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(71) Applicants *(for all designated States except US)*: **ONCOTHERAPY SCIENCE, INC.** [JP/JP]; 2-1, Sakado 3-chome, Takatsu-ku, Kawasaki-shi, Kanagawa, 2130012 (JP). **THE UNIVERSITY OF TOKYO** [JP/JP]; 3-1, Hongo 7-chome, Bunkyo-ku, Tokyo, 1138654 (JP).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **NAKAMURA, Yusuke** [JP/JP]; 6-1, Shirokanedai 4-chome, Minato-ku, Tokyo, 1088639 (JP). **NAKAGAWA, Hidewaki** [JP/JP]; 6-1, Shirokanedai 4-chome, Minato-ku, Tokyo, 1088639 (JP).

(74) Agents: **SHIMIZU, Hatsushi** et al.; Kantetsu Tsukuba Bldg. 6F, 1-1-1, Oroshi-machi, Tsuchiura-shi, Ibaraki 3000847 (JP).

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(54) **Title:** COMPOSITIONS AND METHODS FOR TREATING PANCREATIC CANCER

(57) **Abstract:** The invention features a method for inhibiting growth of a cancer cell by contacting the cell with a composition of a siRNA that inhibits expression of PCDH1, CDH3 or GPR107. Methods of treating cancer are also within the invention. The invention also features products, including nucleic acid sequences and vectors as well as to compositions comprising them, useful in the provided methods. The invention also provides a method for inhibiting of tumor cell, for example pancreatic cancer cell, particularly pancreatic ductal adenocarcinoma (PDACa).

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DESCRIPTION

COMPOSITIONS AND METHODS FOR TREATING PANCREATIC CANCER

This application claims the benefit of U.S. Provisional Application Serial No.60/555,809 filed March 24, 2004, the contents of which are hereby incorporated by reference in its entirety.

Technical Field

The present invention relates to the field of biological science, more specifically to the field of cancer research. In particular, the present invention relates a composition comprising a nucleic acid capable of inhibiting expression of the genes encoding PCDH1, CDH3 or GPR107. In some embodiments, the compound is a small interfering RNA (siRNA) corresponding to a subsequence from these genes.

Background Art

Pancreatic ductal adenocarcinoma (PDACa) is the fifth leading cause of cancer death in the western world and has one of the highest mortality rates of any malignancy, with a 5-year survival rate only 4%. In USA, each year, estimated 30,700 patients are diagnosed with pancreatic cancer and nearly 30,000 will die of these diseases. The vast majority of patients are diagnosed at an advanced stage of disease at which it has no response to current therapies and the patients can survive for few months. Only surgical resection can offer the possibility of cure, but only 10-20% of patients with PDACa can undergo potentially curative resection and even after curative surgery, 80-90% of the patients relapse and die of the disease. Some improvements in surgical outcome or quality of life occur in patients who also receive chemotherapy including gemcitabine and/or radiation, although the impact on long-term survival has been minimal due to the intense resistance of PDACa to any treatment. At this point, management of most patients focuses on palliation.

Therefore, establishment of a novel molecular therapy for PDACa and identification of novel therapeutic molecular targets for PDACa are urgent issues for pancreatic cancer treatment now.

Disclosure of the Invention

The present invention based on the surprising discovery that inhibiting expression of PCDH1, CDH3 or GPR107 is effective in inhibiting the cellular growth of various cancer cells, including those involved in PDACa. The inventions described in this application are based in part on this discovery.

The invention provides methods for inhibiting cell growth. Among the methods provided are those comprising contacting a cell with a composition comprising a small interfering RNA (siRNA) that inhibits expression of PCDH1, CDH3 or GPR107. The invention also provides methods for inhibiting tumor cell growth in a subject. Such methods include administering to a subject a composition comprising a small interfering RNA (siRNA) that hybridizes specifically to a sequence from PCDH1, CDH3 or GPR107. Another aspect of the invention provides methods for inhibiting the expression of the PCDH1, CDH3 or GPR107 gene in a cell of a biological sample. Expression of the gene may be inhibited by introduction of a double stranded ribonucleic acid (RNA) molecule into the cell in an amount sufficient to inhibit expression of the PCDH1, CDH3 or GPR107 gene. Another aspect of the invention relates to products including nucleic acid sequences and vectors as well as to compositions comprising them, useful, for example, in the provided methods. Among the products provided are siRNA molecules having the property to inhibit expression of the PCDH1, CDH3 or GPR107 gene when introduced into a cell expressing said gene. Among such molecules are those that comprise a sense strand and an antisense strand, wherein the sense strand comprises a ribonucleotide sequence corresponding to a PCDH1, CDH3 or GPR107 target sequence, and wherein the antisense strand comprises a ribonucleotide sequence which is complementary to said sense strand. The sense and the antisense strands of the molecule hybridize to each other to form a double-stranded molecule.

As used herein, the term "organism" refers to any living entity comprised of at least one cell. A living organism can be as simple as, for example, a single eukaryotic cell or as complex as a mammal, including a human being.

As used herein, the term "biological sample" refers to a whole organism or a subset of its tissues, cells or component parts (e.g. bodily fluids, including but not limited to blood,

mucus, lymphatic fluid, synovial fluid, cerebrospinal fluid, saliva, amniotic fluid, amniotic cord blood, urine, vaginal fluid and semen). "Biological sample" further refers to a homogenate, lysate, extract, cell culture or tissue culture prepared from a whole organism or a subset of its cells, tissues or component parts, or a fraction or portion thereof. Lastly, "biological sample" refers to a medium, such as a nutrient broth or gel in which an organism has been propagated, which contains cellular components, such as proteins or nucleic acid molecules.

The invention features methods of inhibiting cell growth. Cell growth is inhibited by contacting a cell with a composition of a small interfering RNA (siRNA) of PCDH1, CDH3 or GPR107. The cell is further contacted with a transfection-enhancing agent. The cell is provided *in vitro*, *in vivo* or *ex vivo*. The subject is a mammal, *e.g.*, a human, non-human primate, mouse, rat, dog, cat, horse, or cow. The cell is a pancreatic ductal cell. Alternatively, the cell is a tumor cell (*i.e.*, cancer cell) such as a carcinoma cell or an adenocarcinoma cell. For example, the cell is a pancreatic ductal adenocarcinoma cell. By inhibiting cell growth is meant that the treated cell proliferates at a lower rate or has decreased viability than an untreated cell. Cell growth is measured by proliferation assays known in the art.

By the term "siRNA" is meant a double stranded RNA molecule which prevents translation of a target mRNA. Standard techniques of introducing siRNA into the cell are used, including those in which DNA is a template from which RNA is transcribed. The siRNA includes a sense PCDH1, CDH3 or GPR107 nucleic acid sequence, an anti-sense PCDH1, CDH3 or GPR107 nucleic acid sequence or both. The siRNA may comprise two complementary molecules or may be constructed such that a single transcript has both the sense and complementary antisense sequences from the target gene, e.g., a hairpin, which, in some embodiments, leads to production of micro RNA (miRNA).

The method is used to alter gene expression in a cell in which expression of PCDH1, CDH3 or GPR107 is up-regulated, e.g., as a result of malignant transformation of the cells. Binding of the siRNA to an PCDH1, CDH3 or GPR107 transcript in the target cell results in a reduction in PCDH1, CDH3 or GPR107 production by the cell. The length of the oligonucleotide is at least about 10 nucleotides and may be as long as the naturally-occurring PCDH1, CDH3 or GPR107 transcript. Preferably, the oligonucleotide is about 19 to about 25 nucleotides in length. Most preferably, the oligonucleotide is less than

about 75, about 50, or about 25 nucleotides in length. Examples of siRNA oligonucleotides of PCDH1, CDH3 or GPR107 which inhibit PCDH1, CDH3 or GPR107 expression in mammalian cells include oligonucleotides containing target sequences, for example, nucleotides of SEQ ID NOs: 22, 23 or 24, respectively.

Methods for designing double stranded RNA having the ability to inhibit gene expression in a target cell are known. (See for example, US Patent No. 6,506,559, herein incorporated by reference in its entirety). For example, a computer program for designing siRNAs is available from the Ambion website (http://www.ambion.com/techlib/misc/siRNA_finder.html). The computer program available from Ambion, Inc. selects nucleotide sequences for siRNA synthesis based on the following protocol.

Selection of siRNA Target Sites

1. Beginning with the AUG start codon of the transcript, scan downstream for AA dinucleotide sequences. Record the occurrence of each AA and the 3' adjacent 19 nucleotides as potential siRNA target sites. Tuschl et al.,Targeted mRNA degradation by double-stranded RNA in vitro. *Genes Dev* 13(24): 3191-7 (1999), don't recommend designing siRNA to the 5' and 3' untranslated regions (UTRs) and regions near the start codon (within 75bases) as these may be richer in regulatory protein binding sites. UTR-binding proteins and/or translation initiation complexes may interfere with binding of the siRNA endonuclease complex.
2. Compare the potential target sites to the appropriate genome database (human, mouse, rat, etc.) and eliminate from consideration any target sequences with significant homology to other coding sequences. It is suggested to use BLAST, which can be found on the NCBI server at: www.ncbi.nlm.nih.gov/BLAST/
3. Select qualifying target sequences for synthesis. Selecting several target sequences along the length of the gene to evaluate is typical.

Also included in the invention are isolated nucleic acid molecules that include the nucleic acid sequence of target sequences, for example, nucleotides of SEQ ID NOs: 22, 23 and 24 or a nucleic acid molecule that is complementary to the nucleic acid sequence of nucleotides of SEQ ID NOs: 22, 23 and 24. As used herein, an "isolated nucleic acid" is a nucleic acid removed from its original environment (e.g., the natural environment if

naturally occurring) and thus, synthetically altered from its natural state. In the present invention, isolated nucleic acid includes DNA, RNA, and derivatives thereof. When the isolated nucleic acid is RNA or derivatives thereof, base "t" should be replaced with "u" in the nucleotide sequences. As used herein, the term "complementary" refers to Watson-Crick or Hoogsteen base pairing between nucleotides units of a nucleic acid molecule, and the term "binding" means the physical or chemical interaction between two nucleic acids or compounds or associated nucleic acids or compounds or combinations thereof. Complementary nucleic acid sequences hybridize under appropriate conditions to form stable duplexes containing few or no mismatches. For the purposes of this invention, two sequences having 5 or fewer mismatches are considered to be complementary. Furthermore, the sense strand and antisense strand of the isolated nucleotide of the present invention, can form double stranded nucleotide or hairpin loop structure by the hybridization. In a preferred embodiment, such duplexes contain no more than 1 mismatch for every 10 matches. In an especially preferred embodiment, where the strands of the duplex are fully complementary, such duplexes contain no mismatches. The nucleic acid molecule is less than 3851, 3205, or 6840 nucleotides in length for PCDH1, CDH3 or GPR107, respectively. For example, the nucleic acid molecule is less than about 500, about 200, or about 75 nucleotides in length. Also included in the invention is a vector containing one or more of the nucleic acids described herein, and a cell containing the vectors. The isolated nucleic acids of the present invention are useful for siRNA against PCDH1, CDH3 or GPR107, or DNA encoding the siRNA. When the nucleic acids are used for siRNA or coding DNA thereof, the sense strand is preferably longer than about 19 nucleotides, and more preferably longer than 21 nucleotides.

The invention is based in part on the discovery that the gene encoding PCDH1, CDH3 or GPR107 is over-expressed in pancreatic ductal adenocarcinoma (PDACa) compared to non-cancerous pancreatic tissue. The cDNA of PCDH1, CDH3 or GPR107 is 3851, 3205 or 6840 nucleotides in length. The nucleic acid and polypeptide sequences of PCDH1, CDH3 or GPR107 are shown in SEQ ID NO: 1 and 2, 3 and 4 or 5 and 6, respectively. The sequence data are also available via following accession numbers.

PCDH1(CFUPC): L11370, NM_002587

CDH3: X63629, NM_001793

GPR107: NM_032925, NM_020960, (KIAA1624: R39794) AB046844

Transfection of siRNAs comprising SEQ ID NOs: 22, 23 and 24 resulted in a growth inhibition of PDACa cell lines. PCDH1 (CFUPC) belongs to the protocadherin family, the largest subgroup of cadherin superfamily of calcium-dependent cell-cell adhesion molecules. Many of the protocadherin are highly expressed in the central nervous system and they are likely to play roles in neuronal circuit development and the modulation of synaptic transmission (Sano K, Tanihara H, Heimark RL, Obata S, Davidson M, St John T, Taketani S, Suzuki S. Protocadherins: a large family of cadherin-related molecules in central nervous system. *EMBO J.*, 12:2249-56, 1993.Frank M, and Kemler R. Protocadherins. *Curr Opin Cell Biol.*, 14:557-62, 2002). However, PCDH1 is abundant in pancreatic cancer cells, but not in central nervous system (Figure 3A), and its function remains unknown.

CDH3 is also a classical member of the cadherin family (Shimoyama Y, Yoshida T, Terada M, Shimosato Y, Abe O, Hirohashi S. Molecular cloning of a human Ca2+-dependent cell-cell adhesion molecule homologous to mouse placental cadherin: its low expression in human placental tissues. *J Cell Biol.*, 109:1787-94. 1989) and they link to catenins and cytoskeletons through its conserved intracellular domain, mediating signal-transduction that control cell polarity, differentiation, motility and cell growth (Christofori G. Changing neighbors, changing behavior: cell adhesion molecules-mediated signaling during tumor progression. *EMBO J.*, 22, 2318-2323, 2003). However, different form E-cadherin or N-cadherin, the function of CDH3 still remains unclear. Its expression is observed in mammary glands and ovary, and loss of expression was reported in breast cancer and prostate cancer, although the expression of P-cadherin in breast cancer correlates with poor prognosis (Peralta Soler A, Knudsen KA, Salazar H, Han AC, Keshgegian AA. P-cadherin expression in breast carcinoma indicates poor survival. *Cancer,* 86:1263-1272. 1999).

