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(54) Title: NOVEL USE OF MIG12 AND OIP5 GENES

(57) Abstract: Provided is a composition for cancer diagnosis or anticancer drug screening, comprising an MIG 12 gene or MIG 12 gene-expressed protein, a composition for cancer diagnosis or anticancer drug screening, comprising an OIP 5 gene or OIP5 gene-expressed protein, a composition for treatment of cancer, comprising an inhibitor of the aforesaid genes or an inhibitor of the aforesaid proteins and a pharmaceutically acceptable carrier, and a cancer diagnosis kit comprising at least one of an MIG 12 gene, an OIP5 gene, and an MIG 12- or OIP5-expressed protein. MIG12 and OIP5 genes of the present invention are expressed at relatively high levels in cancer cell lines and increase a proliferation rate of normal cells. Therefore, inhibition of expression of these genes via RNAi using siRNA or shRNA leads to suppression of cancer cell growth. For these reasons, MIGl 2 and OIP5 genes can be used as a target gene for diagnosis or cancer therapy of various cancers.

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NOVEL USE OF MIG12 AND OIP5 GENES

TECHNICAL FIELD

The present invention relates to a composition for cancer diagnosis or anticancer drug screening, comprising an MIG12 gene or MIG12 gene-expressed protein, a composition for cancer diagnosis or anticancer drug screening, comprising an OIP5 gene or OIP5 gene-expressed protein, a composition for treatment of cancer, comprising an inhibitor of the aforesaid genes or an inhibitor of the aforesaid proteins and a pharmaceutically acceptable carrier, and a cancer diagnosis kit comprising at least one of an MIG12 gene, an OIP5 gene, and an MIG12- or OIP5-expressed protein.

BACKGROUND ART

The completion of human genome project which allows understanding of human diseases at the molecular level, identification of target molecules for diseases and further understanding of molecular mechanism of diseases opened the era of post-genomics. The information and insights obtained from human genomics is leading to technologies for development of personalized medicine for human diseases. For personalized medicine, patients will be treated by selecting the appropriate drugs. Also, the pharmacogenomic technologies involving the identification of biomarkers, targeted therapy, disease-specific mode of action of drugs, clinical and genomic information of patients, genome epidemic, and bioinformatic analysis should be complementarily integrated. Especially, the technologies involving prediction of the drug action in each individual and identification of biomarkers for diagnosis and therapeutic targets for disease and development of personalized drugs are crucial.

There has been progress in studying the molecular mechanisms and pathophysiology of human cancers. These mechanisms have been exploited for identification of new targets for development of drugs with greater antitumor activity and less toxicity. Many studies confirmed the potential use of yeast to study the genes associated with key cellular pathways in cancer and disease. Also, a high-throughput assay for analysis of growth interference phenotypes of yeast was carried out.

The fission yeast *Schizosaccharomyces pombe* is well-suited for the study of cross-species gene functions involved in basic cellular processes such as cell cycle progression, cellular morphogenesis, and cellular response to environmental changes. Deletion or

overexpression of a gene playing a crucial role in cell cycle and growth gives an observable phenotype, which makes *S. pombe* a powerful model organism to investigate cancer-related genes. In *S. pombe*, the overexpression of a heterologous gene may cause growth defects or morphological changes by interfering in the cell cycle or basic metabolic pathways, which can be used as a simple indication of a gene functioning in the dysregulation of basic cellular processes. The overexpression of human heterologous genes in *S. pombe*, which produces observable phenotypes, can provide insights into cellular functions relevant to cancer. Therefore, the investigation of the overexpression effects of the heterologous human gene is a powerful method to find clues associated with carcinogenesis.

RNAi is a naturally occurring process in eukaryotes by which double-stranded RNAs (dsRNA) trigger the sequence-specific degradation of homologous mRNAs resulting in preventing expression of their protein products. Initiation of RNAi involves the cleavage of long dsRNA stretches into small fragments of 21-30 bps, termed siRNAs (for small interfering RNAs), which are then bound to the RNA-induced silencing complex (RISC). This complex binds to and cleaves mRNAs which match the siRNA that is bound to it. siRNA can trigger the RNAi response in mammalian cells without activating the antiviral response. RNA interference is an efficient method of identifying targets for gene therapy in a variety of diseases. RNAi has become a widespread tool for reverse genetics in invertebrate model systems and has rapidly emerged as a novel therapeutic approach to treat human diseases.

A short hairpin RNA (shRNA) is a sequence of RNA that makes a tight hairpin turn that can be used to silence gene expression via RNA interference for a relatively long time. shRNA, which consists of siRNA sense sequence, and 3-10 bases of linker-antisense siRNA sequence, uses a vector introduced into cells and utilizes the U6 promoter to ensure that the shRNA is always expressed. This vector is usually passed on to daughter cells, allowing the gene silencing to be inherited. The shRNA hairpin structure is cleaved by the cellular machinery into siRNA. shRNAs can also be cloned into lentivirus or adenovirus and used for *in vivo* animal study.

OIP5 (GenBank NM_007280) was identified as Opa (*Neisseria gonorrhoeae* opacity-associated)-interacting protein. OIP5 is homologous to OIP4, which is a protein antigen that is expressed in tumors and also in some normal tissues, including testes and endometrium. OIP5, a cancer-testis specific gene, was confirmed to be up-regulated in the tumor tissue compared to normal tissue in gastric cancer. It was suggested that OIP5 may be a novel immunotherapy

target for patients with gastric cancer. Recently, OIP5 was reported as LINT-25 since it interacted with LAP2α, lamina associated polypeptide 2α. LINT-25 protein levels were up-regulated during G1 phase in proliferating cells and upon cell cycle exit in quiescence, senescence and differentiation. Upon cell cycle exit LINT-25 accumulated in heterochromatin foci and transient expression of LINT-25 in proliferating cells caused loss of LAP2α and subsequent cell death.

MIG12 (GenBank NM_021242) was isolated as the gene whose protein product interacts with Mid1. Mig12 is mainly expressed in the neuroepithelial midline, urogenital apparatus, and digits during embryonic development. Endogenous Mig12 protein is partially detected in the polymerized tubulin fraction after microtubule stabilization. When co-transfected with Mid1, Mig12 is massively recruited to thick filamentous structures composed of tubulin. These microtubule bundles are resistant to high doses of depolymerizing agents and are composed of acetylated tubulin, thus representing stabilized microtubule arrays. The relevance of MIG12 to cancer has not been reported.

DISCLOSURE OF THE INVENTION

TECHNICAL PROBLEM

Therefore, the present invention has been made in view of the above problems, and it is an object of the present invention to provide a novel use of MIG12 and OIP5 genes.

More specifically, an object of the present invention is to provide a composition for diagnosing cancer or for screening anticancer drugs comprising an MIG12 gene or a protein expressed from the MIG12 gene; a composition for diagnosing cancer or for screening anticancer drugs comprising an OIP5 gene or a protein expressed from the OIP5 gene; a composition for preventing or treating cancer comprising an inhibitor of the aforesaid genes or an inhibitor of the aforesaid proteins and a pharmaceutically acceptable carrier; and a kit for diagnosing cancer comprising one or more selected from an MIG12 gene, an OIP5 gene, a protein expressed from MIG12 gene, and a protein expressed from OIP5 gene.

It is another object of the present invention to provide a use of an MIG12 gene and a protein expressed from the MIG12 gene for diagnosing cancer or for screening anticancer drugs; a use of an OIP5 gene and a protein expressed from the OIP5 gene for diagnosing cancer or for screening anticancer drugs; a method for diagnosing cancer or screening

anticancer drugs using the same; a use of an inhibitor of the aforesaid gene or the proteins expressed from the aforesaid genes; and a method for preventing or treating cancer using the same.

TECHNICAL SOLUTION

For the purposes of the present invention, it is noted that, as used in this specification, the singular forms "a," "an," and "the" include plural referents unless expressly and unequivocally limited to one referent.

The term "or" is used interchangeably with the term "and/or" unless the context clearly indicates otherwise.

In accordance with an aspect of the present invention, the above and other objects can be accomplished by the provision of a composition for diagnosing cancer, comprising an MIG 12 or OIP5 gene.

As used herein, the term "MIG 12 gene" or "OIP5 gene" refers to a full-length DNA or RNA of the MIG 12 or OIP5 genes, or a portion or variant thereof.

Besides the genes, the composition of the present invention may further comprise buffer or distilled water to stably maintain a structure of nucleic acid.

As can be confirmed from Examples 2 and 3 which will be illustrated hereinafter, the expression level of MIG12 gene or OIP5 gene is specifically increased in cancer cells or cancer cell lines. Thus, the diagnosis of cancer can be performed by analysis of the overexpression of MIG12 gene or OIP5 gene, using the genes of the present invention.

In accordance with another aspect of the present invention, there is provided a use of an MIG12 or OIP5 gene for diagnosing cancer and a method for diagnosing cancer, comprising confirming the reaction between a sample obtained from a subject and an MIG12 or OIP5 gene.

In the method for diagnosing cancer according to the present invention, for confirming the reaction between a sample obtained from a subject and an MIG12 gene or an OIP5 gene, it is possible to use conventional methods used in the cases of DNA-DNA, DNA-RNA or DNA-protein, for example, DNA chip, protein chip, PCR (polymerase chain reaction), Northern blotting, Southern blotting, ELISA (Enzyme Linked Immunosorbent assay), yeast two-hybrid assay, 2-D gel electrophoresis, *in vitro* binding assay, etc. For example, incidence of cancer (carcinogenesis) in individuals can be determined by examining whether a gene of interest is

expressed at a high level in a subject, through hybridization of an intact or partial gene as a probe with the nucleic acid isolated from a body fluid of the subject, and detection of a hybridization product according to any conventional method known in the art, such as reverse transcription-polymerase chain reaction (RT-PCR), Southern blotting, Northern blotting, and the like. When the probe is labeled with a suitable label material such as radioisotopes, enzymes, and the like, it is possible to readily confirm the presence of the target gene.

In accordance with a further aspect of the present invention, there is provided a composition for diagnosing cancer, comprising a protein expressed from an MIG12 gene or OIP5 gene.

In addition to the aforesaid protein, the composition of the present invention may further comprise buffer or distilled water to stably maintain a structure of the protein.

As discussed above, the expression level of MIG12 or OIP5 gene is specifically increased in cancer cells. Therefore, the presence of cancer can be diagnosed by analysis of the overexpression of MIG12 or OIP5 gene or the corresponding protein, using the protein expressed from the MIG12 gene or OIP5 gene.

In accordance with a still further aspect of the present invention, there is provided a use of the aforesaid proteins for diagnosing cancer, and a method for diagnosing cancer, comprising confirming the reaction between the gene-expressed protein and a subject-derived sample.

In the cancer diagnosis method according to the present invention, confirmation of the reaction between the subject-derived sample and the protein may be carried out by using any conventional methods used in the cases of DNA-protein, RNA-protein or protein-protein, for example, DNA chip, protein chip, PCR (polymerase chain reaction), Northern blotting, Southern blotting, Western blotting, ELISA (Enzyme Linked Immunosorbent assay), histoimmunostaining, yeast two-hybrid assay, 2-D gel electrophoresis, *in vitro* binding assay, etc. For example, the analysis of individual carcinogenesis can be carried out by examining whether a gene of interest is expressed at a high level in a subject, through hybridization of the gene-expressed intact or partial protein as a probe with the nucleic acid or protein isolated from a body fluid of the subject, and detection of a hybridization product according to any conventional method known in the art, such as reverse transcription-polymerase chain reaction (RT-PCR), Western blotting, and the like. When the probe is labeled with a suitable label material such as radioisotopes, enzymes, the like, it is possible to readily confirm the presence

of the target gene.

Alternatively, the composition of the present invention may comprise an antibody specific for the aforesaid protein, instead of that protein. As the cancer cells exhibit overexpression of the MIG12 or OIP5 gene, amounts of the proteins expressed from these genes also increase. Therefore, as disclosed in Example 1 which will follow, when antibodies directed against the proteins expressed from these genes are used, it is possible to diagnose cancer by detection of the protein through an antigen-antibody reaction.

Monoclonal antibodies directed against the aforesaid protein may be produced by any conventional method for production of monoclonal antibodies known in the art or otherwise may be commercially available. Quantitative analysis of monoclonal antibodies against the aforesaid proteins may be generally carried out by color development using secondary antibodies conjugated with a certain enzyme (such as alkaline phosphatase (AP) or horseradish peroxidase (HRP)) and substrates thereof, or otherwise may be carried out using the protein-specific monoclonal antibodies which are directly conjugated with AP or HRP enzymes. Alternatively, instead of monoclonal antibodies, it may also be possible to use polyclonal antibodies which recognize the aforesaid protein. These polyclonal antibodies may be obtained by any conventional method for production of antiserum known in the art.

Further, the present invention provides a composition for screening anticancer drugs, comprising an MIG12 or OIP5 gene. Further, the present invention provides a use of the same composition for screening anticancer drugs, and a method for screening anticancer drugs, comprising contacting a test material with the composition as a target material, and confirming the reaction between the target material and the test material to determine whether the test material activates or suppresses the expression of the MIG12 or OIP5 gene.

In the screening method according to the present invention, the reaction between the composition and the test material can be confirmed by any conventional method used in the cases of DNA-DNA, DNA-RNA, DNA-protein, DNA-compound. For example, mention may be made of a hybridization test to confirm binding of the gene to the test material *in vitro*, a method of determining a gene expression level involving reacting of mammalian cells with the test material, and subjecting the reaction product to Northern blot analysis, quantitative PCR, or quantitative real-time PCR, and a method of determining an expression level of a reporter gene involving linking the reporter gene to the gene of interest, introducing the resulting gene construct into a cell and reacting the cell with a test material. In this case, the composition of

the present invention may further comprise distilled water or buffer to stably maintain a structure of the nucleic acid, in addition to the aforesaid gene.

Further, the present invention provides a method for screening anticancer drugs, comprising inducing phenotypic changes of a model cell or animal cell using the aforesaid composition as a target material and determining whether the test material activates or suppresses the expression of the gene.

In the anticancer drug screening method of the present invention, candidate materials having direct inhibitory effects on the gene expression and protein function may be screened by cell-based analysis through phenotype assay (including growth rate, morphological changes and temperature sensitivity) using a model organism or animal cell with overexpression of the aforesaid gene.

Further, the present invention provides a composition for screening anticancer drugs, comprising a protein expressed from an MIG12 or OIP5 gene. Further, the present invention provides a use of the same composition for screening anticancer drugs and a method for screening anticancer drugs, comprising a test material with the aforesaid composition as a target material, and confirming the reaction between the target material and the test material to determine whether the test material activates or suppresses the function of the protein.

In the screening method according to the present invention, the reaction between the composition and the test material can be confirmed by any conventional method used in the cases of protein-protein, DNA-compound and the like. For example, mention may be made of a method of determining activity of a test material after reaction of the protein with the test material, yeast two-hybrid assay, detection of phage-displayed peptide clones bound to the aforesaid protein, high throughput screening (HTS) using natural products and chemical libraries, drug hit HTS (HTS), cell-based screening, and DNA array screening. In addition to the protein component, the composition of the present invention may further comprise buffer or reaction liquid to stably maintain a structure or physiological activity of the corresponding protein. For *in vivo* experiments, the composition of the present invention may comprise cells expressing the aforesaid protein, or cells harboring a plasmid that expresses the aforesaid protein under a promoter capable of controlling a transcription rate.

In the screening method of the present invention, the test material may be a material which is suspected to have the possibility of being a cancer metastasis inhibitor according to a conventional criteria and strategy for selection, or may be a material which is randomly

selected, such as individual nucleic acids, proteins, extracts or natural products, compounds, and the like.

There may be two types of test materials which are obtained through the screening method of the present invention: a material capable of enhancing the gene expression or protein function and a material capable of inhibiting the gene expression or protein function. The former material may be an anticancer drug candidate via development of an inhibitor against the test material, whereas the latter material may be directly an anticancer drug candidate. These anticancer drug candidates will serve as a leading compound during a subsequent development process of anticancer drugs. A novel anticancer drug can be created by structural modification and optimization of the leading compound such that the leading compound can exhibit inhibitory effects on function of the gene or expression products thereof.

Further, the present invention provides siRNA of an MIG12 gene (hereinafter, referred to as MIG12 siRNA or siMIG12) or siRNA of an OIP5 gene (hereinafter, referred to as OIP5 siRNA or siOIP5).

siRNA is prepared by simultaneous synthesis of a sense strand of the gene and an anti-sense strand exhibiting practical gene expression inhibition in cells. Thereafter, two strands synthesized are allowed to form a double-stranded RNA, followed by intracellular incorporation.

For convenience, unless otherwise specified, the siRNA sequence of the present invention refers to a sense strand of a gene of interest.

A candidate siRNA sequence region of the MIG12 gene (NM_021242) may have a nucleotide sequence consisting of 19-30 contiguous nucleotides as set forth in SEQ ID NO: 14, 15, 16, 17, or 18. The nucleotide sequence of SEQ ID NO: 14, 15, 16, 17, or 18 is a partial sequence of the MIG12 gene (NM_021242) of SEQ ID NO: 1 and has a nucleotide sequence consisting of from nucleotide 845 to nucleotide 950, a nucleotide sequence consisting of from nucleotide 970 to nucleotide 1030, a nucleotide sequence consisting of from nucleotide 1080 to nucleotide 1160, a nucleotide sequence consisting of from nucleotide 1220 to nucleotide 1290, or a nucleotide sequence consisting of from nucleotide 1320 to nucleotide 1400, each sequence comprising 61-106 nucleotides. That is, a nucleotide sequence complementary to the aforesaid nucleotide sequence may be a sequence which is capable of efficiently inducing RNAi.

The MIG12 siRNA may comprise a nucleotide sequence corresponding to an mRNA

sequence derived from a nucleotide sequence of 19 to 30 contiguous nucleotides, among one or more sequences selected from SEQ ID NOS: 14, 15, 16, 17 and 18.

The MIG12 siRNA may have a nucleotide sequence of SEQ ID NO: 3 or 4. The sequence of SEQ ID NO: 3 may be a sequence which has a 19-bp nucleotide sequence corresponding to an mRNA sequence derived from a nucleotide sequence ranging from nucleotide 1331 to nucleotide 1349 of the MIG12 gene of SEQ ID NO: 1 and contains two overhang nucleotides of TT, whereas the sequence of SEQ ID NO: 4 may be a sequence which has a 19-bp nucleotide sequence corresponding to an mRNA sequence derived from a nucleotide sequence ranging from nucleotide 1123 to nucleotide 1141 of the MIG12 gene of SEQ ID NO: 1 and contains two overhang nucleotides of TT. These sequences may have superior gene expression inhibition (RNAi effects) on the anti-sense strand complementary to the sequence of SEQ ID NO: 3 or 4.

