DESCRIPTION

Novel oncogene, recombinant protein derived therefrom, and uses thereof

Field of the Invention

The present invention relates to a novel oncogene from human, which is involved in development of human cervical cancer, a recombinant protein derived from the oncogene and uses thereof in medical applications.

Background Art

There are many documents reporting that chromosome instability is involved in development of a cancer. In addition, it has been recently demonstrated that a defect in a molecule controlling a checkpoint during the G2/M phase in a cell cycle causes chromosome instability. However, since in many carcinoma cells, a gene defect in a molecule controlling a checkpoint in the cell is not frequently observed, a mechanism of inducing chromosome instability, which is substantially involved in onaet of the cancer, remains still unclear in many aspects.

It is widely known that development of cervical cancer involves infection with a human papilloma virus (HPV) such as types of HPV-16 or HPV-18. In a cervical cancer tissue, HPV infection has been observed at a frequency of 90 % or more. In a development mechanism of cervical cancer induced by HPV

infection, E6 and E7 gene products of the virus play an important role. Specifically, it is known that E6 accelerates a process for digesting p53 tumor suppressor protein, while E7 blocks canceration-inhibiting activity of pRB (retinoblastoma) tumor suppressor protein that is a Rb gene product, which two steps result in tumorigensis. A specific oncogenic protein activated by HPV infection has not, however, been identified yet. Particularly, E6 and E7, viral gene products of HPV, induce chromosome instability and carcerize a cell, but detail of its mechanism directly related thereto is left substantially unknown in variety of aspects. For approaching to treatment of cervical cancer, it is, therefore, very important to identify an oncogenic protein as a target for HPV and an encoding oncogene thereof. Cervical cancer progresses from a precancerous state, i. e., dysplasia (epithelial dysplasia of cervical squamous cell) to invasive cancer. Depending on a case, the disease may remain in the dysplasia stage without progressing to cancer. On the other hand, there are considerable cases where dysplasia may rapidly progress to an advanced cancer. In view of the situation, it may be important for more accurate cancer diagnosis to identify a molecule directly involved in development of cervical cancer.

Disclosure of Invention

As described above, in the process where HPV infection of cervical epithelial cell induces development of an invasive

cancer via a precancerous state, dysplasia, its direct origin would be considered to be a mechanism where an expression-inhibiting activity of p53 tumor suppressor protein or pRB tumor suppressor protein, which has inhibited expression of some oncogene, is damaged, and the damege leads the oncogene to a high-level expression state. Therefore, the full nucleotide sequence of the oncogene and an oncogenic protein encoded thereby must be first identified, which opens a way for developing means for inhibiting the biochemical functions of the oncogenic protein and further means for blocking a cancerization mechanism advanced by the oncogenic protein.

Furthermore, identification of the full nucleotide sequence of the oncogene and the amino acid sequence of the oncogenic protein encoded therein may allow us to produce a nucleic acid probe for detecting expression of an mRNA transcribed from the oncogene or to generate a specific antibody to the oncogenic protein with use of a recombinant oncogenic protein thereof. In other words, it may allow us to develop diagnosis means utilizing the nucleic acid probe or specific antibody, which is useful for diagnosing an early step of developing course to an invasive cancer via a precancerous state, dysplasia, that is caused by HPV infection in uterine cervix.

For solving the above problems, an aim of the present invention is to identify the full nucleotide sequence of a novel oncogene from Human and the amino acid sequence of an oncogenic

protein encoded therein, which is directly involved in a cancerization mechanism in, for example, cervical cancer caused by HPV infection into cervical epithelial cell and, and also to provide a full-length polynucleotide encoding a peptide chain of the oncogenic protein, that is derived from the novel oncogene, which can be used for recombinant production of the oncogenic protein, as well as the peptide chain of the oncogenic protein recombinantly produced therewith.

We have intensely studied for solving the above problems, and finally have found and cloned a gene increasing expression in a cervical cancer cell when adding an environmental hormone thereto. We have concluded that the gene cloned is one of oncogenes, because

(1) the gene is highly expressed in a carcinoma cell;

(2) cervical cancer is caused by HPV infection, and expression of E6 and E7 proteins by transducing E6 and E7 genes from HPV to the cell enhance the expression of said gene;

(3) p53 protein inhibits activity of the promoter region in said gene;

(4) lack or mutation of p53 protein is indeed involved in development of cervical cancer;

(5) expression of said gene can be inhibited by a double strand of interfering short-chain RNA(siRNA) to arrest growth of the cancer, and after further investigation, have achieved the present invention.

Thus, an oncogene polynucleotide according to the present invention is a novel oncogene polynucleotide derived from human involving development of cervical cancer, comprising a nucleotide sequence encoding an amino acid sequence of SEQ. ID. No.1. In particular, it is the polynucleotide, wherein the nucleotide sequence encoding the amino acid sequence of SEQ. ID. No.1 is a nucleotide sequence of SEQ. ID. No.2.

The present invention also provides an invention of a peptide or its salts produced recombinantly, based on the above oncogene polynucleotide according to the present invention. Specifically, the recombinant peptide of the present invention is a recombinant peptide or its salts, comprising the amino acid sequence of SEQ. ID. No.1 or a partial amino acid sequence of the amino acid sequence. In particular, it may be a recombinant oncogenic protein comprising the amino acid sequence of SEQ. ID. No.1. The present invention also provides a recombinant vector comprising a polynucleotide encoding the recombinant peptide, which is usable for preparing said recombinant peptide therewith. For example, when aimed is the recombinant oncogenic protein of the present invention consisting of the amino acid sequence of SEQ. ID. No.1, said recombinant vector therefor is a recombinant vector containing an oncogene polynucleotide comprising a nucleotide sequence encoding the amino acid sequence of SEQ. ID. No.1, particularly containing a polynucleotide wherein the nucleotide sequence encoding the amino acid sequence

of SEQ. ID. No.1 is a nucleotide sequence of SEQ. ID. No.2.

The present invention also provides a transformed cell produced by transforming a host cell using said recombinant vector; for example, a transformed cell produced by transforming a host cell with the recombinant vector targeted to said recombinant oncogenic protein of the present invention. Therefore, a process for producing the recombinant peptide or its salts of the present invention is

a process for producing a recombinant peptide or its salts derived from an oncogene of the present invention comprising the steps of:

culturing the above transformed cell to allow the transformed cell to produce the recombinant peptide of the present invention; and

collecting the recombinant peptide produced from the culture. In particular, it may be preferably a process for producing a recombinant oncogenic protein of the present invention comprising the steps of:

culturing said transformed cell in which the full-length gene DNA has been transformed to allow the transformed cell to produce the recombinant oncogenic protein of the present invention; and

collecting the recombinant oncogenic protein produced from the culture.

With use of the above recombinant peptide of the present

invention, the present invention provides an invention of an antibody that is a specific antibody generated using the recombinant peptide as an immunogen. For example, an antibody of the present invention may be an antibody exhibiting a specific reactivity to the partial amino acid sequence of 623 to 1185 region of the amino acid sequence of SEQ. ID. No.1.

Furthermore, the present invention provides an antibody reagent kit for an antigen-antibody reaction comprising said specific antibody, available for detecting an oncogenic protein comprising the amino acid sequence of SEQ. ID. No.1 or a peptide fragment derived from the oncogenic protein. Alternatively, the present invention provides a diagnosis kit being usable for detection of an oncogenic protein comprising the amino acid sequence of SEQ. ID. No.1 or a peptide fragment derived from the oncogenic protein by means of an antigen-antibody reaction, comprising the specific antibody of the present invention.

The present invention also provides an antisense polynucleotide comprising a complementary nucleotide sequence to a partial nucleotide sequence of the nucleotide sequence of SEQ. ID. No.2, which is a DNA fragment having at least a length selected from the region of 15 to 300 bases. In addition, as for a probe hybridization kit according to the present invention, it also provides a probe hybridization kit available for detecting an mRNA comprising the nucleotide sequence of SEQ. ID. No.2, its partial nucleotide sequence therein or cDNA

prepared by the mRNA, comprising the above antisense polynucleotide as the DNA probe. Alternatively, the present invention provides also a diagnosis kit available for detecting expression of mRNA comprising the nucleotide sequence of SEQ. ID. No.2, which is translated into an oncogenic protein comprising the amino acid sequence of SEQ. ID. No.1, by means of a probe hybridization method, comprising said antisense polynucleotide as the hybridization probe.

On the other hand, the present invention also provides a primer pair for PCR amplification of cDNA comprising the nucleotide sequence of SEQ. ID. No.2, consisting of paired primers of:

a nucleotide sequence :

5'-TTGGATCCATGACATCCAGATTTGGGAAAACATACAGTAGG-3'; and

a nucleotide sequence :

5'-TTGAATTCCTAGCAATGTTCCAAATATTCAATCACTCTAGA-3', and also the present invention provides a primer pair for PCR amplifying a partial chain in cDNA comprising the nucleotide sequence of SEQ. ID. No.2, consisting of paired primers of:

5'-GAATTCATAGGCACAGCGCTGAACTGTGTG-3'; and

5'-TTGAATTCCTAGCAATGTTCCAAATATTCA-3'.

Otherwise, as for an invention of a double strand of an short-chain interfering RNA, the present invention provides a double strand of an short-chain interfering RNA capable of inhibiting expression of mRNA comprising the nucleotide sequence

of SEQ. ID. No.2 in a cervical cancer cell, wherein the double strand of the siRNA has a nucleotide sequence: CGGACTACCCTTAGCACAA. In addition, it may be also applied to a pharmaceutical composition for inhibiting expression of mRNA comprising the nucleotide sequence of SEQ. ID. No.2 in a cervical cancer cell to arrest growth of the carcinoma cell, comprising said double strand of short-chain interfering RNA of the present invention.

Brief Description of Drawings

FIG. 1 shows comparison of an amino acid sequence between a dWAPL of Drosophia reported and an hWAPL of the present invention.

FIG. 2 shows an alignment of amino acids identical or similar to those in the dWAPL in a partial amino acid sequence of 623 to 1185 amino acids lying on the C-terminus side for the hWAPL of the present invention.

FIG. 3 shows very high homology between the amino acid sequences of a human WAPL protein and a mouse WAPL protein.

FIG. 4 shows the results of Western blotting analysis for extracts from Saos-2 cell (lanes 2, 3) and NIH3T3 cell (lane 1) by using an anti-hWAPL-N antibody and an anti-hWAPL-C antibody.

FIG. 5 shows (A): Northern blotting for evaluating an expression level of an hWAPL protein in carcinoma cells (T) of

ovarian cancer, pulmonary cancer, colorectal carcinoma, corpus uteri cancer and cervical cancer in comparison with normal cells (N) corresponding thereto; (B): the results of real-time PCR for confirming expression of hWAPL gene in carcinoma cells (T) of cervical cancer, corpus uteri cancer, ovarian cancer, breast cancer, gastric cancer, renal cancer, colorectal carcinoma normal cells in comparison with (N) corresponding thereto; and (C): the results of RT-PCR detection of mRNA expression of E6/E7 gene derived from HPV in the carcinoma cells (T).

FIG. 6 shows (A): Western blotting for confirming expression of the hWAPL protein, which is induced by E6 and E7 recombinant proteins derived from HPV 16, and cleavage of the p53 suppressor protein thereby in HDK1 cell; and (B): Western blotting for confirming inhibition of transcription of E6 and E7 genes, increase in a p53 suppressor protein level and inhibition of expression of the hWAPL protein by BPV-derived E2.

FIG. 7 shows (A): chromosome instability and increase of polyploid; and (B): increase in a frequency of micronuclei formation induced by over-expression of a GFP-hWAPL fused protein in HeLa cell.

FIG. 8 shows chromosome instability and increase in a frequency of multinucleation induced by over-expression of a GFP-hWAPL fused protein in a HeLa cell.

FIG. 9 shows cancerization induction of NIH 3T3

fibroblast by the hWAPL protein, specifically (A): formation of a focus structure in culturing a HA-hWAPL 3T3 cell strain; (B): formation of a tumor in a site in a nude mouse, to which the HA-hWAPL 3T3 cell strain has been injected; (C): over-expression of the HA-tagged hWAPL protein in a tumor forming region; and (D): heterotypic mitosis in a cancerized cell.

FIG. 10 shows the results of evaluating cell growth inhibiting effect by hWAPL siRNA in SiHa cell derived from HPV16 positive cervical cancer, displayed with a plot of the cell number ($\times 10^3$ in unit) (in ordinate) to a time from transduction of the siRNA (hour in unit) (in abscissa).

Best Mode for Carrying out the Invention

The present invention will be described in detail hereafter.

We have searched for a human-derived protein which is inducing factor for chromosome instability, assuming that chromosome instability would be considerably involved in cancerization mechanism in a cervical cancer cell. We have searched particularly for a protein having high potentiality for inducing heteroploidy or isogene formation among chromosome instability events.

We have noticed that dWAPL protein has been reported as a protein controlling a heterochromatin structure during an interkinesis in a meiosis process, among a variety of proteins

derived from Drosophila melanogaster (fruit-fly) whose genomic genes have been most studied among animals. Specifically, we have found that when such a function that the protein exhibits for controlling the heterochromatin structure is expressed in a mitosis process in a normal cell, heteroploidy or isogene formation may be occasionally induced. We have first studied whether a protein corresponding to such a dWAPL is actually encoded on a human genomic gene. Based on the nucleotide sequence of the dWAPL gene reported (GenBank accession No.U40214), we have searched for fragments showing significant similarity from cDNA fragments registered in GenBank as gene fragments originated from human and have selected the KIAA0261 fragment as that comprising a nucleotide sequence similar to the dWAPL gene.

For identifying a full-length cDNA comprising the KIAA0261 fragment, we have searched in the EST database for a human-derived expression-tag nucleotide sequence, which may be a fragment comprising an un-translation part which is presumably an upstream nucleotide sequence lying in the 5'-side of the KIAA0261 fragment, and have selected EST clones: BE410177, BF79516 and BE257022.

We have determined an upstream nucleotide sequence lying in the 5'-side of the KIAA0261 fragment, using a 5'-RACE method with reference to the EST clones. Furthermore, since there is high probability that a protein having a function similar to the dWAPL is actually expressed in a cell having a meiosis process,

we have cloned a full-length cDNA comprising the nucleotide sequence of the KIAA0261 fragment as well as the upstream nucleotide sequence determined above in the 5'-side thereof, using a commercially available cDNA library, i. e., a human testicular cDNA kit (Marathon-Ready™ cDNA Kit; Clontech Inc.) as a template.

Practical sequencing has indicated that the coding region in the cloned full-length cDNA has 3570 base pairs, deducing an amino acid sequence with 1190 amino acids corresponding thereto. It is referred to "human WAPL (hWAPL)" as a human-derived protein similar to the dWAPL protein, and the corresponding gene is referred to as "hWAPL gene". The full-length nucleotide sequence that is corresponding to the ORF in the hWAPL gene is represented by SEQ. ID. No.2, and the deduced amino acid sequence of the hWAPL protein is represented by SEQ. ID. No.1. Comparison of the amino acid sequence encoded by the ORF of the hWAPL gene of the present invention, i. e., the deduced amino acid sequence of the hWAPL protein, with the amino acid sequence of the dWAPL protein indicates, as shown in FIG. 1, that there is an identity of 35 % and a similarity of 53 % for a partial sequence of amino acids 623 to 1185 lying in region of the C-terminus. The amino acids exhibiting such an identity and a similarity are shown in FIG. 2.

In addition, we have cloned a cDNA encoding a mouse-derived homologue thereto, a mouse WAPL protein, assuming

that some mammals other than human may have also a corresponding protein. After sequencing it, the amino acid sequences encoded therein were compared. In practice, it has been confirmed that the human WAPL protein and the mouse WAPL protein shows very high homology to each other. FIG. 3 shows the result of comparative alignment. We have registered the the full-length nucleotide sequence for coding region in the human WAPL gene under an accession No. AB065003 in DDBJ/EMBL/GenBank.

We have conducted Western blotting analysis for extracts from Saos-2 cell and NIH3T3 cell, using an anti-hWAPL-N antibody and an anti-hWAPL-C antibody, which are antibodies specific to the peptide chain in the human WAPL protein, as explained in Example 6. As shown in FIG. 4, the results have revealed a band of a protein reactive to the antibody with approximately 140 kDa. Thus, it has been confirmed that the human WAPL protein is actually present in the human-derived cell in some extent.

Furthermore, based on the following various confirmation methods:

Example 2: expression of the hWAPL gene in a human cancer tissue;

Example 3: induction of expression of the hWAPL gene by E6 and E7 derived from HPV type 16;

Example 4: inhibition of promoter activity of the hWAPL gene product by the p53 suppressor protein;

Example 7: induction of chromosome instability by the

hWAPL protein;

Example 8: induction of cancerization of NIH 3T3 fibroblast by the hWAPL protein, it can be concluded that the hWAPL gene is an oncogene at least involved in a mechanism of development of cervical cancer.

