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(54) **BETA-CATENIN NUCLEAR LOCALIZING PROTEIN**

ZELLKERNLOKALISIERENDES PROTEIN FÜR BETA-CATENIN

PROTEINE LOCALISANTE NUCLEAIRE DE BETA-CATENINE

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Description

Technical Field

[0001] The present invention relates to a novel protein that binds to β-catenin and localizes β-catenin into the nucleus, DNA encoding the protein, antibody recognizing the protein, therapeutic agent comprising the protein, DNA, or antibody, and diagnostic agent comprising the antibody.

Background Art

[0002] The adenomatous polyposis coli (APC) gene is a gene isolated as a causative gene of familial adenomatous polyposis (FAP) (Kinzler, K.W. and Vogelstein, B., Cell 87: 159 (1996)). However, abnormality of the APC gene is not only reported in FAP but also in 70 to 80% of the cases of sporadic colon cancer. The onset of colon cancer is considered to be triggered by stepwise mutations of multiple genes including besides APC, such genes as K-ras, p53, and DCC. Since mutations in the APC gene are found in the earliest stage among these genes, it is considered that the abnormality of the APC gene has to be caused first in the onset of colon cancer.

[0003] In order to clarify the mechanism underlying carcinogenesis associated with the APC gene abnormality, it is necessary to determine the functions of the gene product, APC. APC, which is a protein about 300 kDa in size, has been reported to bind with β-catenin, glycogen synthase kinase-3β (GSK-3β), as well as DLG in cells (Rubinfeld, B. et al., Science 262: 1731 (1993) ; Su, L. K. et al., Science 262: 1734 (1993); Rubinfeld, B. et al., Science 272: 1023 (1996); Matsumine, A. et al., Science 272: 1020 (1996)). Regarding functions of the APC, it has been reported that the intracellular level of β-catenin is rapidly reduced when wild-type APC is expressed in colon cancer cell line SW480 having mutations in the APC gene (Munemitsu, S. et al., Proc. Natl. Acad. Sci. USA 92: 3046 (1995)). The central region containing a 7-repetitive sequence structure is essential for the function of APC and coincides with a region where mutations are found in many colon cancer cases. It has also been reported that the intracellular β-catenin level is elevated in these colon cancer cells (Munemitsu, S. et al., Proc. Natl. Acad. Sci. USA 92: 3046 (1995); Rubinfeld, B. et al., Cancer Res. 57: 4624 (1997)).

[0004] β-Catenin is also known as a membrane-skeletal protein for cell adhesion molecule cadherin and also reported to participate in the signal transduction of Wnt proteins described below (Cadigan, K. M. and Nusse, R., Genes Dev. 11: 3286 (1997)). The Wnt gene family is a large gene family which has a variety of functions in the processes of early embryogenesis and morphogenesis of animals; the family consists of about 20 types of genes in mouse and the genes are conserved among a variety of animals including African clawed frog (*Xenopus laevis*), fruit fly (*Drosophila melanogaster*), and nematoda (*Caenorhabditis elegans*). When Wnt binds to its receptor Frizzled, the activity of glycogen synthase kinase-3β (GSK-3β) is inhibited through an intracellular signaling molecule Dishevelled (Dsh). Since the phosphorylation of β-catenin mediated by GSK-3β causes the degradation of β-catenin, the inhibition of GSK-3β activity results in accumulation of β-catenin in cells. β-Catenin binds to a transcription factor (hereinafter abbreviated as TCF) belonging to the Lef/Tcf family to form a complex and thereby activates the TCF as a transcription factor. Thus, the accumulation of β-catenin results in the formation of the β-catenin/TCF complex, which translocates to the nucleus and thereby stimulates the transcription of target genes. Among Tcfs, Tcf-4 is specifically expressed in the epithelium of colon, and thus it is believed that β-catenin mainly forms a complex with Tcf-4 in colon cancer (Korinek, V. et al., Science 275: 1784 (1997)). In addition, it has been reported that there are some colon cancer cells and melanoma cells where the APC gene is wild-type but the β-catenin gene has mutation and is not regulated by GSK-3β (Morin, P. J. et al., Science 275: 1787 (1997); Rubinfeld, B. et al., Science 275: 1790 (1997)). It has been estimated that, β-catenin constantly accumulates in these cells, which results in transcriptional activation by the β-catenin/TCF complex.

[0005] Based on the above-described findings, β-Catenin may be greatly involved in the onset of colon cancer. Therefore, a substance capable of inhibiting the function of β-catenin through the binding thereto may be associated with the onset of colon cancer and the like and may be useful for the treatment, diagnosis, and such thereof. As a molecule binding to β-catenin, a protein, Axin, has been reported which negatively regulates the signal transduction system of Wnt proteins (Zeng, L. et al., Cell 90: 181 (1997)). Axin binds to GSK-3β and thereby stimulates the phosphorylation of β-catenin (Ikeda, S. et al., EMBO J. 17: 1371 (1998)). Furthermore, it has been reported that Axin also binds to APC and β-catenin to stimulate the degradation of β-catenin and thereby reducing the level of β-catenin in cells (Kishida, S. et al., J. Biol. Chem. 273: 10823 (1998); Rubinfeld, B. et al., Current Biology 8: 573 (1998); Nakamura, T. et al., Genes Cells 3: 395 (1998)). However, no protein that binds to β-catenin and has the function to localize β-catenin into the nucleus is known.

[0006] The bc19 protein (Willis T. G. et al. , Blood 91: 1871 (1998)) is a product of a gene that has been cloned from the translocation site of chromosome 1 in the CEMO-1 cell line, which was established from a patient with precursor B-cell acute lymphoblastic leukemia, and the expression level of the gene is abnormally high in CEMO-1 cells. However, its association with β-catenin, such as binding to β-catenin, remains unknown.

[0007] It is desired in the art to isolate and clarify the structure of proteins and DNA encoding the protein that are useful in the treatment and diagnosis of cancer by elucidating the mechanism of the onset of cancer (including colon cancer, wherein β-catenin plays a role) through the analysis of proteins that binds to β-catenin and regulate its function and genes encoding them.

Disclosure of the Invention

[0008] An objective of the present invention is to provide a protein that binds to β-catenin and has the activity to localize β-catenin into the nucleus, DNA encoding the protein, antibody recognizing the protein, therapeutic agent comprising the protein or the DNA, and diagnostic agent comprising the antibody, all of which are useful for the treatment and diagnosis of cancer.

[0009] The present invention relates to:

(1) a protein comprising the amino acid sequence of amino acid residues 292 to 439 in the amino acid sequence represented by SEQ ID NO: 2;
(2) the protein according to above (1), wherein the protein comprises the amino acid sequence represented by SEQ ID NO: 2 or 4;
(3) a protein comprising the amino acid sequence represented by SEQ ID NO: 10;
(4) a protein comprising the amino acid sequence represented by SEQ ID NOs: 10 and 12;
(5) a protein consisting of the amino acid sequence, wherein one or more amino acids are added, deleted, or substituted in the amino acid sequence of the protein according to any one of above (1) to (4), and said protein binding to β-catenin and having the activity to localize β-catenin into the nucleus;
(6) a protein consisting of the amino acid sequence having a homology of 60% or more to the amino acid sequence of the protein according to any one of above (1) to (4), and said protein binding to β-catenin and having the activity to localize β-catenin into the nucleus;
(7) the protein according to above (3) or (4), wherein the protein consists of the amino acid sequence having a homology of 60% or more to the amino acid sequence represented by SEQ ID NO: 2, and binds to β-catenin and has the activity to localize β-catenin into the nucleus;
(8) a protein comprising the amino acid sequence represented by SEQ ID NO: 12;
(9) a polypeptide comprising 5 to 60 continuous amino acid residues in the amino acid sequence of the protein according to any one of above (1) to (8);
(10) a DNA encoding the protein or the polypeptide of any one of above (1) to (9);
(11) a DNA comprising the nucleotide sequence of nucleotides 874 to 1317 in the nucleotide sequence represented by SEQ ID NO: 1;
(12) the DNA according to above (11), wherein the DNA comprises the nucleotide sequence represented by SEQ ID NO: 1 or 3;
(13) a DNA encoding a protein that binds to β-catenin and has the activity to localize β-catenin into the nucleus, and comprising the nucleotide sequence of the following [1] or [2]:

[1] the nucleotide sequence represented by SEQ ID NO: 9; and
[2] the nucleotide sequence represented by SEQ ID NOs: 9 and 11;

(14) a DNA hybridizing to the DNA of any one of above (10) to (13) under stringent conditions, which encodes a protein that binds to β-catenin and has the activity to localize β-catenin into the nucleus;
(15) a DNA comprising the nucleotide sequence represented by SEQ ID NOs: 9 and 11, and consisting of the nucleotide sequence having a homology of 60% or more to the nucleotide sequence of SEQ ID NO: 2, and encoding a protein that binds to β-catenin and has the activity to localize β-catenin into the nucleus;
(16) a DNA comprising the nucleotide sequence represented by SEQ ID NO: 11;
(17) a recombinant DNA obtainable by inserting the DNA according to any one of above (10) to (16) into a vector;
(18) a transformant obtainable by introducing the recombinant DNA according to above (17) into a host cell;
(19) a process for producing the protein or the polypeptide according to any one of above (1) to (9), which comprises the steps of:

culturing the transformant of above (18) in a culture medium;
producing and accumulating the protein or the polypeptide according to any one of above (1) to (9) in the culture; and
recovering the protein or the polypeptide from the culture;

(20) an oligonucleotide comprising 10 to 60 continuous nucleotides of the nucleotide sequence of the DNA according to any one of above (10) to (16) or a nucleotide sequence complementary thereto, or a derivative of the oligonucleotides;

(21) a method of detecting or quantifying, at the mRNA level, the expression level of the protein according to any one of above (3) to (7), using the DNA according to any one of above (10) to (16), or the oligonucleotide or derivative thereof according to above (20) ;

(22) a method of detecting a disease wherein the expression level of the protein according to any one of above (3) to (7) is increased or decreased in patient compared to normal healthy subject by measuring and comparing the expression level of the protein in normal healthy subject and test subject at the mRNA level by using the DNA according to any one of above (10) to (16), or the oligonucleotide or derivative thereof according to above (20);

(23) a diagnostic agent for a disease that exhibits increased or decreased expression level of the protein according to any one of above (3) to (7) in patient compared to normal healthy subject upon a measurment and comparison of the expression level of the protein at the mRNA level, wherein the diagnostic agent comprises the DNA according to any one of above (10) to (16), or the oligonucleotide or derivative thereof according to above (20);

(24) a method of detecting a mutation in a gene encoding the protein according to any one of above (3) to (7) using the DNA according to any one of above (10) to (16), or the oligonucleotide or derivative thereof according to above (20) ;

(25) a method of detecting a disease which has a mutation in a gene encoding the protein according to any one of above (3) to (7) using the DNA according to any one of above (10) to (16), or the oligonucleotide or derivative thereof according to above (20);

(26) a diagnostic agent for a disease which has a mutation in a gene encoding the protein according to any one of above (3) to (7), comprising the DNA according to any one of above (10) to (16), or the oligonucleotide or derivative thereof according to above (20) ;

(27) a method of inhibiting the expression of the protein according to any one of above (3) to (7) at the transcriptional level using the DNA according to any one of above (10) to (16), or the oligonucleotide or derivative thereof according to above (20);

(28) a method of inhibiting the nuclear localization of β-catenin, using an oligonucleotide or a DNA selected from the group of: (i) the DNA according to any one of above (10) to (16) ; (ii) the oligonucleotide or derivative thereof according to above (20); (iii) a DNA encoding bcl9 protein; (iv) an oligonucleotide comprising 10 to 60 continuous nucleotides of the nucleotide sequence of the DNA encoding bcl9 protein; and (v) an oligonucleotide comprising a nucleotide sequence complementary to the oligonucleotide of (iv), or a derivative thereof;

(29) a therapeutic agent for cancer comprising an oligonucleotide or a DNA selected from the group of: (i) the DNA according to any one of above (10) to (16) ; (ii) the oligonucleotide or derivative thereof according to above (20) ; (iii) a DNA encoding bcl9 protein; (iv) an oligonucleotide comprising 10 to 60 continuous nucleotides of the nucleotide sequence of the DNA encoding bcl9 protein; and (v) an oligonucleotide comprising a nucleotide sequence complementary to the oligonucleotide of (iv), or a derivative thereof;

(30) a vector for gene therapy comprising the DNA according to any one of above (10) to (16);

(31) a non-human transgenic animal generated by introducing the DNA according to any one of above (10) to (16);

(32) a method of using the non-human transgenic animal according to above (31), or a non-human transgenic animal generated by introducing a DNA encoding bcl9 protein as an animal model for carcinogenesis;

(33) a method of evaluating a therapeutic agent for cancer using the transgenic animal according to above (31), or a transgenic animal generated by introducing a DNA encoding bcl9 protein;

(34) a genetically defective non-human animal, wherein the function of a protein to bind to β-catenin and to localize β-catenin into the nucleus is lost or lowered due to the deletion of all or a part of the DNA according to any one of above (10) to (16);

(35) a method of screening for a substance that inhibits the binding of bcl9 protein and β-catenin, comprising the steps of:

comparing the binding of bcl9 protein and β-catenin in the absence [1] and presence [2] of a test sample; and selecting a substance that inhibits the binding of the bcl9 protein and β-catenin from the test sample;

(36) a method of screening for a substance that inhibits the binding of β-catenin and the protein according to any one of above (1) to (7), comprising the steps of:

comparing the binding of β-catenin and the protein in the absence [1] and presence [2] of a test sample; and selecting a substance that inhibits the binding of β-catenin and the protein according to any one of above (1) to (7) from the test sample;

(37) a compound obtainable by the screening method according to above (35) or (36), or a pharmaceutically acceptable salt thereof;
(38) a therapeutic agent for cancer comprising the compound or pharmaceutically acceptable salt thereof according to above (37) ;
(39) an antibody recognizing the protein or the polypeptide according to any one of above (1) to (9);
(40) a method of immunologically detecting or quantifying the protein according to any one of above (1) to (7) using the antibody according to above (39);
(41) a neutralizing antibody that binds to the protein according to any one of above (1) to (7), and thereby inhibiting the activity of the protein to bind to β-catenin to localize β-catenin into the nucleus;
(42) a neutralizing antibody that binds to bcl9 protein, and thereby inhibiting the activity of the bcl9 protein to bind to β-catenin to localize β-catenin into the nucleus;
(43) a method of detecting a disease wherein the expression level of the protein according to any one of above (3) to (7) is increased or decreased in patient compared to normal healthy subject, by quantifying and comparing the expression level of the protein in normal healthy subject and a test subject by using the antibody according to above (39);
(44) a diagnostic agent comprising the antibody according to above (39), for a disease wherein the expression level of the protein according to any one of above (3) to (7) is increased or decreased in patient compared to normal healthy subjects;
(45) a method of inhibiting the transcriptional activation by a complex of β-catenin and transcription factor belonging to the Lef/Tcf family by inhibiting the function of the protein according to any one of above (1) to (7) by using the antibody according to above (41);
(46) a method of inhibiting the transcriptional activation by a complex of β-catenin and transcription factor belonging to the Lef/Tcf family by inhibiting the function of bcl9 protein by using the antibody according to above (42); and
(47) a therapeutic agent for cancer comprising the antibody according to above (41) or (42).

[0010] The proteins or the polypeptides of the present invention include:

(a) a protein comprising the amino acid sequence of amino acid residues 292 to 439 in the amino acid sequence represented by SEQ ID NO: 2;
(b) the protein according to (a), wherein the protein comprises the amino acid sequence represented by SEQ ID NO: 2 or 4;
(c) a protein comprising the amino acid sequence represented by SEQ ID NO: 10;
(d) a protein comprising the amino acid sequence represented by SEQ ID NOs: 10 and 12;
(e) a protein consisting of the amino acid sequence, wherein one or more amino acids are added, deleted, or substituted in the amino acid sequence of the protein according to any one of above (a) to (d), and said protein binding to β-catenin and having the activity to localize β-catenin into the nucleus;
(f) a protein consisting of the amino acid sequence having a homology of 60% or more to the amino acid sequence of the protein according to any one of above (a) to (d), and said protein binding to β-catenin and having the activity to localize β-catenin into the nucleus;
(g) the protein according to above (c) or (d), wherein the protein consists of the amino acid sequence having a homology of 60% or more to the amino acid sequence represented by SEQ ID NO: 2, and binds to β-catenin and has the activity to localize β-catenin into the nucleus;
(h) a protein comprising the amino acid sequence represented by SEQ ID NO: 12; and
(i) a polypeptide comprising 5 to 60 continuous amino acid residues in the amino acid sequence of the protein according to any one of above (a) to (h).

[0011] The above-mentioned addition, deletion or substitution of amino acid (s) can be performed by introducing a site specific mutation into a DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO: 2 by site-directed mutagenesis as described in the literature (Zoller M. J. and Smith M., Nucleic Acids Res. 10: 6487 (1982); Dalbadie-McFarland G. et al., Proc. Natl. Acad. Sci. USA 79: 6409 (1982); Wells J. A. et al., Gene 34: 315 (1985); Carter P. et al., Nucleic Acids Res. 13: 4431 (1985); Kunkel T. A., Proc. Natl. Acad. Sci. USA 82: 488 (1985), etc.).

[0012] The number of amino acids to be added, deleted, or substituted are not specifically limited; however it is a number that can be added, deleted, or substituted by conventional methods, such as the above-mentioned site-directed mutagenesis, which number is one to several tens, preferably 1 to 20, more preferably 1 to 10, and further more preferably 1 to 5.

[0013] Alternatively, PCR can be performed using a pair of PCR primers which has a sequence introduced with a desired mutation (addition, deletion, or substitution) at respective 5'-terminus (Ho S.N. et al., Gene 77: 51 (1989)) to introduce a mutation into a DNA encoding the protein comprising the amino acid sequence represented by SEQ ID NO:

2. Specifically, first, using the DNA as a template, PCR is performed with a sense primer that corresponds to the 5'-terminus of the DNA and an antisense primer that corresponds to the sequence immediately before (5'-terminal to) a mutation site and contains a sequence complementary to a mutated sequence to amplify fragment A (a mutation introduced at its 3'-end) extending from the 5'-end of the DNA to the mutation site. Next, using the same DNA as a template, PCR is performed with a sense primer that corresponds to the sequence immediately after (3'-terminal to) the mutation site and comprises the mutated sequence on its 5'-terminus, and an antisense primer that corresponds to the 3'-terminus of the DNA to amplify fragment B extending from the mutation site to the 3'-end of the DNA that is introduced with a mutation at its 5'-end. These amplified fragments are purified respectively. Then, by mixing these fragments and conducting PCR without adding a template or primers, the sense strand of the fragment A and the antisense strand of the fragment B hybridize due to their common mutation sites, and the PCR reaction proceeds the hybridized strand as a template and primers to amplify the DNA introduced with the mutation.

[0014] On the other hand, a DNA encoding a partial fragment, a kind of deletion mutant, of the β-catenin nuclear localizing protein can be obtained by performing PCR with a set of primers that correspond to the nucleotide sequence at both ends of an arbitrary DNA fragment of a DNA encoding the protein having the amino acid sequence represented by SEQ ID NO: 2 using the DNA as a template.

[0015] The protein of the present invention preferably has a homology of at least 60% or more, normally 80% or more, and particularly 95% or more to the amino acid sequence represented by SEQ ID NO: 2 so as to retain the function to localize β-catenin into the nucleus.

[0016] The homology of amino acid sequences and nucleotide sequences can be determined using BLAST, an algorithm developed by Karlin and Altschul (Proc. Natl. Acad. Sci. USA 90: 5873 (1993)), or FASTA (Methods Enzymol. 183: 63 (1990)). Programs called BLASTN and BLASTX have been developed based on the BLAST algorithm (J. Mol. Biol. 215: 403 (1990)). For BLASTN analysis of a nucleotide sequence based on BLAST, the parameters are set, for example, at score = 100, and wordlength = 12. For BLASTX analysis of an amino acid sequence based on BLAST, the parameters are set, for example, score = 50, and wordlength = 3. When the BLAST and Gapped BLAST programs are used, default parameters in both programs are used. Specific procedures to perform these analyses are known to those skilled in the art (http://www.ncbi.nlm.nih.gov.).

[0017] An example of the protein comprising the amino acid sequence, wherein one or more amino acid residues are added, deleted, or substituted in the amino acid sequence represented by SEQ ID NO: 2, binding to β-catenin and having the activity to localize β-catenin into the nucleus includes a protein consisting of the amino acid sequence represented by SEQ ID NO: 4, which is a partial fragment corresponding to the residues 245 to 564 of the amino acid sequence represented by SEQ ID NO: 2.

[0018] The DNA of the present invention includes:

(j) a DNA encoding the above protein or polypeptide of the present invention,
(k) a DNA comprising the nucleotide sequence of nucleotides 874 to 1317 in the nucleotide sequence represented by SEQ ID NO: 1;
(l) the DNA according to above (k), wherein the DNA comprises the nucleotide sequence of SEQ ID: 1 or 3;
(m) a DNA encoding a protein that binds to β-catenin and has the activity to localize β-catenin into the nucleus, and comprising the nucleotide sequence of the following [1] or [2]:

[1] the nucleotide sequence represented by SEQ ID NO: 9; and
[2] the nucleotide sequence represented by SEQ ID NOs: 9 and 11;

(n) a DNA hybridizing to the DNA of any one of above (j) to (m) under stringent conditions, and encoding a protein that binds to β-catenin and has the activity to localize β-catenin into the nucleus;
(o) a DNA comprising the nucleotide sequence represented by SEQ ID NOs: 9 and 11, and consisting of the nucleotide sequence having a homology of 60% or more to the nucleotide sequence of SEQ ID NO: 2, and encoding a protein that binds to β-catenin and has the activity to localize β-catenin into the nucleus; or
(p) a DNA comprising the nucleotide sequence represented by SEQ ID NO: 11.

[0019] A DNA hybridizing under stringent conditions means, for instance, those DNAs that can be obtained by colony hybridization method, plaque hybridization method, southern blot hybridization method, and such using the DNA of the present invention, such as the DNA having the nucleotide sequence represented by SEQ ID NO: 1, or partial fragment thereof, as a probe. Specifically, such DNA include those identified by immobilizing a DNA derived from a colony or plaque on a filter, subjecting the filter to hybridization in the presence of 0.7 to 1.0 mol/L NaCl at 65°C, and then washing the filter with 0.1 to 2x SSC solution (1x SSC: 150 mmol/L NaCl, 15 mmol/L sodium citrate) at 65°C. Hybridization can be performed according to methods described in "Molecular Cloning, A Laboratory Manual, Second Edition", Cold Spring Harbor Laboratory Press (1989) (hereinafter abbreviated as Molecular Cloning, Second Edition), "Current Protocols in

Molecular Biology", Supplement 1-38, John Wiley & Sons (1987-1997) (hereinafter abbreviated as Current Protocols in Molecular Biology), "DNA cloning 1: Core Techniques, A Practical Approach, Second Edition", Oxford University (1995), and the like. Specifically, hybridizable DNAs include DNAs that have a homology of at least 60% or more, preferably 70% or more, more preferably 80% or more, further more preferably 90% or more, still further more preferably 95% or more, and most preferably 98% or more to the nucleotide sequence of SEQ ID NO: 1.

[0020] The oligonucleotides of the present invention include those comprising a sequence of 10 to 60 nucleotides in the nucleotide sequence of the DNA of the present invention. However, oligonucleotides consisting of known nucleotide sequences, such as ESTs or genome draft sequences that are already published, are excluded from the oligonucleotides of the present invention.

[0021] The present invention is described below in detail. In the following description, a protein that binds to β-catenin and has the activity to localize β-catenin into the nucleus is referred to as a "β-catenin nuclear localizing protein", and the β-catenin nuclear localizing protein of the present invention having a novel amino acid sequence is referred to as a "bcl9 like protein (hereinafter abbreviated as B9L protein)" due to its homology to bcl9.

1. DNA encoding β-catenin nuclear localizing protein, and oligonucleotide comprising partial sequence of the DNA

1) Preparation of DNA encoding B9L protein

[0022] DNAs encoding the B9L protein of the present invention (hereinafter abbreviated as B9L DNA) include a full-length B9L cDNA, a genomic DNA corresponding to the full-length B9L cDNA, the region within the full-length B9L cDNA that encodes the B9L protein (DNA comprising the nucleotide sequence represented by SEQ ID NO: 1), and a DNA encoding the amino acid sequence represented by SEQ ID NO: 2.

[0023] A B9L cDNA can be obtained by the yeast two-hybrid system (Fields S. et al., Nature 340: 245 (1989)).

[0024] The yeast two-hybrid system is a method for detecting the binding between a protein X of interest (generally called "bait" in the method) and a protein Y to be tested utilizing an yeast transcription factor Z , such as GAL4, that separately has a DNA binding domain (BD) and a transcriptional activation domain (AD). First, a plasmid (bait plasmid) that can express X in a host yeast cell as a fusion protein with the DNA binding domain of a transcription factor Z (hereinafter referred to as BD-X) is prepared. Next, another plasmid that can express Y in a host yeast cell as a fusion protein with the transcriptional activation domain of the transcription factor Z (hereinafter referred to as AD-Y) is prepared. Both plasmids are introduced into a host yeast cell to simultaneously express BD-X and AD-Y. Yeast cells having a genotype that can express a reporter gene under the control of a promoter which activates upon the binding of the transcription factor Z are used as the host. When the protein Y has a characteristic to bind to the protein X, BD-X and AD-Y bind to form a complex, which then binds to the promoter via the DNA binding domain (BD), and activates transcription through the transcriptional activation domain (AD) to express the reporter gene. Therefore, the expression of the reporter gene can be utilized as a marker to detect the binding between the proteins X and Y. A transformant carrying a cDNA encoding a protein Y that binds to the protein X can be isolated by screening transformants using proteins derived from a cDNA library as test protein Y and the expression of the reporter gene as a marker. Furthermore, a cDNA of interest can be cloned by isolating plasmid from the transformant.

[0025] A procedure for the cloning of B9L cDNA according to the above-mentioned method is specifically described below, using an armadillo domain of mouse β-catenin (hereinafter abbreviated as mβ-catenin arm) and yeast GAL4 as an example of bait X and transcription factor Z, respectively.

(1) Preparation of bait plasmid

[0026] In the present invention, mβ-catenin arm (which corresponds to the amino acid sequence of the residues 128 to 683 in mouse β-catenin) was used as bait. To prepare a bait plasmid, a DNA encoding mβ-catenin arm (hereinafter abbreviated as mβ-catenin arm DNA) which serves as the bait is necessary. Since the entire nucleotide sequence of mouse β-catenin cDNA is known to those skilled in the art (GenBank accession No: M90364; Buts, S. et al, Science 257: 1142 (1992)), the nucleotide sequence corresponding to mβ-catenin arm DNA can readily be recognized. Accordingly, an mβ-catenin arm DNA can be amplified and isolated by the RT-PCR method shown below (McPherson, M. J. et al., "PCR, A practical Approach," Oxford University Press (1991)).

