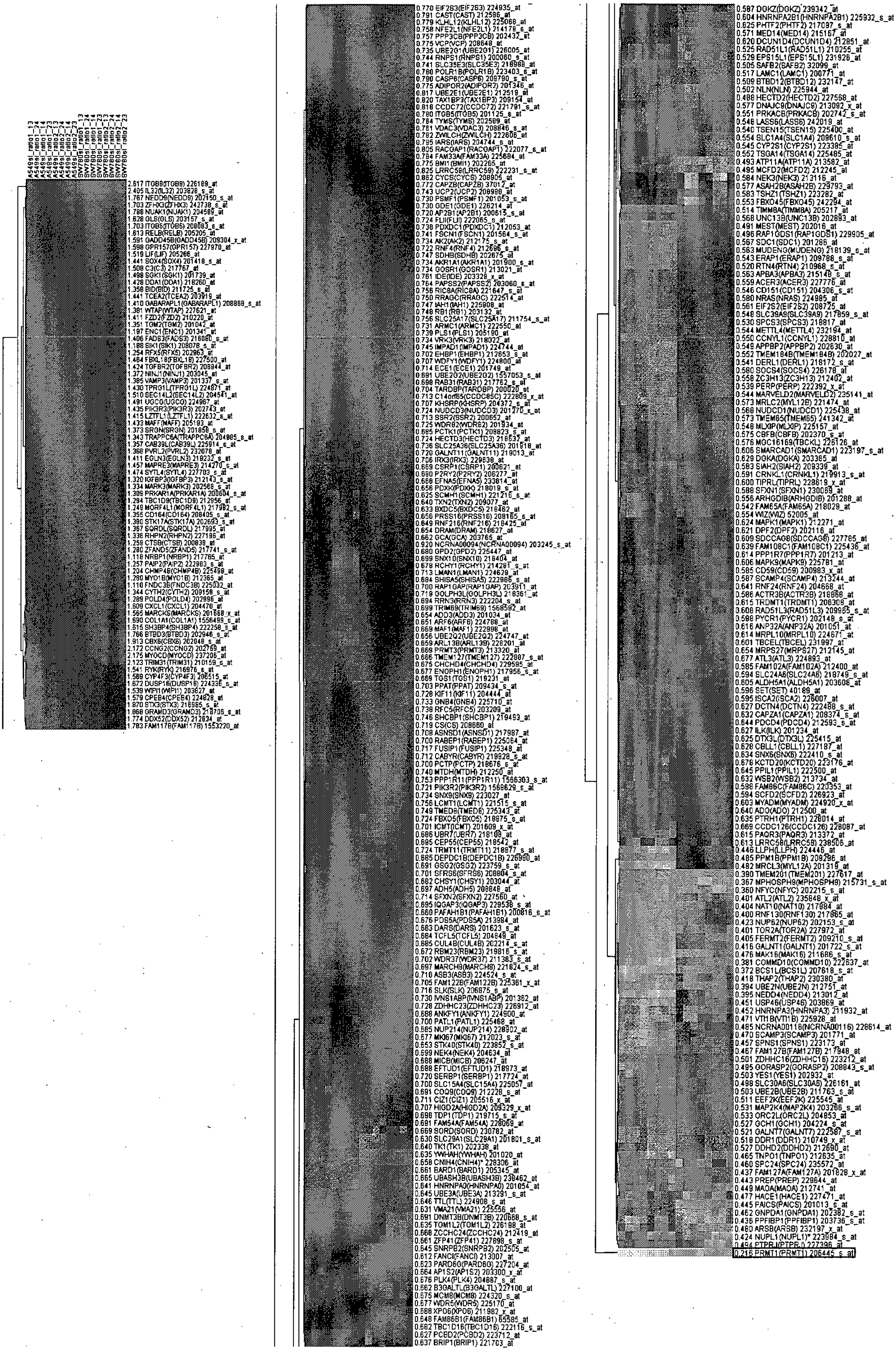
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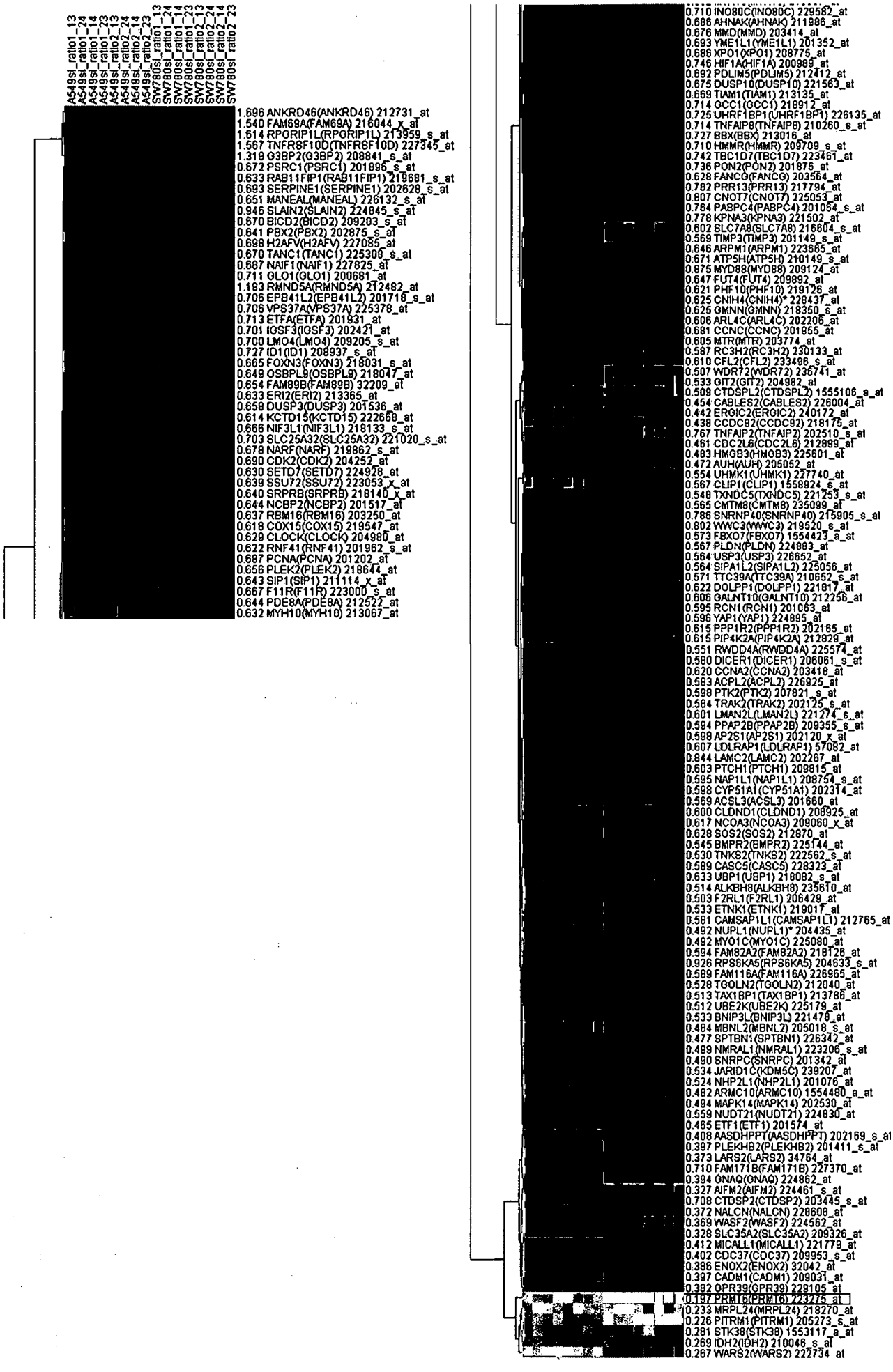
Fig. 5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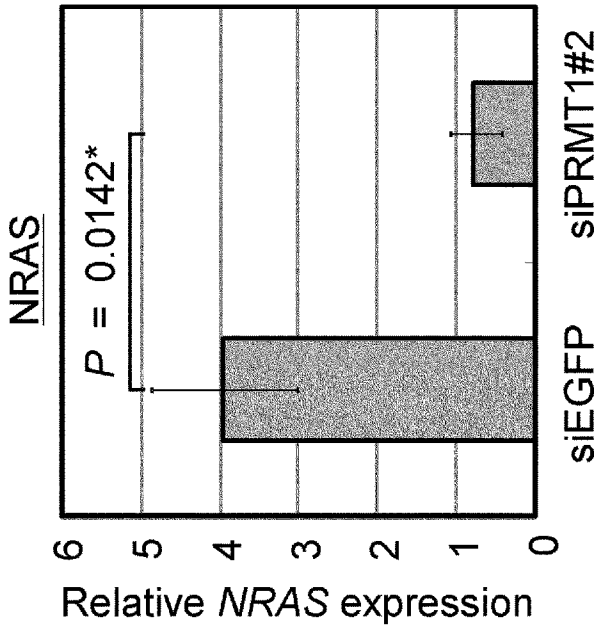
