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(54) Title: METHOD FOR DIAGNOSING AND TREATING RENAL CELL CARCINOMA

(57) Abstract: Objective methods for detecting and diagnosing renal cell carcinoma (RCC) are described herein. In one embodiment, the diagnostic method involves determining the expression level of RCC-associated gene that discriminates between RCC cells and normal cells. The present invention further provides methods of screening for therapeutic agents useful in the treatment of renal cell carcinoma, methods of treating renal cell carcinoma and method of vaccinating a subject against renal cell carcinoma.



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DESCRIPTION

Method for Diagnosing and Treating Renal Cell Carcinoma

This application claims the benefit of U.S. Provisional Application Serial No.60/703,640 filed July 28, 2005 and No.60/799,960 filed May 11, 2006, the contents of which are hereby incorporated by reference in their entirety.

FIELD OF THE INVENTION

The invention relates to methods of diagnosing and treating renal cell carcinoma.

BACKGROUND OF THE INVENTION

Renal cell carcinoma (RCC), the third most common malignancy of the genitourinary system, accounting for 2-3% of all human malignancies. At present, surgical resection is the most effective treatment for localized RCC tumors; however, no satisfactory treatment is available for patients with advanced-stage RCC. Some therapies for RCCs have achieved a response rate of ~20%, but severely adverse reactions are frequent and prognosis for patients does not appear to have improved overall (Ljungberg B, *et al.*, (1999) B J U Int. 84: 405-11; Levy DA, *et al.* (1998) J Urol 159: 1163-7.). Although tumor stage is considered to be the most informative prognostic factor, little is known about the underlying molecular mechanisms of renal carcinogenesis.

RCC tumors are characterized on the basis of histological features as clear cell (80%), papillary (~10%), chromophobe (< 5%), or granular, spindle, or cyst-associated carcinomas (5-15%). Each of these histological subtypes exhibits unique clinical behavior, with clear-cell and granular types tending to show more aggressive clinical phenotypes. For the instant study, the most predominant type, clear-cell carcinoma, was chosen. Specifically, the present inventors performed a large-scale analysis of gene-expression profiles in such tumors. Similar studies reported by other groups have already identified some genes that might be useful for prognostic purposes or for classification of RCCs (Takahashi M, *et al.* (2001) Proc Natl Acad Sci USA 98: 9754-9; Young AN, *et al.* (2001) Am J Pathol. 158: 1639-51; Skubitz KM & Skubitz AP. (2002) J Lab Clin Med 140: 52-64; Takahashi M, *et al.* (2003) Oncogene 22: 6810-8; Higgins JP, *et al.* (2003) Am J Pathol 162: 925-32). Some studies of the gene-expression profiles of kidney cancers have discovered genes that might be candidates as diagnostic markers or prognosis profiles. However, these data, derived from tumor masses,

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cannot adequately reflect expressional changes that arise during renal carcinogenesis because RCC cells exist as a solid mass containing various cellular components. Therefore, previous published expression profiles of RCC are likely to reflect heterogenous profiles.

cDNA microarray technologies have enabled the discovery of comprehensive profiles of gene expression in normal and malignant cells, and the comparison of gene expression in malignant to corresponding normal cells (Okabe *et al.*, (2001) Cancer Res 61:2129-37; Kitahara *et al.*, (2001) Cancer Res 61: 3544-9; Lin *et al.*, (2002) Oncogene 21:4120-8; Hasegawa *et al.*, (2002) Cancer Res 62:7012-7.). This approach enables the disclosure of the complex nature of cancer cells, and understanding of the mechanism of carcinogenesis.

Identification of genes that are deregulated in tumors can lead to more precise and accurate diagnosis of individual cancers, and to develop novel therapeutic targets (Bienz and Clevers, (2000) Cell 103:311-20.). To disclose mechanisms underlying tumors from a genome-wide point of view, and discover target molecules for diagnosis and development of novel therapeutic drugs, the present inventors have analyzed the expression profiles of a number of tumor cells using a cDNA microarray of 23040 genes (Okabe *et al.*, (2001) Cancer Res 61:2129-37; Kitahara *et al.*, (2001) Cancer Res 61:3544-9; Lin *et al.*, (2002) Oncogene 21:4120-8; Hasegawa *et al.*, (2002) Cancer Res 62:7012-7.).

Studies designed to reveal mechanisms of carcinogenesis have already facilitated the identification of molecular targets for certain anti-tumor agents. For example, inhibitors of farnesyltransferase (FTIs) which were originally developed to inhibit the growth-signaling pathway related to Ras, whose activation depends on posttranslational farnesylation, have been demonstrated to be effective in treating Ras-dependent tumors in animal models (Sun J., *et al.*, (1998) Oncogene.;16:1467-73.). Human clinical trials using a combination of anti-cancer drugs and the anti-HER2 monoclonal antibody, trastuzumab, with the aim of antagonizing the proto-oncogene receptor HER2/neu, have achieved improved clinical response and overall survival of breast-cancer patients (Molina MA., *et al.*, (2001) Cancer Res. ;61:4744-9). Finally, a tyrosine kinase inhibitor, STI-571, which selectively inactivates bcr-abl fusion proteins, has been developed to treat chronic myelogenous leukemias wherein constitutive activation of bcr-abl tyrosine kinase plays a crucial role in the transformation of leukocytes. Agents of these kinds are designed to suppress oncogenic activity of specific gene products (O'Dwyer ME & Druker BJ. (2000) Curr Opin Oncol.;12:594-7.). Accordingly, it is apparent that gene products commonly up-regulated in cancerous cells may serve as

potential targets for developing novel anti-cancer agents.

It has been further demonstrated that CD8⁺ cytotoxic T lymphocytes (CTLs) recognize epitope peptides derived from tumor-associated antigens (TAAs) presented on the MHC Class I molecule, and lyse tumor cells. Since the discovery of the MAGE family as the first example of TAAs, many other TAAs have been discovered using immunological approaches (Boon. (1993) *Int J Cancer* 54: 177-80; Boon and van der Bruggen. (1996) *J Exp Med* 183: 725-9; van der Bruggen *et al.*, (1991) *Science* 254: 1643-7; Brichard *et al.*, (1993) *J Exp Med* 178: 489-95; Kawakami *et al.*, (1994) *J Exp Med* 180: 347-52.). Some of the newly discovered TAAs are currently undergoing clinical development as targets of immunotherapy. TAAs discovered so far include MAGE (van der Bruggen *et al.*, (1991) *Science* 254: 1643-7.), gp100 (Kawakami *et al.*, (1994) *J Exp Med* 180: 347-52.), SART (Shichijo *et al.*, (1998) *J Exp Med* 187: 277-88.), and NY-ESO-1 (Chen *et al.*, (1997) *Proc Natl Acad Sci USA* 94: 1914-8.). On the other hand, gene products demonstrated to be specifically over-expressed in tumor cells, have been shown to be recognized as targets inducing cellular immune responses. Such gene products include p53 (Umano *et al.*, (2001) *Brit J Cancer* 84: 1052-7.), HER2/neu (Tanaka *et al.*, (2001) *Brit J Cancer* 84: 94-9.), CEA (Nukaya *et al.*, (1999) *Int J Cancer* 80: 92-7.), and so on.

In spite of significant progress in basic and clinical research concerning TAAs (Rosenberg *et al.*, (1998) *Nature Med* 4: 321-7; Mukherji *et al.*, (1995) *Proc Natl Acad Sci USA* 92: 8078-82; Hu *et al.*, (1996) *Cancer Res* 56: 2479-83.), only limited number of candidate TAAs for the treatment of adenocarcinomas, *e.g.*, colorectal cancer, are currently available. TAAs abundantly expressed in cancer cells yet whose expression is restricted to cancer cells would be promising candidates as immunotherapeutic targets. Further, identification of new TAAs inducing potent and specific antitumor immune responses is expected to encourage clinical use of peptide vaccination strategies for various types of cancer (Boon and van der Bruggen, (1996) *J Exp Med* 183: 725-9; van der Bruggen *et al.*, (1991) *Science* 254: 1643-7; Brichard *et al.*, (1993) *J Exp Med* 178: 489-95; Kawakami *et al.*, (1994) *J Exp Med* 180: 347-52; Shichijo *et al.*, (1998) *J Exp Med* 187: 277-88; Chen *et al.*, (1997) *Proc Natl Acad Sci USA* 94: 1914-8; Harris, (1996) *J Natl Cancer Inst* 88: 1442-55; Butterfield *et al.*, (1999) *Cancer Res* 59: 3134-42; Vissers *et al.*, (1999) *Cancer Res* 59: 5554-9; van der Burg *et al.*, (1996) *J Immunol* 156: 3308-14; Tanaka *et al.*, (1997) *Cancer Res* 57: 4465-8; Fujie *et al.*, (1999) *Int J Cancer* 80: 169-72; Kikuchi *et al.*, (1999) *Int J Cancer* 81:

459-66; Oiso *et al.*, (1999) Int J Cancer 81: 387-94.).

It has been repeatedly reported that peptide-stimulated peripheral blood mononuclear cells (PBMCs) from certain healthy donors produce significant levels of IFN- γ in response to the peptide, but rarely exert cytotoxicity against tumor cells in an HLA-A24 or -A0201 restricted manner in ^{51}Cr -release assays (Kawano *et al.*, (2000) Cancer Res 60: 3550-8; Nishizaka *et al.*, (2000) Cancer Res 60: 4830-7; Tamura *et al.*, (2001) Jpn J Cancer Res 92: 762-7 (2001)). However, both HLA-A24 and HLA-A0201 are popular HLA alleles in the Japanese, as well as the Caucasian populations (Date *et al.*, (1996) Tissue Antigens 47: 93-101; Kondo *et al.*, (1995) J Immunol 155: 4307-12; Kubo *et al.*, (1994) J Immunol 152: 3913-24; Imanishi *et al.*, (1992) Proceeding of the eleventh International Histocompatibility Workshop and Conference Oxford University Press, Oxford, 1065; Williams *et al.*, (1997) Tissue Antigen 49: 129-33.). Thus, antigenic peptides of carcinomas presented by these HLAs may be especially useful for the treatment of carcinomas among Japanese and Caucasian. Further, it is known that the induction of low-affinity CTL *in vitro* usually results from the use of peptide at a high concentration, generating a high level of specific peptide/MHC complexes on antigen presenting cells (APCs), which will effectively activate these CTL (Alexander-Miller *et al.*, (1996) Proc Natl Acad Sci USA 93: 4102-7.).

Accordingly, in an effort to understand the carcinogenic mechanisms associated with cancer and identify potential targets for developing novel anti-cancer agents, large scale analyses of gene expression patterns in purified populations of renal cancer cells were performed using a cDNA microarray representing 27,436 genes. More particularly, using a combination of cDNA microarray and laser beam microdissection, precise genome-wide expression profiles of 15 surgical specimens of renal cell carcinoma were examined. The findings discussed in detail herein suggest that these identified genes may play key roles in tumor cell growth proliferation, and are therefore promising targets for development of anti-cancer drugs.

SUMMARY OF THE INVENTION

Invention involves the discovery of a pattern of gene expression correlated with renal cell carcinoma (RCC). The genes that are differentially expressed in renal cell carcinoma are collectively referred to herein as "RCC nucleic acids" or "RCC polynucleotides" and the

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corresponding encoded polypeptides are referred to as "RCC polypeptides" or "RCC proteins."

To characterize the molecular mechanisms involved in renal carcinogenesis, the present inventors analyzed genome-wide gene expression profiles of 15 surgical specimens of renal cell carcinoma (RCC), as compared to normal renal cortex, through the combined use of cDNA microarrays representing 27,436 human genes and laser microbeam microdissection (LMM). The present inventors identified 251 genes that were commonly up-regulated and 721 genes that were down-regulated in renal cancer cells. Among the up-regulated genes were 74 genes whose functions are currently unknown, whereas 189 genes whose functions are unknown were identified as commonly down-regulated in renal carcinoma cells. The results of semi-quantitative RT-PCR experiments with 19 representatives of candidate genes supported the reliability of our microarray analysis. These data should provide useful information for finding candidate genes whose products might serve as molecular targets for treatment of RCCs.

Accordingly, the present invention provides a method of diagnosing or determining a predisposition to renal cell carcinoma in a subject by determining an expression level of a RCC-associated gene in a patient derived biological sample, such as tissue sample. Herein, the term "RCC-associated gene" refers to a gene that is characterized by an expression level which differs in an RCC cell as compared to a normal cell. A normal cell is one obtained from normal renal tissue. In the context of the present invention, an RCC-associated gene is one or more of the genes numbered RCC 1-972. An alteration, *e.g.*, an increase or decrease of the level of expression of a gene as compared to a normal control level of the gene indicates that the subject suffers from or is at risk of developing RCC.

In the context of the present invention, the phrase "control level" refers to a protein expression level detected in a control sample and includes both a normal control level and a RCC control level. A control level can be a single expression pattern derived from a single reference population or from a plurality of expression patterns. For example, the control level can be a database of expression patterns from previously tested cells. A "normal control level" refers to a level of gene expression detected in a normal, healthy individual or in a population of individuals known not to be suffering from RCC. A normal individual is one with no clinical symptoms of renal cell carcinoma. On the other hand, an "RCC control level" refers to an expression profile of an RCC-associated genes found in a population

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suffering from RCC.

An increase in the expression level of one or more genes numbered RCC 1-251 detected in a test sample as compared to a normal control level indicates that the subject (from which the sample was obtained) suffers from or is at risk of developing RCC. In contrast, a decrease in the expression level of one or more genes numbered RCC 252-972 detected in a test sample compared to a normal control level indicates said subject suffers from or is at risk of developing RCC.

Alternatively, expression of a panel of RCC-associated genes in a sample can be compared to an RCC control level of the same panel of genes. A similarity between a sample expression and an RCC control expression indicates that the subject (from which the sample was obtained) suffers from or is at risk of developing RCC.

According to the present invention, gene expression level is deemed "altered" when gene expression is increased or decreased 10%, 25%, 50% compared to the control level. Alternately, the gene expression may be also be deemed to be altered if it is increased or decreased 1, 2, 5 or more fold compared to the control level. Expression is determined by detecting hybridization, *e.g.*, on an array, of an RCC-associated gene probe to a gene transcript of the patient-derived tissue sample.

In the context of the present invention, the patient derived tissue sample is any tissue obtained from a test subject, *e.g.*, a patient known to or suspected of having RCC. For example, the tissue may contain an epithelial cell. More particularly, the tissue may be an epithelial cell from a renal cell carcinoma (RCC).

The present invention also provides an RCC reference expression profile composed of a gene expression level of two or more of RCC 1-972. Alternatively, the RCC reference expression profile may be composed of two or more of RCC 1-251 or RCC 252-972.

The present invention further provides methods of identifying an agent that inhibits or enhances the expression or activity of an RCC-associated gene, *e.g.* RCC 1-972, by contacting a test cell expressing a RCC-associated gene with a test agent and determining the expression level or activity of the RCC associated gene or the activity of its gene product. The test cell may be an epithelial cell, such as an epithelial cell obtained from a renal cell carcinoma. A decrease in the expression level of an up-regulated RCC-associated gene (*e.g.* RCC 1-251) or the activity its gene product as compared to a control level of the gene indicates that the test

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agent is an inhibitor of the RCC-associated gene and may be used to reduce a symptom of RCC. Alternatively, an increase in the expression level or activity of a down-regulated RCC-associated gene (*e.g.*, RCC 252-972) or the activity its gene product as compared to a control level of the gene indicates that said test agent is an enhancer of expression or function of the RCC-associated gene and may be used to reduce a symptom of RCC.

The present invention also provides a kit including a detection reagent which binds to one or more RCC nucleic acids or RCC polypeptides. Also provided is an array of nucleic acids that binds to one or more RCC nucleic acids.

Therapeutic methods of the present invention include a method of treating or preventing renal cell carcinoma in a subject including the step of administering to the subject an antisense composition. In the context of the present invention, the antisense composition reduces the expression of a specific target gene. For example, the antisense composition may contain a nucleotide that is complementary to an up-regulated RCC-associated gene sequence selected from the group consisting of RCC 1-251. Alternatively, the present method may include the step of administering to a subject a small interfering RNA (siRNA) composition. In the context of the present invention, the siRNA composition reduces the expression of an up-regulated RCC-associated gene, for example nucleic acids selected from the group consisting of RCC 1-251, in particular C6700V2 (RCC 81), B7032N (RCC 126) and B9320 (RCC173).

In yet another method, the treatment or prevention of renal cell carcinoma in a subject may be carried out by administering to a subject a ribozyme composition. Accordingly, the present invention encompasses nucleic acid-specific ribozyme compositions that reduce the expression of an up-regulated RCC-associated gene (*e.g.*, RCC 1-251). Other therapeutic methods of the present invention include those in which a subject is administered a compound that increases the expression of one or more of the down-regulated RCC-associated genes disclosed herein (*e.g.*, RCC 252-972) or the activity of a polypeptide encoded by one or more of these genes..

The present invention also includes vaccines and vaccination methods. For example, a method of treating or preventing RCC in a subject may involve administering to the subject a vaccine containing an RCC polypeptide encoded by an RCC nucleic acid selected from the group consisting of RCC 1-251 or an immunologically active fragment such a polypeptide. In the context of the present invention, an immunologically active fragment is a polypeptide that

is shorter in length than the full-length naturally-occurring protein and yet which induces an immune response analogous to that induced by the full-length protein. For example, an immunologically active fragment is preferably at least 8 residues in length and capable of stimulating an immune cell such as a T cell or a B cell. Immune cell stimulation can be measured by detecting cell proliferation, elaboration of cytokines (*e.g.*, IL-2), or production of an antibody.

The present invention is also based on the surprising discovery that inhibiting expression of C6700, B7032N or B9320 is effective in inhibiting the cellular growth of various cancer cells, including those involved in renal cell carcinoma. The inventions described in this application are based in part on this discovery.

The present invention also provides methods for inhibiting cell growth. Among the methods provided are those including the step of contacting a cell with a composition that includes a small interfering RNA (siRNA) that inhibits the expression of C6700, B7032N or B9320. The present invention also provides methods for inhibiting tumor cell growth in a subject. Such methods include administering to a subject a composition that includes a small interfering RNA (siRNA) that hybridizes specifically to a sequence selected from C6700, B7032N and B9320. In another aspect, the present invention provides methods for inhibiting the expression of one or more of the C6700, B7032N and B9320 genes 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 gene of interest (*e.g.*, the C6700, B7032N or B9320 gene).

Another aspect of the present invention relates to products, including nucleic acid sequences and vectors as well as to compositions including such, useful, for example, in the performing the instant methods. Among the products provided are siRNA molecules that inhibit the expression of one or more of the C6700, B7032N and B9320 genes when introduced into a cell expressing the target gene. Among such molecules are those that are composed of a sense strand and an antisense strand, wherein the sense strand is a ribonucleotide sequence corresponding to a C6700, B7032N or B9320 target sequence, and the antisense strand is a ribonucleotide sequence which is complementary to the sense strand. The sense and the antisense strands of the molecule hybridize to each other to form a double-stranded molecule.

The present invention also provides methods of inhibiting cell growth. Cell growth may be inhibited by contacting a cell with a composition of a small interfering RNA (siRNA) of C6700, B7032N or B9320. The cell may further be contacted with a transfection-enhancing agent. The cell may be provided *in vitro*, *in vivo* or *ex vivo*. The subject is preferably a mammal, *e.g.*, a human, non-human primate, mouse, rat, dog, cat, horse, or cow. The cell may be a genitourinary system, such as a renal cell. Alternatively, the cell may be a tumor cell (*i.e.*, cancer cell) such as a carcinoma cell or an adenocarcinoma cell. For example, the cell may be a renal cell carcinoma cell, more particularly, a clear cell. In the context of the present invention, the phrase "inhibiting cell growth" means that the treated cell proliferates at a lower rate or has a decreased viability as compared to an untreated cell. Cell growth can be measured by proliferation assays known in the art.

The present invention further provides a novel transcriptional variant, *C6700V2*, which serves as a candidate diagnostic marker for renal cell carcinoma as well as a promising potential target for developing new strategies for diagnosis and effective anti-cancer agents. In a preferred embodiment, the *C6700V2* polypeptide includes a 1151 amino acid (SEQ ID NO: 66) protein encoded by the open reading frame of SEQ ID NO: 65.

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.

One advantage of the methods described herein is that the disease is identified prior to detection of overt clinical symptoms of renal cell carcinoma. Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 presents photographic images of premicrodissected (lane a), postmicrodissected (lane b), and the microdissected cells (lane c) of clear cell RCC (left panel) and normal renal cortex cells (right panel) using Laser Microbeam Microdissection (LMM).

Figure 2 (a) depicts the results of semi-quantitative RT-PCR validation assays of highly expressed genes. Expression of 21 genes (Accession No. NM_018092, AA632745, NM_007250, W86513, BC077726, AA156409, W57613, CR749811, AW972553, AL832896, AK021778, AK026403, AK025204, NM_000677, AF070609, AI290343, AA442590, NM_000552, BC000234, BC034014 and NM_004567) and *FDFT1* (internal control) was examined by semi-quantitative RT-PCR. Signals of the microarray corresponded to the results of semi-quantitative RT-PCR experiments. Normal renal cortex was prepared from 11 patients with RCC used in this microarray. RPTEC means renal proximal tubule epithelial cell. Figure 2(b) depicts the expression of C6700 in 11 RCC cell lines measured by semi-quantitative RT-PCR.

Figure 3 depicts the results of Northern blot analysis of the expression pattern of C6700 in renal cell carcinoma cell lines and human normal-tissues.

Figure 4 depicts the results of Multiple northern blot analysis of 3545 bases-transcript in various normal human tissues using a specific probe (upper panel), and the expression of F5749 in normal human tissues using common sequence of 3545 bases- and 4533 bases-transcript as a probe (bottom panel).

Figure 5 depicts the results of Northern blot analyses of the C8919 transcript in various human tissues.

Figure 6 depicts the results of Northern blot analysis of the B7032N transcript in various human tissues (left panel) and the results Northern blot analysis of the B7032N transcript in RCC cell lines (Caki-1, Caki-2, 786-O, A498, ACHN, 769-P, A704, RXF-631L, OS-RC-2, TUHR10TKB and TUHR14TKB), RPTEC (renal proximal tubule epithelial cells) and normal human organs (heart, lung, liver, kidney, testis and brain) (right panel).

Figure 7 depicts the results of Northern blot analysis of the B9320 transcript in various human tissues(left panel) and the results of Northern blot analysis of the B9320 transcript in RCC cell lines (Caki-1, Caki-2, 786-O, and A498), RPTEC (renal proximal tubule epithelial cells) and normal human organs (heart, lung, liver, kidney, brain and testis) (right panel).

Figure 8 depicts the genomic structure of C6700. C6700 has two different variants, designated as V1 and V2. Figure 8(a) depicts the genomic structure, wherein the open

triangle indicates the presence of a stop codon at inframe and asolid triangle indicates the first methionine. Figure 8(b) depicts the results of semi-quantitative RT-PCR analysis of SEMA5B using RNAs prepared from four RCC cell lines (786-O, A704, OS-RC-2 and TUHR14TKB) and fetal brain poly A (+) RNA.

5 Figure 9 depicts the growth-inhibitory effects of small-interfering RNAs (siRNAs) designed to reduce the expression of C6700 in RCC cells. Figure 9(a) depicts the results of semi-quantitative RT-PCR demonstrating the suppression of endogenous expression of C6700 in RCC cell line, OC-RC-2. $\beta 2$ -MG was used as an internal control. si-#2 vector revealed knockdown effect; si-Scramble, and si-Mock failed to show any effect on the level of the
10 C6700 transcript. Transfection with si-#2 vector resulted in reduction of the number of colonies (Figure 9(c)), and numbers of viable cells (Figure 9 (b)) compared with the cells transfected with si-Scramble and si-Mock ($p < 0.005$ and $p < 0.001$, respectively; unpaired t-test).

 Figure 10 depicts the expression and subcellular localization of exogenous
15 B7032N protein in COS7 cells. Figure 10(a) depicts the exogenous expression of B7032N protein by Western blot at 24 and 48 hours after transfection and Figure 10(b) depicts the subcellular localization of Exogenous B7032 protein in COS7 cells.

 Figure 11 depicts the siRNA knockdown effect of B7032N on RCC cell growth and cell viability. Figure 11(a) depicts the effects of RXF-631L cells to si- B7032N (#4) or a
20 control siRNA (si-Scramble), analyzed by semi-quantitative RT-PCR (top left), colony formation assays (bottom left) and MTT assays (right). RT-PCR experiments for examining the B7032N transcript level in the cells treated with siRNA. $\beta 2$ -MG expression level was as quantitative control. si B7032N-#4 vectors revealed knockdown effect; si-Scramble failed to show any effect on the level of the B7032N transcript. Transfection with B7032N-#4 vector
25 resulted in reduction of the number of colonies, and numbers of viable cells, compared with the cells transfected with si-Scramble ($p < 0.0001$; unpaired t-test). (b) Effect of A498 cells to si-B7032N (#4) or a control siRNA (si-Scramble), analyzed by semi-quantitative RT-PCR (top left), colony formation assays (bottom left) and MTT assays (right). RT-PCR
30 experiments for examining the B7032N transcript level in the cells treated with siRNA. $\beta 2$ -MG expression level was as quantitative control. si-B7032N-#4 vectors revealed knockdown effect; si-Scramble failed to show any effect on the level of the B7032N transcript. Transfection with si-B7032N-#4 vector resulted in reduction of the number of colonies, and

numbers of viable cells, compared with the cells transfected with si-Scramble ($p < 0.0001$; unpaired t-test).