[0027] Specifically, RNA is isolated from a mouse tissue or cells expressing β-catenin; cDNA is synthesized from the RNA; PCR is carried out using the cDNA as a template, a sense primer containing the nucleotide sequence corresponding to the 5' end of mβ-catenin arm DNA, and an antisense primer containing a nucleotide sequence complementary to the 3' end of the nucleotide sequence. When the 5' end of each primer for amplification is designed to have a sequence of a restriction-enzyme recognition site of a cloning vector for bait plasmid as described below, then the amplified fragment can be efficiently inserted into the cloning vector for bait plasmid as described below by utilizing the restriction enzyme sites. If the primers are intended to have the restriction-enzyme recognition sequences for cloning, the primers are

designed such that codons of the transcriptional activation domain of the transcription factor are in frame with those of mβ-catenin arm when inserted into the cloning vector.

[0028] The vector (to be preferably used to insert the mβ-catenin arm DNA prepared by the above-mentioned method) includes a vector capable of replicating in yeast *Saccharomyces cerevisiae*, and which has an appropriate marker gene for transformation, e.g., genes for amino acid biosynthesis such as TRP1 and LEU2, and can express the DNA-binding domain of GAL4 (hereinafter abbreviated as GAL4 BD) under the regulation of a promoter for expression in yeast, e.g., alcohol dehydrogenase (ADH) promoter. In such cases, it is preferable to use a vector having appropriate restriction enzyme sites at a C-terminal portion of GAL4 BD for the insertion of mβ-catenin arm DNA, and capable of replicating in *Escherichia coil* because of convenience to handle, e.g., to purify the vector DNA, as well as having a detectable marker for transformation in *E. coli*, e.g., the ampicillin-resistance gene. Such vectors include pGBT9 (Clontech), pAS1 (Durfee, T. et al., Genes and Development 7: 555 (1993)), pAS2-1 (Clontech), and the like.

[0029] The mβ-catenin arm DNA prepared above is isolated and then inserted at a restriction enzyme site on the C-terminal side of GAL4 BD in the vector in frame of codon.

(2) Preparation of cDNA library for two-hybrid system

[0030] In order to prepare a cDNA library for the expression of a fusion protein with the transcriptional activation domain of GAL4, the vector to be used can preferably replicate in yeast *Saccharomyces cerevisiae*, has an appropriate marker gene for transformation, e.g., genes for amino acid biosynthesis in yeast, such as TRP1 and LEU2, and can express the transcriptional activation domain of GAL4 (hereinafter abbreviated as GAL4 AD) under the regulation of a promoter for expression in yeast, e.g., alcohol dehydrogenase (ADH) promoter. In such cases, it is preferable to use a vector having appropriate restriction enzyme sites at a C-terminal portion of GAL4 AD, and capable of replicating also in *E. coli* because of convenience to handle, e.g., to purify the vector DNA, as well as having a detectable marker for transformation in *E. coli*, e.g., the ampicillin-resistance gene. Such vectors include pGAD (Chien, C. T. et al., Proc. Natl. Acad. Sci. USA 88: 9578 (1991)), pGAD424 (Clontech), pACT (Durfee, T. et al., Genes and Development 7: 555 (1993)), pACT2-1 (Clontech), and the like.

[0031] Proteins interacting with β-catenin in cells are predicted to be expressed in the same cells and tissues as β-catenin. β-catenin is reported as being widely expressed in adult and embryo tissues. Thus, it is possible to prepare a cDNA library by preparing cDNA from mouse tissues and cells where β-catenin is predicted to be expressed and inserting it at a restriction enzyme site on the C-terminal side of GAL4 AD in the above-mentioned vector for the expression of fusion protein. In such cases, when the cDNA and GAL4 AD are in the same orientation and are in frame, then the fusion protein between GAL4 AD and the protein encoded by the cDNA can be expressed. Alternatively, it is possible to use a commercially available library usable in the yeast two-hybrid system, e.g., MATCHMAKER cDNA library (Clontech).

(3) Screening of cDNA by yeast two-hybrid system

[0032] An yeast to be used for the introduction of the bait plasmid prepared in (1) and the cDNA library prepared in (2) includes yeasts belonging to *Saccharomyces cerevisiae,* into which the above-mentioned bait plasmid and cDNA library can be introduced, and further, it is required: (a) that the maker gene for transformation in the plasmid to be introduced and the gene of transcription factor GAL4 used in the two-hybrid system are incapable of being expressed due to their deletions or mutations; and (b) that a nucleotide sequence to which GAL4 BD can bind has been inserted in the promoter region of an appropriate reporter gene. In this case, it is preferable to use a reporter gene of which transcription is readily detected when initiated by the binding with the bait, for example, genes for amino acid biosynthesis, e.g., HIS3, etc. (in this case, the gene should be different from that used as the maker for transformation of the bait plasmid); the *E. coli* β-galactosidase gene lacZ that is detectable in yeast, and the like. For example, the host yeast includes *Saccharomyces cerevisiae* CG1945 strain (Clontech), HF7C strain (Clontech), Y153 strain (Durfee, T. et al., Genes and Development 7: 555 (1993)), CGY1: : 171 strain (Gill, G. and Ptashne, M., Cell 51: 121 (1987)), and the like.

[0033] The bait plasmid prepared in (1) and the cDNA library prepared in (2) can be introduced into this host yeast to select transformants containing the cDNA encoding a protein capable of binding to mβ-catenin arm using the expression of a reporter gene as a marker. For example, colonies grown on a minimum medium without histidine are selected when the HIS3 gene for histidine biosynthesis is used as the reporter gene, or colonies expressing blue color in the presence of X-gal is selected when the *E. coli* lacZ gene is used as the reporter gene.

[0034] Since selected colonies of transformant contain both types of plasmids, the bait plasmid and cDNA library, only the plasmid of cDNA library is isolated according to the method as described in references (Glover, D. M. and Hames, B. D., "DNA Cloning 2, Expression Systems, (A Practical Approach Series 149), Second Edition," Oxford University Press (1995); Chien, C. T. et al., Proc. Natl. Acad. Sci. USA 88: 9578 (1991)). Specifically, a whole DNA comprising the plasmid is isolated from the colony followed by the transformation of *E. coli* therewith. In this case, the host *E. coli* to be used is a strain that does not express a gene corresponding to the marker gene contained in the cDNA library, so that

the expression of the maker gene in a transformant introduced with the maker gene can be detected. Some transformants introduced with the marker gene derived from the cDNA library are selected from the transformants, and plasmid DNAs are isolated from the selected transformants to obtain cDNA clones.

(4) Analysis of nucleotide sequences of cDNA clones

[0035] Nucleotide sequences of the cDNA clones obtained in (3) can be determined, using the intact cDNA clones or alternatively after fragments of cDNA moiety are digested with appropriate restriction enzymes and subcloned into appropriate cloning vectors, e.g., pUC118, by a commonly used method for analyzing nucleotide sequence, e.g., the dideoxy-sequencing method by Sanger et al., (Proc. Natl. Acad. Sci. USA 74: 5463 (1977)) or DNA sequencer provided by Perkin Elmer, etc.

[0036] The novelty of the resulting nucleotide sequence of cDNA can be determined, by verifying that the sequence of cDNA does not exhibit significant homology to nucleotide sequences of known genes deposited in databases in the search of nucleotide sequence databases, such as GenBank, EMBL, and DDBJ, using a program for homology search, such as BLAST.

[0037] When the nucleotide sequence is novel, then, as described in (2), the cDNA clone obtained in (3) should encode a fusion protein wherein the B9L protein is connected to the C-terminus of GAL4 AD in frame. Accordingly, amino acid sequence of a protein encoded by the cDNA can be deduced by translating the revealed nucleotide sequence of cDNA to amino acid sequence in the same frame as the translational frame of GAL4 AD.

[0038] Further, known genes exhibiting homology to the protein encoded by the cDNA can be selected by searching amino acid sequence databases, such as Genpept, PIR, and Swiss-Prot, for this amino acid sequence with a program for homology search, such as BLAST, FASTA, and FrameSearch.

[0039] A cDNA having a novel nucleotide sequence as obtained above includes, for example, a cDNA comprising the nucleotide sequence represented by SEQ ID NO: 3. The cDNA encodes a protein comprising a novel amino acid sequence represented by SEQ ID NO: 4.

[0040] However, cDNAs obtained in this way are likely to be a non-full-length cDNA that fail to encode the entire protein of interest. In such cases, the entire amino acid sequence of the protein of interest can be revealed by obtaining a full-length cDNA with method shown in following (5), and translating the nucleotide sequence of the obtained full-length cDNA to the amino acid sequence in the same frame as translating the non-full-length cDNA. The nucleotide sequence of each codon of the region encoding the B9L protein is not restricted to those used in the cDNA, and includes any nucleotide sequence of any codons encoding the same amino acids so long as they encode as a whole the amino acid sequence of the entire B9L protein.

(5) Cloning of full-length B9L cDNA

[0041] When it is predicted that the length of B9L cDNA obtained in (3) is not full length based on a nucleotide sequence analysis in (4) as well as information on the length of mRNA obtained by Northern blot hybridization as described below, the full-length B9L cDNA can be prepared by the following method.

(5-1) Screening of cDNA library

[0042] The cDNA library prepared in (2) which expresses the fusion protein, or a cDNA library prepared from tissues or cells expressing β-catenin where the B9L protein is presumed to be co-expressed or cells where β-catenin mRNA is detected by Northern blotting as described below, etc., is screened by colony hybridization or plaque hybridization using, as a probe, the whole cDNA obtained in (3) or a part thereof, and then cDNA clones with the length that are presumed to be full-length are selected from among the positive clones. The preparation of cDNA library and hybridization can be performed by methods described in "Molecular Cloning, Second Edition,". Alternatively, it is possible to use commercially available cDNA libraries from Clontech or others. The nucleotide sequence of full-length mouse B9L cDNA and the amino acid sequence of entire mouse B9L protein can be revealed by determining the nucleotide sequence of the resulting cDNA clones by the same method as described in (4).

(5-2) Rapid-amplification of cDNA ends (RACE) method

[0043] Complementary DNAs are prepared from tissues or cells that are predicted to express the B9L protein, and then an adapter oligonucleotide is added to both ends of the cDNAs. Complementary DNA fragments containing a part extended to the 5' or 3' direction from the cDNA obtained in (3) can be obtained by 5'-RACE (rapid amplification of cDNA ends) or 3'-RACE (Frohman, M. A., Proc. Natl. Acad. Sci. USA 85: 8998 (1988)) wherein PCR is carried out using a primer from the nucleotide sequence of this adapter and a primer designed based on the nucleotide sequence of the

cDNA clone obtained in (3). The nucleotide sequence of the full-length B9L cDNA can be revealed by determining the nucleotide sequence of the resulting cDNA according to the same method as described in (4).

(5-3) Use of EST nucleotide sequence

[0044] When the nucleotide sequence of B9L cDNA determined in (4) is analyzed by searching public nucleotide sequence databases for homology, identical sequences to the cDNA may be found among partial sequences of random cDNA clones, ESTs, even when there is no identical nucleotide sequence among known genes. In such cases, these ESTs and other ESTs containing nucleotide sequences identical to the ESTs and ESTs derived from the same clone are all collected together as the ESTs derived from the same gene. Sometimes a longer nucleotide sequence extended to the 5' or 3' direction as compared with the cDNA obtained in (4) may be found by assembling the nucleotide sequences of these ESTs that are presumed to be derived from B9L cDNA. In such cases, RT-PCR can be performed using cDNAs or a cDNA library that is prepared from a mouse tissue or cell which is expected to express the B9L protein as a template, and a sense primer comprising the nucleotide sequence of the 5'-end of the nucleotide sequence obtained by assembling those ESTs or an antisense primer comprising a nucleotide sequence complementary to the nucleotide sequence of the 3'-end to obtain a cDNA fragment that comprises a sequence extended to the 5'- or 3'-direction as compared with that of the cDNA obtained in (4). When the nucleotide sequence of the cDNA fragment is determined, and seem to correspond to the 5'- or 3'-end of the full-length B9L cDNA, it can be assembled with the nucleotide sequence of the cDNA obtained in (4) to reveal the nucleotide sequence of the full-length mouse B9L cDNA. When many EST clones considered to be derived from mouse B9L cDNA are found in known nucleotide sequence databases, the nucleotide sequence of the full-length mouse B9L cDNA may be determined, without RT-PCR, by assembling the nucleotide sequence of the EST clones. (5-4) Preparation of full-length B9L cDNA

[0045] A full-length B9L cDNA clone can be prepared by amplifying the full-length B9L cDNA by PCR using cDNAs or a cDNA library prepared from mouse tissue or cell, which are expected to express the B9L protein, according to the same steps as in (3) as a template and primers that are designed based on the revealed nucleotide sequence of the 5'- and 3'-ends of the B9L full-length cDNA.

[0046] Alternatively, the B9L full-length cDNA can be prepared by transforming *E. coli* with a full-length B9L cDNA clone that is prepared by (1) ligating the full-length B9L cDNA clone obtained in (5-1) or the partial cDNA of 5'- or 3'-end obtained in (5-2) or (5-3) with the originally obtained B9L cDNA using the restriction enzyme sites within the cDNAs, or (2) cloning the full-length B9L cDNA clone prepared by the above PCR into an appropriate cloning vector; culturing the *E.coli;* and preparing plasmid DNAs from the cultured *E. coli* .

[0047] *Escherichia coli* MM294/pEGFP-C2B9L, a transformant comprising pEGFP-C2B9L, which is a plasmid DNA obtained as above comprising a DNA consisting of the nucleotide sequence represented by SEQ ID NO: 1, has been deposited under the accession number FERM BP-7291 in the International Patent Organism Depositary of the Independent Administrative Institution, National Institute of Advanced Industrial Science and Technology (Tsukuba Central 6, 1-1-1 Higashi, Tsukuba-shi, Ibaraki, Japan 305-8566) (the former National Institute of Bioscience and Human-Technology, National Institute of Advanced Industrial Science and Technology (1-1-3 Higashi, Tsukuba-shi, Ibaraki, Japan 305-8566)) on September 6, 2000.

[0048] Alternatively, the B9L full-length cDNA can be chemically synthesized using DNA synthesizer based on its nucleotide sequence. Such DNA synthesizers include DNA synthesizer model 392 from Perkin Elmer that utilizes phosphoramidite method, and the like.

(6) Isolation of DNA encoding human B9L protein

[0049] It is important to obtain B9L protein and a DNA encoding the protein of human than those of the mouse to analyze the mechanism underlying the onset of human colon cancer as well as to treat and diagnose the cancer. In general, proteins from different species having the same function often have amino acid sequences not identical but exhibiting homology to each other. Accordingly, the DNAs encoding the proteins are also predicted to exhibit homology to each other. In addition, mutations are accumulating in genes during the evolution of organisms, and therefore it can be assumed that the closer the phylogenetic lineage of the species, the higher the homology may be. Accordingly, it is possible to obtain B9L cDNA from other mammal, for example, human B9L cDNA, by utilizing the mouse B9L cDNA obtained in (3) or its nucleotide sequence information, according to a method as described below. When the cDNA obtained according to the method is not a full-length cDNA, the human DNA B9L can be obtained using similar methods as described in (5).

[0050] Alternatively, without obtaining mouse B9L DNA, human B9L cDNA can be directly obtained via same procedures as described in (1) to (5) of yeast two-hybrid system using human cDNA library and bait plasmid wherein the armadillo domain of human β-catenin (J. Cell Biol. 127: 2601 (1994)) serves as the bait.

(6-1) Screening of cDNA library

[0051] cDNA clones of human B9L protein can be obtained from a cDNA library that is prepared using the same method as (5-1) from human tissue corresponding to the mouse tissue which expresses mouse B9L protein or human cells derived therefrom, or from a commercially available human cDNA library of such human tissue or cells by colony hybridization or plaque hybridization under a relatively stringent condition using mouse B9L DNA labeled with radioisotope, digoxigenin, and the like as a probe.

[0052] Herein, the relatively stringent condition varies depending on the homology between human and mouse B9L cDNAs, and by performing southern blot hybridization using mouse B9L cDNA as a probe against human chromosome DNA digested with restriction enzyme under several conditions of different stringency, the most stringent condition where a hybridization band is clearly detected is selected. For instance, the relatively stringent condition for a hybridization solution without formamide can be determined by performing hybridization under several conditions where the salt concentration of hybridization solution is fixed at 1 mol/l and where the temperature gradationally varies between 68 and 42°C, and washing in 2x SSC containing 0.5% sodium dodecyl sulfate (SDS) at the same temperature as the hybridization. Alternatively, the relatively stringent condition for a formamide-containing hybridization solution can be determined by performing hybridization at fixed temperature of 42°C, fixed salt concentration of 6x SSC, and a formamide concentration gradationally varying between 50 to 0%, and washing at 50°C in 6x SSC containing 5% SDS.

(6-2) Isolation of DNA encoding human B9L protein utilizing EST and human genomic nucleotide sequence

[0053] The nucleotide sequence of EST clones expected to be derived from human B9L cDNA or human genomic DNA sequence comprising the exon(s) of human B9L genomic DNA can be identified by searching for nucleotide sequences of human DNA that has a high homology (specifically 80% or more) with the nucleotide sequence of the mouse B9L cDNA obtained in (4), particularly at the protein coding region, in nucleotide sequence databases such as GenBank, using a homology search program such as BLAST. Such nucleotide sequences include human ESTs with GenBank accession number U46365 and R24762, and the working draft sequences of human genomic DNA with GenBank accession number AP000877, AP002357, and AP000909.

[0054] By comparing the human genomic sequences with the nucleotide sequence of mouse B9L cDNA and human ESTs, the exon sequence encoding human B9L protein, i.e., the sequence of human B9L cDNA, can be obtained. Such exon sequences of human B9L genomic DNA include the nucleotide sequence represented by SEQ ID NOs: 5 to 8. These EST clones can be obtained as EST clones of the Integrated Molecular Analysis of Genome Expression Consortium (I.M.A.G.E. Consortium) from ATCC. Alternatively, human B9L cDNA may be obtained by preparing primers that are designed based on the sequence of homologous human EST or exon of human genomic DNA, and amplifying DNA fragments by RT-PCR as described in (3) using mRNA prepared from human tissue or cell that are expected to express the B9L protein as a template.

(7) Isolation of B9L genomic DNA

[0055] Genomic DNA of mouse or human B9L gene can be obtained by screening according to methods such as plaque hybridization, described in "Molecular Cloning, second edition" using the mouse or human B9L cDNA obtained in (3) or (6) as a probe against a genomic DNA library prepared from chromosomal DNA isolated from a cell or tissue of mouse or human. The exon-intron structure of the B9L gene can be identified through the comparison of the nucleotide sequences of the B9L genomic DNA and the B9L cDNA. Furthermore, using the 5'-end portion of the cDNA as a probe, the nucleotide sequence of regions of the genomic gene that regulate transcription, such as the promoter of the B9L gene, can be identified. Such sequence is useful for analyzing the mechanism of transcriptional regulation of the B9L gene. Furthermore, clones may be prepared wherein the B9L gene on the chromosome is inactivated or substituted with arbitrary sequence using the technique of homologous recombination (Kuehn M. R. et al., Nature 326: 295 (1987); Thomas K. R. and Capecchi M. R., Cell 51: 503 (1987)).

(8) Preparation of B9L oligonucleotides

[0056] An oligonucleotide comprising a partial sequence of the B9L DNA of the present invention or sequence complementary thereto (hereinafter abbreviated as B9L oligonucleotide) can be prepared by the DNA synthesizer described above in (5).

[0057] Specifically, such B9L oligonucleotides include DNAs comprising a sequence identical to 10 to 60 continuous nucleotides of the nucleotide sequence represented by SEQ ID NO: 1 or DNAs comprising a sequence complementary thereto. For the use of such DNAs as sense or antisense primers, oligonucleotides whose melting temperature and number of nucleotides does not extremely change is preferable.

[0058] Furthermore, derivatives of the above oligonucleotides (hereinafter referred to as oligonucleotide derivative) may be also used as an oligonucleotide of the present invention.

[0059] Example of the oligonucleotide derivatives include oligonucleotide derivatives wherein the phosphodiester bond of the oligonucleotide has been converted to a phosphorothioate bond; oligonucleotide derivatives wherein the phosphodiester bond in the oligonucleotide has been converted to a N3'-P5' phosphoramidite bond; oligonucleotide derivatives wherein the ribose and phosphodiester bond in the oligonucleotide has been converted to a peptide-nucleic acid bond; oligonucleotide derivatives wherein uracil of the oligonucleotide has been substituted with C-5 propynyluracil; oligonucleotide derivatives wherein uracil of the oligonucleotide has been substituted with C-5 thiazoleuracil; oligonucleotide derivatives wherein cytosine of the oligonucleotide has been substituted with C-5 propynylcytosine; oligonucleotide derivatives wherein cytosine of the oligonucleotide has been substituted with phenoxazine-modified cytosine; oligonucleotide derivatives wherein ribose of the oligonucleotide has been substituted with 2'-*o*-propylribose; oligonucleotide derivatives wherein ribose of the oligonucleotide has been substituted with 2'-methoxyethoxyribose, and the like (Yokoyama K., Saibo Kogaku (Cell Technology) 16: 1463 (1997)).

2) Preparation of DNA encoding bcl9 protein and oligonucleotide comprising partial nucleotide sequence of the DNA

[0060] bcl9 protein has approximately 37% homology throughout the sequence at the amino acid sequence level to the mouse B9L protein obtained by the above-described methods. According to the present invention, the bcl9 protein was revealed to bind to β-catenin, and thus was considered as a β-catenin nuclear localizing protein. The nucleotide sequence of the full-length cDNA encoding human bcl9 protein and the amino acid sequence encoded by the cDNA are known in the art (Willis T. G. et al., Blood 91: 1871 (1998)). A DNA or oligonucleotide that encodes the bcl9 protein can be obtained from human cDNA or genomic DNA by the methods described in above (5-4), (7), and (8). Furthermore, bcl9 DNA from animals other than human, such as mouse, can be obtained using the same method as described in above (6).

2. Process for producing β-catenin nuclear localizing protein or partial peptide thereof, and method for measuring the activity thereof

[0061] A β-catenin nuclear localizing protein of the present invention, such as B9L protein or bcl9 protein, or a partial peptide thereof can be produced by expressing a DNA encoding the β-catenin nuclear localizing protein or partial peptide thereof prepared in above 1 in host cells according to methods described in "Molecular Cloning, Second Edition"; Glover D. M. and Hames B. D., "DNA Cloning 1: Core Techniques, A Practical Approach, Second Edition", Oxford University Press (1995), etc. Although a method is described below taking the B9L protein as an example, the bcl9 protein and partial peptides thereof can be also produced by the same method.

[0062] Specifically, the B9L protein of the present invention can be produced by preparing a recombinant vector wherein B9L DNA has been inserted into downstream of a promoter in an appropriate expression vector, introducing the vector to host cells to obtain a transformant expressing the B9L protein, and then culturing the transformant.

[0063] The expression vector to be used is a vector that is capable of autonomous replication or being integrated into chromosome in host cells and contains a promoter directing transcription from B9L DNA to mRNA in host cells.

[0064] Any host cells can be used including prokaryotic cells, yeast cells, animal cells, insect cells, plant cells, and the like, as far as the cells can express the gene of interest. Animal individuals and plant bodies are also usable.

[0065] When prokaryotes such as bacteria are used as host cells, then expression vectors to be used are capable of autonomous replication in the host prokaryote and B9L DNA should be placed downstream of a promoter containing ribosome-binding sequence. It is preferable that the distance between the ribosome-binding sequence and the initiation codon has been adjusted appropriately (for example, 6 to 18 nucleotides for a vector of *E. coli* host). It is preferable to place a transcription termination sequence immediately downstream of B9L DNA, although it is not essential in the invention. In addition, the vector should be designed to contain sequences for the expression of marker gene, such as drug-resistance genes, for the convenience of selection of transformants.

[0066] Any promoter can be used, as far as it has the ability to direct the expression in host cells. For example, when *E. coli* is used as a host, the promoters include promoters derived from *E. coli* and phage, such as *trp* promoter (P*trp*), *lac* promoter (P*lac*), P_L promoter, T7 promoter, P_R promoter, etc. It is also possible to use artificially designed or modified promoters, such as promoters wherein two P*trp* are tandemly connected to each other, or tac promoter, T7-*lac* promoter, let I promoter, etc. When *Bacillus subtilis* is used as a host, the promoters include promoters derived from SPO1 and SPO2 that are *Bacillus subtilis* phages, as well as PenP promoter.

[0067] The expression vector is exemplified, for example, by pSE280 (Invitrogen), pGEMEX-1 (Promega), pQE-8 (QIAGEN), pKYP200 (Agric. Biol. Chem. 48: 669 (1984)), pLSA1 (Agric. Biol. Chem. 53: 277 (1989)), pGEL1 (Proc. Natl. Acad. Sci. USA 82: 4306 (1985)), pBluescript II SK(-) (Stratagene), pKK223-3 (Amersham Pharmacia Biotech), pGEX-5X-3 (Amersham Pharmacia Biotech), and pET14 (Novagen).

[0068] The host cells can be microorganisms belonging to the genus *Escherichia*, the genus *Serratia,* the genus *Bacillus,* the genus *Brevibacterium*, the genus *Corynebacterium,* the genus *Microbacterium*, the genus *Pseudomonas,* and the like, for example, *Escherichia coli* XL1-Blue, *Escherichia coli* XL2-Blue, *Escherichia coli* DH1, *Escherichia coli* MC1000, *Escherichia coli* KY3276, *Escherichia coli* W1485, *Escherichia coli* JM109, *Escherichia coli* HB101, *Escherichia coli* No.49, *Escherichia coli* W3110, *Escherichia coli* NY49, *Serratia ficaria, Serratia fonticola, Serratia liquefaciens, Serratia marcescens, Bacillus subtilis, Bacillus amyloliquefaciens, Brevibacterium ammoniagenes, Brevibacterium immariophilum* ATCC14068, *Brevibacterium saccharolyticum* ATCC14066, *Corynebacterium glutamicum* ATCC13032, *Corynebacterium glutamicum* ATCC14067, *Corynebacterium glutamicum* ATCC13869, *Corynebacterium acetoacidophilum* ATCC13870, *Microbacterium ammoniaphilum* ATCC15354, *Pseudomonas* sp. D-0110.