GPR107 (KIAA1624) is one of the G protein-coupled receptors (GPCR) with seven transmembranes. A large percentage of today's prescription drugs target one or more GPCRs with most major therapeutic area being served to some extent by several GPCR-based drugs. Clearly, GPCRs are in the highest rank in the terms of drug discovery potential. GPR107 is expressed without restriction in normal heart, placenta, skeletal muscle, prostate, testis, ovary, spinal cord as shown in Northern blot analysis (Figure 3C). This is not abundant in major vital organs, suggesting that targeting for these molecules

would be expected to lead less toxicity in human body.

Methods of inhibiting cell growth

The present invention relates to inhibiting cell growth, i.e, cancer cell growth by inhibiting expression of PCDH1, CDH3 or GPR107. Expression of PCDH1, CDH3 or GPR107 is inhibited, for example, by small interfering RNA (siRNA) that specifically target the PCDH1, CDH3 or GPR107 gene. PCDH1, CDH3 or GPR107 targets include, for example, nucleotides of SEQ ID NOs: 22, 23 and 24.

In non-mammalian cells, double-stranded RNA (dsRNA) has been shown to exert a strong and specific silencing effect on gene expression, which is referred as RNA interference (RNAi) (Sharp PA. RNAi and double-strand RNA. Genes Dev. 1999 Jan 15;13(2):139-41.). dsRNA is processed into 20-23 nucleotides dsRNA called small interfering RNA (siRNA) by an enzyme containing RNase III motif. The siRNA specifically targets complementary mRNA with a multicomponent nuclease complex (Hammond SM, Bernstein E, Beach D, Hannon GJ. An RNA-directed nuclease mediates post-transcriptional gene silencing in Drosophila cells. Nature. 2000 Mar 16;404(6775):293- 6; Hannon GJ. RNA interference. Nature. 2002 Jul 11;418(6894):244-51.). In mammalian cells, siRNA composed of 20 or 21-mer dsRNA with 19 complementary nucleotides and 3' terminal noncomplementary dimmers of thymidine or uridine, have been shown to have a gene specific knock-down effect without inducing global changes in gene expression (Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature. 2001 May 24;411(6836):494-8.). In addition, plasmids containing small nuclear RNA (snRNA) U6 or polymerase III H1-RNA promoter effectively produce such short RNA recruiting type III class of RNA polymerase III and thus can constitutively suppress its target mRNA Miyagishi M, Taira K. U6 promoter-driven siRNAs with four uridine 3' overhangs efficiently suppress targeted gene expression in mammalian cells.Nat Biotechnol. 2002 May;20(5):497-500. ; Brummelkamp TR, Bernards R, Agami R. A System for Stable Expression of Short Interfering RNAs in Mammalian Cells Science. 296(5567):550-553, April 19, 2002.).

The growth of cells are inhibited by contacting a cell, with a composition containing a siRNA of PCDH1, CDH3 or GPR107. The cell is further contacted with a transfection agent. Suitable transfection agents are known in the art. By inhibition of cell growth is meant the cell proliferates at a lower rate or has decreased viability compared to a cell not exposed to the composition. Cell growth is measured by methods known in the art such as, the MTT cell proliferation assay.

The siRNA of PCDH1, CDH3 or GPR107 is directed to a single target of PCDH1, CDH3 or GPR107 gene sequence. Alternatively, the siRNA is directed to multiple target of PCDH1, CDH3 or GPR107 gene sequences. For example, the composition contains siRNA of PCDH1, CDH3 or GPR107 directed to two, three, four, or five or more target sequences of PCDH1, CDH3 or GPR107. By PCDH1, CDH3 or GPR107 target sequence is meant a nucleotide sequence that is identical to a portion of the PCDH1, CDH3 or GPR107 gene. The target sequence can include the 5' untranslated (UT) region, the open reading frame (ORF) or the 3' untranslated region of the human PCDH1, CDH3 or GPR107 gene. Alternatively, the siRNA is a nucleic acid sequence complementary to an upstream or downstream modulator of PCDH1, CDH3 or GPR107 gene expression. Examples of upstream and downstream modulators include, a transcription factor that binds the PCDH1, CDH3 or GPR107 gene promoter, a kinase or phosphatase that interacts with the PCDH1, CDH3 or GPR107 polypeptide, a PCDH1, CDH3 or GPR107 promoter or enhancer. siRNA of PCDH1, CDH3 or GPR107 which hybridize to target mRNA decrease or inhibit production of the PCDH1, CDH3 or GPR107 polypeptide product encoded by the PCDH1, CDH3 or GPR107 gene by associating with the normally single-stranded mRNA transcript, thereby interfering with translation and thus, expression of the protein. Thus, siRNA molecules of the invention can be defined by their ability to hybridize specifically to mRNA or cDNA from a PCDH1, CDH3 or GPR107 gene under stringent conditions. For the purposes of this invention the terms "hybridize" or "hybridize specifically" are used to refer the ability of two nucleic acid molecules to hybridize under "stringent hybridization conditions." The phrase "stringent hybridization conditions" refers to conditions under which a nucleic acid molecule will hybridize to its target sequence, typically in a complex mixture of nucleic acids, but not detectably to other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An

extensive guide to the hybridization of nucleic acids is found in Tijssen, *Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Probes*, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, stringent conditions are selected to be about 5-10°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength pH. The T_m is the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m, 50% of the probes are occupied at equilibrium). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal is at least two times background, preferably 10 times background hybridization. Exemplary stringent hybridization conditions can be as following: 50% formamide, 5x SSC, and 1% SDS, incubating at 42°C, or, 5x SSC, 1% SDS, incubating at 65°C, with wash in 0.2x SSC, and 0.1% SDS at 50°C.

The siRNA of the invention is less than about 500, about 200, about 100, about 50, or about 25 nucleotides in length. Preferably the siRNA is about 19 to about 25 nucleotides in length. Exemplary nucleic acid sequence for the production of PCDH1, CDH3 or GPR107 siRNA include the sequences of nucleotides of SEQ ID NOs: 22, 23 or 24 as the target sequence, respectively. Furthermore, in order to enhance the inhibition activity of the siRNA, nucleotide "u" can be added to 3'end of the antisense strand of the target sequence. The number of "u"s to be added is at least about 2, generally about 2 to about 10, preferably about 2 to about 5. The added "u"s form single strand at the 3'end of the antisense strand of the siRNA.

The cell is any cell that expresses or over-expresses PCDH1, CDH3 or GPR107. The cell is an epithelial cell such as a pancreatic ductal cell. Alternatively, the cell is a tumor cell such as a carcinoma, adenocarcinoma, blastoma, leukemia, myeloma, or sarcoma. The cell is a pancreatic ductal adenocarcinoma.

An siRNA of PCDH1, CDH3 or GPR107 is directly introduced into the cells in a form that is capable of binding to the mRNA transcripts. Alternatively, the DNA encoding the siRNA of PCDH1, CDH3 or GPR107 is in a vector.

Vectors are produced for example by cloning a PCDH1, CDH3 or GPR107 target sequence into an expression vector operatively-linked regulatory sequences flanking the

PCDH1, CDH3 or GPR107 sequence in a manner that allows for expression (by transcription of the DNA molecule) of both strands (Lee, N.S., Dohjima, T., Bauer, G., Li, H., Li, M.-J., Ehsani, A.,Salvaterra, P., and Rossi, J. (2002) Expression of small interfering RNAs targeted against HIV-1 rev transcripts in human cells. Nature Biotechnology 20 : 500-505.). An RNA molecule that is antisense to PCDH1, CDH3 or GPR107 mRNA is transcribed by a first promoter (e.g., a promoter sequence 3' of the cloned DNA) and an RNA molecule that is the sense strand for the PCDH1, CDH3 or GPR107 mRNA is transcribed by a second promoter (e.g., a promoter sequence 5' of the cloned DNA). The sense and antisense strands hybridize *in vivo* to generate siRNA constructs for silencing of the PCDH1, CDH3 or GPR107 gene. Alternatively, two constructs are utilized to create the sense and anti-sense strands of a siRNA construct. Cloned PCDH1, CDH3 or GPR107 can encode a construct having secondary structure, e.g., hairpins, wherein a single transcript has both the sense and complementary antisense sequences from the target gene.

A loop sequence consisting of an arbitrary nucleotide sequence can be located between the sense and antisense sequence in order to form the hairpin loop structure. Thus, the present invention also provides siRNA having the general formula 5'-[A]-[B]-[A']-3', wherein [A] is a ribonucleotide sequence corresponding to a sequence that specfically hybridizes to an mRNA or a cDNA from PCDH1, CDH3 or GPR107. In preferred embodiments, [A] is a ribonucleotide sequence corresponding to a sequence selected from the group consisting of nucleotides of SEQ ID NOs: 22, 23 and 24,

[B] is a ribonucleotide sequence consisting of 3 to 23 nucleotides, and

[A'] is a ribonucleotide sequence consisting of the complementary sequence of [A]

The region [A] hybridizes to [A'], and then a loop consisting of region [B] is formed. The loop sequence may be preferably about 3 to about 23 nucleotide in length. The loop sequence, for example, can be selected from group consisting of following sequences (http://www.ambion.com/techlib/tb/tb_506.html). Furthermore, loop sequence consisting of 23 nucleotides also provides active siRNA (Jacque, J.-M., Triques, K., and Stevenson, M. (2002) Modulation of HIV-1 replication by RNA interference. Nature 418 : 435-438.).

CCC, CCACC or CCACACC: Jacque, J. M., Triques, K., and Stevenson, M (2002) Modulation of HIV-1 replication by RNA interference. Nature, Vol. 418: 435-438.

UUCG: Lee, N.S., Dohjima, T., Bauer, G., Li, H., Li, M.-J., Ehsani, A., Salvaterra,

P., and Rossi, J. (2002) Expression of small interfering RNAs targeted against HIV-1 rev transcripts in human cells. Nature Biotechnology 20 : 500-505. Fruscoloni, P., Zamboni, M., and Tocchini-Valentini, G. P. (2003) Exonucleolytic degradation of double-stranded RNA by an activity in Xenopus laevis germinal vesicles. Proc. Natl. Acad. Sci. USA 100(4): 1639-1644.

UUCAAGAGA: Dykxhoorn, D. M., Novina, C. D., and Sharp, P. A. (2002) Killing the messenger: Short RNAs that silence gene expression. Nature Reviews Molecular Cell Biology 4: 457-467.

For example, preferable siRNAs having hairpin loop structure of the present invention are shown below. In the following structure, the loop sequence can be selected from group consisting of CCC, UUCG, CCACC, CCACACC, and UUCAAGAGA. Preferable loop sequence is UUCAAGAGA ("ttcaagaga" in DNA (SEQ ID NO:35)).

GACAUCAAUGACAACACAC-[B]-GUGUGUUGUCAUUGAUGUC (for target sequence of SEQ ID NO:22)

GGAGACAGGCUGGUUGUUG-[B]-CAACAACCAGCCUGUCUCC (for target sequence of SEQ ID NO:23)

GUGGCUCUACCAGCUCCUG-[B]-CAGGAGCUGGUAGAGCCAC (for target sequence of SEQ ID NO:24)

The regulatory sequences flanking the PCDH1, CDH3 or GPR107 sequence are identical or are different, such that their expression can be modulated independently, or in a temporal or spatial manner. siRNAs are transcribed intracellularly by cloning the PCDH1, CDH3 or GPR107 gene templates into a vector containing, e.g., a RNA polymerase III transcription unit from the small nuclear RNA (snRNA) U6 or the human H1 RNA promoter. For introducing the vector into the cell, transfection-enhancing agent can be used. FuGENE (Roche Diagnostices), Lipofectamine 2000 (Invitrogen), Oligofectamine (Invitrogen), and Nucleofector (Wako pure Chemical) are useful as the transfection-enhancing agent.

Oligonucleotides and oligonucleotides complementary to various portions of PCDH1, CDH3 or GPR107 mRNA were tested *in vitro* for their ability to decrease production of PCDH1, CDH3 or GPR107 in tumor cells (e.g., using the pancreatic cell line such as pancreatic ductal adenocarcinoma(PDACa) cell line) according to standard

methods. A reduction in PCDH1, CDH3 or GPR107 gene product in cells contacted with the candidate siRNA composition compared to cells cultured in the absence of the candidate composition is detected using specific antibodies of PCDH1, CDH3 or GPR107 or other detection strategies. Sequences which decrease production of PCDH1, CDH3 or GPR107 in *in vitro* cell-based or cell-free assays are then tested for there inhibitory effects on cell growth. Sequences which inhibit cell growth *in vitro* cell-based assay are test *in vivo* in rats or mice to confirm decreased PCDH1, CDH3 or GPR107 production and decreased tumor cell growth in animals with malignant neoplasms.