Figure 12 depicts the growth-promoting effect of exogenous B7032N in NIH3T3 cells. Figure 12(a) depicts the results of Western blot analysis of cells expressing exogenous B7032N at high level or those transfected with mock vector. Exogenous introduction of B7032N expression were validated with anti-HA-tag monoclonal antibody. Beta-actin served as a loading control. Figure 11(b) depicts the *in vitro* growth of NIH3T3- B7032N cells. NIH3T3 cells transfected with B7032N (B7032N -A, -B, -C, -D) and mock (Mock-A, -B, -C), as measured by MTT assay.

Figure 13 depicts the expression and subcellular localization of exogenous B9320 protein in COS7 cells. Figure 12(a) depicts the exogenous expression of B9320 protein as demonstrated by Western blot at 24 and 48 hours after transfection and Figure 13(b) depicts the subcellular localization of Exogenous B9320 protein in COS7 cells.

Figure 14 depicts the siRNA knockdown effect of B9320 on RCC cell growth and cell viability. Figure 14(a) depicts the effects of Caki-2 cells to si-B9320 (#2) or a control siRNA (si-Scramble) analyzed by semi-quantitative RT-PCR (top left), colony formation assays (bottom left) and MTT assays (right). RT-PCR experiments for examining the B9320 transcript level in the cells treated with siRNA. β 2-MG expression level was as quantitative control. si- B9320-#2 vectors revealed knockdown effect; si-Scramble failed to show any effect on the level of the B9320 transcript. Transfection with si-B9320-#2 vector resulted in reduction of the number of colonies, and numbers of viable cells, compared with the cells transfected with si-Scramble ($p < 0.0001$; unpaired t-test). Figure 14(b) depicts the effects of A498 cells to si-B9320-#2 or a control siRNA (si-Scramble), analyzed by semi-quantitative RT-PCR (top left), colony formation assays (bottom left) and MTT assays (right). RT-PCR experiments for examining the B9320 transcript level in the cells treated with siRNA. β 2-MG expression level was as quantitative control. si-B9320-#2 vectors revealed knockdown effect; si-Scramble failed to show any effect on the level of the B9320 transcript. Transfection with si-B9320-#2 vector resulted in reduction of the number of colonies, and numbers of viable cells, compared with the cells transfected with si-Scramble ($p < 0.0001$; unpaired t-test).

Figure 15 depicts the growth-promoting effects of exogenous B9320 in NIH3T3 cells. Figure 15(a) depicts the results of Western blot analysis of cells expressing exogenous B9320 at high level or those transfected with mock vector. Exogenous introduction of B9320

expression were validated with anti-HA-tag monoclonal antibody. Beta-actin served as a loading control. Figure 15(b) depicts the *in vitro* growth of NIH3T3- B9320 cells. NIH3T3 cells transfected with B9320 (B9320-1, -2, -3) and mock (Mock-1, -2, -3), as measured by MTT assay.

5 DETAILED DESCRIPTION OF THE INVENTION

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

Generally, the gene-expression analysis of RCC as compared to normal renal cell by using tumor mass is distorted because RCC cells exist as a solid mass containing various cellular components. As such, the results include “noisy data”. Therefore Laser capture microdissection (LCM), or Laser microbeam microdissection (LMM), a method for isolating pure cell populations, was used herein to obtain specific cancer cells and normal epithelial cells (Kitahara *et al.*, (2001) Cancer Res, 61: 3544-9; Gjerdrum *et al.*, (2001) J Mol Diagn, 3: 105-10.).

15 The present invention is based in part on the discovery of changes in expression patterns of multiple nucleic acids between epithelial cells and renal cells obtained from patients with RCC. The differences in gene expression were identified using a comprehensive cDNA microarray system.

20 The gene-expression profiles of cancer cells from 15 RCCs were analyzed using cDNA microarray representing 27,648 genes coupled with laser microdissection. By comparing expression patterns between cancer cells from patients diagnosed with and normal renal cells purely selected with Laser Microdissection, 251 genes were identified as commonly up-regulated in RCC cells, and 721 genes were identified as being commonly down-regulated in RCC cells. In addition, selection was made of candidate molecular markers having the potential to detect cancer-related proteins in serum or urine of patients, and some potential targets for development of signal-suppressing strategies in human RCC were discovered.

30 The differentially expressed genes identified herein find diagnostic utility as markers of RCC and as RCC gene targets, the expression of which may be altered to treat or alleviate a symptom of RCC.

The genes whose expression level is modulated (*i.e.*, increased or decreased) in RCC patients are summarized in Tables 4-5 and are collectively referred to herein as "RCC-

associated genes", "RCC nucleic acids" or "RCC polynucleotides" and the corresponding encoded polypeptides are referred to as "RCC polypeptides" or "RCC proteins". Unless indicated otherwise, "RCC" refers to any of the sequences disclosed herein. (*e.g.*, RCC 1-972). Genes that have been previously described are presented along with a database accession
5 number.

By measuring expression of the various genes in a sample of cells, RCC can be diagnosed. Similarly, measuring the expression of these genes in response to various agents can identify agents for treating RCC.

The present invention involves determining (*e.g.*, measuring) the expression of at least
10 one, and up to all the RCC sequences listed in Tables 4-5. Using sequence information provided by the GeneBankTM database entries for known sequences, the RCC-associated genes can be detected and measured using techniques well known to one of ordinary skill in the art. For example, sequences within the sequence database entries corresponding to RCC sequences can be used to construct probes for detecting RNA sequences corresponding to
15 RCC-associated genes in, *e.g.*, Northern blot hybridization analysis. Probes typically include at least 10, 20, 50, 100, or 200 nucleotides of a reference sequence. As another example, the sequences can be used to construct primers for specifically amplifying the RCC nucleic acid in, *e.g.*, amplification-based detection methods, such as reverse-transcription based polymerase chain reaction.

20 Expression level of one or more RCC-associated genes in a test cell population, *e.g.*, a patient derived tissues sample, is then compared to the expression level(s) of the same genes in a reference population. The reference cell population includes one or more cells for which the compared parameter is known, *i.e.*, renal cell carcinoma cells (*e.g.*, RCC cells) or normal renal cortex cells (*e.g.*, non-RCC cells).

25 Whether or not a pattern of gene expression in a test cell population as compared to a reference cell population indicates RCC or a predisposition thereto depends upon the composition of the reference cell population. For example, if the reference cell population is composed of non-RCC cells, a similarity in gene expression pattern between the test cell population and the reference cell population indicates the test cell population is non-RCC.
30 Conversely, if the reference cell population is made up of RCC cells, a similarity in gene expression profile between the test cell population and the reference cell population indicates that the test cell population includes RCC cells.

A level of expression of a RCC marker gene in a test cell population is considered altered if it varies from the expression level of the corresponding RCC marker gene in a reference cell population by more than 1.0, 1.5, 2.0, 5.0, 10.0 or more fold.

Differential gene expression between a test cell population and a reference cell population can be normalized to a control nucleic acid, *e.g.* a housekeeping gene. For example, a control nucleic acid is one which is known not to differ depending on the cancerous or non-cancerous state of the cell. The expression levels of a control nucleic acid can be used to normalize signal levels in the test and reference populations. Exemplary control genes include, but are not limited to, *e.g.*, β -actin, glyceraldehyde 3-phosphate dehydrogenase and ribosomal protein P1.

The test cell population can be compared to multiple reference cell populations. Each of the multiple reference populations may differ in the known parameter. Thus, a test cell population may be compared to a first reference cell population known to contain, *e.g.*, RCC cells, as well as a second reference population known to contain, *e.g.*, non-RCC cells (normal cells). The test cell may be included in a tissue type or cell sample from a subject known to contain, or suspected of containing, RCC cells.

The test cell is obtained from a bodily tissue or a bodily fluid, *e.g.*, biological fluid (such as blood, sputum, or urine, for example). For example, the test cell may be purified from renal tissue. Preferably, the test cell population includes an epithelial cell. The epithelial cell is preferably from a tissue known to be or suspected to be a renal cell carcinoma.

Cells in the reference cell population should be derived from a tissue type similar to that of the test cell. Optionally, the reference cell population is a cell line, *e.g.* a RCC cell line (*i.e.*, a positive control) or a normal non-RCC cell line (*i.e.*, a negative control). Alternatively, the control cell population may be derived from a database of molecular information derived from cells for which the assayed parameter or condition is known.

The subject is preferably a mammal. Exemplary mammals include, but are not limited to, *e.g.*, a human, non-human primate, mouse, rat, dog, cat, horse, or cow.

Expression of the genes disclosed herein can be determined at the protein or nucleic acid level using methods known in the art. For example, Northern hybridization analysis using probes which specifically recognize one or more of these nucleic acid sequences can be used to determine gene expression. Alternatively, gene expression may be measured using

reverse-transcription-based PCR assays, *e.g.*, using primers specific for the differentially expressed gene sequences. Expression may also be determined at the protein level, *i.e.*, by measuring the level of a polypeptide encoded by a gene described herein, or the biological activity thereof. Such methods are well known in the art and include, but are not limited to, *e.g.*, immunoassays that utilize antibodies to proteins encoded by the genes. The biological activities of the proteins encoded by the genes are generally well known.

As used herein, the term "organism" refers to any living entity of at least one cell. A living organism can be as simple as, for example, a single eukaryotic cell or as complex as, for example, 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). The term "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, the term "biological sample" may also refer 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.

Diagnosing Renal Cell Carcinoma

In the context of the present invention, RCC is diagnosed by measuring the expression level of one or more RCC nucleic acid sequences from a test population of cells, (*i.e.*, a patient derived biological sample). Preferably, the test cell population contains an epithelial cell, *e.g.*, a cell obtained from renal tissue. Gene expression can also be measured from blood or other bodily fluids such as urine. Other biological samples can be used for measuring protein levels. For example, the protein level in blood or serum derived from a subject to be diagnosed can be measured by immunoassay or other conventional biological assay.

Expression of one or more RCC-associated genes, *e.g.*, RCC 1-972 is determined in the test cell or biological sample and compared to the normal control expression level associated with the particular RCC-associated gene(s) assayed. A normal control level is an expression profile of a RCC-associated gene typically found in a population known not to be suffering from RCC. An alteration (*i.e.*, an increase or decrease) in the level of expression in

the patient-derived tissue sample of one or more RCC-associated genes indicates that the subject is suffering from or is at risk of developing RCC. For example, an increase in expression of one or more up-regulated RCC genes, *i.e.*, RCC 1-251, in the test population as compared to the normal control level indicates that the subject is suffering from or is at risk of developing RCC. Conversely, a decrease in expression of one or more down-regulated genes, *i.e.*, RCC 252-972, in the test population as compared to the normal control level indicates that the subject is suffering from or is at risk of developing RCC.

Alteration of one or more of the RCC-associated genes in the test population as compared to the normal control level indicates that the subject suffers from or is at risk of developing RCC. For example, alteration of at least 1%, 5%, 25%, 50%, 60%, 80%, 90% or more of the panel of RCC-associated genes (RCC 1-251, RCC 252-972, or RCC 1-972) indicates that the subject suffers from or is at risk of developing RCC.

The expression levels of RCC-associated genes in a biological sample can be estimated by quantifying mRNA corresponding to a protein encoded by an RCC-associated gene. Quantification methods for mRNA are known to those skilled in the art. For example, the levels of mRNAs corresponding to RCC-associated genes can be estimated by Northern blotting or RT-PCR. Since the nucleotide sequences of RCC-associated genes are known, anyone skilled in the art can design probes or primers to quantify RCC-associated genes. For example, an illustrative *C6700V2* specific primer set is set forth in the nucleotide sequences of SEQ ID NOs: 38 and 12. Alternatively, both of *C6700V1* and *C6700V2* may be amplified using a primer set that anneals to common nucleotide sequences. When amplified product of such a primer set includes a variant specific region (*i.e.* deletion of exon 21), the amplified product may be distinct from each other based on sequence length. For example, primers of the nucleotide sequence of SEQ ID NOs: 83 and 84 may be used as primer set for both variants. Furthermore, the amplified product may also be detected using a variant specific probe.

In addition, the expression level of the RCC-associated genes can be analyzed based on the activity or quantity of a protein encoded by the genes. A method for determining the quantity of a protein encoded by an RCC-associated gene is described below. For example, immunoassay methods may be used to determine the presence of such proteins in biological materials. As noted above, any biological materials can be used as the biological sample for the determination of the protein or its activity, so long as the marker gene (*i.e.*, the RCC-

associated gene) is expressed in the sample of a renal cancer patient. For example, a renal tubule is an example of a suitable biological sample. However, bodily fluids such as blood and urine may be also analyzed. On the other hand, a suitable method can be selected for the determination of the activity of a protein encoded by an RCC-associated gene according to the activity of the protein to be analyzed.

Expression levels of RCC-associated genes in a biological sample may be estimated and compared with those in a normal sample (*e.g.*, a sample derived from a non-diseased subject). When such a comparison shows that the expression level of the genes is higher (RCC-associated genes shown in table 4, *i.e.* 1-251) or lower (RCC-associated genes shown in table 5, *i.e.* 252-972) than those in the normal sample, the subject is judged to be afflicted with RCC. The expression level of the RCC-associated genes in the biological samples from a normal subject and subject to be diagnosed may be determined at the same time. Alternatively, normal ranges of the expression levels can be determined by a statistical method based on the results obtained from analyzing the expression level of the genes in samples previously collected from a control group. A result obtained by comparing the sample of a subject is compared with the normal range; when the result does not fall within the normal range, the subject is judged to be afflicted with or is at risk of developing RCC.

In the context of the present invention, a diagnostic agent for diagnosing a cell proliferative disease, such as RCC, is also provided. Examples of diagnostic agents of the present invention include compounds that binds to a polynucleotide or a polypeptide of an RCC-associated gene. Preferably, an oligonucleotide that hybridizes to the polynucleotide of RCC-associated genes or an antibody that binds to the polypeptide encoded by RCC-associated genes is used as such a compound. The present method of diagnosing RCC may also be used to assess the efficacy of treatment of RCC in a subject. According to the method, a biological sample, such as a test cell population, is obtained from a subject undergoing treatment for RCC. The method for assessment can be conducted according to conventional methods of diagnosing RCC.

If desired, biological samples may be obtained from the subject at various time points before, during and/or after treatment. The expression level of RCC-associated genes, in the biological sample is then determined and compared to a control level derived, for example, from a reference cell population which includes cells whose state of RCC (*i.e.*, cancerous cell or non-cancerous cell) is known. The control level is determined in a biological sample that

has not been exposed to the treatment.

If the control level is derived from a biological sample which contains no cancerous cell, a similarity between the expression level in the subject-derived biological sample and the control level indicates that the treatment of interest is efficacious. However, a difference in the expression level of the RCC-associated genes in the subject-derived biological sample and the control level indicates a less favorable clinical outcome or prognosis.

Identifying Agents That Inhibit Or Enhance RCC-Associated Gene Expression:

An agent that inhibits the expression of an RCC-associated gene or the activity of its gene product can be identified by contacting a test cell population expressing a RCC-associated up-regulated gene with a test agent and then determining the expression level or activity of the RCC-associated gene. A decrease in the level of expression if the RCC-associated gene or in the level of activity of its gene product in the presence of the agent as compared to the control level (or compared to the expression or activity level in the absence of the test agent) indicates that the agent is an inhibitor of an RCC-associated up-regulated gene and may therefore proved to be useful in inhibiting the onset or progression of RCC.

Alternatively, an agent that enhances the expression of an RCC-associated down-regulated gene or the activity of its gene product can be identified by contacting a test cell population expressing a RCC-associated gene with a test agent and then determining the expression level or activity of the RCC-associated down-regulated gene. An increase in the level of expression of the RCC-associated gene or in the level of activity of its gene product as compared to a control expression level or activity (or compared to the level in the absence of the test agent) of the RCC-associated gene indicates that the test agent augments expression of the RCC-associated down-regulated gene or the activity of its gene product.

The test cell population may be any cell expressing the RCC-associated genes. For example, the test cell population may contain an epithelial cell, such as a cell derived from renal tissue. Furthermore, the test cell may be an immortalized cell line derived from an adenocarcinoma cell. Alternatively, the test cell may be a cell that has been transfected with a RCC-associated gene or which has been transfected with a regulatory sequence (e.g. promoter sequence) from a RCC-associated gene operably linked to a reporter gene.

Assessing Efficacy Of Treatment Of RCC In A Subject:

The differentially expressed RCC-associated genes identified herein also allow for the course of treatment of RCC to be monitored. In this method, a test cell population is provided from a subject undergoing treatment for RCC. If desired, test cell populations are obtained from the subject at various time points, before, during, and/or after treatment. Expression of one or more of the RCC-associated genes in the cell population is then determined and compared to a reference cell population that includes cells whose RCC state is known. In the context of the present invention, the reference cells should not been exposed to the treatment.

If the reference cell population contains no RCC cells, a similarity in the expression of an RCC-associated gene between the test cell population and the reference cell population indicates that the treatment of interest is efficacious. However, a difference in the expression of an RCC-associated gene between the test population and a normal control reference cell population indicates a less favorable clinical outcome or prognosis. Similarly, if the reference cell population contains RCC cells, a difference between the expression of a RCC-associated gene in the test cell population and the reference cell population indicates that the treatment of interest is efficacious, while a similarity in the expression of a RCC-associated gene in the test population and a normal control reference cell population indicates a less favorable clinical outcome or prognosis.

Additionally, the expression level of one or more RCC-associated genes determined in a subject-derived biological sample obtained after treatment (*i.e.*, post-treatment levels) can be compared to the expression level of the one or more RCC-associated genes determined in a subject-derived biological sample obtained prior to treatment onset (*i.e.*, pre-treatment levels). If the RCC-associated gene is an up-regulated gene, a decrease in the expression level in a post-treatment sample indicates that the treatment of interest is efficacious while an increase or maintenance in the expression level in the post-treatment sample indicates a less favorable clinical outcome or prognosis. Conversely, if the RCC-associated gene is a down-regulated gene, an increase in the expression level in a post-treatment sample may indicate that the treatment of interest is efficacious while an increase or maintenance in the expression level in the post-treatment sample indicates a less favorable clinical outcome or prognosis. As used herein, the term "efficacious" refers to a treatment leads to a reduction in the expression of a pathologically up-regulated gene, an increase in the expression of a pathologically down-regulated gene or a decrease in the size, prevalence, or metastatic potential of renal cell carcinoma in a subject. When treatment of interest is applied prophylactically, "efficacious" means that the treatment retards or prevents a renal cell

carcinoma from forming or retards, prevents, or alleviates a symptom of clinical RCC. Assessment of renal cell carcinoma may be made using standard clinical protocols.

Efficaciousness can be determined in association with any known method for diagnosing or treating RCC. RCC can be diagnosed for example, by identifying symptomatic anomalies, *e.g.*, weight loss, abdominal pain, back pain, anorexia, nausea, vomiting and generalized malaise, weakness, and jaundice.

Selecting A Therapeutic Agent For Treating RCC In A Particular Individual:

Differences in the genetic makeup of individuals can result in differences in their relative abilities to metabolize various drugs. An agent that is metabolized in a subject to act as an anti-RCC agent can manifest itself by inducing a change in gene expression pattern in the subject's cells from that characteristic of a cancerous state to a gene expression pattern characteristic of a non-cancerous state. Accordingly, the differentially expressed RCC-associated genes disclosed herein allow for a putative therapeutic or prophylactic inhibitor of RCC to be tested in a test cell population from a selected subject in order to determine if the agent is a suitable inhibitor of RCC in the subject.

To identify an inhibitor of RCC, that is appropriate for a specific subject, a test cell population from the subject is exposed to a therapeutic agent, and the expression of one or more of RCC 1-972 genes is determined.

In the context of the present invention, the test cell population may contain an RCC cell expressing an RCC-associated gene. Preferably, the test cell is an epithelial cell. For example, a test cell population can be incubated in the presence of a candidate agent and the pattern of gene expression of the test sample can be measured and compared to one or more reference profiles, *e.g.*, an RCC reference expression profile or a non-RCC reference expression profile.

A decrease in the expression of one or more of up-regulated RCC genes, *i.e.*, RCC 1-251, or an increase in expression of one or more of the down-regulated RCC genes, *i.e.*, RCC 252-972, in a test cell population relative to a reference cell population containing RCC indicates that the agent is therapeutic.

In the context of the present invention, the test agent can be any compound or composition. Exemplary test agents suitable for use in the context of the present invention include, but are not limited to, immunomodulatory agents.

Screening Assays For Identifying Therapeutic Agents:

The differentially expressed RCC-associated genes disclosed herein can also be used to identify candidate therapeutic agents for treating RCC. The method of the present invention involves screening a candidate therapeutic agent to determine if it can convert an expression profile of one or more RCC-associated genes, such as RCC 1-972, characteristic of a RCC state to a gene expression pattern characteristic of a RCC state.

In the present invention, RCC 1-972 are useful for screening of therapeutic agent for treating or preventing RCC.

In the instant method, a cell is exposed to a test agent or a plurality of test agents (sequentially or in combination) and the expression of one or more RCC 1-972 in the cell is measured. The expression profile of the RCC-associated gene(s) assayed in the test population is compared to expression level of the same RCC-associated gene(s) in a reference cell population that is not exposed to the test agent.

An agent capable of stimulating the expression of an under-expressed gene or suppressing the expression of an overexpressed gene has potential clinical benefit. Such agents may be further tested for the ability to prevent RCC onset and progression in animals or test subjects.

In a further embodiment, the present invention provides methods for screening candidate agents which are potential targets in the treatment of RCC. As discussed in detail above, by controlling the expression levels of marker genes or the activities of their gene products, one can control the onset and progression of RCC. Thus, candidate agents, which are potential targets in the treatment of RCC, can be identified through screening methods that use such expression levels and activities of as indices of the cancerous or non-cancerous state. In the context of the present invention, such screening may include, for example, the following steps:

- a) contacting a test compound with a polypeptide encoded by a polynucleotide selected from the group consisting of RCC 1-972,
- b) detecting the binding activity between the polypeptide and the test compound; and
- c) selecting the test compound that binds to the polypeptide.

A polypeptide encoded by a marker gene to be used for screening may be a

recombinant polypeptide or a protein derived from the nature or a partial peptide thereof. The polypeptide to be contacted with a test compound can be, for example, a purified polypeptide, a soluble protein, a form bound to a carrier or a fusion protein fused with other polypeptides.

As a method of screening for proteins, for example, that bind to a polypeptide encoded by a marker gene, many methods well known to those skilled in the art can be used. Such a screening can be conducted by, for example, immunoprecipitation method, specifically, in the following manner. The marker gene is expressed in host (*e.g.*, animal) cells and so on by inserting the gene to an expression vector for foreign genes, examples of which include, but are not limited to, pSV2neo, pcDNA I, pcDNA3.1, pCAGGS and pCD8. The promoter to be used for the expression may be any promoter that can be used commonly, including, for example, the SV40 early promoter (Rigby in Williamson (ed.), (1982) Genetic Engineering, vol. 3. Academic Press, London, 83-141.), the EF- α promoter (Kim *et al.*, (1990) Gene 91: 217-23.), the CAG promoter (Niwa *et al.*, (1991) Gene 108: 193-9), the RSV LTR promoter (Cullen, (1987) Methods in Enzymology 152: 684-704.) the SR α promoter (Takebe *et al.*, (1988) Mol Cell Biol 8: 466-72.), the CMV immediate early promoter (Seed and Aruffo, (1987) Proc Natl Acad Sci USA 84: 3365-9.), the SV40 late promoter (Gheysen and Fiers, (1982) J Mol Appl Genet 1: 385-94.), the Adenovirus late promoter (Kaufman *et al.*, (1989) Mol Cell Biol 9: 946-58.), the HSV TK promoter and so on. The introduction into host cells of the foreign gene to be expressed can be performed according to any methods, for example, the electroporation method (Chu *et al.*, (1987) Nucleic Acids Res 15: 1311-26.), the calcium phosphate method (Chen and Okayama, (1987) Mol Cell Biol 7: 2745-52.), the DEAE dextran method (Lopata *et al.*, (1984) Nucleic Acids Res 12: 5707-17; Sussman and Milman, (1984) Mol Cell Biol 4: 1641-3.), the Lipofectin method (Derijard B, (1994) Cell 76: 1025-37; Lamb *et al.*, (1993) Nature Genetics 5: 22-30; Rabindran *et al.*, (1993) Science 259: 230-4.) and so on. The polypeptide encoded by the marker gene can be expressed as a fusion protein having a recognition site (epitope) of a monoclonal antibody by introducing the epitope of the monoclonal antibody, whose specificity has been revealed, to the N- or C-terminus of the polypeptide. A commercially available epitope-antibody system can be used (Experimental Medicine 13: 85-90 (1995)). Vectors which can express a fusion protein with, for example, β -galactosidase, maltose binding protein, glutathione S-transferase, green fluorescence protein (GFP) and so on through the use of multiple cloning sites are commercially available.

A fusion protein prepared by introducing only small epitopes made up of several to a dozen amino acids so as not to change the property of the polypeptide by the fusion is also contemplated herein. Epitopes, such as polyhistidine (His-tag), influenza aggregate HA, human c-myc, FLAG, Vesicular stomatitis virus glycoprotein (VSV-GP), T7 gene 10 protein (T7-tag), human simple herpes virus glycoprotein (HSV-tag), E-tag (an epitope on monoclonal phage) and such, and monoclonal antibodies recognizing them can be used as the epitope-antibody system for screening proteins binding to the polypeptide encoded by marker genes (Experimental Medicine 13: 85-90 (1995)).