[Fig. 6]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4
5
6
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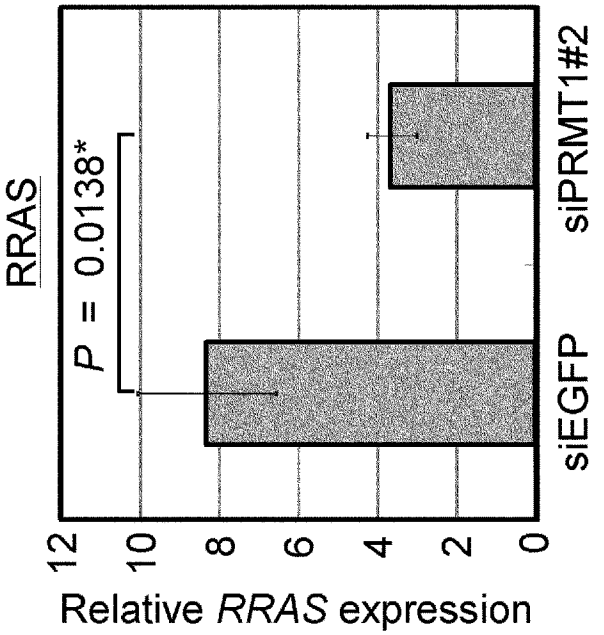
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8
10
12