A candidate siRNA sequence region of the OIP5 gene (NM_007280) may have a nucleotide sequence consisting of 19-30 contiguous nucleotides as set forth in SEQ ID NO: 19, 20, 21, 22, 23, 24, 25, or 26. The nucleotide sequence of SEQ ID NO: 19, 20, 21, 22, 23, 24, 25, or 26 is a partial sequence of the OIP5 gene (NM_007280) of SEQ ID NO: 2 and has a nucleotide sequence consisting of from nucleotide 1 to nucleotide 60, a nucleotide sequence consisting of from nucleotide 120 to nucleotide 180, a nucleotide sequence consisting of from nucleotide 380 to nucleotide 460, a nucleotide sequence consisting of from nucleotide 518 to nucleotide 580, a nucleotide sequence consisting of from nucleotide 720 to nucleotide 780, a nucleotide sequence consisting of from nucleotide 850 to nucleotide 890, a nucleotide sequence consisting of from nucleotide 920 to nucleotide 980, or a nucleotide sequence consisting of from nucleotide 1040 to nucleotide 1100, each sequence comprising 41-81 nucleotides. That is, a nucleotide sequence complementary to the aforesaid nucleotide sequence may be a sequence which is capable of efficiently inducing RNAi.

The OIP5 siRNA may comprise a nucleotide sequence corresponding to an mRNA sequence derived from a nucleotide sequence of 19 to 30 contiguous nucleotides, among one or more sequences selected from SEQ ID NOS: 19, 20, 21, 22, 23, 24, 25, and 26.

The OIP5 siRNA may have a nucleotide sequence of SEQ ID NO: 5 or 6. The sequence of SEQ ID NO: 5 may be a sequence which has a 19-bp nucleotide sequence corresponding to an mRNA sequence derived from a nucleotide sequence ranging from nucleotide 426 to nucleotide 444 of the OIP5 gene of SEQ ID NO: 2 and contains two overhang nucleotides of

TT, whereas the sequence of SEQ ID NO: 6 may be a sequence which has a 19-bp nucleotide sequence corresponding to an mRNA sequence derived from a nucleotide sequence ranging from nucleotide 417 to nucleotide 435 of the OIP5 gene of SEQ ID NO: 2 and contains two overhang nucleotides of TT. These sequences may have superior gene expression inhibition (RNAi effects) on the anti-sense strand complementary to the sequence of SEQ ID NO: 5 or 6.

Further, siRNA of the present invention may be chemically modified in order to prevent rapid degradation which may occur by the action of *in vivo* nucleases. siRNA has a double-stranded structure, and therefore is relatively stable as compared to single-stranded ribonucleic acid or antisense oligonucleotide molecules. However, siRNA is readily susceptible to *in vivo* nuclease degradation, so it is possible to lower a degradation rate of siRNA via chemical modification thereof. Chemical modification of siRNA to secure that siRNA is chemically stable and resistant against rapid degradation may be carried out by any conventional method well known to those skilled in the art. The most conventional method used in chemical modification of siRNA is boranophosphate or phosphorothioate modification. These materials form stable nucleoside-nucleoside association of siRNA molecules, thereby conferring resistance to nucleic acid degradation. Even though it is resistant to nucleic acid degradation, the boranophosphate-modified ribonucleic acid is not synthesized by a chemical reaction and is synthesized only by incorporation of boranophosphate into ribonucleic acid via *in vitro* transcription. The boranophosphate modification is a relatively easy method, but has a disadvantage associated with difficulty of site-directed modification at a certain position of target molecules or compounds. On the other hand, the phosphorothioate modification is advantageous for introduction of sulfur elements into a desired site, but excessive phosphothioation may result in problems associated with decreased efficiency, toxicity, and formation of non-specific RNA-induced silencing complex (RISC). For these reasons suffered by the above-mentioned two modification methods, it may be preferred to employ a method which is designed to provide nuclease resistance via introduction of chemical modification only at a termination position of ribonucleic acid (a region beyond 3'-terminal). Further, chemical modification of a ribose ring is also known to enhance the nuclease resistance of nucleic acid. Particularly, modification at 2'-postion of the ribose ring leads to stabilization of siRNA molecules. However, the stability of siRNA molecules increases only with correct introduction of a methyl group into the above-specified position of the ribose ring. Further, introduction of excessively large numbers of methyl groups may result in loss of ribonucleic

acid-mediated interference. Chemical modifications may also be made to improve the *in vivo* pharmacokinetic retention time and efficiency (Mark et al., Molecular Therapy, 13:644-670, 2006).

In addition to chemical modifications, a safe and efficient delivery system is still required to increase intracellular delivery efficiency of siRNA molecules. For this purpose, siRNA of the present invention may be incorporated in the form of a complex with a nucleic acid delivery system into a pharmaceutical composition for treating cancer.

The nucleic acid delivery system for intracellular delivery of nucleic acid materials may be broadly divided into a viral vector system and a non-viral vector system. The most conventionally and widely used system is a viral vector system because it provides a high delivery efficiency and a long retention time *in vivo*. Among a variety of viral vectors, a retroviral vector, an adenoviral vector, an adeno-associated viral vector, and the like are widely employed. These viral vector systems are efficient in intracellular delivery of ribonucleic acid, but suffer from various problems associated with safety concerns, such as difficulty of recombination of the ribonucleic acid into a virus having *in vivo* activity, elicitation of immune response, undesirable random insertion of the ribonucleic acid into host chromosomes, and the like. On the other hand, the non-viral vector system exhibits various advantages, such as low toxicity and immune response, feasibility of repeated administration, convenient formation of a complex of the delivery system with ribonucleic acid, and easy industrial-scale production, as compared to the viral vector system. Further, organ/cell-targeted nucleic acid delivery to affected cells or tissue lesions can be carried out by conjugation of a specific ligand with the non-viral vector. Examples of the non-viral vector that can be used in the present invention may include various formulations such as liposomes, cationic polymers, micelles, emulsions, nanoparticles, and the like. The nucleic acid delivery system can significantly enhance delivery efficiency of the desired nucleic acid into animal cells and can deliver nucleic acid into any type of animal cells, depending upon the desired uses and applications of the nucleic acids.

siRNA of the present invention is a short double-stranded RNA (dsRNA) consisting of 19-30 bp nucleotides. Intracellular incorporation of such siRNA exhibits inhibitory effects on expression of the MIG12 gene or OIP5 gene without causing non-specific inhibition, specifically showing anticancer activity to kill cancer cells. Further, siRNA of the present invention can be used for functional study of related genes.

Further, the present invention provides an shRNA of an MIG12 (hereinafter, referred to as MIG12 shRNA or shMIG12) or an shRNA of OIP5 gene (hereinafter, referred to as OIP5 shRNA or shOIP5).

The aforementioned shRNA (small hairpin RNA) is a sequence of RNA that makes a tight hairpin turn that can be used to silence gene expression via RNA interference. shRNA, which consists of siRNA sense sequence and 3-10 bases of linker-antisense siRNA sequence, uses a plasmid vector introduced into cells and utilizes the U6 promoter to ensure that the shRNA is always expressed. The loop sequence of the resulting shRNA is cleaved by Dicer, an RNase III enzyme, to generate mature siRNA which will exhibit RNAi effects. shRNAs can also be cloned into lentivirus or adenovirus and used for *in vivo* animal study. shRNA exhibits RNAi effects for a relatively long time, as compared to siRNA.

Similar to MIG12 siRNA, the MIG12 shRNA may also comprise a nucleotide sequence corresponding to an mRNA sequence derived from a nucleotide sequence of 19 to 30 contiguous nucleotides, among one or more sequences selected from SEQ ID NOS: 14, 15, 16, 17 and 18 of the MIG12 gene.

The MIG12 shRNA may comprise an RNA nucleotide sequence of SEQ ID NO: 4 and an antisense RNA sequence which is linked via a 3-10 bp loop to and is reverse complementary to the sequence of SEQ ID NO: 4. The RNA nucleotide sequence of SEQ ID NO: 4 is a 19-bp nucleotide sequence which corresponds to an mRNA sequence derived from a nucleotide sequence ranging from nucleotide 1123 to nucleotide 1141 of the MIG12 gene of SEQ ID NO: 1 and may have superior gene expression inhibition (RNAi effects) on an anti-sense strand reverse complementary to the sequence of SEQ ID NO: 4.

As used herein, the term "RNA nucleotide sequence" refers to an RNA nucleotide sequence part with exception of a short sequence consisting of two bases (TT) from the nucleotide sequence of SEQ ID NO: 4.

A top strand for construction of an expression vector to express the MIG12 shRNA may have a sequence as set forth in SEQ ID NO: 27.

Similar to OIP5 siRNA, the OIP5 shRNA may comprise a nucleotide sequence corresponding to an mRNA sequence derived from a nucleotide sequence of 19 to 30 contiguous nucleotides, among one or more sequences selected from SEQ ID NOS: 19, 20, 21, 22, 23, 24, 25, and 26.

The OIP5 shRNA may comprise an RNA nucleotide sequence of SEQ ID NO: 5 and an

antisense RNA sequence which is linked via a 3-10 bp loop to and is reverse complementary to the sequence of SEQ ID NO: 5. The RNA nucleotide sequence of SEQ ID NO: 5 is a 19-bp nucleotide sequence which corresponds to an mRNA sequence derived from a nucleotide sequence ranging from nucleotide 426 to nucleotide 444 of the OIP5 gene of SEQ ID NO: 2 and may have superior gene expression inhibition (RNAi effects) on an anti-sense strand reverse complementary to the sequence of SEQ ID NO: 5.

As used herein, the term "RNA nucleotide sequence" refers to an RNA nucleotide sequence part with exception of a short sequence consisting of two bases (TT) from the nucleotide sequence of SEQ ID NO: 5.

A top strand for construction of an expression vector to express the OIP5 shRNA may have a sequence as set forth in SEQ ID NO: 28.

Expression constructs/vectors comprising shRNA (small hairpin RNA) having the siRNA nucleotide sequence to enhance the intracellular retention and transfection effects of siRNA are known to those skilled in the art. For example, US Patent Application Nos. 2004/106567 A1 and 2004/0086884 disclose a variety of delivery mechanisms, such as viral and non-viral vectors comprising shRNA, liposome delivery systems, *in vivo* injection systems of plasmid DNAs, artificial viral envelope and polylysine conjugates, as well as plenty of information about the expression constructs/vectors.

Further, the present invention provides a composition for treating cancer, comprising an inhibitor of an MIG12 or OIP5 gene.

As used herein, the term "inhibitor" encompasses a gene-expression inhibitor.

The composition may further comprise one or more pharmaceutically acceptable carriers.

As can be confirmed from Examples which will be illustrated hereinafter, the gene of the present invention is expressed at a high level in cancer cells, and administration of an inhibitor against the aforementioned gene (hereinafter, referred to as "gene inhibitor") can suppress the gene expression to thereby inhibit incidence of cancer.

Therefore, the present invention provides a use of a gene inhibitor for treating cancer, and a method for treating cancer, comprising administering a therapeutically effective amount of the gene inhibitor to a subject in need thereof. As used herein, term "treating cancer" or "treatment of cancer" or "cancer treatment" encompasses prevention and inhibition of cancer.

In the present invention, the gene inhibitor may be an antisense oligonucleotide for mRNA of the MIG12 or OIP5 gene.

The antisense oligonucleotide has been successfully employed to achieve gene-specific inhibition both *in vivo* and *in vitro*. The antisense oligonucleotide is a short synthetic DNA strand (or DNA analog) which is antisense (or complementary) to a certain DNA or RNA target. The antisense oligonucleotide is proposed to prevent expression of the protein encoded by a DNA or RNA target. For this purpose, the antisense oligonucleotide binds to the target to thereby halt the protein expression at a transcription, translation or splicing stage. Further, the antisense oligonucleotide has been successfully used in cell culture and animal models of disease (Hogrefe, 1999). Additional modification of antisense oligonucleotides to secure that oligonucleotide is more stable and resistant against *in vivo* nuclease degradation may be carried out by any conventional method well known to those skilled in the art. As used herein, the term "antisense oligonucleotide" encompasses double-stranded DNAs (dsDNAs) or single-stranded DNAs (ssDNAs), dsRNAs or ssRNAs, DNA/RNA hybrids, DNA and RNA analogs, and oligonucleotides having modifications of base, sugar, and/or backbone. The oligonucleotide may be chemically modified to improve the stability thereof and increase the resistance against nuclease degradation, according to a conventional method known in the art. These modification methods are conventionally known in the art, and may include, but are not limited to, modifications of the oligonucleotide backbone, sugar moieties and bases.

Further, the gene inhibitor may be siRNA (Small Interfering RNA) of the MIG12 or OIP5 gene.

siRNA is prepared by simultaneous synthesis of a sense strand of the gene and an anti-sense strand exhibiting practical gene expression inhibition in cells. Thereafter, two strands synthesized are allowed to form a double-stranded RNA, followed by intracellular incorporation.

For convenience, unless otherwise specified, the siRNA sequence of the present invention refers to a sense strand of a gene of interest.

A candidate siRNA sequence region of the MIG12 gene (NM_021242) may have a nucleotide sequence consisting of 19-30 contiguous nucleotides as set forth in SEQ ID NO: 14, 15, 16, 17, or 18. The nucleotide sequence of SEQ ID NO: 14, 15, 16, 17, or 18 is a partial sequence of the MIG12 gene (NM_021242) of SEQ ID NO: 1 and has a nucleotide sequence consisting of from nucleotide 845 to nucleotide 950, a nucleotide sequence consisting of from nucleotide 970 to nucleotide 1030, a nucleotide sequence consisting of from nucleotide 1080 to nucleotide 1160, a nucleotide sequence consisting of from nucleotide 1220 to nucleotide

1290, or a nucleotide sequence consisting of from nucleotide 1320 to nucleotide 1400, each sequence comprising 61-106 nucleotides. That is, a nucleotide sequence complementary to the aforesaid nucleotide sequence may be a sequence which is capable of efficiently inducing RNAi.

The MIG12 siRNA may comprise a nucleotide sequence corresponding to an mRNA sequence derived from a nucleotide sequence of 19 to 30 contiguous nucleotides, among one or more sequences selected from SEQ ID NOS: 14, 15, 16, 17 and 18.

The MIG12 siRNA may have a nucleotide sequence of SEQ ID NO: 3 or 4. The sequence of SEQ ID NO: 3 may be a sequence which has a 19-bp nucleotide sequence corresponding to an mRNA sequence derived from a nucleotide sequence ranging from nucleotide 1331 to nucleotide 1349 of the MIG12 gene of SEQ ID NO: 1 and contains two overhang nucleotides of TT, whereas the sequence of SEQ ID NO: 4 may be a sequence which has a 19-bp nucleotide sequence corresponding to an mRNA sequence derived from a nucleotide sequence ranging from nucleotide 1123 to nucleotide 1141 of the MIG12 gene of SEQ ID NO: 1 and contains two overhang nucleotides of TT. These sequences may have superior gene expression inhibition (RNAi effects) on the anti-sense strand complementary to the sequence of SEQ ID NO: 3 or 4.

A candidate siRNA sequence region of the OIP5 gene (NM_007280) may have a nucleotide sequence consisting of 19-30 contiguous nucleotides as set forth in SEQ ID NO: 19, 20, 21, 22, 23, 24, 25, or 26. The nucleotide sequence of SEQ ID NO: 19, 20, 21, 22, 23, 24, 25, or 26 is a partial sequence of the OIP5 gene (NM_007280) of SEQ ID NO: 2 and has a nucleotide sequence consisting of from nucleotide 1 to nucleotide 60, a nucleotide sequence consisting of from nucleotide 120 to nucleotide 180, a nucleotide sequence consisting of from nucleotide 380 to nucleotide 460, a nucleotide sequence consisting of from nucleotide 518 to nucleotide 580, a nucleotide sequence consisting of from nucleotide 720 to nucleotide 780, a nucleotide sequence consisting of from nucleotide 850 to nucleotide 890, a nucleotide sequence consisting of from nucleotide 920 to nucleotide 980, or a nucleotide sequence consisting of from nucleotide 1040 to nucleotide 1100, each sequence comprising 41-81 nucleotides. That is, a nucleotide sequence complementary to the aforesaid nucleotide sequence may be a sequence which is capable of efficiently inducing RNAi.

The OIP5 siRNA may comprise a nucleotide sequence corresponding to an mRNA sequence derived from a nucleotide sequence of 19 to 30 contiguous nucleotides, among one

or more sequences selected from SEQ ID NOS: 19, 20, 21, 22, 23, 24, 25, and 26.

The OIP5 siRNA may have a nucleotide sequence of SEQ ID NO: 5 or 6. The sequence of SEQ ID NO: 5 may be a sequence which has a 19-bp nucleotide sequence corresponding to an mRNA sequence derived from a nucleotide sequence ranging from nucleotide 426 to nucleotide 444 of the OIP5 gene of SEQ ID NO: 2 and contains two overhang nucleotides of TT, whereas the sequence of SEQ ID NO: 6 may be a sequence which has a 19-bp nucleotide sequence corresponding to an mRNA sequence derived from a nucleotide sequence ranging from nucleotide 417 to nucleotide 435 of the OIP5 gene of SEQ ID NO: 2 and contains two overhang nucleotides of TT. These sequences may have superior gene expression inhibition (RNAi effects) on the anti-sense strand complementary to the sequence of SEQ ID NO: 5 or 6.