[0069] Any method for introducing recombinant vectors can be used, as long as such methods have the ability to introduce DNAs to the above-mentioned host cells. Such methods include, for example, electroporation (Dower, W. J. et al., Nucleic Acids Res. 16: 6127 (1988)), methods using calcium ion (Cohen, S. N. et al., Proc. Natl. Acad. Sci. USA 69: 2110 (1972) ; Reid, J. D. et al., Gene 17: 107 (1982)), and protoplast method (Unexamined Published Japanese Patent Application No. (JP-A) Sho 63-248394; Chan, S. and Cohen, S. N., Mol. Gen. Genet. 168: 111 (1979)).

[0070] When yeast is used as a host cell, expression vectors to be utilized include vectors containing a promoter directing transcription in host yeast, B9L DNA, transcription termination sequence, and a sequence capable of expressing a maker gene for transformation in yeast (e.g., drug resistance genes and genes for amino acid biosynthesis such as TRP1, HIS3, and LEU2). Further, it is preferable to use an expression vector capable of autonomous replication and capable of expressing a drug-resistance gene that can be utilized as a marker for transformation in *E. coli* for the convenience of preparation and maintenance of the vector.

[0071] Any promoter can be used as long as it is operable in yeast. Such promoters include, for example, promoters of the alcohol dehydrogenase gene ADH1 and genes involved in galactose metabolism, e.g., GAL1, GAL10'; promoter of the acid phosphatase gene PHO5; promoter of the phosphoglycerate kinase gene PGK; promoter of the glycelaldehyde-3-phosphate dehydrogenase gene GAP; promoters of genes for heat shock proteins; promoter of α-mating factor gene MFα1; and promoter of the copper-metallothionein gene CUP1 derived from *Saccharomyces cerevisiae*; as well as promoter of alcohol oxidase gene AOX1 derived from *Pichia pastoris.*

[0072] The host cells include yeast strains belonging to the genus *Saccharomyces*, the genus *Schizosaccharomyces,* the genus *Pichia,* and the like, specifically, include *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Pichia pastoris*, etc.

[0073] Any method for introducing recombinant vectors can be used, as far as such methods have the ability to introduce DNAs to yeast. Such methods include, for example, electroporation (Becker, D. M. and Guarente, L., Methods. Enzymol. 194: 182 (1991)), spheroplast method (Shortel, D. et al., Proc. Natl. Acad. Sci. USA 81: 4889 (1984)), lithium acetate method (Ito, H. et al, Journal of Bacteriology 153: 163 (1983)), etc.

[0074] When animal cells are used as hosts, expression vectors to be utilized include vectors containing a promoter directing transcription in host animal cells, B9L DNA, and signal sequences for transcription termination and polyadenylation of the transcripts. Further, it is preferable to use an expression vector capable of autonomous replication and capable of expressing a drug-resistance gene that can be utilized as a marker for transformation in *E. coli* for the convenience of preparation and maintenance of the vector. Any promoter can be used, as far as it has the ability to direct the transcription in animal cells. Such promoters include virus-derived sequences, such as SV40 early promoter, promoter and enhancer elements of human cytomegalovirus IE (immediate early) gene, LTRs originating from retroviruses such as Rous sarcoma virus, human T cell leukemia virus I, Moloney murine leukemia virus, etc.; or promoters from genes, such as metallothionein gene, β-actin gene, elongation factor-1, and the like, derived from animal cells. Furthermore, it is possible to use artificial promoters wherein multiple promoter elements as listed above have been combined together, e.g., sRα promoter created by combining SV40 early promoter and LTR from human T cell leukemia virus I.

[0075] Cells wherein B9L DNA has been integrated in the host chromosomal DNA and which constitutively expresses B9L can be selected by introducing a B9L expression vector containing a sequence for the expression of a drug-resistance gene against a drug, such as G418 or hygromycin, into the host cells and culturing the cells in the presence of the drug. Furthermore, in order to increase the amount of B9L protein produced in host cells, a vector for constitutive expression of the B9L protein, which contains a sequence for the expression of the dihydrofolate reductase (dhfr) gene, is introduced into host cells, and the cells are cultured while the concentration of methotrexate, a dhfr inhibitor, is successively being increased; and thus it is possible to successfully achieve the amplification of the copy number of B9L DNA together with that of the dhfr gene. Such host cells, in which the gene amplification utilizing the dhfr gene is achieved, can be cells that have no functional dhfr gene, for example, CHO/dhfr$^-$ (ATCC: CRL-9096) and the like.

[0076] Vectors to be used for the preparation of the above-mentioned B9L expression vector specifically include, for example, pEGFP-C2 (Clontech), pAGE107 (Japanese Unexamined Patent Application No. 22979/91; Miyaji, H., Cytotechnology 3: 133, (1990)), pAS3-3 (Japanese Unexamined Patent Application No. 227075/90), pCDM8 (Seed, B., Nature 329: 840 (1987)), pcDNA3.1(+)(Invitrogen), pREP4 (Invitrogen), pBK-RSV (Stratagene), pSVK3 (Amersham

Pharmacia Biotech), pcDNA1.1/Amp (Invitrogen), and pAMo (Sasaki, K., J. Biol. Chem. 268: 22782 (1993)).

[0077] Expression vectors for B9L proteins in animal cells include pEGFP-C2B9L wherein the mouse B9L full-length cDNA is inserted at the *EcOR*I-*Sal*I site of the pEGFP-C2 vector. *Escherichia coli* MM294/pEGFP-C2B9L, a transformant comprising pEGFP-C2B9L, has been deposited under the accession number FERM BP-7291 in the International Patent Organism Depositary of the Independent Administrative Institution National Institute of Advanced Industrial Science and Technology (Tsukuba Central 6, 1-1-1 Higashi, Tsukuba-shi, Ibaraki, Japan 305-8566) (the former National Institute of Bioscience and Human-Technology, National Institute of Advanced Industrial Science and Technology (1-1-3 Higashi, Tsukuba-shi, Ibaraki, Japan 305-8566)) on September 6, 2000.

[0078] The host cells include the following cell lines: HeLa, Namalwa, and 293, which are derived from human; COS-1 and COS-7, which are kidney cells form African green monkey; CHO and BHK, which are derived from hamster; NHI3T3, which is derived from mouse embryo cell; mouse myeloma, SP2/0 and NS0; and rat myeloma YB2/0.

[0079] Any method for introducing recombinant vectors can be used, as far as such methods have the ability to introduce DNAs to animal cells. Such methods include, for example, electroporation (Miyaji, H. et al., Cytotechnology 3: 133 (1990)), calcium phosphate method (Japanese Unexamined Patent Application No. 227075/90), lipofection method (Felgner, P. L. et al., Proc. Natl. Acad. Sci. USA 84: 7413 (1987)), etc.

[0080] When insect cells are used as host cells, the baculovirus expression system (O' Reilly, D. R. et al., "Baculovirus Expression Vectors: A Laboratory Manual", W. H. Freeman and Company, New York (1992); Luckow, V. A. and Summers, M. D. et al., Bio/Technology 6: 47 (1988)) can be utilized. Specifically, after inserting B9L DNA in a vector called transfer vector, both vector and baculovirus are concurrently introduced into insect cells; the resulting homologous recombination provides a recombinant baculovirus in which B9L DNA has been inserted downstream of the polyhedrin gene promoter that is a highly efficient promoter; then, the recombinant baculovirus can be infected again to the insect cells, and thereby achieving the expression of B9L protein.

[0081] Such baculovirus to be utilized includes *Autographa californica* nuclear polyhedrosis virus, *Bombyx mori* nuclear polyhedrosis virus, etc. The insect cells to be used can be Sf9 and Sf21 that are cells derived from *Spodoptera frugiperda* (O' Reilly, D. R. et al., "Baculovirus Expression Vectors: A Laboratory Manual", W. H. Freeman and Company, New York (1992)), High5 (Invitrogen) which is a cell derived from *Trichoplusia ni* and the like. Alternatively, silkworm larvae *per se* are also usable. The transfer vector contains the polyhedrin promoter and a sequence derived from baculovirus for directing homologous recombination, also sequences for the maintenance and replication of vector as well as for the insertion of foreign genes (a sequence capable of autonomous replication in *E. coli* and a sequence of drug resistance gene), and the like for the convenience of gene manipulation in *E. coli.* Specifically such vectors include pVL1392, pVL1393, pBluebac4 (both from Invitrogen), etc.

[0082] B9L protein can be produced using animal individuals. For example, B9L protein can be produced in an animal body in which B9L DNA has been introduced according to a known method (Colman, A., American Journal of Clinical Nutrition 63: 639S (1996); Rosen, J. M. et al, American Journal of Clinical Nutrition 63: 627S (1996); Wright, G. et al., Bio/Technology 9: 830 (1991)).

[0083] Any promoter can be used, as far as it has the ability to direct the expression in animals. For example, α-casein promoter, β-casein promoter, β-lactoglobulin promoter, whey acidic protein promoter, and the like that are promoters specific to mammary gland cells are preferably used.

[0084] When plant cells or plant bodies are used as hosts, B9L can be produced according to known methods (Izawa, T., Sosiki Baiyou (Tissue Culture) 20: 6 (1994); Hashimoto, T., Sosiki Baiyou (Tissue Culture) 21: 14 (1995); Miele, L., Trends in Biotechnology 15: 45 (1997)).

[0085] Any promoter can be used for the expression of B9L DNA, as far as it has the ability to direct gene expression in plant cells. Such promoters include, for example, 35S promoter of cauliflower mosaic virus, actin-1 promoter of rice, etc. Furthermore, intron 1 of the maize alcohol dehydrogenase gene and the like can be inserted between the promoter and B9L DNA to be expressed to increase the expression efficiency of the B9L DNA.

[0086] The host cells can be plant cells derived from potato, tobacco, maize, rice, rape, soybeans, tomato, wheat, barley, rye, alfalfa, flax, etc.

[0087] Any method can be used for introducing recombinant vectors, as far as the method has the ability to introduce DNAs to plant cells. Such methods include, for example, methods using *Agrobacterium*, electroporation (Miyaji, H., Cytotechnology 3: 133 (1990)), methods using particle gun (gene gun), etc.

[0088] Plant cells or organs in which B9L DNA has been introduced can be cultured on a large scale using jar fermenter. Also, plant cells containing introduced genes can be regenerated to create plant bodies (transgenic plant) in which B9L DNA has been introduced.

[0089] Microorganisms, animal cells, or transformants derived from a plant cell, which contain a recombinant vector containing the B9L DNA of the present invention as an insert, can be cultured according to a typical culture method, then B9L protein is allowed to produce and accumulate therein, and the B9L protein is recovered from the culture in order to produce the B9L protein.

[0090] Media to be used for the cultivation of transformants obtained using animal cells as hosts include commonly

used RPMI1640 medium (The Journal of the American Medical Association 199: 519 (1967)), Eagle's MEM (Eagle, H. , Science 122: 501 (1952)), Dulbecco's modified Eagle's medium (Dulbecco, R. and Freeman, G., Virology 8: 396 (1959)), 199 medium (Proceeding of the Society for the Biological Medicine 73: 1 (1950)) and these media containing fetal calf serum or the like. If desired, an antibiotic such as penicillin or streptomycin may be added to the medium. Generally, the cultivation can be performed under a condition such as at a pH of 6 to 8, at 30 to 40°C, in the presence of 5% CO_2 for 1 to 7 days.

[0091] Media to be used for the cultivation of transformants obtained using insect cells as host cells include commonly used TNM-FH medium (Pharmingen); Sf-900 II SFM medium (Life-Technologies); ExCell400 and ExCell405 (both from JRH Biosciences); Grace' s Insect Medium (Grace, T. D. C., Nature 195: 788 (1962)), and the like. The preferable culture condition is at pH of 6 to 7; culture temperature is at 25 to 30°C, and culturing is carried out for 1 to 5 days. Further, if desired, an antibiotic, such as gentamicin, may be added to the medium during the culture.

[0092] When the transformant is an animal individual or a plant body, it is possible to produce B9L protein by breeding or cultivating it according to the usual method, allowing the B9L protein to produce and accumulate and recovering the B9L protein from the animal individual or plant body.

[0093] Specifically, in the case of an animal individual, for example, it is possible to produce the B9L protein by breeding a non-human transgenic animal containing B9L DNA, allowing the B9L protein encoded by the recombinant DNA to be produced and accumulated in the animal body, and recovering the B9L protein from the animal. The sites for the production and accumulation of the protein in the animal include, for example, milk and egg of the animal.

[0094] In the case of plant body, for example, it is possible to produce the B9L protein by cultivating transgenic plant containing B9L DNA, allowing the B9L protein encoded by the recombinant DNA to be produced and accumulated in the plant, and recovering the B9L protein from the plant.

[0095] Any of natural media and synthetic media can be used for the culture of transformant obtained using, as a host, a prokaryote such as *E. coli* or eukaryote such as yeast, as far as it contains carbon source, nitrogen source, inorganic salts, and the like which the organism can assimilate; and the culture of the transformant is achieved efficiently in it.

[0096] Any carbon source that is assimilated by the organisms can be used, including glucose, fructose, sucrose, and molasses containing thereof; carbohydrates such as starch and starch hydrolysate; organic acids such as acetic acid and propionic acid; alcohols such as ethanol and propanol.

[0097] Nitrogen source that can be utilized includes ammonia; ammonium chloride; ammonium salts of inorganic and organic acids, such as ammonium sulfate, ammonium acetate, ammonium phosphate; other nitrogen-containing compounds; as well as, peptone, meat extract, yeast extract, corn steep liquor, casein hydrolysate, soybean cake and soybean cake hydrolysate, various fermentation microorganisms, and digests thereof.

[0098] Such inorganic salts include potassium dihydrogenphosphate, dipotassium hydrogenphosphate, magnesium phosphate, magnesium sulfate, sodium chloride, ferrous sulfate, manganese sulfate, copper sulfate, calcium carbonate, etc.

[0099] The culture is generally carried out under an aerobic condition such as by shaking culture or submerged spinner culture under aeration. It is preferable to perform the culture at 15 to 40°C and generally for 16 to 96 hours. The pH should be maintained at 3.0 to 9.0 during the culture. The adjustment of pH can be conducted using an inorganic or organic acid, alkaline solution, urea, calcium carbonate, ammonia, etc. If desired, antibiotic such as ampicillin or tetracycline may be added to the medium during the culture.

[0100] In the case of culturing microorganisms transformed with an expression vector using an inducible promoter, if desired, an inducer can be added to the medium. For example, in the case of culturing microorganisms transformed with an expression vector using *lac* promoter, IPTG or the like can be added to the medium; and in the case of culturing microorganisms transformed with an expression vector using trp promoter, indoleacrylic acid or the like can be added to the medium.

[0101] The following general methods for the isolation and purification of proteins can be used to isolate and purify B9L protein accumulated in the culture of the above-mentioned transformant.

[0102] When B9L protein is secreted from the cells, the B9L protein accumulates in the medium. Accordingly, after the culture is completed, medium alone, from which the cells have been removed, can be recovered by techniques such as centrifugal separation. It is possible to obtain purified sample from the medium using general methods singly or in combination for the isolation and purification of proteins; specifically, solvent extraction; salting out using ammonium sulfate or the like; desalting; organic-solvent precipitation; anion exchange chromatography using resins, such as DEAE Sepharose, DIAION HPA-75 (Mitsubishi Chemical), and Mono-Q (Amersham Pharmacia Biotech); cation exchange chromatography using resins such as SP Sepharose (Amersham Pharmacia Biotech) ; hydrophobic chromatography using resins such as butyl-Sepharose and phenyl-Sepharose; gel filtration using molecular sieve; affinity-chromatography; chromato-focusing; electrophoretic techniques such as isoelectric focusing; etc.

[0103] When B9L protein is accumulated in cells of transformant, after the culture is completed, the cells of transformant are recovered from the culture by techniques, such as centrifugation, subsequently suspended in a buffer and then disrupted using a sonicator, French press, or the like to give cell-free extract.

[0104] When B9L protein is soluble in the cells, purified sample can be obtained from the supernatant after centrifuging the cell-free extract by the same method as used for the purification and isolation from the above-mentioned medium. Alternatively, when B9L protein is present as inclusion bodies in cells, the cell-free extract is treated by centrifugation, and then the inclusion bodies of the B9L protein can be recovered as precipitated fraction. The inclusion bodies of the B9L protein is solubilized by a protein denaturant, and then the resulting solution is dialyzed as to contain no protein denaturant or so dialyzed or diluted that such a low level of protein denaturant does not denature the protein to restore the normal tertiary structure of the B9L protein. Subsequently, purified sample can be obtained by the same method for the isolation and purification as described above.

[0105] In addition, B9L protein can be produced by in vitro transcription-translation system according to known methods (Kigawa, T., J. Biomolecular NMR 6: 129; Spirin, A. S., Science 242: 1162; Kigawa, T. and Yokoyama, S., J. Biochem. 110: 166 (1991)). Specifically, B9L DNA is ligated downstream of a promoter, such as SP6, T7, or T3 and an RNA polymerase specific to each promoter is allowed to react thereto for the synthesis of large amount of B9L RNA *in vitro*. Thus, the B9L protein can be produced by a cell-free translation system, e.g., translation system utilizing rabbit reticulocyte lysate or wheat germ extract.

[0106] The structural analysis for the purified B9L protein can be carried out by commonly used methods in protein chemistry, for example, a method as described in "Protein Structural Analysis for Gene Cloning" (H. Hirano, Tokyo Kagaku Doujin, 1993).

[0107] Whether a B9L protein or bc19 protein, or derivatives or partial fragments thereof wherein amino acids are substituted, deleted, or added to the amino acid sequence of the protein or proteins having homology to the protein at the amino acid sequence level (hereinafter referred to as B9L/bcl9 analogues) binds to β-catenin or not can be determined by detecting the transcription of reporter gene in the yeast two-hybrid system described in section 1 using a fusion protein expression vector comprising DNAs encoding a transcriptional activation domain and the protein, and a β-catenin bait plasmid.

[0108] Alternatively, the binding can be examined by the following steps: directly mixing a B9L/bcl9 analogue and β-catenin *in vitro* to allow binding reaction, or expressing a B9L/bcl9 analogue in cells to allow their binding in cells; then performing immunoprecipitation against the reaction mixture or cell lysate using an antibody against β-catenin; and detecting, by immunoblotting or the like, whether the B9L/bcl9 analogue is present in the precipitates. It can be also examined without the use of antibodies by the following steps: preparing a fusion protein of a B9L/bcl9 analogue and a protein or peptide, such as GST, that facilitates purification; performing binding reaction using β-catenin labeled with ^{35}S or the like; purifying the fusion protein of the B9L/bcl9 analogue; and detecting, by autoradiography or the like, whether the labeled β-catenin is present in the purified fraction.

[0109] The ability of B9L/bcl9 analogues to cause localization of β-catenin into the nucleus can be examined by fixing cells wherein both β-catenin and a B9L/bcl9 analogue are forcedly expressed, and cells wherein β-catenin alone is forcedly expressed, and detecting β-catenin in the cells, respectively, by immunostaining with anti-β-catenin antibody. In this case, normal β-catenin is restrained by degradation with GSK-3β in the cell. Therefore, use of phosphorylation site mutants that escape degradation and thus accumulates, for example, a β-catenin mutant wherein serine 33 is replaced with tyrosine, will increase the intracellular level of β-catenin and enables a more readily detection of β-catenin in the cell. β-catenin does not have a particular intracellular localization and distributes ubiquitously within cells wherein β-catenin alone is forcedly expressed. However, when B9L/bcl9 analogues have the activity to localize β-catenin into the nucleus, β-catenin is detected more in the nucleus than in the cytoplasm. Such B9L/bcl9 analogues with β-catenin nuclear localizing activity include B9L protein, and partial fragment thereof encoded by cDNA clones obtained by the yeast two-hybrid system in the present invention (corresponding to the region comprising the amino acid residues from 245 to 564 of the amino acid sequence of mouse B9L protein; represented by SEQ ID NO: 4).

[0110] B9L protein and bc19 protein increase the level of β-catenin localizing to the nucleus. Thus, the proteins are expected to promote transcriptional activation mediated by the β-catenin/TCF complex.

[0111] The effect of B9L/bcl9 analogues, including B9L and bcl9 proteins themselves, on transcriptional activation through the β-catenin/TCF complex following Wnt signal transduction can be examined using a plasmid, for example, pTOPFLASH and pTOPCAT (both described in Korinek V. et al., Science 275: 1784 (1997)), wherein a reporter gene, such as luciferase, chloramphenicol acetyltransferase, or β-galactosidase, is placed downstream of a promoter that contains a TCF binding sequence so that the transcription is activated by the_β-catenin/TCF complex. The above reporter gene expression plasmid and an expression plasmid of a mutant β-catenin, for example, wherein serine 33 is substituted with tyrosine, that can constitutively bind to a protein belonging to the TCF/Lef family to activate transcription are introduced into animal cells. Then, by measuring and comparing the expression levels of the reporter gene with and without further introduction of an expression plasmid of B9L/bcl9 analogue, it can be determined whether the B9L/bcl9 analogue can further promote the transcriptional activation by the β-catenin/TCF complex.

3. Preparation of antibody recognizing β-catenin nuclear localizing protein

(1) Preparation of polyclonal antibody

[0112] A polyclonal antibody can be prepared by immunizing animals using the full-length or partial protein of a β-catenin nuclear localizing protein obtained by the method described in the above section 2 or a partial peptide of a β-catenin nuclear localizing protein prepared by chemical synthesis with peptide synthesizer, and the like as an antigen.

[0113] Rabbits, goats, rats, mice, hamsters, and the like can be used as such animals for immunization. The dose of the antigen is preferably 50 to 100 μg per animal.

[0114] To use a partial peptide as the antigen, the partial peptide is preferably covalently conjugated with a carrier protein, such as KLH or bovine thioglobulin.

[0115] After the first administration, the antigen is administered 3 to 10 times at 1 to 2-week intervals. 3 to 7 days after each time of administration, blood is collected from the venous plexus of eyegrounds. Then the serum is tested for the reactivity to the antigen used for the immunization by a method of enzyme immuno-assay (Ishikawa, E., "Kousomeneki Sokuteihou (Methods of Enzyme Immuno-Assay)", Igakushoin (1978); Harlow, E. and Lane, D., "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press (1988), etc.

[0116] The polyclonal antibody can be obtained by collecting the sera from non-human mammals that have exhibited sufficiently high antibody titers in their sera against the antigen used for the immunization, and separating and purifying the sera.

[0117] Such methods for the separation and purification include centrifugal separation, salting out with 40 to 50% saturated ammonium sulfate, precipitation by caprylic acid (Harlow, E. and Lane, D., "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory, (1988)), and a procedure for processing using singly or in combination chromatographic methods, e.g., using DEAE-Sepharose column, anion exchange column, protein-A or -G column, gel filtration column, etc.

(2) Preparation of monoclonal antibody

(2-1) Preparation of antibody-producing cells

[0118] Rats, of which sera have exhibited sufficiently high titers of antibody against the antigen used for the immunization as describe above in (1), are provided as the source of antibody-producing cells.

[0119] 3 to 7 days after the final administration of the antigen substance to the rats which have exhibited such antibody titers, spleens are excised.

[0120] The spleens are sectioned into small pieces in MEM and crushed by forceps. After centrifugation at 1200 rpm for 5 minutes, the supernatant is discarded.

[0121] The resulting precipitated fraction of spleen cells is treated with Tris-ammonium chloride buffer (pH 7.65) for 1 to 2 minutes to remove red blood cells, then the spleen cells are washed 3 times with MEM. The spleen cells prepared are used as antibody-producing cells. (2-2) Preparation of myeloma cells

[0122] Myeloma cell to be used is a cell line established from mouse or rat. For example, 8-azaguanine resistant mouse (BALB/c-derived) myeloma cell lines that are usable include P3-X63Ag8-U1(P3-U1) (Yelton, D. E., Curr. Topics Microbiol. Immunol. 81: 1 (1978) ; Kohler, G. and Milstein, C., Eur. J. Immunol. 6: 511 (1976)), SP2/0-Ag14(SP-2) (Shulman, M. et al., Nature 276: 269 (1978)), P3-X63-8653(653) (Seeger, R. C. et al., J. Immunol. 123: 1548 (1979)), P3-X63-Ag(X63) (Kohler, G. and Milstein, C., Nature 256: 495 (1975)), and the like. Cells of these lines are passaged in 8-azaguanine medium [RPMI1640 medium containing 1.5 mmol/L glutamine, 5x 10^{-5} mol/L 2-mercaptomethanol, 10 μg/ml gentamicin, and 10% fetal calf serum (CSL) (hereinafter referred to as normal medium) further containing 15 μg/ml 8-azaguanine], but 3 to 4 days before the cell fusion the cells are cultured in the normal medium. 2x 10^7 or more cells are used for the fusion.

(2-3) Preparation of hybridoma

[0123] The antibody-producing cells prepared as described in (2-1) and myeloma cells in (2-2) are washed well with MEM or PBS (1.83 g of disodium phosphate, 0.21 g of potassium dihydrogenphosphate, 7.65 g of sodium chloride, 1 L of distilled water; pH 7.2), the cells are mixed with each other at a ratio of the numbers of antibody-producing cells : myeloma cells = 5 to 10: 1. After the mixture was subjected to centrifugation at 1200 rpm for 5 minutes, the supernatant is discarded.

[0124] The mixed cells prepared from the precipitated fraction are well dispersed. While the cells are being stirred at 37°C, 0.2 to 1 ml (per 10^8 antibody-producing cells) of solution of 2 g PEG-1000, 2 ml MEM, and 0.7 ml DMSO is added to the cell mixture; then 1 to 2 ml of MEM is added thereto several times at 1 to 2-minute intervals.

[0125] After the addition, the cells are prepared by further adding MEM so that the total volume becomes 50 ml.

[0126] The suspension prepared is subjected to centrifugation at 900 rpm for 5 minutes, and then the supernatant is discarded.

[0127] The cells from the resulting precipitated fraction are gently dispersed and then suspended by gentle pipetting with a measuring pipette in 100 ml of HAT medium (a medium wherein 10^{-4} mol/L hypoxanthine, $1.5x\ 10^{-5}$ mol/L thymidine, and $4x\ 10^{-7}$ mol/L aminopterin have been added to the normal medium).

[0128] A 100 μl aliquot of the suspension was dispensed into each well of a 96-well culture plate. Then the cells are cultured in an incubator with 5% CO_2 at 37°C for 7 to 14 days.

[0129] After the culture is completed, an aliquot of the culture supernatant is utilized for the selection of hybridomas specifically reacting to the antigen used for the immunization according to the enzyme immuno-assay method as described in "Antibodies-A Laboratory Manual" (Harlow, E. and Lane, D., Cold Spring Harbor Laboratory Press, Chapter14 (1988)), etc. to obtain the above-mentioned antibody-producing cells.