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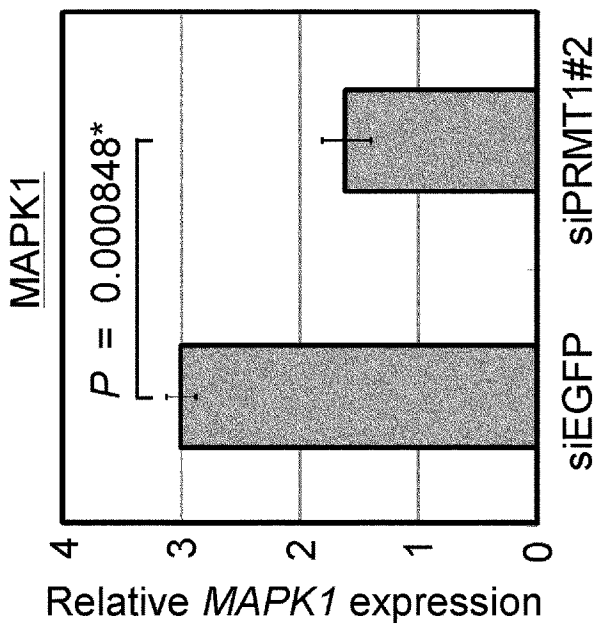
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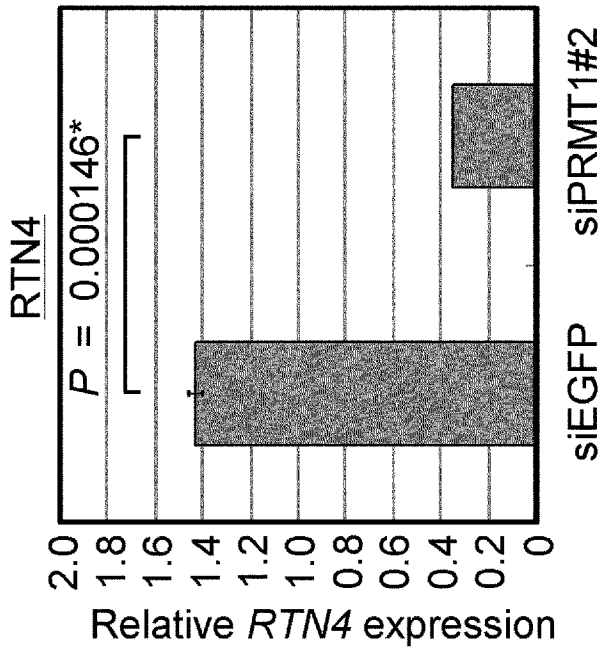
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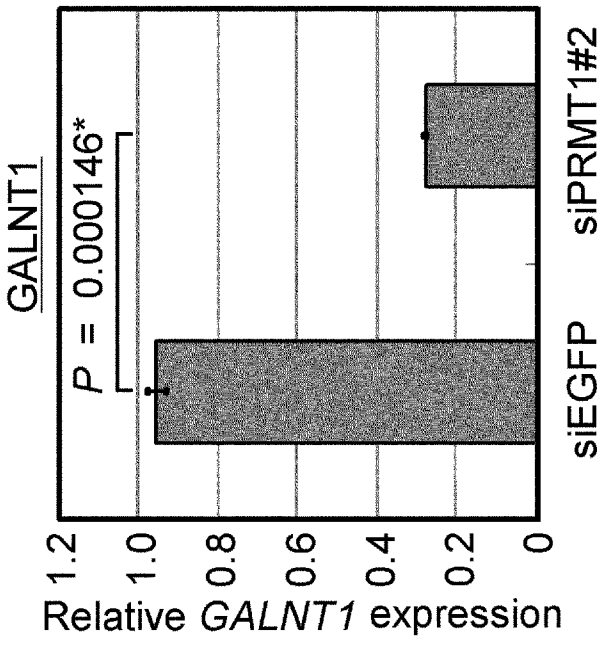