In immunoprecipitation, an immune complex is formed by adding these antibodies to cell lysate prepared using an appropriate detergent. The immune complex consists of the polypeptide encoded by the marker genes, a polypeptide having the binding ability with the polypeptide, and an antibody. In addition to antibodies against the above epitopes, immunoprecipitation can be also conducted using antibodies against the polypeptide encoded by the marker genes, such antibodies optionally prepared as described above.

An immune complex can be precipitated, for example, by Protein A sepharose or Protein G sepharose when the antibody is a mouse IgG antibody. If the polypeptide encoded by the marker genes is prepared as a fusion protein with an epitope, such as GST, an immune complex can be formed in the same manner as in the use of the antibody against the polypeptide, using a substance specifically binding to these epitopes, such as glutathione-Sepharose 4B.

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

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

As a method for screening proteins that bind to a polypeptide encoded by a marker gene, for example, West-Western blotting analysis (Skolnik *et al.*, (1991) Cell 65: 83-90.) can be used. Specifically, a protein binding to a polypeptide encoded by a marker gene can be obtained by preparing a cDNA library from cells, tissues, organs (for example, tissues such as testis or ovary), or cultured cells (*e.g.*, Caki-1, Caki-2, 786-O, A704, OS-RC-2, TUHR14TKB and A498) expected to express a protein that binds to a polypeptide encoded by a marker gene using a phage vector (*e.g.*, ZAP), expressing the protein on LB-agarose, fixing the protein expressed on a filter, reacting the purified and the labeled polypeptide with the above filter, and detecting the plaques expressing proteins bound to the polypeptide encoded by the marker genes according to the label. The polypeptide encoded by the marker gene may be labeled by utilizing the binding between biotin and avidin, or by utilizing an antibody that specifically binds to the polypeptide encoded by the marker gene, or a peptide or polypeptide (for example, GST) that is fused to the polypeptide encoded by the marker gene. Methods using radioisotope or fluorescence and such may be also used.

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

In the two-hybrid system, a polypeptide of the invention is fused to an SRF-binding region or GAL4-binding region and expressed in yeast cells. A cDNA library is prepared from cells expected to express a protein that binds to a polypeptide of the invention, such that the library, when expressed, is fused to the VP16 or GAL4 transcriptional activation region. The cDNA library is then introduced into the above yeast cells and the cDNA derived from the library is isolated from the positive clones detected (when a protein binding to the polypeptide of the invention is expressed in yeast cells, the binding of the two activates a reporter gene; making positive clones detectable). A protein encoded by the cDNA can be prepared by introducing the cDNA isolated above to *E. coli* and expressing the protein.

Examples of reporter genes include, but are not limited to, Ade2 gene, lacZ gene, CAT gene, luciferase gene and such, in addition to the HIS3 gene.

A compound that binds to a polypeptide encoded by a marker gene can also be

screened using affinity chromatography. For example, a polypeptide encoded by a marker gene may be immobilized on a carrier of an affinity column, and a test compound, containing a protein capable of binding to the polypeptide encoded by the marker gene, is applied to the column. A test compound herein may be, for example, cell extracts, cell lysates, *etc.* After
5 loading the test compound, the column is washed, and compounds bound to the polypeptide can be prepared.

When the test compound is a protein, the amino acid sequence of the obtained protein is analyzed, an oligo DNA is synthesized based on the sequence, and cDNA libraries are screened using the oligo DNA as a probe to obtain a DNA encoding the protein.

10 A biosensor using the surface plasmon resonance phenomenon may be used as a means for detecting or quantifying the bound compound in the present invention. When such a biosensor is used, the interaction between the polypeptide of the invention and a test compound can be observed real-time as a surface plasmon resonance signal, using only a minute amount of polypeptide and without labeling (for example, BIAcore, Pharmacia).
15 Therefore, it is possible to evaluate the binding between the polypeptide encoded by the marker genes and a test compound using a biosensor such as BIAcore.

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

Alternatively, the present invention provides a method of screening for a compound
25 for treating or preventing RCC using a polypeptide encoded by a marker gene including the steps as follows:

- a) contacting a test compound with a polypeptide encoded by a polynucleotide selected from the group consisting of RCC 1-972;
- b) detecting the biological activity of the polypeptide of step (a); and
- 30 c) selecting a compound that suppresses the biological activity of the polypeptide encoded by the polynucleotide selected from the group consisting of RCC 1-251 as

compared to the biological activity detected in the absence of the test compound, or enhances the biological activity of the polypeptide encoded by the polynucleotide selected from the group consisting of RCC 252-972 as compared to the biological activity detected in the absence of the test compound.

5 A polypeptide for use in the screening method of the present invention can be obtained as a recombinant protein using the nucleotide sequence of the marker gene. Any polypeptides can be used for screening so long as they retain the biological activity of the polypeptide encoded by the marker genes. Based on the information regarding the marker gene and its encoded polypeptide, one skilled in the art can select any biological activity of
10 the polypeptide as an index for screening and any suitable measurement method to assay for the selected biological activity.

The compound isolated by this screening is a candidate agonist or antagonist of a polypeptide encoded by a marker gene. The term "agonist" refers to molecules that activate the function of the polypeptide by binding thereto. Likewise, the term "antagonist" refers to
15 molecules that inhibit the function of the polypeptide by binding thereto. Moreover, a compound isolated by this screening is a candidate inhibitor or stimulator of the *in vivo* interaction of a polypeptide encoded by a marker gene with molecules (including DNAs and proteins).

When the biological activity to be detected in the present method is cell
20 proliferation, it can be detected, for example, by preparing cells which express the polypeptide encoded by the marker gene, culturing the cells in the presence of a test compound, and determining the speed of cell proliferation, measuring the cell cycle and such, as well as by measuring the colony forming activity as described in the Examples.

In a further embodiment, the present invention provides methods for screening
25 compounds for treating or preventing RCC. As discussed in detail above, by controlling the expression levels of the marker genes, one can control the onset and progression of RCC. Thus, compounds that may be used in the treatment or prevention of RCC can be identified through screenings that use the expression levels of marker genes as indices. In the context of the present invention, such screening may include, for example, the following steps:

- 30 a) contacting a candidate compound with a cell expressing one or more marker genes, wherein the one or more marker genes are selected from the group consisting of

RCC 1-972; and

- b) selecting the candidate compound that reduces the expression level of one or more marker genes selected from the group consisting of RCC 1-251, or elevates the expression level of one or more marker genes selected from the group consisting of
5 RCC 252-972.

Cells expressing a marker gene include, for example, cell lines established from RCC; such cells can be used for the above screening of the present invention (*e.g.*, Caki-1, Caki-2, 786-O, A704, OS-RC-2, TUHR14TKB and A498). Expression levels can be estimated by methods well known to one skilled in the art. In the method of screening, a
10 compound that reduces or enhances the expression level of one or more marker genes is a candidate agent for use in the treatment or prevention of RCC.

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

- a) contacting a candidate compound with a cell into which a vector having the
15 transcriptional regulatory region of one or more marker genes and a reporter gene that is expressed under the control of the transcriptional regulatory region has been introduced, wherein the one or more marker genes are selected from the group consisting of RCC 1-972
- b) measuring the expression or activity of said reporter gene; and
- c) selecting the candidate compound that reduces the expression or activity level of said
20 reporter gene when said marker gene is an up-regulated marker gene selected from the group consisting of RCC 1-251, or that enhances the expression level of said reporter gene when said marker gene is a down-regulated marker gene selected from the group consisting of RCC 252-972, as compared to a level detected in the absence
25 of the test compound.

Suitable reporter genes and host cells are well known in the art. A reporter construct suitable for the screening method of the present invention can be prepared by using the transcriptional regulatory region of a marker gene. When the transcriptional regulatory region of a marker gene is known to those skilled in the art, a reporter construct can be
30 prepared by using the previous sequence information. When the transcriptional regulatory region of a marker gene remains unidentified, a nucleotide segment containing the

transcriptional regulatory region can be isolated from a genome library based on the nucleotide sequence information of the marker gene.

Examples of supports that may be used for binding proteins include insoluble polysaccharides, such as agarose, cellulose and dextran; and synthetic resins, such as polyacrylamide, polystyrene and silicon; preferably commercial available beads and plates (e.g., multi-well plates, biosensor chip, *etc.*) prepared from the above materials may be used. When using beads, they may be filled into a column.

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

The binding between proteins is preferably carried out in a buffer, examples of which include, but are not limited to, phosphate buffer and Tris buffer. However, any buffer that does not inhibit the binding between the proteins is suitable.

In the present invention, a biosensor using the surface plasmon resonance phenomenon may be used as a mean for detecting or quantifying the bound protein. When such a biosensor is used, the interaction between the proteins can be observed real-time as a surface plasmon resonance signal, using only a minute amount of polypeptide and without labeling (for example, BIAcore, Pharmacia).

Alternatively, the polypeptide encoded by the marker gene may be labeled, and the label of the bound protein may be used to detect or measure the bound protein. Specifically, after pre-labeling one of the proteins, the labeled protein is contacted with the other protein in the presence of a test compound, and then bound proteins are detected or measured according to the label after washing.

Labeling substances such as radioisotope (e.g., ^3H , ^{14}C , ^{32}P , ^{33}P , ^{35}S , ^{125}I , ^{131}I), enzymes (e.g., alkaline phosphatase, horseradish peroxidase, β -galactosidase, and β -glucosidase), fluorescent substances (e.g., fluorescein isothiocyanate (FITC), rhodamine) and biotin/avidin, may be used for the labeling of a protein in the present method. When the protein is labeled with radioisotope, the detection or measurement can be carried out by liquid scintillation. Alternatively, proteins labeled with enzymes can be detected or measured by adding a substrate of the enzyme to detect the enzymatic change of the substrate, such as

generation of color, with absorptiometer. Further, in case where a fluorescent substance is used as the label, the bound protein may be detected or measured using fluorophotometer.

When using an antibody in the context of the present screening methods, it is preferable that the antibody be labeled with one of the labeling substances mentioned above, and detected or measured based on the labeling substance. Alternatively, an antibody against a polypeptide encoded by a marker gene or actin may be used as a primary antibody to be detected with a secondary antibody that is labeled with a labeling substance. Furthermore, an antibody bound to a protein in the screening of the present invention may be detected or measured using protein G or protein A column.

Any test compound, for example, cell extracts, cell culture supernatant, products of fermenting microorganism, extracts from marine organism, plant extracts, purified or crude proteins, peptides, non-peptide compounds, synthetic micromolecular compounds and natural compounds can be used in the screening methods of the present invention. In the context of the present invention, the test compound can be also obtained using any of the numerous approaches in combinatorial library methods known in the art, including (1) biological libraries, (2) spatially addressable parallel solid phase or solution phase libraries, (3) synthetic library methods requiring deconvolution, (4) the "one-bead one-compound" library method and (5) synthetic library methods using affinity chromatography selection. The biological library methods using affinity chromatography selection is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam (1997) *Anticancer Drug Des.* 12: 145-67). Examples of methods for the synthesis of molecular libraries can be found in the art (DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90: 6909-13; Erb *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91: 11422-6; Zuckermann *et al.* (1994) *J. Med. Chem.* 37: 2678-85; Cho *et al.* (1993) *Science* 261: 1303-5; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33: 2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33: 2061; Gallop *et al.* (1994) *J. Med. Chem.* 37: 1233-51). Libraries of compounds may be presented in solution (see Houghten (1992) *Bio/Techniques* 13: 412-21) or on beads (Lam (1991) *Nature* 354: 82-4), chips (Fodor (1993) *Nature* 364: 555-6), bacteria (US Pat. No. 5,223,409), spores (US Pat. No. 5,571,698; 5,403,484, and 5,223,409), plasmids (Cull *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89: 1865-9) or phage (Scott and Smith (1990) *Science* 249: 386-90; Devlin (1990) *Science* 249: 404-6; Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87: 6378-82; Felici (1991) *J. Mol. Biol.* 222: 301-10; US

Pat. Application 2002103360).

A compound isolated by the screening methods of the present invention is a candidate for the development of drugs that inhibit or stimulate the activity of a polypeptide encoded by a marker gene, for treating or preventing diseases attributed thereto, for example, cell proliferative diseases, such as RCC. A compound in which a part of the structure of the compound obtained by the present screening methods of the present invention is converted by addition, deletion and/or replacement, is included as the compounds obtained by the screening methods of the present invention.

Pharmaceutical Compositions For Treating Or Preventing RCC:

The present invention provides compositions for treating or preventing renal cell carcinoma that contain any of the compounds selected by the above-described screening methods of the present invention.

When administering a compound isolated by the method of the present invention as a pharmaceutical for humans and other mammals, such as mice, rats, guinea-pigs, rabbits, cats, dogs, sheep, pigs, cattle, monkeys, baboons, and chimpanzees, the isolated compound can be directly administered or can be formulated into a dosage form using known pharmaceutical preparation methods. For example, according to the need, the drugs can be taken orally, as sugar-coated tablets, capsules, elixirs and microcapsules, or non-orally, in the form of injections of sterile solutions or suspensions with water or any other pharmaceutically acceptable liquid. For example, the compounds can be mixed with pharmaceutically acceptable carriers or media, specifically, sterilized water, physiological saline, plant-oils, emulsifiers, suspending agents, surfactants, stabilizers, flavoring agents, excipients, vehicles, preservatives, binders, and such, in a unit dose form required for generally accepted drug implementation. The amount of active ingredients contained in such a preparation makes a suitable dosage within the indicated range acquirable.

Examples of additives that can be mixed to tablets and capsules include, but are not limited to, binders such as gelatin, corn starch, tragacanth gum and arabic gum; excipients such as crystalline cellulose; swelling agents such as corn starch, gelatin and alginic acid; lubricants such as magnesium stearate; sweeteners such as sucrose, lactose or saccharin; and flavoring agents such as peppermint, Gaultheria adenoitrix oil and cherry. When the unit-dose form is a capsule, a liquid carrier, such as an oil, can be further included in the above ingredients. Sterile composites for injections can be formulated following normal drug

implementations using vehicles such as distilled water suitable for injections.

Physiological saline, glucose, and other isotonic liquids including adjuvants, such as D-sorbitol, D-mannose, D-mannitol, and sodium chloride, can be used as aqueous solutions for injections. These can be used in conjunction with suitable solubilizers, such as alcohol, for example, ethanol, polyalcohols such as propylene glycol and polyethylene glycol, and
5 non-ionic surfactants, such as Polysorbate 80 (TM) and HCO-50.

Sesame oil or soy-bean oil can be used as an oleaginous liquid and may be used in conjunction with benzyl benzoate or benzyl alcohol as a solubilizer and may be formulated with a buffer, such as phosphate buffer and sodium acetate buffer; a pain-killer, such as
10 procaine hydrochloride; a stabilizer, such as benzyl alcohol and phenol; and/or an anti-oxidant. A prepared injection may be filled into a suitable ampoule.

Methods well known to those skilled in the art may be used to administer the pharmaceutical composition of the present invention to patients, for example as an intraarterial, intravenous, or percutaneous injection or as an intranasal, intramuscular or oral
15 administration. The dosage and method of administration vary according to the body-weight and age of a patient and the administration method; however, one skilled in the art can routinely select a suitable method of administration. If said compound is encodable by a DNA, the DNA can be inserted into a vector for gene therapy and the vector administered to a patient to perform the therapy. The dosage and method of administration vary according to
20 the body-weight, age, and symptoms of the patient; however, one skilled in the art can suitably select them.

For example, although the dose of a compound that binds to a protein of the present invention and regulates its activity depends on the symptoms, the dose is generally about 0.1 mg to about 100 mg per day, preferably about 1.0 mg to about 50 mg per day and more
25 preferably about 1.0 mg to about 20 mg per day, when administered orally to a normal adult human (weight 60 kg).

When administering the compound parenterally, in the form of an injection to a normal adult human (weight 60 kg), although there are some differences according to the patient, target organ, symptoms and method of administration, it is convenient to
30 intravenously inject a dose of about 0.01 mg to about 30 mg per day, preferably about 0.1 to about 20 mg per day and more preferably about 0.1 to about 10 mg per day. In the case of other animals too, the appropriate dosage amount may be routinely calculated by converting

to 60 kg of body-weight.

Assessing The Prognosis Of A Subject With Renal Cell Carcinoma:

The present invention also provides a method of assessing the prognosis of a subject with RCC including the step of comparing the expression of one or more RCC-associated gene in a test cell population to the expression of the same RCC-associated genes in a reference cell population derived from patients over a spectrum of disease stages. By comparing the gene expression of one or more RCC-associated gene in the test cell population and the reference cell population(s), or by comparing the pattern of gene expression over time in test cell populations derived from the subject, the prognosis of the subject can be assessed.

For example, a decrease in expression of one or more down-regulated RCC-associated gene, such as RCC 252-972, as compared to a normal control or an increase in the expression of one or more up-regulated RCC-associated genes, such as RCC 1-251, as compared to a normal control indicates less favorable prognosis. Conversely, similarity between the expression of one or more of RCC associated genes (*e.g.*, RCC 1-972) as compared to normal control indicates a more favorable prognosis for the subject. Preferably, the prognosis of a subject can be assessed by comparing the expression profile of RCC 1-972.

Kits:

The present invention also includes a RCC-detection reagent, *e.g.*, a nucleic acid that specifically binds to or identifies one or more RCC nucleic acids, such as oligonucleotide sequences which are complementary to a portion of an RCC nucleic acid or an antibody that binds to a protein encoded by an RCC nucleic acid. The detection reagents may be packaged together in the form of a kit. For example, the detection reagents may be packaged in separate containers, *e.g.*, a nucleic acid or antibody (either bound to a solid matrix or packaged separately with reagents for binding them to the matrix), a control reagent (positive and/or negative), and/or a detectable label. Instructions (*e.g.*, written, tape, VCR, CD-ROM, *etc.*) for carrying out the assay may also be included in the kit. The assay format of the kit may be a Northern hybridization or a sandwich ELISA, both of which are known in the art.

For example, an RCC detection reagent may be immobilized on a solid matrix such as a porous strip to form at least one RCC detection site. The measurement or detection region of the porous strip may include a plurality of sites, each containing a nucleic acid. A test strip may also contain sites for negative and/or positive controls. Alternatively, control sites may

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

Alternatively, the kit may contain a nucleic acid substrate array of one or more nucleic acids. The nucleic acids on the array specifically identify one or more nucleic acid sequences represented by RCC 1-972. The expression of 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 40 or 50 or more of the nucleic acids represented by RCC 1-972 may be identified by virtue of the level of binding to an array test strip or chip. The substrate array can be on, *e.g.*, a solid substrate, *e.g.*, a "chip" as described in U.S. Patent No.5,744,305, the contents of which are incorporated by reference herein in its entirety.

Arrays And Pluralities:

The present invention also includes a nucleic acid substrate array of one or more nucleic acids. The nucleic acids on the array specifically correspond to one or more nucleic acid sequences represented by RCC 1-972. The level of expression of 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 40 or 50 or more of the nucleic acids represented by RCC 1-972 may be identified by detecting nucleic acid binding to the array.

The present invention also includes an isolated plurality (*i.e.*, a mixture of two or more nucleic acids) of nucleic acids. The nucleic acids may be in a liquid phase or a solid phase, *e.g.*, immobilized on a solid support such as a nitrocellulose membrane. The plurality includes one or more of the nucleic acids represented by RCC 1-972. In various embodiments, the plurality includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 40 or 50 or more of the nucleic acids represented by RCC 1-972.

Methods Of Inhibiting Renal Cell Carcinoma:

The present invention further provides a method for treating or alleviating a symptom of RCC in a subject by decreasing the expression of one or more of RCC 1-251 (or the activity of its gene product) or increasing expression of one or more of RCC 252-972 (or the activity of its gene product). Suitable therapeutic compounds can be administered prophylactically or therapeutically to subject suffering from or at risk of (or susceptible to)

developing RCC. Such subjects can be identified using standard clinical methods or by detecting an aberrant level of expression of one or more of RCC 1-972 (or the activity of a corresponding gene product). Examples of suitable therapeutic agents include, but are not limited to, inhibitors of cell cycle regulation, cell proliferation, and protein kinase activity.

5 The therapeutic method of the present invention may include the step of increasing the expression, function, or both of one or more gene products of genes whose expression is decreased ("down-regulated" or "under-expressed" genes) in a RCC cell relative to normal cells of the same tissue type from which the RCC cells are derived. In these methods, the subject is treated with an effective amount of a compound that increases the amount of one or
10 more of the under-expressed genes in the subject. Administration can be systemic or local. Suitable therapeutic compounds include, but are not limited to, a polypeptide product of an under-expressed gene, a biologically active fragment thereof, and a nucleic acid encoding an under-expressed gene and having expression control elements permitting expression in the RCC cells; for example, an agent that increases the level of expression of such gene
15 endogenous to the RCC cells (*i.e.*, which up-regulates the expression of the under-expressed gene or genes). Administration of such compounds counters the effects of aberrantly-under expressed gene or genes in the subject's renal cells and improves the clinical condition of the subject.

 Alternatively, the therapeutic method of the present invention may include decreasing
20 the expression, function, or both, of one or more gene products of genes whose expression is aberrantly increased ("up-regulated" or "over-expressed" gene) in RCC cells. Expression may be inhibited in any of several ways known in the art. For example, expression can be inhibited by administering to the subject a nucleic acid that inhibits, or antagonizes, the expression of the over-expressed gene or genes, *e.g.*, an antisense oligonucleotide or small
25 interfering RNA which disrupts expression of the over-expressed gene or genes.

Antisense Nucleic Acids:

 As noted above, antisense nucleic acids corresponding to the nucleotide sequence of RCC 1-251 can be used to reduce the expression level of the RCC 1-251. Antisense nucleic acids corresponding to RCC 1-251 that are up-regulated in renal cell carcinoma are useful for
30 the treatment of renal cell carcinoma. Specifically, the antisense nucleic acids of the present invention may act by binding to the RCC 1-251 or mRNAs corresponding thereto, thereby inhibiting the transcription or translation of the genes, promoting the degradation of the

mRNAs, and/or inhibiting the expression of proteins encoded by the RCC 1-251, thereby inhibiting the function of the proteins. The term "antisense nucleic acids" as used herein encompasses both nucleotides that are entirely complementary to the target sequence and those having a mismatch of one or more nucleotides, so long as the antisense nucleic acids can specifically hybridize to the target sequences. For example, the antisense nucleic acids of the present invention include polynucleotides that have a homology of at least 70% or higher, preferably at 80% or higher, more preferably 90% or higher, even more preferably 95% or higher over a span of at least 15 continuous nucleotides. Algorithms known in the art can be used to determine the homology.

The antisense nucleic acid of the present invention act on cells producing the proteins encoded by the RCC-associated marker genes by binding to the DNAs or mRNAs encoding the proteins, inhibiting their transcription or translation, promoting the degradation of the mRNAs, and inhibiting the expression of the proteins, thereby resulting in the inhibition of the protein function.

An antisense nucleic acid of the present invention can be made into an external preparation, such as a liniment or a poultice, by admixing it with a suitable base material which is inactive against the nucleic acid.

Also, as needed, the antisense nucleic acids of the present invention can be formulated into tablets, powders, granules, capsules, liposome capsules, injections, solutions, nose-drops and freeze-drying agents by adding excipients, isotonic agents, solubilizers, stabilizers, preservatives, pain-killers, and such. These can be prepared by following known methods.

The antisense nucleic acids of the present invention can be given to the patient by direct application onto the ailing site or by injection into a blood vessel so that it will reach the site of ailment. An antisense-mounting medium can also be used to increase durability and membrane-permeability. Examples include, but are not limited to, liposomes, poly-L-lysine, lipids, cholesterol, lipofectin or derivatives of these.

The dosage of the antisense nucleic acid of the present invention can be adjusted suitably according to the patient's condition and used in desired amounts. For example, a dose range of 0.1 to 100 mg/kg, preferably 0.1 to 50 mg/kg can be administered.

The antisense nucleic acids of the present invention inhibit the expression of a protein of the present invention and are thereby useful for suppressing the biological activity of a

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protein of the invention. In addition, expression-inhibitors, *e.g.*, the antisense nucleic acids of the invention, are useful in that they can inhibit the biological activity of a protein of the present invention.

The antisense nucleic acids of present invention include modified oligonucleotides. For example, thioated nucleotides may be used to confer nuclease resistance to an oligonucleotide.

An siRNA against a marker gene can be used to reduce the expression level of the marker gene. Herein, the term "siRNA" refers to a double stranded RNA molecule which prevents translation of a target mRNA. Standard techniques of introducing siRNA into the cell are used, including those in which DNA is a template from which RNA is transcribed. The siRNA includes a sense RCC 1-251 *e.g.* C6700 (RCC 81), B7032N (RCC126) or B9320 (RCC173) nucleic acid sequence, an anti-sense RCC 1-251 *e.g.* C6700, B7032N or B9320 nucleic acid sequence or both. The siRNA may be composed of 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 microRNA (miRNA).

Binding of the siRNA to a transcript corresponding to one of the RCC 1-251 in the target cell results in a reduction in the protein production by the cell. The length of the oligonucleotide is at least 10 nucleotides and may be as long as the naturally-occurring transcript. Preferably, the oligonucleotide is less than 75, 50, 25 nucleotides in length. Most preferably, the oligonucleotide is 19-25 nucleotides in length.