[0130] A specific example of the enzyme immuno-assay method is as follows:

[0131] An appropriate plate is coated with a purified sample of the full-length protein of the present invention or a partial fragment thereof used as an antigen for the immunization. The hybridoma culture supernatant or purified antibody obtained in (2-4) as described below is reacted as a primary antibody, and an anti-rat immunoglobulin antibody labeled with biotin, enzyme, chemically-luminescent substance, radioisotope, or the like is further reacted as a secondary antibody in the plate. Subsequently, a reaction is carried out according to the label substance, and cells exhibiting the specific reactivity to the protein of the present invention are selected as hybridomas producing monoclonal antibody against the protein of the present invention.

[0132] The hybridomas are cloned twice by limiting dilution method [with HT medium (HAT medium without aminopterin) in the first cloning, and with the normal medium in the second]. Cells that stably exhibit high antibody titers are selected as hybridoma lines producing monoclonal antibody against the protein of the present invention. (2-4) Preparation of monoclonal antibody

[0133] The hybridoma cells obtained in (2-3) producing monoclonal antibody against the protein of the present invention are intraperitoneally injected (5 to 20 x 10^6 cells per mouse) to 8 to 10-weeks old mice or nude mice which have been subjected to intraperitoneal administration of 0.5 ml pristane (2,6,10,14-tetramethylpentadecane), and have been bred for 2 weeks. The hybridomas form ascites carcinoma in 10 to 21 days.

[0134] The ascites is collected from each mouse having ascites tumor and then is subjected to centrifugation at 3000 rpm for 5 minutes to remove the solid material.

[0135] The monoclonal antibodies can be purified and prepared from the resulting supernatant by the same method as used for the preparation of polyclonal antibody.

[0136] Subtyping of antibody can be performed using a typing kit for mouse or rat monoclonal antibody. The quantity of protein can be calculated according to the Lowry method or by absorbance at 280 nm. 4. Method for detecting and quantifying mRNA encoding β-catenin nuclear localizing protein and method for diagnosing disease with altered protein expression due to mRNA level

[0137] Messenger RNA of β-catenin nuclear localizing protein can be detected using DNAs encoding the β-catenin nuclear localizing protein, such as B9L DNA comprising the nucleotide sequence represented by SEQ ID NO: 1 or bcl9 DNA; partial fragments of the DNA, for example, a fragment comprising 200 bp or more continuous nucleotides of the nucleotide sequence represented by SEQ ID NO: 1; or an oligonucleotide of the β-catenin nuclear localizing protein.

[0138] Methods for detecting mRNAs of β-catenin nuclear localizing proteins include northern blotting, *in situ* hybridization, quantitative PCR, differential hybridization, DNA chip, RNase protection assay, and the like. It is possible to examine the expression level of B9L protein in tissues or cells at the mRNA level according to these methods.

[0139] The above methods can be used to examine which tissue or cell types express β-catenin nuclear localizing proteins, and what kind of stimulus leads to changes in the expression level within cells.

[0140] Samples to be used in the method include biological samples such as organs, tissues or blood that are collected from human or animals, or primary culture cells or cell lines that are established from these biological samples.

[0141] Northern blotting is a method wherein total RNA or mRNA is extracted from sample (hereinafter referred to as "sample-derived RNA"); separated by gel electrophoresis; transferred to a membrane, such as nylon filter; hybridization is conducted with a probe prepared from a DNA encoding β-catenin nuclear localizing protein, or a DNA or oligonucleotide comprising a partial sequence of the DNA by labeling with radioisotope, digoxigenin, biotin, or the like; then washed to detect mRNA encoding β-catenin nuclear localizing protein as a band to which the labeled probe has been specifically bound. The method can be performed based on the method and conditions described in "Molecular Cloning, Second Edition". The intensity of the band, i.e., the amount of bound labeled probe reflects the amount of mRNA of β-catenin nuclear localizing protein. Therefore, β-catenin nuclear localizing protein can be quantified at the mRNA expression level by detecting, on the same filter, mRNA band of constitutively expressed actin, G3PDH (glyceraldehydes 3-phosphate dehydrogenase), or the like, which expression level does not alter due to the kind of tissue or clinical condition, and using the intensity of the band for normalization. The size of mRNA of a β-catenin nuclear localizing protein can be determined by loading a set of labeled RNA molecular markers on the same gel in the electrophoresis, and comparing

the migrated position of the band with the positions of the molecular markers.

[0142] *In situ* hybridization is a method wherein paraffin embedded slice or cryostat slice is prepared from a sample organ or tissue; hybridized with a labeled probe prepared from a DNA encoding β-catenin nuclear localizing protein, or a DNA or oligonucleotide comprising a partial sequence of the DNA; and then washed to detect cells or the site expressing the β-catenin nuclear localizing protein in detail. The method can be performed according to the method and conditions described in "Current Protocols in Molecular Biology".

[0143] Quantitative PCR (Delidow B.C. et al., Gene Anal. Tech. 6: 120 (1989)) is a method wherein PCR is performed using cDNA synthesized from sample-derived RNA using oligo-dT primer and reverse transcriptase (hereinafter referred to as sample-derived cDNA) as a template with oligonucleotide primers designed based on the nucleotide sequence of cDNA encoding β-catenin nuclear localizing protein to specifically amplify a DNA fragment derived from mRNA encoding the β-catenin nuclear localizing protein. The amount of the amplified DNA fragment reflects the amount of mRNA in the sample that encodes the β-catenin nuclear localizing protein. Thus, mRNA encoding β-catenin nuclear localizing protein can be quantified by using the result of PCR conducted for cDNA of actin, G3PDH (glyceraldehydes 3-phosphate dehydrogenase), or the like, which is constantly expressed among tissues regardless of the clinical conditions, as a control. The oligonucleotide primers are designed so as to specifically bind to a cDNA encoding the β-catenin nuclear localizing protein at an annealing temperature that does not permit hybridization between the primers or intraprimer hybridization, and to be released at a denaturation temperature. Quantification of the amplified DNA fragment must be done within numbers of PCR cycles wherein the amplified products are exponentially increasing. Such number of PCR cycles can be determined by performing PCR for a single sample with different numbers of cycles and analyzing the amount of the amplified DNA fragments with respect to the increase in the number by gel electrophoresis.

[0144] Differential hybridization (Lennon G.G. and Lehrach H., Trends Genet. 7: 314 (1991)) and DNA chip (Shalon D. et al., Genome Res. 6: 639 (1996)) are methods wherein a DNA encoding β-catenin nuclear localizing protein, or a DNA or oligonucleotide comprising a partial sequence of the DNA is immobilized onto a matrix, such as filter, slide glass, and silicon; hybridization is performed with a labeled cDNA probe that is synthesized from sample-derived RNA using oligo-dT primers, labeled dNTP, and reverse transcriptase; and washing is performed to detect the changes in the expression level of the β-catenin nuclear localizing protein in a sample to be measured. In both methods, difference in the mRNA expression level of the β-catenin nuclear localizing protein between the sample to be measured and a control sample can be detected by immobilizing a DNA for actin or G3PDH onto a filter or matrix as an internal control. Furthermore, for accurate quantification, different labeled dNTP may be used for preparing the labeled cDNA probes from the sample to be measured and the control sample, and the two labeled cDNA probe may be simultaneously hybridized on a single filter or matrix.

[0145] In RNase protection assay (Pape M.E. et al., Genet. Anal. Tech. Appl. 8: 206 (1991)), first, a promoter sequence, such as T7 promoter and SP6 promoter, is ligated to the 3'-end of a DNA encoding β-catenin nuclear localizing protein; and then labeled antisense RNA of the β-catenin nuclear localizing protein is synthesized using *in vitro* transcription system with labeled NTP and promoter specific RNA polymerase. The labeled antisense RNA is hybridized with total RNA or mRNA prepared from the sample to allow formation of RNA-RNA hybrid with mRNA of the β-catenin nuclear localizing protein in the sample. Then, digested with ribonuclease, and bands protected from the digestion by the ribonuclease due to hybrid formation is detected by gel electrophoresis. The expression of the β-catenin nuclear localizing protein can be quantified at the mRNA level by quantifying the protected band.

[0146] The above methods can be used for diagnosing diseases wherein the mRNA expression level of β-catenin nuclear localizing protein is increased or decreased in patient compared to normal healthy subject by quantifying and comparing the expression of the β-catenin nuclear localizing protein at the mRNA level between biological samples, such as organ, tissue, and blood, that are collected by biopsy from subject and healthy normal subject (serving as control) or primary culture cells cultured from the samples.

[0147] Such diseases include cancers, for instance, colon cancer. In cancer such as colon cancer, the expression of β-catenin nuclear localizing protein increases, and enhances the localization of β-catenin into the nucleus and transcriptional activation by β-catenin/TCF complex which can be a cause of cell canceration. Therefore, the expression of β-catenin nuclear localizing protein is considered to be increased in some cancer cells, such as colon cancer cells, compared to normal cells. Thus, when the expression of β-catenin nuclear localizing protein is diagnosed to be increased by the above method, the subject may suffer cancer. 5. Method for detecting mutations in gene encoding β-catenin nuclear localizing protein, and diagnostic method for diseases having mutations in the gene

[0148] The relationship between diseases, such as colon cancer, and the existence of a mutation in gene encoding β-catenin nuclear localizing protein can be evaluated using a DNA encoding the β-catenin nuclear localizing protein, or a DNA or oligonucleotide comprising a partial sequence of the DNA by comparing the nucleotide sequence of genomic DNA of the β-catenin nuclear localizing protein in a group of patients suffering the disease and a control group of normal healthy subjects, each group comprising 10 to 100 people.

[0149] Samples to be tested include genomic DNA extracted from biological samples such as organ, tissue, and blood, collected from human, or primary culture cells established from the samples; and cDNA prepared from total RNA or

mRNA extracted from the samples (hereinafter referred to as sample DNA).

[0150] The existence of mutations can be detected by amplifying a DNA fragment using the sample DNA as a template with primers designed based on the nucleotide sequence of B9L DNA, determining the nucleotide sequence of the amplified DNA fragment, and comparing the determined sequence to that of normal B9L DNA.

[0151] Methods of screening for the presence of a disease-related mutation in the B9L gene include single strand conformation polymorphism (SSCP) analysis (Sheffield V.C. et al., Genomics 16: 325 (1993)), mismatch digestion and denaturing gradient gel electrophoresis.

[0152] SSCP analysis is a method wherein primers that enable amplification of B9L DNA fragments smaller than 200 bp are designed based on the nucleotide sequence of DNA encoding B9L protein; PCR is performed using sample-derived DNA as a template and the primers; denaturing the amplified DNA fragments; and conducting electrophoresis on a non-denaturing polyacrylamide gel. The amplified DNA fragments can be detected as a band by labeling a primer with radioisotope or fluorescent dye in PCR, or staining the amplified DNA fragments with silver. As the mobility in a non-denaturing gel shifts by the difference in the nucleotide sequence, a mutation can be detected by comparing the mobility of the amplified fragments of sample DNA to that of B9L DNA having a normal nucleotide sequence.

[0153] Mismatch digestion is a method wherein PCR is performed using sample DNA as a template with primers designed based on the nucleotide sequence of B9L DNA; hybridizing the amplified DNA fragments with a B9L DNA having a normal nucleotide sequence that is labeled with radioisotope or fluorescent dye; and treating the hybridized DNA with osmium tetraoxide or T4 phage endonuclease VII (Dean M., Nat. Genet. 9: 103 (1995)) to digest the DNA at mismatched sites. The method is one of the most sensitive methods for detecting mutations, and is applicable to sample DNAs consisting of kilobases.

[0154] Denaturing gradient gel electrophoresis (DGGF) (Fischer S.G. and Lerman L.S., Proc. Natl. Acad. Sci. USA 80: 1579 (1983) ; Cariello N.F. and Skopek T.R., Mutat. Res. 288: 103 (1993)) is a method wherein DNA fragments are amplified using sample DNA as a template with primers that are designed based on the nucleotide sequence of B9L DNA; and the amplified fragments are electrophoresed on a gel with a concentration gradient of a chemical denaturant or temperature gradient. The presence of a mutation can be detected through the mobility that shifts with the change in temperature or denaturant concentration when the denaturation of the DNA fragment occurs due to a mutation in the nucleotide sequence. Addition of poly (G:C) to the terminus of the primers increases the sensitivity of detection (Sheffield V.C. et al., Proc. Natl. Acad. Sci. USA 86: 232 (1989)).

[0155] Mutations detected by the above methods can be statistically analyzed according to the method described in "Handbook of Human Genetics Linkage", The John Hopkins University Press (1994) to identify single nucleotide polymorphisms (SNPs) linked to a disease.

[0156] Once a mutation is identified as being linked to a disease such as cancer, such disease can be diagnosed by analyzing chromosomal DNA by southern hybridization with an oligonucleotide probe that can hybridize to the mutation site. Alternatively, diagnosis can be conducted by performing PCR with a oligonucleotide primer having a normal sequence corresponding to the mutation site in the disease at the 3'end, which utilizes the fact that the amplification by PCR does not occur unless the 3'-end is matched. Alternatively, diagnosis can be conducted through the nucleotide sequence analysis of the mutation site with B9L oligonucleotide sequence primers.

[0157] In addition, abnormality in B9L gene, such as deletion, change in the copy number, chromosome translocation, can be detected by performing southern hybridization on chromosomal DNA digested with appropriate restriction enzyme using a DNA encoding the B9L protein or a DNA comprising a partial nucleotide sequence of the DNA as a probe.

[0158] The protein truncation test (PTT) (van der Luijt R. et al., Genomics 20: 1 (1994)) is another method for screening a mutation in DNA. The method enables specific detection of frame shift mutations, which causes a stop codon in the middle of the translation frame resulting in truncation of a protein, mutation at splicing sites, nonsense mutation, and the like. In PTT, a particular primer wherein the T7 promoter sequence and eukaryotic translation initiation sequence are linked at the 5'-end of the nucleotide sequence of a DNA encoding B9L protein is designed to amplify cDNA fragments by RT-PCR from sample-derived RNA using the primer. mRNA is transcribed from the cDNA using *in vitro* transcription system that contains T7 RNA polymerase, and protein is produced from the mRNA using *in vitro* translation system. SDS-PAGE is conducted with the protein to compare its molecular size with that of the B9L protein translated from an mRNA of normal B9L protein. Thus, a mutation that causes deletion in the protein can be detected.

6. Determination of chromosomal location of gene encoding β-catenin nuclear localizing protein

[0159] Chromosomal location of a gene encoding β-catenin nuclear localizing protein can be determined by methods such as radiation hybrid (Science 250: 245 (1990)) and *in situ* hybridization (Annals of Human Genetics 45: 135 (1981); Cell 52: 51 (1988)), using the DNA encoding the protein.

[0160] Radiation hybrid is a method wherein PCR is conducted against multiple DNA panels comprising human chromosome fragments (fragments assigned to a specific chromosomal location by analysis using chromosome markers), such as Gene-Bridge 4, to specifically amplify a gene encoding β-catenin nuclear localizing protein, and the amplification

result is analyzed to determine detailed chromosomal location of the gene.

[0161] In *in situ* hybridization, human chromosome preparations are hybridized with a DNA encoding human B9L protein as a probe, and hybridized signal is detected to determine the location of the signal on the preparation. This method enables to determine not only the chromosome number that contains the gene of β-catenin nuclear localizing protein, but also the physical location on the chromosome. The probe may be labeled with radioisotope ^{3}H or biotin so that the signal can be detected by autoradiography or avidin labeled with fluorescent dye fluorescein isothiocyanate (FITC), respectively.

[0162] Alternatively, instead of directly detecting the chromosomal location of the gene of β-catenin nuclear localizing protein as in the above methods, the STS (sequence-tagged site) database (containing information of primers that are derived from the nucleotide sequences of a variety of ESTs, chromosomal DNA fragments that are amplified by the primers, and chromosomal location of the fragments) can be searched for a sequence that is homologous to the nucleotide sequence of the DNA encoding the B9L protein. When an STS having an identical nucleotide sequence to a part of the DNA encoding β-catenin nuclear localizing protein is discovered, the STS is considered to be corresponding to the gene encoding the β-catenin nuclear localizing protein on the chromosome. Thus, the chromosomal location of the STS is presumed to be the location of the gene.

[0163] In addition, a nucleotide sequence of a human genomic DNA in databases is normally accompanied with information on its chromosomal location. For instance, human genomic DNA sequences with GenBank accession numbers AP000877 and AP000909, which comprise the exon of human B9L genomic DNA obtained in above section 1. (6-2), are described to be located on human chromosome 11q23 in the database. Therefore, human B9L gene is concluded to be located on human chromosome 11q23.

[0164] The bcl9 gene has been reported to be located on human chromosome 1q21.

[0165] The information on the chromosomal location of genes encoding β-catenin nuclear localizing protein is useful for examining the relationship between the genes and diseases. For example, identification of regions wherein LOH (loss of heterozygosity: a chromosome deletion found in one of the pair of a gene) is detected at high frequency in many cancers as a chromosomal region highly expected to contain a tumor suppressor gene is in progress (inactivation of a tumor suppressor gene is considered to occur through a mutation in the other pair of the tumor suppressor gene that is within the region with LOH, and leads to the onset of cancer). When the region coincides with the chromosomal location of the gene of β-catenin nuclear localizing protein, the protein may be involved in the onset of cancer which has LOH in this region. In such cases, when the association of the β-catenin nuclear localizing protein with the cancer is clarified by analyzing mutation in the gene or expression of the β-catenin nuclear localizing protein, then diagnosis and treatment of such cancer can be performed using a DNA encoding the β-catenin nuclear localizing protein, the β-catenin nuclear localizing protein, or an antibody recognizing the protein.

7. Treatment of cancer by inhibiting the activity of β-catenin nuclear localizing protein

[0166] In cancer cells that have mutation in APC gene or β-catenin gene such as colon cancer cells, β-catenin accumulates due to the defect in the regulation of β-catenin degradation, and the transcriptional activation by a β-catenin/TCF complex cannot be suppressed. This derepression may be associated with the onset of cancer. It is considered that the transcriptional activation by β-catenin/TCF complex can be suppressed through the suppression of transfer of accumulated β-catenin into the nucleus by inhibiting the β-catenin nuclear localizing function of β-catenin nuclear localizing protein or suppressing the expression of the β-catenin nuclear localizing protein. Substances having such functions may be used as therapeutic agents for cancer.

(1) Inhibitor of β-catenin nuclear localizing protein and method of screening for the inhibitor

[0167] An inhibitor of β-catenin nuclear localizing protein is defined as a substance that inhibits the nuclear localization of β-catenin through inhibiting the binding between the β-catenin nuclear localizing protein and β-catenin. [0168]

[0168] Such a substance that inhibits the binding between a β-catenin nuclear localizing protein and β-catenin can be screened by contacting the β-catenin nuclear localizing protein and β-catenin, and comparing the bound amount of β-catenin nuclear localizing protein and β-catenin in the presence and absence of a test compound.

[0169] For example, the amount of β-catenin bound to the β-catenin nuclear localizing protein can be estimated by mixing and binding the β-catenin nuclear localizing protein with β-catenin that is labeled with ^{35}S or the like in the presence and absence of a test compound, isolating the β-catenin nuclear localizing protein using an antibody or the like, and measuring the amount of the labeled β-catenin in the isolated material. When the amount of bound β-catenin is decreased in the presence of a test compound compared to that in the absence, the compound is considered to be an inhibitor of the binding between β-catenin and the β-catenin nuclear localizing protein.

[0170] Whether such an inhibitor that inhibits the binding between β-catenin and a β-catenin nuclear localizing protein can block the nuclear localization of β-catenin can be examined by fixing cells that forcedly express both β-catenin and

the β-catenin nuclear localizing protein to which cells a test compound has been added or without addition, and detecting β-catenin by immunostaining using an anti-β-catenin antibody. In this case, as normal β-catenin is degraded by GSK-3β in cells. By using a mutated β-catenin such as a mutant wherein the serine 33 is substituted with tyrosine, that has a mutation at a phosphorylation site, and the mutated β-catenin accumulates without degradation. Therefore, the intracellular level of β-catenin increases and a detection of β-catenin becomes easy by using the mutated β-catenin. β-catenin itself does not have a particular intracellular localization tendency, and evenly distributes in cells upon forced expression of β-catenin alone. However, when a B9L/bcl9 protein analogue has a β-catenin nuclear localizing function, larger amount of β-catenin is found in the nucleus compared to the cytoplasm.

[0171] β-catenin and β-catenin nuclear localizing protein used for the above screening may be purified from tissue or cells expressing the proteins. However, large quantity of these proteins can be obtained by culturing a transformant that is transformed with a DNA encoding the proteins, and purifying them according to standard methods. The screening of the present invention can also be performed using cultured cells expressing the proteins, or processed cultured cells. However, when intact cells are used, it is preferable to use cells that secrete the proteins. Herein, processed cultured cells include concentrates of the cells, dried materials of the cells, cells obtained by centrifugation of the culture supernatant, dried cells, freeze-dried cells, detergent treated cells, sonicated cells, mechanically crushed cells, solvent treated cells, enzyme treated cells, protein fractions of the cells, fixed cells, and enzyme preparations extracted from the cells.

(2) Method for inhibiting the expression of β-catenin nuclear localizing protein

[0172] Methods for inhibiting the expression of a β-catenin nuclear localizing protein include: suppression of translation of the β-catenin nuclear localizing protein from mRNA by administering an antisense DNA or antisense oligonucleotide against the protein, or an antisense RNA expression vector; inhibition of transcription by administering an oligonucleotide that binds to the promoter of the gene of the β-catenin nuclear localizing protein to form a triple helix; degradation of mRNA of the β-catenin nuclear localizing protein by administering a ribozyme; and inhibition of transcription by administering a compound that specifically inhibits the transcription of the gene of β-catenin nuclear localizing protein.

8. Vector for gene therapy

[0173] For the treatment of diseases that are caused due to decreased expression levels of β-catenin nuclear localizing protein, the β-catenin nuclear localizing protein or a vector for gene therapy that produces the protein in human body can be administered.

[0174] Such vectors for gene therapy include recombinant virus vectors producing a β-catenin nuclear localizing protein. The recombinant virus vectors can be constructed by inserting the full-length cDNA of the human β-catenin nuclear localizing protein obtained according to the method described in section 1 downstream of a promoter in a virus vector. Alternatively, if necessary, a DNA fragment of a suitable size that comprises the region encoding the β-catenin nuclear localizing protein is prepared to insert the fragment downstream of a promoter in a virus vector. The recombinant virus vectors are defective in genes that encode proteins necessary for virus packaging. For example, retroviruses, such as mouse Moloney's leukemia virus, lack proteins, such as gag, pol, and env; lentiviruses, such as HIV, lack proteins, such as gag, pol, env, vpr, vpu, vif, tat, rev, and nef; adenoviruses lack proteins, such as E1A and E1B; and adeno-associated viruses lack proteins, such as Rep (p5, p19, p40), and Vp (Cap).

[0175] A recombinant virus vector is introduced into a suitable packaging cell. Any cells that can provide proteins required for virus packaging which are lost in the recombinant virus vector can be used for such a purpose, including, for example, HEK293 cells derived from human kidney, and mouse NIH 3T3 fibroblasts.

[0176] Any virus vector can be used so long as it can produce recombinant virus in the above packaging cells and contains a promoter at a position to enable transcription of a B9L gene in target cells. A plasmid vector such as MFG (Riviere I. et al., Proc. Natl. Acad. Sci. USA 92: 6733 (1995)), pBabepuro (Morgrnstern J.P. and Land H., Nucleic Acids Res. 18: 3587 (1990)), LL-CG, CL-CG, CS-CG, and CLG (Miyoshi H. et al., J. Virol. 72: 8150 (1998)), and pAdexl (Kanegae Y. et al., Nucleic Acids Res. 23: 3816 (1995)) can be used. Any promoter that can direct expression in human tissue can be used, including, for example, cytomegalovirus immediate early (IE) gene promoter,SV40 early promoter, retrovirus LTR, metallothionein promoter, heat shock protein promoters, and the like. In addition, the enhancer of the cytomegalovirus IE gene may be used with the promoter.

[0177] The above recombinant virus vector can be introduced into the above packaging cells by the calcium phosphate method, lipofection (Felgner P.L. et al., Proc. Natl. Acad. Sci. USA 84: 7413 (1987)), and the like.

[0178] Alternatively, gene therapy without the use of a virus vector includes transfection method of therapeutic gene (US 5589466) wherein naked plasmid DNA is'directly injected as a technique to directly transfer the DNA into desired tissue. Specifically, a β-catenin nuclear localizing protein can be expressed in tissue, such as colon cancer tissue, for which treatment is required by injecting an expression vector containing a DNA encoding the protein using a syringe or the like.

9. Method for detecting and determining β-catenin nuclear localizing protein using antibody

[0179] Methods for detecting a β-catenin nuclear localizing protein by an antibody that immunologically recognizes the protein include immunohistochemistry such as tissue-immunostaining and immunocytochemistry; flow cytometry; western blotting; enzyme immunoassay (EIA) such as sandwich ELISA; and radioimmunoassay (RIA), which can be performed according to the literature ("Monoclonal Antibody Experiment Manual", edited by Sakuji Toyama and Tomie Yasu, Kodan-sha Scientific (1987); "Biochemical Experiment Seminar Series 5: Immunobiochemical Study", Tokyo-Kagaku Dojin (1986) ; Goding J.W., "Monoclonal Antibodies: Principles and Practice, Third edition", Academic Press (1996) ; Harlow E. and Lane D., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory (1988)).

[0180] Immunohistochemistry is a method wherein tissue or cells are fixed; reacted with an antibody recognizing a β-catenin nuclear localizing protein followed by an anti-immunoglobulin antibody or fragment thereof that is labeled with fluorescent dye, enzyme, biotin, colloidal gold, radioisotope, or the like; then, if necessary, the labeled antibody is visualized; and the tissue or cells are examined under a microscope to detect the β-catenin nuclear localizing protein in the tissue or the cells. For fluorescent labeling, fluorescein isothiocyanate (FITC), tetramethylrhodamine isothiocyanate, or the like is used and are detected with a fluorescent microscope. For enzyme labeling, peroxidase, alkaline phosphatase, or the like is used, which can be detected by observing the specimen under a light microscope after the chromogenic reaction caused by the addition of a substrate allowing color to develop by the function of the enzyme. For biotin labeling, avidin that is conjugated with an enzyme such as peroxidase, is reacted, and then similar procedures used for enzyme-labeled antibody are performed. For colloidal gold labeling, the signal is detected by examining with an electron microscope. For radioisotope labeling, ^{125}I or the like may be used, and the detection can be conducted by coating with photosensitive emulsion and observing the silver grain developed by radiation with a light microscope.