Methods of treating malignant tumors

Patients with tumors characterized as over-expressing PCDH1, CDH3 or GPR107 are treated by administering siRNA of PCDH1, CDH3 or GPR107. siRNA therapy is used to inhibit expression of PCDH1, CDH3 or GPR107 in patients suffering from or at risk of developing, for example, pancreatic ductal adenocarcinoma (PDACa). Such patients are identified by standard methods of the particular tumor type. Pancreatic ductal adenocarcinoma (PDACa) is diagnosed for example, by CT, MRI, ERCP, MRCP, computer tomography, or ultrasound. Treatment is efficacious if the treatment leads to clinical benefit such as, a reduction in expression of PCDH1, CDH3 or GPR107, or a decrease in size, prevalence, or metastatic potential of the tumor in the subject. When treatment is applied prophylactically, "efficacious" means that the treatment retards or prevents tumors from forming or prevents or alleviates a symptom of clinical symptom of the tumor. Efficaciousness is determined in association with any known method for diagnosing or treating the particular tumor type.

siRNA therapy is carried out by administering to a patient a siRNA by standard vectors encoding the siRNAs of the invention and/or gene delivery systems such as by delivering the synthetic siRNA molecules. Typically, synthetic siRNA molecules are chemically stabilized to prevent nuclease degradation *in vivo*. Methods for preparing chemically stabilized RNA molecules are well known in the art. Typically, such molecules comprise modified backbones and nucleotides to prevent the action of ribonucleases. Other modifications are also possible, for example, cholesterol-conjugated siRNAs have shown improved pharmacological properties. (Song *et al. Nature Med.* 9:347-351 (2003)). Suitable gene delivery systems may include liposomes, receptor-mediated delivery systems,

or viral vectors such as herpes viruses, retroviruses, adenoviruses and adeno-associated viruses, among others. A therapeutic nucleic acid composition is formulated in a pharmaceutically acceptable carrier. The therapeutic composition may also include a gene delivery system as described above. Pharmaceutically acceptable carriers are biologically compatible vehicles which are suitable for administration to an animal, e.g., physiological saline. A therapeutically effective amount of a compound is an amount which is capable of producing a medically desirable result such as reduced production of a PCDH1, CDH3 or GPR107 gene product, reduction of cell growth, e.g., proliferation, or a reduction in tumor growth in a treated animal.

Parenteral administration, such as intravenous, subcutaneous, intramuscular, and intraperitoneal delivery routes, may be used to deliver siRNA compositions of PCDH1, CDH3 or GPR107. For treatment of pancreatic tumors, direct infusion the celiac artery, splenic artery, or common hepatic artery, is useful.

Dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular nucleic acid to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Dosage for intravenous administration of nucleic acids is from approximately 10^6 to 10^{22} copies of the nucleic acid molecule.

The polynucleotides are administered by standard methods, such as by injection into the interstitial space of tissues such as muscles or skin, introduction into the circulation or into body cavities or by inhalation or insufflation. Polynucleotides are injected or otherwise delivered to the animal with a pharmaceutically acceptable liquid carrier, e.g., a liquid carrier, which is aqueous or partly aqueous. The polynucleotides are associated with a liposome (e.g., a cationic or anionic liposome). The polynucleotide includes genetic information necessary for expression by a target cell, such as a promoters.

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

case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Brief Description of the Drawings

Figure 1 depicts photographs showing the results of validation of over expression of PCDH1 (A) and CDH3 (B) in the PDACa cells by RT-PCR. The microdissected normal pancreatic ductal epithelial cells (Normal) and vital organs (lung, heart, liver, kidney and bone marrow) form the same individual were compared by semiquantitative RT-PCR.

Figure 2 depicts photographs showing the result of immunohistochemistry in PDACa tissues. Over-expression of CDH3 protein was observed in pancreatic ductal adenocarcinoma, but not in normal pancreatic duct.

Figure 3 depicts photographs of Northern blot analysis showing the expression pattern in normal adult tissues of each target genes for pancreatic cancer. (A) PCDH1, (B) CDH3 and (C) GPR107.

Figure 4 depicts photographs showing the effect of Knocking-down endogenous PCDH1 in PDACa cell, PK-45P, by siRNA. Figure 4 (A) shows the results of RT-PCR. It validated knockdown effect of PCDH1 mRNA by transfection of siRNA expression vector 410si, but not by EGFPsi. The 410si was designed specifically for PCDH1 mRNA sequence, and EGFP was for EGFP mRNA sequence. RNA was harvested 48 hours after transfection and analyzed. ACTB was used to normalize input cDNA. Figure 4 (B) is a photograph showing the results of Colony formation assay. It showed drastic decrease of colony numbers in the cells one week after transfection with 410si that was validated to knock down PCDH1 effectively by RT-PCR. Figure 4 (C) is a bar chart showing the results MTT assay. It also showed drastic decreased number of the grown cells transfected with 410si but not by EGFPsi.

Figure 5 depicts photographs showing the effect of Knocking-down endogenous CDH3 in PDACa cell, KLM-1, by siRNA. Figure 5 (A) shows the results of RT-PCR. It validated knockdown effect of CDH3 mRNA by transfection of siRNA expression vectors si24 but not by EGFPsi. The si24 was designed specifically

for CDH3 mRNA sequence, and EGFPsi was for EGFP mRNA sequence. RNA was harvested 48 hours after transfection and analyzed. ACTB was used to normalize input cDNA. Figure 5 (B) is a photograph showing the results of Colony formation assay. It showed drastic decrease of colony numbers in the cells one week after transfection with si24 that was validated to knock down CDH3 effectively by RT-PCR. Figure 5 (C) is a bar chart showing the results MTT assay. It also showed drastic decreased number of the grown cells transfected with si24, but not by EGFPsi.

Figure 6 depicts photographs showing the effect of Knocking-down endogenous GPR107 in PDACa cell, KLM-1, by siRNA. Figure 6 (A) shows the results of RT-PCR. It validated knockdown effect of GPR107 mRNA by transfection of siRNA expression vectors 1003si, but not by and EGFPsi. The 1003si was designed specifically for GPR107 mRNA sequence, and EGFPsi was for EGFP mRNA sequence. RNA was harvested 48 hours after transfection and analyzed. ACTB was used to normalize input cDNA. Figure 6 (B) is a photograph showing the results of Colony formation assay. It showed decrease of colony numbers in the cells one week after transfection with 1003si that was validated to knock down GPR107 effectively by RT-PCR. Figure 6 (C) is a bar chart showing the results MTT assay. It also showed decreased number of the grown cells transfected with 1003si, but not by EGFPsi.

Best Mode for Carrying out the Invention

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

[Example 1] General Methods

Cell lines and tissue specimens

Human Pancreatic cell lines PK45P, KLM1 and MIA-PaCa2 (ATCC Number: CRL-1420) were obtained from the Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University. All these cells are publicly available.

Isolation of over-expressing genes in PDACa cells by using cDNA microarray

Fabrication of the cDNA microarray slides has been described (Ono K, Tanaka T,

Tsunoda T, Kitahara O, Kihara C, Okamoto A, Ochiai K, Takagi T, and Nakamura Y. *Cancer Res.*, 60: 5007-5011, 2000). For each analysis of expression profiles it was prepared duplicate sets of cDNA microarray slides containing approximately 23,040 DNA spots, to reduce experimental fluctuation. Briefly, total RNA was purified from PDACa cells and normal pancreatic duct epithelium microdissected from 18 pancreatic cancer tissues. T7-based RNA amplification was carried out to obtain adequate RNA for microarray experiments. Aliquots of amplified RNA from PDACa cells and normal duct epithelium were labeled by reverse transcription with Cy5-dCTP and Cy3-dCTP, respectively (Amersham Biosciences). Hybridization, washing, and detection were carried out as described previously (Ono K, Tanaka T, Tsunoda T, Kitahara O, Kihara C, Okamoto A, Ochiai K, Takagi T, and Nakamura Y. *Cancer Res.*, 60: 5007-5011, 2000). Subsequently, among the up-regulated genes, it was focused three genes, PCDH1, CDH3 and GPR107 because its expression ratio was greater than 5.0 in more than 50% of informative cancers and their expression level in normal vital major organs was relatively low according to the our previous data of gene expression in 29 normal human tissues (Saito-Hisaminato A, Katagiri T, Kakiuchi S, Nakamura T, Tsunoda T, Nakamura Y. Genome-wide profiling of gene expression in 29 normal human tissues with a cDNA microarray. *DNA Res.*, 9: 35-45, 2002).

Semiquantitative RT-PCR for PCDH1 and CDH3

RNA from the microdissected PDACa cells and normal pancreatic ductal epithelial cells were subject to two-round amplification by T7-based *in vitro* transcription (Epicentre Technologies) and synthesized to single-strand cDNA. It was prepared appropriate dilutions of each single-stranded cDNA for subsequent PCR amplification by monitoring β-actin (ACTB) as a quantitative control. The primer sequences the present inventors used were 5'-AGAAGGAGACCAAGGACCTGTAT-3' (SEQ.ID.NO.7) and
5'-AGAACTTTATTGTCAGGGTCAAGG-3' (SEQ.ID.NO.8) for PCDH1,
5'-CTGAAGGCGGCTAACACAGAC-3' (SEQ.ID.NO.9) and
5'-TACACGATTGTCCTCACCCTTC-3' (SEQ.ID.NO.10) for CDH3, and
5'-CATCCACGAAACTACCTTCAACT-3' (SEQ.ID.NO.11) and
5'-TCTCCTTAGAGAGAAGTGGGGTG-3' (SEQ.ID.NO.12) for ACTB. All reactions involved initial denaturation at 94°C for 2 min followed by 21 cycles (for ACTB)

or 28-32 cycles (for PCDH1 and CDH3) at 94°C for 30 s, 58°C for 30 s, and 72°C for 1 min, on a GeneAmp PCR system 9700 (PE Applied Biosystems).

Immunohistochemistry

Formalin-fixed and paraffin-embedded PDACa sections were immunostained using a mouse anti-CDH3 monoclonal antibody (BD Transduction Laboratories) for CDH3 expression. Deparaffinized tissue sections were placed in 10 mM citrate buffer, pH 6.0, and heated to 108°C in an autoclave for 15 minutes for antigen retrieval. Sections were incubated with a 1:10 dilution or a 1:100 dilution of primary antibody for CDH3, respectively, in a humidity chamber for an hour at room temperature, and developed with peroxidase labeled-dextran polymer followed by diaminobenzidine (DAKO Envision Plus System; DAKO Corporation, Carpinteria, CA). Sections were counterstained with hematoxylin. For negative controls, primary antibody was omitted.

Northern blot analysis

Human multiple-tissue Northern blots (Clontech) were hybridized with a [$\tilde{\alpha}^{32}$P] dCTP-labeled PCR product amplified by the primers described above. Pre-hybridization, hybridization and washing were performed according to the supplier's recommendations. The blots were auto-radiographed with intensifying screens at -80°C for 5 days.

Construction of psiU6BX Plasmid

The DNA flagment encoding siRNA was inserted into the GAP at nucleotide 485-490 as indicated (-) in the following plasmid sequence (SEQ ID No: 26).

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GACGGATCGGGAGATCTCCCGATCCCCTATGGTGCACTCTCAGTACAATCTGCTCTGGAT
CCACTAGTAACGGCCGCCAGTGTGCTGGAATTCGGCTTGGGGATCAGCGTTTGAGTAAGA
GCCCGCGTCTGAACCCTCCGCGCCGCCCCGGCCCCAGTGGAAAGACGCGCAGGCAAAACG
CACCACGTGACGGAGCGTGACCGCGCGCCGAGCGCGCGCCAAGGTCGGGCAGGAAGAGGG
CCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAAT
TAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTA
ATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCT
TACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAA
CACC------TTTTTACATCAGGTTGTTTTTCTGTTTGGTTTTTTTTTTACACCACGTTT
ATACGCCGGTGCACGGTTTACCACTGAAAACACCTTTCATCTACAGGTGATATCTTTTAA
CACAAATAAAATGTAGTAGTCCTAGGAGACGGAATAGAAGGAGGTGGGGCCTAAAGCCGA
ATTCTGCAGATATCCATCACACTGGCGGCCGCTCGAGTGAGGCGGAAAGAACCAGCTGGG
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GCTCTAGGGGGTATCCCCACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGG

TTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCT

TCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCC

CTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTG

ATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGT

CCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGG

TCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGC

TGATTTAACAAAAATTTAACGCGAATTAATTCTGTGGAATGTGTGTCAGTTAGGGTGTGG

AAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGC

AACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCT

CAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCC

CAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGA

GGCCGCCTCTGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGG

CTTTTGCAAAAAGCTCCCGGGAGCTTGTATATCCATTTTCGGATCTGATCAAGAGACAGG

ATGAGGATCGTTTCGCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTG

GGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGC

CGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGG

TGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGT

TCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGG

CGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCAT

CATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCA

CCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCA

GGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAA

GGCGCGCATGCCCGACGGCGAGGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAA

TATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGC

GGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGA

ATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGC

CTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGGTTCGAAATGACCGAC

CAAGCGACGCCCAACCTGCCATCACGAGATTTCGATTCCACCGCCGCCTTCTATGAAAGG

TTGGGCTTCGGAATCGTTTTCCGGGACGCCGGCTGGATGATCCTCCAGCGCGGGGATCTC

ATGCTGGAGTTCTTCGCCCACCCCAACTTGTTTATTGCAGCTTATAATGGTTACAAATAA

AGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGT

TTGTCCAAACTCATCAATGTATCTTATCATGTCTGTATACCGTCGACCTCTAGCTAGAGC

TTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCA

CACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAA

CTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAG

CTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCC

GCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCT

CACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATG

TGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTC
CATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGA
AACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCT
CCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTG
GCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAG
CTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTAT
CGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAAC
AGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAAC
TACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTC
GGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTTTTTTT
GTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTT
TCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGA
TTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATC
TAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCT
ATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATA
ACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCA
CGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGA
AGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGA
GTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTG
GTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGA
GTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTT
GTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCT
CTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCA
TTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAAT
ACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGA
AAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCC
AACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGG
CAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTC
CTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTT
GAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCA
CCTGACGTC

snRNA U6 gene is reported to be transcribed by RNA polymerase III, which produce short transcripts with uridines at the 3' end. The genomic fragment of the snRNA U6 gene containing the promoter region was amplified by PCR using a set of primers,

5'-GGGGATCAGCGTTTGAGTAA-3' (SEQ ID No: 27), and

5'-TAGGCCCCACCTCCTTCTAT-3' (SEQ ID No: 28) and human placental DNA as a template. The product was purified and cloned into pCR plasmid vector using a

TA cloning kit according to the supplier's protocol (Invitrogen). The *Bam*HI, *Xho*I fragment containing the snRNA U6 gene was purified and cloned into nucleotide 1257 to 56 fragment of pcDNA3.1(+) plasmid, which was amplified by PCR with a set of primer, 5'-TGCGGATCCAGAGCAGATTGTACTGAGAGT-3' (SEQ ID No: 29) and

5'- CTCTATCTCGAGTGAGGCGGAAAGAACCA-3' (SEQ ID No: 30). The ligated DNA was used for a template of PCR with primers,

5'-TTTAAGCTTGAAGACTATTTTTACATCAGGTTGTTTTTCT-3' (SEQ ID No: 31) and

5'-TTTAAGCTTGAAGACACGGTGTTTCGTCCTTTCCACA-3' (SEQ ID No: 32). The product was digested with HindIII, which was subsequently self-ligated to produce psiU6BX vector plasmid. For the control, psiU6BX-EGFP was prepared by cloning double-stranded oligonucleotides of

5'- CACCGAAGCAGCACGACTTCTTCTTCAAGAGAGAAGAAGTCGTGCT GCTTC-3' (SEQ ID No: 33) and

5'- AAAAGAAGCAGCACGACTTCTTCTCTTGAAGAAGAAGTCGTGCT GCTTC -3' (SEQ ID No: 34) into the BbsI site in the psiU6BX vector.

siRNA-expressing constructs

The nucleotide sequences of the siRNAs were designed using an siRNA design computer program available from the Ambion website. (http://www.ambion.com/techlib/misc/siRNA_finder.html). Briefly, nucleotide sequences for siRNA synthesis are selected using the following protocol.