Further, siRNA of the present invention may be chemically modified in order to prevent rapid degradation which may occur by the action of *in vivo* nucleases. siRNA has a double-stranded structure, and therefore is relatively stable as compared to single-stranded ribonucleic acid or antisense oligonucleotide molecules. However, siRNA is readily susceptible to *in vivo* nuclease degradation, so it is possible to lower a degradation rate of siRNA via chemical modification thereof. Chemical modification of siRNA to secure that siRNA is chemically stable and resistant against rapid degradation may be carried out by any conventional method well known to those skilled in the art. The most conventional method used in chemical modification of siRNA is boranophosphate or phosphorothioate modification. These materials form stable nucleoside-nucleoside association of siRNA molecules, thereby conferring resistance to nucleic acid degradation. Even though it is resistant to nucleic acid degradation, the boranophosphate-modified ribonucleic acid is not synthesized by a chemical reaction and is synthesized only by incorporation of boranophosphate into ribonucleic acid via *in vitro* transcription. The boranophosphate modification is a relatively easy method, but has a disadvantage associated with difficulty of site-directed modification at a certain position of target molecules or compounds. On the other hand, the phosphorothioate modification is advantageous for introduction of sulfur elements into a desired site, but excessive phosphothioation may result in problems associated with decreased efficiency, toxicity, and formation of non-specific RNA-induced silencing complex (RISC). For these reasons suffered by the above-mentioned two modification methods, it may be preferred to employ a method which is designed to provide nuclease resistance via introduction of chemical modification only at a termination position of ribonucleic acid (a region beyond 3'-terminal). Further,

chemical modification of a ribose ring is also known to enhance the nuclease resistance of nucleic acid. Particularly, modification at 2'-postion of the ribose ring leads to stabilization of siRNA molecules. However, the stability of siRNA molecules increases only with correct introduction of a methyl group into the above-specified position of the ribose ring. Further, introduction of excessively large numbers of methyl groups may result in loss of ribonucleic acid-mediated interference. Chemical modifications may also be made to improve the *in vivo* pharmacokinetic retention time and efficiency (Mark et al., Molecular Therapy, 13:644-670, 2006).

In addition to chemical modifications, a safe and efficient delivery system is still required to increase intracellular delivery efficiency of siRNA molecules. For this purpose, siRNA of the present invention may be incorporated in the form of a complex with a nucleic acid delivery system into a pharmaceutical composition for treating cancer.

The nucleic acid delivery system for intracellular delivery of nucleic acid materials may be broadly divided into a viral vector system and a non-viral vector system. The most conventionally and widely used system is a viral vector system because it provides a high delivery efficiency and a long retention time *in vivo*. Among a variety of viral vectors, a retroviral vector, an adenoviral vector, an adeno-associated viral vector, and the like are widely employed. These viral vector systems are efficient in intracellular delivery of ribonucleic acid, but suffer from various problems associated with safety concerns, such as difficulty of recombination of the ribonucleic acid into a virus having *in vivo* activity, elicitation of immune response, undesirable random insertion of the ribonucleic acid into host chromosomes, and the like. On the other hand, the non-viral vector system exhibits various advantages, such as low toxicity and immune response, feasibility of repeated administration, convenient formation of a complex of the delivery system with ribonucleic acid, and easy industrial-scale production, as compared to the viral vector system. Further, organ/cell-targeted nucleic acid delivery to affected cells or tissue lesions can be carried out by conjugation of a specific ligand with the non-viral vector. Examples of the non-viral vector that can be used in the present invention may include various formulations such as liposomes, cationic polymers, micelles, emulsions, nanoparticles, and the like. The nucleic acid delivery system can significantly enhance delivery efficiency of the desired nucleic acid into animal cells and can deliver nucleic acid into any type of animal cells, depending upon the desired uses and applications of the nucleic acids.

siRNA of the present invention is a short double-stranded RNA (dsRNA) consisting of 19-30 bp nucleotides. Intracellular incorporation of such siRNA exhibits inhibitory effects on expression of the MIG12 gene or OIP5 gene without causing non-specific inhibition, specifically showing anticancer activity to kill cancer cells. Further, siRNA of the present invention can be used for functional study of related genes.

Further, the gene inhibitor may be shRNA of an MIG12 or OIP5 gene.

The aforementioned shRNA (small hairpin RNA) is a sequence of RNA that makes a tight hairpin turn that can be used to silence gene expression via RNA interference. shRNA, which consists of siRNA sense sequence and 3-10 bases of linker-antisense siRNA sequence, uses a plasmid vector introduced into cells and utilizes the U6 promoter to ensure that the shRNA is always expressed. The loop sequence of the resulting shRNA is cleaved by Dicer, an RNase III enzyme, to generate mature siRNA which will exhibit RNAi effects. shRNAs can also be cloned into lentivirus or adenovirus and used for *in vivo* animal study. shRNA exhibits RNAi effects for a relatively long time, as compared to siRNA.

Similar to MIG12 siRNA, the MIG12 shRNA may also comprise a nucleotide sequence corresponding to an mRNA sequence derived from a nucleotide sequence of 19 to 30 contiguous nucleotides, among one or more sequences selected from SEQ ID NOS: 14, 15, 16, 17 and 18 of the MIG12 gene.

The MIG12 shRNA may comprise an RNA nucleotide sequence of SEQ ID NO: 4 and an antisense RNA sequence which is linked via a 3-10 bp loop to and is reverse complementary to the sequence of SEQ ID NO: 4. The RNA nucleotide sequence of SEQ ID NO: 4 is a 19-bp nucleotide sequence which corresponds to an mRNA sequence derived from a nucleotide sequence ranging from nucleotide 1123 to nucleotide 1141 of the MIG12 gene of SEQ ID NO: 1 and may have superior gene expression inhibition (RNAi effects) on an anti-sense strand reverse complementary to the sequence of SEQ ID NO: 4.

As used herein, the term "RNA nucleotide sequence" refers to an RNA nucleotide sequence part with exception of a short sequence consisting of two bases (TT) from the nucleotide sequence of SEQ ID NO: 4.

A top strand for construction of an expression vector to express the MIG12 shRNA may have a sequence as set forth in SEQ ID NO: 27.

Similar to OIP5 siRNA, the OIP5 shRNA may comprise a nucleotide sequence corresponding to an mRNA sequence derived from a nucleotide sequence of 19 to 30

contiguous nucleotides, among one or more sequences selected from SEQ ID NOS: 19, 20, 21, 22, 23, 24, 25, and 26.

The OIP5 shRNA may comprise an RNA nucleotide sequence of SEQ ID NO: 5 and an antisense RNA sequence which is linked via a 3-10 bp loop to and is reverse complementary to the sequence of SEQ ID NO: 5. The RNA nucleotide sequence of SEQ ID NO: 5 is a 19-bp nucleotide sequence which corresponds to an mRNA sequence derived from a nucleotide sequence ranging from nucleotide 426 to nucleotide 444 of the OIP5 gene of SEQ ID NO: 2 and may have superior gene expression inhibition (RNAi effects) on an anti-sense strand reverse complementary to the sequence of SEQ ID NO: 5.

As used herein, the term "RNA nucleotide sequence" refers to an RNA nucleotide sequence part with exception of a short sequence consisting of two bases (TT) from the nucleotide sequence of SEQ ID NO: 5.

A top strand for construction of an expression vector to express the OIP5 shRNA may have a sequence as set forth in SEQ ID NO: 28.

Expression constructs/vectors comprising shRNA (small hairpin RNA) having the siRNA nucleotide sequence to enhance the intracellular retention and transfection effects of siRNA are known to those skilled in the art. For example, US Patent Application Nos. 2004/106567 A1 and 2004/0086884 disclose a variety of delivery mechanisms, such as viral and non-viral vectors comprising shRNA, liposome delivery systems, *in vivo* injection systems of plasmid DNAs, artificial viral envelope and polylysine conjugates, as well as plenty of information about the expression constructs/vectors.

siRNA has a promising potential as a very strong inhibitor drug on *in vivo* expression of a certain gene, in view of the long retention time upon cell culture and under *in vivo* conditions, *in vivo* transfection capacity and resistance against serum degradation (Bertrand et al., 2002).

siRNA even at a relatively low concentration can achieve inhibitory effects on gene expression comparable to or higher than those obtained by the antisense oligonucleotide and therefore is proposed as a substitute for the antisense oligonucleotide (Thompson, 2002). siRNAs are widely utilized for inhibition of gene expression in animal models of disease. Those skilled in the art will appreciate that it is possible to synthesize and modify the antisense oligonucleotide and siRNA as desired, using any conventional method known in the art (see Andreas Henschel, Frank Buchholz1 and Bianca Habermann (2004) DEQOR: a web-based tool for the design and quality control of siRNAs. Nucleic Acids Research 32 (Web Server

Issue):W113-W120). Further, it will be apparent to those skilled in the art that there are a variety of regulatory sequences (for example, constitutive or inducible promoters, tissue-specific promoters or functional fragments thereof, etc.) which are useful for the antisense oligonucleotide, siRNA, or shRNA expression construct/vector.

The antisense oligonucleotide, siRNA, or shRNA of the present invention, which is used for treatment of cancer, may be administered in the form of a composition further comprising pharmaceutically acceptable carrier(s). Examples of suitable pharmaceutically acceptable carriers may include water, saline, PBS (phosphate buffered saline), dextrin, glycerol, and ethanol. These materials may be used alone or in any combination thereof. Additionally, the composition of the present invention may be appropriately formulated by a conventional method known in the art, such that it is possible to achieve fast, sustained or delayed release of active ingredients after administration of the composition to a subject including mammals or humans.

The gene inhibitor may be antisense oligonucleotides, siRNAs, or shRNAs, as well as any material capable of inhibiting expression of a gene of interest, such as low-molecular weight compounds, natural products, bioactive proteins, *in vivo* proteins, novel proteins, and synthetic and naturally-occurring chemicals. Therefore, it is possible to use compounds known as the inhibitor of the aforementioned gene in the art, and materials identified by a screening method using the same gene.

Further, the present invention provides a composition for treating cancer, comprising an inhibitor against the protein expressed from an MIG12 or OIP5 gene (hereinafter, referred to as "protein inhibitor").

Suppression of the gene of the present invention results in subsequent inhibition of the corresponding protein expression to thereby inhibit development and/or progress of cancer. Consequently, it is possible to inhibit development of cancer via suppression of expression of the corresponding protein from the MIG12 or OIP5 gene.

Therefore, the present invention provides a use of a protein inhibitor for treating cancer, and a method for treating cancer, comprising administering a therapeutically effective amount of the same protein inhibitor to a subject in need thereof. As used herein, term "treating cancer" or "treatment of cancer" or "cancer treatment" encompasses prevention and inhibition of cancer.

The protein inhibitor may be an antibody directed against the protein expressed from the

gene of the present invention. Monoclonal antibodies directed against the aforesaid protein may be produced by any conventional method for production of monoclonal antibodies known in the art or otherwise may be commercially available. Alternatively, instead of monoclonal antibodies, it may also be possible to use polyclonal antibodies which recognize the aforesaid protein. These polyclonal antibodies may be obtained by any conventional method for production of antiserum known in the art.

For purpose of desired administration, the composition of the present invention may be formulated into a variety of dosage forms by further inclusion of one or more pharmaceutically acceptable carriers in combination with the above-mentioned active ingredient. When the inhibitor against the protein of the present invention is an antibody, the pharmaceutically acceptable carrier may be comprised of a minimum amount of an additive to improve a shelf life or effectiveness of a binding protein, such as a wetting agent, emulsifier, preservative or buffer.

Further, the anticancer composition of the present invention may be used in combination with one or more anticancer drugs. The anticancer composition may further comprise chemotherapeutic agents well-known to those skilled in the art, for example, alkylating agents such as cyclophosphamide, aziridine, alkyl alkone sulfonate, nitrosourea, dacarbazine, carboplatin, cisplatin, and the like, antibiotics such as mitomycin C, anthracycline, doxorubicin (Adriamycin), and the like, antimetabolic agents such as methotrexate, 5-fluorouracil (5-FU) and cytarabine, plant-derived agents such as Vinca alkaloids, and hormones.

The composition of the present invention may further comprise pharmaceutically and physiologically acceptable additives, in addition to the active ingredient. Examples of such additives may include excipients, disintegrating agents, sweeteners, binders, coating agents, blowing agents, lubricants, glidants, solubilizers, etc.

The composition of the present invention may further comprise one or more pharmaceutically acceptable carriers besides the active ingredient, to be formulated to a pharmaceutical composition appropriately.

For formulation of the composition into a liquid preparation, a pharmaceutically acceptable carrier which is sterile and biocompatible may be used such as saline, sterile water, Ringer's solution, buffered physiological saline, albumin infusion solution, dextrose solution, maltodextrin solution, glycerol, and ethanol. These materials may be used alone or in any

combination thereof. If necessary, other conventional additives may be added such as antioxidants, buffers, bacteriostatic agents, and the like. Further, diluents, dispersants, surfactants, binders and lubricants may be additionally added to the composition to prepare injectable formulations such as aqueous solutions, suspensions, and emulsions, or oral formulations such as pills, capsules, granules, and tablets. Furthermore, the composition may be preferably formulated into a desired dosage form, depending upon diseases to be treated and ingredients, using any appropriate method known in the art, as disclosed in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA.

Dosage forms of the composition of the present invention may include granules, powders, coated tablets, tablets, capsules, suppositories, syrups, juice, suspensions, emulsions, drops or injectable liquid formulations and sustained-release formulations of active ingredient(s).

The pharmaceutical composition can be administered via a conventional route, for example intravenously, intraarterially, intraperitoneally, intramuscularly, intrathoracically, percutaneously, intranasally, locally, rectally, orally, intraocularly, intradermally, or by inhalation.

As used herein, the term "therapeutically effective amount" refers to an amount of an active ingredient that is required to exert anticancer effects. As will be apparent to those skilled in the art, the effective dose of the active ingredient in accordance with the present invention may vary depending upon various factors such as kinds of disease, severity of disease, kinds and contents of active ingredients and other components contained in the composition, kinds of dosage forms, age, weight, health, sex and dietary habits of patients, administration times and routes, release rates of the composition, treatment duration, and co-administered drugs. For adults, when the gene or protein inhibitor is administrated one or more times a day, the dosage is preferably in a range of 0.01 ng/kg to 10 mg/kg for siRNA, 0.01 ng/kg to 10 mg/kg for shRNA, 0.01 ng/kg to 10 mg/kg for antisense oligonucleotide to mRNA of the aforesaid gene, 0.1 ng/kg to 10 mg/kg for a compound, 0.1 ng/kg to 10 mg/kg for a monoclonal antibody directed against the aforesaid protein, 0.1 ng/kg to 10 mg/kg for a low-molecular weight compound, 0.1 ng/kg to 10 mg/kg for a natural product, and 0.1 ng/kg to 10 mg/kg for bioactive proteins, respectively.

Further, the present invention provides a kit for cancer diagnosis, comprising at least one of an MIG12 gene, an OIP5 gene, and a protein expressed from the MIG12 or OIP5 gene.

In addition to the diagnostic composition of the present invention, the cancer diagnosis kit may further comprise a material for application of DNA chips, protein chips, etc., which are used to examine the reaction of the composition with a test sample.

In one embodiment of the present invention, the MIG12 gene may have a nucleotide sequence of SEQ ID NO: 1 or its variant sequence with deletion, substitution or insertion of one or more bases.

In another embodiment of the present invention, the OIP5 gene may have a nucleotide sequence of SEQ ID NO: 2 or its variant sequence with deletion, substitution or insertion of one or more bases.

Further, it will be apparent to those skilled in the art that the aforementioned sequences of MIG12 gene, OIP5 gene, and siRNA or shRNA of the MIG12 and OIP5 genes are provided for illustration only and are not necessarily representative of the sequences for the purpose of limiting the invention. Sequences having substantial sequence identity or homology to the above-mentioned sequences also fall within the scope of the present invention. As used herein, the term "substantial sequence identity" or "substantial sequence homology" is used to indicate that a sequence of interest exhibits substantial structural or functional equivalence with another sequence. Differences may be due to inherent variations in codon usage among different species, for example. Structural differences are considered *de minimis* if there is a significant amount of sequence overlap or similarity between two or more different sequences or if the different sequences exhibit similar physical characteristics even if the sequences differ in length or structure.

The matters relating to genetic engineering technologies will be more explicit from the written text described in Sambrook et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N. Y. (2001) and Frederick M. Ausubel et al., Current Protocols In Molecular Biology, volume 1, 2, 3, John Wiley & Sons, Inc. (1994).

ADVANTAGEOUS EFFECTS

Upon overexpression of MIG12 and OIP5 genes of the present invention in model cells, these genes are implicated in cell cycle regulation and growth factors, are expressed at high levels in cancer cell lines, and increase a cell growth rate. Therefore, inhibition of expression of these genes leads to suppression of cancer cell growth. For these reasons, MIG12 and OIP5 genes can be used as a target gene for diagnosis or cancer therapy of various cancers. As a

result, it is expected that the present invention will be used for research and development of personalized anticancer drugs having low adverse side effects, such as cancer target gene-specific therapeutic agents, for example biopharmaceuticals such as siRNA/shRNA drugs, antibody drugs and protein drugs, and cancer-targeted drugs such as low-molecular weight synthetic drugs and natural products which target MIG12 and OIP5 proteins. Further, the present invention will contribute to the development of a source technology which forms the basis of study of new cancer-related mechanisms.

DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates a schematic diagram of cloning a human gene from a plasmid (Entry clone) with insertion of the human gene into a destination vector, *S. pombe*;

FIG. 2 illustrates phenotypic changes observable upon overexpression of a human gene MIG12 in *S. pombe*. FIG. 2A: Cell growth inhibition, FIG. 2B: Morphological changes of cells, FIG. 2C: Intracellular changes of DNA levels, and FIG. 2D: Confirmation of human gene expression;

FIG. 3 illustrates phenotypic changes observable upon overexpression of a human gene OIP5 in *S. pombe*. FIG. 3A: Cell growth inhibition, FIG. 3B: Morphological changes of cells, FIG. 3C: Intracellular changes of DNA levels, and FIG. 3D: Confirmation of human gene expression;

FIG. 4 illustrates RT-PCR measurement of mRNA levels for MIG12 and OIP5 genes in liver and stomach cancer cell lines;

FIG. 5 illustrates comparison of expression levels of an OIP5 gene in cancer tissues and normal tissues of colorectal cancer patients;

FIG. 6 illustrates comparison of expression levels of an OIP5 mRNA in cancer tissues of colorectal cancer patients, using RT-PCR;

FIG. 7 illustrates a graph for the results of proliferation assay after expression of MIG12 and OIP5 genes in animal cells (NIH3T3), as experiments for prediction of the relationship between genes and carcinogenesis;

FIG. 8 illustrates energy profiles of MIG12 and OIP5 genes;

FIG. 9 illustrates a graph showing a degree of cell growth inhibition in various stomach, liver, lung and colorectal cancer cell lines, as the results of siRNA experiments to confirm the potentiality of MIG12 and OIP5 genes as a cancer target gene;

FIG. 10 illustrates cell photographs showing cell growth inhibition in a variety of stomach and liver cancer cell lines, as the results of siRNA experiments to confirm the potentiality of MIG12 and OIP5 genes as a cancer target gene;

FIG. 11 illustrates FACS analysis results for measurement of cell apoptosis due to expression inhibition of the corresponding gene after siRNA experiments;

FIG. 12 illustrates changes in expression levels of apoptosis-related genes through RNAi experiments using siMIG12 or siOIP5;

FIG. 13 illustrates shRNA design (A) and experimental results (B);

FIG. 14 illustrates a graph showing cell growth inhibition and inhibition degree after overexpression of MIG12 and OIP5 genes in fission yeast *S. pombe* as a model cell; and

FIG. 15 illustrates investigation results for the development feasibility of an HTS screening system which is intended to target MIG12 and OIP5 genes, using a 96-well plate.