The method is also used to alter gene expression in a cell in which expression of C6700, B7032N or B9320 is up-regulated, *e.g.*, as a result of malignant transformation of the cells. Binding of the siRNA to a C6700, B7032N or B9320 transcript in the target cell results in a reduction in C6700, B7032N or B9320 production by the cell. The length of the oligonucleotide is at least about 10 nucleotides and may be as long as the naturally-occurring C6700, B7032N or B9320 transcript. In the present invention, the target sequence of the siRNA may be selected from the nucleotide sequence of C6700V2. Most of the nucleotide sequences are shared between two variants of C6700, V1 and V2. Accordingly, effective siRNA for suppression of one variant may also suppress another variant. Thus, the siRNAs suppressing the both of C6700V1 and C6700V2 may be used for treating or preventing cancers. Alternatively, C6700V2 specific siRNAs may also be used for treating or preventing

cancers. Examples of siRNA oligonucleotides of C6700 which inhibit C6700 expression in mammalian cells include those oligonucleotides containing target sequences, for example, nucleotides of SEQ ID NOs: 43, 47 or 81 which may suppress both of *C6700V1* and *C6700V2*. In the present invention, the target sequence of the siRNA may be selected from the nucleotide sequence of B7032N. For example, the siRNA oligonucleotides containing nucleotides of SEQ ID NO: 106 as a target sequence for B7032N. In the present invention, the target sequence of siRNA may be selected from the nucleotide sequence of B9320V2. Most of the nucleotide sequence are shared between two variants of B9320 V1 and V2. Accordingly, effective siRNA for suppression of one variant may also suppress another variant. Thus, the siRNAs suppressing the both of B9320 V1 and B9320 V2 may be used for treating or preventing cancers. Alternatively, B9320 V2 specific siRNAs may also be used for treating or preventing cancers. Examples of siRNA oligonucleotides of B9320 which inhibit B9320 expression in mammalian cells include oligonucleotides containing target sequences, for example, nucleotides of SEQD NO: 110 which may suppress B9320 V2.

Methods for designing suitable 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.*, (1999) *Genes Dev* 13: 3191-7, don't recommend designing siRNA to the 5' and 3' untranslated regions (UTRs) and regions near the start codon (within 75 bases) as these may be richer in regulatory protein binding sites. 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 (Altschul SF, *et*

al., (1997) Nucleic Acids Res.;25:3389-402; (1990) J Mol Biol.;215:403-10.), 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.

5 Also included in the present invention are isolated nucleic acid molecules that include the nucleic acid sequence of target sequences, for example, nucleotides of SEQ ID NOs: 43, 47, 81, 106 or 110 and a nucleic acid molecule that is complementary to the nucleic acid sequence of nucleotides of SEQ ID NOs: 43, 47, 81, 106 or 110. As used herein, the term “isolated nucleic acid” refers to a nucleic acid removed from its original environment (*e.g.*,
10 the natural environment if naturally occurring) and thus, is synthetically altered from its natural state. In the present invention, examples of isolated nucleic acid include 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
15 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
20 complementary.

In the context of the present invention, 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
25 the strands of the duplex are fully complementary, such duplexes contain no mismatches. The nucleic acid molecule is preferably less than 4725 or 4494 nucleotides in length for C6700, less than 3503 nucleotides in length for B7032N, or less than 2539 or 3427 nucleotides in length for B9320. 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
30 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 C6700, B7032N or B9320, or DNA encoding the siRNA. When the nucleic acids are used for siRNA or coding DNA

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thereof, the sense strand is preferably longer than about 19 nucleotides, and more preferably longer than 21 nucleotides.

The present invention is based in part on the discovery that the transcriptional variant *C6700V2* is over-expressed in renal cell carcinoma (RCC) compared to non-cancerous renal
5 sells. *C6700* has two different transcriptional variants. The cDNA of *C6700V1* or *V2* is 4725 or 4494 nucleotides in length. The nucleic acid and polypeptide sequences of *C6700V1* or *V2* are shown in SEQ ID NO: 63 and 64, or 65 and 66, respectively. The sequence data are also available via following accession numbers.

C6700V1 :AY358124

10 *C6700V2* :BC077726

Transfection of siRNAs of SEQ ID NOs: 43, 47 or 81 resulted in a growth inhibition of RCC cell lines. *C6700*, designed Sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (Semaphorin 5B), *SEMA5B*, located on the chromosome 3p21.1. Members of the semaphorin protein family,
15 such as *SEMA5B*, are involved in axonal guidance during neural development (Adams, *et al.*, (1996) *Mech. Dev.* 57: 33-45.). *C6700* was up-regulated in three RCC cell lines (A704, OS-RC-2 and TUHR14TKB) of more eight RCC cell lines (ACHN, 769-P, RXF-631L, TUHR10TKB, Caki-1, Caki-2, 786-O and A-498) compared with renal proximal tubule epithelial cell by RT-PCR and Northern blot analyses (Figure 2b and 3, right panel). *C6700*
20 has two different transcriptional variants consisting of 23 exons, corresponding to *C6700V1* and *C6700V2*, respectively (Figure 8a). Herein, it was discovered that the *V2* variant is specifically overexpressed in RCC cells (Figure 8b).

The present invention is based in part on the discovery that *B7032N* is over-expressed in renal cell carcinoma (RCC) compared to non-cancerous renal is cells. *B7032N* is 3503
25 nucleotides in length. The nucleic acid and polypeptide sequence of *B7032N* is shown in SEQ ID NO: 112 and 113. The sequence data are also available via following accession numbers.

NM_004567

Transfection of siRNA of SEQ ID NO: 106 resulted in a growth inhibition of RCC
30 cell lines. *B7032N*, designed PFKFB4 (6-phosphofructo-2-kinase/fructose-2, 6-biphosphatase 4) gene is up-regulated in clinical RCC cases (Figure 2a) as well as RCC cell

lines (data not shown) by semi-quantitative RT-PCR analysis, but its expression was undetectable in any of normal vital organs examined. Subsequent northern blot analysis confirmed that approximately 3.5kb B7032N transcript was up-regulated in 2 of 11 bladder cancer cell lines, but not expressed in normal organs except testis and pancreas (Figure 6).

5 The present invention is based in part on the discovery that the transcriptional variant B9320V2 is over-expressed in renal cell carcinoma (RCC) compared to non-cancerous renal cells. B9320 has two different transcriptional variants. The cDNA of B9320V1 or V2 is 2539 or 3427 nucleotides in length. The nucleic acid and polypeptide sequences of B9320V1 or V2 are shown in SEQ ID NO: 115 and 116, or 118 and 119, respectively. The sequence data are
10 also available via following accession numbers.

B9320V1 : BC034014

B9320V2 : NM_153350

Transfection of siRNA of SEQ ID NO: 110 resulted in a growth inhibition of RCC cell lines. B9320, designed FBXL16 (F-box and leucine-rich repeat protein 16) gene is up-
15 regulated in clinical RCC case (Figure 2a) as well as RCC cell lines (data not shown) by semi-quantitative RT-PCR analysis, but its expression was undetectable in any of normal vital organs examined. Subsequent northern blot analysis confirmed that approximately B9320V1 (2.4kb) and B9320V2 (4kb) of two B9320 transcripts were significantly up-regulated in 2 of 4 bladder cancer cell lines. The B9320V1 transcript was expressed in kidney and thyroid,
20 whereas B9320V2 transcript was not expressed in normal organs except brain, testis, spinal cord and pancreas (Figure 7).

Structure Of siRNA Composition:

The present invention relates to inhibiting cell growth, *i.e.*, cancer cell growth by inhibiting expression of C6700, B7032N or B9320. Expression of C6700, B7032N or B9320
25 is inhibited, for example, by small interfering RNA (siRNA) that specifically target the C6700, B7032N or B9320 gene. C6700, B7032N or B9320 targets include, for example, nucleotides of SEQ ID NOs: 43, 47, 81, 106 or 110.

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
30 (RNAi) (Sharp PA. (1999) Genes Dev.;13:139-41.). 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, *et al.*, (2000) *Nature*.;404:293- 6; Hannon GJ. (2002) *Nature*.;418:244-51.). In mammalian cells, siRNA composed of 20 or 21-mer dsRNA with 19 complementary nucleotides and 3' terminal noncomplementary dimers of thymidine or uridine, have been shown to have a gene specific knock-down effect without inducing global changes in gene expression (Elbashir SM, *et al.*, (2001) *Nature*.;411:494-8.). 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 and Taira K. (2002) *Nat Biotechnol.*;20:497-500.; Brummelkamp TR, *et al.*, (2002) *Science*. 296:550-3.).

In the context of the present invention, the growth of cells may be inhibited by contacting a cell, with a composition containing a siRNA of C6700, B7032N or B9320. 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 C6700, B7032N or B9320 may be directed to a single target of C6700, B7032N or B9320 gene sequence. Alternatively, the siRNA may be directed to multiple target of C6700, B7032N or B9320 gene sequences. For example, the composition may contain siRNA of C6700, B7032N or B9320 directed to two, three, four, or five or more target sequences of C6700, B7032N or B9320. By C6700, B7032N or B9320 target sequence is meant a nucleotide sequence that is identical to a portion of the C6700, B7032N or B9320 gene. The target sequence can include the 5' untranslated (UT) region, the open reading frame (ORF) or the 3' untranslated region of the human C6700, B7032N or B9320 gene. Alternatively, the siRNA is a nucleic acid sequence complementary to an upstream or downstream modulator of C6700, B7032N or B9320 gene expression. Examples of upstream and downstream modulators include, a transcription factor that binds the C6700, B7032N or B9320 gene promoter, a kinase or phosphatase that interacts with the C6700, B7032N or B9320 polypeptide, a C6700, B7032N or B9320 promoter or enhancer. siRNA of C6700, B7032N or B9320 which hybridize to target mRNA decrease or inhibit production of the C6700, B7032N or B9320 polypeptide product encoded by the C6700, B7032N or B9320 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 present invention can be defined by their ability to hybridize specifically to mRNA or cDNA from a C6700, B7032N or B9320 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, (1993) *Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Probes*, "Overview of principles of hybridization and the strategy of nucleic acid assays". 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.

An siRNA of the invention is preferably 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 C6700, B7032N or B9320 siRNA include the sequences of nucleotides of SEQ ID NOs: 43, 47, 81, 106 or 110 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 C6700 gene as transcriptional variant of C6700V2, B7032N or B9320. The cell is an epithelial cell such as a renal cell. Alternatively, the cell is a tumor cell such as a carcinoma, adenocarcinoma, blastoma, leukemia, myeloma, or sarcoma. The cell is a renal cell carcinoma.

5 An siRNA of C6700, B7032N or B9320 may be directly introduced into the cells in a form that is capable of binding to the mRNA transcripts. Alternatively, the DNA encoding the siRNA of C6700, B7032N or B9320 may be contained in a vector.

Vectors are produced, for example, by cloning a C6700, B7032N or B9320 target sequence into an expression vector operatively-linked regulatory sequences flanking the
10 C6700, B7032N or B9320 sequence in a manner that allows for expression (by transcription of the DNA molecule) of both strands (Lee, N.S., *et al.*, (2002) Nature Biotechnology 20 : 500-5.). An RNA molecule that is antisense to C6700, B7032N or B9320 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 C6700, B7032N or B9320 mRNA is transcribed by a
15 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 C6700, B7032N or B9320 gene. Alternatively, two constructs can be utilized to create the sense and anti-sense strands of a siRNA construct. Cloned C6700, B7032N or B9320 can encode a construct having secondary structure, *e.g.*, hairpins, wherein a single transcript has both the sense and
20 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 specifically
25 hybridizes to an mRNA or a cDNA from C6700, B7032N or B9320. In preferred embodiments, [A] is a ribonucleotide sequence corresponding to a sequence selected from the group consisting of nucleotides of SEQ ID NOs: 43, 47, 81, 106 or 110

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

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

30 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 nucleotides in length. The loop

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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, JM., *et al.*, (2002) Nature 418: 435-8).

CCC, CCACC or CCACACC: Jacque, JM., *et al.*, (2002) Nature, 418: 435-8.

5 UUCG: Lee, NS., *et al.*, (2002) Nature Biotechnology 20: 500-5. Fruscoloni, P., *et al.*, (2003) Proc. Natl. Acad. Sci. USA 100: 1639-44.

UUCAAGAGA: Dykxhoorn, DM., *et al.*, (2003) Nature Reviews Molecular Cell Biology 4: 457-67.

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 ("tcaagaga" in DNA).

CTACCTCTTCAGACTCAGC-[B]-GCTGAGTCTGAAGAGGTAG (for target sequence of SEQ ID NO: 43)

15 CCATGTGAGCACTTGGATG-[B]-CATCCAAGTGCTCACATGG (for target sequence of SEQ ID NO: 47)

GCAGCAACGTTGCAGCACA-[B]-TGTGCTGCAACGTTGCTGC (for target sequence of SEQ ID NO: 81),

GAGGACAATCCAGACGGCT-[B]-AGCCGTCTGGATTGTCCTC (for target sequence of SEQ ID NO: 106),

20 GGAGCTCTACAACGTGCTG-[B]-CAGCACGTTGTAGAGCTCC (for target sequence of SEQ ID NO: 110)

The regulatory sequences flanking the C6700, B7032N or B9320 sequence can be identical or different, such that their expression can be modulated independently, or in a temporal or spatial manner. siRNAs may be transcribed intracellularly by cloning the C6700, B7032N or B9320 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 Diagnostics), Lipofectamine 2000 (Invitrogen), Oligofectamine (Invitrogen), and Nucleofector (Wako pure Chemical) are useful as the transfection-enhancing agent.

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Oligonucleotides and oligonucleotides complementary to various portions of C6700, B7032N or B9320 mRNA were tested *in vitro* for their ability to decrease production of C6700, B7032N or B9320, respectively, in tumor cells (*e.g.*, using the renal cell line such as renal cell carcinoma(RCC) cell line) according to standard methods. A reduction in C6700, B7032N or B9320 gene product in cells contacted with the candidate siRNA composition as compared to cells cultured in the absence of the candidate composition can be detected using specific antibodies of C6700, B7032N or B9320 or other detection strategies. Sequences which decrease production of C6700, B7032N or B9320 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 C6700, B7032N or B9320 production and decreased tumor cell growth in animals with malignant neoplasms.

Methods Of Treating Malignant Tumors:

Patients with tumors characterized as over-expressing C6700, B7032N or B9320 may be treated by administering siRNA of C6700, B7032N or B9320. siRNA therapy may be used to inhibit expression of C6700, B7032N or B9320 in patients suffering from or at risk of developing, for example, renal cell carcinoma (RCC). Such patients may be identified by standard methods of the particular tumor type. Renal cell carcinoma (RCC) may be diagnosed for example, by CT, MRI, ERCP, MRCP, computer tomography, or ultrasound. Treatment is deemed efficacious if it leads to a clinical benefit such as, a reduction in expression of up-regulated genes, 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 may be 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 include 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.*, (2003) *Nature Med.* 9:347-51.). 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.

5 A therapeutic nucleic acid composition may be 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
10 medically desirable result such as reduced production of up-regulated gene products, reduction of cell growth, *e.g.*, proliferation, or a reduction in tumor growth in a treated animal.

Parenteral administration, via intravenous, subcutaneous, intramuscular, and intraperitoneal delivery routes, may be used to deliver siRNA compositions of C6700, B7032N or B9320. For treatment of renal cell carcinomas, direct infusion renal artery is
15 particularly preferred.

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
20 nucleic acid molecule.

The polynucleotides of the present invention may be 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 generally injected or otherwise delivered to the animal in combination
25 with a pharmaceutically acceptable liquid carrier, *e.g.*, a liquid carrier, which is aqueous or partly aqueous. The polynucleotides may further be associated with a liposome (*e.g.*, a cationic or anionic liposome). The polynucleotides of the present invention include genetic information necessary for expression by a target cell, such as promoters.

The antisense oligonucleotide or siRNA of the invention inhibit the expression of a
30 polypeptide of the present invention and is thereby useful for suppressing the biological activity of a polypeptide of the invention. Also, expression-inhibitors, *e.g.*, the antisense

oligonucleotide or siRNA of the invention, are useful in the point that they can inhibit the biological activity of the polypeptide of the invention. Therefore, a composition that includes an antisense oligonucleotide or siRNA of the present invention is useful for treating a renal cell carcinoma.

5 Alternatively, function of one or more gene products of the genes over-expressed in RCC can be inhibited by administering a compound that binds to or otherwise inhibits the function of the gene products. For example, the compound may be an antibody which binds to the over-expressed gene product or gene products.

10 The present invention refers to the use of antibodies, particularly antibodies against a protein encoded by an up-regulated marker gene, or a fragment of such an antibody. As used herein, the term "antibody" refers to an immunoglobulin molecule having a specific structure, that interacts (*i.e.*, binds) only with the antigen that was used for synthesizing the antibody (*i.e.*, the gene product of an up-regulated marker gene) or with an antigen closely related thereto. Furthermore, an antibody may be a fragment of an antibody or a modified antibody,
15 so long as it binds to one or more of the proteins encoded by the marker genes. For instance, the antibody fragment may be Fab, F(ab')₂, Fv, or single chain Fv (scFv), in which Fv fragments from H and L chains are ligated by an appropriate linker (Huston J. S. *et al.*, (1988) Proc. Natl. Acad. Sci. U.S.A. 85:5879-83.). More specifically, an antibody fragment may be generated by treating an antibody with an enzyme, such as papain or pepsin. Alternatively, a
20 gene encoding the antibody fragment may be constructed, inserted into an expression vector, and expressed in an appropriate host cell (see, for example, Co MS. *et al.*, (1994) J. Immunol. 152:2968-76; Better M. and Horwitz AH. (1989) Methods Enzymol. 178:476-96; Pluckthun A. and Skerra A. (1989) Methods Enzymol. 178:497-515; Lamoyi E. (1986) Methods Enzymol. 121:652-63; Rousseaux J. *et al.*, (1986) Methods Enzymol. 121:663-9; Bird RE.
25 and Walker BW. (1991) Trends Biotechnol. 9:132-7.).

 An antibody may be modified by conjugation with a variety of molecules, such as polyethylene glycol (PEG). The present invention provides such modified antibodies. The modified antibody can be obtained by chemically modifying an antibody. Such modification methods are conventional in the field.

30 Alternatively, an antibody may be a chimeric antibody, having a variable region derived from a nonhuman antibody and a constant region derived from a human antibody, or a humanized antibody, having a complementarity determining region (CDR) derived from a

nonhuman antibody, a frame work region (FR) derived from a human antibody, and a constant region. Such antibodies can be prepared by using known technologies.

Cancer therapies directed at specific molecular alterations that occur in cancer cells have been validated through clinical development and regulatory approval of anti-cancer drugs such as trastuzumab (Herceptin) for the treatment of advanced breast cancer, imatinib methylate (Gleevec) for chronic myeloid leukemia, gefitinib (Iressa) for non-small cell lung cancer (NSCLC), and rituximab (anti-CD20 mAb) for B-cell lymphoma and mantle cell lymphoma (Ciardiello F and Tortora G. (2001) Clin Cancer Res.;7:2958-70. Review.; Slamon DJ, *et al.*, (2001) N Engl J Med.;344:783-92; Rehwald U, *et al.*, (2003) Blood.;101:420-4; Fang G, *et al.*, (2000) Blood, 96, 2246-53.). These drugs are clinically effective and better tolerated than traditional anti-cancer agents because they target only transformed cells. Hence, such drugs not only improve survival and quality of life for cancer patients, but also validate the concept of molecularly targeted cancer therapy. Furthermore, targeted drugs can enhance the efficacy of standard chemotherapy when used in combination with it (Gianni L. (2002) Oncology, 63 Suppl 1, 47-56; Klejman A, *et al.*, (2002) Oncogene, 21, 5868-76.). Therefore, future cancer treatments will probably involve combining conventional drugs with target-specific agents aimed at different characteristics of tumor cells such as angiogenesis and invasiveness.

These modulatory methods can be performed *ex vivo* or *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g., by administering the agent to a subject). The method involves administering a protein or combination of proteins or a nucleic acid molecule or combination of nucleic acid, molecules as therapy to counteract aberrant expression of the differentially expressed genes or aberrant activity of their respective gene products.

Diseases and disorders that are characterized by increased (relative to a subject not suffering from the disease or disorder) expression levels or biological activities of genes and gene products, respectively, may be treated with therapeutics that antagonize (*i.e.*, reduce or inhibit) activity of the over-expressed gene or genes. Therapeutics that antagonize activity can be administered therapeutically or prophylactically.

Accordingly, therapeutics that may be utilized in the context of the present invention include, *e.g.*, (i) a polypeptide of an RCC-associated gene or analogs, derivatives, fragments or homologs thereof; (ii) antibodies to an over-expressed gene or gene product; (iii) nucleic

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acids encoding the over-expressed or under-expressed gene or genes; (iv) antisense nucleic acids or nucleic acids that are "dysfunctional" (*i.e.*, due to a heterologous insertion within the nucleic acids of one or more over-expressed gene or genes); (v) small interfering RNA (siRNA); or (vi) modulators (*i.e.*, inhibitors, agonists and antagonists that alter the interaction
5 between an over/under-expressed polypeptide and its binding partner). The dysfunctional antisense molecules are utilized to "knockout" endogenous function of a polypeptide by homologous recombination (see, *e.g.*, Capecchi, (1989) Science 244: 1288-92.).

Diseases and disorders that are characterized by decreased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with
10 therapeutics that increase (*i.e.*, are agonists to) activity. Therapeutics that up-regulate activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to, a polypeptide (or analogs, derivatives, fragments or homologs thereof) or an agonist that increases bioavailability.

Increased or decreased levels can be readily detected by quantifying peptide and/or
15 RNA, by obtaining a patient tissue sample (*e.g.*, from biopsy tissue) and assaying it *in vitro* for RNA or peptide levels, structure and/or activity of the expressed peptides (or mRNAs of a gene whose expression is altered). Methods that are well-known within the art include, but are not limited to, immunoassays (*e.g.*, by Western blot analysis, immunoprecipitation followed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis,
20 immunocytochemistry, *etc.*) and/or hybridization assays to detect expression of mRNAs (*e.g.*, Northern assays, dot blots, in situ hybridization, *etc.*).

Prophylactic administration occurs prior to the manifestation of overt clinical symptoms of disease, such that a disease or disorder is prevented or, alternatively, delayed in its progression.

Therapeutic methods of the present invention may include the steps of contacting a
25 cell with an agent that modulates one or more of the activities of the gene products of the differentially expressed genes. Examples of agents that modulate protein activity include, but are not limited to, a nucleic acid or a protein, a naturally-occurring cognate ligand of these proteins, a peptide, a peptidomimetic, or other small molecule. For example, a suitable agent
30 may stimulate one or more protein activities of one or more differentially under-expressed gene.

The present invention also relates to a method of treating or preventing renal cell

carcinoma in a subject including the steps of administering to said subject a vaccine containing a polypeptide encoded by a nucleic acid selected from the group consisting of RCC 1-251 (*i.e.*, an up-regulated RCC gene), an immunologically active fragment of such a polypeptide, or a polynucleotide encoding such a polypeptide or fragment. Administration of the polypeptide induces an anti-tumor immunity in a subject. To induce anti-tumor immunity, a polypeptide encoded by a nucleic acid selected from the group consisting of RCC 1-251, an immunologically active fragment of such a polypeptide, or a polynucleotide encoding such a polypeptide or fragment thereof is administered to a subject in need thereof. The polypeptide or the immunologically active fragments thereof are useful as vaccines against RCC. In some cases the proteins or fragments thereof may be administered in a form bound to the T cell receptor (TCR) or presented by an antigen presenting cell (APC), such as macrophage, dendritic cell (DC), or B-cells. Due to the strong antigen presenting ability of DC, the use of DC is most preferable among the APCs.

In the present invention, vaccine against RCC refers to a substance that has the ability to induce anti-tumor immunity upon inoculation into animals. According to the present invention, polypeptides encoded by RCC 1-251 or fragments thereof were suggested to be HLA-A24 or HLA-A*0201 restricted epitopes peptides that may induce potent and specific immune response against RCC cells expressing RCC 1-251. Thus, the present invention also encompasses a method of inducing anti-tumor immunity using the polypeptides.

In general, anti-tumor immunity includes immune responses such as follows:

- induction of cytotoxic lymphocytes against tumors,
- induction of antibodies that recognize tumors, and
- induction of anti-tumor cytokine production.

Therefore, when a certain protein induces any one of these immune responses upon inoculation into an animal, the protein is determined to have anti-tumor immunity inducing effect. The induction of the anti-tumor immunity by a protein can be detected by observing *in vivo* or *in vitro* the response of the immune system in the host against the protein.

For example, a method for detecting the induction of cytotoxic T lymphocytes is well known. Specifically, a foreign substance that enters the living body is presented to T cells and B cells by the action of antigen presenting cells (APCs). T cells that respond to the antigen presented by an APC in an antigen specific manner differentiate into cytotoxic T cells

(or cytotoxic T lymphocytes; CTLs) due to stimulation by the antigen, and then proliferate (this is referred to as activation of T cells). Therefore, CTL induction by a certain peptide can be evaluated by presenting the peptide to a T cell via an APC, and detecting the induction of CTL. Furthermore, an APC has the effect of activating CD4⁺ T cells, CD8⁺ T cells,
5 macrophages, eosinophils, and NK cells. Since CD4⁺ T cells and CD8⁺ T cells are also important in anti-tumor immunity, the anti-tumor immunity inducing action of the peptide can be evaluated using the activation effect of these cells as indicators.