[0181] Flow cytometry is a method wherein cells collected from a test subject are reacted with an antibody recognizing a β-catenin nuclear localizing protein followed by an anti-immunoglobulin antibody or fragment thereof that is labeled with a fluorescent dye such as FITC or phycoerythrin; and then the fluorochrome is measured on a flow cytometer to detect the expression of the β-catenin nuclear localizing protein in the cells.

[0182] Western blotting is a method wherein tissue sample or cells collected from a test subject, or homogenate thereof are separated by SDS-polyacrylamide gel electrophoresis ; then blotted on a PVDF membrane or nitrocellulose membrane; reacting the membrane with an antibody recognizing a β-catenin nuclear localizing protein or a fragment thereof, followed by an anti-immunoglobulin antibody or fragment thereof that is labeled with an enzyme such as peroxidase or alkaline phosphatase, or radioisotope, such as ^{125}I, to detect a band corresponding to the β-catenin nuclear localizing protein. When enzyme labeling is used, the detection is conducted by visualizing the band of the β-catenin nuclear localizing protein via the addition of a substrate that develops color through the reaction with the enzyme, or by autoradiography on an X-ray film via the addition of a substrate that emits light through the reaction with the enzyme. When radioisotope labeling is used, the band is detected by autoradiography on an X-ray film.

[0183] In sandwich ELISA, a variation of enzyme-linked immunoassays, two monoclonal antibodies with different antigen-recognition sites that recognize a β-catenin nuclear localizing protein are prepared, and then one of the monoclonal antibodies or fragment thereof is adsorbed on a plate and the other or fragment thereof is labeled with an enzyme, such as peroxidase or alkaline phosphatase. Cell homogenate is prepared from tissue or cells collected from a test subject and used as the test sample. The test sample is reacted with the antibody-adsorbed plate, the enzyme labeled anti-β-catenin nuclear localizing protein antibody or fragment thereof is reacted, a substrate that develops color by the enzyme is added to develop color, and the intensity of the color is measured on a spectrophotometer to detect or determine the β-catenin nuclear localizing protein in the sample.

[0184] Radioimmunoassay is a method wherein similar procedures as in enzyme immunoassay are carried out using an antibody labeled with radioisotope, such as ^{125}I, instead of enzyme are used to detect or determine a β-catenin nuclear, localizing protein in the sample by measuring the radioactivity with a scintillation counter.

[0185] In addition to the above sandwich assay, competitive assay is known for enzyme immunoassays and radioimmunoassay, wherein a preparation of a β-catenin nuclear localizing protein, instead of antibody, is labeled ; a proper amount of the labeled β-catenin nuclear localizing protein preparation and a test sample are reacted with an antibody recognizing the β-catenin nuclear localizing protein that is immobilized on a plate; and the enzyme activity or radioactivity on the plate is measured to detect or determine the β-catenin nuclear localizing protein in the sample.

10. Pharmaceutical composition

[0186] The above β-catenin nuclear localizing protein, compounds, and antibodies can be administered alone as therapeutic agents. However, generally, it is preferably provided as a pharmaceutical compositions by mixing them with one or more pharmaceutically acceptable carriers, and formulating by any conventional method of pharmaceutics. The agent is preferably administered via the most efficient method for the treatment, including oral administration and parenteral administration such as intraoral injection, intrarespiratory injection, intrarectal injection, subcutaneous injection, intra-

muscular injection and intravenous injection. Formulation for administration includes spray, capsule, tablet, granule, syrup, emulsion, suppository, injection, ointment and taping.

[0187] Appropriate formulations for oral administration include emulsion, syrup, capsule, tablet, powder and granule. For example, liquid preparations, such as emulsion and syrup, may be prepared using water; sugars, such as sucrose, sorbitol and fructose; glycols such as polyethylene glycol and propylene glycol; oil such as sesame oil, olive oil and soybean oil; antiseptics such as *p*-hydroxybenzoate esters; flavors such as strawberry flavor and peppermint; and the like as additives. Capsules, tablets, powder, granules and the like may be prepared using excipients such as lactose, glucose, sucrose and mannitol; disintegrants such as starch and sodium arginate; lubricants such as magnesium stearate and talc; binding agents such as polyvinyl alcohol, hydroxypropyl cellulose and gelatin; detergents such as fatty acid esters; plasticizers such as glycerin; and the like as additives.

[0188] An appropriate formulation for parenteral administration includes injection, suppository and spray. For example, injection may be prepared using a carrier consisting of a salt solution, glucose solution, or mixture of both; and the like. Suppository may be prepared using a carrier, such as cacao butter, hydrogenated fat or carbonate. Spray may be prepared from the protein as such or by using a carrier that disperses the protein as fine particles to facilitate absorption, but which does not stimulate oral cavity or respiratory mucosa of the recipient. Specifically, example of such carrier includes lactose and glycerin. It is possible to prepare aerosol or dry powder depending on the characteristic of the protein and the used carrier. The components described above as additives for oral administration can be also added for parenteral agents.

[0189] Dosage and frequency of administration may vary depending on the desired therapeutic effect, method of administration, duration of treatment, age, body weight, and the like, but normally it is 10 μg/kg to 100 mg/kg per day for an adult.

Brief Description of the Drawings

[0190]

Fig. 1 shows the comparison of the amino acid sequences of mouse B9L and human bcl9. Amino acid sequences of mouse B9L (upper) and human bcl9 (lower) are represented in single letter notation. * denotes identical residues between human bcl9 and mouse B9L, and - indicates the absence of a corresponding residue. The β-catenin binding site is underlined, and sequence similar to the nuclear localization signal is double underlined.

Fig. 2 shows the binding of β-catenin and B9L in cells. + under each lane indicates the expressed gene. Results of Western blotting using anti-β-catenin antibody as the primary antibody on immunoprecipitated cell lysates from COS-7 cells co-expressing β-catenin S33Y and GFP-B9L (center and right lanes), and β-catenin S33Y and GFP as a control (left lane) are shown. Antibodies used for immunoprecipitation are indicated on the top of each lane. Left and center: anti-GFP antibody; and right: anti-β-catenin antibody.

Best Mode for Carrying out the Invention

[0191] The present invention will be described below in detail with reference to Examples.

[Example 1] Cloning of B9L cDNA

[0192] A gene encoding a protein binding to mouse mβ-catenin arm was cloned by the yeast two-hybrid system.

(1) Preparation of bait plasmid for mβ-catenin arm

[0193] The nucleotide sequence of mouse β-catenin cDNA and the amino acid sequence of mouse β-catenin encoded by the cDNA are publicly known (GenBank accession No: M90364; Science 257: 1142 (1992)). Mouse β-catenin contains a repetitive sequence, which is called armadillo domain (mβ-catenin arm) in the region of residues 128 to 683 in its amino acid sequence. A DNA fragment of mouse β-catenin encoding this portion of mβ-catenin arm was amplified and isolated by PCR using cDNA from mouse cells as a template. The PCR primers were designed based on the nucleotide sequence of the portion of the cDNA encoding the above-mentioned mβ-catenin arm. The amplified DNA fragment was sequenced to confirm that it encodes mβ-catenin arm, and then the fragment was inserted into a vector pGBT9 (Clontech) between *Bam*HI/*Sal*I sites to prepare a plasmid for the expression of GAL4-β-catenin fusion protein in which β-catenin is fused with GAL4 BD.

(2) Screening using the two-hybrid system

[0194] Screening was carried out with MATCHMAKER mouse fetal (Swiss Webster/NIH mouse; 17-day embryo) cDNA library, which is a library to be used for the two-hybrid system and provided by Clontech. This cDNA library contains vector pGAD10 (Clontech) with cDNA insert and, as a selection marker, LEU2 gene involved in leucine biosynthesis in yeast, and can express fusion proteins of GAL4 AD and cDNA-encoding proteins through ADH1 promoter. Specific method for the screening was conducted according to the manual attached to the library from Clontech as follows.

[0195] Specifically, both mouse fetal cDNA library for the two-hybrid system and plasmid GAL4-β-catenin prepared in (1) were introduced in yeast *Saccharomyces cerevisiae* HF7C strain (Clontech). HF7C strain is a yeast strain that is tryptophan-, leucine-, and histidine-auxotrophic. The strain has HIS3 gene on the chromosome wherein the gene is involved in histidine biosynthesis and has been ligated downstream of GAL1 promoter to which GAL4 BD can bind, as well as *E. coli*-derived β-galactosidase gene lacZ ligated downstream of a nucleotide sequence to which GAL4 BD can bind as reporter genes (Gene 212: 197 (1998)). A transformant containing both plasmid GAL4-β-catenin and cDNA clone of a protein that binds to mβ-catenin arm and expresses the respective fusion proteins, is non-auxotrophic for histidine and is positive in β-galactosidase activity. It is due to the fact that GaL4 BD and GAL4 AD come close to each other by the binding of mβ-catenin arm and to activate the transcription of HIS3 gene and lacZ gene in downstream of the nucleotide sequence to which GAL4 BD binds. Finally, a colony, indicating positive for β-galactosidase activity, was selected by growing 1.2x 10^6 transformants on a medium without leucine, histidine, and tryptophan.

[0196] Plasmid DNA was recovered from the clone (selected colony), and the nucleotide sequence of the inserted cDNA fragment was determined as represented by SEQ ID NO: 3. Homology search for the nucleotide sequence was performed in the nucleotide sequence databases, and several mouse ESTs were found to be identical to the sequence. However, known gene with identical nucleotide sequence could not be found. Therefore, the above cDNA fragment isolated by the two-hybrid system was revealed to have a novel nucleotide sequence that encodes a protein binding to mouse β-catenin. The amino acid sequence of the protein encoded by the cDNA fragment is represented by SEQ ID NO: 4. The cDNA fragment comprising the nucleotide sequence represented by SEQ ID NO: 3 lacks a stop codon and the entire sequence encodes a protein; thus it was considered to be a part of full-length cDNA. The full-length cDNA was expected to contain extended sequences on both 5' and 3' ends of the nucleotide sequence represented by SEQ ID NO: 3. Thus, 5'-RACE and 3'-RACE were performed to amplify cDNA fragments containing nucleotide sequences further extending to the 5' and 3' directions, respectively, and their sequences were determined. By assembling the nucleotides sequence of the cDNA clone obtained by the two-hybrid system and those by 5'- and 3'-RACE, the nucleotide sequence represented by SEQ ID NO: 1 was obtained. The nucleotide sequence encodes a protein consisting of 1494 amino acids represented by SEQ ID: 2. The nucleotide sequence of the cDNA fragment isolated by the two-hybrid system corresponds to the nucleotides 733 to 1692 in the nucleotide sequence represented by SEQ ID NO: 1, and the amino acid sequence encoded by the cDNA fragment corresponds to the residues 245 to 564 in the amino acid sequence represented by SEQ ID NO: 2. This amino acid sequence was used for homology search against the amino acid sequence database in NCBI (National Center for Biotechnology Information) with the BLAST2 homology analysis program. As shown in Fig. 1, the sequence showed an overall homology of 37% to the bcl9 protein (Accession number CAA73942; Willis T. G. et al., Blood 91: 1871 (1998)). Thus, the β-catenin binding protein comprising the amino acid sequence represented by SEQ ID NO: 2 was dubbed B9L protein (bcl9 like protein). The above bcl9 protein is derived from human and the amino acid sequence of mouse bcl9 protein is not identified yet. However, there are a couple of EST clones (Accession numbers AI550007, and AI426858) in the GenBank nucleotide sequence database that are considered as the nucleotide sequence of a cDNA encoding mouse bcl9 protein, which nucleotide sequence of the clones exhibit a homology of 90% or more to the human bcl9 protein (the homology of the nucleotide sequence corresponding to that of B9L protein is 40% or lower). Thus, the obtained mouse B9L protein seems not to be the mouse bcl9 protein. The B9L protein is a proline-rich protein like the bcl9 protein, and contains a sequence similar to the nuclear localization signal.

[0197] By northern blot hybridization using the above cDNA as a probe against mRNA prepared from mouse embryo (day-13.5), an mRNA band of 7.5 kb was detected.

(3) Human B9L gene and its chromosomal location

[0198] Nucleotide sequences of human DNAs having homology to the nucleotide sequence of mouse B9L cDNA obtained in (2) was searched in nucleotide sequence databases including GenBank using homology search programs such as BLAST. As a result, human EST clones (GenBank accession numbers U46365 and R24762) that were predicted to be derived from human B9L cDNA, and working draft sequences of human genomic DNA (GenBank accession numbers AP000877, AP002357, and AP000909) comprising an exon sequence of human B9L genomic DNA were identified. These working draft sequences were compared with the nucleotide sequences of mouse B9L cDNA and human ESTs to obtain sequences of exons encoding human B9L as represented by SEQ ID NOs: 5 to 8. SEQ ID NOs: 5 to 8 are exons corresponding to the nucleotides 27 to 412, 413 to 532, 1183 to 3115, and 3116 to 3397, respectively,

in the sequence of mouse B9L cDNA (SEQ ID NO: 1). SEQ ID NO: 9 represents a partial nucleotide sequence of human B9L cDNA derived from SEQ ID NOs: 5 and 6 (corresponding to the nucleotides 27 to 532 in the nucleotide sequence of mouse B9L cDNA represented by SEQ ID NO: 1). SEQ ID NO: 10 represents a partial amino acid sequence of human B9L protein encoded by the nucleotide sequence represented by SEQ ID NO: 9 (corresponding to the residues 10 to 177 in the amino acid sequence of mouse B9L protein represented by SEQ ID NO: 2). SEQ ID NO: 11 represents a partial nucleotide sequence of human B9L cDNA derived from SEQ ID NOs: 7 and 8 (corresponding to the nucleotides 1183 to 3397 in the sequence of mouse B9L cDNA represented by SEQ ID NO: 1). SEQ ID NO: 12 represents a partial amino acid sequence of human B9L protein encoded by the nucleotide sequence represented by SEQ ID NO: 11 (corresponding to the residues 395 to 1132 in the amino acid sequence of mouse B9L protein represented by SEQ ID NO: 2).

[0199] AP000877 and AP000909 were described to be located on human chromosome 11q23 in the database. Thus, human B9L gene was considered to be located on human chromosome 11q23.

[Example 2] Binding of B9L protein and β-catenin *in vitro*

[0200] Direct binding of B9L protein and β-catenin was confirmed as follows. Protein consisting of the amino acid sequence of SEQ ID NO: 4, encoded by the B9L cDNA fragment obtained by the two-hybrid screening described in Example 1 was used in Examples 2 to 5 as B9L protein.

[0201] ^{35}S-labeled B9L protein was synthesized from the B9L cDNA fragment obtained by the two-hybrid method of Example 1 by *in vitro* transcription and translation using ^{35}S-labeled methionine and TNT®-coupled reticulocyte lysate system (Promega). *E. coli* transformed with GST-β-catenin fusion protein (hereinafter abbreviated as GST-β-catenin) expression plasmid (Nakamura et al., Genes to Cells 3: 395 (1998)) wherein mouse β-catenin DNA was inserted into the cloning site of *E. coli* glutathione-S-transferase (GST) expression plasmid vector pGEX5X-1 (Amersham Pharmacia Biotech), and pGEX5X-1, as a control, were cultured to prepare bacterial lysates, respectively. Glutathione-Sepharose 4B (Amersham Pharmacia Biotech) was added to the lysates to isolate GST-β-catenin or GST by adsorption. The glutathione-Sepharose 4B to which GST-β-catenin or GST had been adsorbed was reacted with the above ^{35}S-labeled B9L protein in buffer A (10 mM Tris-HCl (pH 8.0), 140 mM NaCl, 1 mM EGTA, 10 μg/ml leupeptin, 10 μg/ml aprotinin) containing 0.1% Triton X-100 at 4°C for 2 hr. The glutathione-Sepharose 4B was washed well with buffer A, and then SDS-PAGE sample buffer was added to elute bound proteins into the sample buffer. The eluate and ^{35}S-labeled B9L protein were used as a sample for SDS-PAGE, and then subjected to autoradiography. The result showed that a band corresponding to the ^{35}S-labeled B9L protein was detected in *E. coli* expressing GST-β-catenin, whereas no band was detected in *E. coli* expressing GST. Therefore, it was confirmed that the B9L protein directly binds to GST-β-catenin *in vitro* and that the site responsible for the binding with the B9L protein is located within the β-catenin moiety of the GST-β-catenin.

[Example 3] Binding of B9L protein and β-catenin in animal cells

[0202] The B9L cDNA fragment obtained in Example 1 was inserted into pEGFP-C2, green fluorescent protein (GFP) expression vector (Clontech), to construct an expression plasmid for GFP-B9L fusion protein (hereinafter abbreviated as GFP-B9L). Mutated mouse β-catenin S33Y cDNA wherein serine 33, the target site of GSK-3β-mediated phosphorylation, is substituted with tyrosine was subcloned into animal cell expression plasmid vector pMKITneo (Nakamura et al., Genes to cells 3: 395 (1998)) to construct an expression plasmid for S33Y β-catenin. These plasmids were transfected using LipofectAMINE (Life Technologies) into monkey kidney cell line COS-7 cells (ATCC: CRL-1651) for forced expression. After 24 hr, the cells were harvested to prepare cell lysates by adding buffer A containing 1% Triton X-100. The cell lysates were reacted with anti-GFP antibody or anti-β-catenin antibody at 4°C for 1 hr to form an immune complex between GFP-B9L or S33Y β-catenin and the respective antibody, and then Protein G-Sepharose 4B (Amersham Pharmacia Biotech), which is capable of binding to IgG, was added to the reaction solution to adsorb the immune complex. The Protein G-Sepharose 4B was washed well with lysis buffer A, and SDS-PAGE sample buffer was added to elute the immune complex. SDS-PAGE was conducted using the eluate as a sample, transferred onto polyvinylidene fluoride (PVDF) membrane, Immobilon-P (Millipore), and S33Y β-catenin was detect by Western blotting using anti-β-catenin antibody (Transduction Laboratory, raised in mice) and alkaline phosphatase-labeled anti-mouse IgG antibody as primary and secondary antibodies, respectively. As shown in Figure 2, a band was detected with anti-β-catenin antibody in the immunoprecipitate obtained using anti-GFP antibody, which was used to precipitate GFP-B9L. Thus, the result confirmed that GFP-B9L is bounds to β-catenin in cells as well.

[Example 4] Effect of B9L protein on subcellular localization of β-catenin

[0203] The plasmids for the expression of GFP-B9L and S33Y β-catenin constructed in Example 3 were transfected

alone or together into mouse NIH 3T3 cells using Effectene (QIAGEN), and were forcedly expressed. GFP expression vector pEGFP-C2, as a control, was also transfected into mouse NIH 3T3 cells and was forcedly expressed. After 24 hours, the cells were fixed with formaldehyde, stained with anti-β-catenin antibody, reacted with rhodamine isothiocyanate (RITC)-labeled anti-rabbit IgG antibody, and observed by fluorescence microscopy to detect S33Y β-catenin in the cell. GFP-B9L was directly detected by observation of GFP under fluorescence microscopy (with the use of FITC filter).

[0204] The result revealed that S33Y β-catenin was evenly distributed in the cytoplasm and the nucleus within cells expressing only S33Y β-catenin, whereas both S33Y β-catenin and GFP-B9L were localized in the nucleus in cells expressing both proteins. Thus, it was indicated that the B9L protein has a characteristic to bind to β-catenin to localize β-catenin into the nucleus. GFP-B9L was also localized to the nucleus by the expression of GFP-B9L alone. Due to the fact that, when GFP was expressed alone, GFP evenly distributed in the cytoplasm and nucleus, the nuclear localization of GFP-B9L was confirmed to be independent to the attached GFP and depends on the presence of the B9L protein.

[0205] The full-length B9L cDNA obtained in Example 1 was inserted into the *Eco*RI-*Sal*I site of pEGFP-C2 to prepare plasmid pEGFP-C2B9L for the expression in animal cells, cotransfected with S33Y β-catenin expression plasmid into mouse 3T3 cells and the plasmids were forcedly expressed as described above, and S33Y β-catenin was detected in the cells. Similar to the B9L protein fragment obtained by the two hybrid system, both S33Y β-catenin and GFP-B9L (full-length) were localized in the nucleus. Thus, the full-length B9L protein was also confirmed to bind to β-catenin and localizes β-catenin into the nucleus.

[Example 5] Binding form of B9L protein and β-catenin

(1) Binding region in β-catenin [0206]

[0206] As described in Example 1, the B9L protein was obtained as a protein binding to the armadillo domain of β-catenin (β-catenin arm) . To determine which region of the β-catenin arm binds to the B9L protein, partial fragments of the β-catenin arm were expressed as described below, and the binding of the respective β-catenin fragments to the B9L protein was examined. The bait plasmid encoding the β-catenin arm prepared in Example 1 was used as a template for PCR to amplify DNA encoding (a) almost the entire β-catenin arm (corresponding to residues 141 to 664 in the amino acid sequence of mouse β-catenin) ; (b) N-terminal half of the β-catenin arm (corresponding to residues 141 to 390 in the amino acid sequence of mouse β-catenin, i.e., armadillo repeats 1-6) ; and (c) C-terminal half of the β-catenin arm (corresponding to residues 391 to 664 in the amino acid sequence of mouse β-catenin, i.e., armadillo repeats 7-12) using a pair of sense and antisense primers represented by SEQ ID NOs: 13 and 16; 13 and 14; and 15 and 16, respectively. The amplified products were inserted into pGBT9 vector to prepared respective bait plasmids.

[0207] The respective bait plasmids were cotransfected with the B9L cDNA fragment-expressing clone obtained by the two-hybrid system in Example 1 into yeast HF7C strain, and the binding between the B9L protein and the respective β-catenin partial fragments were examined by the existence of β-galactosidase activity in the transformants. The result showed that the almost entire region and the N-terminal half (armadillo repeats 1-6) of the β-catenin arm were able to bind to the B9L protein but no binding could be detected for the C-terminal half of the β-catenin arm (armadillo repeats 7-12). Thus, it was considered that the B9L protein binds to the N-terminal region of β-catenin arm (armadillo repeats 1-6).

(2) Binding region in B9L protein

[0208] As described in Example 1, the B9L protein was suggested to bind to the β-catenin arm through the region of residues 245 to 564 in its amino acid sequence, due to the fact that a protein having the residues 245 to 564 in the amino acid sequence of the B9L protein represented by SEQ ID NO: 2 was isolated as a protein that binds to the β-catenin arm. In order to further examine which region of the B9L protein binds to the β-catenin arm, two-hybrid system was performed as follows. DNA fragments encoding residues 245-291, 292-439, 440-564, 245-439, and 292-564, respectively, in the amino acid sequence of the B9L protein represented by SEQ ID NO: 2 were amplified by PCR, and inserted into the pGAD424 vector (Clontech) to prepared expression plasmids for the two-hybrid system which express the respective B9L partial fragments as a fusion protein with GAL4 AD. Each plasmid was cotransfected with the β-catenin arm bait plasmid prepared in Example 1 into yeast HF7C strain, and the binding of each B9L protein partial fragment and β-catenin arm was examined based on the presence or absence of β-galactosidase activity in the transformants. The result showed that the B9L protein fragments comprising residues 292-439, 245-439, or 292-564 in the amino acid sequence represented by SEQ ID NO: 2 was capable of binding to the β-catenin arm, whereas no binding could be detected for the fragment of residues 245-291 or 440-564, which lacks residues 292-439. Thus, the B9L protein was indicated to bind to the β-catenin arm at the region of residues 292-439 in the amino acid sequence represented by SEQ ID NO: 2.

[Example 6] Binding actvity of bcl9 protein and β-catenin [0209]

[0209] The region determined to be the binding region with β-catenin in Example 5, residues 292-439 in the amino acid sequence of the B9L protein represented by SEQ ID NO: 2, corresponds to the residues 244-410 of protein bcl9 which is homologous to B9L protein. Whether this region of the bcl9 protein has the activity to bind to β-catenin was tested using similar method as described in Example 5.

[0210] Specifically, DNA fragment encoding the residues 244-410 in the amino acid sequence of human bcl9 protein was amplified by PCR, and inserted into pGAD424 vector (Clontech) to prepaerd an expression plasmid for two-hybrid system which expresses the partial fragment (244-410) of the bcl9 protein as a fusion protein with GAL4 AD. The plasmid was cotransfected with the β-catenin arm bait plasmid prepared in Example 1 into yeast HF7C strain. β-galactosidase activity was confirmed in the resulting transformant. Thus, the bcl9 protein was revealed to bind to β-catenin at the region of residues 244-410 in the amino acid sequence. The bcl9 protein has a sequence resembling the nuclear localization signal, and thus the protein was also predicted to bind to β-catenin and has the activity to localize β-catenin into the nucleus. Therefore, the bcl9 protein, DNAs encoding the bcl9 protein, antibodies recognizing the bcl9 protein, and oligonucleotides comprising a partial nucleotide sequence of a DNA encoding the bcl9 protein can be used for similar purposes as the B9L protein, DNAs encoding the B9L protein, antibodies recognizing the B9L protein, and oligonucleotides comprising a partial nucleotide sequence of a DNA encoding the B9L protein. The bcl9 protein is reported to be expressed abnormally high in CEMO-1 cell line, which was established from a patient with precursor-B-cell acute lymphoblastic leukemia (Willis T.G. et al., Blood 91: 1871 (1998)). This supports the hypothesis that the bcl9 protein is involved in tumor development by enhancing the transcriptional activation by β-catenin/TCF complex through localizing β-catenin into the nucleus like the B9L protein.

Industrial Applicability

[0211] The present invention provides novel β-catenin nuclear localizing protein and DNA encoding the protein. The use of the β-catenin nuclear localizing protein and DNA encoding the protein enables development of diagnostic and therapeutic agents for diseases, such as cancer, relating to nuclear localization of β-catenin.

"Sequence List free text"

[0212] SEQ ID NO: 13, Description of artificial sequence: sense primer for armadillo repeat 1 (downstream of Asn141) of mouse β-catenin comprising the *MunI* recognition site at the 5'-terminus.

[0213] SEQ ID NO: 14, Description of artificial sequence: antisense primer for armadillo repeat 6 (upstream of Asp390) of mouse β-catenin comprising the *Sal*I recognition site at the 5'-terminus.

[0214] SEQ ID NO: 15, Description of artificial sequence: sense primer for armadillo repeat 7 (downstream of Ala391) of mouse β-catenin comprising the *Mun*I recognition site at the 5'-terminus.

[0215] SEQ ID NO: 16, Description of artificial sequence: antisense primer for armadillo repeat 12 (upstream of Glu664) of mouse β-catenin comprising the SalI recognition site at the 5'-terminus.