MODE FOR INVENTION

The present invention will be described in more detail with reference to the following Examples.

These and other objects, advantages and features of the present invention will become apparent from the detailed embodiments given below which are made in conjunction with the following Examples. The present invention may be embodied in different forms and should not be misconstrued as being limited to the embodiments set forth herein, and those skilled in the art will appreciate that various modifications, additions and substitutions are possible without departing from the scope and spirit of the invention as disclosed in the accompanying claims. Therefore, it should be understood that the embodiments disclosed herein are provided only for illustrating the present invention and should not be construed as limiting the scope and spirit of the present invention.

Example 1: Effects of MIG12 and OIP5 overexpression on cells

Fission yeast *S. pombe* expressed phenotypes of overexpression and/or deletion of the genes involved in cell cycle and signal transduction since they caused deregulation in signal transduction resulting in growth inhibition and morphological changes. The phenotypic analysis for growth inhibition and morphological changes was performed by overexpression of MIG12 and OIP5 in *S. pombe*.

1-1. Construction of fission yeast expressiong MIG12 and OIP5

Gateway Cloning Technology was chosen for the rapid cloning of human cDNA into the *S. pombe* expression vector. For the overexpression of human cDNA in *S. pombe*, the destination vector, pDES173 (KCTC 10757BP), was constructed by inserting a conversion cassette into pSLF173, an *S. pombe* expression vector containing an nmt1 inducible promoter with an HA tag at the 5'-end of the cloning site (http://www.atcc.org/ common/products/ vectors.cfm). The gene under the nmt1 promoter is expressed in the absence of thiamine and subject to transcriptional repression in the presence of thiamine in *S. pombe*. The genes of MIG12 and OIP5 human cDNAs in the pDONR201 donor vector (21C Frontier Human cDNA Bank (http://kugi.kribb.re.kr:8080/genbank/)) were successfully transferred into the *S. pombe* pDES173 by the clonase reaction (FIG. 1).

Human genes in the pDES173 expression vector were introduced into *S. pombe* haploid strain ED665 (h-, ade6-M210 leu1-32 ura4-D18) (Fanters, University of Glasgow, UK) by the method of lithium acetate. ED665 was cultured in Edinburgh Minimal Medium (EMM, Edinburgh Minimal Media) supplemented with 75 μg/mL of adenine, 75 μg/mL of uracil, and/or 250 μg/mL of leucine.

1-2. Growth inhibition by overexpression of MIG12 or OIP5

To overexpress human genes cloned into pDES173 under the nmt1 promoter, cells were grown in minimal media containing adenine and leucine in the presence (EMM+AL+Thia) and absence (EMM+AL) of thiamine, a repressor of nmt1 promoter. The cells containing the expression vector only were used as a control. Growth defects were examined by plating cells containing human cDNA in a plate with and without thiamine, incubating them for 3 days and comparing the numbers and sizes of colonies in two plates. The growth inhibition of colonies overexpressing MIG12 and OIP5 indicates overexpression of these genes induces cellular function of human genes relevant to cancer, such as growth inhibition and morphological changes of *S. pombe* (FIGS. 2A and 3A).

1-3. Phenotypic changes by expression of MIG12 and OIP5

The phenotypic analysis was performed based on the hypothesis that the overexpression of genes involved in carcinogenesis induced growth inhibition and morphological changes. To

observe phenotypes of cells overexpressing MIG12 and OIP5, exponentially growing cells grown in the presence of thiamine were washed 3 times, transferred to fresh media with or without thiamine, and grown for 8 hours. After reinoculating *S. pombe* containing MIG12 or OIP5 genes in fresh media without thiamine and growing for 9 to 16 hours in the absence of thiamine, overexpression phenotypes were analyzed for growth inhibition and morphological changes. The expression of human genes MIG12 and OIP5 induced abnormal multi-septa formation of *S. pombe*, mostly caused by defects in the mitotic checkpoint and cytokinesis (FIGS. 2B and 3B).

1-4. Changes in regulation of cell cycle

To detect septa and nuclei, cells were stained with 10 $\mu\ell$ of Calcofluor (200 μg/mL) and DNA-specific fluorochrome (DAPI, 4, 6-diamidino-2-phenylindole dihydrochloride, 20 μg/mL) for 15 min. After washing, cells were analyzed by fluorescent microscopy (Zeiss). To analyze the DNA contents in single cells by flow cytometry, *S. pombe* cells were fixed in 80% ethanol for 16 to 24 hours and treated with propidium iodide (50 mg/mL) and RNase (200 mg/mL). The amount of DNA was analyzed by FACScan^{TM} (Becton Dickinson, Franklin Lakes, NJ., USA) in 1 x 10^4 cells. When the human genes MIG12 and OIP5 were overexpressed in *S. pombe* cells, FACS analysis showed the cell population with multisepta with each individual nucleus increased (FIGS. 2C and 3C) indicating that they may have defects in mitotic control and/or cytokinesis.

1-5. Expression of human gene in *S. pombe*

Here, human cDNAs were cloned into pDES173 under the nmt1 promoter to be expressed as HA-tagged proteins at the N-terminal region in the absence of thiamine. The overexpression of the human genes was clearly confirmed by Western blot analysis using an HA antibody (FIGS. 2D and 3D). The phenotypic analysis indicated that the overexpression of MIG12 and OIP5 genes induced growth inhibition and morphological changes of *S. pombe*. Therefore, MIG12 and OIP5 genes can be used as potential diagnostic markers and therapeutic targets of cancer.

Example 2: Expression of MIG12 and OIP5 in cancer cell lines

The simple validation of a gene for relevance to cancer is to examine expression level of

mRNA of the gene in cancer tissues or cancer cell lines. Comparison of mRNA levels between cancer and normal tissues provides clues that a gene of interest is involved in progression and regulation of carcinogenesis. Here, oncogenic validation was performed by examining the mRNA levels of the genes in various cancer cell lines.

The mRNA levels of MIG12 and OIP5 genes in the various cancer cell lines can be measured by RT-PCR. The stomach cancer cell lines used are Sun620, Sun216, Sun484, Sun601 and Sun638 (Korean Cell Line Bank, Seoul National University, Korea). The liver cancer cell lines used are Huh7, SH-J1, and Ck-1a (by courtesy of Dr. Dae Ghon Kim, Chonnam National University, Korea), Chang (ATCC #CCL-62), Snu790 and Snu354 (Korean Cell Line Bank, Seoul National University, Korea). And as a normal cell control, IMR90 (human fetal lung fibroblasts, ATCC #CCL-186) was used.

Total RNA was purified using the Qiagen RNeasy Mini kit according to the instructions of the manufacturer. RT-PCR was performed with an RNA template (1 μg/reaction) and specific primers (Table 1) by using the One-step RT-PCR PreMix kit (iNtRON Biotechnology, Inc., Korea). PCR was carried out according to the instructions of the manufacturer: one cycle (45℃ for 30 min, 94℃ for 5 min), 30 cycles (94℃ for 30 sec, 52℃ for 30 sec, 72℃ for 1 min), and one final extension cycle (72℃ for 5 min).

Table 1

	N-terminus	C-terminus
MIG12	SEQ ID NO: 10 5'-TGATGCAAATCTGCGACACC-3'	SEQ ID NO: 11 5'-CAGTGGCCCCAATTGCCGAA-3'
OIP5	SEQ ID NO: 12 5'-GGCTGGGGGCTGAGGAGCCA-3'	SEQ ID NO: 13 5'-CACTTCACTCAGAATCTTCA-3'

The genes of MIG12 and OIP5 were highly expressed in most liver and stomach cancer cell lines compared to those in a fibroblast cell line, IMR90. OIP5 exhibited much higher expressions in stomach cancer cell lines than liver cancer cell lines (FIG. 4). These results indicate that the genes expressing at a high level in most of the cell lines may be associated with a common mechanism of liver and stomach cancers.

Example 3

3-1: Microarray analysis of OIP5 in colorectal cancer

The colorectal cancer tissues were obtained from 66 patients of stage II and stage III at Samsung Medical Center (Seoul, Korea). Total RNAs were extracted from cancer and normal tissues, using the Qiagen kit as described in the manual and used for microarray analysis of 48K Illumina chips. The quality of total RNA samples was assessed using an Experion (Bio-Rad Laboratories). RNA samples were labeled according to the chip manufacturers recommended protocols. 0.5 μg of total RNA from each sample was labeled by using the Illumina TotalPrep RNA Amplification Kit (Ambion) in a process of cDNA synthesis and *in vitro* transcription. Single-stranded RNA (cRNA) was generated and labeled by incorporating biotin-NTP (Ambion). A total of 1.5 μg of biotin-labeled cRNA was hybridized at 58℃ for 16 hours to Illumina's Sentrix Human-6 v2 Expression BeadChip (Illumina). The hybridized biotinylated cRNA was detected with streptavidin-Cy3 and quantitated using Illumina's BeadArray Reader Scanner (Illumina) according to manufacturer's instructions.

Microarray data of a total of 66 tumors and nine contiguous normal mucosa samples were processed and analyzed by Illumina BeadStudio version 3.0 software (Illumina). Data normalization was performed using quantile normalization, and fold changes and statistical significance were determined using the Avadis Prophetic version 3.3 (Strand Genomics). Scanned data was normalized by a quantile-quantile normalization method and log-transformed by base two. T-test was used to infer the significance of gene expression difference between tumor and normal tissues. Java Treeview (http://jtreeview.sourceforge.net/) was used to visualize the pattern of gene expression in tumors compared to normal tissues.

The up-regulating genes and down-regulating genes were shown in red and green respectively. GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) and ACTB (Homo sapiens actin, beta) were used as controls. The expression profile of OIP5 in colorectal cancer tissues was examined and its expression level in tumor was compared with that of normal tissue (FIG. 5). OIP5 was significantly up-regulated about 3.7-fold in colon tumors. OIP5 was up-regulated over two fold in 58 out of 66 tumors. When compared with the expression level of OIP5 in Stage II and III colorectal cancer, no difference was noticed. Also, the expression levels of OIP5 among tumors of reoccurred and non-reoccurred patients were not very different.

3-2. Expression analysis of OIP5 in colorectal cancer tissues by RT-PCR

The mRNA levels of OIP5 in 10 cancer tissues randomly chosen among 66 colorectal cancer tissues were examined by reverse transcription-polymerase chain reaction (RT-PCR) (FIG. 6). The OIP5 mRNA levels were higher in 80% (8/10) colorectal cancer tissues compared to normal tissue. This result was consistent with that of microarray analysis. The fact that OIP5 expressed at a high level in most stomach and liver cancer cell lines tested suggests that OIP5 may be associated with a common mechanism of carcinogenesis of various cancers and can be used in diagnostics and therapeutics of cancer and screening of anticancer drugs.

Example 4: Increase of proliferation rate of cells by overexpression of OIP5 and MIG12

Oncogenic genes can increase the proliferation rate of cells. The proliferation rates of NIH3T3 cells transfected with MIG12 and OIP5 genes for 72 hours were examined as an indication of their oncogenic properties (FIG. 4).

NIH3T3 cells grown in 60 to 70% confluency in a 60 mm dish were transfected with 7 μg of DNA and 15 $\mu \ell$ of Lipofectamine™ 2000 reagent (Invitrogen, Carlsbad, CA, U.S.A) for 24 hours at 37℃ in 5% CO_2. Cells were harvested and counted using a hemacytometer. Approximately 5 x 10^3 cells were plated and cell numbers were counted every 12 hours for 3 days.

The transient expression of the MIG12 and OIP5 genes resulted in a two-fold increase in the proliferation rate (FIG. 7). The increase of the proliferation rate by transient expression of a gene was coincident with foci formation and colony-forming assay.

Example 5: Design and Synthesis of siRNA

RNA interference, in which a sequence-specific gene-silencing process is mediated by small interfering RNA (siRNA), is an efficient method of identifying targets for gene therapy in a variety of diseases. Since OIP5 is highly expressed in cancer tissues, which is related to carcinogenesis, a decrease of OIP5 level may induce growth inhibition of cancer cells. To validate MIG12 and OIP5 as a target in cancer therapeutics, RNAi experiments using siRNA in various cancer cells including colorectal cancer, liver cancer, stomach cancer, colorectal cancer and lung cancer were organized.

Two different siRNAs for OIP5 were designed based on the program (ref) 5′-CAU UGAAGGUUCACUCAAATT-3′ siOIP5-2, 5′-CCUAGUUGGCAUUGAAGGUTT-3′ GFP

control, 5′-CACACCACATCTTTCCGTCAC-3′. RNA oligonucleotides were custom-synthesized by Samchully Pharm. Co., Ltd. (Seoul, Korea) with an overhang of two thymidine residues (dTdT) at the 3′ end. The RNA oligonucleotides were dissolved in Tris-EDTA (10 mM Tris-HCl, pH 8.0; and 1 mM EDTA) as 200 μM solutions and were stored at –20℃. Double-stranded siRNA molecules were generated by mixing the corresponding pair of sense and antisense RNA oligonucleotides in annealing buffer (30 mM HEPES-KOH, pH 7.9; 100 mM potassium acetate; and 2 mM magnesium acetate) at 20℃ and then by incubating the reaction mixture at 95℃ for 2 min, followed by gradually cooling to room temperature. The siRNAs were then aliquoted and stored at –20℃.

To find out most favorable siRNA target regions for MIG12 and OIP5, we calculated a relative binding energy pattern of candidate siRNAs so that cellular RISC (RNA-induced silencing complex) may bind and unwind siRNA double helix with ease. As shown in FIG. 8, the target regions were determined by the relative binding energy pattern search algorithm and siRNAs in the regions were designed and applied to our experiments.

The relative binding energy pattern search algorithm determined the MIG12 (NM_011242) siRNA regions as those comprising SEQ ID NOS: 14, 15, 16, 17, and 18 extending contiguous 19-30 sequences and their complementaries. Sequences of SEQ ID NOS: 14, 15, 16, 17, and 18 are parts of MIG12 (NM_011242) sequences having both sense and complementary antisense strands in the range of nucleotides 845-950, 970-1030, 1080-1160, 1220-1290, and 1320-1400, respectively. In other words, the regions for MIG12 candidate siRNAs include sequences of SEQ ID NOS: 14, 15, 16, 17, and 18 and the sequences comprising 19-30 contiguous nucleotide sequences and their complementaries.

The relative binding energy pattern search algorithm determined the OIP5 (NM_021242) siRNA regions as those comprising SEQ ID NOS: 19, 20, 21, 22, 23, 24, 25 and 26 extending contiguous 19-30 sequences and their complementaries. Sequences of SEQ ID NOS: 19, 20, 21, 22, 23, 24, 25 and 26 are parts of OIP5 (NM_021242) sequences having both sense and complementary antisense strands in the range of nucleotides 1-60, 120-180, 380-460, 518-580, 720-780, 850-890, 920-980 and 1040-1100, respectively. In other words, the regions for OIP5 candidate siRNAs include sequences of SEQ ID NOS: 19, 20, 21, 22, 23, 24, 25 and 26 and the sequences comprising 19-30 contiguous nucleotide sequences and their complementaries.

Utilizing the relative binding energy profile described above, siMIG12 was designed to have a sequence of SEQ ID NO: 3 or 4 and siOIP5 was designed to have a sequence of SEQ

ID NO: 5 or 6. Sequences of SEQ ID NO: 3 and 4 have 1331st to 1349th nucleotides and 1123rd to 1141st nucleotides, respectively, of MIG12 mRNA with two overhang nucleotides of TT. Sequences of SEQ ID NO: 5 and 6 have 426th to 444th nucleotides and 417th to 435th nucleotides, respectively, of OIP5 mRNA with two overhang nucleotides of TT.

The cancer-related gene, named RABGTTA (Rab geranylgeranyl transferase alpha-subunit gene), was targeted using siRNAs that have sequences of SEQ ID NOS: 7 and 8. As a negative control, GFP (Green Fluorescent Protein) which is not involved in cellular functions was targeted using the siRNA with a sequence of SEQ ID NO: 9.

All siRNAs for the targeted genes were introduced into cells by synthesizing both sense and anti-sense strands followed by combining two in order to have double helix ribonucleotide bond.

The siRNAs designed were synthesized by Samchully Pharm. Co., Ltd. (Seoul, Korea).

Example 6: Growth inhibition of cancer cells by RNAi of MIG12 or OIP5 genes

To determine whether RNAi of MIG12 or OIP5 genes can affect growth inhibition of cancer cells and validate them as potential targets in cancer therapeutics, various cancer cells including colorectal cancer, liver cancer, stomach cancer, and lung cancer were treated with siOIP5-1 and siOIP5-2 and growth inhibition of cells was determined using SRB assay, flow cytometry analysis and mRNA measurements. The knockdown of OIP5 was confirmed by RT-PCR and growth inhibition of cells was observed under a microscope.

6-1. Growth inhibition of cancer cells by RNAi of MIG12 or OIP5 genes

The cancer cell lines used are Sun638 (stomach cancer, Korean Cell Line Bank, Seoul National University, Korea), HCC (liver cancer, Korean Cell Line Bank, Seoul National University, Korea), Huh7 (liver cancer, by courtesy of Dr. Dae Ghon Kim, Chonnam National University, Korea), Ck-1a (by courtesy of Dr. Dae Ghon Kim, Chonnam National University, Korea), A549 (lung cancer, Korean Cell Line Bank, Seoul National University, Korea), colo205 (colorectal cancer, Korean Cell Line Bank, Seoul National University, Korea), sw620 (colorectal cancer, Korean Cell Line Bank, Seoul National University, Korea), DL-D1 (colorectal cancer, Korean Cell Line Bank, Seoul National University, Korea), and KM120 (colorectal cancer, Korean Cell Line Bank, Seoul National University, Korea).