A method for evaluating the inducing action of CTL using dendritic cells (DCs) as an APC is well known in the art. A DC is a representative APC having the strongest CTL
10 inducing action among APCs. In this method, the test polypeptide is initially contacted with a DC, and then this DC is contacted with T cells. Detection of T cells having cytotoxic effects against the cells of interest after the contact with DC shows that the test polypeptide has an activity of inducing the cytotoxic T cells. Activity of CTL against tumors can be detected, for example, using the lysis of ⁵¹Cr-labeled tumor cells as the indicator. Alternatively, the
15 method of evaluating the degree of tumor cell damage using ³H-thymidine uptake activity or LDH (lactose dehydrogenase)-release as the indicator is also well known.

Apart from DC, peripheral blood mononuclear cells (PBMCs) may also be used as the APC. The induction of CTL has been reported to be enhanced by culturing PBMC in the presence of GM-CSF and IL-4. Similarly, CTLs have been shown to be induced by culturing
20 PBMC in the presence of keyhole limpet hemocyanin (KLH) and IL-7.

Test polypeptides confirmed to possess CTL inducing activity by these methods are deemed to be polypeptides having DC activation effect and subsequent CTL inducing activity. Therefore, polypeptides that induce CTLs against tumor cells are useful as vaccines against tumors. Furthermore, APCs that have acquired the ability to induce CTLs against tumors
25 through contact with the polypeptides are also useful as vaccines against tumors. Furthermore, CTLs that have acquired cytotoxicity due to presentation of the polypeptide antigens by APC can also be used as vaccines against tumors. Such therapeutic methods for tumors using anti-tumor immunity due to APCs and CTLs are referred to as cellular immunotherapy.

Generally, when using a polypeptide for cellular immunotherapy, efficiency of the
30 CTL-induction is known to be increased by combining a plurality of polypeptides having different structures and contacting them with DC. Therefore, when stimulating DC with protein fragments, it is advantageous to use a mixture of multiple types of fragments.

Alternatively, the induction of anti-tumor immunity by a polypeptide can be confirmed by observing the induction of antibody production against tumors. For example, when antibodies against a polypeptide are induced in a laboratory animal immunized with the polypeptide, and when growth of tumor cells is suppressed by those antibodies, the polypeptide is deemed to have the ability to induce anti-tumor immunity.

Anti-tumor immunity is induced by administering the vaccine of this invention, and the induction of anti-tumor immunity enables treatment and prevention of RCC. Therapy against cancer or prevention of the onset of cancer includes any of the following steps, such as inhibition of the growth of cancerous cells, involution of cancer, and suppression of the occurrence of cancer. A decrease in mortality or morbidity in individuals having cancer, decrease the levels of tumor markers in the blood, alleviation of detectable symptoms accompanying cancer, and such are also included in the therapy or prevention of cancer. Such therapeutic and preventive effects are preferably statistically significant. For example, in observation, at a significance level of 5% or less, wherein the therapeutic or preventive effect of a vaccine against cell proliferative diseases is compared to a control without vaccine administration. For example, Student's t-test, the Mann-Whitney U-test, or ANOVA may be used for statistical analyses.

The above-mentioned protein having immunological activity or a vector encoding the protein may be combined with an adjuvant. An adjuvant refers to a compound that enhances the immune response against the protein when administered together (or successively) with the protein having immunological activity. Examples of adjuvants include, but are not limited to, cholera toxin, salmonella toxin, alum, and such, but are not limited thereto. Furthermore, the vaccine of this invention may be combined appropriately with a pharmaceutically acceptable carrier. Examples of such carriers include, but are not limited to, sterilized water, physiological saline, phosphate buffer, culture fluid, and such. Furthermore, the vaccine may contain as necessary, stabilizers, suspensions, preservatives, surfactants, and such. The vaccine can be administered systemically or locally. Vaccine administration may involve a single administration, or multiple booster administrations.

When using an APC or CTL as the vaccine of this invention, tumors can be treated or prevented, for example, by the *ex vivo* method. More specifically, PBMCs of the subject receiving treatment or prevention are collected, the cells are contacted with the polypeptide *ex vivo*, and following the induction of APCs or CTLs, the cells may be administered to the

subject. APCs can be also induced by introducing a vector encoding the polypeptide into PBMCs *ex vivo*. APCs or CTLs induced *in vitro* can be cloned prior to administration. By cloning and growing cells having high activity of damaging target cells, cellular immunotherapy can be performed more effectively. Furthermore, APCs and CTLs isolated in this manner may be used for cellular immunotherapy not only against individuals from whom the cells are derived, but also against similar types of tumors from other individuals.

Furthermore, a pharmaceutical composition for treating or preventing a cell proliferative disease, such as cancer, including a pharmaceutically effective amount of the polypeptide of the present invention is provided. The pharmaceutical composition may be used for raising anti tumor immunity.

Pharmaceutical Compositions For Inhibiting RCC Or Malignant RCC:

In the context of the present invention, suitable formulations include those suitable for oral, rectal, nasal, topical (including buccal and sub-lingual), vaginal or parenteral (including intramuscular, sub-cutaneous and intravenous) administration, or for administration by inhalation or insufflation. Preferably, administration is intravenous. The formulations are optionally packaged in discrete dosage units.

Pharmaceutical formulations suitable for oral administration include capsules, cachets or tablets, each containing a predetermined amount of the active ingredient. Suitable formulations also include powders, granules, solutions, suspensions and emulsions. The active ingredient is optionally administered as a bolus electuary or paste. Tablets and capsules for oral administration may contain conventional excipients such as binding agents, fillers, lubricants, disintegrant and/or wetting agents. A tablet may be made by compression or molding, optionally with one or more formulational ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, lubricating, surface active and/or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may be coated according to methods well known in the art. Oral fluid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may

include edible oils), and/or preservatives. The tablets may optionally be formulated so as to provide slow or controlled release of the active ingredient therein. A package of tablets may contain one tablet to be taken on each of the month.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which optionally contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient as well as aqueous and non-aqueous sterile suspensions which optionally include suspending agents and/or thickening agents. The formulations may be presented in unit dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, saline, water-for-injection, immediately prior to use. Alternatively, the formulations may be presented for continuous infusion. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Formulations suitable for rectal administration include suppositories with standard carriers such as cocoa butter or polyethylene glycol. Formulations suitable for topical administration in the mouth, for example buccally or sublingually, include lozenges, containing the active ingredient in a flavored base such as sucrose and acacia or tragacanth, and pastilles including the active ingredient in a base such as gelatin and glycerin or sucrose and acacia. For intra-nasal administration the compounds of the invention may be used as a liquid spray, a dispersible powder or in the form of drops. Drops may be formulated with an aqueous or non-aqueous base which may optionally include one or more dispersing agents, solubilizing agents and/or suspending agents.

For administration by inhalation, the compounds of the present invention can be conveniently delivered from an insufflator, nebulizer, pressurized packs or other convenient means of delivering an aerosol spray. Pressurized packs may include a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount.

Alternatively, for administration by inhalation or insufflation, the compounds may take the form of a dry powder composition, for example a powder mix of the compound and a suitable powder base such as lactose or starch. The powder composition may be presented in

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unit dosage form, for example, as capsules, cartridges, gelatin or blister packs from which the powder may be administered with the aid of an inhalator or insufflators.

Other formulations include implantable devices and adhesive patches; which release a therapeutic agent.

5 When desired, the above described formulations, adapted to give sustained release of the active ingredient, may be employed. The pharmaceutical compositions may also contain other active ingredients such as antimicrobial agents, immunosuppressants and/or preservatives.

10 It should be understood that in addition to the ingredients particularly mentioned above, the formulations of this invention may include other agents conventional in the art with regard to the type of formulation in question. For example, formulations suitable for oral administration may include flavoring agents.

Preferred unit dosage formulations are those containing an effective dose, as recited below, or an appropriate fraction thereof, of the active ingredient.

15 For each of the aforementioned conditions, the compositions, *e.g.*, polypeptides and organic compounds can be administered orally or via injection at a dose ranging from about 0.1 to about 250 mg/kg per day. The dose range for adult humans is generally from about 5 mg to about 17.5 g/day, preferably about 5 mg to about 10 g/day, and most preferably about 100 mg to about 3 g/day. Tablets or other unit dosage forms of presentation provided in
20 discrete units may conveniently contain an amount which is effective at such dosage or as a multiple of the same, for instance, units containing about 5 mg to about 500 mg, usually from about 100 mg to about 500 mg.

 The dose employed will depend upon a number of factors, including the age and sex of the subject, the precise disorder being treated, and its severity. Also the route of
25 administration may vary depending upon the condition and its severity. In any event, appropriate and optimum dosages may be routinely calculated by those skilled in the art, taking into consideration the above-mentioned factors.

 Aspects of the present invention are further described in the following examples, which are not intended to limit the scope of the invention described in the claims. The
30 following examples illustrate the identification and characterization of genes differentially expressed in RCC cells.

Genome-wide cDNA Microarray Analysis of Gene-Expression Profiles of Renal cell carcinoma Using Clear Cell RCC Cells and Normal Renal Cortex Cells Purely Selected by Laser Microdissection:

Tumor markers and targets for therapeutic intervention were identified by analyzing gene-expression profiles using a cDNA microarray representing 27,648 genes. Renal cell carcinomas exist as a solid mass containing various cellular components.. Therefore, cancer cells were purified from 15 renal cell carcinomas by means of laser microbeam microdissection (LMM). Gene expression profiles were examined and compared with those of normal purified renal cortex cells. These cell populations were thusly rendered homogenous (more than 95% purified cells). As a result, 251 genes were identified to be commonly up-regulated in renal cell carcinoma cells. Among these up-regulated genes, Cyclin-D1 (*CCND1*) which had already been reported as over-expressed in RCCs (Hedberg Y., *et al.*, (1999) Int. J. Cancer, 84: 268-72, 1999.) was included. This gene was over-expressed in 9 of 15 informative RCC cases in the present invention. 721 genes were identified as being commonly down-regulated in renal cell carcinoma cells.

The present invention overcomes a number of limitations of earlier methods. Accordingly, the gene expression profile of the present invention constitutes a highly accurate cancer reference. First, because of various cellular components are present in the normal as well as cancer tissues, a microarray analysis using clinical samples had previously proven to be difficult. In particular, renal cell carcinoma has a characteristic of existing as a solid mass containing various cellular components. Therefore, the analysis of gene-expression profiles using bulk renal cell carcinoma and normal renal cortex cells is significantly influenced by the proportions of cells mixed in the tissues examined may mask the significant increase or decrease of genes that are involved in renal cell carcinoma. Hence, in the present invention, LMM systems were used to purify cancer and normal epithelial cells from surgical specimens to a high degree of purity (95% or higher). Because it is possible to microdissect even a single cell with LMM, this technology is critical for an accurate microarray analysis of renal cell carcinoma specimens.

Careful purification of cancer cells as well as normal renal cortex cells, subsequent RNA isolation, and cDNA microarray analysis resulted in the identification of 251 genes whose expression was commonly up-regulated (genes which were able to obtain expression data in more than 50% cancer cases and whose expression ratio (Cy5/Cy3 intensity ratio) by

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more than 5.0; genes which were calculated in 33 to 50% cases and which expressed the expression ratio of more than 5.0 in all of that cases were also evaluated.

The profiles obtained and described herein represent an improvement over earlier profiles, because they were obtained by analyzing highly purified populations of cancerous cells (renal cell carcinoma) and compared to a highly purified population of the most relevant normal control, *i.e.*, normal renal cortex cells. Earlier methods and profiles were hampered by a high percentage of contaminating cells, which reduced the accuracy and reliability of earlier profiles. This present profile is the first one of precise and genome-wide gene expression profiles in large-scale renal cell carcinoma. These data identify molecular targets for therapeutic modulation for the treatment of renal cell carcinoma and specific novel tumor markers for early and accurate diagnosis of the cancer or a precancerous condition.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

EXAMPLES

MATERIALS AND METHODS

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

Tissue obtained from diseased tissue and normal tissues was evaluated to identify genes which are differently expressed or a disease state, *e.g.*, RCC. The assays were carried out as follows.

Patients, tissue samples and cell lines

Renal cell carcinomas were obtained with informed consent from 15 patients (9 males and 6 females; clear cell renal cell carcinomas including pT1 10cases, pT2 2cases, and pT3 3cases, average age 61.3 in a range of 36 to 75 years old) concerning which all patients had given informed consent (Table 1). Clinical information was obtained from medical

records and each tumor was diagnosed according to histopathological subtype and grade by pathologists. Clinical stage of each patient was judged according to the General Rules for Clinical Pathological Studies on Renal Cell Carcinoma from Japanese Urological Association. All samples were immediately frozen and stored at -80°C. Cell lines derived from renal cell carcinomas (Caki-1, Caki-2, 786-O, A498, ACHN, 769-P, A704, RXF-631L, OS-RC-2, 5 TUHR10TKB and TUHR14TKB) cells and renal proximal tubule epithelial cell (RPTEC) were grown in monolayers in appropriate media supplemented with 10% fetal bovine serum and 1% antibiotic/antimycotic solution, and maintained at 37°C in air containing 5% CO₂.

Table 1 Clinicopathological features of RCC patients

Sample No.	Age	Gender	pT
1	57	M	1
2	70	M	2
3	68	M	1
4	73	F	1
5	54	M	1
6	71	F	1
7	69	F	3
8	47	F	1
9	75	M	3
10	41	M	1
11	54	F	2
12	74	F	3
13	66	M	1
14	62	M	1
15	36	M	1

Tissue Samples and LMM

10 Samples were embedded in TissueTek OCT medium (Sakura) and then stored at -80°C until use. Frozen specimens were serially sectioned in 8-μm slices with a cryostat and stained with hematoxylin and eosin to define the analyzed regions. To avoid cross-contamination of cancer and noncancerous cells, these two populations were prepared by EZ Cut LMM System (SL Microtest GmbH) followed the manufacture's protocol with several 15 modifications. To minimize the effects during storage process and tissue collection, the cancer tissues were carefully handled by the same procedure. To check the quality of RNAs, total RNA extracted from the residual tissue of each case were electrophoresed under the degenerative agarose gel, and confirmed their quality by a presence of ribosomal RNA bands.

RNA Extraction and T7-Based RNA Amplification

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Total RNA was extracted from each population of laser captured cells into 350µl RLT lysis buffer (QIAGEN). The extracted RNA was treated for 30 minutes at room temperature with 30 units of DNase I (QIAGEN). After inactivation at 70°C for 10 min, the RNAs were purified with an RNeasy Mini Kit (QIAGEN) according to the manufacturer's
5 recommendations. All of the DNase I treated RNA was subjected to T7-based amplification using Ampliscribe T7 Transcription Kit (Epicentre Technologies). Two rounds of amplification yielded 41.5-163.4 µg of amplified RNAs (aRNAs) for each sample, whereas when the present inventors amplified RNAs from normal renal cortex from 11 kidney cancer patients, total of 477.0 µg were yielded. 2.5µg aliquots of aRNA from each cancerous cells
10 and normal kidney cortex cells were reverse-transcribed in the presence of Cy5-dCTP and Cy3-dCTP (Amersham Biosciences), respectively.

cDNA microarray

To obtain cDNAs for spotting on the glass slides, the present inventors performed RT-PCR for each gene, as described previously (Kitahara O, *et al.*, (2001) Cancer Res;61:3544-9).
15 The PCR products were spotted on type VII glass slides (GE Healthcare, Amersham Biosciences, Buckinghamshire UK) with a high-density Microarray Spotter Lucidea (GE Healthcare, Amersham Biosciences); 9,216 genes were spotted in duplicate on a single slide. Three different sets of slides (a total of 27,648 gene spots) were prepared; each of which included the same 52 housekeeping genes and two negative control genes as well. The cDNA
20 probes were prepared from aRNA in the manner described previously (Okabe H, *et al.*, (2001) Cancer Res;61:2129-37). For hybridization experiments, 7.5 µg of amplified RNAs (aRNAs) from each cancerous tissue and from the control were reversely transcribed in the presence of Cy5-dCTP and Cy3-dCTP (GE Healthcare, Amersham Biosciences) respectively. Hybridization, washing and detection of signals were carried out as described previously
25 (Okabe H, *et al.*, (2001) Cancer Res;61:2129-37).

Hybridization and Acquisition of Data

Hybridization and washing were performed according to protocols described previously except that all processes were carried out with an Automated Slide Processor (Amersham Biosciences) (Ono K., *et al.*, (2000) Cancer Res, 60: 5007-11.).

30 Signal intensities of Cy3 and Cy5 from the 27,648 spots were quantified and analyzed by substituting backgrounds, using ArrayVision software (Imaging Research, Inc., St.

Catharines, Ontario, Canada). Subsequently the fluorescent intensities of Cy5 (tumor) and Cy3 (control) for each target spot were adjusted so that the mean Cy3/Cy5 ratio of 52 housekeeping genes on the array was equal to one. Because data derived from low signal intensities are less reliable, the present inventors determined a cut-off value on each slide as described previously (Ono, K., *et al.*, (2000) Cancer Res, 60: 5007-11.) and excluded genes from further analysis when both Cy3 and Cy5 dyes yielded signal intensities lower than the cut-off (Saito-Hisaminato, A., *et al.*, (2002) DNA Res, 9: 35-45.). For other genes the present inventors calculated the Cy5/Cy3 ratio using the raw data of each sample.

Identification of commonly up or down-regulated genes in RCCs

The relative expression ratio of each gene (Cy5/Cy3 intensity ratio) was classified into one of four categories: (A) up-regulated (expression ratio >5.0); (B) down-regulated (expression ratio <0.2); (C) unchanged (expression ratio between 0.2 and 5.0); and (D) not expressed (or slight expression but under the cutoff level for detection). These categories were used to detect a set of genes for which changes in the expression ratios were common among samples. To detect candidate genes that were commonly significantly up or down-regulated in each group, the overall expression patterns of 27,648 genes were first screened to select genes with expression ratios >5.0 or <0.2 that were present in >50% of the groups categorized.

Semi-quantitative RT-PCR

The 19 up-regulated genes were selected and their expression levels were examined by applying the semi-quantitative RT-PCR experiments. A 1-μg aliquot of aRNA from each sample was reverse-transcribed for single-stranded cDNAs using random primer and Superscript II (Life Technologies, Inc.). Each cDNA mixture was diluted for subsequent PCR amplification with the primer sets that were shown in Table 2. To normalize the expression of each gene, farnesyl-diphosphate farnesyltransferase 1 (*FDFT1*) was selected as internal control, from among the 52 housekeeping genes because it showed the smallest Cy5/Cy3 fluctuations in the instant microarray data. PCR reactions were optimized for the number of cycles to ensure product intensity within the linear phase of amplification.

Table2. The primer sequences for semi-quantitative RT-PCR

LMMID	Accession No.	Forward primer	SEQ ID NO	Reverse primer	SEQ ID NO
A6149N	NM_018092	5'-GCTCTTATCAAGAA TAACAACCTCCA-3'	1	5'-AGTTATAGCGTCCA GGTCCAAC-3'	2

B0732	AA632745	5'-CAGAGTGCTTCCTC CATCTCTTA-3'	5	5'-CATAAGAAGCATCG CTGAGAATC-3'	6
B8940	NM_007250	5'-GAATCAAACCTCAAG GCTGTGAAC-3'	7	5'-GAATGTGCCTCAAA CTCAGAAAC-3'	8
C6314	W86513	5'-TCACCTGGTTTCAG TACTGCTCT-3'	9	5'-GAAGGCACAGGTCT TGTAATAATG-3'	10
C6700	BC077726	5'-TGACCTGTGCTTAG AAGTCCTTT-3'	11	5'-GTGTGTGTTTCCTGA ACAGATGAA-3'	12
C7670	AA156409	5'-AAACAACATCTGAC TTACGGGAG-3'	13	5'-CAACATGAAGATTC TGAAGGGTC-3'	14
C8379	W57613	5'-CCTCTACTTCCCAT TCCATTTCT-3'	15	5'-TGCACTACATATGG TCAGAGTGG-3'	16
C8919	CR749811	5'-GTTGCAGTTAGACG AAGTGGTTC-3'	17	5'-CCAAAAAGTTGTGC TAGTGTGTG-3'	18
C9099	AW972553	5'-ATGATCTGCCCACT CACCTC-3'	19	5'-AGGAGGCTAAAGG CAATGAATAG-3'	20
D9553	AL832896	5'-GTGAATGAATGAGT GTGTCATGG-3'	21	5'-GTGTTAGTGTCACT CTCAGCCAG-3'	22
F0887	AK021778	5'-GCCCTCAGAAGAGA AACCTGTA-3'	23	5'-TCACCACTATGGTC GATCTTTCT-3'	24
F4886	AK026403	5'-GCAGTCAGAAGGC AGGTTTC-3'	25	5'-TTCTTCCCACAACA TCTCTATGC-3'	26
F5749	AK025204	5'-GCAAATTCGGTAAA ACCCATC-3'	27	5'-CGAGACAGAAATA GGTTGCTGG-3'	28
A0707	NM_000677	5'-CCTGAAGGGTGCCT AGTTGA-3'	67	5'-ACTCAAAAACATCC ACAGGTGA-3'	68
A4587	AF070609	5'-GGCTGTGGTGCAGT AACCAT-3'	69	5'-CATCTACAAAGTAA TGCTTCCCAGT-3'	70
B4578	AI290343	5'-TCCCTCACGTTATT GGAAGC-3'	71	5'-TCCACATCCTTCCT CAAAGG-3'	72
D9765	AA442590	5'-ATGATGGCCATTTT GATGCT-3'	73	5'-GCTCTCCACGTTGG TAGGTC-3'	74
A3412	NM_000552	5'-CACCAATGGCTCTG TTGTGT-3'	75	5'-TAAGAGCTCAGCCT TTATTGTGG-3'	76
C4971	BC000234	5'-GCTACTACATGATT GGTGAGCAG-3'	77	5'-CTTTAATTGAGGTC ACAGGCATC-3'	78
B7032N	NM_004567	5'-CTTCCAGACTCATC TGTCAGAGC-3'	117	5'-GCAACTCCTAACTC CCCTCTGTA-3'	87
B9320	BC000234	5'-CAGAGAGCCAGAG AGTGAGAGAG-3'	88	5'-GCATATACAGGAGA ATGAGGTCG-3'	89
FDFT1	NM_004462	5'-AGTGAAATGCAGGT GAGAAGAAC-3'	29	5'-TCATTCTAGCCAGG ATCATACTAAG-3'	30

Since C6700 has two different transcripts, semiquantitative RT-PCR was performed to confirm which variants was overexpressed in RCC cells using the following primers;

C6700FV1 5'-GTCCCCACTGCTTTGAGATG-3' (SEQ ID NO:37),

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C6700RV1 5'-GTGTGTGTTTCCTGAACAGATGAA-3' (SEQ ID NO:12);

C6700FV2 5'-GACTGTGCAGGGTTCAATCTC-3' (SEQ ID NO:38),

C6700RV2 5'-GTGTGTGTTTCCTGAACAGATGAA-3' (SEQ ID NO:12);

β 2-microglobulin (β 2MG) 5'-TTAGCTGTGCTCGCGCTACT-3' (SEQ ID NO:39),

5 5'-TCACATGGTTCACACGGCAG-3' (SEQ ID NO:40) for the internal control.

To examine the expression level of C6700V1 and C6700V2 in RCC cells (786-O, A704, OS-RC-2 and TUHR14TKB), RT-PCR experiments were performed using the following primer sets; 5'-CTGGAAACAGCAGCCAGAG-3' (SEQ ID NO:83) and 5'-

10 CAGTGCTGGCAAGACAGGTA-3' (SEQ ID NO:84). PCR reactions were optimized for the number of cycles to ensure product intensity within the logarithmic phase of amplification.

To further confirm the expression level of B9320 variant transcripts by RT-PCR, the following primer sets were used: variant1: 5'-GGAGTGGGAACCGCAGAAC-3' (SEQ ID NO: 90), and 5'-GACCCCCACCTTCAAATCAC-3'(SEQ ID NO: 91), Variant2: 5'-GCTCGTGCTCGACAGGTGTGTA-3'(SEQ ID NO: 92) and 5'-

15 CAGGTCATGGCCGGGTTC-3'(SEQ ID NO: 93). PCR reactions were optimized for the number of cycles to ensure product intensity within the logarithmic phase of amplification.

Northern-blot analysis

A 1- μ g aliquot of each mRNA isolated from four renal cell carcinomas cell lines and RPTEC cells using Micro-FastTrack (Invitrogen), and human normal-tissue polyA (+) RNAs
20 (Clontech, Palo Alto, California), heart, liver, lung, kidney, brain and testis were separated on a 1% denaturing agarose gel, transferred to a nylon membrane. This northern blots and Human multiple-tissue northern blots (Clontech, Palo Alto, CA) were also hybridized with an [α ³²P]-dCTP-labeled amplification product of candidate genes by RT-PCR. Pre-hybridization, hybridization and washing were performed according to the supplier's recommendations. The
25 blots were autoradiographed with intensifying screens at -80 °C for 14 days. Specific probes were prepared by PCR using a specific primer set as shown in Table 3.