The plasmid pGEMhWAPL comprising the full-length cDNA of the hWAPL gene, which is used in producing the transformant containing the full-length cDNA of the WAPL gene; Escherichia coli DH5 pGEMhWAPL strain that was obtained in Example 1, as described below, have been deposited on January 7, 2003 as an original deposition date, in International Patent Organism Depositary (National Institute of Bioscience and Human-Technology) in National Institute of Advanced Industrial Science and Technology at Chuo 6th, 1-1-1, Higashi, Tsukuga, Ibaragi, 305-8566, under a deposit number of FERM BP-8269 in the International Depositary Authority under the Budapest Treaty,

We have also confirmed that expression of the hWAPL protein as a hWAPL gene product is inhibited by using a double strand of short-chain interfering RNA (siRNA). Specifically, a double strand of short-chain interfering RNA (siRNA) exhibiting such expression inhibition activity may include, for example, that having a nucleotide sequence: CGGACTACCCTTAGCACAA. In such a case, further growth of the carcinoma cell is also inhibited, so that development of the cancer can be arrested.

As for a protein having an amino acid sequence identical or substantially identical to the amino acid sequence of SEQ. ID. No.1 of the present invention (hereinafter, sometimes referred to as "a protein of the present invention"), examples thereof may include an amino acid sequence having a homology of about 70 % or more, preferably about 80 % or more, more preferably 90 % or more, most preferably about 95 % or more with the amino acid sequence of SEQ. ID. No.1.

As for the protein comprising an amino acid sequence identical or substantially identical to the amino acid sequence of the SEQ. ID. No.1, preferred may be such a peptide that has an amino acid sequence substantially identical to the amino acid sequence of SEQ. ID. No.1 and possesses a substantially equivalent activity to the protein having the amino acid sequence of SEQ. ID. No.1.

The substantially equivalent activity may include, for instance, activity of the protein comprising the amino acid sequence of SEQ. ID. No.1, such as a function for inducing cancer, enzymic activity, transcribing activity and binding activity with a binding protein therefor.

The term "substantially equivalent" as used herein means that these activities are identical in nature (for example, bio-chemically or pharmacologically).

Examples of the amino acid sequence being identical or substantially identical to the amino acid sequence of SEQ. ID.

No.1 include

(i) the amino acid sequence of SEQ. ID. No.1;

(ii) an amino acid sequence which has deletions of 1 to 30, preferably 1 to 20, more preferably 1 to 10 amino acids from the amino acid sequence of SEQ. ID. No.1;

(iii) an amino acid sequence which has addition of 1 to 30, preferably 1 to 20, more preferably 1 to 10 amino acids to the amino acid sequence of SEQ. ID. No.1;

(iv) an amino acid sequence which has insertion of 1 to 30, preferably 1 to 20, more preferably 1 to 10 amino acids into the amino acid sequence of SEQ. ID. No.1;

(v) an amino acid sequence which has replacements of 1 to 30, preferably 1 to 20, more preferably 1 to 10 amino acids in the amino acid sequence of SEQ. ID. No.1 with other amino acids; and

(vi) an amino acid sequence which has combinational modification of two or more selected from the above (ii) to (v).

An example of a protein of the present invention may be a protein comprising the amino acid sequence of SEQ. ID. No.1.

A partial peptide of the present invention may be any of partial peptides of the protein of the present invention described above, and generally preferred is a peptide that is consisted of at least 5 or more, preferably at least 10 or more amino acids, and further preferably has activity equivalent to a protein of the present invention.

In a protein of the present invention or its partial peptide (hereinafter, sometimes referred to as "proteins of the present invention"), the left end is N-terminus (amino terminus) while the right end is C-terminus (carboxy terminus) according to conventional notation for peptide.

In a protein of the present invention including a protein comprising amino acid sequence of the SEQ. ID. No.1, C-terminal may be selected from carboxy (-COOH), carboxylate ($-COO^-$), amide ($-CONH_2$) and ester (-COOR).

Examples of R for the ester include C_{1-6}-alkyls such as methyl, ethyl, n-propyl, isopropyl and n-butyl; C_{3-8}-cycloalkyls such as cyclopentyl and cyclohexyl; C_{6-12}-aryls such as phenyl and α-naphthyl; phenyl-C_{1-2}-alkyls such as benzyl and phenethyl; C_{7-14}-aralkyls such as α-naphthyl-C_{1-2}-alkyls including α-naphthylmethyl; and pivaloyloxymethyl commonly used as ester for oral application.

When a protein of the present invention has a carboxy group (or carboxylate) in a position other than C-terminus, a protein in which the carboxy group is amidated or esterified is also a protein of the present invention. The ester here may be the C-terminal ester described above.

Furthermore, examples of a protein of the present invention include a protein in which an amino group in an N-terminal amino acid residue (for example, methionine residue) is protected by a protective group such as C_{1-6}-acyl including

C_{1-6}-alkanoyl such as formyl and acetyl; a protein in which N-terminal glutamic acid residue formed by in vivo cleavage is converted into form of pyroglutamic acid; a protein in which a substituent on a side chain in an amino acid residue in the molecule (for example, -OH, -SH, amino, imidazole, indole and guanidino) is protected by an appropriate protective group (for example, such as C_{1-6}-acyls including typically C_{1-6}-alkanoyl such as formyl and acetyl); and a conjugated protein such as a so-called glucoprotein in which a sugar chain is linked.

A salt of a protein of the present invention may be a salt with a physiologically acceptable acid (for example, inorganic and organic acids) or base (for example, an alkali metal salt), particularly preferably a physiologically acceptable acid-addition salt. Examples of such a salt include salts with inorganic acids such as hydrochloric acid, phosphoric acid, hydrobromic acid and sulfuric acid, and with organic acids such as acetic acid, formic acid, propionic acid, fumaric acid, maleic acid, succinic acid, tartaric acid, citric acid, malic acid, oxalic acid, benzoic acid, methanesulfonic acid and benzenesulfonic acid. Hereinafter, such a salt is also included in a protein of the present invention.

A protein of the present invention or its salt may be prepared by a known process for purification of a protein from human or warm-blooded mammalian cells, or alternatively by culturing a transformant produced by transformation with a DNA

encoding the protein described below.

When producing from a human or mammal tissue or cell, the human or mammal tissue or cell is homogenized and extracted with an acid. The extract is then purified by combined chromatographical procedures such as reverse phase chromatography and ion-exchange chromatography to isolate a desired product.

A polynucleotide encoding an oncogenic protein of the present invention may be any polynucleotide comprising the above nucleotide sequence encoding an oncogenic protein of the present invention (DNA or RNA, preferably DNA). The polynucleotide may be a DNA or RNA such as an mRNA encoding an oncogenic protein of the present invention, which may be single or double stranded. When being double stranded, it may be a double-stranded DNA, a double-stranded RNA or a hybrid of DNA:RNA. When being single stranded, it may be a sense chain, i. e., a coding chain, or an antisense chain, i. e., a non-coding chain.

A polynucleotide encoding a protein of the present invention may be used to quantify an mRNA of a protein of the present invention, according to a known method or its modification, for example, a method described in Experimental Medicine Extra Edition "Novel PCRs and their applications", 15(7), 1997.

A DNA encoding a protein of the present invention may be any DNA comprising the above nucleotide sequence encoding

a protein of the present invention, and may be a genome DNA, a genome DNA library, the above cell/tissue-derived cDNA, the above cell/tissue-derived cDNA library or a synthetic DNA.

A vector used for a library may be a bacteriophage, plasmid, cosmid or phagemid. A preparation of a total RNA or mRNA fraction from the cell or tissue may be used for amplification by a direct Reverse Transcriptase Polymerase Chain Reaction (hereinafter, referred to as "RT-PCR").

A nucleotide sequence available for a probe DNA of the present invention may be any sequence such as a DNA sequence which comprises a nucleotide sequence hybridizable with the nucleotide sequence of SEQ. ID. No.2 under high stringent conditions and encodes a protein having an activity substantially equivalent to that of a protein comprising the amino acid sequence of SEQ. ID. No.1.

A nucleotide sequence hybridizable with the nucleotide sequence of SEQ. ID. No.2 under high stringent conditions may be a nucleotide sequence having a homology of about 70 % or more, preferably about 80 % or more, more preferably about 90 % or more, further preferably about 95 % or more with the nucleotide sequence of SEQ. ID. No.2.

Hybridization may be conducted in accordance with a known method or its modification, for example, a method described in Molecular Cloning 2nd (J. Sambrook et al., Cold Spring Harbor Lab. Press, 1989). When using a commercially available library,

hybridization can be conducted in accordance with an attached manual. More preferably, it can be conducted under high stringent conditions.

High stringent conditions may include, for example, a sodium concentration of about 19 to 40 mM, preferably about 19 to 20 mM and a temperature of about 50 to 70 °C, preferably about 60 to 65 °C, most preferably a sodium concentration of about 19 mM and a temperature of about 65 °C.

More specifically, a DNA encoding a protein comprising amino acid sequence of the SEQ. ID. No.1 may be a DNA comprising the nucleotide sequence of SEQ. ID. No.2.

A DNA encoding a partial peptide of the present invention may be any DNA comprising a nucleotide sequence encoding the above partial peptide of the present invention, and may be a genome DNA, a genome DNA library, the above cell/tissue-derived cDNA, the above cell/tissue-derived cDNA library or a synthetic DNA.

A DNA encoding a partial peptide of the present invention may be a DNA comprising a partial nucleotide sequence of a DNA comprising the nucleotide sequence of SEQ. ID. No.2, or a DNA comprising a partial nucleotide sequence of a DNA which comprises a nucleotide sequence hybridizable with the nucleotide sequence of SEQ. ID. No.2 under high stringent conditions and encodes a protein having an activity substantially equivalent to that of a protein comprising the amino acid sequence of SEQ. ID. No.1.

The nucleotide sequence hybridizable with the nucleotide sequence of SEQ. ID. No.2 is defined as described above.

A hybridization method and the high stringent conditions may be as described above.

A polynucleotide comprising a part of a DNA sequence encoding a protein of the present invention or its partial peptide (hereinafter, sometimes referred to as "a protein of the present invention") or a part of a nucleotide sequence complementary to the DNA may encompass a DNA as well as an RNA encoding a protein of the present invention or its partial peptide.

According to the present invention, an antisense polynucleotide (nucleic acid) capable of inhibiting replication or expression of a protein gene of the present invention designed and synthesized on the basis of nucleotide sequence data on a DNA encoding the cloned or determined protein of the present invention. Such a polynucleotide (nucleic acid) can be hybridized with an RNA of a protein gene of the present invention to inhibit synthesis or activities of the RNA, or regulate or control expression of a protein gene of the present invention via interaction with an RNA related to a protein of the present invention. A polynucleotide complementary to a selected sequence in the RNA related to a protein of the present invention and a polynucleotide specifically hybridizable with the RNA related to a protein of the present invention are useful for

regulating or controlling in vivo or in vitro expression of a protein gene of the present invention, and for treatment or diagnosis of a disease. The term "corresponding to" as used herein means homology or complementation to a particular sequence of a nucleotide including a gene, a nucleotide sequence or a nucleic acid. The term "corresponding to" in terms of relationship between a nucleotide, a nucleotide sequence or a nucleic acid and a peptide (protein) generally refers to an amino acid in a protein (peptide), a command derived from a sequence of a nucleotide (nucleic acid) or its complementary sequence. Examples of a preferable target domain may include a 5'-terminal hairpin loop, a 5'-terminal 6-base pair repeat, a 5'-terminal untranslation domain, a protein translation initiating codon, a protein coding domain, an ORF translation stop codon, a 3'-terminal non-translation domain, a 3'-terminal palindrome domain and a 3'-terminal hairpin loop in a protein gene of the present invention, but any domain in a protein gene of the present invention may be selected as a target.

Relationship between a given nucleic acid a polynucleotide complementary to at least a part of a target domain and between a target and a hybridizable polynucleotide can be called "antisense". Examples of an antisense polynucleotide include a polynucleotide comprising 2-deoxy-D-ribose, a polynucleotide comprising D-ribose, other types of polynucleotides as an N-glycoside of purine or pyrimidine base

and other polymers comprising a non-nucleotide structure (for example, a commercially available protein nucleic acid and a synthetic-sequence specific nucleic acid polymer) or other polymer having a special bond although the polymer comprises a nucleotide having a configuration which can accept base pairing or base attachment as observed in a DNA or RNA. These may be a double-stranded DNA, a single-stranded DNA, a double-stranded RNA, a single-stranded RNA or a DNA:RNA hybrid. They may further include unmodified polynucleotides (or unmodified oligonucleotide), those having a known modification such as a tag known in the art, a capping, methylation, replacement of at least one natural nucleotide with an analogue and an intramolecular nucleotide modification; those having a non-charged bond (for example, methylphosphonate, phosphotriester, phosphoramidate and carbamate); those having a charged bond or sulfur-containing bond (for example, phosphorothioate and phosphorodithioate); those having a side chain group including a protein (a nuclease, a nuclease inhibitor, toxine, an antibody, a signal peptide and poly-L-lysine) or a sugar (for example, a monosaccharide); those comprising an intercurrent compound (for example, acridine and psoralen); those comprising a chelating compound (for example, a metal, a radioactive metal, boron and an oxidizing metal); those comprising an alkylating agent; and those comprising a modified bond (for example, an α-anomer type nucleic acid). The terms

"nucleoside", "nucleotide" and "nucleic acid" as used herein may include not only those containing purine and pyrimidine bases but also those further containing another modified heterocyclic base. Such a modified substance may contain methylated purine and pyrimidine, acylated purine and pyrimidine or other heterocycles. A modified nucleoside and a modified nucleotide may be modified in a sugar moiety; for example, one or more hydroxyls may be replaced with a halogen or aliphatic group, or may be converted into another functional group such as ether and amine.

An antisense polynucleotide (nucleic acid) of the present invention is an RNA, DNA or modified nucleic acid (RNA, DNA). Examples of a modified nucleic acid include, but not limited to, a sulfur derivative or thiophosphate derivative of a nucleic acid and those resistant to decomposition by a polynucleosideamide or an oligonuclesideamide. An antisense nucleic acid of the present invention may be preferably designed, for example, such that the antisense nucleic acid is made more stable in a cell, the antisense nucleic acid has a higher permeability in a cell, it has affinity to a target sense chain, or if it is toxic, the antisense nucleic acid is made less toxic.

A variety of such modifications are well known in the art, and have been disclosed in, for example, J. Kawakami et al., Pharm Tech Japan, Vol. 8, pp.247, 1992; Vol. 8, pp.395, 1992; S. T. Crooke et al. ed., Antisense Research and Applications,

CRC Press, 1993.

An antisense nucleic acid of the present invention may comprise a converted and/or modified sugar, base and/or bond, and thus may be provided as a special form such as a liposome and a microsphere, may be applied in gene therapy or may be provided as an adduct. Examples of such an adduct include a polycationic adduct such as polylysine acting as a neutralizer to a charge in a phosphate structure and a hydrophobic adduct such as a lipid enhancing interaction with a cell membrane or increasing an uptake of a nucleic acid (for example, a phospholipid and cholesterol). Examples of a lipid preferable for addition include cholesterol and its derivatives (for example, cholesteryl chloroformate and cholic acid). It may be attached to a 3'- or 5'-terminal in a nucleic acid via a base, sugar or intramolecular nucleoside bond. Another available group may be, for example, a capping group specifically located at a 3'- or 5'-terminal in a nucleic acid for preventing decomposition by a nuclease such as exonuclease and RNase. Examples of such a capping group include, but not limited to, those known as a hydroxyl-protecting group in the art such as glycols including polyethyleneglycol and tetraethyleneglycol.

Inhibition of activity of antisense nucleic acid can be determined using a transformant of the present invention, an in vivo or in vitro gene expression system of the present invention, or an in vivo or in vitro translation system in a

protein of the present invention. The nucleic acid may be applied to a cell by any of various known methods.

A DNA encoding a protein of the present invention may be labeled by a known method; for example, isotope labeling, fluorescent labeling (for example, fluorescent labeling with fluorescein), biotinylation and enzyme labeling.

A DNA fully encoding a protein of the present invention may be cloned by amplification by a known PCR using a synthetic DNA primer comprising a partial nucleotide sequence in a protein of the present invention, or selecting a DNA integrated in an appropriate vector by hybridization with that labeled with a DNA fragment or synthetic DNA encoding a partial or full-length of a protein of the present invention. Hybridization may be conducted by, for example, a method described in Molecular Cloning 2nd, J. Sambrook et al., Cold Spring Harbor Lab. Press, 1989. When using a commercially available library, hybridization can be conducted in accordance with an attached manual.

A sequence of a DNA may be transformed using a known kit such as Mutan™-super Express Km (Takara Shuzo Co., Ltd.), Mutan™-K(Takara Shuzo Co., Ltd.), by a known method such as ODA-LA PCR, Gapped duplex method and Kunkel method or variation thereof.

Depending on an application, a DNA encoding a cloned peptide may be used as such or if desired used after digestion by a restriction enzyme or addition of a linker. The DNA may

have ATG as a translation initiating codon at the 5'-terminal and TAA, TGA or TAG as a translation stop codon at the 3'-terminal. The translation initiating codon or the translation stop codon may be added using an appropriate synthetic DNA adapter.