Twenty-four hours before siRNA transfection, 1 x 10^5 cells were seeded in 6-well plates

in RPMI-minimal essential medium (OptiMEM; Invitrogen, Grand Island, NY, USA) containing 10% Fetal Bovine Serum (FBS) with no antibiotics. Cells were seeded per 6-well plate to give 30% confluency at the time of the transfection. The siMIG12 (SEQ ID NOS: 3 and 4), siOIP5 (SEQ ID NOS: 5 and 6), siRABGGTA (SEQ ID NOS: 7 and 8), and siGFP (SEQ ID NO: 9) were transfected at a final concentration of 50 nM using HiPerFect (Gibco BRL, USA) according to the manufacturer's recommendations. The cells received siGFP sequence were used as a control. After incubating the cells transfected with siRNA for 72 hours, SRB staining and microscopic observations were carried out (FIGS. 9 and 10). The knockdown of OIP5 was confirmed by RT-PCR and growth inhibition of cells was observed under a microscope.

When various cancer cells were treated with siMIG12 and siOIP5-1, significant inhibition of cell growth occurred compared with siGFP as shown in FIGS. 9 and 10. siMIG12 showed strong effects on Hur7, SNU387, SNU638, colo205 and KM12C, while siOIP5 showed strong effects on SNU387, SNU638, A549 and KM12C, thus suggesting cancer cell specific-RNAi effects of siMIG12 and siOIP5. Introduction of both siMIG12 and siOIP5 into SNU638 (stomach cancer) caused growth inhibition (FIG. 10) and induced apoptosis as shown in FIG. 11. However, CK-1a (liver cancer) was not affected by either siMIG12 or siOIP5. The knockdown of RABGTTA, a subunit of a Rab geranylgeranyl transferase, did not cause growth inhibition of cancer cells.

6-2. Induction of apoptosis by siMIG12 or siOIP5

Since knockdown of MIG12 or OIP5 caused growth inhibition in various cancer cells, whether or not knockdown of MIG12 or OIP5 derived induction of apoptosis of SNU638 cells was examined by Flow cytometry analysis (FIG. 11). After 72 hours with transfection of siMIG12 or siOIP5, the sub-G1 portion of DNA increased compared to the cells treated with siGFP, indicating both siMIG12 and siOIP5 induced apoptosis of SNU638 cells.

Apoptosis is an essential physiological process for the selective elimination of cells, which is involved in a variety of biological events. RT-PCR of the genes involved in apoptosis was performed to examine their changes in mRNA levels during RNAi of MIG12 or OIP5 (FIG. 12). We detected increased mRNA levels of Bax and Bak, pro-apoptotic factors, which trigger the release of caspases from death antagonists via heterodimerization and also by inducing the release of mitochondrial apoptogenic factors into the cytoplasm via acting on the

mitochondrial permeability transition pore, thereby leading to caspase activation. Also, the expression level of Granzyme B increased when cells were treated with siOIP5. Granzyme B, a serine protease of cytotoxic T-lymphocytes (CTL) and NK cells, is able to directly activate caspases 3, 7, 8 and 10. Also, mRNAs of caspases 8, 9, 2, 5, 10, 4 were increased. They are pro-apoptotic species proteins. These results suggest that knockdown of MIG12 or OIP5 via siMIG12 or siOIP5 can trigger apoptosis of cancer cells.

Example 7: Construction of plasmid containing shMIG12 and shOIP5

To observe the RNAi effect by shMIG12 or shOIP5, a plasmid containing shRNA was constructed (FIG. 13A). After transfection of cells with the plasmid containing shRNA, growth inhibition of cancer cells was examined. The shRNA sequences of top and down complementary strands were designed. Each strand (67 mer/each) consisting of a restriction enzyme site, a target sense siRNA sequence, a loop sequence and a nonsense siRNA sequence, which is gatcc-siRNA sense-loop-siRNA reverse-TTTTTTACGCGTg, was designed and synthesized. Sequences of SEQ ID NOS: 27 and 28 represent top shRNA strands of siMIG12 containing a sequence of SEQ ID NO: 4 and siOIP5 containing a sequence of SEQ ID NO: 5 respectively. The sequence of SEQ ID NO: 29 represents a top shRNA strand of siGFP containing a sequence of SEQ ID NO: 9. The annealed oligonucleotides were cloned into pENTR-U6-BHRNX (Neurogenex) and used for RNAi experiments as shown in FIG. 13A.

Example 8: Inhibition of cancer cell growth by shMIG12 or shOIP5

The plasmid containing shMIG12 or shOIP5 was introduced into Huh7 (liver cancer cell line) allowing cell growth for 72 hours and growth inhibition was examined by SRB staining. The cells transfected with shMIG12 or shOIP5 showed growth inhibition, while the cells with shGFP were not affected, thus indicating that shMIG12 or shOIP5 can be developed as cancer therapeutics.

Example 9: Application of *S. pombe* cells overexpressing MIG12 or OIP5

When MIG12 or OIP5 was overexpressed in *S. pombe*, cell growth was inhibited, suggesting that MIG12 and OIP5 might be involved in cell signaling.

To establish a screening system for identifying targeting MIG12 or OIP5, the plasmids containing MIG12 or OIP5 cloned into the pDES173 expression vector under nmt1 promoter

were introduced into *S. pombe*. MIG12 or OIP5 under the nmt1 promoter is subject to transcriptional repression in the presence of thiamine (EMM+AL+Thia) and overexpressed in the absence of thiamine (EMM+AL) in *S. pombe*. Therefore, *S. pombe* overexpressing MIG12 or OIP5 in the absence of thiamine can be exploited to identify compounds or molecules relieving growth inhibition of cells using a chemical library.

9-1. Growth inhibition by overexpression of MIG12 or OIP5 in *S. pombe*

Growth inhibition by overexpression of MIG12 or OIP5 in *S. pombe* was described in Example 3-1.

9-2. Quantitative measurement of growth inhibition effects by MIG12 or OIP5 in 96-well plates

To measure the degree of growth inhibition, exponentially growing cells in the presence of thiamine were washed 3 times, transferred to a fresh media with or without thiamine and grown for 24 hours, and optical densities of cells were measured (FIG. 14). To establish an HTS screening system, cells serially diluted to 1:4 in a 96-well plate were grown for 24 hours (FIG. 15A) and spotted in the plate (FIG. 15B). The cells overexpressing MIG12 or OIP5 in the media lacking thiamine (EMM+AL) clearly showed growth inhibition of cells. This result suggests that simple procedure of growing cells in a 96-well plate and adding compounds of a chemical library can be applicable for identification of the compounds relieving growth inhibition by overexpression of MIG12 or OIP5.

INDUSTRIAL APPLICABILITY

MIG12 and OIP5 genes of the present invention are expressed at relatively high levels in cancer cell lines and increase a proliferation rate of normal cells. Therefore, inhibition of expression of these genes via RNAi using siRNA or shRNA leads to suppression of cancer cell growth. For these reasons, MIG12 and OIP5 genes can be used as a target gene for diagnosis or cancer therapy of various cancers. As a result, it is expected that the present invention will significantly contribute to the development of biopharmaceuticals and synthetic/natural medicines which are aimed at development of anticancer drugs having low adverse side effects, such as cancer target gene-specific therapeutic agents. Further, the present invention will contribute to the development of a source technology which forms the basis of study of new cancer-related mechanisms.

WHAT IS CLAIMED IS:

1. A composition for diagnosing cancer, comprising an MIG12 gene.

2. A composition for diagnosing cancer, comprising a protein expressed from an MIG12 gene.

3. A composition for diagnosing cancer, comprising an antibody against a protein expressed from an MIG12 gene.

4. A use of an MIG12 gene for diagnosing cancer.

5. A use of a protein expressed from an MIG12 gene for diagnosing cancer.

6. A use of an antibody against a protein expressed from an MIG12 gene for diagnosing cancer.

7. A method for diagnosing cancer, comprising confirming the reaction between a sample obtained from a subject and an MIG12 gene.

8. A method for diagnosing cancer, comprising confirming the reaction between a sample obtained from a subject and a protein expressed from an MIG12 gene.

9. A method for diagnosing cancer, comprising confirming the reaction between a sample obtained from a subject and an antibody against a protein expressed from an MIG12 gene.

10. A composition for screening anticancer drugs, comprising an MIG12 gene.

11. A composition for screening anticancer drugs, comprising a protein expressed from an MIG12 gene.

12. A method for screening anticancer drugs, comprising contacting a test material with a composition of claim 10 as a target material, and examining the resulting reaction to determine whether the test material activates or suppresses the expression of the MIG12 gene.

13. A method for screening anticancer drugs, comprising inducing phenotypic changes of a model cell or animal cell using a composition of claim 10 as a target material and determining whether the test material activates or suppresses the expression of an MIG12 gene

14. A method for screening anticancer drugs, comprising contacting a test material with a composition of claim 11 as a target material, and examining the resulting reaction to determine whether the test material activates or suppresses the function of the protein.

15. A use of an MIG12 gene for screening anticancer drugs.

16. A use of a protein expressed from an MIG12 gene for screening anticancer drugs.

17. An siRNA of an MIG12 gene.

18. The siRNA of claim 17, wherein the siRNA comprises a nucleotide sequence corresponding to an mRNA sequence derived from a nucleotide sequence of 19 to 30 contiguous nucleotides, among one or more sequences selected from SEQ ID NOS: 14, 15, 16, 17 and 18.

19. The siRNA of claim 18, wherein the siRNA has a nucleotide sequence of SEQ ID NO: 3 or 4.

20. The siRNA of any one of claims 17 to 19, wherein the siRNA is chemically modified.

21. An shRNA of an MIG12 gene.

22. The shRNA of claim 21, wherein the shRNA comprises a nucleotide sequence corresponding to an mRNA sequence derived from a nucleotide sequence of 19 to 30 contiguous nucleotides, among one or more sequences selected from SEQ ID NOS: 14, 15, 16, 17 and 18.

23. The shRNA of claim 22, wherein the shRNA has an RNA nucleotide sequence of SEQ ID NO: 4 and an antisense RNA sequence which is linked via a 3-10 bp loop to and is reverse complementary to the sequence of SEQ ID NO: 4.

24. The shRNA of claim 23, wherein a top strand for constructing an expression vector to express the shRNA has a nucleotide sequence of SEQ ID NO: 27.

25. A composition for preventing or treating cancer, comprising an inhibitor of an MIG12 gene.

26. The composition of claim 25, wherein the inhibitor is an siRNA or shRNA of the MIG12 gene.

27. The composition of claim 26, wherein the siRNA comprises a nucleotide sequence corresponding to an mRNA sequence derived from a nucleotide sequence of 19 to 30 contiguous nucleotides, among one or more sequences selected from SEQ ID NOS: 14, 15, 16, 17 and 18.

28. The composition of claim 27, wherein the siRNA has a nucleotide sequence of SEQ ID NO: 3 or 4.

29. The composition of any one of claims 26 to 28, wherein the siRNA is chemically modified.

30. The composition of claim 26, wherein the shRNA comprises a nucleotide sequence corresponding to an mRNA sequence derived from a nucleotide sequence of 19 to 30 contiguous nucleotides, among one or more sequences selected from SEQ ID NOS: 14, 15, 16, 17 and 18.

31. The composition of claim 30, wherein the shRNA has an RNA nucleotide sequence of SEQ ID NO: 4 and an antisense RNA sequence which is linked via a 3-10 bp loop to and is reverse complementary to the sequence of SEQ ID NO: 4.

32. The composition of claim 31, wherein a top strand for constructing an expression vector to

express the shRNA has a nucleotide sequence of SEQ ID NO: 27.

33. The composition of claim 25, wherein the inhibitor of an MIG12 gene is one or more selected from a small molecule, a natural product, and a useful protein for the human body.

34. A composition for preventing or treating cancer, comprising an inhibitor of the protein expressed from an MIG12 gene.

35. The composition of claim 34, wherein the inhibitor is an antibody against the protein expressed from an MIG12 gene.

36. A use of an inhibitor of an MIG12 gene for preventing or treating cancer.

37. The use of claim 36, wherein the inhibitor is an siRNA or shRNA of the MIG12 gene.

38. The use of claim 37, wherein the siRNA comprises a nucleotide sequence corresponding to an mRNA sequence derived from a nucleotide sequence of 19 to 30 contiguous nucleotides, among one or more sequences selected from SEQ ID NOS: 14, 15, 16, 17 and 18.

39. The use of claim 38, wherein the siRNA has a nucleotide sequence of SEQ ID NO: 3 or 4.

40. The use of any one of claims 37 to 39, wherein the siRNA is chemically modified.

41. The use of claim 40, wherein the shRNA comprises a nucleotide sequence corresponding to an mRNA sequence derived from a nucleotide sequence of 19 to 30 contiguous nucleotides, among one or more sequences selected from SEQ ID NOS: 14, 15, 16, 17 and 18.

42. The use of claim 41, wherein the shRNA has an RNA nucleotide sequence of SEQ ID NO: 4 and an antisense RNA sequence which is linked via a 3-10 bp loop to and is reverse complementary to the sequence of SEQ ID NO: 4.

43. The use of claim 42, wherein a top strand for constructing an expression vector to express

the shRNA has a nucleotide sequence of SEQ ID NO: 27.

44. The use of claim 36, wherein the inhibitor of an MIG12 gene is one or more selected from a small molecule, a natural product, and a useful protein for the human body.

45. A use of an inhibitor of the protein expressed from an MIG12 gene for preventing or treating cancer.

46. The use of claim 45, wherein the inhibitor is an antibody against the protein expressed from an MIG12 gene.

47. A method for preventing or treating cancer, comprising administering a therapeutically effective amount of an inhibitor of an MIG12 gene to a subject in need thereof.

48. The method of claim 47, wherein the inhibitor is an siRNA or shRNA of the MIG12 gene.

49. The method of claim 48, wherein the siRNA comprises a nucleotide sequence corresponding to an mRNA sequence derived from a nucleotide sequence of 19 to 30 contiguous nucleotides, among one or more sequences selected from SEQ ID NOS: 14, 15, 16, 17 and 18.

50. The method of claim 49, wherein the siRNA has a nucleotide sequence of SEQ ID NO: 3 or 4.

51. The method of any one of claims 48 to 50, wherein the siRNA is chemically modified.

52. The method of claim 48, wherein the shRNA comprises a nucleotide sequence corresponding to an mRNA sequence derived from a nucleotide sequence of 19 to 30 contiguous nucleotides, among one or more sequences selected from SEQ ID NOS: 14, 15, 16, 17 and 18.

53. The method of claim 52, wherein the shRNA has an RNA nucleotide sequence of SEQ ID

NO: 4 and an antisense RNA sequence which is linked via a 3-10 bp loop to and is reverse complementary to the sequence of SEQ ID NO: 4.

54. The method of claim 53, wherein a top strand for constructing an expression vector to express the shRNA has a nucleotide sequence of SEQ ID NO: 27.

55. A method for preventing or treating cancer, comprising administering a therapeutically effective amount of an inhibitor of the protein expressed from an MIG12 gene to a subject in need thereof.

56. The method of claim 55, wherein the inhibitor is an antibody against the protein expressed from an MIG12 gene.

57. A composition for screening anticancer drugs, comprising an OIP5 gene.

58. A composition for screening anticancer drugs, comprising a protein expressed from an OIP5 gene.

59. A method for screening anticancer drugs, comprising contacting a test material with a composition of claim 57 as a target material, and examining the resulting reaction to determine whether the test material activates or suppresses the expression of the OIP5 gene.

60. A method for screening anticancer drugs, comprising inducing phenotypic changes of a model cell or animal cell using a composition of claim 57 as a target material and determining whether the test material activates or suppresses the expression of an MIG12 gene.

61. A method for screening anticancer drugs, comprising contacting a test material with a composition of claim 58 as a target material, and examining the resulting reaction to determine whether the test material activates or suppresses the function of the protein.

62. A use of an OIP5 gene for screening anticancer drugs.

63. A use of a protein expressed from an OIP5 gene for screening anticancer drugs.

64. An siRNA of an OIP5 gene.

65. The siRNA of claim 64, the siRNA comprises a nucleotide sequence corresponding to an mRNA sequence derived from a nucleotide sequence of 19 to 30 contiguous nucleotides, among one or more sequences selected from SEQ ID NOS: 19, 20, 21, 22, 23, 24, 25, and 26.

66. The siRNA of claim 65, wherein the siRNA has a nucleotide sequence of SEQ ID NO: 5 or 6.

67. The siRNA of any one of claims 64 to 66, wherein the siRNA is chemically modified.

68. An shRNA of an OIP5 gene.

69. The siRNA of claim 68, the shRNA comprises a nucleotide sequence corresponding to an mRNA sequence derived from a nucleotide sequence of 19 to 30 contiguous nucleotides, among one or more sequences selected from SEQ ID NOS: 19, 20, 21, 22, 23, 24, 25, and 26.

70. The shRNA of claim 69, wherein the shRNA has an RNA nucleotide sequence of SEQ ID NO: 5 and an antisense RNA sequence which is linked via a 3-10 bp loop to and is reverse complementary to the sequence of SEQ ID NO: 5.

71. The shRNA of claim 70, wherein a top strand for constructing an expression vector to express the shRNA has a nucleotide sequence of SEQ ID NO: 28.

72. A composition for preventing or treating cancer, comprising an inhibitor of an OIP5 gene.

73. The composition of claim 72, wherein the inhibitor is an siRNA or shRNA of an OIP5 gene.

74. The composition of claim 73, the siRNA comprises a nucleotide sequence corresponding

to an mRNA sequence derived from a nucleotide sequence of 19 to 30 contiguous nucleotides, among one or more sequences selected from SEQ ID NOS: 19, 20, 21, 22, 23, 24, 25, and 26.

75. The composition of claim 74, wherein the siRNA has a nucleotide sequence of SEQ ID NO: 5 or 6.

76. The composition of claim 73, wherein the siRNA is chemically modified.

77. The composition of claim 73, the shRNA comprises a nucleotide sequence corresponding to an mRNA sequence derived from a nucleotide sequence of 19 to 30 contiguous nucleotides, among one or more sequences selected from SEQ ID NOS: 19, 20, 21, 22, 23, 24, 25, and 26.

78. The composition of claim 77, wherein the shRNA has an RNA nucleotide sequence of SEQ ID NO: 5 and an antisense RNA sequence which is linked via a 3-10 bp loop to and is reverse complementary to the sequence of SEQ ID NO: 5.