Table 3. The sequence of primers for Northern probe

LMMID	Accession No.	Forward Primer	SEQ ID NO	Reverse primer	SEQ ID NO
C6700	BC077726	5'-CTGTGGACCTA CTGGGCATT-3'	31	5'-GTGTGTGTTTCCTG AACAGATGAA-3'	12

F5749V1	AK025204	5'-TTTGCCTTTTGTG TGTTCGCTT-3'	32	5'-CCAGAAGCCTGC AGTATACCA-3'	33
F5749V2		5'-CTGGGATATGG CAGCAATGTA-3'	34	5'-ATATGCTGGCACT GTGTGGTC-3'	35
B7032N	NM_004567	5'-GGGTCAGAAAG TGTCTGGACTT-3'	94	5'-GCAACTCCTAACT CCCCTCTGTA-3'	87
B9320	BC034104 NM_153350	5'-CAACAGCCCAA AGATTTTCC-3'	95	5'-GGTCACGTGATAA AATAGCACAA-3'	96
C8919	CR749811	5'-TCCAACCAAGT GGACACTGA-3'	36	5'-CCAAAAAGTTGTG CTAGTGTGTG-3'	18

Construction of the expression vectors

An open reading frame sequence of B7032N and B9320 was obtained by PCR using KOD-Plus DNA polymerase (Toyobo, Osaka, Japan) with the primers as follows; B7032N - forward, 5'-AACGAATTCATGGCGTCCCCACGGGA-3' (SEQ ID NO: 97) (underline indicate EcoRI restriction enzyme site) and 5'-CAACTCGAGCTGGTGAGCAGGCACCGTG-3' (SEQ ID NO: 98) (underlines indicate XhoI restriction enzyme site), and B9320-forward, 5'-CAAGAATTCATGTCGAGCCCGGGCATC-3' (SEQ ID NO,99) and 5'-GCTGAATTCACCTCAATGACGAGGCAGCGG-3' (SEQ ID NO,100) (underlines indicate EcoRI restriction enzyme sites).. The PCR products of B7032N were inserted into the EcoRI and XhoI sites of pCAGGSsnH3F expression vectors. The PCR products of B9320 were inserted into the EcoRI sites of pCAGGSsnH3F expression vectors. DNA sequences of these constructs were confirmed by DNA sequencing.

Immunocytochemical analysis.

COS7 cells were seeded at 5×10^4 per well for exogenous expression. After 24 and 48 hours, the cells were transiently transfected with 1mg pCAGGS-HA-B7032N or pCAGGS-HA-B9320 into COS7 cells using FuGENE 6 transfection reagent (Roche) according to the manufacturer's instructions. Then, cells were fixed with PBS (-) containing 4% paraformaldehyde for 15 min at 4°C, and rendered permeable with PBS containing 0.1% Triton X-100 for 2.5 min at 4°C. Subsequently the cells were covered with 3% BSA in PBS(-) for 12 hours at 4°C to block non-specific hybridization. Next, B7032N-HA-transfected COS7 cells and B9320-HA-transfected COS7 cells were incubated with a rat anti-HA antibody (SANTA CRUZ) at 1:1000 dilution. After washing with PBS(-), the transfected-cells were stained by an AlexaFluoro 488-conjugated anti-rat secondary antibody (Molecular Probe) at 1:3000 dilution. Nuclei were counter-stained with 4',6'-diamidine-2'-phenylindole

dihydrochloride (DAPI). Fluorescent images were obtained under a TCS SP2 AOBS microscope (Leica, Tokyo, Japan).

Western blotting analysis.

B7032N protein and B9320 protein were exogenously expressed by transfection into
5 COS7 cell using pCAGGS-nHA expression vector as described above respectively. Whole
cell lysates were harvested 24 and 48 hours after the transfection, respectively. The
transfected-cells were lysed in lysis buffer (50mmol/L Tris (pH 7.5), 150 mmol/L NaCl, 10
mmol/L CHAPS, and 0.1% Protease Inhibitor Cocktail SetIII (Calbiochem)). After
homogenization, the cell lysates were incubated on ice for 30 minutes and centrifuged at
10 14,000 rpm for 15 minutes to separate only supernatant from cell debris. The amount of total
protein was estimated by protein assay kit (Bio-Rad, Hercules, CA), and then proteins were
mixed with SDS-sample buffer and boiled before loading at 10% SDS-PAGE gel. After
electrophoresis, the proteins were blotted onto nitrocellulose membrane (GE Healthcare).
Membranes including proteins were blocked by blocking solution and incubated with a rat
15 anti-HA monoclonal antibody (SANTA CRUZ) for detection of exogenous B7032N protein
and B9320 protein. Finally the membrane was incubated with HRP conjugated secondary rat
antibody and protein bands were visualized by ECL detection reagents (GE Healthcare).

Establishment of NIH3T3 cells stably expressing PFKFB4.

B7032N expression vector, B9320 expression vector or mock vector was transfected
20 into NIH3T3 cells using FUGENE6 as describe above. Transfected cells were incubated in
the culture medium containing 0.9 mg/ml of geneticin (G418) (Invitrogen). Clonal NIH3T3
cells were subcloned by limiting dilution. Expression of HA-tagged B7032N or HA-tagged
B9320 was assessed by western blot analysis using anti-HA monoclonal antibody. Eventually,
several clones were established and designated as B7032N-NIH3T3 or B9320-NIH3T3. To
25 investigate growth-promoting effect of B7032N or B9320, 5×10^3 cells each of four
independent B7032N-NIH3T3 cells (B7032N-A, -B, -C, and -D) and three independent
Mock-NIH3T3 cells (Mock-A, -B, and -C), or four independent B9320-NIH3T3 cells
(B9320-1, -2, -3) three independent Mock-NIH3T3 cells (Mock-1, -2, and -3) were seeded,
and the number of cells were counted by Cell-counting kit-8 (DOJINDO, Kumamoto, Japan)
30 according to the manufacturer's instruction everyday for five days. These experiments were
done in triplicate.

Construction of C6700, B7032N and B9320 specific-siRNA expression vector using

psiU6X3.0

A vector-based RNAi system was established using psiU6BX siRNA expression vector for C6700 and PsiH1BX siRNA expression vector for B7032N and B9320 according to the previous report (WO2004076623; Shimokawa T, *et al.* Cancer Res. 2003;63:6116-20.).

A siRNA expression vector against C6700, B7032N and B9320 (psiU6BX-C6700, psiH1BX-B7032N or psiH1BX-B9320) was prepared by cloning of double-stranded oligonucleotides in Table 6 into the *Bbs*I site in the psiU6BX vector. A control plasmid, psiU6BX-Mock, Luc, Scramble and EGFP was prepared by cloning double-stranded oligonucleotides of into the *Bbs*I site in the psiU6BX3.0 vector.

Gene-silencing effect of C6700, B7032N and B9320

Human RCC cells line OS-RC-2 for C6700, RXF-631L and A498 for B7032N, Caki-2 and A498 for B9320, were plated onto 10-cm dishes (1×10^6 cells/dish) and transfected with psiU6BX-Mock, Luc, Scramble and EGFP as negative controls, psiU6BX-C6700, psiU6BX-B7032N and psiU6BX-B9320 using FuGENE6 reagent (Roche) using Lipofectamine 2000 (Invitrogen) according to the supplier's recommendations. Total RNAs were extracted from the cells at 10 days after the transfection of each construct, and then the knockdown effect of siRNAs was confirmed by semi-quantitative RT-PCR using specific primers for common regions of C6700 as above mentioned. The transfected OS-RC-2 cells were selected in medium containing 0.7 mg/ml of neomycin (Geneticin; Gibco BRL, Carlsbad, CA). Next, total RNA was extracted from the cells at 5 days after Geneticin selection, and then the knockdown effect of siRNAs was examined by a semi-quantitative RT-PCR using specific primer sets; forward, 5'-AACTTAGAGGTGGGGAGCAG-3' (SEQ ID NO:85) and reverse, 5'-CACAACCATGCCTTACTTTATC-3' (SEQ ID NO:86) as an internal control, and 5'-TGACCTGTGCTTAGAAGTCCTTT-3' (SEQ ID NO:11) and 5'-GTGTGTGTTCTCCTGAACAGATGAA-3' (SEQ ID NO:12) for C6700; forward, 5'-CGGTGGTCTCTCATCCTTGT-3' (SEQ ID NO: 101) and reverse, 5'-GCAACTCCTAACTCCCCTCTGTA-3' (SEQ ID NO: 87) for B7032N; forward, 5'-GCCTCGAGGTTATGCTTGAA-3' (SEQ ID NO,102) and reverse, 5'-ATGCAGTCACTCACGCTCAG-3' (SEQ ID NO,103) for B9320. In 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, cell viability was evaluated using Cell-counting kit-8 (DOJINDO, Kumamoto, Japan) at 7 or 14 days after the transfection according to the protocol of the supplier. After 21 days of the incubation, these

cells were fixed with 4% paraformaldehyde, and stained with Giemsa solution for colony formation assay.

RESULTS

Identification of commonly up- or down-regulated genes in RCC

5 To avoid contamination of the surrounding non-cancerous cells or non-normal cells, laser-microbeam microdissection was carried out to select only cancer cells in tumor mass and originate from normal renal cortex cells. As shown in Figure 1, the representative examples of RCCs were microdissected from each clinical specimen. This allows us the subsequent gene expression profiles to be obtained more preciously.

10 To clarify mechanisms underlying carcinogenesis of RCC, genes commonly up- or down-regulated in RCCs were initially investigated. Gene expression profiles in 15 RCCs identified 972 genes with commonly altered expression; 251 genes that revealed expression ratios of >5.0 in more than 50% of informative cases (see Materials and methods) (Table 4), whereas 721 genes whose expression were reduced to less than 0.2 in RCC cells, compared to
15 normal kidney cortex cells (Table 5). Among these up-regulated genes, Cyclin-D1 (*CCND1*) which had already been reported as over-expressed in RCCs (Hedberg Y., *et al.*, (1999) *Int. J. Cancer*, 84: 268-72.) was included. This gene was over-expressed in 9 of 15 informative RCC cases in the present invention. Among these up-regulated genes, the biological functions of 182 genes were already known to some extent. Of them, HIG2 (Hypoxia
20 inducible gene 2), NNMT (Nicotinamide N-methyltransferase), IGFBP3 (Insulin-like growth factor binding protein 3), VEGF (Vascular endothelial growth factor), and VWF (Von Willebrand factor) had already been reported as genes involved in renal tumorigenesis (Togashi A, *et al.*, (2005) *Cancer Res.*;65:4817-26; Yao M, *et al.*, (2005) *J Pathol.*;205:377-87; Cheung CW, *et al.*, (2004) *Kidney Int.*;65:1272-9; Staehler M, *et al.*, (2005) *Curr Drug*
25 *Targets.*;6:835-46; Braybrooke JP, *et al.*, (2000) *Clin Cancer Res.*;6:4697-704.), supporting the high quality of our microarray data. The up-regulated genes represented a variety of functions including genes associated with signal-transduction pathways (ADORA3, EDA2R), or genes involved in various metabolic pathways (SCD, ENPP3), transport systems (SLC1A3, ABCG1), angiogenesis (VEGF), apoptosis (FTS), and cell adhesion (CDH2). In particular,
30 92 of the up-regulated genes were expressed at a level more than 10-fold higher than in normal cortex cells; for example, ADORA3 (Adenosine A3 receptor), SLC1A3 (Solute carrier family 1, member 3) and STC2 (Stanniocalcin 2) were up-regulated more than 10 folds in

more than 90% of the informative cases. Among these down-regulated genes, 532 genes had been functionally characterized in some extent. They included WT1 (Wilms tumors 1), CDKN1C (Cyclin-dependent kinase inhibitor 1C), and GAS1 (Growth arrest-specific 1), which have been implicated their roles in growth suppression or apoptosis (Niu Z, *et al.*, (2005) J Urol;174:1460-2; Kikuchi T, *et al.*, (2002) Oncogene;21:2741-9; Watanabe H, *et al.*, (1998) Proc Natl Acad Sci U S A.;95:1392-7; Evdokiou A & Cowled PA. (1998) Int J Cancer.;75 :568-77.). In particular, WT1 and CDKN1C were significantly down-regulated in all of 15 RCC cases, and GAS1 was also significantly down-regulated in 14 of 15 cases, indicating down-regulation of those genes may be related to RCC-tumorigenesis.

Table 4 Up-regulated genes in RCC

ASSIGNMENT NO	LMMID	ACCESSION NO	SYMBOL	TITLE
1	F0661	AY048757	ABCG1	ATP-binding cassette, sub-family G (WHITE), member 1
2	C4404	NM 052947	ALPK2	Alpha-kinase 2
3	B8656	AY260577	C14orf58	Chromosome 14 open reading frame 58
4	B3940	K02765	C3	Complement component 3
5	F0981	AK021624	C5orf13	Chromosome 5 open reading frame 13
6	A0206	M73554	CCND1	Cyclin D1 (PRAD1: parathyroid adenomatosis 1)
7	E0525	BM988473	CCND1	Cyclin D1 (PRAD1: parathyroid adenomatosis 1)
8	C8039	Z22970	CD163	CD163 antigen
9	B8048	BQ448718	CDC2L6	Cell division cycle 2-like 6 (CDK8-like)
10	A0692	X57548	CDH2	Cadherin 2, type 1, N-cadherin (neuronal)
11	F2312	U57962	CG018	Hypothetical gene CG018
12	A5065	BC036661	CMKOR1	Chemokine orphan receptor 1
13	F2133	AJ224864	CMRF-35H	Leukocyte membrane antigen
14	D9371	H83622	COMMMD10	COMM domain containing 10
15	A3153	M57730	EFNA1	Ephrin-A1
16	F8999	AK025273	EGLN3	Egl nine homolog 3 (<i>C. elegans</i>)
17	A2065N	AK124656	ENO2	Enolase 2 (gamma, neuronal)
18	C7756	H03641	FAM13A1	Family with sequence similarity 13, member A1
19	A0797	J04162	FCGR3B	Fc fragment of IgG, low affinity IIIb, receptor for (CD16)
20	B8373	R27957	FCHO2	FCH domain only 2
21	E0847	NM 024830	FLJ12443	Hypothetical protein FLJ12443
22	F3997	AL049987	FLJ40092	FLJ40092 protein
23	A2464	U63917	GPR30	G protein-coupled receptor 30
24	F6022	AK022479	HDHD1A	Haloacid dehalogenase-like hydrolase domain containing 1A

25	C8023	M81141	HLA-DQB1	Major histocompatibility complex, class II, DQ beta 1
26	A1710	NM_002133	HMOX1	Heme oxygenase (decycling) 1
27	A1803	BC000013	IGFBP3	Insulin-like growth factor binding protein 3
28	A3181	NM_002193	INHBB	Inhibin, beta B (activin AB beta polypeptide)
29	A7770	R55185	IRX3	Iroquois homeobox protein 3
30	A2404	M15395	ITGB2	Integrin, beta 2 (antigen CD18 (p95), lymphocyte function-associated antigen 1; macrophage antigen 1 (mac-1) beta subunit)
31	B8882	BC005832	KIAA0101	KIAA0101
32	F2379	AB002365	KIAA0367	KIAA0367
33	C5287	N91945	KIAA0746	KIAA0746 protein
34	C7200	AB058765	KIAA1862	KIAA1862 protein
35	D7516	AI074524	LRRK2	Leucine-rich repeat kinase 2
36	A6527	T40467	MAPRE2	Microtubule-associated protein, RP/EB family, member 2
37	F8140	AW976457	MBNL1	Muscleblind-like (Drosophila)
38	C4971	BC000234	NNMT	Nicotinamide N-methyltransferase
39	E0657	CR600491	NOL3	Nucleolar protein 3 (apoptosis repressor with CARD domain)
40	A4224	BC045651	P2RY5	Purinergic receptor P2Y, G-protein coupled, 5
41	A1757	M24486	P4HA1	Procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha polypeptide I
42	C6771	NM_002610	PDK1	Pyruvate dehydrogenase kinase, isoenzyme 1
43	A4010	BC002536	PFKP	Phosphofructokinase, platelet
44	E1191	AA187749	PFKP	Phosphofructokinase, platelet
45	A5123	NM_000292	PHKA2	Phosphorylase kinase, alpha 2 (liver)
46	A4486	AF059617	PLK2	Polo-like kinase 2 (Drosophila)
47	A6551	AA811043	RNASET2	Ribonuclease T2
48	A4132	NM_005063	SCD	Stearoyl-CoA desaturase (delta-9-desaturase)
49	A5240	BQ027924	SLC15A4	Solute carrier family 15, member 4
50	E0623	AL162079	SLC16A1	Solute carrier family 16 (monocarboxylic acid transporters), member 1
51	E1497	BU625507	SLC16A3	Solute carrier family 16 (monocarboxylic acid transporters), member 3
52	F2465	U88878	TLR2	Toll-like receptor 2
53	B8387	BC007372	TRIM52	Tripartite motif-containing 52
54	A0478N	BC065522	VEGF	Vascular endothelial growth factor
55	A3412	NM_000552	VWF	Von Willebrand factor
56	F9001	AL137588	ZNF395	Zinc finger protein 395
57	A5918	BX648117	ZNF6	Zinc finger protein 6 (CMPX1)
58	B6343	T79802		MRNA full length insert cDNA clone EUROIMAGE 1534000

59	B0741	BM991954		Transcribed locus, weakly similar to XP_311626.1 Anopheles gambiae str. PEST ENSANGG00000020300 gene
60	B6492	AK057151		CDNA FLJ32589 fis, clone SPLEN2000443
61	F7562	AI146812		qb92e02.x1 Soares_fetal_heart NbHH19W Homo sapiens cDNA clone IMAGE:1707578 3', mRNA sequence.
62	F8824	AI916271		Transcribed locus
63	E1182	CR599886	ADSL	Adenylosuccinate lyase
64	A8343	AK123519	ARHGAP26	Rho GTPase activating protein 26
65	C0227	N49962	BCL2	B-cell CLL/lymphoma 2
66	C8205	BX648568	CARD8	Caspase recruitment domain family, member 8
67	F0691	NM_004932	CDH6	Cadherin 6, type 2, K-cadherin (fetal kidney)
68	C1993	AA831021	CLK4	CDC-like kinase 4
69	A6942	AA521342	DDX31	DEAD (Asp-Glu-Ala-Asp) box polypeptide 31
70	B6050	BG621779	EGFR	Epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)
71	A8639	AI368204	ENPP3	Ectonucleotide pyrophosphatase/phosphodiesterase 3
72	F0200	AL832950	FLJ31033	Hypothetical protein FLJ31033
73	D0587	AA872040	INHBB	Inhibin, beta B (activin AB beta polypeptide)
74	A8946	AY423763	KLHL17	Kelch-like 17 (Drosophila)
75	A9042	NM_022349	MS4A6A	Membrane-spanning 4-domains, subfamily A, member 6A
76	C6251	AA904502	MSL3L1	Male-specific lethal 3-like 1 (Drosophila)
77	A6149N	NM_018092	NETO2	Neuropilin (NRP) and tolloid (TLL)-like 2
78	A1815	NM_002664	PLEK	Pleckstrin
79	A8911	AK095793	PRKXP1	Protein kinase, X-linked, pseudogene 1
80	C9471	AK090411	RGPR	Regucalcin gene promotor region related protein
81	C6700	BC077726	SEMA5B	Sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B
82	B8658	CA429220	SKP2	S-phase kinase-associated protein 2 (p45)
83	F0306	NM_003044	SLC6A12	Solute carrier family 6 (neurotransmitter transporter, betaine/GABA), member 12
84	B8485	CA308403	WIP149	WD40 repeat protein Interacting with phosphoinositides of 49kDa

85	E1336	XM 040486	ZMAT1	Zinc finger, matrin type 1
86	B0756	AA653431		Transcribed locus
87	C7780	AA159605		Transcribed locus
88	D7481	BX392279		Transcribed locus, strongly similar to XP_496781.1 transposon-derived Buster3 transposase-like [Homo sapiens]
89	D7317	AI015709		Transcribed locus
90	D8458	AA830668		oc52h12.s1 NCI CGAP GCB1 Homo sapiens cDNA clone IMAGE:1353383 3', mRNA sequence.
91	D8466	AI619500		Transcribed locus
92	A0707	NM 000677	ADORA3	Adenosine A3 receptor
93	F2294	AK024900	AP2B1	Adaptor-related protein complex 2, beta 1 subunit
94	D3390	AL832660	C7orf29	Chromosome 7 open reading frame 29
95	D9553	AL832896	CAPN12	Calpain 12
96	A1150	NM 000560	CD53	CD53 antigen
97	F5859	AK023507	CMKOR1	Chemokine orphan receptor 1
98	F2310	AB002367	DCAMKL1	Doublecortin and CaM kinase-like 1
99	C2017	H88117	DKFZP564J102	DKFZP564J102 protein
100	B0726	BC034919	EDA2R	Ectodysplasin A2 receptor
101	C8054	NM_001445	FABP6	Fatty acid binding protein 6, ileal (gastrotropin)
102	A1051	BM662950	FCER1G	Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide
103	B7439	N51406	FLJ14503	Hypothetical protein FLJ14503
104	B9480	AB018345	KIAA0802	KIAA0802
105	F6208	XM 058513	LRRK2	Leucine-rich repeat kinase 2
106	E1387	D87448	TOPBP1	Topoisomerase (DNA) II binding protein 1
107	A9518N	AA570186		Hypothetical gene supported by AK096951; BC066547
108	F5778	AK021801		CDNA FLJ11739 fis, clone HEMBA1005497
109	C4457	BM665350	AIG1	Androgen-induced 1
110	A2811	X00570	APOC1	Apolipoprotein C-I
111	C9189	BC065544	C14orf106	Chromosome 14 open reading frame 106
112	B8942	AA868809	CHC1L	Chromosome condensation 1-like
113	A5716	U78556	CRA	Cisplatin resistance associated
114	A1687	U29171	CSNK1D	Casein kinase 1, delta
115	D3230	AA780074	CSS3	Chondroitin sulfate synthase 3
116	C2022	H88362	EHBP1	EH domain binding protein 1
117	C7370	BC037568	EOMES	Eomesodermin homolog (Xenopus laevis)
118	A2115	BQ949386	FCGR1A	Fc fragment of IgG, high affinity Ia, receptor for (CD64)
119	C6584	BC042143	FLJ32009	Hypothetical protein FLJ32009
120	A2839	M36284	GYPC	Glycophorin C (Gerbich blood group)

121	A2715	BC035802	GZMK	Granzyme K (serine protease, granzyme 3; tryptase II)
122	A3113	M60445	HDC	Histidine decarboxylase
123	D9621	NM_178229	IQGAP3	IQ motif containing GTPase activating protein 3
124	D3104	AK091335	MARCH-I	Membrane-associated RING-CH protein I
125	A2753N	BC009924	NPTX2	Neuronal pentraxin II
126	B7032N	AA398096 NM_004567	PFKFB4	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4
127	C9099	BM724257	PLEKHM2	Pleckstrin homology domain containing, family M (with RUN domain) member 2
128	B8547	BC033746	PNCK	Pregnancy upregulated non-ubiquitously expressed CaM kinase
129	F3374	AF195765	RAMP	RA-regulated nuclear matrix-associated protein
130	A4587	AF070609	SLC1A3	DKFZP547J0410 protein
131	B4440	AB040120	SLC39A8	Solute carrier family 39 (zinc transporter), member 8
132	B4578	AI290343	STC2	Stanniocalcin 2
133	A1064	NM_024164	TPSB2	Tryptase alpha/beta 1
134	B8105	AI023320		Hypothetical LOC387790
135	E0400	N46844		CDNA: FLJ20892 fis, clone ADKA03430
136	B7272	BQ268701		ir34g09.x1 HR85 islet Homo sapiens cDNA clone IMAGE:6547217 3', mRNA sequence.
137	B1075	BU623171		CDNA clone IMAGE:3029742, partial cds
138	F5749	AK025204	ABI3BP	ABI gene family, member 3 (NESH) binding protein
139	B8361	AI741514	ALPK2	Alpha-kinase 2
140	A4505	S60415	CACNB2	Calcium channel, voltage-dependent, beta 2 subunit
141	F2918	AF376061	CARD12	Caspase recruitment domain family, member 12
142	A3560	L06797	CXCR4	Chemokine (C-X-C motif) receptor 4
143	B7437	AK024965	DAB2	Disabled homolog 2, mitogen-responsive phosphoprotein (Drosophila)
144	B1960	AI821270	DARS	Aspartyl-tRNA synthetase
145	D9765	AA442590	ENPP3	Ectonucleotide pyrophosphatase/phosphodiesterase 3
146	B9275	BU736022	FABP7	Fatty acid binding protein 7, brain
147	A6168	AK123603	FLJ12443	Hypothetical protein FLJ12443
148	A9014	AK023320	FTS	Fused toes homolog (mouse)
149	B1834	AW978675	GNRH1	Gonadotropin-releasing hormone 1 (luteinizing-releasing hormone)
150	F2358	AK021481	GPC6	Glypican 6
151	C0773	BX537890	KLF7	Kruppel-like factor 7 (ubiquitous)
152	B6517	BC024278	LOC255326	Hypothetical protein LOC255326