SEQUENCE LISTING

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ccc agc tcc tcc cag atg atg tcc ttc cct cct cgg ctg cag caa cct 3696
Pro Ser Ser Ser Gln Met Met Ser Phe Pro Pro Arg Leu Gln Gln Pro
1220 1225 1230

cac ggt gcc atg gcc ccc acc ggg gcc ggg ggc cca ggc ctg cag cag 3744
His Gly Ala Met Ala Pro Thr Gly Ala Gly Gly Pro Gly Leu Gln Gln
1235 1240 1245

cac tac cct tca ggc atg gcc ctg ccc ccc gag gac ctg ccc acc cag 3792
His Tyr Pro Ser Gly Met Ala Leu Pro Pro Glu Asp Leu Pro Thr Gln
1250 1255 1260

cca ccg ggt ccc ata ccc ccc cag cag cac cta atg ggc aaa ggc atg 3840
Pro Pro Gly Pro Ile Pro Pro Gln Gln His Leu Met Gly Lys Gly Met
1265 1270 1275 1280

act ggc cgc atg ggc gac gca tac cca ccc ggg gtg ctc cct ggg gtg 3888
Thr Gly Arg Met Gly Asp Ala Tyr Pro Pro Gly Val Leu Pro Gly Val

1285 1290 1295

gca tcg gta ctg aat gac cca gag ctg agt gag gtg atc cgg ccc acc 3936
Ala Ser Val Leu Asn Asp Pro Glu Leu Ser Glu Val Ile Arg Pro Thr
1300 1305 1310

cct acc ggc att cct gag ttc gac tta tcc agg atc atc ccc tct gag 3984
Pro Thr Gly Ile Pro Glu Phe Asp Leu Ser Arg Ile Ile Pro Ser Glu
1315 1320 1325

aaa cca agc agc acc ctc cag tac ttc ccc aag agc gag aac cag ccc 4032
Lys Pro Ser Ser Thr Leu Gln Tyr Phe Pro Lys Ser Glu Asn Gln Pro
1330 1335 1340

ccc aag gcc cag ccc ccc aat ctg cat ctc atg aac ctg cag aac atg 4080
Pro Lys Ala Gln Pro Pro Asn Leu His Leu Met Asn Leu Gln Asn Met
1345 1350 1355 1360

atg gca gag cag acc ccg tct cga ccc ccc aac ctc ccg ggc caa cag 4128
Met Ala Glu Gln Thr Pro Ser Arg Pro Pro Asn Leu Pro Gly Gln Gln
1365 1370 1375

ggg gtc cag cgg ggg ctc agc atg tcc atg tgc cac cct gga cag atg 4176
Gly Val Gln Arg Gly Leu Ser Met Ser Met Cys His Pro Gly Gln Met
1380 1385 1390

tcc ttg ctg ggc agg aca ggt gtg ccc cca caa cag ggc atg gtg ccc 4224
Ser Leu Leu Gly Arg Thr Gly Val Pro Pro Gln Gln Gly Met Val Pro
1395 1400 1405

cat ggc ctg cac cag ggg gtc atg tcc cct cca caa ggc ctc atg acc 4272
His Gly Leu His Gln Gly Val Met Ser Pro Pro Gln Gly Leu Met Thr
1410 1415 1420

cag cag aat ttt atg ctg atg aag cag agg ggt gtg ggg ggc gag gtc 4320
Gln Gln Asn Phe Met Leu Met Lys Gln Arg Gly Val Gly Gly Glu Val
1425 1430 1435 1440

tac acc cag cct ccc cac atg ctc tcc cca cag ggc tcc ctc atg ggc 4368

Tyr Thr Gln Pro Pro His Met Leu Ser Pro Gln Gly Ser Leu Met Gly
1445 1450 1455

ccc cca ccc cag cag aac ctc atg gtg tcc cac cct ctg cgt cag cgc 4416
Pro Pro Pro Gln Gln Asn Leu Met Val Ser His Pro Leu Arg Gln Arg
1460 1465 1470

agt gtg tct ctg gac agc cag atg ggc tac ctg cca acg ccg ggc agc 4464
Ser Val Ser Leu Asp Ser Gln Met Gly Tyr Leu Pro Thr Pro Gly Ser
1475 1480 1485

atg gcc aat cta ccc ttc 4482
Met Ala Asn Leu Pro Phe
1490

<210> 2
<211> 1494
<212> PRT
<213> Mus musculus

<400> 2

Met Arg Ile Leu Ala Asn Lys Thr Arg Leu Pro His Pro Arg Arg Arg
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Glu Ala Pro Gly Ser Pro Pro Leu Ser Pro Arg Gly His Cys Pro Pro
20 25 30

Ala Pro Ala Lys Pro Met His Pro Glu Asn Lys Leu Thr Asn His Gly
35 40 45

Lys Thr Gly Asn Gly Gly Ala Gln Ser Gln His Gln Asn Val Asn Gln
50 55 60

Gly Pro Thr Cys Asn Leu Gly Ser Lys Gly Val Gly Ala Gly Ser His
65 70 75 80

Gly Ala Lys Ala Asn Gln Ile Ser Pro Ser Asn Ser Ser Leu Lys Asn
85 90 95

Pro Gln Ala Gly Val Ser Pro Phe Ser Ser Leu Lys Gly Lys Val Lys
100 105 110

Arg Glu Arg Ser Val Ser Val Asp Ser Gly Glu Gln Arg Glu Ala Gly
115 120 125

Thr Pro Ser Leu Asp Ser Glu Ala Lys Glu Val Ala Pro Arg Ser Lys
130 135 140

Arg Arg Cys Val Leu Glu Arg Lys Gln Pro Tyr Ser Gly Asp Glu Trp
145 150 155 160

Cys Ser Gly Pro Asp Ser Glu Glu Asp Asp Lys Pro Ile Ala Ala Ala
165 170 175

His Asn Cys Asn Val Ala Asp Pro Ala Met Val Thr Pro Gln Leu Gly
180 185 190

Pro Gly Gln Thr Ala Gln Leu Pro Leu Ser Glu Ser Ser Ala Pro Gly
195 200 205

Pro Gln His Gly Pro Gln Pro Gly Leu Arg Pro Asp Val Pro Gly Gly
210 215 220

Gly Gly Gly Gly Val Pro Gly Lys Pro Pro Ser Gln Phe Val Tyr Val
225 230 235 240

Phe Thr Thr His Leu Ala Asn Thr Ala Ala Glu Ala Val Leu Gln Gly
245 250 255

Arg Ala Glu Ser Ile Leu Ala Tyr His Gln Gln Asn Val Pro Arg Ala
260 265 270

Lys Leu Asp Gln Ala Pro Lys Val Pro Pro Thr Pro Glu Pro Leu Pro
275 280 285

Leu Asn Thr Pro Ser Ala Gly Thr Pro Gln Ser Gln Pro Pro Pro Leu
290 295 300

Pro Pro Pro Pro Pro Ala Pro Gly Ser Ala Pro Pro Ala Leu Pro Pro
305 310 315 320

Glu Gly Pro Pro Glu Asp Thr Ser Gln Asp Leu Ala Pro Asn Ser Val
325 330 335

Gly Ala Ala Ser Thr Gly Gly Gly Thr Gly Gly Thr His Pro Asn Thr
340 345 350

Pro Thr Ala Ala Thr Ala Asn Asn Pro Leu Pro Pro Gly Gly Asp Pro
355 360 365

Gly Ser Ala Pro Gly Ser Ala Leu Leu Gly Glu Ala Thr Pro Thr Gly
370 375 380

Asn Gly Gln Arg Asn Leu Val Gly Ser Glu Gly Leu Ser Lys Glu Gln
385 390 395 400

Leu Glu His Arg Glu Arg Ser Leu Gln Thr Leu Arg Asp Ile Glu Arg
405 410 415

Leu Leu Leu Arg Ser Gly Glu Thr Glu Pro Phe Leu Lys Gly Pro Pro
420 425 430

Gly Gly Ala Gly Glu Gly Gly Pro Pro Ala Gln Ala Pro Ser Ala Ala
435 440 445

Gln Pro Pro Pro Ser Ala Pro Pro Gly Gly Leu Lys Lys Tyr Glu Glu
450 455 460

Pro Leu Gln Ser Met Ile Ser Gln Thr Gln Ser Leu Gly Gly Pro Pro
465 470 475 480

Leu Glu His Glu Val Pro Gly His Pro Gln Gly Gly Asp Met Gly Gln
485 490 495

Gln Met Asn Met Met Met Gln Arg Leu Gly Gln Asp Ser Leu Thr Pro
500 505 510

Glu Gln Val Ala Trp Arg Lys Leu Gln Glu Glu Tyr Tyr Glu Glu Lys
515 520 525

Arg Arg Lys Glu Glu Gln Ile Gly Leu His Gly Gly Arg Pro Leu Gln
530 535 540

Asp Met Val Gly Met Gly Gly Met Met Gly Arg Gly Pro Pro Pro Pro
545 550 555 560

Tyr His Ser Lys Pro Gly Asp Gln Cys Ala Pro Gly Met Gly Ala Gln
565 570 575

Leu Arg Gly Pro Met Asp Val Gln Asp Pro Met Gln Leu Arg Pro Gly
580 585 590

Pro Pro Phe Pro Gly Pro Arg Phe Pro Gly Asn Gln Met Gln Arg Val
595 600 605

Pro Gly Phe Gly Gly Met Gln Ser Met Pro Met Glu Val Pro Met Asn
610 615 620

Ala Met Gln Arg Pro Val Arg Pro Gly Met Ala Trp Asn Glu Asp Leu
625 630 635 640

Pro Pro Ile Gly Gly Pro Ser Asn Phe Ala Gln Asn Ala Val Pro Tyr
645 650 655

Pro Gly Gly Gln Gly Glu Ala Glu Arg Phe Met Thr Pro Arg Val Arg
660 665 670

Glu Glu Leu Leu Arg His Gln Leu Leu Glu Lys Arg Ser Met Gly Met
675 680 685

Gln Arg Pro Leu Gly Met Ala Gly Ser Gly Met Gly Gln Ser Met Glu
690 695 700

Met Glu Arg Met Ile Gln Ala His Arg Gln Met Asp Pro Ala Met Phe
705 710 715 720

Pro Gly Gln Met Thr Gly Gly Asp Gly Leu Ala Gly Thr Pro Met Gly
725 730 735

Ile Glu Phe Gly Gly Gly Arg Gly Leu Leu Ser Pro Pro Met Gly Gln
740 745 750

Ser Gly Leu Arg Glu Val Asp Pro Pro Met Gly Pro Gly Asn Leu Asn
755 760 765

Met Asn Met Asn Val Asn Met Asn Met Asn Met Asn Leu Asn Val Gln
770 775 780

Met Thr Pro Gln Gln Gln Met Leu Met Ser Gln Lys Met Arg Gly Pro
785 790 795 800

Gly Asp Met Met Gly Pro Gln Gly Leu Ser Pro Glu Glu Met Ala Arg
805 810 815

Val Arg Ala Gln Asn Ser Ser Gly Met Met Gly Gly Pro Gln Lys Met
820 825 830

Leu Met Pro Ser Gln Phe Pro Asn Gln Gly Gln Gln Gly Phe Ser Gly
835 840 845

Gly Gln Gly Pro Tyr Gln Ala Met Pro Gln Asp Met Gly Asn Thr Pro
850 855 860

Asp Met Phe Ser Pro Asp Gln Ser Ser Val Pro Met Gly Thr Val Gly
865 870 875 880

Thr Ala Arg Leu Ser His Met Pro Leu Pro Pro Ala Ser Asn Pro Pro
885 890 895

Gly Ser Val His Leu Ala Ser Asn Arg Gly Leu Gly Arg Arg Pro Ser
900 905 910

Asp Leu Thr Ile Ser Ile Asn Gln Met Gly Ser Pro Gly Met Gly His
915 920 925

Leu Lys Ser Pro Thr Leu Ser Gln Val His Ser Pro Leu Val Thr Ser
930 935 940

Pro Ser Ala Asn Leu Lys Ser Pro Gln Thr Pro Ser Gln Met Val Pro
945 950 955 960

Leu Pro Ser Ala Asn Pro Pro Gly Pro Leu Lys Ser Pro Gln Val Leu
965 970 975

Ser Ser Ser Leu Gly Val Arg Ser Pro Thr Gly Ser Pro Ser Arg Leu
980 985 990

Lys Ser Pro Ser Met Ala Val Pro Ser Pro Gly Trp Val Ala Ser Pro
995 1000 1005

Lys Thr Ala Met Pro Ser Pro Gly Val Ser Gln Asn Lys Gln Pro Pro
1010 1015 1020

Leu Ser Ile Asn Ser Ser Ser Thr Leu Gly Asn Val Glu Gln Gly Ala
1025 1030 1035 1040

Leu Pro Pro Ser Ala Pro Arg Asn Ser Ser Ser Ala Pro Pro Ala Asn
1045 1050 1055

Pro Ser Ser Gly Leu Met Asn Pro Ser Leu Pro Phe Thr Ser Ser Pro
1060 1065 1070

Asp Pro Thr Pro Ser Gln Asn Pro Leu Ser Leu Met Met Ser Gln Met
1075 1080 1085

Ser Lys Tyr Ala Met Pro Ser Ser Thr Pro Leu Tyr His Asn Ala Ile
1090 1095 1100

Lys Thr Ile Ala Thr Ser Asp Asp Glu Leu Leu Pro Asp Arg Pro Leu
1105 1110 1115 1120

Leu Pro Pro Pro Pro Pro Pro Gln Gly Ser Gly Pro Gly Ile Ser Asn
1125 1130 1135

Asn Gln Pro Asn Gln Met His Met Asn Pro Ala Ala Ala Gln Ser Pro
1140 1145 1150

Met Gly Met Asn Leu Pro Gly Gln Gln Pro Leu Ser His Glu Pro Pro
1155 1160 1165

Pro Thr Met Leu Pro Ser Pro Thr Pro Leu Gly Ser Asn Ile Pro Leu
1170 1175 1180

His Pro Asn Ala Gln Gly Thr Gly Gly Ser Ser Gln Asn Ser Met Met
1185 1190 1195 1200

Met Ala Pro Gly Gly Pro Asp Ser Leu Asn Ala Pro Cys Gly Pro Val
1205 1210 1215

Pro Ser Ser Ser Gln Met Met Ser Phe Pro Pro Arg Leu Gln Gln Pro
1220 1225 1230

His Gly Ala Met Ala Pro Thr Gly Ala Gly Gly Pro Gly Leu Gln Gln
1235 1240 1245

His Tyr Pro Ser Gly Met Ala Leu Pro Pro Glu Asp Leu Pro Thr Gln
1250 1255 1260

Pro Pro Gly Pro Ile Pro Pro Gln Gln His Leu Met Gly Lys Gly Met
1265 1270 1275 1280

Thr Gly Arg Met Gly Asp Ala Tyr Pro Pro Gly Val Leu Pro Gly Val
1285 1290 1295

Ala Ser Val Leu Asn Asp Pro Glu Leu Ser Glu Val Ile Arg Pro Thr
1300 1305 1310

Pro Thr Gly Ile Pro Glu Phe Asp Leu Ser Arg Ile Ile Pro Ser Glu
1315 1320 1325

Lys Pro Ser Ser Thr Leu Gln Tyr Phe Pro Lys Ser Glu Asn Gln Pro
1330 1335 1340

Pro Lys Ala Gln Pro Pro Asn Leu His Leu Met Asn Leu Gln Asn Met
1345 1350 1355 1360

Met Ala Glu Gln Thr Pro Ser Arg Pro Pro Asn Leu Pro Gly Gln Gln
1365 1370 1375

Gly Val Gln Arg Gly Leu Ser Met Ser Met Cys His Pro Gly Gln Met
1380 1385 1390

Ser Leu Leu Gly Arg Thr Gly Val Pro Pro Gln Gln Gly Met Val Pro
1395 1400 1405

His Gly Leu His Gln Gly Val Met Ser Pro Pro Gln Gly Leu Met Thr
1410 1415 1420

Gln Gln Asn Phe Met Leu Met Lys Gln Arg Gly Val Gly Gly Glu Val
1425 1430 1435 1440

Tyr Thr Gln Pro Pro His Met Leu Ser Pro Gln Gly Ser Leu Met Gly
1445 1450 1455

Pro Pro Pro Gln Gln Asn Leu Met Val Ser His Pro Leu Arg Gln Arg
1460 1465 1470

Ser Val Ser Leu Asp Ser Gln Met Gly Tyr Leu Pro Thr Pro Gly Ser
1475 1480 1485

Met Ala Asn Leu Pro Phe
1490

<210> 3
<211> 960
<212> DNA
<213> Mus musculus

<220>
<221> CDS

<222> (1).. (960)

<400> 3

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Leu Ala Asn Thr Ala Ala Glu Ala Val Leu Gln Gly Arg Ala Glu Ser
 1               5                  10                  15

atc ctt gcc tac cac cag cag aat gtg cct cgg gcc aag ctg gat cag   96
Ile Leu Ala Tyr His Gln Gln Asn Val Pro Arg Ala Lys Leu Asp Gln
            20                  25                  30

gcc cct aaa gtg cca ccc acc cca gaa cca cta ccc ctg aat acg cca  144
Ala Pro Lys Val Pro Pro Thr Pro Glu Pro Leu Pro Leu Asn Thr Pro
        35                  40                  45

tca gca ggt aca cca cag tcc cag cca cct cct ttg cca ccg cca ccc  192
Ser Ala Gly Thr Pro Gln Ser Gln Pro Pro Pro Leu Pro Pro Pro Pro
    50                  55                  60

cca gcc cct ggc agt gcc cct cct gct ctg ccc ccg gag ggg cct cct  240
Pro Ala Pro Gly Ser Ala Pro Pro Ala Leu Pro Pro Glu Gly Pro Pro
65                  70                  75                  80

gaa gac acc agt cag gac ctg gcc ccc aac tca gtg gga gct gcc agt  288
Glu Asp Thr Ser Gln Asp Leu Ala Pro Asn Ser Val Gly Ala Ala Ser
                85                  90                  95

aca ggt ggt ggg act ggg ggt acc cac cct aac acc cca acg gct gcc  336
Thr Gly Gly Gly Thr Gly Gly Thr His Pro Asn Thr Pro Thr Ala Ala
            100                 105                 110

acc gct aac aac cct ctg cct cct gga gga gac cct ggc agt gcc cct  384
Thr Ala Asn Asn Pro Leu Pro Pro Gly Gly Asp Pro Gly Ser Ala Pro
        115                 120                 125

ggc tcc gcc cta ttg ggg gag gcc acg ccc acc gga aat ggg cag agg  432
Gly Ser Ala Leu Leu Gly Glu Ala Thr Pro Thr Gly Asn Gly Gln Arg
    130                 135                 140
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aac ctg gtg ggc tct gag ggc ctg tcc aaa gag cag ctg gag cac cgg   480
Asn Leu Val Gly Ser Glu Gly Leu Ser Lys Glu Gln Leu Glu His Arg
145                 150                 155                 160

gag cgc tcc ctc cag aca ctg cgg gac atc gag agg ctg ctg ctc cgc   528
Glu Arg Ser Leu Gln Thr Leu Arg Asp Ile Glu Arg Leu Leu Leu Arg
                165                 170                 175

agt ggg gag act gag ccc ttc ctc aag ggg ccc ccg gga gga gct ggt   576
Ser Gly Glu Thr Glu Pro Phe Leu Lys Gly Pro Pro Gly Gly Ala Gly
            180                 185                 190

gag gga ggc cca ccg gca caa gcg ccc tct gct gct caa ccg cct ccc   624
Glu Gly Gly Pro Pro Ala Gln Ala Pro Ser Ala Ala Gln Pro Pro Pro
        195                 200                 205

tcc gcc cct cct ggg ggg ctg aag aag tat gag gag cct ctg cag tca   672
Ser Ala Pro Pro Gly Gly Leu Lys Lys Tyr Glu Glu Pro Leu Gln Ser
    210                 215                 220

atg atc tca cag aca cag agc cta gga ggt ccc cct ctg gag cat gaa   720
Met Ile Ser Gln Thr Gln Ser Leu Gly Gly Pro Pro Leu Glu His Glu
225                 230                 235                 240

gtg ccg ggg cac cct cag ggt gga gac atg gga cag caa atg aac atg   768
Val Pro Gly His Pro Gln Gly Gly Asp Met Gly Gln Gln Met Asn Met
                245                 250                 255

atg atg cag agg ctg ggc cag gac agt ctg acg ccc gag cag gtg gcc   816
Met Met Gln Arg Leu Gly Gln Asp Ser Leu Thr Pro Glu Gln Val Ala
            260                 265                 270

tgg cgc aaa ctg cag gaa gag tac tac gag gag aag cgg cgg aaa gag   864
Trp Arg Lys Leu Gln Glu Glu Tyr Tyr Glu Glu Lys Arg Arg Lys Glu
        275                 280                 285

gag cag att gga ttg cac gga ggc cgc cct ctg cag gac atg gtg gga   912
Glu Gln Ile Gly Leu His Gly Gly Arg Pro Leu Gln Asp Met Val Gly
    290                 295                 300
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atg ggg ggt atg atg ggg agg ggg ccc cca cct cct tac cac agc aaa 960
Met Gly Gly Met Met Gly Arg Gly Pro Pro Pro Tyr His Ser Lys
305 310 315 320

<210> 4
<211> 320
<212> PRT
<213> Mus musculus

<400> 4

Leu Ala Asn Thr Ala Ala Glu Ala Val Leu Gln Gly Arg Ala Glu Ser
1 5 10 15

Ile Leu Ala Tyr His Gln Gln Asn Val Pro Arg Ala Lys Leu Asp Gln
20 25 30

Ala Pro Lys Val Pro Pro Thr Pro Glu Pro Leu Pro Leu Asn Thr Pro
35 40 45

Ser Ala Gly Thr Pro Gln Ser Gln Pro Pro Pro Leu Pro Pro Pro Pro
50 55 60

Pro Ala Pro Gly Ser Ala Pro Pro Ala Leu Pro Pro Glu Gly Pro Pro
65 70 75 80

Glu Asp Thr Ser Gln Asp Leu Ala Pro Asn Ser Val Gly Ala Ala Ser
85 90 95

Thr Gly Gly Gly Thr Gly Gly Thr His Pro Asn Thr Pro Thr Ala Ala
100 105 110

Thr Ala Asn Asn Pro Leu Pro Pro Gly Gly Asp Pro Gly Ser Ala Pro
115 120 125

Gly Ser Ala Leu Leu Gly Glu Ala Thr Pro Thr Gly Asn Gly Gln Arg
130 135 140

Asn Leu Val Gly Ser Glu Gly Leu Ser Lys Glu Gln Leu Glu His Arg
145 150 155 160

Glu Arg Ser Leu Gln Thr Leu Arg Asp Ile Glu Arg Leu Leu Leu Arg
165 170 175

Ser Gly Glu Thr Glu Pro Phe Leu Lys Gly Pro Pro Gly Gly Ala Gly
180 185 190

Glu Gly Gly Pro Pro Ala Gln Ala Pro Ser Ala Ala Gln Pro Pro Pro
195 200 205

Ser Ala Pro Pro Gly Gly Leu Lys Lys Tyr Glu Glu Pro Leu Gln Ser
210 215 220

Met Ile Ser Gln Thr Gln Ser Leu Gly Gly Pro Pro Leu Glu His Glu
225 230 235 240

Val Pro Gly His Pro Gln Gly Gly Asp Met Gly Gln Gln Met Asn Met
245 250 255

Met Met Gln Arg Leu Gly Gln Asp Ser Leu Thr Pro Glu Gln Val Ala
260 265 270

Trp Arg Lys Leu Gln Glu Glu Tyr Tyr Glu Glu Lys Arg Arg Lys Glu
275 280 285

Glu Gln Ile Gly Leu His Gly Gly Arg Pro Leu Gln Asp Met Val Gly
290 295 300

Met Gly Gly Met Met Gly Arg Gly Pro Pro Pro Tyr His Ser Lys
305 310 315 320

<210> 5
<211> 386
<212> DNA
<213> Homo sapiens

<400> 5

gttaccccac cccaggagga gagaagctcc agggagcccg ccgctgtccc cccgcggtca 60
ttgccccccc gccccagcca agccaatgca cccagaaaat aaattgacca atcatggcaa 120
gacagggaat ggcggggccc aatctcagca ccagaatgtg aaccaaggac ccacctgcaa 180
cgtgggctcg aagggcgtgg gggcggggaa ccatgggggc aaggccaacc agatctcgcc 240
tagcaactca agtctgaaga acccccaggc aggggtgccc cctttcagct cgctcaaggg 300
caaggtgaag agggaccgga gtgtgtctgt ggactctgga gagcagcgag aggctgggac 360
cccatccctg gattcagagg ccaaag 386

<210> 6
<211> 120
<212> DNA,
<213> Homo sapiens

<400> 6
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acgaatggtg ctctggaccg gacagtgagg aggacgacaa gcccattggg gccacccaca 120

<210> 7
<211> 1933
<212> DNA
<213> Homo sapiens

<400> 7

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gagcgactgc tgctccgcag cggagagact gagctcttcc tcaaggggcc cccaggagga 120
gcgggtgagg gagggccacc agcacaagcc ccccctccac cccagcagca acccatggcc 180
cctcccagtg ggctgaaaaa atatgaggac cctttgcagt ccatgatttc acagacacag 240
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gggcagcaga tgaacatgat gatacagagg ctgggccagg acagcctcac gcctgagcag 360
gtggcctggc gcaagctgca ggaggagtac tacgaagaga aacggcggaa agaggaacag 420
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agggggcccc cgcctcctta ccacagcaag cctggggatc agtggccacc tggaatgggt 540
gcgcagctgc gggggcccat ggatgttcaa gatcccatgc agctccgggg cggacctccc 600
tttcctgggc cccgtttccc aggcaaccag atacaacggg tacctgggtt tgggggcatg 660
cagagtatgc ccatggaggt gcccatgaat gccatgcaga ggcccgtgag accaggcatg 720
ggctggaccg aagacttgcc ccctatgggg ggacccagca attttgccca gaacaccatg 780

ccctacccag gtgggcaggg tgaggcggag cgattcatga ctccccgggt ccgtgaggag 840
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gcaggcagtg gcatgggaca gagcatggag atggagcgga tgatgcaggc gcaccgacag 960
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ctgagggagg tggacccacc catggggcca ggcaacctca acatgaacat gaatgtcaac 1140
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cctcctggga ccgtgcattc agccccaaac cgggggctag gcaggcggcc ttcggacctc 1560
accatcagta ttaatcagat gggctcaccg ggcatggggc acttgaagtc gcccaccctt 1620
agccaggtgc actcaccccc ggtcacctcg ccctctgcca acctcaagtc accccagact 1680
ccctcacaga tggtgccctt gccttctgcc aacccgccag gacctctcaa gtcgccccag 1740
gtcctcggct cctccctcag tgtccgttca cccactggct cgcccagcag gctcaagtct 1800
ccttccatgg cggtgccttc tccaggctgg gttgcctcac ctaagacggc catgcccagc 1860
ccgggggtct cccagaacaa gcagccgcct ctcaacatga actcttccac caccctgagc 1920
aacatggaac agg 1933