Selection of siRNA Target Sites:

1. Starting with the AUG start codon of the each gene transcript, scan downstream for an AA dinucleotide sequences. The occurrence of each AA and the 3' adjacent 19 nucleotides are recorded as potential siRNA target sites. Tuschl et al. don't recommend against designing siRNA to the 5' and 3' untranslated regions (UTRs) and regions near the start codon (within 75bases) as these may be richer in regulatory protein binding sites. UTR-binding proteins and/or translation initiation complexes may interfere with binding of the siRNA endonuclease complex.

2. The potential target sites are compared to the appropriate genome database (human, mouse, rat, etc.) to eliminate target sequences with significant homology to other

coding sequences.

3. Qualifying target sequences are selected for synthesis. Several target sequences along the length of the gene are selected for evaluation.

The oligonucleotides used for siRNAs of PCDH1, CDH3 or GPR107 are shown below. Each oligionucleotide is a combination of a sense nucleotide sequence and an antisense nucleotide sequence of the target sequence. The nucleotide sequences of the hairpin loop structure and target sequence are shown in SEQ ID NO:19 to SEQ ID NO:21 and SEQ ID NO:22 to SEQ ID NO:24, respectively (endonuclease recognition cites are eliminated from each hairpin loop structure sequence).

Insert sequence of siRNA expression vectors for PCDH1

410si:

5'-CACCGACATCAATGACAACACACTTCAAGAGAGTGTGTTGTCATTGATGTC-3' (SEQ ID NO: 13) and

5'-AAAAGACATCAATGACAACACACTCTCTTGAAGTGTGTTGTCATTGATGTC-3' (SEQ ID NO:14)

Insert sequence of siRNA expression vectors for CDH3

si24:

5'-CACCGGAGACAGGCTGGTTGTTGTTCAAGAGACAACAACCAGCCTGTCTCC-3' (SEQ ID NO: 15) and

5'-AAAAGGAGACAGGCTGGTTGTTGTCTCTTGAACAACAACCAGCCTGTCTCC-3' (SEQ ID NO: 16)

Insert sequence of siRNA expression vectors for GPR107

1003si:

5'-CACCGTGGCTCTACCAGCTCCTGTTCAAGAGACAGGAGCTGGTAGAGCCAC-3' (SEQ ID NO: 17) and

5'-AAAAGTGGCTCTACCAGCTCCTGTCTCTTGAACAGGAGCTGGTAGAGCCAC-3' (SEQ ID NO: 18)

Insert sequence of siRNA expression vectors for control

EGFPsi: (control)

5'- CACCGAAGCAGCACGACTTCTTCTTCAAGAGAGAAGAAGTCGTGCTGCTTC -3' (SEQ ID NO: 33) and

5'-AAAAGAAGCAGCACGACTTCTTCTCTCTTGAAGAAGAAGTCGTGCTGCTTC-3' (SEQ ID NO: 34)

Sequence ID NO of each sequences are listed in Table1

gene	siRNA	effect	insert seq SEQ ID NO		hairpin siRNA	target SEQ ID NO	position
PCDH1	410si	+	13	14	19	22	595-613
CDH3	si24	+	15	16	20	23	556-574
GPR107	1003si	+	17	18	21	24	1570-1588
control	EGFPsi	-	33	34		25	

colony formation / MTT assay

Human PDACa cell lines among PK45P, KLM1 and MIA-PaCa2, were plated onto 10-cm dishes (5 X 10^5 cells/dish) and transfected with psiU6BX containing EGFP target sequence (EGFP) and psiU6BX containing target sequence using Lipofectamine 2000 (Invitrogen) or FuGENE6 (Roche), according to manufacture's instruction. Cells were selected by 500 mg/ml Geneticin for one week, and preliminary cells were harvested 48 hours after transfection and analyzed by RT-PCR to validate knockdown effect on PCDH1, CDH3 and GPR107. The primers of RT-PCR were the same ones described above. These cells were also stained by Giemsa solution and performed MTT assay to evaluate the colony formation and the cell number, respectively.

[Example 2] Reduction of the expression of the genes PCDH1, CDH3 or GPR107 and growth suppression of cancer cells by siRNA

In previous study, it was generated precise expression profiles of PDACa by combining laser microdissection with genome-wide cDNA microarrays with 27,000 genes spotted. The present inventors identified more than 200 genes as up-regulated genes in PDACa cells comparing with the expression pattern of normal pancreatic ductal epithelium that was thought to be the origin of PDACa (Nakamura T, Furukawa Y, Nakagawa H, Tsunoda T, Ohigashi H, Murata K, Ishikawa O, Ohgaki, Kashimura N, Miyamoto M, Hirano S, Kondo S, Katoh H, Nakamura Y, and Katagiri T. Genome-wide cDNA microarray analysis of gene-expression profiles in pancreatic cancers using populations of tumor cells and normal ductal epithelium cells selected for purity by laser microdissection. *Oncogene*, 2004 Feb 9, Epub ahead of print). Based on these expression profile of PDACa cells, the present inventors selected three over-expressing genes, and PCDH1 and CDH3

were validated their overexpression in PDACa by RT-PCR using the cDNA from microdissected PDACa cells (Figure 1A, B) or immunohistochemistry (Figure 2).

(1) PCDH1 (Protocadherin 1) (Genbank Accession No.NM_002587; SEQ ID No.1, 2)

To investigate the growth or survival effect of PCDH1 on PDACa cells, the present inventors knocked down their endogenous expression of PCDH1 specifically by mammalian vector-based RNA interference (RNAi) technique in PDACa cell line. PCDH1 is expressed inrestrictedly in normal heart, placenta, prostate as shown in Northern blot analysis (Figure 3A). This is not abundant in major vital organs, suggesting that targeting for these molecules would be expected to lead less toxicity in human body.

The transfection of the siRNA-producing vectors clearly resulted in reduction of the endogenous expression in one designed siRNA, 410si, for PCDH1 (Figure 4A). This knocking-down effect by the siRNA on PCDH1 mRNA resulted in drastic growth suppression in colony formation assay (Figure 4B) and MTT assay (Figure 4C). These findings strongly suggested that over-expression of PCDH1 in PDACa cells were associated with cancer cell viability. PCDH1 and other protocadherins are supported to have homophilic interaction on the cell surface by means of their cadherin domains and modulate intercellular signal transduction for cytoskeleton conformation, cell motility or cell growth (Sano K, Tanihara H, Heimark RL, Obata S, Davidson M, St John T, Taketani S, Suzuki S. Protocadherins: a large family of cadherin-related molecules in central nervous system. *EMBO J.*, 12:2249-56, 1993, Frank M, and Kemler R. Protocadherins. *Curr Opin Cell Biol.*, 14:557-62, 2002.). According to our data, PCDH1 is likely to modulate positive signal for pancreatic cancer cell growth through its homophilic interaction in cell-cell adhesion.

(2) CDH3 (P-cadherin) (Genbank Accession No.NM_001793; SEQ ID No.3, 4)

The present inventors validated CDH3 over-expression in PDACa cells by RT-PCR (Figure 1B) and immunohistochemistry (Figure 2), and according to the microarray data and RT-PCR (Figure 1B), CDH3 over-expression was one of the most predominant patterns among more than 200 up-regulated genes in our PDACa profiles. CDH3 is expressed inrestrictedly in normal thymus, prostate, ovary, trachea as shown in Northern blot analysis (Figure 3B). This is not abundant in major vital organs, suggesting that targeting for these molecules would be expected to lead less toxicity in human body.

To investigate the growth or survival effect of CDH3 on PDACa cells, the present inventors knocked down their endogenous expression of CDH3 specifically by mammalian vector-based RNA interference (RNAi) technique in PDACa cell line. The transfection of the siRNA-producing vectors clearly resulted in reduction of the endogenous expression in one designed siRNA, si24, for CDH3 (Figure 5A). This knocking-down effect by the siRNA on CDH3 mRNA resulted in drastic growth suppression in colony formation assay (Figure 5B) and MTT assay (Figure 5C). These findings strongly suggested that over-expression of CDH3 in PDACa cells were associated with cancer cell viability as well as cell-cell interaction, and this molecule may involve signal transduction from cell-cell interaction. PDACa is extremely aggressive and high expression of CDH3 in PDACa may be associated with their aggressiveness and metastatic potential as well.

(3) GPR107 (G protein-coupled receptor 107) (Genbank Accession No. AB046844; SEQ ID No.5, 6)

The present inventors identified this orphan GPCR as a target for pancreas cancer, which function and ligands are unknown. GPR107 is expressed inrestrictedly in normal heart, placenta, skeletal muscle, testis, ovary, spinal cord as shown in Northern blot analysis (Figure 3C). To investigate the growth or survival effect of GPR107 on PDACa cells, the present inventors knocked down their endogenous expression of GPR107 specifically by siRNA in PDACa cell line. The transfection of the siRNA-producing vectors clearly resulted in reduction of the endogenous expression in one designed siRNA, 1003si, for GPR107 (Figure 6A). This knocking-down effect by the siRNA on GPR107 mRNA resulted in growth suppression in colony formation assay (Figure 6B) and MTT assay (Figure 6C). These findings strongly suggested that over-expression of GPR107 in PDACa cells were associated with cancer cell viability.

In conclusion, the present inventors identified three membrane-type molecules over-expressed in PDACa cells and all of them are likely to be associated with cancer cell growth, suggested these membrane-type molecules are ideal molecular targets for deadly pancreatic cancer treatment.

Industrial Applicability

The present inventors have shown that the cell growth is suppressed by small interfering RNA (siRNA) that specifically target the PCDH1, CDH3 or GPR107 gene.

Thus, this novel siRNAs are useful target for the development of anti-cancer pharmaceuticals. For example, agents that block the expression of PCDH1, CDH3 or GPR107 or prevent its activity may find therapeutic utility as anti-cancer agents, particularly anti-cancer agents for the treatment of pancreatic cancer, such as pancreatic ductal adenocarcinoma (PDACa).

While the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and scope of the invention.

CLAIMS

1. A method for treating or preventing pancreatic cancer in a subject comprising administering to said subject a composition comprising a small interfering RNA (siRNA) that inhibits expression of *PCDH1, CDH3 or GPR107.*

2. The method of claim 1, wherein said siRNA comprises a sense nucleic acid sequence and an anti-sense nucleic acid sequence that specifically hybridizes to a sequence from *PCDH1, CDH3 or GPR107.*

3. The method of claim 1, wherein the pancreatic cancer is a pancreatic ductal adenocarcinoma (PDACa).

4. The method of claim 2, wherein said siRNA comprises a ribonucleotide sequence corresponding to a sequence selected from the group consisting of SEQ ID NOs: 22, 23 and 24 as the target sequence.

5. The method of claim 4, wherein said siRNA has the general formula 5'-[A]-[B]-[A']-3', wherein [A] is a ribonucleotide sequence corresponding to a sequence selected from the group consisting of nucleotides of SEQ ID NOs: 22, 23 and 24. [B] is a ribonucleotide loop sequence consisting of 3 to 23 nucleotides, and [A'] is a ribonucleotide sequence consisting of the complementary sequence of [A].

6. The method of claim 1, wherein said composition comprises a transfection-enhancing agent.

7. A double-stranded molecule comprising a sense strand and an antisense strand, wherein the sense strand comprises a ribonucleotide sequence corresponding to a target sequence selected from the group consisting of SEQ ID NOs: 22, 23 and 24, and wherein the antisense strand comprises a ribonucleotide sequence which is complementary to said sense strand, wherein said sense strand and said antisense strand hybridize to each other to form said double-stranded molecule, and wherein said double-stranded molecule, when introduced into a cell expressing the *PCDH1, CDH3 or GPR107* gene, inhibits expression of said gene.

8. The double-stranded molecule of claim 7, wherein said target sequence comprises at least about 10 contiguous nucleotides from the nucleotide sequences selected

from the group of SEQ ID NOs: 1, 3, and 5.

9. The double-stranded molecule of claim 8, wherein said target sequence comprises from about 19 to about 25 contiguous nucleotides from the nucleotide sequences selected from the group of SEQ ID NOs: 1, 3, and 5.

10. The double-stranded molecule of claim 9, wherein said double-stranded molecule is a single ribonucleotide transcript comprising the sense strand and the antisense strand linked via a single-stranded ribonucleotide sequence.