153	F3337	AK026747	LOC54103	Hypothetical protein LOC54103
154	D5370	AA907927	MDS009	X 009 protein
155	D4999	AA971400	MGC47816	Hypothetical protein MGC47816
156	E0087	BX484485	MLL3	B melanoma antigen family, member 4
157	B8611	W60419	POLI	Polymerase (DNA directed) iota
158	A6389	BC084567	POLS	Polymerase (DNA directed) sigma
159	C2006	H87865	RFP2	Ret finger protein 2
160	A0949	NM_005505	SCARB1	Scavenger receptor class B, member 1
161	B3918	AK027663	STC2	Stanniocalcin 2
162	F3366	AL080215	TFPI	Tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor)
163	A6906	BC050423	TMEM22	Transmembrane protein 22
164	C4733	AA342991		Similar to Polyadenylate-binding protein 4 (Poly(A)-binding protein 4) (PABP 4) (Inducible poly(A)-binding protein) (iPABP) (Activated-platelet protein-1) (APP-1)
165	D7225	AA827683		Transcribed locus, moderately similar to NP_775735.1 l(3)mbt-like 4 (Drosophila) [Homo sapiens]
166	C1701	H60869		yr46b12.s1 Soares fetal liver spleen 1NFLS Homo sapiens cDNA clone IMAGE:208319 3', mRNA sequence.
167	D9545	AI076475		Hypothetical gene supported by AK026189
168	C0801	AA205305	ANKRA2	Ankyrin repeat, family A (RFXANK-like), 2
169	A8990	NM_013448	BAZ1A	Bromodomain adjacent to zinc finger domain, 1A
170	B9465	BC039999	C9orf76	Chromosome 9 open reading frame 76
171	A6965	R78576	COG6	Component of oligomeric golgi complex 6
172	F3077	NM_001003927	EVI2A	Ecotropic viral integration site 2A
173	B9320	BC036680 BC034014 NM_153350	FBXL16	F-box and leucine-rich repeat protein 16
174	C9574	Y18046	FGFR1OP	FGFR1 oncogene partner
175	A6659	AK096712	FLJ11850	Hypothetical protein FLJ11850
176	F4018	AK022473	FTO	Fatso
177	C7773	AF430643	GBP5	Guanylate binding protein 5
178	B7466	AA128378	KIAA0303	Microtubule associated serine/threonine kinase family member 4
179	A3867	AF013249	LAIR1	Leukocyte-associated Ig-like receptor 1
180	C5174	AL832259	LOC284749	Hypothetical protein LOC284749
181	D3452	BX482647	PARP14	Poly (ADP-ribose) polymerase family, member 14

182	B1210	AA398762	PHKA2	Phosphorylase kinase, alpha 2 (liver)
183	A3613	CR608325	PLA2G7	Phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)
184	A2498	L11932	SHMT2	Serine hydroxymethyltransferase 2 (mitochondrial)
185	E1370	NM_000544	TAP2	Transporter 2, ATP-binding cassette, sub-family B (MDR/TAP)
186	B7455N	BX112530	TLK1	Tousled-like kinase 1
187	F4886	AK026403	TLN2	Talin 2
188	A0504	NM_005428	VAV1	Vav 1 oncogene
189	C6417	BU742664		Full length insert cDNA clone ZE12B03
190	C1799	BQ007156		Transcribed locus
191	C4116	XM_496823		Similar to RIKEN cDNA A630077B13 gene; RIKEN cDNA 2810048G17
192	B0732	AA632745		Transcribed locus
193	B8870	NM_018685	ANLN	Anillin, actin binding protein (scraps homolog, Drosophila)
194	D3310	NM_019043	APBB1IP	Amyloid beta (A4) precursor protein-binding, family B, member 1 interacting protein
195	B3966	BC047724	C10orf128	Chromosome 10 open reading frame 128
196	B3130	BQ025155	C10orf9	Chromosome 10 open reading frame 9
197	C4454	AK026746	C14orf140	Chromosome 14 open reading frame 140
198	C0579	BX648468	DKFZP564J0863	DKFZP564J0863 protein
199	B7451N	AY358175	GBGT1	Globoside alpha-1,3-N-acetylgalactosaminyltransferase 1
200	F3828	U79242	GRM5	Glutamate receptor, metabotropic 5
201	A2681	NM_001884	HAPLN1	Hyaluronan and proteoglycan link protein 1
202	A6379	BM464532	HIST1H1C	Histone 1, H1c
203	F0266	NM_000878	IL2RB	Interleukin 2 receptor, beta
204	F6738	AK022173	LAF4	Lymphoid nuclear protein related to AF4
205	A8928	R38549	LOC150271	Hypothetical LOC388889
206	C7670	AA156409	MCPH1	Microcephaly, primary autosomal recessive 1
207	C5756	AK090397	MGC15875	Hypothetical protein MGC15875
208	C6664	AI142832	MGC34923	Hypothetical protein MGC34923
209	D8440	AA826148	NRCAM	Neuronal cell adhesion molecule
210	A3896	BC015050	OIP5	Opa-interacting protein 5
211	C9148	AA282540	OSBPL8	Oxysterol binding protein-like 8
212	E1852	AA258620	PLXNC1	Plexin C1
213	F0887	AK021778	PTPRK	Protein tyrosine phosphatase, receptor type, K
214	B9282	R60655	RNF32	Ring finger protein 32
215	B7289N	AF146761	SLAMF8	SLAM family member 8
216	F3858	AB020701	SORBS1	Sorbin and SH3 domain containing 1

217	F4051	M31165	TNFAIP6	Tumor necrosis factor, alpha-induced protein 6
218	C2049	N24715		Full length insert cDNA clone YX74D05
219	C8379	W57613		Transcribed locus, weakly similar to NP_062553.1 hypothetical protein FLJ11267 [Homo sapiens]
220	C9252	AK126261		Similar to RIKEN cDNA E030024N20 gene
221	D2965	BU622474		Similar to D(1B) dopamine receptor (D(5) dopamine receptor) (D1beta dopamine receptor)
222	C4328	AK023966		CDNA FLJ13904 fis, clone THYRO1001895
223	A2677	Z11502	ANXA13	Annexin A13
224	C3760	BC008718	BIRC5	Baculoviral IAP repeat-containing 5 (survivin)
225	B3180N	BM992179	CYB5	Cytochrome b-5
226	C8944	AK001149	FLJ10287	Hypothetical protein FLJ10287
227	C7777	BU191922	FLJ23861	Hypothetical protein FLJ23861
228	B2874	AA883488	KIAA0408	KIAA0408
229	B8940	BX641066	KLF8	Kruppel-like factor 8
230	D5261	BC033490	LOC285016	Hypothetical protein LOC285016
231	D5688	AL832281	LOC340351	Hypothetical protein LOC340351
232	D3549	BU620736	MAGI-3	Membrane-associated guanylate kinase-related (MAGI-3)
233	A8335	BC028421	MGC33630	Hypothetical protein MGC33630
234	F3431	AK021954	NRCAM	Neuronal cell adhesion molecule
235	D7468	BC010943	OSMR	Oncostatin M receptor
236	B7110	AK124752	PCDH21	Protocadherin 21
237	C0488	AA781195	PRAME	Preferentially expressed antigen in melanoma
238	C8373	R77952	PSMA3	Proteasome (prosome, macropain) subunit, alpha type, 3
239	D3765	AK096798	RDH13	Retinol dehydrogenase 13 (all-trans and 9-cis)
240	B6688	NM_003042	SLC6A1	Solute carrier family 6 (neurotransmitter transporter, GABA), member 1
241	A5720	BQ787632	SPON1	Spondin 1, extracellular matrix protein
242	B9009	AB011123	TNIK	TRAF2 and NCK interacting kinase
243	B0869N	AF274048	UHRF1	Ubiquitin-like, containing PHD and RING finger domains, 1
244	C4641	BF115786	ZCCHC11	Zinc finger, CCHC domain containing 11
245	C8919	CR749811		Similar to Hypothetical protein MGC38937
246	C6314	W86513		Transcribed locus, weakly similar to XP_517256.1 UDP glycosyltransferase 2 family, polypeptide B4 [Pan troglodytes]
247	A9641	BG196354		Transcribed locus
248	D5230	AA938691		Transcribed locus

249	D1218	BQ709650		Transcribed locus
250	B1929	AA663031		ab72b05.s1 Stratagene fetal retina 937202 Homo sapiens cDNA clone IMAGE:852465 3', mRNA sequence.
251	D6557	AK092260		CDNA FLJ34941 fis, clone NT2RP7007480

Table 5 Down-regulated genes in RCC

ASSIGNMENT NO	LMMID	ACCESSION NO	SYMBOL	TITLE
252	C6486	X83618	HMGCS2	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (mitochondrial)
253	A4297	NM_012205	HAAO	3-hydroxyanthranilate 3,4-dioxygenase
254	A1767	M93107	BDH	3-hydroxybutyrate dehydrogenase (heart, mitochondrial)
255	A2694N	AK057510	HPD	4-hydroxyphenylpyruvate dioxygenase
256	B3053	AI732791	XRN1	5'-3' exoribonuclease 1
257	D4971	AA918686	PFKFB2	6-phosphofructo-2-kinase/fructose-2,6- biphosphatase 2
258	A6530	NM_006988	ADAMTS1	A disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 1
259	B3397	AA732989	ADAMTS2	A disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 2
260	A1182	U23435	ABI2	Abl interactor 2
261	B8954	NM_032432	ABLIM2	Actin binding LIM protein family, member 2
262	A1850	L22214	ADORA1	Adenosine A1 receptor
263	F0673	AF062595	AK5	Adenylate kinase 5
264	C7731	AF245505	DKFZp564I 1922	Adlican
265	B3219	T67409	AFM	Afamin
266	A3322	M80899	AHNAK	AHNAK nucleoprotein (desmoyokin)
267	A2323	V00494	ALB	Albumin
268	A2644	BC062476	ADH1C	Alcohol dehydrogenase 1C (class I), gamma polypeptide
269	A1760	BC039065	ADH6	Alcohol dehydrogenase 6 (class V)
270	A2508	X03350	ADH1B	Alcohol dehydrogenase 1B (class I), beta polypeptide
271	A5088	U24266	ALDH4A1	Aldehyde dehydrogenase 4 family, member A1
272	A3081	NM_005589	ALDH6A1	Aldehyde dehydrogenase 6 family, member A1
273	A2056	X02747	ALDOB	Aldolase B, fructose-bisphosphate
274	A2084	BM709336	AIF1	Allograft inflammatory factor 1

275	B3363	AA806986	ATRX	Alpha thalassemia/mental retardation syndrome X-linked (RAD54 homolog, <i>S. cerevisiae</i>)
276	B3404	NM_015120	ALMS1	Alstrom syndrome 1
277	B5424	NM_020039	ACCN2	Amiloride-sensitive cation channel 2, neuronal
278	A4409	NM_005503	APBA2	Amyloid beta (A4) precursor protein-binding, family A, member 2 (X11-like)
279	B9341	BC012984	ALS2CR19	Amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 19
280	F3692	NM_004673	ANGPTL1	Angiopoietin-like 1
281	A3360	NM_031850	AGTR1	Angiotensin II receptor, type 1
282	B5155	W84893	AGTRL1	Angiotensin II receptor-like 1
283	A9039	AK095632	ABTB2	Ankyrin repeat and BTB (POZ) domain containing 2
284	C7993	NM_020349	ANKRD2	Ankyrin repeat domain 2 (stretch responsive muscle)
285	A3006	M62839	APOH	Apolipoprotein H (beta-2-glycoprotein I)
286	A4483	AF049884	ARGBP2	Arg/Abl-interacting protein ArgBP2
287	A2008	BC029050	ARG2	Arginase, type II
288	B4126	AK027126	ASS	Argininosuccinate synthetase
289	A3010	NM_080912	ASGR2	Asialoglycoprotein receptor 2
290	C7318	BM677716	ATP8A2	ATPase, aminophospholipid transporter-like, Class I, type 8A, member 2
291	A3380	L20977	ATP2B2	ATPase, Ca ⁺⁺ transporting, plasma membrane 2
292	D5516	AI261591	ATP6V1G3	ATPase, H ⁺ transporting, lysosomal 13kDa, V1 subunit G isoform 3
293	A0859	M25809	ATP6V1B1	ATPase, H ⁺ transporting, lysosomal 56/58kDa, V1 subunit B, isoform 1 (Renal tubular acidosis with deafness)
294	A5628	NM_001690	ATP6V1A	ATPase, H ⁺ transporting, lysosomal 70kDa, V1 subunit A
295	D1054	AA994979	ATP6V0A4	ATPase, H ⁺ transporting, lysosomal V0 subunit a isoform 4
296	A3090	BC031609	ATP12A	ATPase, H ⁺ /K ⁺ transporting, nongastric, alpha polypeptide
297	C3735	NM_000701	ATP1A1	ATPase, Na ⁺ /K ⁺ transporting, alpha 1 polypeptide
298	F3709	NM_152296	ATP1A3	ATPase, Na ⁺ /K ⁺ transporting, alpha 3 polypeptide
299	D5023	AK131204	BTLA	B and T lymphocyte associated
300	B5356	AA758171	MLL3	B melanoma antigen family, member 4
301	A3079	J04599	BGN	Biglycan
302	A4481	AF053470	BLCAP	Bladder cancer associated protein
303	A1922	CR605506	BAMBI	BMP and activin membrane-bound inhibitor homolog (<i>Xenopus laevis</i>)

304	A2545	BC033873	BST2	Bone marrow stromal cell antigen 2
305	A2691N	BC041846	CDH3	Cadherin 3, type 1, P-cadherin (placental)
306	A3072	NM_199248	CACNB1	Calcium channel, voltage-dependent, beta 1 subunit
307	A3062	BC025687	CAMK4	Calcium/calmodulin-dependent protein kinase IV
308	A4819	D17408	CNN1	Calponin 1, basic, smooth muscle
309	B5410	BC033183	CHST3	Carbohydrate (chondroitin 6) sulfotransferase 3
310	A7810	BC022567	CHST1	Carbohydrate (keratan sulfate Gal-6) sulfotransferase 1
311	A3324	BC057792	CA4	Carbonic anhydrase IV
312	B0852	H23177	CA10	Carbonic anhydrase X
313	A1754	AB119995	CES1	Carboxylesterase 1 (monocyte/macrophage serine esterase 1)
314	C8606	BC063430	CPXM	Carboxypeptidase X (M14 family)
315	C4120	X66417	CSN3	Casein kappa
316	A1912	BC052996	CTNNA2	Catenin (cadherin-associated protein), alpha 2
317	A3822	BC067289	CTSL2	Cathepsin L2
318	C0791	BC051340	CD164L1	CD164 sialomucin-like 1
319	A5148N	NM_006566	CD226	CD226 antigen
320	A3224	J04132	CD3Z	CD3Z antigen, zeta polypeptide (TiT3 complex)
321	E0542	NM_152243	CDC42EP1	CDC42 effector protein (Rho GTPase binding) 1
322	A9130	AF001436	CDC42EP2	CDC42 effector protein (Rho GTPase binding) 2
323	A6126N	H11384	CDC42EP3	CDC42 effector protein (Rho GTPase binding) 3
324	B6737	NM_012127	CIZ1	CDKN1A interacting zinc finger protein 1
325	C7260	BC071790		CDNA clone IMAGE:4611512, partial cds
326	B4933	R12478		CDNA clone IMAGE:4797817, partial cds
327	C5860	BU683028		CDNA FLJ10151 fis, clone HEMBA1003402
328	F3736	AK021754		CDNA FLJ11692 fis, clone HEMBA1004983
329	B3217	AI279516		CDNA FLJ11723 fis, clone HEMBA1005314
330	D1811	AK128814		CDNA FLJ25106 fis, clone CBR01467
331	D4748	AK055811		CDNA FLJ31249 fis, clone KIDNE2005327
332	D3276	AA765718		CDNA FLJ33551 fis, clone BRAMY2009105

333	B3261	AK091765		CDNA FLJ34446 fis, clone HLUNG2002050
334	C9234	AK093732		CDNA FLJ36413 fis, clone THYMU2010816
335	F1221	AL109700		CDNA FLJ37610 fis, clone BRCOC2011398
336	C9594	N71087		CDNA FLJ37676 fis, clone BRHIP2012627
337	C4381	W45330		CDNA FLJ38345 fis, clone FCBBF3028671
338	D6230	AA977738		CDNA FLJ40061 fis, clone TESOP2000083
339	B8241	AK075052		CDNA FLJ90571 fis, clone OVARC1001725, highly similar to Homo sapiens patched related protein TRC8 (TRC8) gene.
340	F2149	AK024898		CDNA: FLJ21245 fis, clone COL01184
341	A5311N	BQ718658		CDNA: FLJ22642 fis, clone HSI06970
342	B3296	BU741595	LHX4	LIM homeobox 4
343	A6754	NM_004378	CRABP1	Cellular retinoic acid binding protein 1
344	E1369	AK025651	PNAS-4	CGI-146 protein
345	C6124	NM_002989	CCL21	Chemokine (C-C motif) ligand 21
346	A1660	BC048282	CLCNKA	Chloride channel Ka
347	F1739	AF216941	CLIC5	Chloride intracellular channel 5
348	B2906	AI808724	CHRN2	Cholinergic receptor, nicotinic, beta polypeptide 2 (neuronal)
349	C8926	BU569535	CHODL	Chondrolectin
350	C8476	R59552	CHRD1	Chordin-like 1
351	A3539	NM_001275	CHGA	Chromogranin A (parathyroid secretory protein 1)
352	A2596	BC000375	CHGB	Chromogranin B (secretogranin 1)
353	A6346	NM_015576	C1orf1	Chromosome 1 open reading frame 1
354	B6193N	NM_030806	C1orf21	Chromosome 1 open reading frame 21
355	B9135	BC066977	C1orf40	Chromosome 1 open reading frame 40
356	F6114	AK025270	C10orf137	Chromosome 10 open reading frame 137
357	F6052	BF967878	C10orf83	Chromosome 10 open reading frame 83
358	F4642	NM_001584	C11orf8	Chromosome 11 open reading frame 8
359	D3719	H08298	C13orf7	Chromosome 13 open reading frame 7
360	F7019	AK001590	C14orf132	Chromosome 14 open reading frame 132
361	D6180	AK096674	C14orf32	Chromosome 14 open reading frame 32
362	F1846	AK023979	C14orf46	Chromosome 14 open reading frame 46
363	F0168	BC015178	C18orf10	Chromosome 18 open reading frame 10
364	B4749	AA715708	C18orf49	Chromosome 18 open reading frame 49
365	F6269	AY327407	C2orf10	Chromosome 2 open reading frame 10
366	D3266	AA617917	C20orf65	Chromosome 20 open reading frame 65
367	B0294	BC029130	C6orf35	Chromosome 6 open reading frame 35
368	A6409	AK091288	C9orf19	Chromosome 9 open reading frame 19
369	D1204	XM_059954	C9orf57	Chromosome 9 open reading frame 57

370	A5356	NM_001002260	C9orf58	Chromosome 9 open reading frame 58
371	B0676	AK126055	C9orf84	Chromosome 9 open reading frame 84
372	B9368	AF504647		Cilia-associated protein (CYS1)
373	C0661	H18687	CLDN11	Claudin 11 (oligodendrocyte transmembrane protein)
374	A2460	AF000959	CLDN5	Claudin 5 (transmembrane protein deleted in velocardiofacial syndrome)
375	A6080	N99340	CLIPR-59	CLIP-170-related protein
376	B8234	AF070632		Clone 24405 mRNA sequence
377	A5341	AF131834		Clone 24841 mRNA sequence
378	C4665	AK022877		Clone TUA8 Cri-du-chat region mRNA
379	A2191	M16967	F5	Coagulation factor V (proaccelerin, labile factor)
380	A3265N	NM_004291	CART	Cocaine- and amphetamine-regulated transcript
381	B0268	AK123393	CCDC3	Coiled-coil domain containing 3
382	A3156	L02870	COL7A1	Collagen, type VII, alpha 1 (epidermolysis bullosa, dystrophic, dominant and recessive)
383	C1520	BC014640	COL14A1	Collagen, type XIV, alpha 1 (undulin)
384	A3963	NM_080542	COLQ	Collagen-like tail subunit (single strand of homotrimer) of asymmetric acetylcholinesterase
385	C0431	BX538105	COLM	Collomin
386	C7997	J03565	CR2	Complement component (3d/Epstein Barr virus) receptor 2
387	F2392	NM_001901	CTGF	Connective tissue growth factor
388	B0555	AA533963	CPNE4	Copine IV
389	A1793	X58022	CRHBP	Corticotropin releasing hormone binding protein
390	F2073	NM_020990	CKMT1	Creatine kinase, mitochondrial 1 (ubiquitous)
391	A1403	J05401	CKMT2	Creatine kinase, mitochondrial 2 (sarcomeric)
392	A1171N	U05569	CRYAA	Crystallin, alpha A
393	D8699	BX117062	CTBP2	C-terminal binding protein 2
394	A7291	BC042692	CTL2	CTL2 gene
395	A0775	L12579	CUTL1	Cut-like 1, CCAAT displacement protein (Drosophila)
396	D4370	AA883874	CXXC4	CXXC finger 4
397	A0260	U47413	CCNG1	Cyclin G1
398	A9564	NM_000076	CDKN1C	Cyclin-dependent kinase inhibitor 1C (p57, Kip2)
399	A2452	BX537488	CSRP1	Cysteine and glycine-rich protein 1
400	B7775	AF169802	CYB5R2	Cytochrome b5 reductase b5R.2
401	D6271	AY161004	COX8C	Cytochrome c oxidase subunit 8C
402	D1185	AA451886	CYP1B1	Cytochrome P450, family 1, subfamily B, polypeptide 1

403	A0920N	NM_000773	CYP2E1	Cytochrome P450, family 2, subfamily E, polypeptide 1
404	C7590	BC001776	CYP27B1	Cytochrome P450, family 27, subfamily B, polypeptide 1
405	D9075	AL832156	CPEB1	Cytoplasmic polyadenylation element binding protein 1
406	C7057	H22566	DACH1	Dachshund homolog 1 (Drosophila)
407	A4586	D86977	DHX38	DEAH (Asp-Glu-Ala-His) box polypeptide 38
408	A0922	NM_004394	DAP	Death-associated protein
409	C2154	AF007144	DIO2	Deiodinase, iodothyronine, type II
410	B5367	BC033736	DPT	Dermatopontin
411	B5268	BQ062201	DCXR	Dicarbonyl/L-xylulose reductase
412	A2257N	BC052998	DDR2	Discoidin domain receptor family, member 2
413	A0283	NM_021120	DLG3	Discs, large homolog 3 (neuroendocrine-dlg, Drosophila)
414	A8605	NM_015393	DKFZP564O0823	DKFZP564O0823 protein
415	A2029	BC034227	D4S234E	DNA segment on chromosome 4 (unique) 234 expressed sequence
416	A0748	M76180	DDC	Dopa decarboxylase (aromatic L-amino acid decarboxylase)
417	A8098	AL713793	DAPP1	Dual adaptor of phosphotyrosine and 3-phosphoinositides
418	A0623	NM_001395	DUSP9	Dual specificity phosphatase 9
419	A3054	U01839	FY	Duffy blood group
420	A0192	M62829	EGR1	Early growth response 1
421	E1348	BX640908	EVI1	Ecotropic viral integration site 1
422	A4665	D12485	ENPP1	Ectonucleotide pyrophosphatase/phosphodiesterase 1
423	B8411	BX412247	EFHD1	EF hand domain containing 1
424	C9877	BQ001493	EHD3	EH-domain containing 3
425	B6831	NM_000118	ENG	Endoglin (Osler-Rendu-Weber syndrome 1)
426	A7247N	AL133118	EMCN	Endomucin
427	D6367	NM_152461	ERN1	Endoplasmic reticulum to nucleus signalling 1
428	A3097	M65199	EDN2	Endothelin 2
429	A8387	AL697428	ENTH	Enthoprotin
430	C0774	AK023744	EPS15L1	Epidermal growth factor receptor pathway substrate 15-like 1
431	A2019N	AA442410	EMP1	Epithelial membrane protein 1
432	A8525	W67837	EMP2	Epithelial membrane protein 2
433	D4636	AF370395	EPS8L1	EPS8-like 1
434	C2324	BQ182775	ECRG4	Esophageal cancer related gene 4 protein
435	A3160N	NM_000125	ESR1	Estrogen receptor 1
436	F3825	BC064700	ESRRG	Estrogen-related receptor gamma

437	B5753	BC010082	ETNK2	Ethanolamine kinase 2
438	C7625	BU684240	EHF	Ets homologous factor
439	A1553	BC023505	ECM1	Extracellular matrix protein 1
440	A4438	AF055015	EYA2	Eyes absent homolog 2 (Drosophila)
441	A7246	N75862	EYA4	Eyes absent homolog 4 (Drosophila)
442	B7281	NM_058186	FAM3B	Family with sequence similarity 3, member B
443	A8682	AL713770	FAM31C	Family with sequence similarity 31, member C
444	F0573	AB032996	FAM40B	Family with sequence similarity 40, member B
445	B5086	CR595341	FLJ90024	Fasting-inducible integral membrane protein TM6P1
446	A0441	CR612744	FABP1	Fatty acid binding protein 1, liver
447	A2542	J02874	FABP4	Fatty acid binding protein 4, adipocyte
448	B6097	W39428	FBXO2	F-box protein 2
449	A5840N	AA738314	FBXO42	F-box protein 42
450	A6380	NM_005141	FGB	Fibrinogen, B beta polypeptide
451	A0075N	D14838	FGF9	Fibroblast growth factor 9 (glia-activating factor)
452	B6082	BX537781	FNDC5	Fibronectin type III domain containing 5
453	A8531	BX537531	FBLN5	Fibulin 5
454	A5736	AF052170	MICAL2	Flavoprotein oxidoreductase MICAL2
455	A9082	H77985	FLJ25476	FLJ25476 protein
456	A5895	AL713743	FLJ42875	FLJ42875 protein
457	C9358	AI126777	FLJ45455	FLJ45455 protein
458	A9984	AK057972	LOC51066	Fls485
459	B5279	BC004107	FST	Follistatin
460	B3525	NM_012188	FOXI1	Forkhead box I1
461	F6422	AW964166	FTCD	Formiminotransferase cyclodeaminase
462	B8503	AF225426	FMN2	Formin 2
463	B4294	NM_012190	FTHFD	Formyltetrahydrofolate dehydrogenase
464	B2754	W94882	C9orf154	FRAS1 related extracellular matrix 1
465	D9934	CA450275	FREQ	Frequenin homolog (Drosophila)
466	A0764	L10320	FBP1	Fructose-1,6-bisphosphatase 1
467	C0328	BE080213		Full-length cDNA clone CS0DE011YI04 of Placenta of Homo sapiens (human)
468	D1157	AW977392		Full-length cDNA clone CS0DI042YD07 of Placenta Cot 25-normalized of Homo sapiens (human)
469	B3229	AK026244	GPR107	G protein-coupled receptor 107
470	A4491	L15388	GRK5	G protein-coupled receptor kinase 5
471	C6906	AK122672	GPCR5A	G protein-coupled receptor, family C, group 5, member A
472	A5937	BC028315	GABARAP L1	GABA(A) receptor-associated protein like 1
473	A3352	NM_000807	GABRA2	Gamma-aminobutyric acid (GABA) A receptor, alpha 2