79. The composition of claim 78, wherein a top strand for constructing an expression vector to express the shRNA has a nucleotide sequence of SEQ ID NO: 28.

80. The composition of claim 72, wherein the inhibitor of an OIP5 gene is one or more selected from a small molecule, a natural product, and a useful protein for the human body.

81. A composition for preventing or treating cancer, comprising an inhibitor of a protein expressed from an OIP5 gene.

82. The composition of claim 81, wherein the inhibitor is an antibody against the protein expressed from the OIP5 gene.

83. The composition of any one of claims 72 to 82, wherein the cancer is one or more selected from stomach cancer, liver cancer, lung cancer, prostate cancer, and colorectal cancer.

84. A use of an inhibitor of an OIP5 gene for preventing or treating cancer.

85. The use of claim 84, wherein the inhibitor is an siRNA or shRNA of the OIP5 gene.

86. The use of claim 85, wherein the siRNA comprises a nucleotide sequence corresponding to an mRNA sequence derived from a nucleotide sequence of 19 to 30 contiguous nucleotides, among one or more sequences selected from SEQ ID NOS: 19, 20, 21, 22, 23, 24, 25, and 26.

87. The use of claim 86, wherein the siRNA has a nucleotide sequence of SEQ ID NO: 5 or 6.

88. The use of claim 85, wherein the siRNA is chemically modified.

89. The use of claim 85, wherein the shRNA comprises a nucleotide sequence corresponding to an mRNA sequence derived from a nucleotide sequence of 19 to 30 contiguous nucleotides, among one or more sequences selected from SEQ ID NOS: 19, 20, 21, 22, 23, 24, 25, and 26.

90. The use of claim 89, wherein the shRNA has an RNA nucleotide sequence of SEQ ID NO: 5 and an antisense RNA sequence which is linked via a 3-10 bp loop to and is reverse complementary to the sequence of SEQ ID NO: 5.

91. The use of claim 90, wherein a top strand for constructing an expression vector to express the shRNA has a nucleotide sequence of SEQ ID NO: 28.

92. The use of claim 84, wherein the inhibitor of an OIP5 gene is one or more selected from a small molecule, a natural product, and a useful protein for the human body.

93. A use of an inhibitor of a protein expressed from an OIP5 gene for preventing or treating cancer.

94. The use of claim 93, wherein the inhibitor is an antibody against the protein expressed from an OIP5 gene for preventing or treating cancer.

95. The use of any one of claims 84 to 94, wherein the cancer is one or more selected from

stomach cancer, liver cancer, lung cancer, prostate cancer, and colorectal cancer.

96. A method for preventing or treating cancer, comprising administering a therapeutically effective amount of an inhibitor of an OIP5 gene to a subject in need thereof.

97. The method of claim 96, wherein the inhibitor of an OIP5 gene is an siRNA of the OIP5 gene or an shRNA of the OIP5 gene.

98. The method of claim 97, wherein the siRNA comprises a nucleotide sequence corresponding to an mRNA sequence derived from a nucleotide sequence of 19 to 30 contiguous nucleotides, among one or more sequences selected from SEQ ID NOS: 19, 20, 21, 22, 23, 24, 25, and 26.

99. The method of claim 98, wherein the siRNA has a nucleotide sequence of SEQ ID NO: 5 or 6.

100. The method of claim 97, wherein the siRNA is chemically modified.

101. The method of claim 97, wherein the shRNA comprises a nucleotide sequence corresponding to an mRNA sequence derived from a nucleotide sequence of 19 to 30 contiguous nucleotides, among one or more sequences selected from SEQ ID NOS: 19, 20, 21, 22, 23, 24, 25, and 26.

102. The method of claim 101, wherein the shRNA has an RNA nucleotide sequence of SEQ ID NO: 5 and an antisense RNA sequence which is linked via a 3-10 bp loop to and is reverse complementary to the sequence of SEQ ID NO: 5.

103. The method of claim 102, wherein a top strand for constructing an expression vector to express the shRNA has a nucleotide sequence of SEQ ID NO: 28.

104. The method of claim 96, wherein the inhibitor of OIP5 gene is one or more selected from a small molecule, a natural product, and a useful protein for the human body.

105. A method for preventing or treating cancer, comprising administering to a subject in need thereof a therapeutically effective amount of an inhibitor of the protein expressed from an OIP5 gene.

106. The method of claim 105, wherein the inhibitor is an antibody against the protein expressed from the OIP5 gene.

107. The method of any one of claims 96 to 106, wherein the cancer is one or more selected from stomach cancer, liver cancer, lung cancer, prostate cancer, and colorectal cancer.

108. A kit for diagnosing cancer, comprising one or more selected from an MIG12 gene, an OIP5 gene, a protein expressed from MIG12 gene, and a protein expressed from the OIP5 gene.

FIGURES

Fig. 1

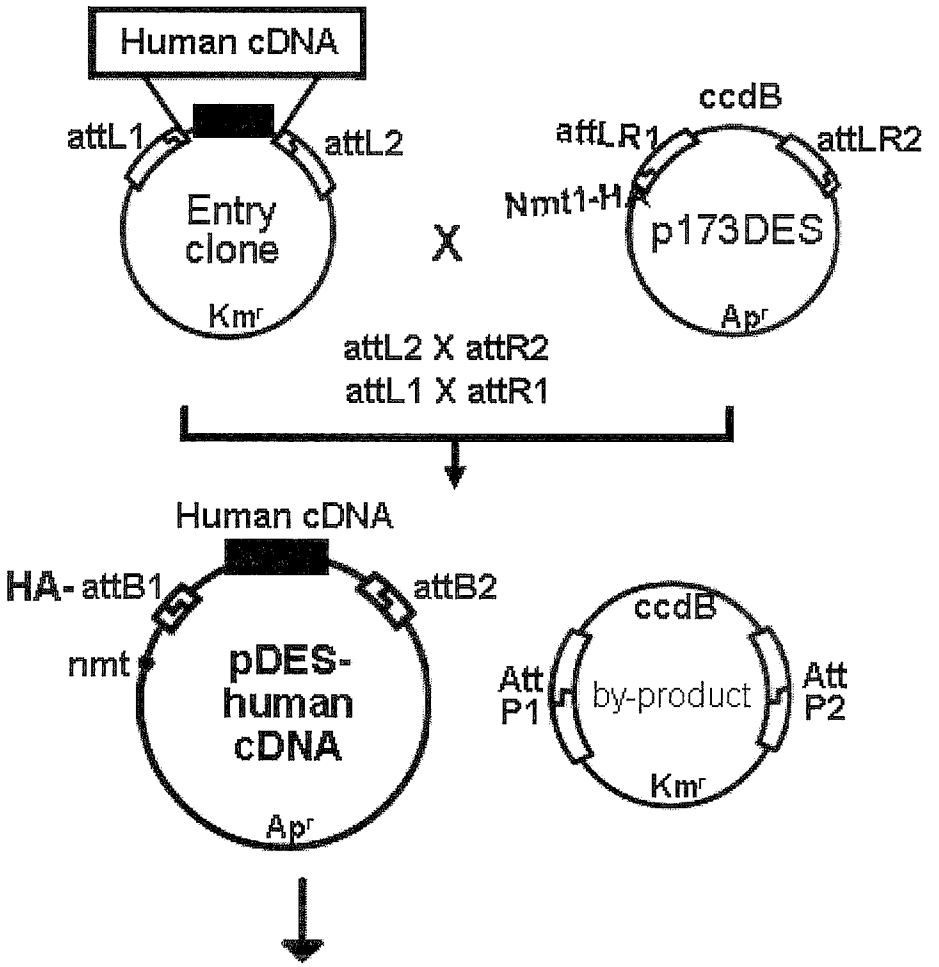


Fig. 2

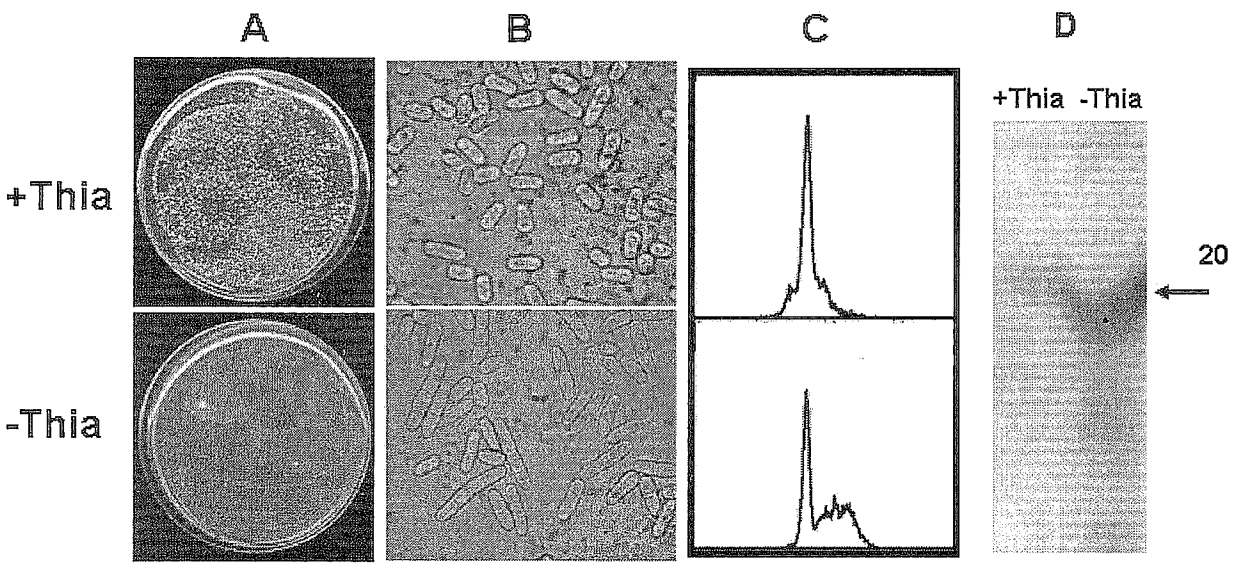


Fig. 3

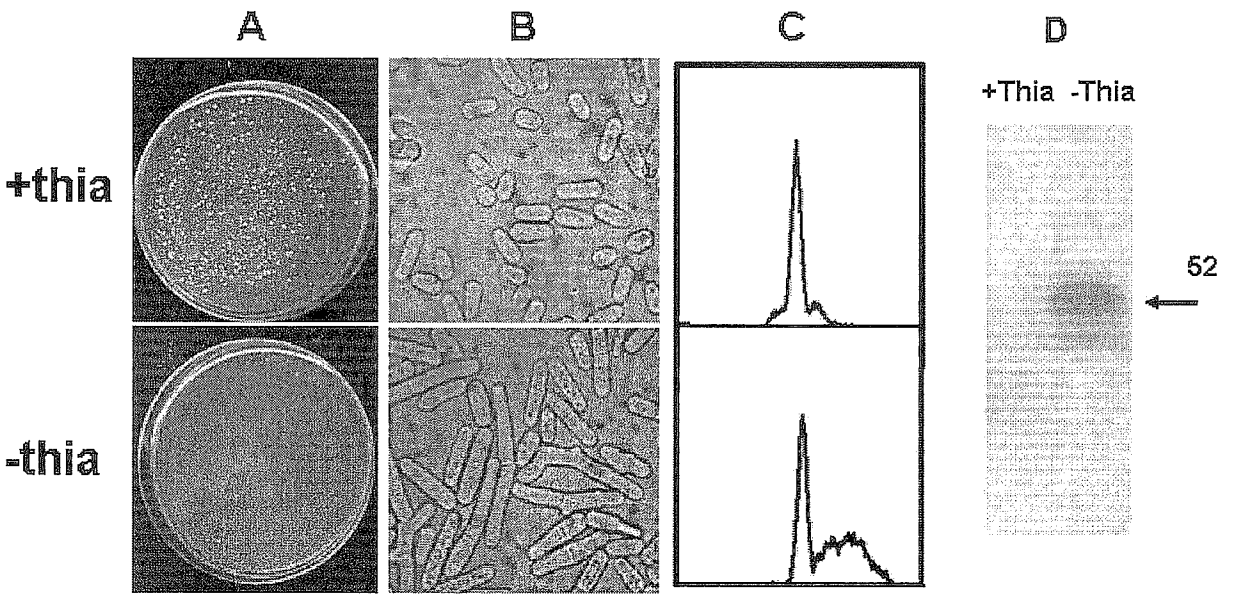


Fig. 4

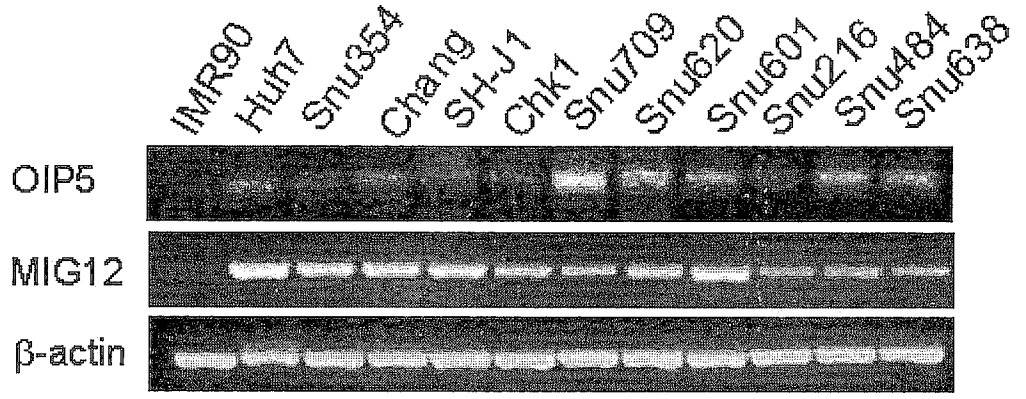
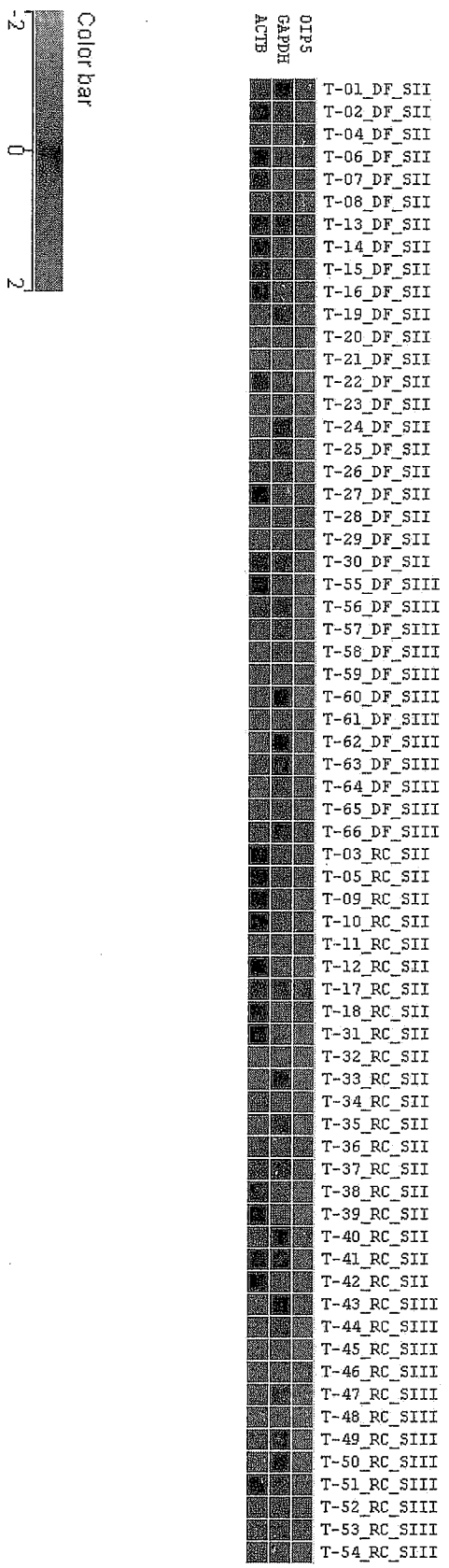

IMR90
Huh7
Snu354
Chang
SH-J1
Chk1
Snu709
Snu620
Snu601
Snu216
Snu484
Snu638
OIP5
MIG12
β-actin

Fig. 5


Color bar
2
0
-2
ACTB
GAPDH
OIP5
T-01_DF_SII
T-02_DF_SII
T-04_DF_SII
T-06_DF_SII
T-07_DF_SII
T-08_DF_SII
T-13_DF_SII
T-14_DF_SII
T-15_DF_SII
T-16_DF_SII
T-19_DF_SII
T-20_DF_SII
T-21_DF_SII
T-22_DF_SII
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T-24_DF_SII
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T-26_DF_SII
T-27_DF_SII
T-28_DF_SII
T-29_DF_SII
T-30_DF_SII
T-55_DF_SIII
T-56_DF_SIII
T-57_DF_SIII
T-58_DF_SIII
T-59_DF_SIII
T-60_DF_SIII
T-61_DF_SIII
T-62_DF_SIII
T-63_DF_SIII
T-64_DF_SIII
T-65_DF_SIII
T-66_DF_SIII
T-03_RC_SII
T-05_RC_SII
T-09_RC_SII
T-10_RC_SII
T-11_RC_SII
T-12_RC_SII
T-17_RC_SII
T-18_RC_SII
T-31_RC_SII
T-32_RC_SII
T-33_RC_SII
T-34_RC_SII
T-35_RC_SII
T-36_RC_SII
T-37_RC_SII
T-38_RC_SII
T-39_RC_SII
T-40_RC_SII
T-41_RC_SII
T-42_RC_SII
T-43_RC_SIII
T-44_RC_SIII
T-45_RC_SIII
T-46_RC_SIII
T-47_RC_SIII
T-48_RC_SIII
T-49_RC_SIII
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T-51_RC_SIII
T-52_RC_SIII
T-53_RC_SIII
T-54_RC_SIII