<210> 8
<211> 282
<212> DNA
<213> Homo sapiens

<400> 8

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gcggcctcat gaaccccagc ctaccattca cttcctcccc agaccccaca ccttcccaga 120
acccccctgtc actgatgatg acccagatgt ccaagtacgc catgcccagc tccacccccgc 180
tctaccacaa tgccatcaag accatcgcca cctcagacga cgagctgctg cccgaccggc 240
ccctgctgcc ccccccacca ccaccgcagg gctccgggcc ag 282

<210> 9
<211> 506
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (2).. (505)

<400> 9

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  Leu Pro His Pro Arg Arg Arg Glu Ala Pro Gly Ser Pro Pro Leu Ser
   1               5                  10                  15

ccc cgc ggt cat tgc ccc cct gcc cca gcc aag cca atg cac cca gaa  97
Pro Arg Gly His Cys Pro Pro Ala Pro Ala Lys Pro Met His Pro Glu
            20                  25                  30

aat aaa ttg acc aat cat ggc aag aca ggg aat ggc ggg gcc caa tct 145
Asn Lys Leu Thr Asn His Gly Lys Thr Gly Asn Gly Gly Ala Gln Ser
        35                  40                  45

cag cac cag aat gtg aac caa gga ccc acc tgc aac gtg ggc tcg aag 193
Gln His Gln Asn Val Asn Gln Gly Pro Thr Cys Asn Val Gly Ser Lys
    50                  55                  60

ggc gtg ggg gcg ggg aac cat ggg ggc aag gcc aac cag atc tcg cct 241
Gly Val Gly Ala Gly Asn His Gly Gly Lys Ala Asn Gln Ile Ser Pro
65                  70                  75                  80

agc aac tca agt ctg aag aac ccc cag gca ggg gtg ccc cct ttc agc 289
Ser Asn Ser Ser Leu Lys Asn Pro Gln Ala Gly Val Pro Pro Phe Ser
                85                  90                  95

tcg ctc aag ggc aag gtg aag agg gac cgg agt gtg tct gtg gac tct 337
Ser Leu Lys Gly Lys Val Lys Arg Asp Arg Ser Val Ser Val Asp Ser
            100                 105                 110

gga gag cag cga gag gct ggg acc cca tcc ctg gat tca gag gcc aaa 385
Gly Glu Gln Arg Glu Ala Gly Thr Pro Ser Leu Asp Ser Glu Ala Lys
        115                 120                 125

gag gtg gcg ccg cgg agt aag cgg cgc tgt gtg ctg gag cgg aag cag 433
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Glu Val Ala Pro Arg Ser Lys Arg Arg Cys Val Leu Glu Arg Lys Gln
130 135 140

ccg tac agt ggg gac gaa tgg tgc tct gga ccg gac agt gag gag gac 481
Pro Tyr Ser Gly Asp Glu Trp Cys Ser Gly Pro Asp Ser Glu Glu Asp
145 150 155 160

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Asp Lys Pro Ile Gly Ala Thr His
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35 40 45

Gln His Gln Asn Val Asn Gln Gly Pro Thr Cys Asn Val Gly Ser Lys
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Leu Arg Asp Ile Glu Arg Leu Leu Leu Arg Ser Gly Glu Thr Glu Leu
20 25 30

ttc ctc aag ggg ccc cca gga gga gcg ggt gag gga ggg cca cca gca 144
Phe Leu Lys Gly Pro Pro Gly Gly Ala Gly Glu Gly Gly Pro Pro Ala
35 40 45

caa gcc ccc cct cca ccc cag cag caa ccc atg gcc cct ccc agt ggg 192
Gln Ala Pro Pro Pro Pro Gln Gln Gln Pro Met Ala Pro Pro Ser Gly
50 55 60

ctg aaa aaa tat gag gac cct ttg cag tcc atg att tca cag aca cag 240
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agc cta ggg ggc ccc ccg ctg gag cat gaa gtg cct ggg cac ccc ccg 288
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85 90 95

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Gly Gly Asp Met Gly Gln Gln Met Asn Met Met Ile Gln Arg Leu Gly
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cag gac agc ctc acg cct gag cag gtg gcc tgg cgc aag ctg cag gag 384
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165 170 175

cct gga atg ggt gcg cag ctg cgg ggg ccc atg gat gtt caa gat ccc 576
Pro Gly Met Gly Ala Gln Leu Arg Gly Pro Met Asp Val Gln Asp Pro
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atg cag ctc cgg ggc gga cct ccc ttt cct ggg ccc cgt ttc cca ggc 624
Met Gln Leu Arg Gly Gly Pro Pro Phe Pro Gly Pro Arg Phe Pro Gly
195 200 205

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Gln Lys Met Arg Gly Pro Gly Asp Leu Met Gly Pro Gln Gly Leu Ser
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Gln Gln Gly Phe Ser Gly Gly Gln Gly Pro Tyr Gln Ala Met Ser Gln
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ccc atg agc aac gtg ggc acc acc cgg ctc agc cac atg cct ctg ccc   1488
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Gly Ser Arg Pro Leu Gln Asp Met Met Gly Met Gly Gly Met Met Val
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Gly Trp Val Ala Ser Pro Lys Thr Ala Met Pro Ser Pro Gly Val Ser
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<210> 16
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Claims

1. A protein selected from the group consisting of:

(a) a protein comprising the amino acid sequence of amino acid residues 292 to 439 in the amino acid sequence represented by SEQ ID NO: 2;
(b) the protein according to (a), wherein the protein comprises the amino acid sequence represented by SEQ ID NO: 2 or 4;
(c) a protein comprising the amino acid sequence represented by SEQ ID NO: 10;
(d) a protein comprising the amino acid sequence represented by SEQ ID NOs: 10 and 12;
(e) a protein consisting of the amino acid sequence, wherein one to five amino acids are added, deleted, or substituted in the amino acid sequence of the protein according to any one of (a) to (d), wherein said protein binds to β-catenin and has the activity to localize β-catenin into the nucleus;
(f) a protein consisting of an amino acid sequence having a homology of 60% or more to the amino acid sequence of the protein according to any one of (a) to (d), wherein said protein binds to β-catenin and has the activity to localize β-catenin into the nucleus; and
(g) the protein according to (c) or (d), wherein the protein consists of an amino acid sequence having a homology of 60% or more to the amino acid sequence represented by SEQ ID NO: 2, and binds to β-catenin and has the activity to localize β-catenin into the nucleus.

2. A DNA selected from the group consisting of:

(a) a DNA encoding the protein of claim 1;
(b) a DNA comprising the nucleotide sequence of nucleotides 874 to 1317 in the nucleotide sequence represented by SEQ ID NO: 1;
(c) the DNA according to (b), wherein the DNA comprises the nucleotide sequence represented by SEQ ID NO: 1 or 3;
(d) a DNA encoding a protein that binds to β-catenin and has the activity to localize β-catenin into the nucleus, and comprising the nucleotide sequence of the following (i) or (ii):

(i) the nucleotide sequence represented by SEQ ID NO: 9; and
(ii) the nucleotide sequence represented by SEQ ID NOs: 9 and 11;

(e) a DNA hybridizing to the DNA of any one of (a) to (d) under stringent conditions, which encodes a protein that binds to β-catenin and has the activity to localize β-catenin into the nucleus;
(f) a DNA comprising the nucleotide sequence represented by SEQ ID NOs: 9 and 11, and consisting of the nucleotide sequence encoding a protein consisting of the amino acid sequence having a homology of 60% or more to the amino acid sequence of SEQ ID NO: 2, wherein said protein binds to β-catenin and has the activity to localize β-catenin into the nucleus; and
(g) a DNA comprising the nucleotide sequence represented by SEQ ID NO: 11.

3. A recombinant DNA obtainable by inserting the DNA according to claim 2 into a vector.

4. A non-human transformant obtainable by introducing the recombinant DNA according to claim 3 into a host cell.

5. A process of producing the protein according to claim 1, which comprises the steps of:

(a) culturing the transformant of claim 4 in a culture medium;
(b) producing and accumulating the protein according to claim 1 in the culture; and
(c) recovering the protein from the culture.

6. An in vitro method of detecting or quantifying, at the mRNA level, the expression level of the protein according to any one of (c) to (g) of claim 1, using the DNA according to claim 2, or an oligonucleotide comprising 10 to 60 continuous nucleotides of the nucleotide sequence of the DNA according to claim 2 or derivative thereof.

7. An in vitro method of detecting a disease wherein the expression level of the protein according to any one of (c) to (g) of claim 1 is increased or decreased in a patient compared to normal healthy subject by measuring and comparing the expression level of the protein in normal healthy subject and test subject at the mRNA level by using the DNA according to claim 2, or an oligonucleotide comprising 10 to 60 continuous nucleotides of the nucleotide sequence of the DNA according to claim 2 or derivative thereof.

8. A diagnostic agent for a disease that exhibits increased or decreased expression level of the protein according to any one of (c) to (g) of claim 1 in a patient compared to normal healthy subject upon a measurement and comparison of the expression level of the protein at the mRNA level, wherein the diagnostic agent comprises the DNA according to claim 2.

9. An in vitro method of detecting a mutation in a gene encoding the protein according to any one of (c) to (g) of claim 1 using the DNA according to claim 2, or an oligonucleotide comprising 10 to 60 continuous nucleotides of the nucleotide sequence of the DNA according to claim 2 or derivative thereof.

10. An in vitro method of detecting a disease which has a mutation in a gene encoding the protein according to any one of (c) to (g) of claim 1 using the DNA according to claim 2, or an oligonucleotide comprising 10 to 60 continuous nucleotides of the nucleotide sequence of the DNA according to claim 2 or derivative thereof.

11. A diagnostic agent for a disease which has a mutation in a gene encoding the protein according to any one of (c) to (g) of claim 1, comprising the DNA according to claim 2.

12. An in vitro method of inhibiting the expression of the protein according to any one of (c) to (g) of claim 1 at the transcriptional level using the DNA according to claim 2, or an oligonucleotide comprising 10 to 60 continuous nucleotides of the nucleotide sequence of the DNA according to claim 2 or derivative thereof.

13. An in vitro method of inhibiting the nuclear localization of β-catenin, using an oligonucleotide or a DNA selected from the group of:

(i) the DNA according to claim 2;
(ii) an oligonucleotide comprising 10 to 60 continuous nucleotides of the nucleotide sequence of the DNA according to claim 2 or derivative thereof;
(iii) a DNA encoding bcl9 protein (Figure 1; lower);
(iv) an oligonucleotide comprising 10 to 60 continuous nucleotides of the nucleotide sequence of the DNA encoding bcl9 protein (Figure 1; lower); and
(v) an oligonucleotide comprising a nucleotide sequence complementary to the oligonucleotide of (iv), or a derivative thereof.

14. A therapeutic agent for cancer comprising the DNA according to claim 2.

15. A vector for gene therapy comprising the DNA according to claim 2.

16. A non-human transgenic animal generated by introducing the DNA according to claim 2.

17. A method of using the non-human transgenic animal according to claim 16, or a non-human transgenic animal generated by introducing a DNA encoding bcl9 protein (Figure 1; lower) as an animal model for carcinogenesis.

18. A method of evaluating a therapeutic agent for cancer using the transgenic animal according to claim 16, or a transgenic animal generated by introducing a DNA encoding bcl9 protein (Figure 1; lower).

19. A genetically defective non-human animal, wherein the function of a protein to bind to β-catenin and to localize β-catenin into the nucleus is lost or lowered due to the deletion of all or a part of the DNA according to claim 2.

20. An in vitro method of screening for a substance that inhibits the binding of bcl9 protein (Figure 1; lower) and β-catenin, comprising the steps of:

(a) comparing the binding of bcl9 protein (Figure 1; lower) and β-catenin in the absence or presence of a test sample; and

(b) selecting a substance that inhibits the binding of the bcl9 protein and β-catenin from the test sample.

21. An in vitro method of screening for a substance that inhibits the binding of β-catenin and the protein according to claim 1, comprising the steps of:

(a) comparing the binding of β-catenin and the protein in the absence or presence of a test sample; and
(b) selecting a substance that inhibits the binding of β-catenin and the protein according to claim 1 from the test sample.

22. An antibody recognizing the protein according to claim 1.

23. A method of immunologically detecting or quantifying the protein according to claim 1 using the antibody according to claim 22.

24. A neutralizing antibody that binds to the protein according to claim 1, and thereby inhibiting the activity of the protein to bind to β-catenin and to localize β-catenin into the nucleus.

25. An in vitro method of detecting a disease wherein the expression level of the protein according to any one of (c) to (g) of claim 1 is increased or decreased in a patient compared to normal healthy subject, by quantifying and comparing the expression level of the protein in normal healthy subject and a test subject by using the antibody according to claim 22.

26. A diagnostic agent comprising the antibody according to claim 22, for a disease wherein the expression level of the protein according to any one of (c) to (g) of claim 1 is increased or decreased in a patient compared to normal healthy subjects.

27. An in vitro method of inhibiting the transcriptional activation by a complex of β-catenin and transcription factor belonging to the Lef/Tcf family by inhibiting the function of the protein according to claim 1 by using the antibody according to claim 24.

28. An in vitro method of inhibiting the transcriptional activation by a complex of β-catenin and transcription factor belonging to the Lef/Tcf family by inhibiting the function of bcl9 protein (Figure 1; lower) by using an antibody that binds to the bcl9 protein, and thereby inhibiting the activity of the bcl9 protein to bind to β-catenin and to localize β-catenin into the nucleus.

29. A therapeutic agent for cancer comprising the antibody according to claim 24.

30. Use of the DNA according to claim 2 or an oligonucleotide comprising 10 to 60 continuous nucleotides of the nucleotide sequence of the DNA according to claim 2 or derivative thereof for the preparation of a diagnostic composition for detecting or quantifying, at the mRNA level, the expression level of the protein according to any one of (c) to (g) of claim 1.

31. Use of the DNA according to claim 2 or an oligonucleotide comprising 10 to 60 continuous nucleotides of the nucleotide sequence of the DNA according to claim 2 or derivative thereof for the preparation of a diagnostic composition for detecting a disease wherein the expression level of the protein according to any one of (c) to (g) of claim 1 is increased or decreased in a patient compared to normal healthy subject wherein the level of the protein in normal healthy subject and test subject at the mRNA level is to be measured and compared.

32. Use of the antibody according to claim 22 for the preparation of a diagnostic composition for detecting a disease wherein the expression level of the protein according to any one of (c) to (g) of claim 1 is increased or decreased in a patient compared to normal healthy subject, wherein the expression level of the protein in normal healthy subject and a test subject, is to be quantified and compared.

33. Use of the DNA according to claim 2 or an oligonucleotide comprising 10 to 60 continuous nucleotides of the nucleotide sequence of the DNA according to claim 2 or derivative thereof for the preparation of a diagnostic composition for detecting a mutation in a gene or a disease which has a mutation and a gene encoding the protein according to any one of (c) to (g) of claim 1.

34. Use of the DNA according to claim 2 or an oligonucleotide comprising 10 to 60 continuous nucleotides of the nucleotide sequence of the DNA according to claim 2 or derivative thereof for the preparation of a pharmaceutical composition for in vivo inhibiting the expression of the protein according to any one of (c) to (g) of claim 1 at the transcriptional level in gene therapy.

35. Use of an oligonucleotide or a DNA selected from the group of:

(i) the DNA according to claim 2;
(ii) an oligonucleotide comprising 10 to 60 continuous nucleotides of the nucleotide sequence of the DNA according to claim 2 or derivative thereof;
(iii) a DNA encoding bcl9 protein (Figure 1; lower);
(iv) an oligonucleotide comprising 10 to 60 continuous nucleotides of the nucleotide sequence of the DNA encoding bcl9 protein (Figure 1; lower); and
(v) an oligonucleotide comprising a nucleotide sequence complementary to the oligonucleotide of (iv), or a derivative thereof;

for the preparation of a pharmaceutical composition for in vivo inhibiting the nuclear localization of β-catenin in gene therapy.

36. Use of an oligonucleotide or a DNA selected from the group of:

(i) the DNA according to claim 2;
(ii) an oligonucleotide comprising 10 to 60 continuous nucleotides of the nucleotide sequence of the DNA according to claim 2 or derivative thereof;
(iii) a DNA encoding bcl9 protein (Figure 1; lower);
(iv) an oligonucleotide comprising 10 to 60 continuous nucleotides of the nucleotide sequence of the DNA encoding bcl9 protein (Figure 1; lower); and
(v) an oligonucleotide comprising a nucleotide sequence complementary to the oligonucleotide of (iv), or a derivative thereof

for the preparation of a pharmaceutical composition for treating a cancer.

37. Use of the antibody according to claim 24 for the preparation of a pharmaceutical composition for in vivo inhibiting the transcriptional activation by a complex of β-catenin and transcription factor belonging to the Lef/Tcf family by inhibiting the function of the protein according to claim 1.

38. Use of an antibody that binds to bcl9 protein (Figure 1; lower), and thereby inhibiting the activity of the bcl9 protein to bind to β-catenin and to localize β-catenin into the nucleus for the preparation of a pharmaceutical composition for in vivo inhibiting the transcriptional activation by a complex of β-catenin and transcription factor belonging to the Lef/Tcf family by inhibiting the function of the bcl9 protein.

Revendications

1. Protéine choisie parmi le groupe consistant en :

(a) une protéine comprenant la séquence d'acides aminés des résidus 292 à 439 dans la séquence d'acides aminés représentée par SEQ ID NO : 2 ;
(b) la protéine selon (a), la protéine comprenant la séquence d'acides aminés représentée par SEQ ID NO : 2 ou 4 ;
(c) une protéine comprenant la séquence d'acides aminés représentée par SEQ ID NO : 10;
(d) une protéine comprenant la séquence d'acides aminés représentée par SEQ ID NO : 10 et 12 ;
(e) une protéine consistant en la séquence d'acides aminés, dans laquelle un à cinq des acides aminés sont ajoutés, délétés, ou substitués dans la séquence d'acides aminés de la protéine selon l'une quelconque de (a) à (d) dans laquelle ladite protéine se lie à la β-caténine et a l'activité pour localiser la β-caténine dans le noyau ;
(f) une protéine consistant en une séquence d'acides aminés ayant une homologie de 60% ou plus avec la séquence de la protéine selon l'une quelconque de (a) à (d) dans laquelle ladite protéine se lie à la β-caténine et a l'activité pour localiser la β-caténine dans le noyau ; et

(g) une protéine selon (c) or (d) dans laquelle la protéine consiste en une séquence d'acides aminés ayant une homologie de 60% ou plus avec la séquence d'acides aminés représentée par SEQ ID NO : 2, et se lie à la β-caténine et a l'activité pour localiser la β-caténine dans le noyau.

2. ADN choisi parmi le groupe consistant en :

(a) un ADN codant pour la protéine de la revendication 1 ;
(b) un ADN comprenant la séquence nucléotidique des nucléotides 874 à 1317 dans la séquence nucléotidique représentée par SEQ ID NO : 1 ;
(c) l'ADN selon (b), dans laquelle l'ADN comprend la séquence nucléotidique représentée par SEQ ID NO : 1 ou 3 ;
(d) un ADN codant une protéine qui se lie à la β-caténine et a l'activité pour localiser la β-caténine dans le noyau, et comprenant la séquence nucléotidique (i) ou (ii) suivante:

(i) la séquence nucléotidique représentée par SEQ ID NO : 9 ; et
(ii) la séquence nucléotidique représentée par SEQ ID NO : 9 et 11 ;

(e) un ADN hybridant avec l'ADN selon l'une quelconque de (a) à (d) dans des conditions stringentes, qui code pour une protéine qui se lie à la β-caténine et a l'activité pour localiser la β-caténine dans le noyau ;
(f) un ADN comprenant la séquence nucléotidique représentée par SEQ ID NO : 9 et 11, et consistant en la séquence nucléotidique codant pour une protéine consistant en la séquence d'acides aminés ayant une homologie de 60% ou plus avec la séquence d'acides aminés de SEQ ID NO : 2, dans laquelle ladite protéine se lie à la β-caténine et a l'activité pour localiser la β-caténine dans le noyau ; et
(g) un ADN comprenant la séquence d'acides nucléiques représentée par SEQ ID NO : 11.

3. ADN recombinant susceptible d'être obtenu par insertion de l'ADN selon la revendication 2 dans un vecteur.

4. Transformant non-humain susceptible d'être obtenu en introduisant l'ADN recombinant selon la revendication 3 dans une cellule hôte.

5. Procédé pour produire la protéine selon la revendication 1, qui comprend les étapes de :

(a) culture du transformant de la revendication 4 dans un milieu de culture ;
(b) production et accumulation de la protéine selon la revendication 1 dans la culture ; et
(c) récupération de la protéine de la culture.

6. Méthode in vitro de détection ou de quantification, au niveau de l'ARNm, du niveau d'expression de la protéine selon l'une quelconque de (c) à (g) de la revendication 1, utilisant l'ADN selon la revendication 2, ou un oligonucléotide comprenant de 10 à 60 nucléotides continus de la séquence nucléotidique d'ADN selon la revendication 2 ou dérivé de celui-ci.

7. Méthode in vitro de détection d'une maladie dans laquelle le niveau d'expression de la protéine selon l'une quelconque de (c) à (g) de la revendication 1 est augmenté ou diminué chez le patient en comparaison avec le sujet normal sain, par mesure et comparaison du niveau d'expression de la protéine chez le sujet normal sain et le sujet test au niveau de l'ARNm en utilisant l'ADN selon la revendication 2, ou un oligonucléotide comprenant de 10 à 60 d'oligonucléotides continus de la séquence nucléotidique de l'ADN selon la revendication 2 ou dérivé de celui-ci.

8. Agent diagnostique pour une maladie qui présente une augmentation ou une diminution du niveau d'expression de la protéine selon l'une quelconque de (c) à (g) de la revendication 1 chez un patient comparé au sujet sain normal, par mesure et comparaison du niveau d'expression de la protéine au niveau de l'ARNm, dans lequel l'agent diagnostique comprend l'ADN selon la revendication 2.

9. Méthode in vitro de détection d'une mutation dans un gène codant la protéine selon l'une quelconque de (c) à (g) de la revendication 1 utilisant l'ADN selon la revendication 2, ou un oligonucléotide comprenant de 10 à 60 nucléotides continus de la séquence nucléotidique de l'ADN selon la revendication 2 ou dérivé de celui-ci.

10. Méthode in vitro de détection d'une maladie qui a une mutation dans un gène codant la protéine selon l'une quelconque de (c) à (g) de la revendication 1 utilisant l'ADN selon la revendication 2, ou un oligonucléotide comprenant

de 10 à 60 nucléotides continus de la séquence nucléotidique de l'ADN selon la revendication 2 ou dérivé de celui-ci.

11. Agent diagnostique pour une maladie qui a une mutation dans un gène codant la protéine selon l'une quelconque de (c) à (g) de la revendication 1, comprenant l'ADN selon la revendication 2.

12. Méthode in vitro d'inhibition de l'expression de la protéine selon l'une quelconque de (c) à (g) de la revendication 1 au niveau transcriptionnel utilisant l'ADN selon la revendication 2, ou un oligonucléotide comprenant de 10 à 60 nucléotides continus de la séquence nucléotidique de l'ADN selon la revendication 2 ou dérivé de celui-ci.

13. Méthode in vitro d'inhibition de la localisation nucléaire de la β-caténine, utilisant un oligonucléotide ou un ADN choisi parmi le groupe de :

(i) l'ADN selon la revendication 2 ;
(ii) un oligonucléotide comprenant de 10 à 60 oligonucléotides continus de la séquence nucléotidique de l'ADN selon la revendication 2 ou dérivé de celui-ci ;
(iii) un ADN codant la protéine bcl9 (Figure 1 bas) ;
(iv) un oligonucléotide comprenant de 10 à 60 oligonucléotides continus de la séquence nucléotidique de l'ADN codant pour la protéine bcl9 (Figure 1 bas) ; et
(v) un oligonucléotide comprenant une séquence nucléotidique complémentaire de l'oligonucléotide de (iv), ou dérivé de celui-ci.

14. Agent thérapeutique pour le cancer comprenant l'ADN selon la revendication 2.

15. Vecteur pour la thérapie génique comprenant l'ADN selon la revendication 2.

16. Animal transgénique non humain généré par introduction l'ADN selon la revendication 2.

17. Méthode utilisant l'animal transgénique non humain selon la revendication 16, ou un animal transgénique non humain généré par introduction d'un ADN codant pour la protéine bcl9 (Figure 1 bas), en tant que modèle animal pour la carcinogénèse.

18. Méthode d'évaluation d'un agent thérapeutique pour le cancer utilisant l'animal transgénique selon la revendication 16, ou un animal transgénique généré par introduction d'un ADN codant pour la protéine bcl9 (Figure 1 bas).

19. Animal non humain génétiquement déficient, dans lequel la fonction d'une protéine pour lier ou localiser la β-caténine dans le noyau est perdue ou diminuée à cause de la délétion de tout ou partie de l'ADN selon la revendication 2.

20. Méthode in vitro de criblage pour une substance qui inhibe la liaison de la protéine bcl9 (Figure 1, bas) et de la β-caténine, comprenant les étapes de :

(a) comparaison de la liaison de la protéine bcl9 (Figure 1 bas) et de β-caténine en présence ou absence d'un échantillon test ; et
(b) sélection d'une substance qui inhibe la liaison de la protéine bcl9 et de la β-caténine, à partir de l'échantillon test.

21. Méthode in vitro de criblage pour une substance qui inhibe la liaison de la β-caténine et de la protéine selon la revendication 1, comprenant les étapes de :

(a) comparaison de la liaison de β-caténine et de la protéine en absence ou en présence d'un échantillon test ; et
(b) sélection d'une substance qui inhibe la liaison de la β-caténine et de la protéine selon la revendication 1, à partir de l'échantillon test.

22. Anticorps reconnaissant la protéine selon la revendication 1.

23. Méthode de détection ou de quantification immunologique de la protéine selon la revendication 1, utilisant l'anticorps selon la revendication 22.