An expression vector of a protein of the present invention may be produced by, for example, (i) excising a desired DNA fragment from a DNA encoding a protein of the present invention and (ii) ligating the DNA fragment in the downstream of a promoter in an appropriate expression vector.

Examples of a vector which can be used include plasmids derived from E. coli (for example, pBR322, pBR325, pUC12 and pUC13); plasmids derived from Bacillus subtilis (for example, pUB110, pTP5 and pC194); plasmids derived from an yeast (for example, pSH19 and pSH15); bacteriophages such as λ-phage; mammalian viruses such as retroviruses, vaccinia viruses and baculoviruses; pA1-11; pXT1; pRc/CMV; pRc/RSV; and pcDNAI/Neo.

A promoter used in the present invention may be any promoter appropriate to a host used in gene expression. For example, when using a mammalian cell as a host, an SR α-promoter, an SV40 promoter, an HIV-LTR promoter, a CMV promoter or an HSV-TK promoter may be used.

Among these, it is preferable to use a CMV (cytomegalovirus) promoter or an SR α-promoter. When a host is an Escherichia coli, a trp promoter, a lac promoter, a recA promoter, a λ-PL promoter, an lpp promoter and a T7 promoter

are preferable; when a host is a Bacillus, an SPO1 promoter, an SPO2 promoter and a penP promoter are preferable; when a host is an yeast, a PHO5 promoter, aPGK promoter, a GAP promoter and an ADH promoter are preferable. When a host is an insect cell, a polyhetrin promoter and a P10 promoter are preferable.

If desired, another expression vector can be used, including those comprising an enhancer, a splicing signal, a poly-A addition signal, a selection marker and/or an SV40 replication origin (hereinafter, sometimes referred to as "SV40ori"). Examples of a selection marker include dihydrofolate reductase (hereinafter, sometimes referred to as "dhfr") gene [methotrexate (MTX) resistant], an ampicillin resistant gene (hereinafter, sometimes referred to as "Ampr") and a neomycin resistant gene (hereinafter, sometimes referred to as "Neor", G418 resistant). In particular, when using the dhfr gene as a selection marker using a dhfr gene deleted Chinese Hamster cell, integration of a desired gene may be selected using a thymidine deficient medium.

As a selection marker, a reporter gene or drug resistance gene is used (New Biochemical Experimental Lectures (Shin Seikagaku Jikken Koza) 2, nucleic acid III, 3.6 Mammalian cell expression vector, p84-103).

Examples of a combination of a drug resistance gene and a drug which can be used include:

(1) a combination of a puromycin-N-acetyltransferase

gene and puromycin;

(2) a combination of an aminoglycoside phosphotransferase gene (APH) and G418;

(3) a combination of a hygromycin-B phosphotransferase gene (HPH) and hygromycin-B; and

(4) a combination of xanthine-guanine phosphoribosyltransferase (XGPRT) and mycophenolate.

When a parent cell strain is a hypoxanthine-guanine phosphoribosyltransferase (HGPRT) or thymidinekinase (TK) deficient strain, a combination of these genes and HAT(hypoxanthine, aminopterin and thymidine) may be used.

Dihydrofolate reductase or ampicillin resistance gene may be used as a selection marker gene.

If necessary, a signal sequence appropriate for a host is added to the N-terminal of a protein of the present invention. When a host is an Escherichia coli, a PhoA signal sequence and an OmpA signal sequence may be used; when a host is a Bacillus, an α-amylase signal sequence and a subtilisin signal sequence may be used; when a host is an yeast, an MFα signal sequence and an SUC2 signal sequence may be used; when a host is a mammalian cell, an insulin signal sequence, an α-interferon signal sequence and an antibody-molecule signal sequence may be used.

A vector comprising a DNA encoding a protein of the present invention thus constructed may be used to produce a transformant.

A host may be, for example, an Escherichia coli, a Bacillus, an yeast, an insect cell, an insect or a mammalian cell.

Examples of an Escherichia coli include Escherichia coli K12 DH1 [Proc. Natl. Acad. Sci. USA), Vol. 60, 160 (1968)], JM103 [Nucleic Acids Research, Vol. 9, 309 (1981)], JA221 [Journal of Molecular Biology, Vol.120, 517 (1978)], HB101 [Journal of Molecular Biology, Vol.41, 459 (1969)] and C600 [Genetics, Vol.39, 440 (1954)].

Examples of a Bacillus include Bacillus subtilis MI114 [Gene, Vol. 24, 255 (1983)] and 207-21 [Journal of Biochemistry, Vol. 95, 87 (1984)].

Examples of an yeast include Saccharomyces cerevisiae AH22, AH22R$^-$, NA87-11A, DKD-5D and 20B-12; Schizosaccharomyces pombe NCYC1913 and NCYC2036; and Pichia pastoris KM71.

In terms of an insect cell, when the virus is AcNPV, an established cell derived from armyworm larva (Spodoptera frugiperda cell; Sf cell), an MG1 cell derived from a midgut of Trichoplusia ni, a High Five™ cell derived from an egg of Trichoplusia ni, a cell derived from Mamestra brassicae or a cell derived from Estigmena acrea may be used. When the virus is BmNPV, an established cell derived from silkworm (Bombyx mori N cell; BmN cell) may be used. Examples of the Sf cell which can be used include an Sf9 cell (ATCC CRL1711) and an Sf21 cell, which have been described in Vaughn, J. L. et al., In Vivo, 13,

213-217 (1977)).

Examples of an insect include silkworm larvae [Maeda et al., Nature, Vol.315, 592 (1985)].

Examples of a mammalian cell include a simian cell COS-7 (COS7), Vero, a Chinese Hamster cell CHO (hereinafter, referred to as "CHO cell"), a dhfr-gene deleted Chinese Hamster cell CHO (hereinafter, referred to as "CHO(dhfr$^-$) cell"), a murine L cell, a murine AtT-20, a murine myeloma cell, a rat GH3 and a human FL cell.

An Escherichia coli may be transformed in accordance with, for example, a method described in Proc. Natl. Acad. Sci. USA), Vol.69, 2110 (1972) or Gene, Vol.17, 107 (1982).

A Bacillus may be transformed in accordance with, for example, a method described in Molecular & General Genetics), Vol.168, 111 (1979).

An yeast may be transformed in accordance with, for example, a method described in Methods in Enzymology, Vol.194, 182-187 (1991) or Proc. Natl. Acad. Sci. USA, Vol.75, 1929 (1978).

An insect cell or an insect may be transformed in accordance with, for example, a method described in Bio/Technology, 6, 47-55 (1988).

A mammalian cell may be transformed in accordance with, for example, a method described in Cell Technology Extra Issue 8, New Cell Technological Experiment Protocols. 263-267 (1995) (Shuju Co. Ltd.) or Virology, Vol.52, 456 (1973).

Thus, a transformant which has been transformed with an expression vector comprising a DNA encoding a protein of the present invention can be obtained.

When culturing a transformant for which a host is an Escherichia coli or Bacillus, an appropriate medium used for culturing is a liquid medium containing a carbon source, a nitrogen source, inorganic materials and so on needed for growing the transformant. Examples of a carbon source include glucose, dextrin, soluble starch and sucrose. Examples of a nitrogen source include inorganic and organic materials such as ammonium salts, nitrates, corn steep liquor, peptone, casein, meat extract, soybean cake and potato extract. Examples of an inorganic material include calcium chloride, sodium dihydrogenphosphate and magnesium chloride. An yeast extract, vitamins and/or growth accelerating factors may be added. The medium desirably has a pH of about 5 to 8.

A preferable example of a medium for culturing an Escherichia coli may be an M9 medium containing glucose and casamino acid [Miller, Journal of Experiments in Molecular Genetics, 431-433, Cold Spring Harbor LaboHumanory, New York, 1972]. If necessary, an additional agent such as 3 β -indolylacrylic acid may be added for efficient promoter activity.

When a host is an Escherichia coli, culturing is generally conducted at about 15 to 43 °C for about 3 to 24 hours

under, if necessary, aeration and/or agitation.

When a host is a Bacillus, culturing is generally conducted at about 30 to 40 °C for about 6 to 24 hours under, if necessary, aeration and/or agitation.

When culturing a transformant whose host is an yeast, examples of a culture medium which can be used include a Burkholder minimum medium [Bostian, K. L. et al, Proc. Natl. Acad. Sci. USA, Vol.77, 4505 (1980)] and an SD medium containing 0.5% of casamino acid [Bitter, G. A. et al., Proc. Natl. Acad. Sci. USA, Vol.81, 5330 (1984)]. A pH of the medium is preferably adjusted to about 5 to 8. Culturing is generally conducted at about 20 to 35 °C for about 24 to 72 hours under, if necessary, aeration and/or agitation.

When culturing a transformant in which a host is an insect cell or insect, a culture may be a Grace's Insect Medium (Grace, T.C.C., Nature, 195, 788 (1962)) containing additives such as 10 % decomplemented bovine serum as appropriate. A pH of the medium is preferably adjusted to about 6.2 to 6.4. Culturing is generally conducted at about 27 °C for about 3 to 5 days under, if necessary, aeration and/or agitation.

When culturing a transformant in which a host is a mammalian cell, examples of a culture include an MEM medium containing about 5 to 10 % fetal bovine serum [Science, Vol.122, 501 (1952)], a DMEM medium [Virology, Vol.8, 396 (1959)], an RPMI 1640 medium [the Journal of the American Medical Association,

Vol.199, 519 (1967)] and a 199 medium [Proceeding of the Society for the Biological Medicine, Vol.73, 1 (1950)]. A pH of the medium is preferably adjusted to about 6 to 8. Culturing is generally conducted at about 30 to 40 °C for about 15 to 60 hours under, if necessary, aeration and/or agitation.

As described above, a protein of the present invention may be produced in an intracellular, cell-membrane or extracellular region of a transformant cell.

A protein of the present invention can be isolated and purified from the above culture by, for example, the following method.

A protein of the present invention can be extracted from cultured bacteria or cells by, as appropriate, a method where after culturing, the bacteria or the cells are collected by a known procedure, they are suspended in a proper buffer, the bacteria or the cells are lysed using ultrasonic, lysozyme and/or freezing and thawing, then they are centrifuged or filtrated to give a crude extract of a protein of the present invention. The buffer may contain a protein modifier such as urea and guanidine hydrochloride and a surfactant such as Triton X-100™. When a peptide is secreted into a culture medium, the bacteria or the cells are separated from the supernatant by a known method after culturing, and then the supernatant is collected.

A protein of the present invention contained in the culture supernatant or the extract thus obtained may be purified

by an appropriate combination of known separation/purification methods. Examples of such known separation/purification methods include methods utilizing a solubility such as salting out and solvent precipitation; methods mainly utilizing a molecular weight difference such as dialysis, ultrafiltration, gel filtration and SDS-polyacrylamide gel electrophoresis; methods utilizing a charge difference such as ion-exchange chromatography; methods utilizing specific affinity such as affinity chromatography; method utilizing a hydrophobicity difference such as reverse phase high performance liquid chromatography; and methods an isoelectric point difference such as isoelectric focusing.

When the protein of the present invention thus obtained is a free form, it can be converted into a salt by a known method or a modification thereof. In reverse, when it is obtained as a salt, it can be converted into a free form or another salt by a known method or a modification thereof.

Before or after purification, a protein of the present invention produced by a recombinant may be attacked by an appropriate protein modifying enzyme to be modified as appropriate or to partially remove a peptide. Examples of a protein modifying enzyme include trypsin, chymotrypsin, arginyl-endopeptidase, protein kinase and glycosidase.

An antibody to a protein of the present invention (hereinafter, sometimes simply referred to as "an antibody of

the present invention") may be either polyclonal or monoclonal as long as it is an antibody which can recognize an antibody to a protein of the present invention.

The antibody to a protein of the present invention can be produced by a known method for preparing an antibody or antiserum using a protein of the present invention as an antigen.

Preparation of a monoclonal antibody

(a) Preparation of a monoclonal antibody producing cell

A protein of the present invention is applied alone or in combination with a carrier and a diluent to a site in a warm-blooded animal where an antibody can be produced. For application, a Freund's complete or incomplete adjuvant may be applied for improving antibody-producing ability. It is generally applied once in 2 to 6 weeks and about 2 to 10 times in total. Examples of a warm-blooded animal include monkey, rabbit, dog, guinea pig, mouse, rat, sheep, goat and poultry, preferably mouse and rat.

For preparing a monoclonal antibody producing cell, an individual exhibiting an antibody titer from a warm-blooded animal immunized by an antigen such as mouse is selected and a spleen or lymph node is isolated 2 to 5 days after final immunization. An antibody producing cell contained in the isolated may be fused to a myeloma cell of the same or a different animal to prepare a monoclonal antibody producing hybridoma. An antibody titer in an antiserum can be determined by, for example,

reacting a labeled peptide described later with the antiserum and then measuring a label activity bound to an antibody. Fusion may be conducted by a known method such as a Kaehler-Milstein method [Nature, 256, 495 (1975)]. Examples of a fusion accelerator include polyethyleneglycol (PEG) and Sendai virus, preferably PEG.

Examples of a myeloma cell include those of a warm-blooded animal such as NS-1, P3U1, SP2/0 and AP-1, preferably P3U1. A preferable number ratio of an antibody producing cell (spleen cell) to a myeloma cell is about 1:1 to 20:1, PEG (preferably PEG1000 to PEG6000) is added to a concentration of about 10 to 80 %, and the cell fusion can be efficiently conducted by incubation at 20 to 40 °C, preferably 30 to 37 °C for 1 to 10 min.

A monoclonal antibody producing hybridoma can be screened by any of various methods; for example, by adding a hybridoma culture supernatant to a solid phase (e. g., microplate) in which a peptide (protein) antigen has been adsorbed directly or in combination with a carrier, then adding an anti-immunoglobulin antibody (when a cell used for cell fusion is a murine cell, an anti-mouse immunoglobulin antibody is used) or protein A labeled with a radioactive agent or enzyme, and finally detecting a monoclonal antibody bound to the solid phase, or alternatively by adding a hybridoma culture supernatant to a solid phase in which an anti-immunoglobulin antibody or protein

A has been adsorbed, adding a peptide labeled with a radioactive agent or enzyme, and finally detecting a monoclonal antibody bound to the solid phase.

A monoclonal antibody can be selected by a known method or its modification. It can be generally using a medium for a mammalian cell containing HAT (hypoxanthine, aminopterin and thymidine). A medium for selection and breeding may be any medium in which a hybridoma can be grown; for example, an RPMI 1640 medium containing 1 to 20%, preferably 0 to 20% fetal bovine serum, a GIT medium containing 1 to 10% fetal bovine serum (Wako Pure Chemicals Co. Ltd.) and a serum-free medium for culturing a hybridoma (SFM-101, Nissui Pharmaceutical Co., Ltd.). A culturing temperature is generally 20 to 40 °C, preferably about 37 °C. A culturing period is generally 5 days to 3 weeks, preferably 1 to 2 weeks. Culturing may be generally conducted under 5 % gaseous carbon dioxide. An antibody titer in a hybridoma culture supernatant can be determined as described for determination of an antibody titer in the above antiserum.

(b) Purification of a monoclonal antibody

A monoclonal antibody can be separated and purified by a known method including separation/purification methods for an immunoglobulin such as salting out, alcohol precipitation, isoelectric precipitation, electrophoresis, an adsorption and desorption method with an ion exchanger (for example, DEAE), ultracentrifugation, gel filtration, and a specific

purification in which an antibody is exclusively collected by an activated adsorbent such as an antigen-binding solid phase, protein A or protein G for dissociating the bond to obtain the antibody.

Preparation of a polyclonal antibody

A polyclonal antibody of the present invention can be prepared in accordance with a known method or its modification. For example, an immunogen (peptide antigen) or its complex with a carrier protein is prepared, a warm-blooded animal is immunized with it as described for a preparation process for the above monoclonal antibody, a product containing an antibody to a protein of the present invention is collected from the immunized animal, and after separation and purification, the antibody can be prepared.

In terms of a complex of an immunogen and a carrier protein for immunizing a warm-blooded animal, any type of carrier proteins and any mix ratio of the carrier to a hapten may be employed as long as an antibody can be efficiently produced to a hapten immunized by crosslinking to the carrier; for example, about 0.1 to 20 parts by weight, preferably about 1 to 5 parts by weight of bovine serum albumin, bovine thyroglobulin, hemocyanin or the like is coupled to 1 part by weight of the hapten.

Various condensing agents may be used for coupling of the hapten with the carrier, including glutaraldehyde,

carbodiimide, activated maleimide ester and activated ester reagents having a thiol and/or a dithiopyridyl groups.

A condensation product is applied alone or in combination of a carrier and a diluent, to a site in a warm-blooded animal where an antibody can be produced. For application, a Freund's complete or incomplete adjuvant may be applied for improving antibody-producing ability. It is generally applied once in 2 to 6 weeks and about 3 to 10 times in total.

A polyclonal antibody can be collected from blood or ascites, preferably blood of a warm-blooded animal immunized as described above.