Fig. 6

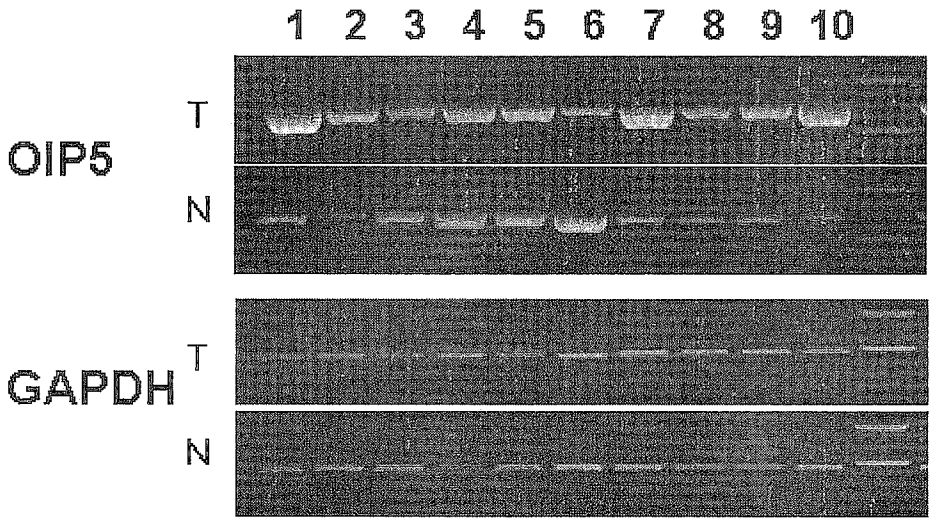


Fig. 7

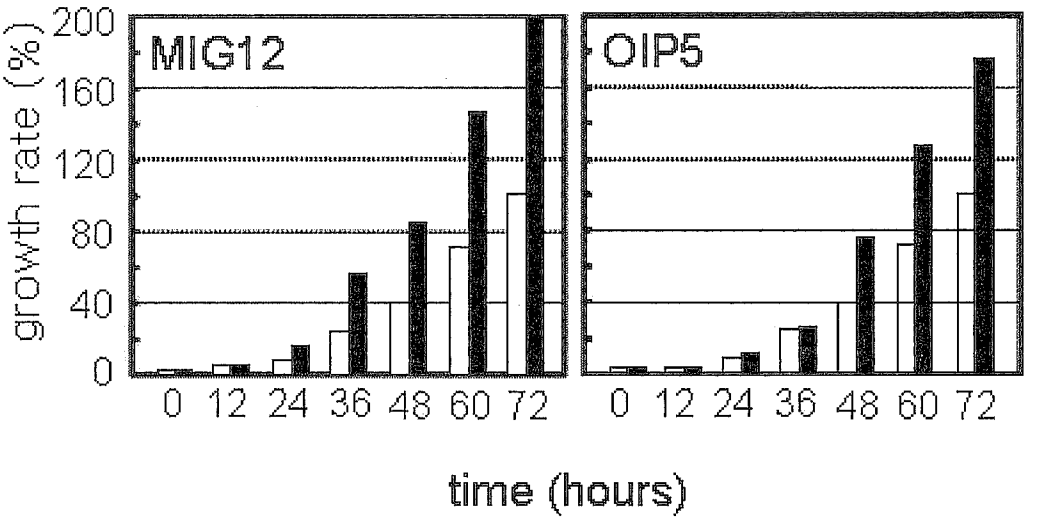

MIG12
OIP5
growth rate (%)
200
160
120
80
40
0
0 12 24 36 48 60 72
0 12 24 36 48 60 72
time (hours)

Fig. 8

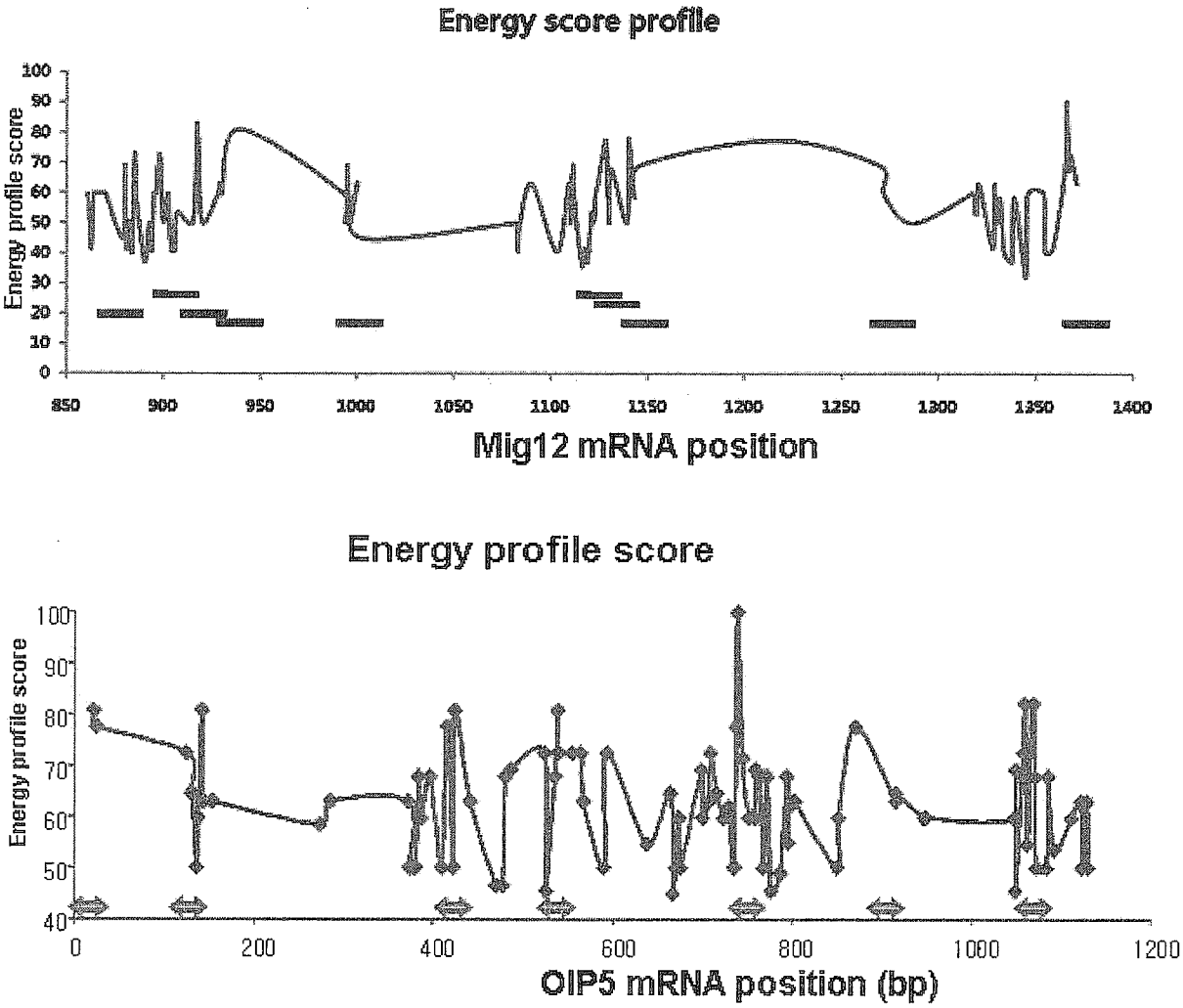

Energy score profile
Energy profile score
100
90
80
70
60
50
40
30
20
10
0
850
900
950
1000
1050
1100
1150
1200
1250
1300
1350
1400
Mig12 mRNA position
Energy profile score
Energy profile score
100
90
80
70
60
50
40
0
200
400
600
800
1000
1200
OIP5 mRNA position (bp)

Fig. 9

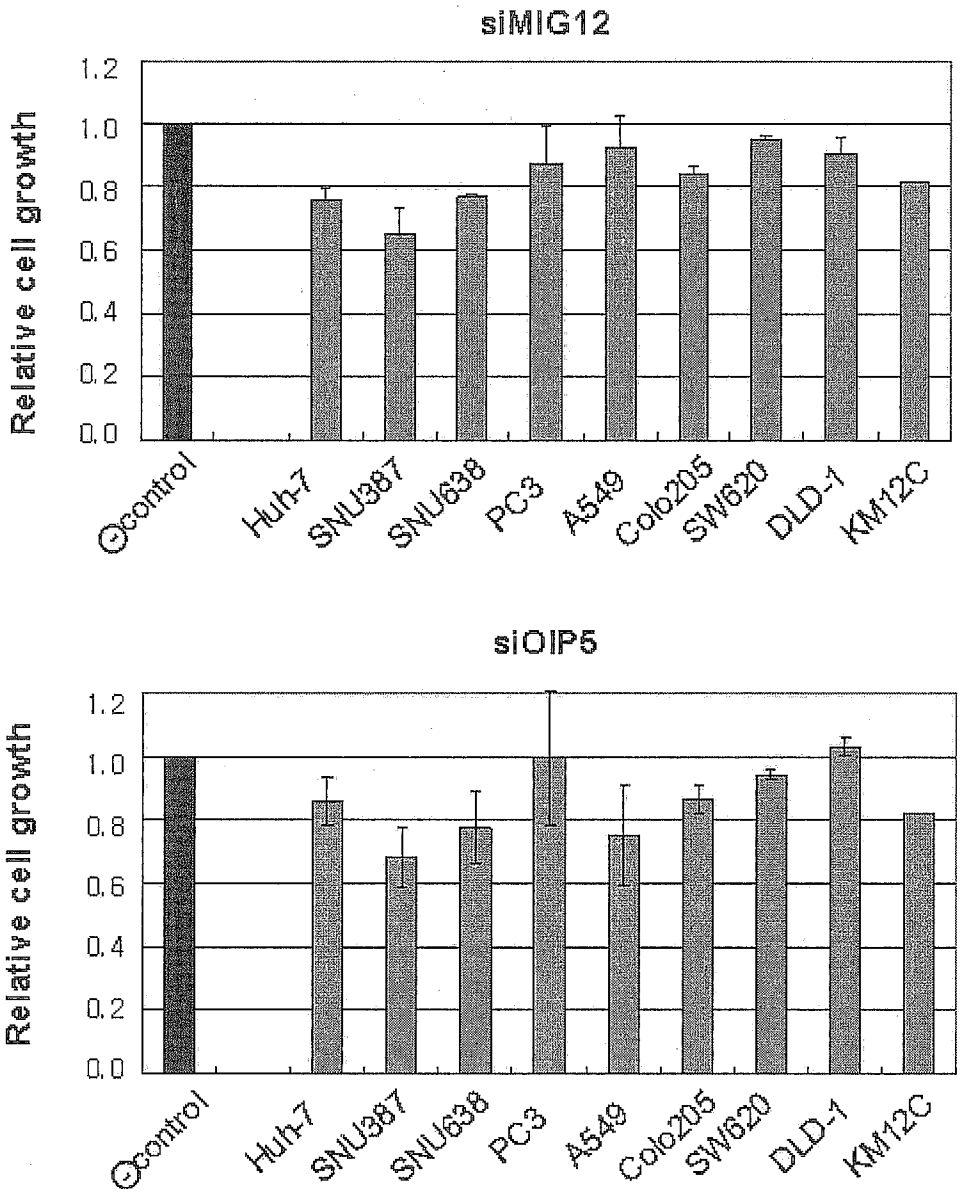

siMIG12
Relative cell growth
1.2
1.0
0.8
0.6
0.4
0.2
0.0
control
Huh-7
SNU387
SNU638
PC3
A549
Colo205
SW620
DLD-1
KM12C
siOIP5
Relative cell growth
1.2
1.0
0.8
0.6
0.4
0.2
0.0
control
Huh-7
SNU387
SNU638
PC3
A549
Colo205
SW620
DLD-1
KM12C