Relative RTN4 expression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0.8
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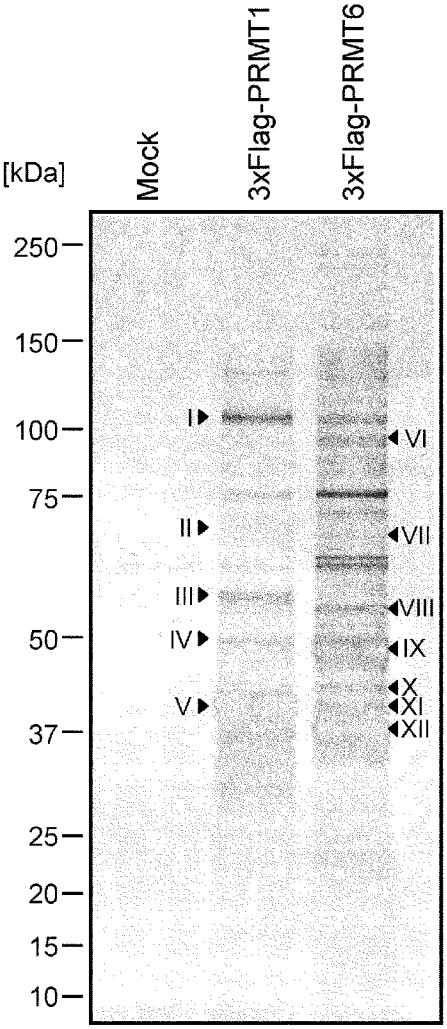
1.2
Relative GALNT1 expression