A polyclonal antibody titer in an antiserum can be determined as described for determination of an antibody titer in an antiserum. The polyclonal antibody can be separated and purified as described for separation and purification of the immunoglobulin in the course of separation/purification of the above monoclonal antibody.

An antisense DNA comprising a nucleotide sequence complementary or substantially complementary to a DNA encoding a protein of the present invention (hereinafter, the latter DNA is sometimes referred to as "a DNA of the present invention", and the former antisense DNA is sometimes referred to as "antisense DNA") may be any antisense DNA comprising a nucleotide sequence complementary or substantially complementary to a DNA of the present invention and capable of inhibiting expression

of the DNA.

A nucleotide sequence substantially complementary to a DNA of the present invention may be, for example, a nucleotide sequence having a homology of about 70 % or more, preferably about 80 % or more, more preferably 90 % or more, most preferably about 95 % or more with the full or partial nucleotide sequence complementary to a DNA of the present invention (i. e., a complementary chain to a DNA of the present invention). Particularly preferred is an antisense DNA having a homology of about 70 % or more, preferably about 80 % or more, more preferably 90 % or more, most preferably about 95 % or more with a complementary chain to a nucleotide sequence of a domain encoding the N-terminal site of a protein of the present invention (for example, a nucleotide sequence near an initiating codon) in the full nucleotide sequence of the complementary chain to a DNA of the present invention. Such an antisense DNA can be prepared using, for example, a known DNA synthesizer.

There will be described applications of an oncogenic protein according to the present invention (partial peptide, including a salt), a DNA of the present invention, a antibody of the present invention and an antisense DNA of the present invention.

(2) Screening method for a compound promoting or inhibiting expression of an oncogenic protein according to the present invention

An oncogenic protein of the present invention, an oligonucleotide of the present invention, a transformant of the present invention or an antibody of the present invention can be used for a screening method for a compound promoting or inhibiting expression of a protein of the present invention.

Specifically, the present invention provides

(i) a method for screening a compound promoting or inhibiting expression of an oncogenic protein of the present invention comprising determining and comparing the amount of expression of an oncogenic protein of the present invention or the amount of an mRNA encoding an oncogenic protein of the present invention when culturing a cell or tissue which can express an oncogenic protein of the present invention in the presence or absence of a test compound.

Examples of a cell or tissue which can express an oncogenic protein of the present invention include a human-derived cell, a warm-blooded animal (e. g., guinea pig, rat, mouse, poultry, rabbit, pig, sheep, bovine and monkey) cell (for example, neural cell, endocrine cell, neuroendocrine cell, glia cell, pancreatic β-cell, marrow cell, hepatic cell, splenic cell, mesangium cell, epidermal cell, epithelial cell, endothelial cell, fibroblast, fibrocyte, myocyte, adipocyte, immunocyte (e.g., macrophage, T-cell, B-cell, natural killer cell, mast cell, neutrophile, basophilic cell, acidophilic leucocyte, monocyte, dendritic cell), mega karyocyte,

synoviocyte, cartilage cell, osteocyte, osteoblast, osteoclast, mammary glandular cell, intersitial cell and their precursor cells, stem cells and carcinoma cells, as well as all tissues where any of these cells is present such as brain, brain sites (for example, olfactory bulb, amygdaloid nucleus, basal cistern, hippocampus, optic thalamus, hypothalamus, cerebral cortex, medula oblongata, cerebellum), spinal cord, pituitary gland, stomach, pancreas, kidney, liver, genital gland, hyroid gland, gallbladder, bone marrow, adrenal gland, skin, muscle, lung, gastrointestinal tract (for example, large intestine and small intestine), blood vessel, heart, thymus gland, spleen, salivary gland, peripheral blood, prostate gland, testicle (spermary), ovary, placenta, uterus, bone, cartilage, joint and skeletal muscle. Here, an established cell or primary culture system may be used. In particular, it is desirable to use the above transformant cell of the present invention.

A method for culturing a cell which can express a protein of the present invention is as described for culturing the above transformant of the present invention.

A test compound may be, in addition to the above test compounds, a DNA library.

The amount of expression of a cancer cell of the present invention can be determined a known method such as an immunochemical method using, for example, an antibody, or alternatively an mRNA encoding an oncogenic protein of the

present invention can be determined by a known method using Northern hybridization, RT-PCR or TaqMan PCR.

The amounts of expression of an mRNA can be compared by hybridization in accordance with a known method or its modification, for example, a method described in Molecular Cloning 2nd, J. Sambrook et al., Cold Spring Harbor Lab. Press, 1989.

Specifically, the amount of an mRNA encoding an oncogenic protein of the present invention is determined according to a known method, i. e., by contacting an RNA extracted from a cell with a polynucleotide of the present invention or its part or an antisense polynucleotide of the present invention and then measuring the amount of mRNA bound to a polynucleotide of the present invention or its part or an antisense polynucleotide of the present invention. A polynucleotide of the present invention or its part or an antisense polynucleotide of the present invention can be labeled with, for example, a radioisotope or dye to facilitate determination of the amount of mRNA bound to a polynucleotide of the present invention or its part or an antisense polynucleotide of the present invention. Examples of a radioisotope include ^{32}P and ^{3}H, and examples of a dye include fluorochromes such as fluorescein, FAM (PE Biosystems Inc.), JOE (PE Biosystems Inc.), TAMRA (PE Biosystems Inc.), ROX (PE Biosystems Inc.), Cy5 (Amersham Inc.) and Cy3 (Amersham Inc.).

The amount of an mRNA can be determined by transforming an RNA extracted from a cell into cDNA using a reverse transcriptase and then measuring the amount of amplified cDNA by PCR using a polynucleotide of the present invention or its part or an antisense polynucleotide of the present invention as a primer.

Thus, a test compound which increases the amount of an mRNA encoding an oncogenic protein of the present invention can be selected as a compound promoting expression of an oncogenic protein of the present invention, while a test compound which reduce the amount of an mRNA encoding an oncogenic protein of the present invention can be selected a compound inhibiting expression of an oncogenic protein of the present invention.

The present invention also provides

(ii) a method for screening a compound promoting or inhibiting promoter activity comprising determining and comparing a reporter activity when culturing, in the presence or absence of a test compound, a transformant obtained by transforming with a recombinant DNA in which a reporter gene is ligated to the downstream of a promoter or enhancer domain for a gene encoding an oncogenic protein of the present invention.

Examples of a reporter gene which can be used include lacZ (β-galactosidase gene), chloramphenicol acetyltransferase (CAT), luciferase, growth factors, β-glucuronidase, alkaline phosphatase, Green fluorescent protein (GFP) and β-lactamase.

By determining the amount of a reporter gene product (e. g., mRNA and protein) using a known method, a test compound which increases the amount of the reporter gene product can be selected as a compound controlling (particularly, promoting) promoter or enhancer activity of a protein of the present invention, i. e., as a compound promoting expression of a protein of the present invention. On the contrary, a test compound which reduces the amount of the reporter gene product can be selected as a compound controlling (particularly, inhibiting) promoter or enhancer activity of a protein of the present invention, i. e., as a compound inhibiting expression of a protein of the present invention.

A test compound may be as described above.

A vector comprising a reporter gene can be constructed or assayed by a known technique (For example, see Molecular Biotechnology 13, 29-43, 1999).

Since a compound inhibiting expression of an oncogenic protein of the present invention can inhibit biological activities of an oncogenic protein of the present invention, it is useful as a safe and low-toxic medical drug for inhibiting biological activities of an oncogenic protein of the present invention. Specifically, it is useful as a prophylactic or therapeutic drug for a cancer such as pulmonary cancer, renal cancer, hepatic carcinoma, non-small cell pulmonary cancer, ovarian cancer, prostatic cancer, gastric cancer, bladder cancer,

breast cancer, cervical cancer, colonical cancer, rectal cancer and pancreatic cancer, particularly cervical cancer.

A compound or its salt obtained using a screening method or screening kit of the present invention may be, for example, a compound selected from the group consisting of a peptide, a protein, a non-peptide compound, a synthetic compound, a fermentation product, a cell extract, a plant extract, an animal-tissue extract and plasma. A salt of the compound may be as described for a salt of a peptide derived from an oncogenic protein of the present invention.

When using a compound obtained using a screening method or screening kit according to the present invention as the above therapeutic or prophylactic agent, it can be used as usual. For example, as described for a medicine comprising a protein of the present invention, it can be prepared as a tablet, a capsule, an elixir, a microcapsule, a sterile solution and a suspension.

A dose of the compound or its salt depends on various factors such as its effect, a target disease, a recipient and a delivery route. For example, in oral administration of a compound inhibiting expression of a protein of the present invention for treating a cancer, its dose is about 0.1 to 100 mg, preferably about 1.0 to 50 mg, more preferably about 1.0 to 20 mg per day to a normal adult (weight: 60 kg). In parenteral administration, although a dose of the compound depends on various factors such as a recipient and a target disease, its

dose is suitably about 0.01 to 30 mg, preferably about 0.1 to 20 mg, more preferably about 0.1 to 10 mg per day by intravenous injection when a compound inhibiting expression of a protein of the present invention is administered to a normal adult (weight: 60 kg) for treating a cancer. For another animal, an amount converted to a weight of 60 kg may be administered.

(3) Assay of an oncogenic protein of the present invention

An antibody of the present invention can specifically recognize an oncogenic protein of the present invention, so that it can be used for assaying the oncogenic protein of the present invention in a test solution, particularly an assay by sandwich immunity measuring method.

Thus, the present invention provides

(i) a process for assaying an oncogenic protein of the present invention in a test solution comprising the steps of competitively reacting an antibody of the present invention with the test solution and a labeled protein of the present invention and then determining a proportion of the labeled protein of the present invention bound to the antibody; and

(ii) a process for assaying an oncogenic protein of the present invention in a test solution comprising the steps of simultaneously or sequentially reacting the test solution with an antibody of the present invention insolubilized on a carrier and another labeled antibody of the present invention and then determining an activity of the labeling agent on the

insolubilized carrier.

In the assay described in (ii), it is desirable that one antibody is an antibody recognizing the N-terminus in an oncogenic protein of the present invention, while the other antibody is an antibody reacting with the C-terminus in an oncogenic protein of the present invention.

A monoclonal antibody to an oncogenic protein of the present invention can be used for assaying an oncogenic protein of the present invention or detection using, for example, tissue staining. For these purposes, an antibody molecule itself can be used, or alternatively, an $F(ab')_2$, Fab' or Fab fraction of the antibody molecule can be used.

There are no particular restrictions to a procedure for assaying an oncogenic protein of the present invention using an antibody of the present invention, and it may be any procedure whereby the amount of an antibody, antigen or antibody-antigen complex corresponding to the amount of an antigen (for example, the amount of a peptide) in a measured solution can be detected by chemical or physical means and a desired value can be calculated using a standard curve plotted using the standard solution containing a known amount of the antigen. For example, nephelometry, a competition assay, an immunoradiometric assay and a sandwich assay are suitably used, and in terms of sensitivity and specificity, a sandwich assay described below is particularly preferable.

Examples of a labeling agent used in an assay using such a labeling material include a radioisotope, an enzyme, a fluorescent substance and a luminescent material. Examples of a radioisotope include [^{125}I], [^{131}I], [^{3}H] and [^{14}C]. Among these, a stable enzyme exhibiting a large specific activity is preferable, including β -galactosidase, β -glucuronidase, alkaline phosphatase, peroxidase and malate dehydrogenase. Examples of a fluorescent substance include fluorescamine and fluorescein isothiocyanate. Examples of a luminescent material include luminol, luminol derivatives, luciferin and lucigenin. A biotin-avidin system may be used for binding an antibody or antigen with a labeling agent.

An antigen or antibody can be insolubilized using physical adsorption, or using a chemical bond commonly used for insolubilizing or immobilizing a peptide or enzyme. Examples of a carrier include insoluble polysaccharides such as agarose, dextran and cellulose; synthetic resins such as polystyrene, polyacrylamide and silicones; and glasses.

In a sandwich assay, an insolubilized monoclonal antibody of the present invention is reacted with a test solution (first reaction), and then reacted with another labeled monoclonal antibody of the present invention (second reaction). Then, an activity of the labeling agent on the insolubilized carrier can be determined to assay the amount of a protein of the present invention in the test solution. The first and the

second reactions may be conducted in the reverse order, simultaneously or sequentially. A labeling agent and an insolubilizing method may be as described above. In an immunity measuring method using a sandwich assay, it is not necessary to use only one antibody for a solid phase or for labeling, but a mixture of two or more antibodies may be used for, for instance, improving measurement sensitivity.

In determination of an oncogenic protein of the present invention by a sandwich assay according to the present invention, monoclonal antibodies of the present invention used in the first and the second reactions is preferably antibodies having a different site to which the oncogenic protein of the present invention is bound. Specifically, in terms of antibodies used in the first and the second reactions, when the antibody used in the second reaction recognizes the C-terminus in the oncogenic protein of the present invention, the antibody used in the first reaction is an antibody which recognizes a site other than the C-terminus, for example the N-terminus.

A monoclonal antibody of the present invention may be used a measurement system other than a sandwich assay, such as a competitive assay, immunometry and nephelometry.

In a competitive assay, an antigen in a test solution and a labeled antigen are competitively reacted with an antibody, the unreacted labeled antigen (F) is separated from the bound labeled antigen (B) (B/F separation) and the labeled amount of

either B or F is determined to assay the antigen amount in the test solution. This reaction assay can be conducted by a liquid phase method where a soluble antibody is used as an antibody and polyethylene glycol and a secondary antibody to the above antibody are used in B/F separation, or alternatively by a solid phase method where a immobilized antibody is used as the first antibody or the first antibody is a soluble one and the second antibody is a immobilized antibody.

In immunometry, an antigen in a test solution and a immobilized antigen are competitively reacted with a given amount of a labeled antibody, the solid and the liquid phases are separated, or alternatively an antigen in a test solution is reacted with an excessive amount of a labeled antibody, a immobilized antigen is added to allow the unreacted labeled antibody to bind to the solid phase and then the solid and the liquid phases are separated. Then, the label amount in either phase is determined to assay the antigen amount in the test solution.

Nephelometry determines the amount of an insoluble precipitate resulting from an antigen-antibody reaction in a gel or solution. Even when the amount of an antigen in a test solution is small so that a small amount of precipitate is formed, laser nephelometry utilizing laser scattering is suitably used.

For applying the individual immunological measuring methods to an assay of the present invention, special conditions

or operations are not necessary. A measuring system for a protein of the present invention may be constructed in the light of common conditions and operations in the individual methods with modifications known to the skilled in the art. Details in these common technical procedures will be found in various reviews and textbooks.

Such literatures may be, for example, "Radioimmunoassay", ed. by Hiroshi Irie, Kodansha, published in 1974; "Radioimmunoassay, 2nd", ed. by Hiroshi Irie, Kodansha, published in 1979; "Enzyme Immunoassay", ed. by Eiji Ishikawa et al., Igaku-Shoin, published in 1978; "Enzyme Immunoassay, 2nd Edition", ed. by Eiji Ishikawa et al., Igaku-Shoin, published in 1982; "Enzyme Immunoassay, 3rd Edition", ed. by Eiji Ishikawa et al., Igaku-Shoin, published in 1987; "Methods in ENZYMOLOGY", Vol.70 (Immunochemical Techniques (Part A)); ibid., Vol. 73 (Immunochemical Techniques (Part B)); ibid., Vol.74 (Immunochemical Techniques (Part C)); ibid., Vol.84 (Immunochemical Techniques (Part D: Selected Immunoassays)); ibid., Vol.92 (Immunochemical Techniques (Part E: Monoclonal Antibodies and General Immunoassay Methods)); and ibid., Vol.121 (Immunochemical Techniques (Part I: Hybridoma Technology and Monoclonal Antibodies)) (these have been published by Academic Press).

When reduction in a level of an oncogenic protein of the present invention is detected by assaying the level using

an antibody of the present invention, for example, it leads to a diagnosis that the subject has a disease associated with insufficiency in the oncogenic protein of the present invention or will probably contract the disease in future.

When increase in a level of an oncogenic protein of the present invention is detected, for example, it leads to a diagnosis that the subject has a disease induced by over-expression of the oncogenic protein of the present invention, including cancers such as pulmonary cancer, renal cancer, hepatic carcinoma, non-small cell pulmonary cancer, ovarian cancer, prostatic cancer, gastric cancer, bladder cancer, breast cancer, cervical cancer, colonical cancer, rectal cancer and pancreatic cancer (in particular, cervical cancer), or will probably contract the disease in future.

An antibody of the present invention may be used to detect an oncogenic protein of the present invention present in a sample such as a body fluid and a tissue. It can be also used for preparing an antibody column used for purification of an oncogenic protein of the present invention, detection of a protein of the present invention in each fraction during purification and analysis of behavior of a protein of the present invention in a test cell.

(4) Gene diagnostic agent

A polynucleotide or antisense polynucleotide of the present invention can be also, for example, used as a nucleic acid probe for detecting an abnormality (gene defect) in a DNA

or mRNA encoding an oncogenic protein of the present invention mainly in a human. For example, it is useful as a gene diagnostic agent for injury, mutation or reduced expression in the DNA or the mRNA or over-expression of the mRNA.