Fig. 10

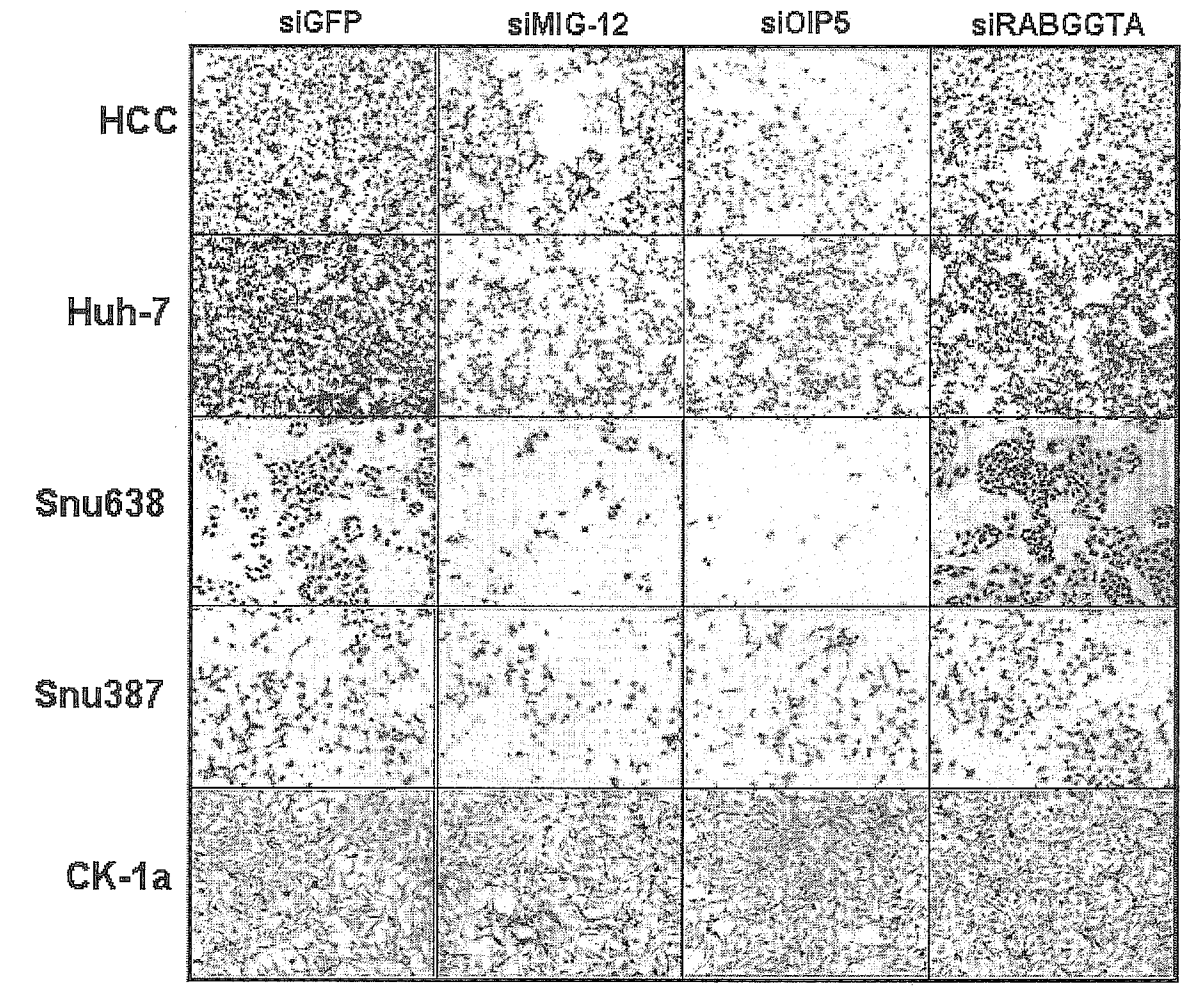

siGFP
siMIG-12
siOIP5
siRABGGTA
HCC
Huh-7
Snu638
Snu387
CK-1a

Fig. 11

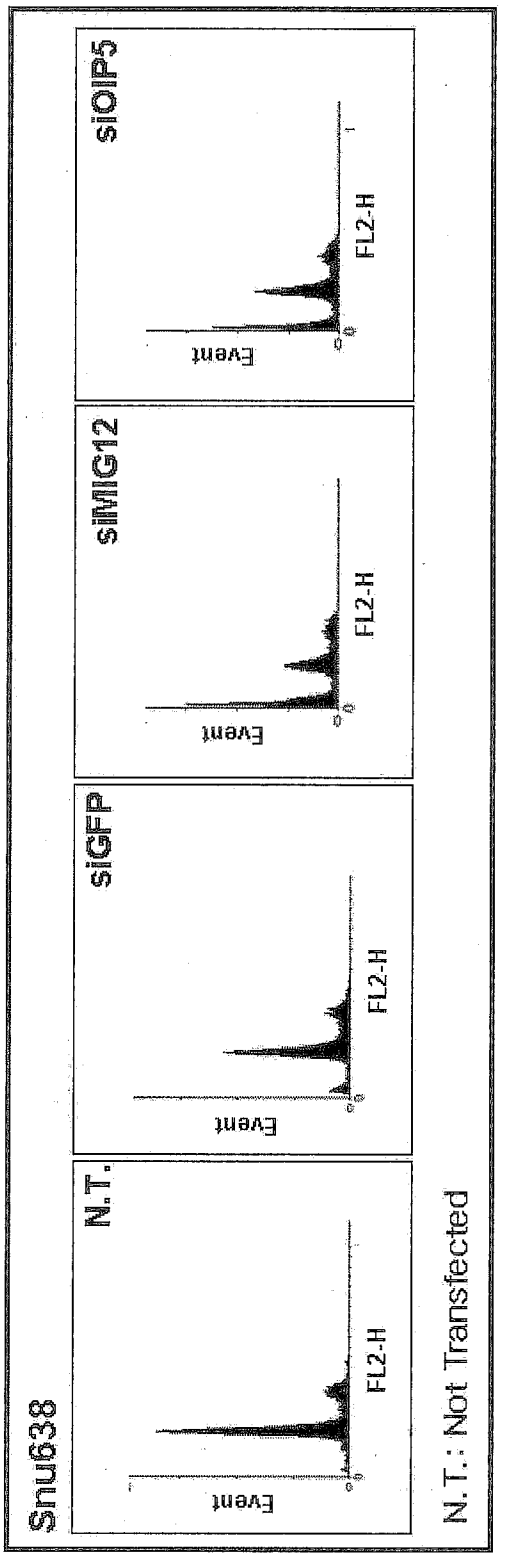

Snu638
N.T.
siGFP
siMIG12
siOIP5
Event
FL2-H
N.T.: Not Transfected

Fig. 12

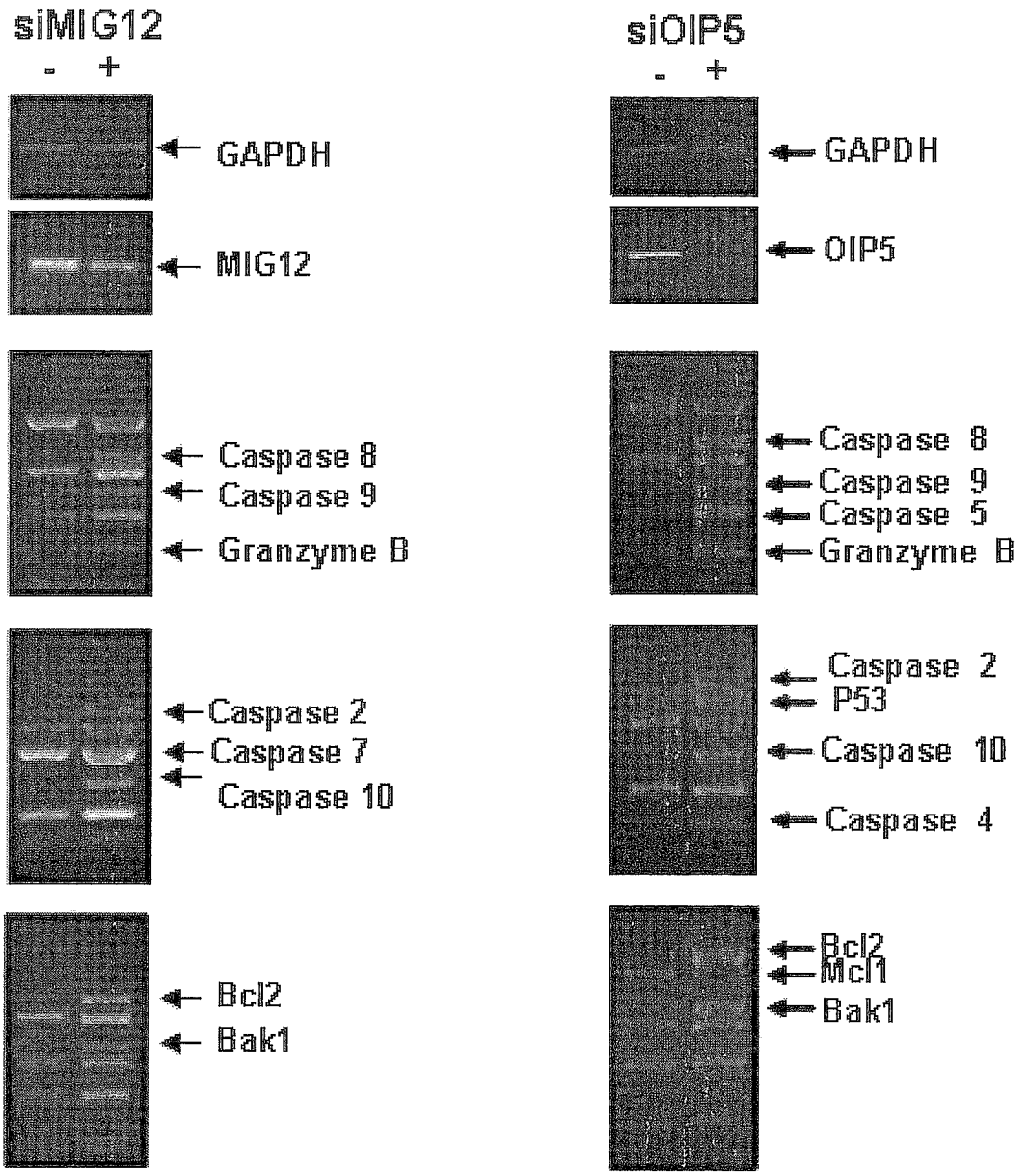
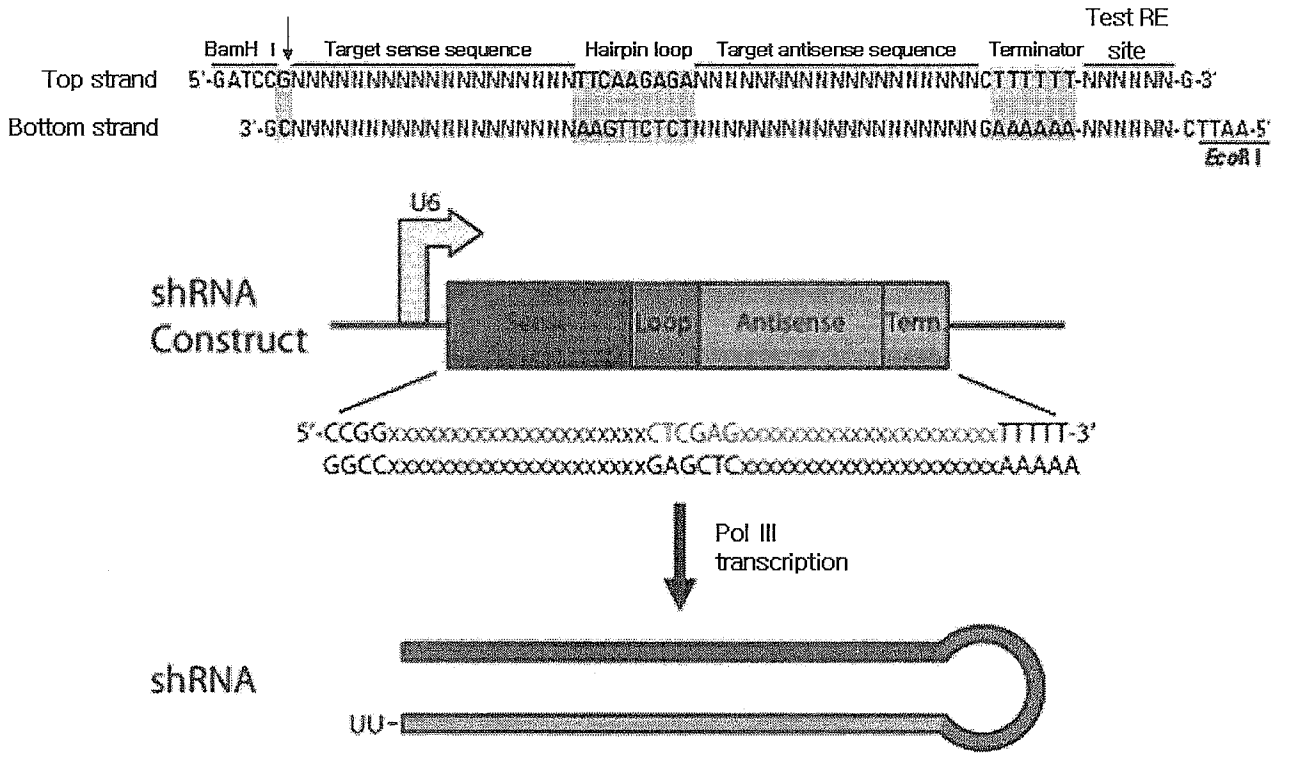

siMIG12
- +
GAPDH
MIG12
Caspase 8
Caspase 9
Granzyme B
Caspase 2
Caspase 7
Caspase 10
Bcl2
Bak1
siOIP5
- +
GAPDH
OIP5
Caspase 8
Caspase 9
Caspase 5
Granzyme B
Caspase 2
P53
Caspase 10
Caspase 4
Bcl2
Mcl1
Bak1

Fig. 13

A.


Test RE
BamH I
Target sense sequence
Hairpin loop
Target antisense sequence
Terminator
site
Top strand
5'-GATCCGNNNNNNNNNNNNNNNNNNTTCAAGAGANNNNNNNNNNNNNNNNNNCTTTTTTT-NNNNNN-G-3'
Bottom strand
3'-GCNNNNNNNNNNNNNNNNNNNNNNAAGTTCTCTNNNNNNNNNNNNNNNNNNGAAAAAA-NNNNNN-CTTAA-5'
EcoR I
U6
shRNA
Construct
Sense
Loop
Antisense
Term
5'-CCGGxxxxxxxxxxxxxxxxxxxCTCGAGxxxxxxxxxxxxxxxxxxxTTTTT-3'
GGCCxxxxxxxxxxxxxxxxxxxGAGCTCxxxxxxxxxxxxxxxxxxxAAAAA
Pol III
transcription
shRNA
UU-

B.

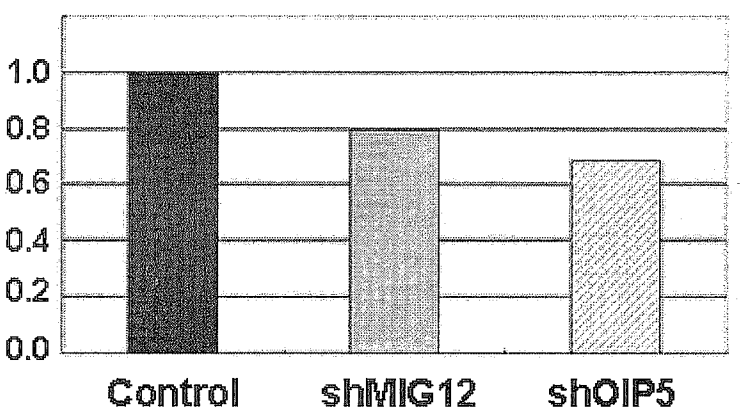

1.0
0.8
0.6
0.4
0.2
0.0
Control
shMIG12
shOIP5

Fig. 14

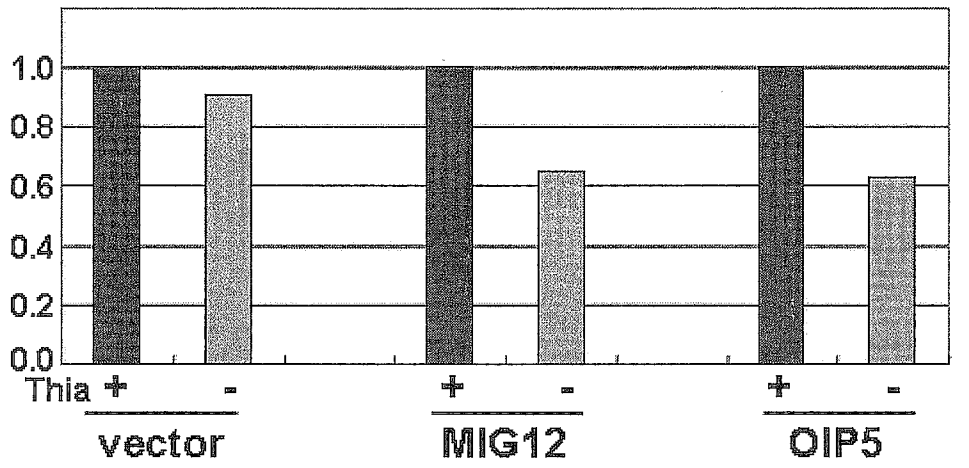
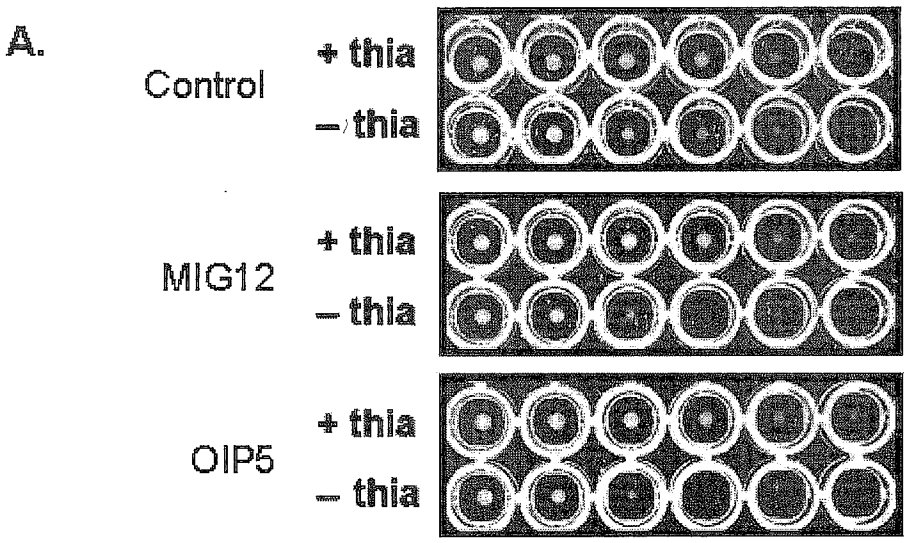
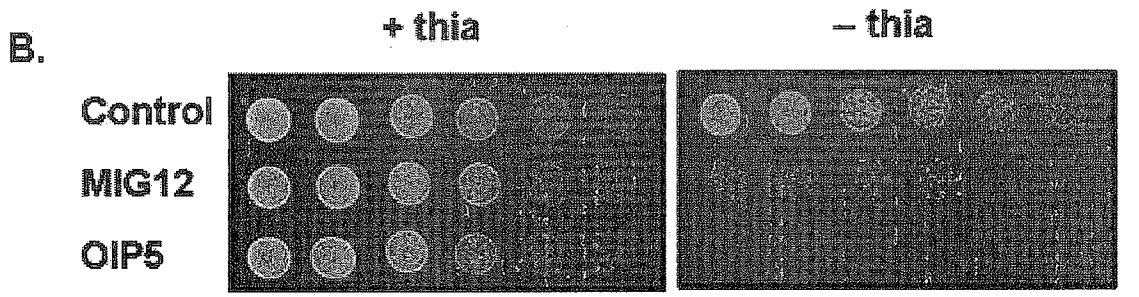


Fig. 15


A.
Control
+ thia
– thia
MIG12
+ thia
– thia
OIP5
+ thia
– thia


B.
+ thia
– thia
Control
MIG12
OIP5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2007/006366

A. CLASSIFICATION OF SUBJECT MATTER

C07K 14/39(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 8 C07K 14/39, A61K 48/00, C07H 17/00

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

N.A.

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

eKIPASS, WPI, USPTO, PAJ, NCBI, INSPECT "cancer, tumor, MIG12, G12-like, THRSPL, FLJ10386, STRAIT11499, OIP5, MID1 interacting protein, composition, siRNA, diagnosis, treat, gene, antibody screen, method, etc."

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A (Group 1)	Y: WO 0073443 A1 (INCYTE GENOMICS, US) 07 Sec 2000 - see p.2, line 13 ~ p.3, line 23; p.5, line 29 ~ p.6, line 15; p.12, lines 12~23; p.13, line 22 ~ P.16, line 21; p.24, line 35 ~ p.27, line 8	1-6,10-46,57-95, & 108
Y (Group 2)	Y: "Genome Wide Expression Analysis of Colorectal Cancer and Cervical Cancer" M.S. Thesis of Michal Sheffer, Weizmann Institute of Science, Mar 2005 - see p.54; and Table 5	57-95,108(partial)
Y (Group 2)	Y2: WO 2004074320 A2 (SAGRES DISCOVERY INC., US) 02 Sep 2004 - see abstract and claims	57-95,108(partial)
Y (Group 2)	Y2: US 20040171037 A1 (Li, J., et al., US) 2 Sep 2004 - see abstract and claims	57-95,108(partial)
A (Group 1)	BMC Cell Biol. Vol.5:9. (Berti, C., et al., Italy) 2004 Feb 29 "Mig12, a novel Opitz syndrome gene product partner, is expressed in the embryonic ventral midline and co-operates with Mid1 to bundle and stabilize microtubules." - see abstract (cited in the application)	1-6,10-46,57-95, & 108

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

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 MARCH 2008 (17.03.2008)	**17 MARCH 2008 (17.03.2008)**

Name and mailing address of the ISA/KR

Korean Intellectual Property Office
Government Complex-Daejeon, 139 Seonsa-ro, Seo-gu, Daejeon 302-701, Republic of Korea

Facsimile No. 82-42-472-7140

Authorized officer

SHIN, Weon Hye

Telephone No. 82-42-481-5591

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2007/006366

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of :

   a. type of material
   - [x] a sequence listing
   - [ ] table(s) related to the sequence listing

   b. format of material
   - [x] on paper
   - [x] in electronic form

   c. time of filing/furnishing
   - [x] contained in the international application as filed
   - [x] filed together with the international application in electronic form
   - [ ] furnished subsequently to this Authority for the purposes of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2007/006366

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 7-9, 47-56 & 96-107
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 7-9 are directed to a method for diagnosing cancer and claims 47-56 & 96-107 to a method for treating cancer. Rule 39.1(iv) PCT does not draw a line with regard to whether the medical method should be practiced on the body or not. Claims 7-9, 47-56 & 96-107, therefore, fall into the category of methods for treatment of the human body or animal body by surgery or therapy as well as diagnostic methods. [Article 17(2)(a)(i), Rule 39.1(iv) PCT]

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This ISA found multiple inventions as follows:
Group 1 : claims 1-6, 10-46 & 108 (partial) feature the MIG12 gene or its product.
Group 2 : claims 57-95 & 108 (partial) feature the OIP5 gene or its product.

The single general concept linking the two Groups together is merely the finding that a gene is oncogenic and a possible target of cancer diagnosis and treatment. WO 0073443 A1 and "M.Sc. Thesis of Michal Sheffer, Weizmann Institute of Science (Mar 2005)", however, respectively describe MIG12 and OIP5 genes as an effective agent of a composition for cancer diagnosis, therapy, etc.. The aforementioned concept, therefore, is not considered to represent any contribution over the prior art.

Hence the inventions listed as Groups 1 & 2 do not relate to a single general inventive concept under PCT Rule 13.1 because the above mentioned common concept fails to make a contribution over the prior art within the meaning of PCT Rule 13.2.

1. ☐ As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2007/006366

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A (Group 2)	Mol Microbiol. Vol.27(1):171-186 (Williams, J.M., et al., US) Jan 1998 "Using the yeast two-hybrid system to identify human epithelial cell proteins that bind gonococcal Opa proteins: intracellular gonococci bind pyruvate kinase via their Opa proteins and require host pyruvate for growth." - see the whole document	1-6,10-46,57-95, & 108(partial)
A	Mol Cancer. Vol.4:34. (Ghiselli, G., et al., US) 2005 Sep 12 "Global gene expression profiling of cells overexpressing SMC3." - see the whole document	1-6,10-46,57-95, & 108

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR2007/006366

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0073443 A1	07.12.2000	AU 200050189 A1	18.12.2000
		CA 2374376 A1	07.12.2000
		EP 1181364 A1	27.02.2002
		JP 2003501026 T2	14.01.2003
		US 2004058358 A1	25.03.2004
		US 6245526 B1	12.06.2001
		WO 200073443 A1	07.12.2000
WO 2004074320 A2	02.09.2004	AU 2004213432 A1	02.09.2004
		CA 2516128 A1	02.09.2004
		EP 01594893 A2	16.11.2005
		US 20070218071 A1	20.09.2007
US 20040171037 A1	02.09.2004	none	