24. Anticorps neutralisant qui lie la protéine selon la revendication 1, et ainsi inhibe l'activité de la protéine pour se lier

à la β-caténine et pour localiser la β-caténine dans le noyau.

25. Méthode in vitro de détection d'une maladie dans laquelle le niveau d'expression de la protéine selon l'une quelconque de (c) à (g) de la revendication 1 est augmenté ou diminué chez le patient par rapport au sujet normal sain, par quantification et comparaison du niveau d'expression de la protéine chez un sujet normal sain et un sujet test, par utilisation l'anticorps selon la revendication 22.

26. Agent diagnostique comprenant un anticorps selon la revendication 22, pour une maladie dans laquelle le niveau d'expression de la protéine selon l'une quelconque de (c) à (g) de la revendication 1 est augmenté ou diminué chez un patient comparé à un sujet normal sain.

27. Méthode in vitro d'inhibition de l'activation transcriptionnelle par un complexe de β-caténine et d'un facteur de transcription appartenant à la famille Lef/Tcf par inhibition de la fonction de la protéine selon la revendication 1 par utilisation de l'anticorps selon la revendication 24.

28. Méthode in vitro d'inhibition de l'activation transcriptionnelle par un complexe de β-caténine et d'un facteur de transcription appartenant à la famille Lef/Tcf par inhibition de la fonction de la protéine bcl9 (figure 1 bas) par utilisation un anticorps qui se lie sur la protéine bcl9, et ainsi inhibe l'activité de la protéine bcl9 pour se lier avec la β-caténine et localiser la β-caténine dans le noyau.

29. Agent thérapeutique pour le cancer comprenant l'anticorps selon la revendication 24.

30. Utilisation de l'ADN selon la revendication 2 ou d'un oligonucléotide comprenant de 10 à 60 nucléotides continus de la séquence nucléotidique de l'ADN selon la revendication 2 ou dérivé de celui-ci, pour la préparation d'un agent diagnostique pour détecter ou quantifier, au niveau de l'ARNm, le niveau d'expression de la protéine selon l'une ou quelconque de (c) à (g) de la revendication 1.

31. Utilisation de l'ADN selon la revendication 2 ou d'un oligonucléotide comprenant de 10 à 60 nucléotides continus de la séquence nucléotidique de l'ADN selon la revendication 2 ou dérivé de celui-ci pour la préparation d'un agent diagnostique pour détecter la présence d'une maladie dans laquelle le niveau d'expression de la protéine selon l'une ou quelconque de (c) à (g) de la revendication 1 est augmenté ou diminué chez un patient comparé à un sujet normal sain dans lequel le niveau de la protéine chez le sujet normal sain et le sujet test, au niveau de l'ARNm, est à mesurer et à comparer.

32. Utilisation de l'anticorps selon la revendication 22 pour la préparation d'un agent diagnostique pour la détection d'une maladie dans laquelle le niveau d'expression de la protéine selon l'une quelconque de (c) à (g) de la revendication 1 est augmenté ou diminué chez un patient comparé au sujet normal sain, le niveau d'expression de la protéine chez le sujet normal sain et le sujet test étant à quantifier et comparer.

33. Utilisation de l'ADN selon la revendication 2 ou d'un oligonucléotide comprenant de 10 à 60 nucléotides continus de la séquence nucléotidique de l'ADN selon la revendication 2 ou dérivé de celui-ci pour la préparation d'un agent diagnostique pour la détection d'une mutation dans un gène ou une maladie qui a une mutation et un gène codant pour la protéine selon l'une quelconque de (c) à (g) de la revendication 1.

34. Utilisation de l'ADN selon la revendication 2 ou d'un oligonucléotide comprenant de 10 à 60 nucléotides continus de la séquence nucléotidique de l'ADN selon la revendication 2 ou dérivé de celui-ci pour la préparation d'une composition pharmaceutique pour inhiber in vivo l'expression de la protéine selon l'une quelconque de (c) à (g) de la revendication 1 à un niveau transcriptionnel en thérapie génique.

35. Utilisation d'un oligonucléotide ou d'un ADN choisi parmi le groupe de :

(i) l'ADN selon la revendication 2 ;
(ii) un oligonucléotide comprenant de 10 à 60 nucléotides continus de la séquence nucléotidique de l'ADN selon la revendication 2 ou dérivé de celui-ci ;
(iii) un ADN codant pour la protéine bcl9 (Figure 1 bas) ;
(iv) un oligonucléotide comprenant de 10 à 60 nucléotides continus de la séquence nucléotidique de l'ADN codant pour la protéine bcl9 (Figure 1 bas) ; et
(v) un oligonucléotide comprenant une séquence nucléotidique complémentaire de l'oligonucléotide de (iv), ou

un dérivé de celui-ci

pour la préparation d'une composition pharmaceutique pour inhiber in vivo la localisation nucléaire de la β-caténine en thérapie génique.

36. Utilisation d'un oligonucléotide ou d'un ADN choisi parmi un groupe de :

(i) l'ADN selon la revendication 2 ;
(ii) un oligonucléotide comprenant de 10 à 60 nucléotides continus de la séquence nucléotidique de l'ADN selon la revendication 2 ou dérivé de celui-ci ;
(iii) un ADN codant pour la protéine bcl9 (Figure 1 bas) ;
(iv) un oligonucléotide comprenant 10 à 60 nucléotides continus de la séquence nucléotidique de l'ADN codant pour la protéine bcl9 (Figure 1 bas) ; et
(v) un oligonucléotide comprenant une séquence nucléotidique complémentaire de l'oligonucléotide de (iv), ou un dérivé de celui-ci ,

pour la préparation d'une composition pharmaceutique pour le traitement d'un cancer.

37. Utilisation d'anticorps selon la revendication 24 pour la préparation d'une composition pharmaceutique pour inhiber in vivo l'activation transcriptionnelle par un complexe de β-caténine et d'un facteur de transcription appartenant à la famille Lef/Tcf par inhibition de la fonction de la protéine selon la revendication 1.

38. Utilisation d'un anticorps qui se lie à la protéine bcl9 (Figure 1 bas), et ainsi inhibe l'activité de la protéine bcl9 pour lier et localiser la β-caténine dans le noyau, pour la préparation d'une composition pharmaceutique pour inhiber in vivo l'activation transcriptionnelle par un complexe β-caténine et facteur de transcription appartenant à la famille Lef/Tcf par inhibition de la fonction de la protéine bcl9.

Patentansprüche

1. Protein, ausgewählt aus der Gruppe bestehend aus:

(a) einem Protein, das die Aminosäuresequenz der Aminosäurereste 292 bis 439 in der Aminosäuresequenz wie dargestellt durch SEQ ID NO: 2 umfasst;
(b) dem Protein gemäß (a), wobei das Protein die Aminosäuresequenz wie dargestellt durch SEQ ID NO: 2 oder 4 umfasst;
(c) einem Protein, das die Aminosäuresequenz wie dargestellt durch SEQ ID NO: 10 umfasst;
(d) einem Protein, das die Aminosäuresequenz wie dargestellt durch die SEQ ID NOs: 10 und 12 umfasst;
(e) einem Protein, bestehend aus der Aminosäuresequenz, in der eine bis fünf Aminosäuren in der Aminosäuresequenz des Proteins gemäß einem von (a) bis (d) hinzugefügt, entfernt oder ausgetauscht wurden, wobei das Protein an β-Catenin bindet und die Aktivität hat, β-Catenin in den Nucleus zu lokalisieren;
(f) einem Protein, bestehend aus einer Aminosäuresequenz mit einer Homologie von 60% oder mehr zu der Aminosäuresequenz des Proteins gemäß einem von (a) bis (d), wobei das Protein an β-Catenin bindet und die Aktivität hat, β-Catenin in den Nucleus zu lokalisieren; und
(g) dem Protein gemäß (c) oder (d), wobei das Protein aus einer Aminosäuresequenz mit einer Homologie von 60% oder mehr zu der Aminosäuresequenz wie dargestellt durch SEQ ID NO: 2 besteht und an β-Catenin bindet und die Aktivität hat, β-Catenin in den Nucleus zu lokalisieren.

2. DNA, ausgewählt aus der Gruppe bestehend aus:

(a) einer DNA, die das Protein gemäß Anspruch 1 codiert;
(b) einer DNA, die die Nucleotidsequenz der Nucleotide 874 bis 1317 in der Nucleotidsequenz wie dargestellt durch SEQ ID NO: 1 umfasst;
(c) der DNA gemäß (b), wobei die DNA die Nucleotidsequenz wie dargestellt durch SEQ ID NO: 1 oder 3 umfasst;
(d) einer DNA, die ein Protein codiert, das an β-Catenin bindet und das die Aktivität hat, β-Catenin in den Nucleus zu lokalisieren, und die die Nucleotidsequenz des folgenden (i) oder (ii) umfasst:

(i) die Nucleotidsequenz wie dargestellt durch SEQ ID NO: 9; und

(ii) die Nucleotidsequenz wie dargestellt durch die SEQ ID NOs: 9 und 11;

(e) einer DNA, die unter stringenten Bedingungen an die DNA gemäß einem von (a) bis (d) hybridisiert, die ein Protein codiert, das an β-Catenin bindet und die Aktivität hat, β-Catenin in den Nucleus zu lokalisieren;
(f) einer DNA, die die Nucleotidsequenz wie dargestellt durch die SEQ ID NOs: 9 und 11 umfasst und aus der Nucleotidsequenz besteht, die ein Protein bestehend aus der Aminosäuresequenz mit einer Homologie von 60% oder mehr zur Aminosäuresequenz von SEQ ID NO: 2 codiert, wobei das Protein β-Catenin bindet und die Aktivität hat, β-Catenin in den Nucleus zu lokalisieren; und
(g) einer DNA, die die Nucleotidsequenz wie dargestellt durch SEQ ID NO: 11 umfasst.

3. Rekombinante DNA, erhältlich durch Einfügen der DNA gemäß Anspruch 2 in einen Vektor.

4. Nicht-menschliche Transformante, erhältlich durch das Einführen der rekombinanten DNA gemäß Anspruch 3 in eine Wirtszelle.

5. Verfahren zur Herstellung des Proteins gemäß Anspruch 1, umfassend die Schritte:

(a) Züchten der Transformante gemäß Anspruch 4 in einem Kulturmedium;
(b) Herstellen und Anreichern des Proteins gemäß Anspruch 1 in der Kultur; und
(c) Gewinnen des Proteins aus der Kultur.

6. In vitro-Verfahren zum Nachweisen oder Quantifizieren, auf mRNA-Ebene, des Expressionsniveaus des Proteins gemäß einem von (c) bis (g) von Anspruch 1, unter Verwendung der DNA gemäß Anspruch 2 oder eines Oligonucleotids, das 10 bis 60 zusammenhängende Nucleotide der Nucleotidsequenz der DNA gemäß Anspruch 2 oder eines Derivates davon umfasst.

7. In vitro-Verfahren zum Nachweisen einer Krankheit, wobei das Expressionsniveau des Proteins gemäß einem von (c) bis (g) von Anspruch 1, in einem Patienten, verglichen mit einem normalen, gesunden Individuum, erhöht oder erniedrigt ist, durch das Messen und Vergleichen des Expressionsniveaus des Proteins in einem normalen, gesunden Individuum und dem Testpatienten auf mRNA-Ebene, unter Verwendung der DNA gemäß Anspruch 2 oder eines Oligonucleotids, das 10 bis 60 zusammenhängende Nucleotide der Nucleotidsequenz der DNA gemäß Anspruch 2 oder eines Derivates davon umfasst.

8. Diagnosemittel für eine Krankheit, die ein erhöhtes oder erniedrigtes Expressionsniveau des Proteins gemäß einem von (c) bis (g) von Anspruch 1 in einem Patienten verglichen mit einem normalen, gesunden Individuum aufweist, durch das Messen und Vergleichen des Expressionsniveaus des Proteins auf mRNA-Ebene, wobei das Diagnosemittel die DNA gemäß Anspruch 2 umfasst.

9. In vitro-Verfahren zum Nachweis einer Mutation in einem Gen, das das Protein gemäß einem von (c) bis (g) von Anspruch 1 codiert, unter Verwendung der DNA gemäß Anspruch 2 oder eines Oligonucleotids, das 10 bis 60 zusammenhängende Nucleotide der Nucleotidsequenz der DNA gemäß Anspruch 2 oder eines Derivates davon umfasst.

10. In vitro-Verfahren zum Nachweis einer Krankheit, die eine Mutation in einem Gen aufweist, das das Protein gemäß einem von (c) bis (g) von Anspruch 1 codiert, unter Verwendung der DNA gemäß Anspruch 2 oder eines Oligonucleotids, das 10 bis 60 zusammenhängende Nucleotide der Nucleotidsequenz der DNA gemäß Anspruch 2 oder eines Derivates davon umfasst.

11. Diagnosemittel für eine Erkrankung, die eine Mutation in einem Gen aufweist, das das Protein gemäß einem von (c) bis (g) von Anspruch 1 codiert, umfassend die DNA gemäß Anspruch 2.

12. In vitro-Verfahren zum Hemmen der Expression des Proteins gemäß einem von (c) bis (g) von Anspruch 1 auf der Transkriptionsebene, unter Verwendung der DNA gemäß Anspruch 2 oder eines Oligonucleotids, das 10 bis 60 zusammenhängende Nucleotide der Nucleotidsequenz der DNA gemäß Anspruch 2 oder eines Derivates davon umfasst.

13. In vitro-Verfahren zum Hemmen der nuclearen Lokalisation von β-Catenin, unter Verwendung eines Oligonucleotids oder einer DNA, ausgewählt aus der Gruppe von:

(i) der DNA gemäß Anspruch 2;
(ii) einem Oligonucleotid, das 10 bis 60 zusammenhängende Nucleotide der Nucleotidsequenz der DNA gemäß Anspruch 2 oder eines Derivates davon umfasst;
(iii) einer DNA, die das Protein bcl9 (Figur 1; unten) codiert;
(iv) einem Oligonucleotid, das 10 bis 60 zusammenhängende Nucleotide der Nucleotidsequenz der DNA, die das Protein bcl9 (Figur 1; unten) codiert, umfasst; und
(v) einem Oligonucleotid, das eine Nucleotidsequenz komplementär zu dem Oligonucleotid von (iv) umfasst oder ein Derivat davon.

14. Therapeutisches Mittel für Krebs, das die DNA gemäß Anspruch 2 umfasst.

15. Vektor für Gentherapie, der die DNA gemäß Anspruch 2 umfasst.

16. Nicht-menschliches transgenes Tier, hergestellt durch das Einführen der DNA gemäß Anspruch 2.

17. Verfahren zur Verwendung des nicht-menschlichen transgenen Tieres gemäß Anspruch 16 oder eines nicht-menschlichen transgenen Tieres, hergestellt durch das Einführen einer DNA, die das Protein bcl9 (Figur 1; unten) codiert, als ein Tier-Modell für Karzinogenese.

18. Verfahren zum Beurteilen eines therapeutischen Mittels für Krebs, unter Verwendung des transgenen Tieres gemäß Anspruch 16 oder eines transgenen Tieres, hergestellt durch das Einführen einer DNA, die das Protein bcl9 (Figur 1; unten) codiert.

19. Genetisch fehlerhaftes nicht-menschliches Tier, wobei die Funktion eines Proteins, an β-Catenin zu binden und β-Catenin in den Nucleus zu lokalisieren, aufgrund der Deletion der gesamten oder eines Teils der DNA gemäß Anspruch 2 verloren gegangen oder erniedrigt ist.

20. In vitro-Verfahren zum Screenen nach einem Stoff, der das Binden von Protein bcl9 (Figur 1; unten) und β-Catenin hemmt, umfassend die Schritte:

(a) Vergleichen des Bindens von Protein bcl9 (Figur 1; unten) und β-Catenin in Ab- oder Anwesenheit einer Testprobe; und
(b) Auswählen eines Stoffes aus der Testprobe, der das Binden von Protein bcl9 und β-Catenin hemmt.

21. In vitro-Verfahren zum Screenen für einen Stoff, der das Binden von β-Catenin und dem Protein gemäß Anspruch 1 hemmt, umfassend die Schritte:

(a) Vergleichen des Bindens von β-Catenin und dem Protein in Abwesenheit oder Anwesenheit einer Testprobe; und
(b) Auswählen eines Stoffes aus der Testprobe, der das Binden von β-Catenin und dem Protein gemäß Anspruch 1 hemmt.

22. Antikörper, der das Protein gemäß Anspruch 1 erkennt.

23. Verfahren zum immunologischen Nachweisen oder Quantifizieren des Proteins gemäß Anspruch 1, unter Verwendung des Antikörpers gemäß Anspruch 22.

24. Neutralisierender Antikörper, der an das Protein gemäß Anspruch 1 bindet und dabei die Aktivität des Proteins, an β-Catenin zu binden und β-Catenin in den Nucleus zu lokalisieren, hemmt.

25. In vitro-Verfahren zum Nachweisen einer Krankheit, bei der das Expressionsniveau des Proteins gemäß einem von (c) bis (g) von Anspruch 1, in einem Patienten verglichen mit einem normalen, gesunden Individuum, erhöht oder erniedrigt ist, durch Quantifizieren und Vergleichen des Expressionsniveaus des Proteins in einem normalen, gesunden Individuum und einem Testpatienten, unter Verwendung des Antikörpers gemäß Anspruch 22.

26. Diagnosemittel, das den Antikörper gemäß Anspruch 22 umfasst, für eine Krankheit, bei der das Expressionsniveau des Proteins gemäß einen von (c) bis (g) von Anspruch 1 in einem Patienten verglichen mit einem normalen, gesunden Individuum erhöht oder erniedrigt ist.

27. In vitro-Verfahren zur Hemmung der transkriptionalen Aktivierung durch einen Komplex von β-Catenin und einem Transkriptionsfaktor, der zur Lef/Tcf-Familie gehört, durch Hemmen der Funktion des Proteins gemäß Anspruch 1 unter Verwendung des Antikörpers gemäß Anspruch 24.

28. In vitro-Verfahren zum Hemmen der transkriptionalen Aktivierung durch einen Komplex von β-Catenin und einem Transkriptionsfaktor, der zur Lef/Tcf-Familie gehört, durch Hemmen der Funktion von Protein bcl9 (Figur 1; unten) unter Verwendung eines Antikörpers, der an das Protein bcl9 bindet und dabei die Aktivität des Proteins bcl9, an β-Catenin zu binden und β-Catenin in den Nucleus zu lokalisieren, hemmt.

29. Therapeutisches Mittel für Krebs, das den Antikörper gemäß Anspruch 24 umfasst.

30. Verwendung der DNA gemäß Anspruch 2 oder eines Oligonucleotids, das 10 bis 60 zusammenhängende Nucleotide der Nucleotidsequenz der DNA gemäß Anspruch 2 oder eines Derivates davon umfasst, für die Herstellung eines Diagnosemittels zum Nachweisen oder Quantifizieren, auf mRNA-Ebene, des Expressionsniveaus des Proteins gemäß einem von (c) bis (g) von Anspruch 1.

31. Verwendung der DNA gemäß Anspruch 2 oder eines Oligonucleotids, das 10 bis 60 zusammenhängende Nucleotide der Nucleotidsequenz der DNA gemäß Anspruch 2 oder eines Derivates davon umfasst, für die Herstellung eines Diagnosemittels zum Nachweisen einer Krankheit, wobei das Expressionsniveau des Proteins gemäß einem von (c) bis (g) von Anspruch 1 in einem Patienten, verglichen mit einem normalen, gesunden Individuum, erhöht oder erniedrigt ist, wobei das Niveau des Proteins im normalen, gesunden Individuum und im Testpatienten auf der mRNA-Ebene gemessen und verglichen werden soll.

32. Verwendung des Antikörper gemäß Anspruch 22 für die Herstellung eines Diagnosemittels zum Nachweisen einer Krankheit, wobei das Expressionsniveau des Proteins gemäß einem von (c) bis (g) von Anspruch 1 in einem Patienten, verglichen mit einem normalen, gesunden Individuum, erhöht oder erniedrigt ist, wobei das Expressionsniveau des Proteins im normalen, gesunden Individuum und im Testpatienten quantifiziert und verglichen werden soll.

33. Verwendung der DNA gemäß Anspruch 2 oder eines Oligonucleotids, das 10 bis 60 zusammenhängende Nucleotide der Nucleotidsequenz der DNA gemäß Anspruch 2 oder eines Derivates davon umfasst, für die Herstellung eines Diagnosemittels zum Nachweisen einer Mutation in einem Gen oder einer Krankheit, die eine Mutation und ein Gen, das das Protein gemäß einem von (c) bis (g) von Anspruch 1 codiert, aufweist.

34. Verwendung der DNA gemäß Anspruch 2 oder eines Oligonucleotids, das 10 bis 60 zusammenhängende Nucleotide der Nucleotidsequenz der DNA gemäß Anspruch 2 oder eines Derivates davon umfasst, für die Herstellung eines Arzneimittels zum in vivo-Hemmen der Expression des Proteins gemäß einem von (c) bis (g) von Anspruch 1 auf transkriptionaler Ebene in der Gentherapie.

35. Verwendung eines Oligonucleotids oder einer DNA, ausgewählt aus der Gruppe von:

(i) der DNA gemäß Anspruch 2;
(ii) einem Oligonucleotid, das 10 bis 60 zusammenhängende Nucleotide der Nucleotidsequenz der DNA gemäß Anspruch 2 oder eines Derivates davon umfasst;
(iii) einer DNA, die das Protein bcl9 (Figur 1; unten) codiert;
(i v) einem Oligonucleotid, das 10 bis 60 zusammenhängende Nucleotide der Nucleotidsequenz der DNA, die das Protein bcl9 (Figur 1; unten) codiert, umfasst; und
(v) einem Oligonucleotid, das eine Nucleotidsequenz komplementär zu dem Oligonucleotid von (iv) umfasst, oder ein Derivat davon;

für die Herstellung eines Arzneimittels zum in vivo-Hemmen der nuclearen Lokalisation von β-Catenin in der Gentherapie.

36. Verwendung eines Oligonucleotids oder einer DNA, ausgewählt aus der Gruppe von:

(i) der DNA gemäß Anspruch 2;
(ii) einem Oligonucleotid, das 10 bis 60 zusammenhängende Nucleotide der Nucleotidsequenz der DNA gemäß Anspruch 2 oder eines Derivates davon umfasst;
(iii) einer DNA, die das Protein bcl9 (Figur 1; unten) codiert;

(iv) einem Oligonucleotid, das 10 bis 60 zusammenhängende Nucleotide der Nucleotidsequenz der DNA, die das Protein bcl9 (Figur 1; unten) codiert, umfasst; und
(v) einem Oligonucleotid, das eine Nucleotidsequenz komplementär zu dem Oligonucleotid von (iv) umfasst, oder ein Derivat davon;

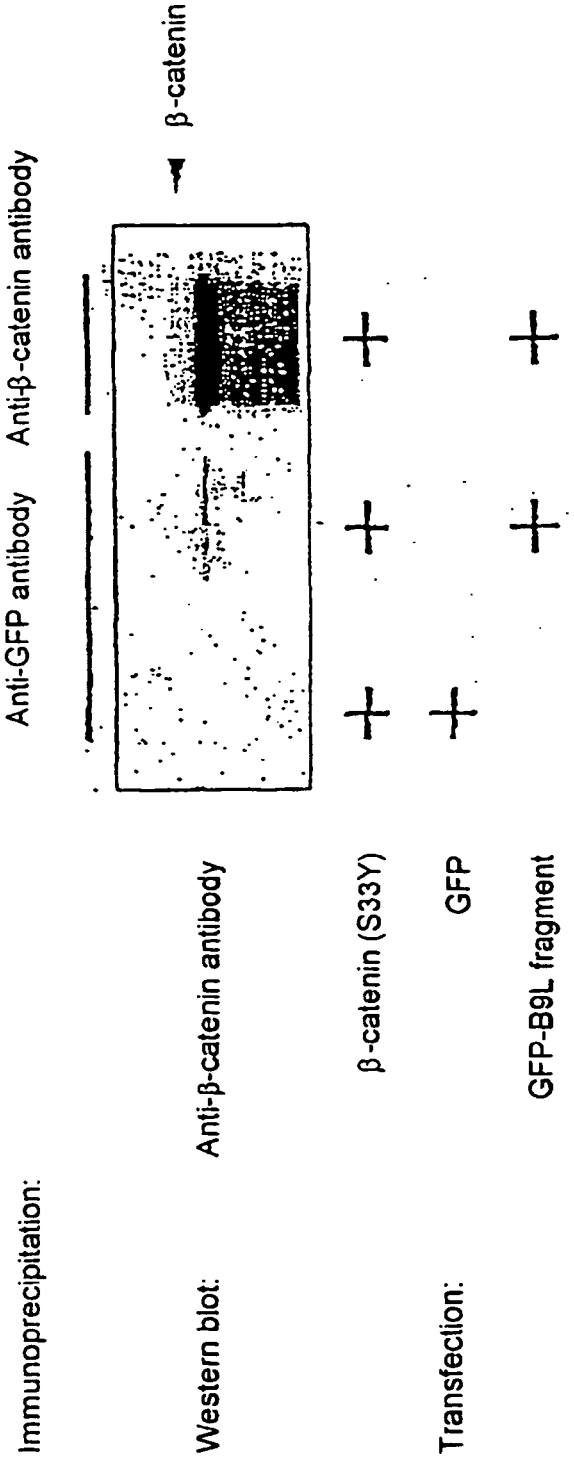
für die Herstellung eines Arzneimittels zur Behandlung von Krebs.

37. Verwendung des Antikörpers gemäß Anspruch 24 für die Herstellung eines Arzneimittels zum in vivo-Hemmen der transkriptionalen Aktivierung durch einen Komplex von β-Catenin und einem Transkriptionsfaktor, der zu der Lef/Tcf-Familie gehört, durch Hemmen der Funktion des Proteins gemäß Anspruch 1.

38. Verwendung eines Antikörpers, der an Protein bcl9 (Figur 1; unten) bindet und dabei die Aktivität des Proteins bcl9, an β-Catenin zu binden und β-Catenin in den Nucleus zu lokalisieren, hemmt, für die Herstellung eines Arzneimittels zum in vivo-Hemmen der transkriptionalen Aktivierung durch einen Komplex von β-Catenin und einem Transkriptionsfaktor, der zu der Lef/Tcf-Familie gehört, durch Hemmen der Funktion des Proteins bcl9.

FIG. 1

FIG. 2


β-catenin
Anti-GFP antibody
Anti-β-catenin antibody
Immunoprecipitation:
Western blot:
Anti-β-catenin antibody
β-catenin (S33Y)
GFP
GFP-B9L fragment
Transfection:
+
+
+
+
+
+

REFERENCES CITED IN THE DESCRIPTION

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