The above gene diagnosis using a polynucleotide or antisense polynucleotide of the present invention can be conducted in accordance with, for example, known Northern hybridization or PCR-SSCP (See, Genomics, Vol.5, pp.874-879 (1989); Proceedings of the National Academy of Sciences of the United States of America, Vol. 86, pp.2766-2770 (1989)).

For example, when reduction in expression of an mRNA is detected by Northern hybridization, it leads to a diagnosis that the subject has a disease associated with insufficiency of an oncogenic protein of the present invention or will probably contract the disease in future.

When over-expression of an mRNA is detected by Northern hybridization, for example, it leads to a diagnosis that the subject probably has a disease induced by over-expression of the oncogenic protein of the present invention, including cancers such as pulmonary cancer, renal cancer, hepatic carcinoma, non-small cellpulmonary cancer, ovarian cancer, prostatic cancer, gastric cancer, bladder cancer, breast cancer, cervical cancer, colonical cancer, rectal cancer and pancreatic cancer (in particular, cervical cancer), or will probably contract the disease in future.

(5) Drug containing an antisense polynucleotide

An antisense polynucleotide of the present invention which complementarily binds to a polynucleotide of the present invention (for example, DNA) and can inhibit expression of the polynucleotide (for example, DNA) is less toxic and can inhibit in vivo activity of the protein of the present invention or the polynucleotide of the present invention (for example, DNA). Thus, it is useful as a prophylactic or therapeutic agent for a disease induced by over-expression of the protein of the present invention, for example, a cancer such as pulmonary cancer, renal cancer, hepatic carcinoma, non-small cellpulmonary cancer, ovarian cancer, prostatic cancer, gastric cancer, bladder cancer, breast cancer, cervical cancer, colonical cancer, rectal cancer and pancreatic cancer.

When using the antisense polynucleotide as the above prophylactic or therapeutic agent, the antisense polynucleotide can be formulated as described for the above polynucleotide of the present invention.

The formulation thus obtained is less toxic and can be orally or parenterally administered to a human or mammal (for example, rat, rabbit, sheep, pig, bovine, cat, dog and monkey).

The antisense polynucleotide can be administered as such or in combination with a physiologically acceptable carrier such as an adjuvant for promoting intake, using a gene gun or a catheter such as a hydrogel catheter.

A dose of the antisense polynucleotide varies depending on many factors such as a target disease, a recipient and a delivery route, and is, for example, about 0.1 to 100 mg per day to an adult (weight: 60 kg) when an antisense nucleotide of the present invention is locally administered to an organ (for example, liver, lung, heart and kidney) for treating a cancer.

Furthermore, the antisense polynucleotide can be used as a diagnostic nucleotide probe for determining the presence of an oncogene DNA of the present invention in a tissue or cell or its expression status.

The present invention also provides

(i) a double strand RNA comprising a part of an RNA encoding a protein of the present invention or an RNA complementary thereto;

(ii) a drug comprising the double strand RNA;

when appropriate,

(iii) a ribozyme comprising a part of an RNA encoding a protein of the present invention; and

(iv) a drug comprising the ribozyme.

These double strand RNA (RNAi; RNA interference method) and the ribozyme can inhibit expression of a polynucleotide (for example, DNA) of the present invention as in the above antisense polynucleotide and can inhibit in vivo activities of an oncogenic protein or polynucleotide (for example, DNA) of the present invention. Thus, it is useful as a prophylactic or therapeutic

agent for a disease induced by over-expression of the oncogenic protein of the present invention, for example, a cancer such as pulmonary cancer, renal cancer, hepatic carcinoma, non-small cellpulmonary cancer, ovarian cancer, prostatic cancer, gastric cancer, bladder cancer, breast cancer, cervical cancer, colonical cancer, rectal cancer and pancreatic cancer, particularly cervical cancer.

The double strand RNA can be designed and produced on the basis of the sequence of the polynucleotide of the present invention in accordance with a known method (See, for example, Nature, Vol.411, p.494, 2001).

The ribozyme can be designed and produced on the basis of the polynucleotide of the present invention in accordance with a known method (See, for example, TRENDS in Molecular Medicine, Vol.7, p.221, 2001). For example, it can be produced by ligating a known ribozyme to a part of an RNA encoding a protein of the present invention. An example of the part of an RNA encoding a protein of the present invention is a part (RNA fragment) near a restriction site on the RNA of the present invention which can be digested by a known ribozyme.

When using the double strand RNA or the ribozyme as the above prophylactic or therapeutic agent, it can be formulated and administered as described for the antisense polynucleotide.

(6) Medical drug comprising an antibody of the present invention

An antibody of the present invention capable of

neutralizing cancerization activity of an oncogenic protein of the present invention can be used as a prophylactic or therapeutic drug for a disease induced by over-expression of the oncogenic protein of the present invention, for example, a cancer such as pulmonary cancer, renal cancer, hepatic carcinoma, non-small cellpulmonary cancer, ovarian cancer, prostatic cancer, gastric cancer, bladder cancer, breast cancer, cervical cancer, colonical cancer, rectal cancer and pancreatic cancer, particularly cervical cancer.

The above prophylactic or therapeutic drug for the disease comprising the antibody of the present invention can be orally or parenterally administered to a human or mammal (for example, rat, rabbit, sheep, pig, bovine, cat, dog and monkey) as such, i. e., as a solution or as an appropriate dosage form of pharmaceutical composition. A dose may vary depending on various factors such as a recipient, a target disease, a symptom and a delivery route, and may be, for example, generally 0.01 to 20 mg/kg, preferably 0.1 to 10 mg/kg, more preferably 0.1 to 5 mg/kg of the antibody of the present invention for a dose, about once to five times a day, preferably one to three times a day. It can be suitably administered by intravenous injection. In another type of parenteral or oral administration, a dose similar to that described above may be employed. If a symptom is particularly severe, a dose may be further increased, depending on the symptom.

An antibody of the present invention can be administered as such or as an appropriate pharmaceutical composition. A pharmaceutical composition used for the above administration comprises the above antibody or its salt and a pharmacologically acceptable carrier, diluent or excipient. Such a composition is provided as a dosage form appropriate for oral or parenteral administration.

Specifically, examples of a composition for oral administration include solid and liquid dosage forms including tablet such as sugar-coated tablet and film-coated tablet), pill, granule, powder, capsule such as soft capsule, syrup, emulsion and suspension. Such a composition is prepared by a known process and comprises a carrier, diluent or excipient commonly used in the art of pharmacy. Examples of a carrier or excipient for tablet include lactose, starch, sucrose and magnesium stearate.

Examples of a composition for parenteral administration include an injection and a suppository. Examples of an injection include an intravenous injection, a subcutaneous injection, an intracutaneous injection, an intramuscular injection and an intravenous drip injection. Such an injection may be prepared by a known method; for example, by dissolving, suspending or emulsifying the above antibody or its salt in a sterile aqueous or oily liquid commonly used for an injection. Examples of an aqueous liquid for injection include saline and an isotonic solution containing glucose or other adjuvants, which may be

combined with an appropriate solubilizing agent including alcohols such as ethanol; polyols such as propyleneglycol and polyethyleneglycol; nonionic surfactants such as polysorbate 80 and HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil). Examples of an oily liquid include sesame oil and soybean oil, which may be combined with a solubilizing agent such as benzyl benzoate and benzyl alcohol. An injection thus prepared is generally filled in an appropriate ampule. A suppository used for rectal administration is prepared by mixing the above antibody or its salt with a common suppository base.

The oral or parenteral pharmaceutical composition is conveniently prepared in a dosage form suitable for a dose of an active ingredient. Examples of such a unit dosage form include tablet, pill, capsule, injection (ampule) and suppository. Each unit dosage form preferably contains generally 5 to 500 mg of the above antibody. In particular, an injection contains 5 to 100 mg, while another dosage form contains 10 to 250 mg.

Each of the above compositions may contain other active ingredients as long as they result in undesirable interaction when being compounded with the antibody.

(7) DNA transferred animal

The present invention provides a non-human mammal, for example, a "knock-in" animal, having a DNA encoding a human-derived oncogenic protein of the present invention (hereinafter, referred to as "an oncogene DNA of the present

invention") or its variant DNA.

Specifically, the present invention provides

(1) a non-human mammal having a human-derived oncogene DNA of the present invention or its variant DNA ("knock-in" animal);

(2) the "knock-in" animal in which the non-human mammal is a rodent;

(3) the "knock-in" mouse or rat in which the rodent is mouse or rat; and

(4) a recombinant vector comprising the human-derived oncogene DNA of the present invention or its variant DNA which can be expressed in the non-human mammal.

A non-human mammal having a human-derived oncogene DNA or its variant DNA of the present invention (hereinafter, referred to as "a DNA transferred animal of the present invention") can be produced by transferring a desired DNA to an unfertilized egg, a fertilized egg, a sperm or a germinal cell including their initial cells, preferably in an embryogenesis stage in development of the non-human mammal (further preferably, in a stage of a single cell or fertilized egg cell and generally up to the 8-cell phase) by an appropriate method such as a calcium phosphate method, an electrical pulse method, lipofection, aggregation, microinjection, a particle gun method and a DEAE-dextran. By the DNA transfer method, a desired foreign DNA of the present invention may be transferred

to a somatic cell, living organ or tissue cell for using it in cell culture or tissue culture. Furthermore, the cell can be fused with the above germinal cell by a known cell-fusion process to produce a DNA transferred animal of the present invention.

Examples of a non-human mammal include bovine, pig, sheep, goat, rabbit, dog, cat, guinea pig, hamster, mouse and rat. Among these, preferred is a rodent, particularly mouse (for example, a pure line such as a C57BL/6 strain and a DBA2 strain, and a hybrid line such as a $B6C3F_1$ strain, a BDF_1 strain, a $B6D2F_1$ strain, a BALB/c strain ad an ICR strain) or rat (for example, Wistar and SD) because its ontogenesis and biological cycles are relatively shorter in the light of producing a disease animal model and it can be easily bred.

A "mammal" in terms of a recombinant vector which can be expressed in a mammal may be, in addition to the above non-human mammal, a human.

A variant DNA to an oncogene derived from a human of the present invention refers not to a DNA of a homologue to an oncogene of the present invention inherent in a non-human mammal, but an artificially mutated one.

A variant DNA of the present invention may be a human-derived oncogene DNA of the present invention whose original nucleotide sequence has been varied (for example, mutation) such as a DNA having addition of a base, deletion or substitution with another base, or an abnormal DNA.

An abnormal DNA refers to a DNA expressing a protein which is similar to a normal oncogenic protein of the present invention but has a different activity; for example, a DNA expressing a peptide in which an activity of a normal oncogenic protein of the present invention is inhibited.

For transferring an oncogene DNA of the present invention to a target non-human animal, it is generally advantageous to use the DNA as a DNA construct bound in the downstream of a promoter capable of expressing the DNA in the non-human animal cell. For example, when transferring a human-derived DNA of the present invention, a DNA construct (for example, a vector) to which a human-derived oncogene DNA of the present invention has been bound can be microinjected to a fertilized egg of a target non-human mammal, for example, a murine fertilized egg, in the downstream of any of various promoters capable of expressing a DNA derived from any of various non-human mammals exhibiting higher homology to the DNA of the present invention (for example, rabbit, dog, cat, guinea pig, hamster, rat and mouse), to produce a DNA transferred non-human mammal capable of highly expressing the oncogene DNA of the present invention.

Examples of an expression vector for an oncogenic protein of the present invention include a E. coli derived plasmid, a Bacillus subtilis derived plasmid, an yeast derived plasmid, a bacteriophage such as λ-phage, a retrovirus such as Moloney leukemia virus and a mammalian virus such as a vaccinia virus

and a baculovirus, preferably a E. coli derived plasmid, a Bacillus subtilis derived plasmid and an yeast derived plasmid.

Examples of a promoter for regulating the DNA expression include

(i) promoters for a DNA derived from a virus (for example, simian virus, cytomegalovirus, Moloney leukemia virus, JC virus, mammary tumor virus and poliovirus);

(ii) promoters derived from various mammals (for example, human, rabbit, dog, cat, guinea pig, hamster, rat and mouse); for example, a promoter for albumin, insulin II, uroplakin II, elastase, erythropoietin, endothelin, muscle cretine kinase, glial fibrillary acidic protein, glutathione S-transferase, platelet-derived growth factor β, keratin K1, K10 and K14, type I and II collagens, cyclic-AMP dependent protein kinase βI subunit, dystrophin, tartrate-resistant alkaline phosphatase, atrial sodium diuretic factor, endothelial receptor tyrosine kinase (generally, abbreviated as "Tie2"), sodium potassium adenosine triphosphatase (Na, K-ATPase), neurofilament light chain, metallothionine I and IIA, metalloproteinase-1 tissue inhibitor, MHC Class I antigen (H-2L), H-ras, renin, dopamin β -hydroxylase, thyroid peroxidase (TPO), peptide chain elongation factor 1α (EF-1α), β-actin, α- and β-myosin heavy chain, myosin light chain 1 and 2, myelin base protein, thyroglobulin, Thy-1, immunoglobulin, variable H-chain part (VNP), serum amyloid P component, myoglobin, troponin C, smooth

muscle α-actin, preproenkephalin A and vasopressin. Among these, preferred are a cytomegalovirus promoter, a promoter of human peptide chain elongation factor 1α (EF-1α), a human and a poultry β-actin promoters capable of higher expression in an entire body.

The above vector preferably comprises a sequence which terminate transcription of a desired messenger RNA in a DNA transferred non-human mammal (generally, called "terminator"). For example, DNA sequences derived from bovine or various mammals can be used, preferably a simian virus SV40 terminator.

Furthermore, if desired, it may be possible to ligate a splicing signal for each DNA, an enhancer region or a part of a eukaryotic DNA intron in the 5'-upstream of the promoter region, between the promoter region and a translation region, or in the 3'-downstream of the translation region for higher expression of a desired oncogene DNA.

A translation region of a normal oncogenic protein of the present invention can be obtained using, as a starting material, a complementary DNA prepared as a whole or partial genome DNA from human-derived liver, kidney or thyroid gland cell, a fibroblast-derived DNA and various commercially available genome DNA libraries, or by a known process from an RNA derived from liver cell, kidney cell, thyroid gland cell or fibroblast. For producing an abnormal DNA induced by mutation, a translation region in the normal peptide obtained from the

above cell or tissue can be mutated by a point mutation induction method to produce a mutant translation region.

The translation region can be produced as a DNA construct which can be expressed in a DNA transferred animal, by a common DNA engineering procedure in which it is ligated to the downstream of the promoter or if desired the upstream of a transcription termination site.

Transfer of a human-derived oncogene DNA of the present invention in a fertilized egg cell stage is conducted such that the DNA is carried in all of germinal and somatic cells in a target non-human mammal. Presence of the oncogene DNA of the present invention in germinal cells in a produced animal after DNA transfer means that all progenies of the produced animal will carry the oncogene DNA of the present invention in all of their germinal and somatic cells. Offsprings of this type of animal inheriting the oncogene DNA of the present invention carry the oncogene DNA of the present invention in all of their germinal and somatic cells.

A non-human mammal to which a human-derived oncogene DNA of the present invention has been transferred can be successively bred under common breeding circumstances as a mammal carrying the oncogene DNA, after confirming that the mammal stably carries the human-derived oncogene DNA after mating.

Transfer of a human-derived oncogene DNA of the present invention in a fertilized egg cell stage is conducted such that

the DNA is carried in all of germinal and somatic cells in a target non-human mammal. Presence of the human-derived oncogene DNA of the present invention in germinal cells in a produced mammal after DNA transfer means that all offsprings of the produced mammal will successively carry the human-derived oncogene DNA of the present invention in all of their germinal and somatic cells. Offsprings of this type of animal inheriting the human-derived oncogene DNA of the present invention successively carry the human-derived oncogene DNA of the present invention in all of their germinal and somatic cells.

A homozygote animal having a transduced DNA in both of homologous chromosomes can be produced and the male and the female animals can be mated for successive breeding such that all offsprings successively carry the DNA.

In a non-human mammal having a human-derived oncogene DNA of the present invention, the normal DNA of the present invention is highly expressed, and an activity of the intrinsic normal DNA may be promoted, sometimes resulting in onset of hyperactivity of the protein of the present invention. Thus, it can be used as a model animal for the disease. For example, a normal DNA transferred animal of the present invention can be used to elucidate a mechanism of hyperactivity of the oncogenic protein of the present invention or of a disease associated with the oncogenic protein of the present invention and investigate a therapy for these diseases.

Furthermore, since a non-human mammal to which a human-derived oncogene DNA of the present invention has been transferred shows increase in the free protein of the present invention, it can be used for screening a therapeutic agent to a disease associated with the oncogenic protein of the present invention.