11. The double-stranded molecule of claim 8, wherein the double-stranded molecule is an oligonucleotide of less than about 100 nucleotides in length.

12. The double-stranded molecule of claim 11, wherein the double-stranded molecule is an oligonucleotide of less than about 75 nucleotides in length.

13. The double-stranded molecule of claim 12, wherein the double-stranded molecule is an oligonucleotide of less than about 50 nucleotides in length.

14. The double-stranded molecule of claim 13, wherein the double-stranded molecule is an oligonucleotide of less than about 25 nucleotides in length.

15. The double-stranded polynucleotide of claim 14, wherein the double stranded molecule is an oligonucleotide of between about 19 and about 25 nucleotides in length.

16. A vector encoding the double-stranded molecule of claim 8.

17. The vector of claim 16, wherein the vector encodes a transcript having a secondary structure and comprises the sense strand and the antisense strand.

18. The vector of claim 17, wherein the transcript further comprises a single-stranded ribonucleotide sequence linking said sense strand and said antisense strand.

19. A vector comprising a polynucleotide comprising a combination of a sense strand nucleic acid and an antisense strand nucleic acid, wherein said sense strand nucleic acid comprises nucleotide sequence of SEQ ID NOs: 22, 23 and 24, and said antisense strand nucleic acid consists of a sequence complementary to the sense strand.

20. The vector of claim 19, wherein said polynucleotide has the general formula

5'-[A]-[B]-[A']-3'

wherein [A] is a nucleotide sequence of SEQ ID NOs: 22, 23 and 24; [B] is a nucleotide sequence consisting of 3 to 23 nucleotides; and [A'] is a nucleotide sequence complementary to [A].

21. A pharmaceutical composition for treating or preventing pancreatic cancer comprising a pharmaceutically effective amount of a small interfering RNA (siRNA) that inhibits expression of *PCDH1, CDH3 or GPR107* as an active ingredient, and a pharmaceutically acceptable carrier..

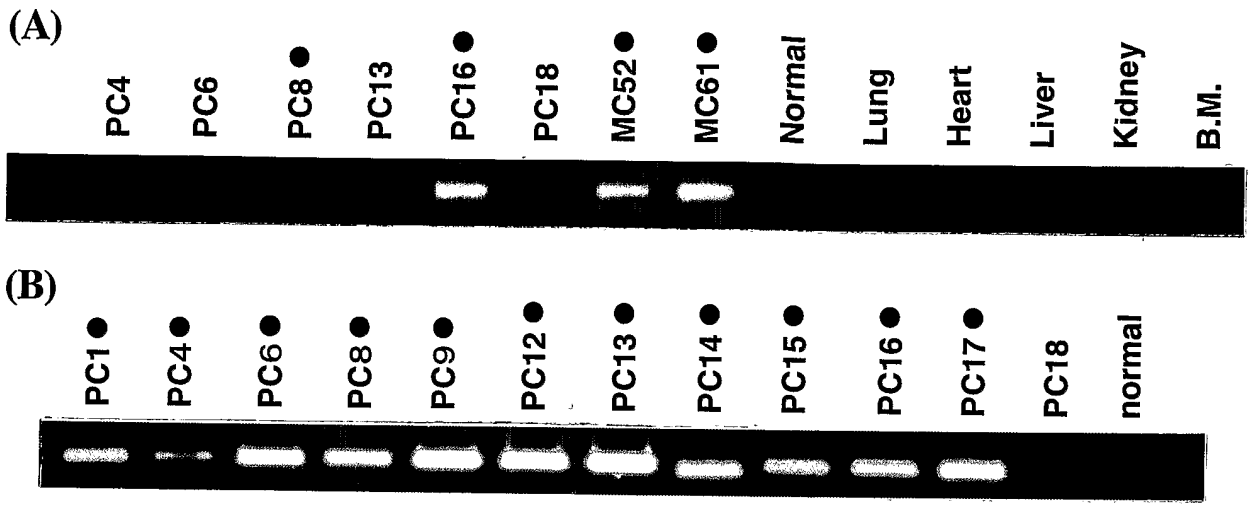
22. The pharmaceutical composition of claim 21, wherein the siRNA comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 22, 23 and 24 as the target sequence.

23. The composition of claim 22, wherein the siRNA has the general formula

5'-[A]-[B]-[A']-3'

wherein [A] is a ribonucleotide sequence corresponding to a nucleotide sequence of SEQ ID NOs: 22, 23 and 24; [B] is a ribonucleotide sequence consisting of 3 to 23 nucleotides; and [A'] is a ribonucleotide sequence complementary to [A].

Fig. 1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PC8
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PC16
PC18
MC52
MC61
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Heart
Liver
Kidney
B.M.
(B)
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PC4
PC6
PC8
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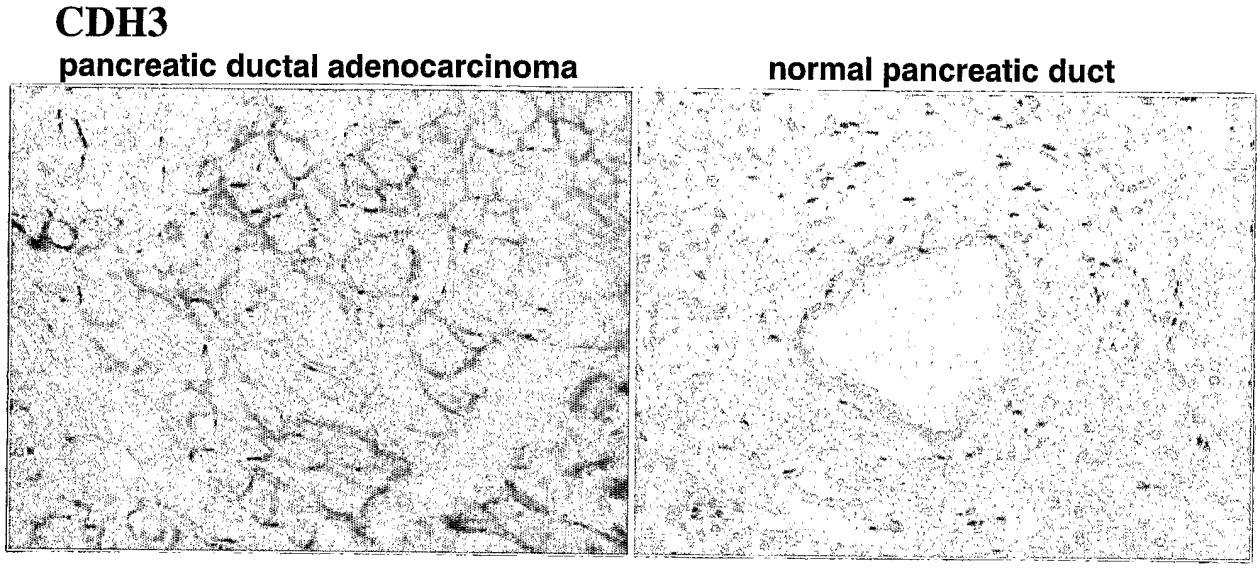
PC17
PC18
normal

Fig. 2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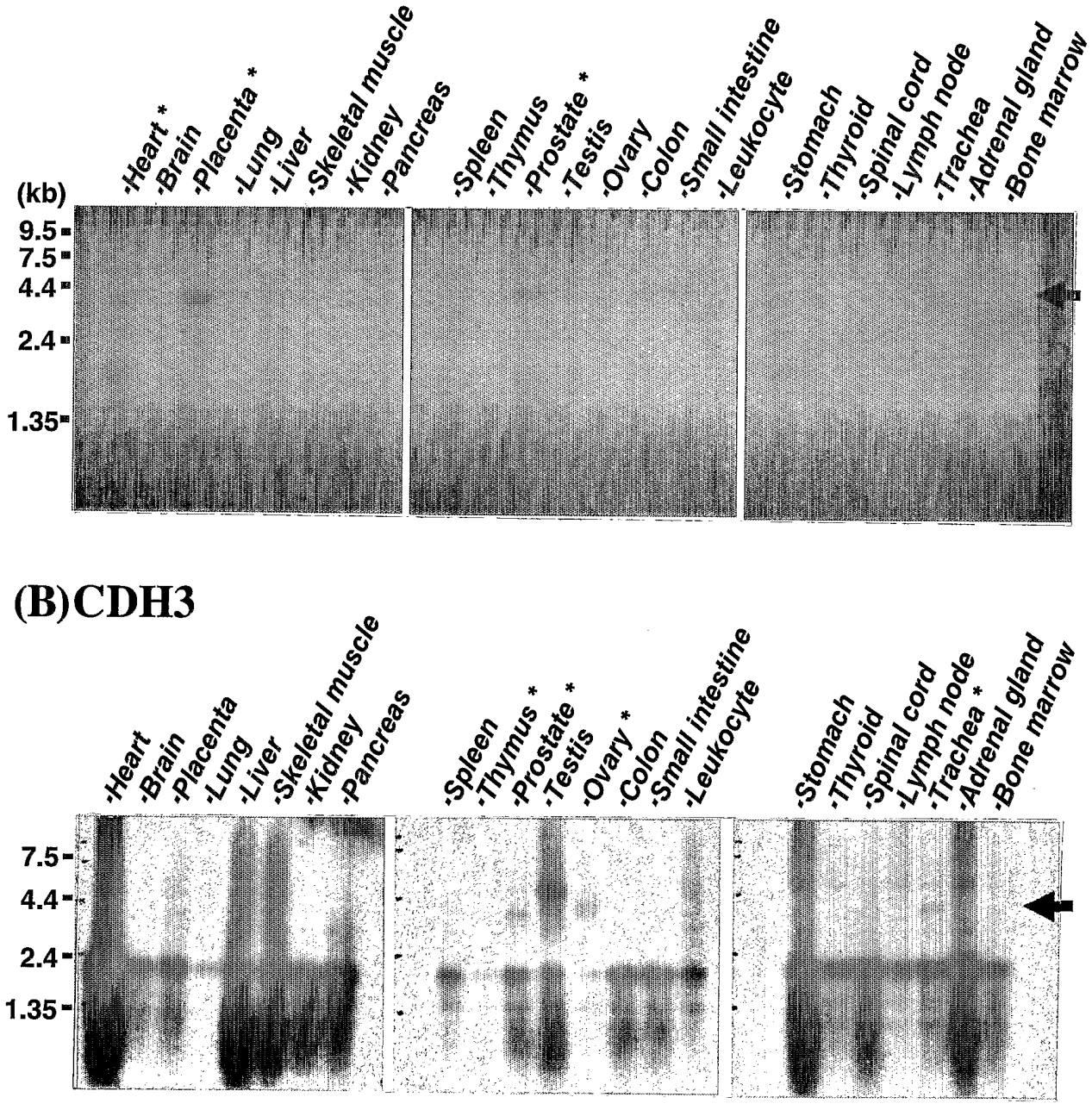
normal pancreatic duct

Fig. 3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·Placenta *
·Lung
·Liver
·Skeletal muscle
·Kidney
·Pancreas
·Spleen
·Thymus
·Prostate *
·Testis
·Ovary
·Colon
·Small intestine
·Leukocyte
·Stomach
·Thyroid
·Spinal cord
·Lymph node
·Trachea
·Adrenal gland
·Bone marrow
(kb)
9.5
7.5
4.4
2.4
1.35
(B)CDH3
·Heart
·Brain
·Placenta
·Lung
·Liver
·Skeletal muscle
·Kidney
·Pancreas
·Spleen
·Thymus *
·Prostate *
·Testis
·Ovary *
·Colon
·Small intestine
·Leukocyte
·Stomach
·Thyroid
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·Trachea *
·Adrenal gland
·Bone marrow
7.5
4.4
2.4
1.35

Fig. 3

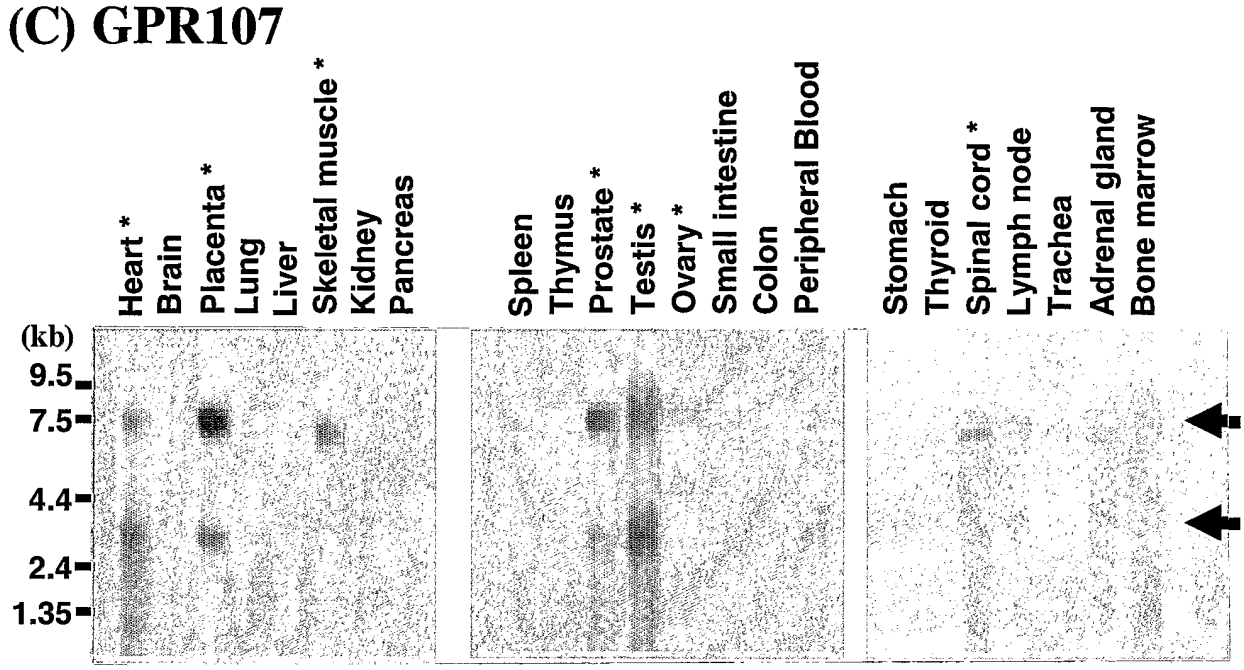
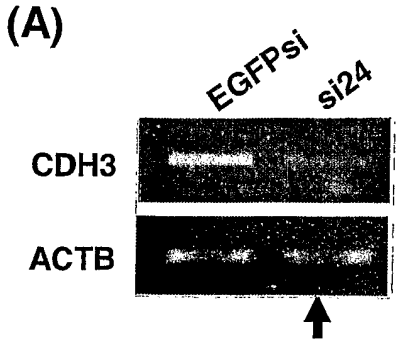