[Fig. 7]



PRMT1 binding proteins

I: SRRT (serrate RNA effector molecule homolog)

II: EIF4B (eukaryotic translation initiation factor 4B)

III: HNRNPK (heterogenous nuclear ribonucleoprotein K)

IV: SERBP1 (SERPINE1 mRNA binding protein 1)

V: PRMT1

PRMT6 binding proteins

VI: MSH2 (mutS homolog 2, colon cancer, nonpolyposis type 1)

VII: EIF4B (eukaryotic translation initiation factor 4B)

VIII: HNRNPK (heterogenous nuclear ribonucleoprotein K)

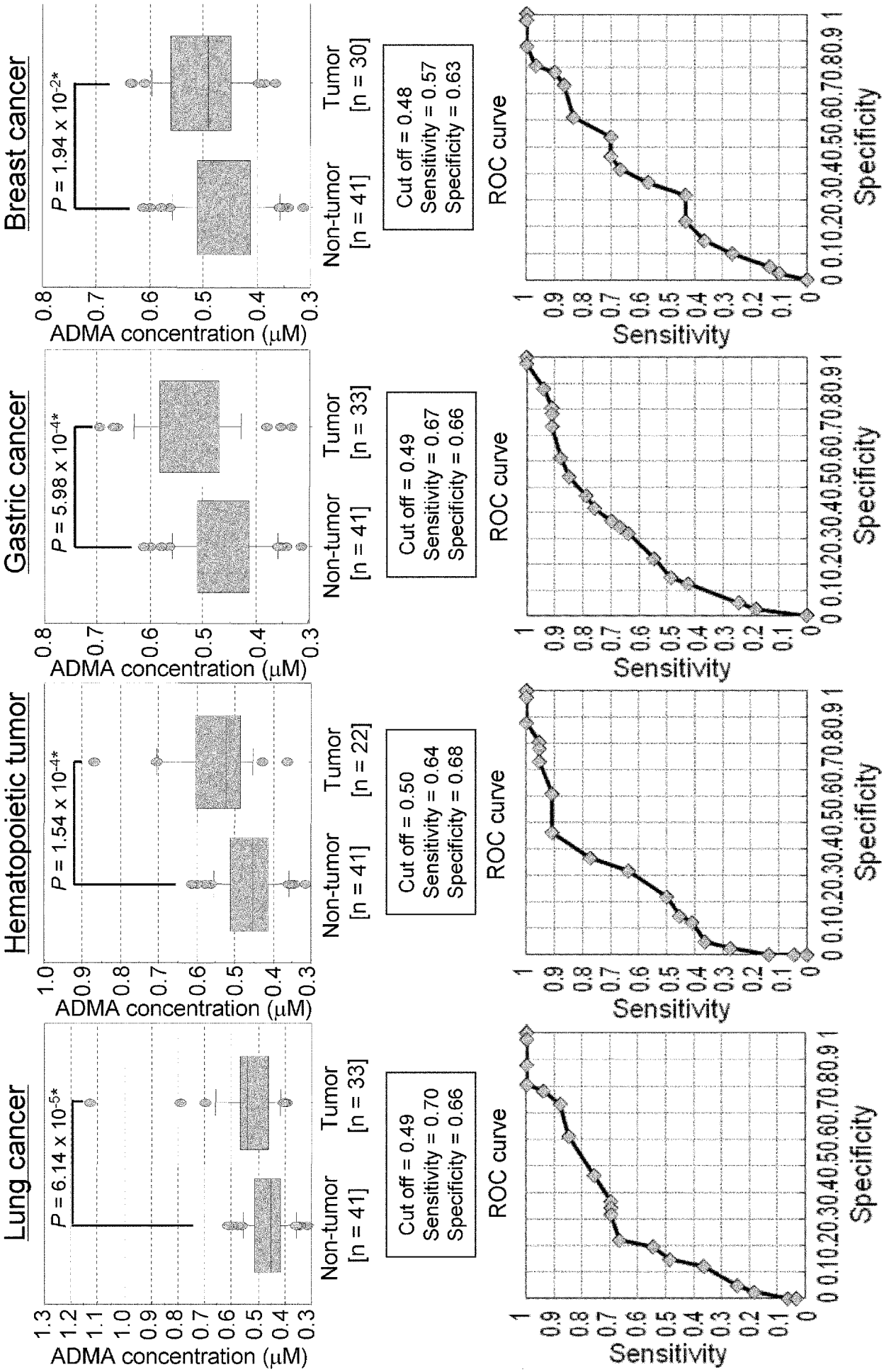
IX: RUVBL1 (RuvB (E.coli homolog)-like 1)

X: EEF1A1 (eukaryotic translation elongation factor 1 alpha 1)

XI: HNRNPD (heterogenous nuclear ribonucleoprotein D)

XII: PRMT6

[Fig. 8]