On the other hand, a non-human mammal having an abnormal DNA of the present invention can be successively bred under common breeding circumstances as an animal carrying the DNA, after confirming that the animal stably carries the transduced DNA after mating. Furthermore, a desired abnormal DNA can be incorporated into the above plasmid to be used as a starting material. A DNA construct with a promoter can be produced by a common DNA engineering procedure. Transfer of an abnormal DNA of the present invention in a fertilized egg cell stage is conducted such that the DNA is carried in all of germinal and somatic cells in a target mammal. Presence of the abnormal DNA of the present invention in germinal cells in a produced animal after DNA transfer means that all offsprings of the produced animal will carry the abnormal DNA of the present invention in all of their germinal and somatic cells. Offsprings of this type of animal inheriting the abnormal DNA of the present invention carry the abnormal DNA of the present invention in all of their germinal and somatic cells. A homozygote animal having a transduced DNA in both of homologous chromosomes can

be produced and the male and the female animals can be mated for successive breeding such that all offsprings successively carry the DNA.

In a non-human mammal having an abnormal DNA of the present invention, the abnormal DNA of the present invention is highly expressed, and an activity of the intrinsic normal DNA may be inhibited, sometimes resulting in a deactivation type refractoriness of the protein of the present invention. Thus, it can be used as a model animal for the disease. For example, an abnormal DNA transferred animal of the present invention can be used to elucidate a mechanism of the deactivation type refractoriness of the oncogenic protein of the present invention and investigate a therapy for the disease.

As a specific application, an animal highly expressing the abnormal DNA of the present invention can be used as a model for elucidating inhibition of a normal peptide activity (dominant negative activity) by the abnormal peptide of the present invention in the deactivation type refractoriness of the protein of the present invention.

Furthermore, since a non-human mammal to which an abnormal DNA of the present invention has been transferred shows increase in the free oncogenic protein of the present invention, it can be used for screening a therapeutic agent to the oncogenic protein or its deactivation type refractoriness.

A DNA transferred animal of the present invention can

be used for investigating a clinical symptom of a disease associated with an oncogenic protein of the present invention such as a deactivation type refractoriness of the protein of the present invention. Furthermore, the animal can give more specific pathologic observation in each organ in a disease model associated with the oncogenic protein of the present invention and can contribute to development of a new therapy and investigation and treatment of a secondary disease due to the above disease.

Each organ can be extracted from a DNA transferred animal of the present invention, chopped and treated with a protease such as trypsin to obtain a free DNA transferred cell. The cell can be then cultured and the cultured cell can be subjected to lineage study. Furthermore, it can be used for identifying a cell producing the oncogenic protein of the present invention and investigating association with apotosis, differentiation or growth or a signal transfer mechanism in them and abnormality in them. Thus, it can be an effective research subject for elucidating the oncogenic protein of the present invention and its activity.

Furthermore, in order to develop a therapeutic agent for a disease associated with an oncogenic protein of the present invention such as a deactivation type refractoriness of the protein of the present invention, a DNA transferred animal of the present invention can be used to provide an effective and

speedy screening method for the therapeutic agent using the above test and assay processes.

A non-human mammal germinal stem cell in which a gene DNA homologue to an oncogene of the present invention is inactivated, is very useful for producing a non-human mammal model insufficiently expressing the oncogene DNA of the present invention. A non-human mammal insufficiently expressing a gene DNA homologue to the oncogene of the present invention does not have various biological activities which can be induced by the oncogenic protein of the present invention. It can be, therefore, used as a model for a disease caused by inactivation of biological activities of the oncogenic protein of the present invention. Thus, it can be useful for elucidating the cause of the disease and investigating a therapy therefor.

(8a) Method for screening a therapeutic or prophylactic compound to a disease caused by deletion or damage in an oncogene DNA of the present invention

A non-human mammal insufficiently expressing a DNA of the present invention can be used for screening a therapeutic or prophylactic compound to a disease caused by deletion or damage in the DNA of the present invention.

Thus, the present invention provides a method for screening a therapeutic or prophylactic compound or its salt to a disease caused by deletion or damage in a DNA of the present invention, comprising the steps of administering a test compound

to a non-human mammal insufficiently expressing the DNA of the present invention and then observing and/or determining change in the animal.

A non-human mammal insufficiently expressing the DNA of the present invention used in the screening process may be as described above.

Examples of a test compound include a peptide, a protein, a non-peptide compound, a synthetic compound, a fermentation product, a cell extract, a plant extract, an animal tissue extract and plasma, which may be a novel or known compound.

(8b) Method for screening a compound promoting or inhibiting a promoter activity to an oncogene DNA of the present invention

The present invention provides a method for screening a compound or its salt promoting or inhibiting a promote activity to an oncogene DNA of the present invention, comprising the steps of administering a test compound to a non-human mammal insufficiently expressing the DNA of the present invention and then detecting expression of a reporter gene.

In the screening method, a non-human mammal insufficiently expressing the DNA of the present invention may be, among the non-human mammals insufficiently expressing the DNA of the present invention, an animal in which the DNA of the present invention is inactivated by transducing a reporter gene and the reporter gene can be expressed under the control of a promoter to the DNA of the present invention.

Examples of a test compound include a peptide, a protein, a non-peptide compound, a synthetic compound, a fermentation product, a cell extract, a plant extract, an animal tissue extract and plasma, which may be a novel or known compound.

A reporter gene which can be used may be as described above; suitably β-galactosidase gene (lacZ), soluble alkaline phosphatase gene and luciferase gene.

In the non-human mammal insufficiently expressing the DNA of the present invention in which the oncogene DNA of the present invention is replaced with the reporter gene, the reporter gene exists under the control of the promoter to the DNA of the present invention, so that a substance encoded by the reporter gene can be traced to detect an activity of the promoter.

For example, when a part of a DNA domain encoding an oncogenic protein of the present invention is substituted with an E. coli derived β-galactosidase gene (lacZ), β-galactosidase is expressed in place of the oncogenic protein of the present invention in a tissue in which the oncogenic protein of the present invention is naturally to be expressed. Thus, for example, by staining with a reagent to be a substrate for β-galactosidase such as 5-bromo-4-chloro-3-indolyl- β -galactopyranoside (X-gal), an expression state of the oncogenic protein of the present invention in a living animal can be conveniently observed. Specifically, a mouse defective in a homologue protein to an

oncogenic protein of the present invention or its tissue section is fixed by, for example, glutaraldehyde, washed with a phosphate-buffered saline (PBS) and reacted with a stain solution containing X-gal at room temperature or about 37 °C for about 30 min to 1 hour. The tissue preparation thus obtained is washed with a 1 mM EDTA/PBS solution to quench the β-galactosidase reaction and then a color can be observed. Alternatively, an mRNA encoding lacZ may be detected as usual.

A compound or its salt obtained using the above screening method is selected from the above test compounds, which can promote or inhibit a promoter activity to an oncogene DNA of the present invention.

The compound selected by the above screening method can form a salt, which may be a salt with a physiologically acceptable acid (for example, an inorganic acid) or base (for example, an organic base), preferably an acid-addition salt. Examples of such a salt include salts with an inorganic acid such as hydrochloric acid, phosphoric acid, hydrobromic acid and sulfuric acid, or with an organic acid such as acetic acid, formic acid, propionic acid, fumaric acid, maleic acid, succinic acid, tartaric acid, citric acid, malic acid, oxalic acid, benzoic acid, methanesulfonic acid and benzenesulfonic acid.

Since a compound or its salt promoting a promoter activity to an oncogene DNA of the present invention promotes the activity of the peptide, it is useful as, for example, a

medical drug such as a prophylactic and a therapeutic agents for a disease associated with insufficiency of an oncogenic protein of the present invention.

On the other hand, a compound or its salt inhibiting a promoter activity to a DNA encoding an oncogenic protein of the present invention is useful as a safe and low-toxic medical drug inhibiting cancer-inducing activity of the oncogenic protein of the present invention, for example a prophylactic or therapeutic agent for a cancer such as pulmonary cancer, renal cancer, hepatic carcinoma, non-small cellpulmonary cancer, ovarian cancer, prostatic cancer, gastric cancer, bladder cancer, breast cancer, cervical cancer, colonical cancer, rectal cancer and pancreatic cancer.

A derivative of the compound selected by the above screening may be also used.

A drug comprising the compound or its salt selected by the screening method can be prepared in a dosage form suitable for delivery to a target cancerous tissue as in a drug comprising a known anticancer drug to a given cancer.

Since the formulation thus prepared is safe and low toxic, it can be administered to a human as a main subject or a mammal in which a similar pharmacological effect would be expected (for example, rat, mouse, guinea pig, rabbit, sheep, pig, bovine, horse, cat, dog and monkey).

A dose of the compound or its salt depends on various

factors such as a target disease, a recipient and a delivery route. For example, in oral administration of a compound inhibiting a promoter activity to a DNA of the present invention for treating a cancer, its dose is about 0.1 to 100 mg, preferably about 1.0 to 50 mg, more preferably about 1.0 to 20 mg per day to a normal adult patient (weight: 60 kg). In parenteral administration, although a dose of the compound depends on various factors such as a recipient and a target disease, its dose is suitably about 0.01 to 30 mg, preferably about 0.1 to 20 mg, more preferably about 0.1 to 10 mg per day by intravenous injection when a compound inhibiting a promoter activity to a DNA of the present invention is administered to a normal adult patient (weight: 60 kg) for treating a cancer. For another animal, an amount converted to a weight of 60 kg may be administered.

Thus, a non-human mammal insufficiently expressing an oncogene DNA of the present invention is considerably useful for screening a compound or its salt promoting or inhibiting a promoter activity to the oncogene DNA of the present invention, and therefore, can significantly contribute to elucidating causes of a variety of diseases due to insufficient expression of the oncogene DNA of the present invention and developing prophylactic or therapeutic agents for the diseases.

Furthermore, using a DNA containing a promoter region for an oncogenic protein of the present invention, a gene encoding a given protein can be ligated to a downstream site, and the

product can be injected in an animal egg cell to produce a so-called transgenic animal (an introgressant animal), so that a mechanism specifically causing in vivo expression of the oncogene while avoiding cancerization. Furthermore, a proper reporter gene can be ligated to the promoter region to establish a transformant cell strain expressing the gene, which can be used as an in vitro search system for a low molecular-weight compound specifically promoting or inhibiting an in vivo production ability of the oncogenic protein of the present invention itself.

When being used for prevention or treatment of a cancer, a compound or its salt regulating activity of an oncogenic protein of the present invention can be combined with another anticancer agent such as ifosfamide, UTF, adriamycin, peplomycin, cisplatin, cyclophosphamide, 5-FU, UFT, methotrexate, mitomycin C and mitoxantrone.

EXAMPLES

The present invention will be more specifically with reference to Examples, These examples are included in the most preferred embodiments of the present invention, but the present invention is not limited to these embodiments.

Example 1

Cloning and sequencing of a cDNA encoding a hWAPL from human

PCR was conducted using a commercially available cDNA library, a human testicular cDNA kit (Marathon-Ready™ cDNA Kit; Clontech Inc.) as a template and using two primers:

primer 1

(sequence: TTGGATCCATGACATCCAGATTTGGGAAAACATACAGTAGG); and

primer 2

(sequence: TTGAATTCCTAGCAATGTTCCAAATATTCAATCACTCTAGA). In the PCR reaction, Advantage 2 polymerase mix (Clontech Inc.) kit was used and according to the instructions in the kit, amplification was conducted with a temperature cycle:

(1) 94 °C for 1 min;

(2) 5 cycles of 94 °C for 10 sec and 72 °C for 2 min; and

(3) 25 cycles of 96 °C for 10 sec and 70 °C for 2 min, and then followed by elongation reaction being carried out at 72 °C for 5 min. After the reaction, the product of PCR amplification thus obtained was cloned to a plasmid vector pGEM-T easy (PROMEGA) in accordance with the instructions for pGEM-T easy (PROMEGA). It was transduced to E. coli DH5 α (Invitrogen) and using an ampicillin resistance gene in the plasmid vector pGEM-T easy, clones carrying the plasmid were selected in an LB agar medium containing ampicillin.

The plasmid carried by each of the selected clones was sequenced to obtain the sequence of the novel cDNA (SEQ. ID. No.2) cloned therein. A novel protein comprising an amino acid sequence (SEQ. ID. No.1) deduced from an ORF in the nucleotide sequence of the cDNA was designated as a human WAPL (hWAPL). A transformant carrying the full-length cDNA of the hWAPL, which

was cloned in the plasmid, was designated as Escherichia coli DH5 pGEMhWAPL.

Example 2

Expression of an hWAPL gene in a human cancerous tissue

Under the consent of patients subjected to surgical excision of a cancerous tissue in Tokyo Medical College Hospital, the samples of surgically excised cancerous tissues were provided. The samples of surgically excised cancerous tissues were examined for the presence of hWAPL gene expression and the expression amount thereof by Northern blotting and real-time PCR.

In the Northern blotting, an mRNA expressed from the hWAPL gene was identified from total RNAs prepared, using a DNA having a nucleotide sequence complementary to the portion of nucleic acid Nos. 2511 to 2813 in the full-length sequence of the hWAPL of SEQ. ID. No.2, as a detection probe. On the other hand, in the real-time PCR, a cDNA of the hWAPL was amplified, using an amplification kit SYBR Green I (TaKaRa Co. Ltd.) and as PCR primers, a pair of primers:

5'-GAATTCATAGGCACAGCGCTGAACTGTGTG-3' and

5'-TTGAATTCCTAGCAATGTTCCAAATATTCA-3'.

Furthermore, human β-actin was used as an intrinsic standard. PCR primers used for amplification of cDNA of human β-actin were a commercially available pair of primers (Clontech) of:

5'-GGGAAATCGTGCGTGACATTAAG-3' and

5'-TGTGTTGGCGTACAGGTCTTTG-3'.

The temperature cycle condition selected for the real-time PCR was as follows:

(a) 95 °C for 30 sec;

(b) 40 cycles of 95 °C for 3 sec and 68 °C for 30 sec; and then

(c) 87 °C for 6 sec.

For the thermal cycle in the PCR reaction, Smart Cycler System (TaKaRa Co. Ltd.) was used. The amount of the double strand cDNA was detected by means of a fluorescent label therein to determine the amount of amplification product.

For the cancerous tissue samples mentioned above, comparative samples of total RNA were prepared from normal cells and carcinoma cells in the individual tissues in accordance with a known procedure (Oikawa et al., Cancer Res., 61, 5707-5709 (2001)). Among the carcinoma cells examined of cervical cancer, corpus uteri cancer, ovarian cancer, gastric cancer, renal cancer, pulmonary cancer, colorectal carcinoma and breast cancer, about 40 % of carcinoma cell samples of cervical cancer demonstrated significant hWAPL gene expression (FIG. 5).

The carcinoma cell samples of cervical cancer were examined for the presence of HPV infection. Specifically, an mRNA region for the HPV-derived E6/E7 gene was detected by RT-PCR with corresponding primers therefor in accordance with a known procedure (Nakagawa et al., J. Med. Virol., 62, 251-258 (2000)), and in all the invasive carcinoma cell samples of cervical cancer,

HPV infection and expression of the E6/E7 gene were confirmed. Exceptionally, some of gastric cancer samples also demonstrated high expression of the hWAPL gene.

Example 3

Induction of hWAPL gene expression by E6 and E7 from HPV 16

An E6 gene from HPV 16 was amplified and isolated by RCR method using a pair of primers

16E6attB1:

5'-AAAAAGCAGGCTCCACCATGTTTCAGGACCCACAGGAGCGACCC-3', and

16E6attB2:

5'-AGAAAGCTGGGTTACAGCTGGGTTTCTCTACGTG-3',

while an E7 gene from HPV 16 was amplified and isolated by RCR method using a pair of primers

16E7attB1:

5'-AAAAAGCAGGCTCCACCATGCATGGAGATACACCTACAT-3' and

16E7attB2:

5'-AGAAAGCTGGGTTATGGTTTCTGAGAACAGATGGGG-3'.

Then, each of these genes were cloned into a retrovirus vector pCLXSN in accordance with a procedure of Naviaux et al. (Naviaux et al., J. Virol., 70, 5701-5705 (1996)), to generate retroviruses LXSN-16E6 and LXSN-16E7 for producing E6 and E7 recombinants, respectively. In addition, the retrovirus LXSN-16E6E7 for producing E6 and E7 recombinants simultaneously was produced.

It has been found that an product of E2 gene derived

from HPV can be bound to a promoter region in the E6 and E7 genes to possess a function for inhibition of the transcription thereof, and that a product of E2 gene derived from a bovine papilloma virus (BPV) has a similar function for inhibiting the transcription. The BPV1 E2 gene fragment was obtained by nested PCR using pBPV-MII as a template. In the nested PCR, the pair of inner primers used was consisted of:

5'-AAAAAGCAGGCTCCACCATGGAGACAGCATGCGAAC-3' and

5'-AGAAAGCTGGGTCAGAAGTCCAAGCTGGCTGTAAAG-3',

while the pair of outer primers was consisted of:

5'-GGGGACAAGTTTGTACAAAAAAGCAGGCT-3'and

5'-GGGGACAAGTTTGTACAAGAAAGCTGGGT-3'.

The BPV1 E2 gene fragment thus obtained was cloned into a retrovirus vector pCMSVpuro, which is based on a general-purpose virus vector pCMSCV (Clontech), to prepare a retrovirus MSCV-puro BPV1E2 for producing the E2 recombinant from BPV. The retrovirus vector pCMSVpuro comprises a puromycin resistance gene as a selection marker.