(C) GPR107
Heart *
Brain
Placenta *
Lung
Liver
Skeletal muscle *
Kidney
Pancreas
Spleen
Thymus
Prostate *
Testis *
Ovary *
Small intestine
Colon
Peripheral Blood
Stomach
Thyroid
Spinal cord *
Lymph node
Trachea
Adrenal gland
Bone marrow
(kb)
9.5
7.5
4.4
2.4
1.35

Fig. 5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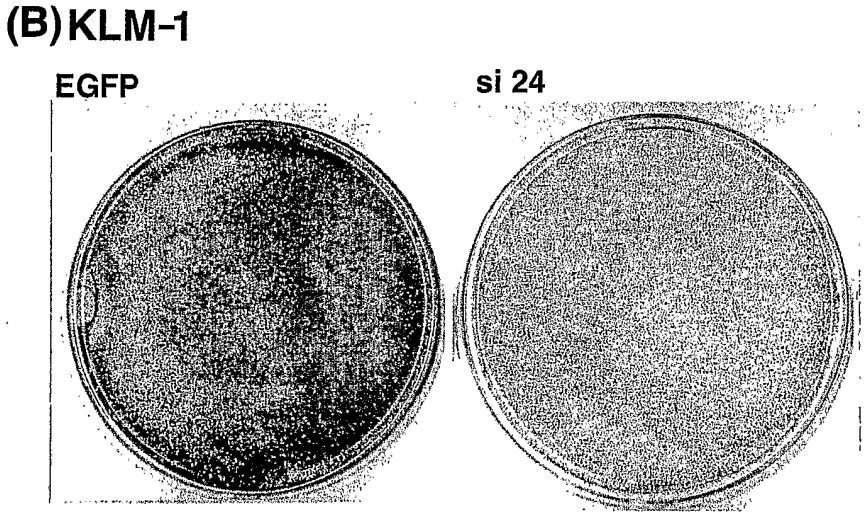
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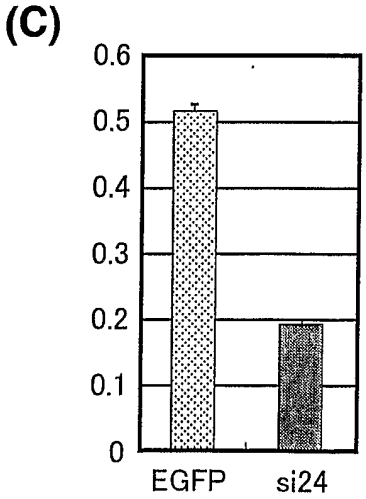
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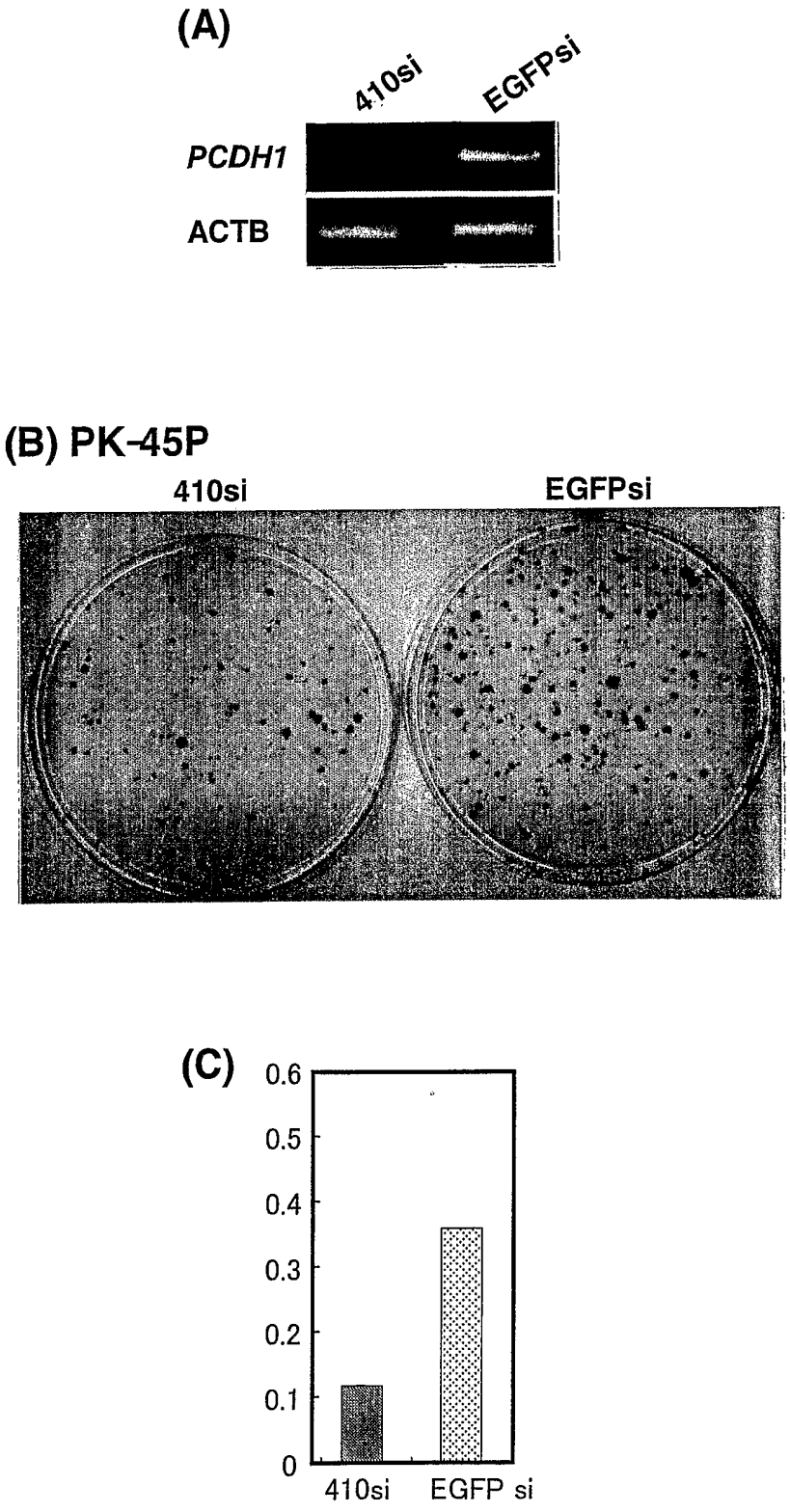
0
EGFP
si24

Fig. 4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ACTB
(B) PK-45P
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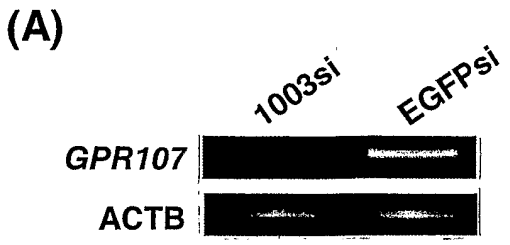
0.2
0.1
0
410si
EGFP si

Fig. 6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GPR107
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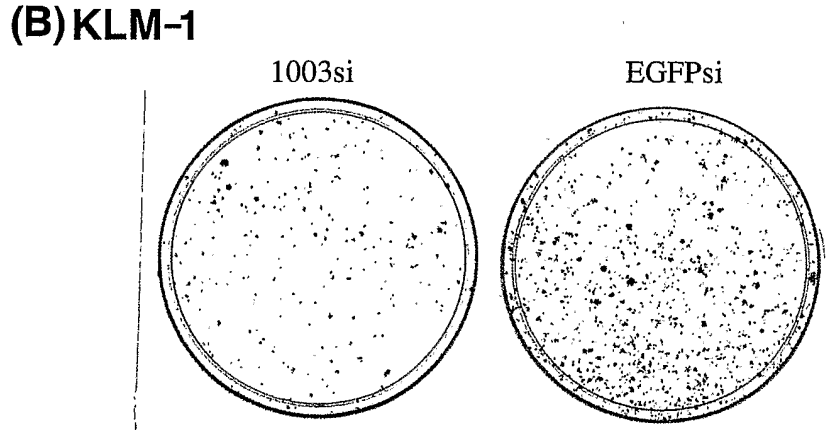
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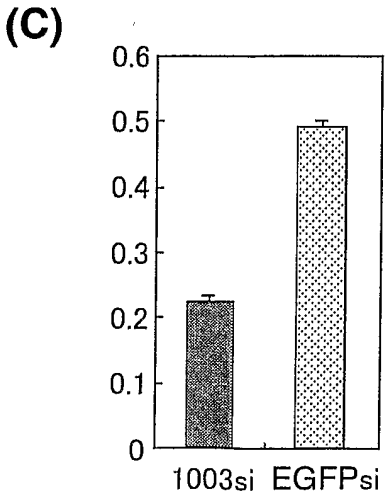
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SEQUENCE LISTING

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THE UNIVERSITY OF TOKYO

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<151> 2004-03-24

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gtg gta cag gac ctg cca cct gca aac aca ttc gtg ggc acc ggg gac 3141
Val Val Gln Asp Leu Pro Pro Ala Asn Thr Phe Val Gly Thr Gly Asp
995 1000 1005

acc acg tcc acg ggc tct gag cag tac tcc gac tac agc tac cgc 3186
Thr Thr Ser Thr Gly Ser Glu Gln Tyr Ser Asp Tyr Ser Tyr Arg
1010 1015 1020

acc aac ccc ccc aaa tac ccc agc aag cag gta ggc cag ccc ttt 3231
Thr Asn Pro Pro Lys Tyr Pro Ser Lys Gln Val Gly Gln Pro Phe
1025 1030 1035

cag ctc agc aca ccc cag ccc cta ccc cac ccc tac cac gga gcc 3276
Gln Leu Ser Thr Pro Gln Pro Leu Pro His Pro Tyr His Gly Ala
1040 1045 1050

atc tgg acc gag gtg tgg gag tga tggagcaggt ttactgtgcc 3320
Ile Trp Thr Glu Val Trp Glu
1055 1060

tgcccgtgtt gggggccagc ctgagccagc agtgggaggt ggggccttag tgcctcaccg 3380

ggcacacgga ttaggctgag tgaagattaa gggagggtgt gctctgtggt ctcctccctg 3440

ccctctcccc actggggaga gacctgtgat ttgccaagtc cctggaccct ggaccagcta 3500

ctgggcctta tgggttgggg gtggtaggca ggtgagcgta agtggggagg gaaatgggta 3560

agaagtctac tccaaaccta ggtctctatg tcagaccaga cctaggtgct tctctaggag 3620

ggaaacaggg agacctgggg tcctgtggat aactgagtgg ggagtctgcc aggggagggc 3680

accttcccat tgtgccttct gtgtgtattg tgcattaacc tcttcctcac cactaggctt 3740

ctggggctgg gtcccacatg cccttgaccc tgacaataaa gttctctatt tttggaaaaa 3800

aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa a 3851

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<212> PRT

<213> Homo sapiens

<400> 2

Met Asp Ser Gly Ala Gly Gly Arg Arg Cys Pro Glu Ala Ala Leu Leu
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Ile Leu Gly Pro Pro Arg Met Glu His Leu Arg His Ser Pro Gly Pro
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Gly Gly Gln Arg Leu Leu Leu Pro Ser Met Leu Leu Ala Leu Leu Leu
35 40 45