Breast cancer
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ADMA concentration (μM)
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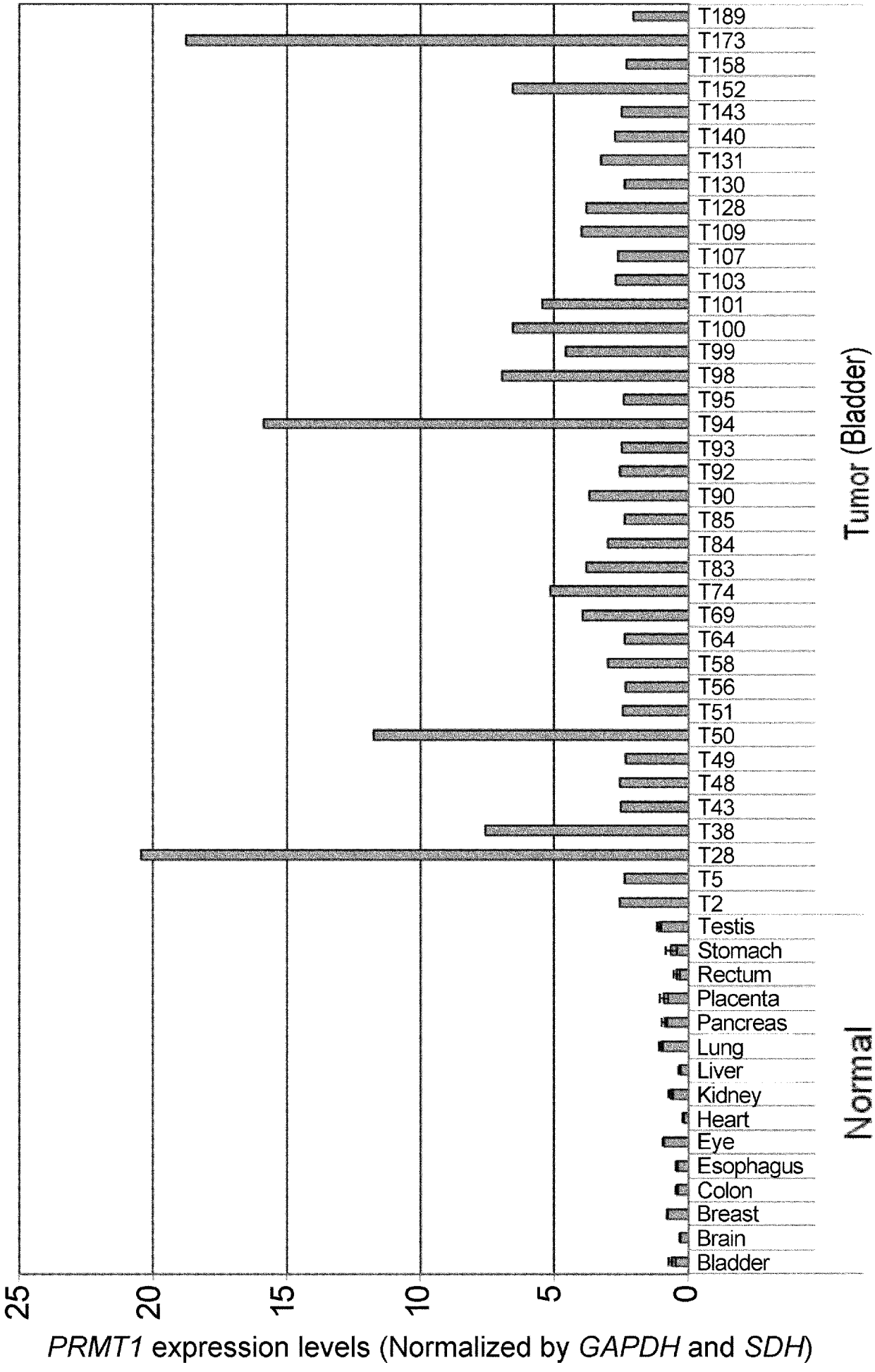
Tumor [n = 30]
Cut off = 0.48
Sensitivity = 0.57
Specificity = 0.63
ROC curve
Sensitivity
Specificity
Gastric cancer
P = 5.98 x 10-4*
ADMA concentration (μM)
Non-tumor [n = 41]
Tumor [n = 33]
Cut off = 0.49
Sensitivity = 0.67
Specificity = 0.66
ROC curve
Sensitivity
Specificity
Hematopoietic tumor
P = 1.54 x 10-4*
ADMA concentration (μM)
Non-tumor [n = 41]
Tumor [n = 22]
Cut off = 0.50
Sensitivity = 0.64
Specificity = 0.68
ROC curve
Sensitivity
Specificity
Lung cancer
P = 6.14 x 10-5*
ADMA concentration (μM)
Non-tumor [n = 41]
Tumor [n = 33]
Cut off = 0.49
Sensitivity = 0.70
Specificity = 0.66
ROC curve
Sensitivity
Specificity