Human epidermal cells HDK1 (BioWhittaker) were infected with the retroviruses LXSN-16E6, LXSN-16E7 and LXSN-16E6E7 for producing HPV 16 derived E6 or E7 recombinants, respectively. As a negative control, a human epidermal cell HDK1 infected with a retrovirus vector pCLXSN was used. A cell line in which continued infection of the retrovirus vector was established was selected by culturing the cells on a medium containing G418

at 50 μg/mL for 3 days. After infection, expression of the hWAPL gene induced by a recombinant protein of the E6 or E7 from HPV 16 was determined by Western blotting using a specific antibody recognizing a region of partial amino acid sequence 50 to 66 (amino acid sequence: CNFKPDIQEIPKKPKVEE) in the oncogenic protein hWAPL (FIG. 6).

At the same time, the amount of expression of p53 suppressor protein was also determined by Western blotting. Then, it was again confirmed that E6 product promoted a cleavage process of the p53 tumor suppressor protein, resulting in expression of the oncogenic protein hWAPL.

Furthermore, when the carcinoma cell lines of cervical cancer; CaSki, SiHa and C33A were infected with MSCV-puro BPV1E2, CaSki and SiHa cell lines producing E6 and E7 from HPV 16 demonstrated increase of the remained p53 tumor suppressor protein as a result of inhibiting transcription of E6 and E7 genes by the E2 from BPV. Concomitantly, expression of the oncogenic protein hWAPL was inhibited (FIG. 6). On the other hand, in the C33A cell line, in which cancerization was induced by mechanism other than such cancerization being originated by HPV infection, the amount of the remaining p53 tumor suppressor protein or the amount of expression of the oncogenic protein hWAPL was not affected. The results indicate that although the increased amount of expression of the oncogenic protein hWAPL is closely associated with cancerization, an independent

mechanism causing increased expression of the oncogenic protein hWAPL may exist, which is different from the mechanism of increased expression of the oncogenic protein hWAPL due to E6 and E7 from HPV.

Example 4

Inhibition of promoter activity of the hWAPL gene by a p53 suppressor protein

It was confirmed that the p53 suppressor protein has a function for inhibition of transcription of the hWAPL gene from its promoter, as follows.

A promoter of hWAPL gene was amplified and isolated by PCR method using a genomic DNA in DLD-1 cell as a template with use of a pair of primers:

primer 1

(sequence: GTGCATCCCACCCACAGTGGAAGACATGG) and

primer 2

(sequence: CCGCTTCCGCCGGTGAATGGTCAGTGCTGG).

A DNA fragment of the PCR product was first cloned to PGEMT-easy (PROMEGA), and sequentially, the fragment digested with a restriction enzyme EcoRI was then cloned to pBluescript (Stratagene). A region comprising a promoter portion of the hWAPL gene, which was inserted into the plasmid, was digested with SalI/XhoI and cloned into the pGL3-Basic vector (PROMEGA).

After purification with Qiagen Plasmid Maxi Kit (Qiagen), the vector obtained was co-transfected with a p53 expression

vector, pHM6 (Roche) as for a control and a pGR3-tK vector for standardization of luciferase assay, using LipofectAmine2000 (Invitrogen) in an HeLa cell. Dual Luciferase Kit (Promega) was used in the luciferase assay for determining the amount of labeled protein, luciferase, which was a reporter gene product transcribed and translated under control of the promoter of hWAPL gene.

As a result, it was confirmed that when transducing the p53 expression vector, increase in the amount of the expressed p53 suppressor protein reduced the amount of the labeled protein luciferase, which was transcribed and translated under control of the promoter of hWAPL gene, by 30 % in comparison with the control. In other words, it is confirmed that the p53 suppressor protein has a function causing reduction in activity of the promoter of the hWAPL gene.

Example 5

Construction of an expression vector for producing the hWAPL recombinant protein

A HindIII and EcoRI restriction sites was transduced respectively at the ends of the region of base Nos. 1 to 3570 in the cDNA having the nucleotide sequence of SEQ. ID. No.2 by site-specific mutagenesis using PCR. The PCR product thus obtained was digested with HindIII/EcoRI, and the corresponding DNA fragment was inserted into an HA-tagged mammalian expression vector, pHM6 (Roche Diagnostics) to construct an expression

vector for producing a recombinant of the HA-tagged hWAPL protein, pHM6-hWAPL. The fragment was also inserted into an mammalian expression vector, phrGFP-N1 (Stratagene) for expressing a fused protein with an hrGFP fusion partner to construct an expression vector for producing a recombinant protein of a GFP fused hWAPL, phrGFP-hWAPL.

The expression vector for producing a recombinant was transfected to a host mammal cell using LipofectAmine 2000 (Invitrogen). After culturing for 2 days following the transfection, selection is conducted using EPICS ALYRA HyperSort (Bechman Coulter) on the basis of the presence of expression of the hrGFP tag. After further culturing and additional selection, a transformed host cell line carrying the desired expression vector for producing the hWAPL recombinant protein.

Example 6

Generation of an antibody specific to the hWAPL protein

For generating an antibody specific to the oncogenic protein hWAPL, a peptide chain ($hWAPL_{50-66}$) having a partial amino acid sequence 50 to 66: CNFKPDIQEIPKKPKVEE that is located in the N-terminal region of the oncogenic protein hWAPL was prepared as an immunogen peptide by chemical synthesis. Furthermore, by recombination was produced a fused polypeptide tagged with 6xH His comprising a partial amino acid sequence 814-1037 lying in the C-terminal region of the oncogenic protein hWAPL.

According to conventional techique, these two immunogen

peptides were used to separately immunize a rabbit to produce polyclonal antibodies specific to these antigen peptides, an anti-hWAPL-N antibody and an anti-hWAPL-C antibody. In Western blotting in Example 3 and so on, the oncogenic protein hWAPL was detected using the anti-hWAPL-N antibody specific to the $hWAPL_{50-66}$ antigen.

Example 7

Induction of chromosome instability by the hWAPL protein

A HeLa cell is infected with the expression vector phrGFP-hWAPL for producing a recombinant proteinof the hrGFP-fused hWAPL. The procedure described in Example 5 is conducted to select a GFP-hWAPL positive cell line producing the GFP-hWAPL-fused protein and a GFP-hWAPL negative cell line not producing the GFP-hWAPL-fused protein. After subculturing for five generations following the selection, the content of a chromosome gene DNA therein is assayed by Laser Scanning Cytomerty in accordance with Buse's procedure (J. Biol. Chem., 274, 7253-7263 (1999)).

The GFP-hWAPL negative cell line demonstrates a chromosome gene content equivalent to the host cell, HeLa cell, in which a half or more is comprised of a normal diploid and a content of a tetraploid is about a half of the diploid. On the other hand, in the GFP-hWAPL positive cell line, a normal diploid exists in a less amount, a major part demonstrates polyploidy, a content of a tetraploid is slightly more than that

of a diploid, and an octoploid also exists at a content of 10.1 %. Thus, it can be judged that over-expression of the hWAPL protein induces chromosome instability (FIG. 7).

Correspondingly, induction of nuclear atypia, in particular, of multi-nucleation is also originated from over-expression of the hWAPL protein. After three generation subculturing, ratios of observed multi-nucleation are only 5 % and 4 % in the GFP-hWAPL negative cell line and the HeLa cell infected with the phrGFP-N1 vector (control), respectively, while being 15.6 % (three times or more) in the GFP-hWAPL positive cell line (FIG. 8).

Furthermore, in association with chromosomal abnormality such as nuclear atypia due to abnormal centromere division during a separation process by mitosis of a chromosome gene, induction of micronuclei formation is originated by over-expression of the hWAPL protein. A frequency of micronuclei formation in the GFP-hWAPL positive cell line is about two times as much as that in the GFP-hWAPL negative cell line (FIG. 7).

Example 8

Induction of cancerization of an NIH 3T3 fibroblast by the hWAPL protein

An NIH 3T3 fibroblast is infected with the expression vector pHM6-hWAPL for producing a recombinant protein of the HA-tagged hWAPL to produce a recombinant cell line

over-expressing the HA-tagged hWAPL protein, an HA-hWAPL 3T3 cell line. As a negative control, an HA-3T3 cell line infected with a pHM6 vector is produced. When culturing on a plate, the HA-3T3 cell line for negative control forms a homogenous confluent-like single cell layer as in the host cell, NIH 3T3 fibroblast, while the HA-hWAPL 3T3 cell line forms a focus structure.

To a nude mouse was subcutaneously injected the HA-hWAPL 3T3 cell lne and the HA-3T3 cell line. Subsequent follow-up indicated that within 10 days, oncogenesis was induced in all the injection sites for the HA-hWAPL 3T3 cell line while no oncogenesis was induced in injection sites for the HA-3T3 cell line. By Western blotting using the anti-HA antibody and the anti-hWAPL-C antibody, it was confirmed that the HA-tagged hWAPL protein was actually over-expressed in the cancerized cell. In the cancerized cell, heterotypic mitosis was observed; for example, three pole division was also observed.

Example 9

Inhibition of carcinoma cell growth by an siRNA targeted to the hWAPL gene

Using an siRNA targeted to the hWAPL gene, it was confirmed that inhibiting expression of the hWAPL protein can result in inhibition of growth of a carcinoma cell.

(1) In vitro inhibition of carcinoma cell growth by an siRNA

Using the Silencer siRNA construction Kit (Ambion),

siRNA were produced which are targeted to the following gene sequence (hWAPL AsiRNA) and to a control (negative control), respectively:

hWAPL AsiRNA: CGGACTACCCTTAGCACAA

negative control: ACTACAACTGGTCGCAACC.

Practically, two synthetic oligomers were prepared for each; specifically, for the hWAPL AsiRNA,

AACGGACTACCCTTAGCACAAcctgtctc and

AATTGTGCTAAGGGTAGTCCGcctgtctc,

and, for the negative control,

AAACTACAACTGGTCGCAACCcctgtctc and

AAGGTTGCGACCAGTTGTAGTcctgtctc.

Then, in each synthetic oligomer, a T7 promoter primer was hybridized at portion of ctgtctc and was treated with Klenow DNA polymerase to prepare completely a double strand DNA. Then, it was transcribed by T7 RNA polymerase, the RNAs of the antisense and sense chains prepared were hybridized each other, and then both cohesive ends were digested with Rnase to prepare a double strand siRNA with blunt ends.

To a SiHa cell derived from cervical cancer, in which hWAPL was highly expressing, was transduced the hWAPL AsiRNA and the negative control siRNA at a concentration of 1 nM for evaluating influence to cell growth.

The siRNAs were transfected to the SiHa cell derived from an HPV16 positive cervical cancer, in which high expression

of the hWAPL was observed. FIG. 10 shows the results of plotting the living cell number ($\times 10^3$) in the ordinate to a time from transduction of the siRNA (hour) in the abscissa. In the figure, "siRNA" for transfection of an siRNA targeting DIF-2, "cont" for transfection of a control siRNA , and "TSA" indicate the results obtained when adding a histone deacetylase inhibitor, Trichostatin A to a culture after transfection of the siRNA, respectively.

Until 20 hours after transfection of the hWAPL AsiRNA, increase in the living tumor cell number can be observed. During the period, comparison with the control may indicate inhibition of tumor growth. Subsequently, the cells to which the hWAPL AsiRNA was transduced show reduction in the living cell number and after 100 hours, a small number of cells were living. In contrast, when progress of deacetylation for acetylated lysines contained in a histon is inhibited by adding Trichostatin A, it attenuates effect of inhibition of cell growth by the hWAPL AsiRNA.

These results indicate that cell death by inhibition of an hWAPL is caused by inhibition of histon modification such as acetylation, and implies that the hWAPL itself may be involved in control of a histon code.

(2) in vivo inhibition effect of tumor growth

To six-week old BALB3T3/nude mice were inoculated 2×10^6 of SiHa cells. From 10 days after inoculation, to the inoculated

tumor cells were injected hWAPL AsiRNA and a negative control siRNA once two days and five times in total, respectively.

Variation of the inoculated tumor size was evaluated for 6 animals in the hWAPL AsiRNA injection group, 6 animals in the negative control siRNA injection group and 5 animals in the untreated group. The results indicated an average tumor size reduction of 33 % in the hWAPL AsiRNA injection group in relation to the untreated group.

Furthermore, it was demonstrated that the nucleotide sequence of the cDNA for the hWAPL, which we cloned, was located in 10q23.31 to q23.32 on the human genome. The nucleotide sequence thereof is shown below, along with the nucleotide sequence of the 5'-untranslation region and promoter region in the elucidated hWAPL gene as well as the nucleotide sequence of cDNA for the above mouse WAPL. In the ORFs in the cDNAs, a region from the initiating codon ATG to the stop codon TAG is written by capitals, while in the promoter region, a region after a putative transcription initiating point is written by capitals on the basis of comparison with EST.

cDNA sequence of the hWAPL

gcgagcggctgttggaggaaggaggtgggggccgggagcgcaaatggcgttgagatggt
ycarggccctgttcaaactccagcactgaccattcaccggcggaagcggcggcgcagga
ggcggcggcggcccagcgggggcacacagcaggctctgttaccagctccagcagtggcg
gccagcgagagctaggcccgsgcccggccggcggcgctcgaggcggggagggaagttgc
ggggccgccgctcctgccccccaaccgggcttcctatttaccgaaagcagagtccctc