Leu Leu Ala Pro Ser Pro Gly His Ala Thr Arg Val Val Tyr Lys Val

50 55 60

Pro Glu Glu Gln Pro Pro Asn Thr Leu Ile Gly Ser Leu Ala Ala Asp
65 70 75 80

Tyr Gly Phe Pro Asp Val Gly His Leu Tyr Lys Leu Glu Val Gly Ala
85 90 95

Pro Tyr Leu Arg Val Asp Gly Lys Thr Gly Asp Ile Phe Thr Thr Glu
100 105 110

Thr Ser Ile Asp Arg Glu Gly Leu Arg Glu Cys Gln Asn Gln Leu Pro
115 120 125

Gly Asp Pro Cys Ile Leu Glu Phe Glu Val Ser Ile Thr Asp Leu Val
130 135 140

Gln Asn Gly Ser Pro Arg Leu Leu Glu Gly Gln Ile Glu Val Gln Asp
145 150 155 160

Ile Asn Asp Asn Thr Pro Asn Phe Ala Ser Pro Val Ile Thr Leu Ala
165 170 175

Ile Pro Glu Asn Thr Asn Ile Gly Ser Leu Phe Pro Ile Pro Leu Ala
180 185 190

Ser Asp Arg Asp Ala Gly Pro Asn Gly Val Ala Ser Tyr Glu Leu Gln
195 200 205

Ala Gly Pro Glu Ala Gln Glu Leu Phe Gly Leu Gln Val Ala Glu Asp
210 215 220

Gln Glu Glu Lys Gln Pro Gln Leu Ile Val Met Gly Asn Leu Asp Arg
225 230 235 240

Glu Arg Trp Asp Ser Tyr Asp Leu Thr Ile Lys Val Gln Asp Gly Gly
245 250 255

Ser Pro Pro Arg Ala Ser Ser Ala Leu Leu Arg Val Thr Val Leu Asp

260 265 270

Thr Asn Asp Asn Ala Pro Lys Phe Glu Arg Pro Ser Tyr Glu Ala Glu
275 280 285

Leu Ser Glu Asn Ser Pro Ile Gly His Ser Val Ile Gln Val Lys Ala
290 295 300

Asn Asp Ser Asp Gln Gly Ala Asn Ala Glu Ile Glu Tyr Thr Phe His
305 310 315 320

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

Gly Leu Ile Thr Val Gln Gly Pro Val Asp Arg Glu Asp Leu Ser Thr
340 345 350

Leu Arg Phe Ser Val Leu Ala Lys Asp Arg Gly Thr Asn Pro Lys Ser
355 360 365

Ala Arg Ala Gln Val Val Val Thr Val Lys Asp Met Asn Asp Asn Ala
370 375 380

Pro Thr Ile Glu Ile Arg Gly Ile Gly Leu Val Thr His Gln Asp Gly
385 390 395 400

Met Ala Asn Ile Ser Glu Asp Val Ala Glu Glu Thr Ala Val Ala Leu
405 410 415

Val Gln Val Ser Asp Arg Asp Glu Gly Glu Asn Ala Ala Val Thr Cys
420 425 430

Val Val Ala Gly Asp Val Pro Phe Gln Leu Arg Gln Ala Ser Glu Thr
435 440 445

Gly Ser Asp Ser Lys Lys Lys Tyr Phe Leu Gln Thr Thr Thr Pro Leu
450 455 460

Asp Tyr Glu Lys Val Lys Asp Tyr Thr Ile Glu Ile Val Ala Val Asp

465 470 475 480

Ser Gly Asn Pro Pro Leu Ser Ser Thr Asn Ser Leu Lys Val Gln Val
485 490 495

Val Asp Val Asn Asp Asn Ala Pro Val Phe Thr Gln Ser Val Thr Glu
500 505 510

Val Ala Phe Pro Glu Asn Asn Lys Pro Gly Glu Val Ile Ala Glu Ile
515 520 525

Thr Ala Ser Asp Ala Asp Ser Gly Ser Asn Ala Glu Leu Val Tyr Ser
530 535 540

Leu Glu Pro Glu Pro Ala Ala Lys Gly Leu Phe Thr Ile Ser Pro Glu
545 550 555 560

Thr Gly Glu Ile Gln Val Lys Thr Ser Leu Asp Arg Glu Gln Arg Glu
565 570 575

Ser Tyr Glu Leu Lys Val Val Ala Ala Asp Arg Gly Ser Pro Ser Leu
580 585 590

Gln Gly Thr Ala Thr Val Leu Val Asn Val Leu Asp Cys Asn Asp Asn
595 600 605

Asp Pro Lys Phe Met Leu Ser Gly Tyr Asn Phe Ser Val Met Glu Asn
610 615 620

Met Pro Ala Leu Ser Pro Val Gly Met Val Thr Val Ile Asp Gly Asp
625 630 635 640

Lys Gly Glu Asn Ala Gln Val Gln Leu Ser Val Glu Gln Asp Asn Gly
645 650 655

Asp Phe Val Ile Gln Asn Gly Thr Gly Thr Ile Leu Ser Ser Leu Ser
660 665 670

Phe Asp Arg Glu Gln Gln Ser Thr Tyr Thr Phe Gln Leu Lys Ala Val

675 680 685

Asp Gly Gly Val Pro Pro Arg Ser Ala Tyr Val Gly Val Thr Ile Asn
690 695 700

Val Leu Asp Glu Asn Asp Asn Ala Pro Tyr Ile Thr Ala Pro Ser Asn
705 710 715 720

Thr Ser His Lys Leu Leu Thr Pro Gln Thr Arg Leu Gly Glu Thr Val
725 730 735

Ser Gln Val Ala Ala Glu Asp Phe Asp Ser Gly Val Asn Ala Glu Leu
740 745 750

Ile Tyr Ser Ile Ala Gly Gly Asn Pro Tyr Gly Leu Phe Gln Ile Gly
755 760 765

Ser His Ser Gly Ala Ile Thr Leu Glu Lys Glu Ile Glu Arg Arg His
770 775 780

His Gly Leu His Arg Leu Val Val Lys Val Ser Asp Arg Gly Lys Pro
785 790 795 800

Pro Arg Tyr Gly Thr Ala Leu Val His Leu Tyr Val Asn Glu Thr Leu
805 810 815

Ala Asn Arg Thr Leu Leu Glu Thr Leu Leu Gly His Ser Leu Asp Thr
820 825 830

Pro Leu Asp Ile Asp Ile Ala Gly Asp Pro Glu Tyr Glu Arg Ser Lys
835 840 845

Gln Arg Gly Asn Ile Leu Phe Gly Val Val Ala Gly Val Val Ala Val
850 855 860

Ala Leu Leu Ile Ala Leu Ala Val Leu Val Arg Tyr Cys Arg Gln Arg
865 870 875 880

Glu Ala Lys Ser Gly Tyr Gln Ala Gly Lys Lys Glu Thr Lys Asp Leu

885 890 895

Tyr Ala Pro Lys Pro Ser Gly Lys Ala Ser Lys Gly Asn Lys Ser Lys
900 905 910

Gly Lys Lys Ser Lys Ser Pro Lys Pro Val Lys Pro Val Glu Asp Glu
915 920 925

Asp Glu Ala Gly Leu Gln Lys Ser Leu Lys Phe Asn Leu Met Ser Asp
930 935 940

Ala Pro Gly Asp Ser Pro Arg Ile His Leu Pro Leu Asn Tyr Pro Pro
945 950 955 960

Gly Ser Pro Asp Leu Gly Arg His Tyr Arg Ser Asn Ser Pro Leu Pro
965 970 975

Ser Ile Gln Leu Gln Pro Gln Ser Pro Ser Ala Ser Lys Lys His Gln
980 985 990

Val Val Gln Asp Leu Pro Pro Ala Asn Thr Phe Val Gly Thr Gly Asp
995 1000 1005

Thr Thr Ser Thr Gly Ser Glu Gln Tyr Ser Asp Tyr Ser Tyr Arg
1010 1015 1020

Thr Asn Pro Pro Lys Tyr Pro Ser Lys Gln Val Gly Gln Pro Phe
1025 1030 1035

Gln Leu Ser Thr Pro Gln Pro Leu Pro His Pro Tyr His Gly Ala
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Met Gly Leu Pro Arg Gly Pro Leu Ala Ser Leu Leu Leu
1 5 10

ctc cag gtt tgc tgg ctg cag tgc gcg gcc tcc gag ccg tgc cgg gcg 157
Leu Gln Val Cys Trp Leu Gln Cys Ala Ala Ser Glu Pro Cys Arg Ala
15 20 25

gtc ttc agg gag gct gaa gtg acc ttg gag gcg gga ggc gcg gag cag 205
Val Phe Arg Glu Ala Glu Val Thr Leu Glu Ala Gly Gly Ala Glu Gln
30 35 40 45

gag ccc ggc cag gcg ctg ggg aaa gta ttc atg ggc tgc cct ggg caa 253
Glu Pro Gly Gln Ala Leu Gly Lys Val Phe Met Gly Cys Pro Gly Gln
50 55 60

gag cca gct ctg ttt agc act gat aat gat gac ttc act gtg cgg aat 301
Glu Pro Ala Leu Phe Ser Thr Asp Asn Asp Asp Phe Thr Val Arg Asn

65 70 75

ggc gag aca gtc cag gaa aga agg tca ctg aag gaa agg aat cca ttg 349
Gly Glu Thr Val Gln Glu Arg Arg Ser Leu Lys Glu Arg Asn Pro Leu
80 85 90

aag atc ttc cca tcc aaa cgt atc tta cga aga cac aag aga gat tgg 397
Lys Ile Phe Pro Ser Lys Arg Ile Leu Arg Arg His Lys Arg Asp Trp
95 100 105

gtg gtt gct cca ata tct gtc cct gaa aat ggc aag ggt ccc ttc ccc 445
Val Val Ala Pro Ile Ser Val Pro Glu Asn Gly Lys Gly Pro Phe Pro
110 115 120 125

cag aga ctg aat cag ctc aag tct aat aaa gat aga gac acc aag att 493
Gln Arg Leu Asn Gln Leu Lys Ser Asn Lys Asp Arg Asp Thr Lys Ile
130 135 140

ttc tac agc atc acg ggg ccg ggg gca gac agc ccc cct gag ggt gtc 541
Phe Tyr Ser Ile Thr Gly Pro Gly Ala Asp Ser Pro Pro Glu Gly Val
145 150 155

ttc gct gta gag aag gag aca ggc tgg ttg ttg ttg aat aag cca ctg 589
Phe Ala Val Glu Lys Glu Thr Gly Trp Leu Leu Leu Asn Lys Pro Leu
160 165 170

gac cgg gag gag att gcc aag tat gag ctc ttt ggc cac gct gtg tca 637
Asp Arg Glu Glu Ile Ala Lys Tyr Glu Leu Phe Gly His Ala Val Ser
175 180 185

gag aat ggt gcc tca gtg gag gac ccc atg aac atc tcc atc atc gtg 685
Glu Asn Gly Ala Ser Val Glu Asp Pro Met Asn Ile Ser Ile Ile Val
190 195 200 205

acc gac cag aat gac cac aag ccc aag ttt acc cag gac acc ttc cga 733
Thr Asp Gln Asn Asp His Lys Pro Lys Phe Thr Gln Asp Thr Phe Arg
210 215 220

ggg agt gtc tta gag gga gtc cta cca ggt act tct gtg atg cag gtg 781
Gly Ser Val Leu Glu Gly Val Leu Pro Gly Thr Ser Val Met Gln Val
225 230 235

aca gcc acg gat gag gat gat gcc atc tac acc tac aat ggg gtg gtt 829
Thr Ala Thr Asp Glu Asp Asp Ala Ile Tyr Thr Tyr Asn Gly Val Val
240 245 250

gct tac tcc atc cat agc caa gaa cca aag gac cca cac gac ctc atg 877
Ala Tyr Ser Ile His Ser Gln Glu Pro Lys Asp Pro His Asp Leu Met
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ttc acc att cac cgg agc aca ggc acc atc agc gtc atc tcc agt ggc 925
Phe Thr Ile His Arg Ser Thr Gly Thr Ile Ser Val Ile Ser Ser Gly

270 275 280 285

ctg gac cgg gaa aaa gtc cct gag tac aca ctg acc atc cag gcc aca 973
Leu Asp Arg Glu Lys Val Pro Glu Tyr Thr Leu Thr Ile Gln Ala Thr
290 295 300

gac atg gat ggg gac ggc tcc acc acc acg gca gtg gca gta gtg gag 1021
Asp Met Asp Gly Asp Gly Ser Thr Thr Thr Ala Val Ala Val Val Glu
305 310 315

atc ctt gat gcc aat gac aat gct ccc atg ttt gac ccc cag aag tac 1069
Ile Leu Asp Ala Asn Asp Asn Ala Pro Met Phe Asp Pro Gln Lys Tyr
320 325 330

gag gcc cat gtg cct gag aat gca gtg ggc cat gag gtg cag agg ctg 1117
Glu Ala His Val Pro Glu Asn Ala Val Gly His Glu Val Gln Arg Leu
335 340 345

acg gtc act gat ctg gac gcc ccc aac tca cca gcg tgg cgt gcc acc 1165
Thr Val Thr Asp Leu Asp Ala Pro Asn Ser Pro Ala Trp Arg Ala Thr
350 355 360 365

tac ctt atc atg ggc ggt gac gac ggg gac cat ttt acc atc acc acc 1213
Tyr Leu Ile Met Gly Gly Asp Asp Gly Asp His Phe Thr Ile Thr Thr
370 375 380

cac cct gag agc aac cag ggc atc ctg aca acc agg aag ggt ttg gat 1261
His Pro Glu Ser Asn Gln Gly Ile Leu Thr Thr Arg Lys Gly Leu Asp
385 390 395

ttt gag gcc aaa aac cag cac acc ctg tac gtt gaa gtg acc aac gag 1309
Phe Glu Ala Lys Asn Gln His Thr Leu Tyr Val Glu Val Thr Asn Glu
400 405 410

gcc cct ttt gtg ctg aag ctc cca acc tcc aca gcc acc ata gtg gtc 1357
Ala Pro Phe Val Leu Lys Leu Pro Thr Ser Thr Ala Thr Ile Val Val
415 420 425

cac gtg gag gat gtg aat gag gca cct gtg ttt gtc cca ccc tcc aaa 1405
His Val Glu Asp Val Asn Glu Ala Pro Val Phe Val Pro Pro Ser Lys
430 435 440 445

gtc gtt gag gtc cag gag ggc atc ccc act ggg gag cct gtg tgt gtc 1453
Val Val Glu Val Gln Glu Gly Ile Pro Thr Gly Glu Pro Val Cys Val
450 455 460

tac act gca gaa gac cct gac aag gag aat caa aag atc agc tac cgc 1501
Tyr Thr Ala Glu Asp Pro Asp Lys Glu Asn Gln Lys Ile Ser Tyr Arg
465 470 475

atc ctg aga gac cca gca ggg tgg cta gcc atg gac cca gac agt ggg 1549
Ile Leu Arg Asp Pro Ala Gly Trp Leu Ala Met Asp Pro Asp Ser Gly