[Fig. 9]


T189
T173
T158
T152
T143
T140
T131
T130
T128
T109
T107
T103
T101
T100
T99
T98
T95
T94
T93
T92
T90
T85
T84
T83
T74
T69
T64
T58
T56
T51
T50
T49
T48
T43
T38
T28
T5
T2
Tumor (Bladder)
Testis
Stomach
Rectum
Placenta
Pancreas
Lung
Liver
Kidney
Heart
Eye
Esophagus
Colon
Breast
Brain
Bladder
Normal
25
20
15
10
5
0
PRMT1 expression levels (Normalized by GAPDH and SDH)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2011/000582

A. CLASSIFICATION OF SUBJECT MATTER

Int.Cl. C12N15/09(2006.01)i, A61K31/713(2006.01)i, A61P35/00(2006.01)i, C12Q1/68(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int.Cl. C12N15/09, A61K31/713, A61P35/00, C12Q1/68

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Published examined utility model applications of Japan 1922-1996
Published unexamined utility model applications of Japan 1971-2011
Registered utility model specifications of Japan 1996-2011
Published registered utility model applications of Japan 1994-2011

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CA/BIOSIS/MEDLINE/WPIDS(STN)
JSTPlus/JMEDPlus/JST7580(JDreamII)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X/ Y/ A	JP 2008-164517 A (Advanced Life Science Institute, Inc.) 2008.07.17, (No Family)	4-6, 15-19,21,24/ 27-32, 36-38/ 25
Y/ A	JP 2008-530974 A (OncoTherapy Science, Inc.) 2008.08.14, & US 2009/0142344 A1 & EP 1866436 A	27-32, 36-38/ 25
Y/ A	JP 2009-502113 A (OncoTherapy Science, Inc.) 2009.01.29, & EP 1920056 A	27-32, 36-38/ 25
P,X	YOSHIMATSU M. et al., Dysregulation of PRMT1 and PRMT6, Type I arginine methyltransferases, is involved in various types of human cancers., International Journal of Cancer, (2011), vol.128, no.3, p.562-573	4-6,15-19,21,24,25 27-32, 36-38

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
07.04.2011	19.04.2011

Name and mailing address of the ISA/JP	Authorized officer
Japan Patent Office 3-4-3, Kasumigaseki, Chiyoda-ku, Tokyo 100-8915, Japan	FUKUMA Nobuko Telephone No. +81-3-3581-1101 Ext. 3448

4B 3539

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2011/000582

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

a. (means)

- ☐ on paper
- ☑ in electronic form

b. (time)

- ☑ in the international application as filed
- ☐ together with the international application in electronic form
- ☐ subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2011/000582

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claims Nos.: 1-3,7-12,33-35
because they relate to subject matter not required to be searched by this Authority, namely:
The subject matter of claims 1-3,7-12,33-35 relate to a method for treatment of the human body by surgery or therapy, which does not require an intentional search by the International Searching Authority in accordance with PCT Article 17(2)(a)(i) and [Rule 39.1(iv)].

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Invention 1: claims 4-6,15-19,21,24,25, 27-32, 36-38 concern kits and methods related to PMRT6.

Invention 2: claims 7-14 concern kits and methods related to ADMA.

Invention 3: claims 20, 22, 23, 26, 28-32, 36-38 concern kits and methods related to PMRT1.

There is no special technical feature between invention 1 and invention 2, because PMRT6 and ADMA differ in structure and biological significance.

Please see continuation in supplemental page.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☑ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

4-6,15-19,21,24,25,27-32,36-38

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☑ No protest accompanied the payment of additional search fees.

The matter common to invention 1 and invention 3 resides in inventions related to PMRT. However this matter is known by WO02/068649 A2 (claims 24,25,47, SEQ ID no.188, p.12, l.33-p.13, l.2). Therefore, this common matter cannot be considered as a special technical feature. Accordingly, claims 4-32, 36-38 have three general inventive concepts and there is no novel special technical feature common to these general concepts.