gcctctctcggctctcacctgccggccctgctctcccgcgcgagggttccgcgcccgcc

cgcgggccgtarggagcgggagaaggcggargcggccccgtggccaaagcacccgccag

gcttccgaggagaatatgaaactggtgtcaaaATGACATCCAGATTTGGGAAAACATAC

AGTAGGAAAGGTGGAAATGGCAGTTCAAAATTCGATGAAGTCTTTTCCAACAAACGGAC

TACCCTTAGCACAAAATGGGGAGAGACCACATTTATGGCTAAATTAGGGCAGAAGAGGC

CCAATTTCAAACCAGATATCCAAGAAATTCCGAAGAAACCTAAAGTGGAAGAAGAAAGT

ACTGGAGATCCTTTTGGATTTGATAGTGATGATGAGTCTCTACCAGTTTCTTCAAAGAA

TTTAGCCCAGGTTAAGTGTTCCTCTTATTCAGAATCTAGTGAAGCTGCTCAGTTGGAAG

AGGTCACTTCAGTACTTGAAGCTAATAGCAAAATTAGTCATGTGGTCGTTGAAGACACT

GTCGTTTCTGATAAATGCTTCCCTTTGGAGGACACTTTACTTGGGAAAGAAAAGAGCAC

AAACCGAATTGTAGAAGATGATGCAAGCATAAGTAGCTGTAATAAATTAATAACTTCAG

ATAAAGTGGAGAATTTTCATGAAGAACATGAAAAGAATAGTCACCATATTCACAAAAAT

GCTGATGACAGTACTAAGAAACCCAATGCAGAAACTACAGTGGCTTCTGAAATCAAGGA

AACAAATGATACTTGGAACTCCCAGTTTGGGAAAAGGCCAGAATCACCATCAGAAATAT

CTCCAATCAAGGGATCTGTTAGAACTGGTTTGTTTGAATGGGATAATGATTTTGAAGAT

ATCAGATCAGAAGACTGTATTTTAAGTTTGGATAGTGATCCCCTTTTGGAGATGAAGGA

TGACGATTTTAAAAATCGATTGGAAAATCTGAATGAAGCCATTGAGGAAGATATTGTAC

AAAGTGTTCTTAGGCCAACCAACTGTAGGACGTACTGTAGGGCCAATAAAACGAAATCC

TCCCAAGGAGCATCAAATTTTGATAAGCTGATGGACGGCACCAGTCAGGCCTTAGCCAA

AGCAAACAGTGAATCGAGTAAAGATGGCCTGAATCAGGCAAAGAAAGGGGGTGTAAGTT

GTGGGACCAGTTTTAGAGGGACAGTTGGACGGACTAGAGATTACACTGTTTTACATCCA

TCTTGCTTGTCAGTTTGTAATGTTACCATACAGGATACTATGGAACGCAGCATGGATGA

GTTCACTGCATCCACTCCTGCAGATTTGGGAGAAGCTGGTCGTCTCAGAAAAAAGGCAG

ATATTGCAACTTCTAAGACTACTACTAGATTTCGACCTAGTAATACTAAATCCAAAAAG

GATGTTAAACTTGAATTTTTTGGTTTTGAAGATCATGAGACAGGAGGTGATGAAGGAGG

TTCTGGAAGTTCTAATTACAAAATTAAGTATTTTGGCTTTGATGATCTCAGTGAAAGCG

AAGATGATGAAGATGATGACTGTCAAGTAGAAAGAAAGACAAGCAAAAAAAGAACTAAA

ACAGCTCCATCACCCTCCTTGCAGCCTCCCCCAGAAAGCAATGATAATTCCCAGGACAG

TCAGTCTGGTACTAACAATGCAGAAAACTTGGATTTTACAGAGGACTTGCCTGGTGTGC

CTGAAAGTGTGAAGAAGCCCATAAATAAACAAGGAGATAAATCAAAGGAAAATACCAGA

AAGATTTTTAGTGGCCCCAAACGGTCACCCACAAAAGCTGTATATAATGCCAGACATTG

GAATCATCCAGATTCAGAAGAACTGCCTGGGCCACCAGTAGTAAAACCTCAGAGTGTCA

CAGTGAGGCTGTCTTCAAAGGAACCAAATCAAAAAGATGATGGAGTTTTTAAGGCTCCT

GCACCACCATCCAAAGTGATAAAAACTGTGACAATACCTACTCAGCCCTACCAAGATAT

AGTTACTGCACTGAAATGCAGACGAGAAGACAAAGAATTATATACTGTTGTTCAGCACG

TGAAGCACTTCAACGATGTTGTAGAATTTGGTGAAAATCAAGAGTTCACTGATGACATT

GAGTACTTGTTAAGTGGCTTAAAGAGCACTCAGCCTCTAAACACACGTTGCCTTAGTGT

TATTAGCTTGGCTACTAAATGTGCCATGCCCAGTTTTCGAATGCACCTGAGAGCACATG

GGATGGTAGCAATGGTCTTTAAAACCTTGGATGATTCCCAGCACCATCAGAATCTGTCC

CTCTGTACAGCTGCCCTCATGTATATACTGAGTAGAGATCGTTTGAACATGGATCTTGA

TAGAGCTAGCTTAGATCTAATGATTCGACTTTTGGAACTGGAACAAGATGCTTCATCAG

CCAAGCTACTGAATGAAAAAGACATGAACAAAATTAAAGAAAAAATCCGAAGGCTCTGT

GAAACTGTACACAACAAGCATCTTGATCTAGAAAATATAACGACTGGGCATTTAGCTAT

GGAGACATTATTATCCCTTACTTCTAAACGAGCAGGAGACTGGTTTAAAGAAGAACTCC

GGCTTTTGGGTGGTCTGGATCATATTGTAGATAAAGTAAAAGAATGTGTGGATCATTTA

AGTAGAGATGAGGATGAAGAGAAACTGGTAGCCTCACTATGGGGAGCAGAGAGATGTTT

ACGAGTTTTAGAAAGTGTAACTGTGCATAATCCCGAAAATCAAAGCTACTTGATAGCAT

ATAAAGATTCCCAACTTATTGTTTCATCAGCTAAAGCATTACAGCATTGTGAAGAACTG

ATTCAGCAGTACAACCGTGCTGAGGACAGCATATGCTTAGCTGACAGTAAGCCTCTGCC

TCACCAGAATGTAACTAACCATGTAGGCAAAGCAGTGGAGGACTGCATGAGGGCCATCA

TCGGGGTGTTGCTTAATTTAACTAATGATAATGAGTGGGGCAGCACCAAAACAGGAGAG

CAGGACGGTCTCATAGGCACAGCGCTGAACTGTGTGCTTCAGGTTCCAAAGTACCTACC

TCAGGAGCAGAGATTTGATATTCGAGTGCTGGGCTTAGGTCTGCTGATAAATCTAGTGG

AGTATAGTGCTCGGAATCGGCACTGTCTTGTCAACATGGAAACATCGTGCTCTTTTGAT

TCTTCCATCTGTAGTGGAGAAGGGGATGATAGTTTAAGGATAGGTGGACAAGTTCATGC

TGTCCAGGCTTTAGTGCAGCTATTCCTTGAGCGAGAGCGGGCAGCCCAGCTAGCAGAAA

GTAAAACAGATGAGTTGATCAAAGATGCTCCCACCACTCAGCATGATAAGAGTGGAGAG

TGGCAAGAAACAAGTGGAGAAATACAGTGGGTGTCAACTGAAAAGACTGATGGTACAGA

AGAGAAACATAAGAAGGAGGAGGAGGATGAAGAACTTGACCTCAATAAAGCCCTTCAGC

ATGCCGGCAAACACATGGAGGATTGCATTGTGGCCTCCTACACGGCACTACTTCTTGGG

TGTCTCTGCCAGGAAAGTCCAATCAATGTAACCACTGTGCGGGAATATCTGCCAGAAGG

AGACTTTTCAATAATGACAGAGATGCTCAAAAAATTTTTGAGTTTTATGAATCTCACTT

GTGCTGTTGGAACAACTGGCCAGAAATCTATCTCTAGAGTGATTGAATATTTGGAACAT

TGCTAGctgctttacctttgcttcaggtgctcggtaatgctggagctatccttagacaa

agaaaagtcaagtcatgaaagaagtccttgaagatataccaagaacattcatcagtatc

attcgtgtttggatttttaaggccacctgatttcttcgtcatgcattcggcatttgcta

aatgacagttactacatcaatctgcaactatcaaaaatgaggggaaaaggttcaggctg

ttaacaattccatgcagtatttaaatacatttactttggcagagtttataccctcccct

tgttttcttgctttattctgggcaagtttgaaggggaaaatttgtgctgctgttagtgc

aactgctgtgtatgttgagccactgttgtcatgccagccaggtgcaaaggcagcttagc

tactgaggtagcgaatgttctgaggacattctagacaacagcttagttccttttcagg

ctcatttgcttttgctttttttgttgaatgattccaatcgtaaataaagctttaataat

tttgtgaatttttttggttgttgttccctgaactactgtctatatttaaaattagatgga

atccaaagatacacgggattaatagtatatttttttattcttgattaggtttgggttat

tgaactatttttttacttttgagaccacaaccatattcaatatcataccataatgtgtca

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Promoter region sequence of the hWAPLgene

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cDNA sequence of the mouse WAPL

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In the description and the drawings, when abbreviating nucleotides and/or amino acids, the abbreviations are those in

accordance with IUPAC-IUB Commission on Biochemical Nomenclature or conventional abbreviations in the art. Examples are listed below. When optical isomers exist in an amino acid, an L-isomer is indicated unless specifically indicated.

DNA: deoxyribonucleic acid;

cDNA: complementary deoxyribonucleic acid;

A: adenine;

T: thymine;

G: guanine;

C: cytosine;

I: inosine;

R: adenine (A) or guanine (G);

Y: thymine (T) or cytosine (C);

M: adenine (A) or cytosine (C);

K: guanine (G) or thymine (T);

S: guanine (G) or cytosine (C);

W: adenine (A) or thymine (T);

B: guanine (G), guanine (G) or thymine (T);

D :adenine (A), guanine (G) or thymine (T);

V :adenine (A), guanine (G) or cytosine (C);

N :adenine (A), guanine (G), cytosine (C) or thymine (T) or unknown or another base;

RNA: ribonucleic acid;

mRNA: messenger ribonucleic acid;

dATP: deoxyadenosine triphosphate;

dTTP: deoxythymidine triphosphate;

dGTP: deoxyguanosine triphosphate;

dCTP: deoxycytidine triphosphate;

ATP: adenosine triphosphate;

EDTA: ethylenediaminetetraacetic acid;

SDS: sodium dodecyl sulfate;

BHA: benzhydrylamine;

pMBHA: p-methylbenzhydrylamine;

Tos: p-toluenesulfonyl;

Bzl: benzyl;

Bom: benzyloxymethyl;

Boc: t-butyloxycarbonyl;

DCM: dichloromethane;

HOBt: 1-hydroxybenzotriazole;

DCC: N,N'-dicyclohexylcarbodiimide;

TFA: trifluoroacetic acid;

DIEA: diisopropylethylamine;

Gly or G: glycine;

Ala or A: alanine;

Val or V: valine;

Leu or L: leucine;

Ile or I: isoleucine;

Ser or S: serine;

Thr or T: threonine;

Cys or C: cysteine;

Met or M: methionine;

Glu or E: glutamic acid;

Asp or D: aspartic acid;

Lys or K: lysine;

Arg or R: arginine;

His or H: histidine;

Phe or F: phenylalanine;

Tyr or Y: tyrosine;

Trp or W: tryptophan;

Pro or P: proline;

Asn or N: asparagine;

Gln or Q: glutamine;

pGlu: pyroglutamic acid;

Tyr(I): 3-iodotyrosine;

DMF: N,N-dimethylformamide;

Fmoc: N-9-fluorenylmethoxycarbonyl;

Trt: trityl;

Pbf:

2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl;

Clt: 2-chlorotrityl;

Bu^t: t-butyl; and

Met(O): methionine sulfoxide.

Industrial Applicability

The full-length nucleotide sequence of an hWAPL oncogene according to the present invention allows for producing a recombinant of an hWAPL oncogenic protein encoded by the hWAPL oncogene, and investigating a cancerization mechanism induced by over-expression of the hWAPL oncogenic protein in a cell strain derived from any of various epithelial cells. Furthermore, in the present invention, a promoter region in the identified hWAPL oncogene may provide a new target in studying cancer prevention or therapy on the basis of a mechanism of inhibition of the cancerization mechanism induced by over-expression of the nWAPL oncogene, by inhibiting transcription of the oncogene. Furthermore, production of a recombinant of the full-length nucleotide sequence of the hWAPL oncogene according to the present invention, the hWAPL oncogenic protein, allows for preparation of a nucleic acid probe or PCR primer for detecting mRNA expression by transcription of the hWAPL oncogene or a specific antibody for detecting a translated hWAPL oncogenic protein peptide. Thus, it allows for using various diagnosis kits which detect over-expression of the hWAPL oncogene directly involved in onset of a cancer.

[Sequence Listing]

<110> NEC Corporation and Dr. Masahiko KURODA

<120> Novel Cancer Gene And Its Use

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<210> 1

<211>

<212> PRT

<213> Human

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Thr Lys Trp Gly Glu Thr Thr Phe Met Ala Lys Leu Gly Gln Lys Arg
35 40 45

Pro Asn Phe Lys Pro Asp Ile Gln Glu Ile Pro Lys Lys Pro Lys Val
50 55 60

Glu Glu Glu Ser Thr Gly Asp Pro Phe Gly Phe Asp Ser Asp Asp Glu
65 70 75 80

Ser Leu Pro Val Ser Ser Lys Asn Leu Ala Gln Val Lys Cys Ser Ser

85 90 95

Tyr Ser Glu Ser Ser Glu Ala Ala Gln Leu Glu Glu Val Thr Ser Val

100 105 110

Leu Glu Ala Asn Ser Lys Ile Ser His Val Val Val Glu Asp Thr Val

115 120 125

Val Ser Asp Lys Cys Phe Pro Leu Glu Asp Thr Leu Leu Gly Lys Glu

130 135 140

Lys Ser Thr Asn Arg Ile Val Glu Asp Asp Ala Ser Ile Ser Ser Cys

145 150 155 160

Asn Lys Leu Ile Thr Ser Asp Lys Val Glu Asn Phe His Glu Glu His

165 170 175

Glu Lys Asn Ser His His Ile His Lys Asn Ala Asp Asp Ser Thr Lys

180 185 190

Lys Pro Asn Ala Glu Thr Thr Val Ala Ser Glu Ile Lys Glu Thr Asn

195 200 205

Asp Thr Trp Asn Ser Gln Phe Gly Lys Arg Pro Glu Ser Pro Ser Glu

210 215 220

Ile Ser Pro Ile Lys Gly Ser Val Arg Thr Gly Leu Phe Glu Trp Asp

225 230 235 240

Asn Asp Phe Glu Asp Ile Arg Ser Glu Asp Cys Ile Leu Ser Leu Asp

245 250 255

Ser Asp Pro Leu Leu Glu Met Lys Asp Asp Asp Phe Lys Asn Arg Leu

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Ala Lys Lys Gly Gly Val Ser Cys Gly Thr Ser Phe Arg Gly Thr Val
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Phe Thr Glu Asp Leu Pro Gly Val Pro Glu Ser Val Lys Lys Pro Ile

515 520 525

Asn Lys Gln Gly Asp Lys Ser Lys Glu Asn Thr Arg Lys Ile Phe Ser

530 535 540

Gly Pro Lys Arg Ser Pro Thr Lys Ala Val Tyr Asn Ala Arg His Trp

545 550 555 560

Asn His Pro Asp Ser Glu Glu Leu Pro Gly Pro Pro Val Val Lys Pro

565 570 575

Gln Ser Val Thr Val Arg Leu Ser Ser Lys Glu Pro Asn Gln Lys Asp

580 585 590

Asp Gly Val Phe Lys Ala Pro Ala Pro Pro Ser Lys Val Ile Lys Thr

595 600 605

Val Thr Ile Pro Thr Gln Pro Tyr Gln Asp Ile Val Thr Ala Leu Lys

610 615 620

Cys Arg Arg Glu Asp Lys Glu Leu Tyr Thr Val Val Gln His Val Lys

625 630 635 640

His Phe Asn Asp Val Val Glu Phe Gly Glu Asn Gln Glu Phe Thr Asp

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CLAIMS

1. An oncogene polynucleotide derived from human involving development of cervical cancer, comprising a nucleotide sequence encoding an amino acid sequence of SEQ. ID. No.1.

2. The polynucleotide as claimed in Claim 1 wherein the nucleotide sequence encoding the amino acid sequence of SEQ. ID. No.1 is a nucleotide sequence of SEQ. ID. No.2.

3. A recombinant peptide or its salts, comprising an amino acid sequence of SEQ. ID. No.1 or a partial amino acid sequence of the amino acid sequence.

4. A recombinant oncogenic protein comprising an amino acid sequence of SEQ. ID. No.1.

5. A recombinant vector comprising a polynucleotide encoding the recombinant peptide as claimed in Claim 3.

6. A recombinant vector comprising the polynucleotide as claimed in Claim 1 or 2.

7. A transformed cell produced by transforming a host cell using the recombinant vector as claimed in Claim 5.

8. A transformed cell produced by transforming a host cell using the recombinant vector as claimed in Claim 6.

9. A process for producing the recombinant peptide as claimed in Claim 3 or its salts comprising the steps of:

culturing the transformed cell as claimed in Claim 7

to allow the transformed cell to produce the recombinant peptide as claimed in Claim 3; and

collecting the recombinant peptide produced from the culture.

10. A process for producing the recombinant oncogenic protein as claimed in Claim 4 comprising the steps of:

culturing the transformed cell as claimed in Claim 8 to allow the transformed cell to produce the recombinant oncogenic protein as claimed in Claim 4; and

collecting the recombinant oncogenic protein produced from the culture.

11. An antibody which is a specific antibody generated using the recombinant peptide as claimed in Claim 3 as an immunogen.

12. The antibody as claimed in Claim 11, wherein the antibody is reactive to a partial amino acid sequence of 623 to 1185 region of the amino acid sequence of SEQ. ID. No.1.

13. An antibody reagent kit for an antigen-antibody reaction comprising the antibody as claimed in Claim 11, available for detecting an oncogenic protein comprising the amino acid sequence of SEQ. ID. No.1 or a peptide fragment derived from the oncogenic protein.

14. A diagnosis kit being usable for detection of an oncogenic protein comprising the amino acid sequence of SEQ. ID. No.1 or a peptide fragment derived from the oncogenic protein

by means of an antigen-antibody reaction, comprising the antibody as claimed in Claim 11.

15. An antisense polynucleotide comprising a complementary nucleotide sequence to a partial nucleotide sequence of the nucleotide sequence of SEQ. ID. No.2, which is a DNA fragment having at least a length selected from the region of 15 to 300 bases.

16. A probe hybridization kit available for detecting an mRNA comprising the nucleotide sequence of SEQ. ID. No.2, its partial nucleotide sequence or cDNA prepared by the mRNA, comprising the antisense polynucleotide as claimed in Claim 15 as the DNA probe therein.

17. A diagnosis kit available for detecting expression of mRNA comprising the nucleotide sequence of SEQ. ID. No.2, which is translated into an oncogenic protein comprising the amino acid sequence of SEQ. ID. No.1, by means of a probe hybridization method, comprising the antisense polynucleotide as claimed in Claim 15 as the hybridization probe.

18. A primer pair for PCR amplification of cDNA comprising the nucleotide sequence of SEQ. ID. No.2, consisting of paired primers of:

a nucleotide sequence :

5'-TTGGATCCATGACATCCAGATTTGGGAAAACATACAGTAGG-3'; and

a nucleotide sequence :

5'-TTGAATTCCTAGCAATGTTCCAAATATTCAATCACTCTAGA-3'.

19. A primer pair for PCR amplifying a partial chain in cDNA comprising the nucleotide sequence of SEQ. ID. No.2, consisting of paired primers of:

5'-GAATTCATAGGCACAGCGCTGAACTGTGTG-3'; and

5'-TTGAATTCCTAGCAATGTTCCAAATATTCA-3'.

20. A double strand of short-chain interfering RNA capable of inhibiting expression of mRNA comprising the nucleotide sequence of SEQ. ID. No.2 in a cervical cancer cell, wherein the siRNA has a nucleotide sequence: CGGACTACCCTTAGCACAA.

21. A pharmaceutical composition for inhibiting expression of mRNA comprising the nucleotide sequence of SEQ. ID. No.2 in a cervical cancer cell to arrest growth of the carcinoma cell, comprising the double strand of short-chain interfering RNA as claimed in Claim 20.