480 485 490

cag gtc aca gct gtg ggc acc ctc gac cgt gag gat gag cag ttt gtg 1597
Gln Val Thr Ala Val Gly Thr Leu Asp Arg Glu Asp Glu Gln Phe Val
495 500 505

agg aac aac atc tat gaa gtc atg gtc ttg gcc atg gac aat gga agc 1645
Arg Asn Asn Ile Tyr Glu Val Met Val Leu Ala Met Asp Asn Gly Ser
510 515 520 525

cct ccc acc act ggc acg gga acc ctt ctg cta aca ctg att gat gtc 1693
Pro Pro Thr Thr Gly Thr Gly Thr Leu Leu Leu Thr Leu Ile Asp Val
530 535 540

aat gac cat ggc cca gtc cct gag ccc cgt cag atc acc atc tgc aac 1741
Asn Asp His Gly Pro Val Pro Glu Pro Arg Gln Ile Thr Ile Cys Asn
545 550 555

caa agc cct gtg cgc cag gtg ctg aac atc acg gac aag gac ctg tct 1789
Gln Ser Pro Val Arg Gln Val Leu Asn Ile Thr Asp Lys Asp Leu Ser
560 565 570

ccc cac acc tcc cct ttc cag gcc cag ctc aca gat gac tca gac atc 1837
Pro His Thr Ser Pro Phe Gln Ala Gln Leu Thr Asp Asp Ser Asp Ile
575 580 585

tac tgg acg gca gag gtc aac gag gaa ggt gac aca gtg gtc ttg tcc 1885
Tyr Trp Thr Ala Glu Val Asn Glu Glu Gly Asp Thr Val Val Leu Ser
590 595 600 605

ctg aag aag ttc ctg aag cag gat aca tat gac gtg cac ctt tct ctg 1933
Leu Lys Lys Phe Leu Lys Gln Asp Thr Tyr Asp Val His Leu Ser Leu
610 615 620

tct gac cat ggc aac aaa gag cag ctg acg gtg atc agg gcc act gtg 1981
Ser Asp His Gly Asn Lys Glu Gln Leu Thr Val Ile Arg Ala Thr Val
625 630 635

tgc gac tgc cat ggc cat gtc gaa acc tgc cct gga ccc tgg aag gga 2029
Cys Asp Cys His Gly His Val Glu Thr Cys Pro Gly Pro Trp Lys Gly
640 645 650

ggt ttc atc ctc cct gtg ctg ggg gct gtc ctg gct ctg ctg ttc ctc 2077
Gly Phe Ile Leu Pro Val Leu Gly Ala Val Leu Ala Leu Leu Phe Leu
655 660 665

ctg ctg gtg ctg ctt ttg ttg gtg aga aag aag cgg aag atc aag gag 2125
Leu Leu Val Leu Leu Leu Leu Val Arg Lys Lys Arg Lys Ile Lys Glu
670 675 680 685

ccc ctc cta ctc cca gaa gat gac acc cgt gac aac gtc ttc tac tat 2173
Pro Leu Leu Leu Pro Glu Asp Asp Thr Arg Asp Asn Val Phe Tyr Tyr

690 695 700

ggc gaa gag ggg ggt ggc gaa gag gac cag gac tat gac atc acc cag 2221
Gly Glu Glu Gly Gly Gly Glu Glu Asp Gln Asp Tyr Asp Ile Thr Gln
705 710 715

ctc cac cga ggt ctg gag gcc agg ccg gag gtg gtt ctc cgc aat gac 2269
Leu His Arg Gly Leu Glu Ala Arg Pro Glu Val Val Leu Arg Asn Asp
720 725 730

gtg gca cca acc atc atc ccg aca ccc atg tac cgt cct cgg cca gcc 2317
Val Ala Pro Thr Ile Ile Pro Thr Pro Met Tyr Arg Pro Arg Pro Ala
735 740 745

aac cca gat gaa atc ggc aac ttt ata att gag aac ctg aag gcg gct 2365
Asn Pro Asp Glu Ile Gly Asn Phe Ile Ile Glu Asn Leu Lys Ala Ala
750 755 760 765

aac aca gac ccc aca gcc ccg ccc tac gac acc ctc ttg gtg ttc gac 2413
Asn Thr Asp Pro Thr Ala Pro Pro Tyr Asp Thr Leu Leu Val Phe Asp
770 775 780

tat gag ggc agc ggc tcc gac gcc gcg tcc ctg agc tcc ctc acc tcc 2461
Tyr Glu Gly Ser Gly Ser Asp Ala Ala Ser Leu Ser Ser Leu Thr Ser
785 790 795

tcc gcc tcc gac caa gac caa gat tac gat tat ctg aac gag tgg ggc 2509
Ser Ala Ser Asp Gln Asp Gln Asp Tyr Asp Tyr Leu Asn Glu Trp Gly
800 805 810

agc cgc ttc aag aag ctg gca gac atg tac ggt ggc ggg gag gac gac 2557
Ser Arg Phe Lys Lys Leu Ala Asp Met Tyr Gly Gly Gly Glu Asp Asp
815 820 825

tag gcggcctgcc tgcagggctg gggaccaaac gtcaggccac agagcatctc 2610

caaggggtct cagttccccc ttcagctgag gacttcggag cttgtcagga agtggccgta 2670

gcaacttggc ggagacaggc tatgagtctg acgttagagt ggttgcttcc ttagcctttc 2730

aggatggagg aatgtgggca gtttgacttc agcactgaaa acctctccac ctgggccagg 2790

gttgcctcag aggccaagtt tccagaagcc tcttacctgc cgtaaaatgc tcaaccctgt 2850

gtcctgggcc tgggcctgct gtgactgacc tacagtggac tttctctctg gaatggaacc 2910

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ctaggttgcc ccttattttt tattttccct gttgcgttgc tatagatgaa gggtgaggac 3150

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<211> 829

<212> PRT

<213> Homo sapiens

<400> 4

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Glu Ala Glu Val Thr Leu Glu Ala Gly Gly Ala Glu Gln Glu Pro Gly
35 40 45

Gln Ala Leu Gly Lys Val Phe Met Gly Cys Pro Gly Gln Glu Pro Ala
50 55 60

Leu Phe Ser Thr Asp Asn Asp Asp Phe Thr Val Arg Asn Gly Glu Thr
65 70 75 80

Val Gln Glu Arg Arg Ser Leu Lys Glu Arg Asn Pro Leu Lys Ile Phe
85 90 95

Pro Ser Lys Arg Ile Leu Arg Arg His Lys Arg Asp Trp Val Val Ala
100 105 110

Pro Ile Ser Val Pro Glu Asn Gly Lys Gly Pro Phe Pro Gln Arg Leu
115 120 125

Asn Gln Leu Lys Ser Asn Lys Asp Arg Asp Thr Lys Ile Phe Tyr Ser
130 135 140

Ile Thr Gly Pro Gly Ala Asp Ser Pro Pro Glu Gly Val Phe Ala Val
145 150 155 160

Glu Lys Glu Thr Gly Trp Leu Leu Leu Asn Lys Pro Leu Asp Arg Glu
165 170 175

Glu Ile Ala Lys Tyr Glu Leu Phe Gly His Ala Val Ser Glu Asn Gly
180 185 190

Ala Ser Val Glu Asp Pro Met Asn Ile Ser Ile Ile Val Thr Asp Gln
195 200 205

Asn Asp His Lys Pro Lys Phe Thr Gln Asp Thr Phe Arg Gly Ser Val
210 215 220

Leu Glu Gly Val Leu Pro Gly Thr Ser Val Met Gln Val Thr Ala Thr
225 230 235 240

Asp Glu Asp Asp Ala Ile Tyr Thr Tyr Asn Gly Val Val Ala Tyr Ser
245 250 255

Ile His Ser Gln Glu Pro Lys Asp Pro His Asp Leu Met Phe Thr Ile
260 265 270

His Arg Ser Thr Gly Thr Ile Ser Val Ile Ser Ser Gly Leu Asp Arg
275 280 285

Glu Lys Val Pro Glu Tyr Thr Leu Thr Ile Gln Ala Thr Asp Met Asp
290 295 300

Gly Asp Gly Ser Thr Thr Thr Ala Val Ala Val Val Glu Ile Leu Asp
305 310 315 320

Ala Asn Asp Asn Ala Pro Met Phe Asp Pro Gln Lys Tyr Glu Ala His
325 330 335

Val Pro Glu Asn Ala Val Gly His Glu Val Gln Arg Leu Thr Val Thr
340 345 350

Asp Leu Asp Ala Pro Asn Ser Pro Ala Trp Arg Ala Thr Tyr Leu Ile
355 360 365

Met Gly Gly Asp Asp Gly Asp His Phe Thr Ile Thr Thr His Pro Glu
370 375 380

Ser Asn Gln Gly Ile Leu Thr Thr Arg Lys Gly Leu Asp Phe Glu Ala
385 390 395 400

Lys Asn Gln His Thr Leu Tyr Val Glu Val Thr Asn Glu Ala Pro Phe
405 410 415

Val Leu Lys Leu Pro Thr Ser Thr Ala Thr Ile Val Val His Val Glu
420 425 430

Asp Val Asn Glu Ala Pro Val Phe Val Pro Pro Ser Lys Val Val Glu
435 440 445

Val Gln Glu Gly Ile Pro Thr Gly Glu Pro Val Cys Val Tyr Thr Ala
450 455 460

Glu Asp Pro Asp Lys Glu Asn Gln Lys Ile Ser Tyr Arg Ile Leu Arg
465 470 475 480

Asp Pro Ala Gly Trp Leu Ala Met Asp Pro Asp Ser Gly Gln Val Thr
485 490 495

Ala Val Gly Thr Leu Asp Arg Glu Asp Glu Gln Phe Val Arg Asn Asn
500 505 510

Ile Tyr Glu Val Met Val Leu Ala Met Asp Asn Gly Ser Pro Pro Thr
515 520 525

Thr Gly Thr Gly Thr Leu Leu Leu Thr Leu Ile Asp Val Asn Asp His
530 535 540

Gly Pro Val Pro Glu Pro Arg Gln Ile Thr Ile Cys Asn Gln Ser Pro
545 550 555 560

Val Arg Gln Val Leu Asn Ile Thr Asp Lys Asp Leu Ser Pro His Thr
565 570 575

Ser Pro Phe Gln Ala Gln Leu Thr Asp Asp Ser Asp Ile Tyr Trp Thr
580 585 590

Ala Glu Val Asn Glu Glu Gly Asp Thr Val Val Leu Ser Leu Lys Lys
595 600 605

Phe Leu Lys Gln Asp Thr Tyr Asp Val His Leu Ser Leu Ser Asp His
610 615 620

Gly Asn Lys Glu Gln Leu Thr Val Ile Arg Ala Thr Val Cys Asp Cys
625 630 635 640

His Gly His Val Glu Thr Cys Pro Gly Pro Trp Lys Gly Gly Phe Ile
645 650 655

Leu Pro Val Leu Gly Ala Val Leu Ala Leu Leu Phe Leu Leu Leu Val
660 665 670

Leu Leu Leu Leu Val Arg Lys Lys Arg Lys Ile Lys Glu Pro Leu Leu
675 680 685

Leu Pro Glu Asp Asp Thr Arg Asp Asn Val Phe Tyr Tyr Gly Glu Glu
690 695 700

Gly Gly Gly Glu Glu Asp Gln Asp Tyr Asp Ile Thr Gln Leu His Arg
705 710 715 720

Gly Leu Glu Ala Arg Pro Glu Val Val Leu Arg Asn Asp Val Ala Pro
725 730 735

Thr Ile Ile Pro Thr Pro Met Tyr Arg Pro Arg Pro Ala Asn Pro Asp
740 745 750

Glu Ile Gly Asn Phe Ile Ile Glu Asn Leu Lys Ala Ala Asn Thr Asp
755 760 765

Pro Thr Ala Pro Pro Tyr Asp Thr Leu Leu Val Phe Asp Tyr Glu Gly
770 775 780

Ser Gly Ser Asp Ala Ala Ser Leu Ser Ser Leu Thr Ser Ser Ala Ser
785 790 795 800

Asp Gln Asp Gln Asp Tyr Asp Tyr Leu Asn Glu Trp Gly Ser Arg Phe
805 810 815

Lys Lys Leu Ala Asp Met Tyr Gly Gly Gly Glu Asp Asp
820 825

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Ala Ala Gly Leu Arg Leu Leu Pro Met Leu Gly Leu Leu Gln Leu Leu
20 25 30

gcc gag cct ggc ctg ggc cgc gtc cat cac ctg gca ctc aag gat gat 145
Ala Glu Pro Gly Leu Gly Arg Val His His Leu Ala Leu Lys Asp Asp
35 40 45

gtg agg cat aaa gtt cat ctg aac acc ttt ggc ttc ttc aag gat ggg 193
Val Arg His Lys Val His Leu Asn Thr Phe Gly Phe Phe Lys Asp Gly
50 55 60

tac atg gtg gtg aat gtc agt agc ctc tca ctg aat gag cct gaa gac 241
Tyr Met Val Val Asn Val Ser Ser Leu Ser Leu Asn Glu Pro Glu Asp
65 70 75 80

aag gat gtg act att gga ttt agc cta gac cgt aca aag aat gat ggc 289
Lys Asp Val Thr Ile Gly Phe Ser Leu Asp Arg Thr Lys Asn Asp Gly
85 90 95

ttt tct tct tac ctg gat gaa gat gtg aat tac tgt att tta aag aaa 337
Phe Ser Ser Tyr Leu Asp Glu Asp Val Asn Tyr Cys Ile Leu Lys Lys
100 105 110

cag tct gtc tct gtc acc ctt tta atc cta gac atc tcc aga agt gag 385

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Val Arg Val Lys Ser Pro Pro Glu Ala Gly Thr Gln Leu Pro Lys Ile
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Gln Gly Phe Pro Ile Glu Gly Trp Ala Val Val Tyr Tyr Ile Thr His
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Lys Asp Val Thr Ile Gly Phe Ser Leu Asp Arg Thr Lys Asn Asp Gly
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