474	A2418	M96789	GJA4	Gap junction protein, alpha 4, 37kDa (connexin 37)
475	A5978	BQ003596	GJA5	Gap junction protein, alpha 5, 40kDa (connexin 40)
476	A1859N	NM_001002295	GATA3	GATA binding protein 3
477	A0697	L20316	GCGR	Glucagon receptor
478	A2059N	M81883	GAD1	Glutamate decarboxylase 1 (brain, 67kDa)
479	A6127	AI356291	GPT2	Glutamic pyruvate transaminase (alanine aminotransferase) 2
480	A2801	NM_002083	GPX2	Glutathione peroxidase 2 (gastrointestinal)
481	A1610	NM_002084	GPX3	Glutathione peroxidase 3 (plasma)
482	A4202	BC053578	GSTA1	Glutathione S-transferase A1
483	A2479	L34041	GPD1	Glycerol-3-phosphate dehydrogenase 1 (soluble)
484	B5381N	D42047	GPD1L	Glycerol-3-phosphate dehydrogenase 1-like
485	A0961	NM_001482	GATM	Glycine amidinotransferase (L-arginine:glycine amidinotransferase)
486	B7749	BC023152	GYG2	Glycogenin 2
487	C0893	BC052210	GARP	Glycoprotein A repetitions predominant
488	A1879	U45955	GPM6B	Glycoprotein M6B
489	C3689	NM_004484	GPC3	Glypican 3
490	B0738	BQ962682	GOLGA3	Golgi autoantigen, golgin subfamily a, 3
491	D8393	AA804745	GBF1	Golgi-specific brefeldin A resistance factor 1
492	A5658	AJ243937	GPSM3	G-protein signalling modulator 3 (AGS3-like, <i>C. elegans</i>)
493	C4884	AA036952	Gup1	GRINL1A complex upstream protein
494	C7089	H14263	GAS1	Growth arrest-specific 1
495	A6083	NM_004293	GDA	Guanine deaminase
496	A5969	AA642294	GNG7	Guanine nucleotide binding protein (G protein), gamma 7
497	B8265	AA156792	HEYL	Hairy/enhancer-of-split related with YRPW motif-like
498	B3924	AK075151	HSPB7	Heat shock 27kDa protein family, member 7 (cardiovascular)
499	A6317	AI205684	HSPA2	Heat shock 70kDa protein 2
500	F1385	AB002320	NEDL1	HECT, C2 and WW domain containing E3 ubiquitin protein ligase 1
501	C6283	BC039071	HHAT	Hedgehog acyltransferase
502	A0451	V00497	HBB	Hemoglobin, beta
503	C1603	BQ446275	HBD	Hemoglobin, delta
504	B2559	CA426475	HBE1	Hemoglobin, epsilon 1
505	A0460	X55656	HBG2	Hemoglobin, gamma A
506	A5532	BC063521	HS6ST1	Heparan sulfate 6-O-sulfotransferase 1

507	D0231	AA527435	LOC63928	Hepatocellular carcinoma antigen gene 520
508	B3992	BC078664	HMGA1	High mobility group AT-hook 1
509	A3112	M60052	HRC	Histidine rich calcium binding protein
510	C7133	NM_139205	HDAC5	Histone deacetylase 5
511	B5415	NM_024017	HOXB9	Homeo box B9
512	C6875	AA043381	HOXD10	Homeo box D10
513	B5353N	BC018099		Homo sapiens, clone IMAGE:4796019, mRNA
514	D3237	BX109466		Homo sapiens, clone IMAGE:5276804, mRNA
515	C3975	BX102181		Homo sapiens, Similar to neuronal thread protein, clone IMAGE:4102657, mRNA
516	A9187	BC034222	HRLP5	H-rev107-like protein 5
517	A2972	X72475		HRV Fab 027-VL
518	A1379	D49742	HABP2	Hyaluronan binding protein 2
519	A2287	AK127945	HYAL2	Hyaluronoglucosaminidase 2
520	A1807N	BC018986	HPGD	Hydroxyprostaglandin dehydrogenase 15-(NAD)
521	A3095	U26726	HSD11B2	Hydroxysteroid (11-beta) dehydrogenase 2
522	C4407	BC050354		Hypothetical gene supported by BC014163
523	D8684	BX091957		Hypothetical LOC387905
524	B9722	BQ773658		Hypothetical LOC402560
525	A8056	XM_496688	LOC152573	Hypothetical protein BC012029
526	D4082	NM_138456	MGC20410	Hypothetical protein BC012330
527	C7564	BC068590	LOC150356	Hypothetical protein BC012882
528	A6664	H19830	DKFZP434G156	Hypothetical protein DKFZp434G156
529	A6611	N58556	DKFZp547K1113	Hypothetical protein DKFZp547K1113
530	C0898	AA319638	DKFZp761L1417	Hypothetical protein DKFZp761L1417
531	D6549	BC004888	FLJ10052	Hypothetical protein FLJ10052
532	A6475	BC032508	FLJ10781	Hypothetical protein FLJ10781
533	B3947	AI075877	FLJ11539	Hypothetical protein FLJ11539
534	C2308	AI281932	FLJ13639	Hypothetical protein FLJ13639
535	B8081	BM981462	FLJ13710	Hypothetical protein FLJ13710
536	F2724	AK024275	FLJ14213	Hypothetical protein FLJ14213
537	B4626	BC052574	FLJ20171	Hypothetical protein FLJ20171
538	B4469	AB081837	FLJ20315	Hypothetical protein FLJ20315
539	A8964	AI091459	FLJ20489	Hypothetical protein FLJ20489
540	D5189	BX101094	FLJ21128	Hypothetical protein FLJ21128
541	F3313	AK025164	FLJ21511	Hypothetical protein FLJ21511
542	B1006	R34577	FLJ21657	Hypothetical protein FLJ21657
543	B5905	T83961	FLJ21986	Hypothetical protein FLJ21986

544	B1851	AA032154	FLJ22655	Hypothetical protein FLJ22655
545	A8061	AA808804	FLJ23469	Hypothetical protein FLJ23469
546	B8353	AY358845	FLJ31166	Hypothetical protein FLJ31166
547	B6208	AA723586	FLJ31951	Hypothetical protein FLJ31951
548	D4414	AA994364	FLJ32421	Hypothetical protein FLJ32421
549	B7458N	BC068446	FLJ34658	Hypothetical protein FLJ34658
550	F6944	AL137326	FLJ37478	Hypothetical protein FLJ37478
551	D5971	AI912733	FLJ40125	Hypothetical protein FLJ40125
552	B4545	AI359551	FLJ90119	Hypothetical protein FLJ90119
553	A1098	AF000560	LOC126208	Hypothetical protein LOC126208
554	C7585	AK095271	LOC128977	Hypothetical protein LOC128977
555	C4878	BC040176	LOC130576	Hypothetical protein LOC130576
556	C9677	AL832661	LOC143381	Hypothetical protein LOC143381
557	D5601	AK056793	LOC145694	Hypothetical protein LOC145694
558	B0277	AA747835	LOC150568	Hypothetical protein LOC150568
559	D9407	CR749484	LOC152519	Hypothetical protein LOC152519
560	C8269	BC060866	LOC163782	Hypothetical protein LOC163782
561	C1869	BC046365	LOC253012	Hypothetical protein LOC253012
562	A6532	AY358336	LOC255743	Hypothetical protein LOC255743
563	B3517	AK097190	LOC285286	Hypothetical protein LOC285286
564	C9503	AA621124	LOC338773	Hypothetical protein LOC338773
565	C9551	AL133596	LOC339692	Hypothetical protein LOC339692
566	D3168	AA776749	LOC57821	Hypothetical protein LOC57821
567	D1155	AY358100	MGC11324	Hypothetical protein MGC11324
568	B1230	AA703185	MGC24039	Hypothetical protein MGC24039
569	D3691	R49126	MGC24039	Hypothetical protein MGC24039
570	B3759	AF067420	MGC27165	Hypothetical protein MGC27165
571	C1982	AI076840	MGC33926	Hypothetical protein MGC33926
572	B0979	BC030666	MGC33993	Hypothetical protein MGC33993
573	F0655	AK026196	MGC4172	Hypothetical protein MGC4172
574	B0742	BX648671	MGC61571	Hypothetical protein MGC61571
575	C0400	BC021290	IMP-2	IGF-II mRNA-binding protein 2
576	A3044	BC075840	IGHG1	Immunoglobulin heavy constant gamma 1 (G1m marker)
577	B1676	BC025985	IGHG4	Immunoglobulin heavy constant gamma 4 (G4m marker)
578	A1032	M87790	IGLC1	Immunoglobulin lambda constant 1 (Mcg marker)
579	A2442	AK074668	ISLR	Immunoglobulin superfamily containing leucine-rich repeat
580	C7503	N90724	IGSF4	Immunoglobulin superfamily, member 4
581	A0560N	NM_000618	IGF1	Insulin-like growth factor 1 (somatomedin C)
582	A0160N	CR621807	IGFBP1	Insulin-like growth factor binding protein 1
583	A1034	BC004312	IGFBP2	Insulin-like growth factor binding protein 2, 36kDa
584	A4474	AF047433	ITGB4BP	Integrin beta 4 binding protein

585	B3893	AY549722	ITLN1	Intelectin 1 (galactofuranose binding)
586	A3829	NM_002182	IL1RAP	Interleukin 1 receptor accessory protein
587	A3037	BC030975	IL1RL1	Interleukin 1 receptor-like 1
588	B7877	AA056734	IQSEC3	IQ motif and Sec7 domain 3
589	A9295	AY335938	IRX1	Iroquois homeobox protein 1
590	F8485	AW452669	IARS	Isoleucine-tRNA synthetase
591	A2888	BI759204	KLK1	Kallikrein 1, renal/pancreas/salivary
592	A3135N	X05332	KLK3	Kallikrein 3, (prostate specific antigen)
593	A3211	M13143	KLKB1	Kallikrein B, plasma (Fletcher factor) 1
594	A6092	AF208070	KLHL3	Kelch-like 3 (Drosophila)
595	B4062	X14640	KRT13	Keratin 13
596	C2083	NM_002277	KRTHA1	Keratin, hair, acidic, 1
597	D0488	AA405685	UHSKerB	Keratin, ultrahigh sulfur, B
598	A9635	BU621583	KIAA0056	KIAA0056 protein
599	B6832	BC002378	KIAA0652	KIAA0652 gene product
600	A5953	N24235	KIAA0789	KIAA0789 gene product
601	C9575	XM_032571	KIAA0888	KIAA0888 protein
602	A8875	R56087	KIAA0984	KIAA0984 protein
603	B8035	AL834240	KIAA1576	KIAA1576 protein
604	C6986	NM_020946	KIAA1608	KIAA1608
605	B1759	AA629743	KIAA1712	KIAA1712
606	B8308	NM_001001936	KIAA1914	KIAA1914
607	B4896	AK124139	KIAA1970	KIAA1970 protein
608	D6871	AB062483	KIF18A	Kinesin family member 18A
609	D6309	BU608866	KIF5A	Kinesin family member 5A
610	C8249	NM_000893	KNG1	Kininogen 1
611	A0005N	NM_153683	KL	Klotho
612	B6617	NM_153486	LDHD	Lactate dehydrogenase D
613	A3061	U07643	LTF	Lactotransferrin
614	A0227	NM_032737	LMNB2	Lamin B2
615	A0184	NM_000426	LAMA2	Laminin, alpha 2 (merosin, congenital muscular dystrophy)
616	A0084	BC075838	LAMB3	Laminin, beta 3
617	C6140	NM_007356	LAMB4	Laminin, beta 4
618	B4847	AA490011	LTBP1	Latent transforming growth factor beta binding protein 1
619	A4381N	U81523	LEFTY2	Left-right determination factor 2
620	F0288	BC080187	LMOD1	Leiomodin 1 (smooth muscle)
621	B6844	CR627284	LEPR	Leptin receptor
622	B6115N	AF097431	LEPRE1	Leucine proline-enriched proteoglycan (leprecan) 1
623	B6180	AF052098	LGI2	Leucine-rich repeat LGI family, member 2
624	F0967	AB006000	LECT1	Leukocyte cell derived chemotaxin 1
625	A1779N	AF025534	LILRB5	Leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 5
626	A6566	AK126536	TSK	Likely ortholog of chicken tsukushi

627	C4755	Z30137	LDB3	LIM domain binding 3
628	A6665	AW450890	LMO3	LIM domain only 3 (rhombotin-like 2)
629	B3695	BC017312	MGC3047	Limitrin
630	D3586	BC041168	LCN12	Lipocalin 12
631	A3736	NM_005577	LPA	Lipoprotein, Lp(a)
632	A8193	AA975742	LRP16	LRP16 protein
633	D8515	CR591759	LUM	Lumican
634	C7457	W44613	LY6K	Lymphocyte antigen 6 complex, locus K
635	A0654	BC000458	MAL	Mal, T-cell differentiation protein
636	F3821	AL117612	MAL2	Mal, T-cell differentiation protein 2
637	B1723	AA634140	ME2	Malic enzyme 2, NAD(+)-dependent, mitochondrial
638	E0706	AW298180	MMP7	Matrix metalloproteinase 7 (matrilysin, uterine)
639	C8140	BC006093	MMP9	Matrix metalloproteinase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)
640	A7972	NM_170677	MEIS2	Meis1, myeloid ecotropic viral integration site 1 homolog 2 (mouse)
641	A6122	AB040529	MAGED4	Melanoma antigen, family D, 4
642	F0049	NM_007289	MME	Membrane metallo-endopeptidase (neutral endopeptidase, enkephalinase, CALLA, CD10)
643	A2000	BC014564	MEST	Mesoderm specific transcript homolog (mouse)
644	C8057	N70019	MT1E	Metallothionein 1E (functional)
645	A6757	T95199	MT1F	Metallothionein 1F (functional)
646	B2396N	N39341	MT1G	Metallothionein 1G
647	A2819	NM_005951	MT1H	Metallothionein 1H
648	A4284	BM458221	MT1X	Metallothionein 1X
649	A0450	NM_005953	MT2A	Metallothionein 2A
650	B9198	AK123132	MSRA	Methionine sulfoxide reductase A
651	C1412	BX648776	LOC253827	Methionine sulfoxide reductase B3
652	A1423	L38486	MFAP4	Microfibrillar-associated protein 4
653	D9341	AI823969	MGST1	Microsomal glutathione S-transferase 1
654	A2162	X91148	MTP	Microsomal triglyceride transfer protein (large polypeptide, 88kDa)
655	D8609	NM_033063	MAP6	Microtubule-associated protein 6
656	A4705	NM_005928	MFGE8	Milk fat globule-EGF factor 8 protein
657	D0470	BC011873	MTRF1L	Mitochondrial translational release factor 1-like
658	A1558N	U66464	MAP4K1	Mitogen-activated protein kinase kinase kinase 1
659	B4246	AF131821	MGLL	Monoglyceride lipase
660	F3349	AL109706		MRNA full length insert cDNA clone EUROIMAGE 362430
661	F2702	AL049990		MRNA; cDNA DKFZp564G112 (from clone DKFZp564G112)

662	A3553	J05581	MUC1	Mucin 1, transmembrane
663	A0720N	L21998	MUC2	Mucin 2, intestinal/tracheal
664	B5338N	AA757932	MINPP1	Multiple inositol polyphosphate histidine phosphatase, 1
665	C2258	AI093859	MLL5	Myeloid/lymphoid or mixed-lineage leukemia 5 (trithorax homolog, Drosophila)
666	A1886	BC029261	MYOC	Myocilin, trabecular meshwork inducible glucocorticoid response
667	D4984	AW977610	MYO1E	Myosin IE
668	A3489	NM_000260	MYO7A	Myosin VIIA (Usher syndrome 1B (autosomal recessive, severe))
669	A3296	M24122	MYL3	Myosin, light polypeptide 3, alkali; ventricular, skeletal, slow
670	A4641	J02854	MYL9	Myosin, light polypeptide 9, regulatory
671	B2506	AL834231	MTPN	Myotrophin
672	B6305	H03606	NPL	N-acetylneuraminate pyruvate lyase (dihydrodipicolinate synthase)
673	B1531	BC063304	NPR1	Natriuretic peptide receptor A/guanylate cyclase A (atrionatriuretic peptide receptor A)
674	A1456	M59305	NPR3	Natriuretic peptide receptor C/guanylate cyclase C (atrionatriuretic peptide receptor C)
675	C7971	AA195950	NRAP	Nebulin-related anchoring protein
676	A4831	D83017	NELL1	NEL-like 1 (chicken)
677	B3699	NM_006617	NES	Nestin
678	F1252	AB023193	NTNG1	Netrin G1
679	A6063	NM_001005388	NFASC	Neurofascin
680	A5027	U89165	NRGN	Neurogranin (protein kinase C substrate, RC3)
681	B9126	NM_020795	NLGN2	Neuroigin 2
682	B5787	NM_182964	NAV2	Neuron navigator 2
683	C9201	NM_000909	NPY1R	Neuropeptide Y receptor Y1
684	B0020	NM_001007156	NTRK3	Neurotrophic tyrosine kinase, receptor, type 3
685	B0149	AF052090	NNT	Nicotinamide nucleotide transhydrogenase
686	C0472	BQ044958	NRK	Nik related kinase
687	C5950	CF146489	NKX3-1	NK3 transcription factor related, locus 1 (Drosophila)
688	A4830	NM_004557	NOTCH4	Notch homolog 4 (Drosophila)
689	B7552	NM_016143	NSFL1C	NSFL1 (p97) cofactor (p47)
690	A0875	L13740	NR4A1	Nuclear receptor subfamily 4, group A, member 1
691	A0805	NM_002505	NFYA	Nuclear transcription factor Y, alpha
692	B6820	XM_497078	NUP188	Nucleoporin 188kDa
693	C7256	NM_021963	NAP1L2	Nucleosome assembly protein 1-like 2

694	B4244	AK128514	NDEL1	NudE nuclear distribution gene E homolog like 1 (A. nidulans)
695	B5969	AF191652	NUDT4	Nudix (nucleoside diphosphate linked moiety X)-type motif 4
696	D4979	AW977053	CAS1	O-acetyltransferase
697	F3395	AB032953	ODZ2	Odz, odd Oz/ten-m homolog 2 (Drosophila)
698	B4288	AK092766	OLFML3	Olfactomedin-like 3
699	B0997	BQ000754	OR7E47P	Olfactory receptor, family 7, subfamily E, member 47 pseudogene
700	A0472	BG569025	ORM1	Orosomucoid 1
701	F2316	AB033090	PAK7	P21(CDKN1A)-activated kinase 7
702	A1275	AF020543	PPT2	Palmitoyl-protein thioesterase 2
703	A3340	M93284	PNLIPRP2	Pancreatic lipase-related protein 2
704	A6261	L02867	HUMPPA	Paraneoplastic antigen
705	A3085	L04308	PTHRI	Parathyroid hormone receptor 1
706	A2970	BM687969	PVALB	Parvalbumin
707	A0709N	W20074	PNPLA4	Patatin-like phospholipase domain containing 4
708	A0574	NM_033018	PCTK1	PCTAIRE protein kinase 1
709	B7105	AK055782	PDLIM2	PDZ and LIM domain 2 (mystique)
710	C7651	BM560961	PDLIM3	PDZ and LIM domain 3
711	F3501	AK021708	PDZRN3	PDZ domain containing RING finger 3
712	A3242N	BC039733	PTX3	Pentaxin-related gene, rapidly induced by IL-1 beta
713	B2142	NM_000277	PAH	Phenylalanine hydroxylase
714	B7947	NM_080672	PHACTR3	Phosphatase and actin regulator 3
715	F5803	AL359590	PGS1	Phosphatidylglycerophosphate synthase
716	A4324	NM_004910	PITPNM1	Phosphatidylinositol transfer protein, membrane-associated 1
717	D6890	AI003542	PDE11A	Phosphodiesterase 11A
718	B8389	AK095203	PDE3A	Phosphodiesterase 3A, cGMP-inhibited
719	A3055	AK095384	PDE4C	Phosphodiesterase 4C, cAMP-specific (phosphodiesterase E1 dunce homolog, Drosophila)
720	A2307	NM_004563	PCK2	Phosphoenolpyruvate carboxykinase 2 (mitochondrial)
721	A2755	BC011262	PHGDH	Phosphoglycerate dehydrogenase
722	A1157N	NM_002667	PLN	Phospholamban
723	A3605	U17033	PLA2R1	Phospholipase A2 receptor 1, 180kDa
724	B6373	BX423161	LHPP	Phospholysine phosphohistidine inorganic pyrophosphate phosphatase
725	B4239	NM_058179	PSAT1	Phosphoserine aminotransferase 1
726	A1014	NM_000301	PLG	Plasminogen
727	F0834	NM_000930	PLAT	Plasminogen activator, tissue
728	B4086	NM_006206	PDGFRA	Platelet-derived growth factor receptor, alpha polypeptide

729	D6878	AI002365	PDGFRB	Platelet-derived growth factor receptor, beta polypeptide
730	A6691	NM_021200	PLEKHB1	Pleckstrin homology domain containing, family B (evectins) member 1
731	A4597	U97519	PODXL	Podocalyxin-like
732	A3208	D29013	POLB	Polymerase (DNA directed), beta
733	F6101	AW978378	KCNN3	Potassium intermediate/small conductance calcium-activated channel, subfamily N, member 3
734	A3070N	BC063109	KCNJ1	Potassium inwardly-rectifying channel, subfamily J, member 1
735	A6181	NM_002241	KCNJ10	Potassium inwardly-rectifying channel, subfamily J, member 10
736	D5416	AF209747	KCNMB2	Potassium large conductance calcium-activated channel, subfamily M, beta member 2
737	A3447N	M55513	KCNA5	Potassium voltage-gated channel, shaker-related subfamily, member 5
738	D3143	AA767342	KCNC3	Potassium voltage-gated channel, Shaw-related subfamily, member 3
739	B0838	NM_002581	PAPPA	Pregnancy-associated plasma protein A, pappalysin 1
740	C8046	NM_002864	PZP	Pregnancy-zone protein
741	C8718	AA206141	PRICKLE1	Prickle-like 1 (Drosophila)
742	D3149	AF338109	PACAP	Proapoptotic caspase adaptor protein
743	F0920	AF098269	PCOLCE2	Procollagen C-endopeptidase enhancer 2
744	D8901	AI262277	PFN2	Profilin 2
745	A1748	U29089	PRELP	Proline arginine-rich end leucine-rich repeat protein
746	A3905	AF117225	PROM1	Prominin 1
747	A3258	U19487	PTGER2	Prostaglandin E receptor 2 (subtype EP2), 53kDa
748	B1655	AA455926	PTGER3	Prostaglandin E receptor 3 (subtype EP3)
749	F0018	NM_000963	PTGS2	Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)
750	C8119	NM_002775	PRSS11	Protease, serine, 11 (IGF binding)
751	A3291	BM805032	PRSS2	Protease, serine, 2 (trypsin 2)
752	B2641	BX094063	PIN4	Protein (peptidyl-prolyl cis/trans isomerase) NIMA-interacting, 4 (parvulin)
753	A9668	AA759203	XTP7	Protein 7 transactivated by hepatitis B virus X antigen (HBxAg)
754	A1816N	BC075800	PRKAR2B	Protein kinase, cAMP-dependent, regulatory, type II, beta
755	A8588	BM683764	PRKWINK4	Protein kinase, lysine deficient 4
756	D8862	NM_032105	PPP1R12B	Protein phosphatase 1, regulatory (inhibitor) subunit 12B

757	B7480	AF407165	PPP1R14C	Protein phosphatase 1, regulatory (inhibitor) subunit 14C
758	A2536	U48707	PPP1R1A	Protein phosphatase 1, regulatory (inhibitor) subunit 1A
759	B6647	XM_350880	PPM1H	Protein phosphatase 1H (PP2C domain containing)
760	A6385	AA663484	PPP2R2B	Protein phosphatase 2 (formerly 2A), regulatory subunit B (PR 52), beta isoform
761	C9379	AL049338	PTPRD	Protein tyrosine phosphatase, receptor type, D
762	A0860	M55671	PROZ	Protein Z, vitamin K-dependent plasma glycoprotein
763	B2346	AA669023	PCDH9	Protocadherin 9
764	B9131	AA626775	PCDHA6	Protocadherin alpha 6
765	A2624	NM_002563	P2RY1	Purinergic receptor P2Y, G-protein coupled, 1
766	A6781	CB529051	G0S2	Putative lymphocyte G0/G1 switch gene
767	A6574N	NM_032932	RAB11FIP4	RAB11 family interacting protein 4 (class II)
768	B5608N	BC011782	RAB3A	RAB3A, member RAS oncogene family
769	A1257	BC006992	RAD51AP1	RAD51 associated protein 1
770	D5783	BX092660	RORA	RAR-related orphan receptor A
771	B9024	BC042688	RASD1	RAS, dexamethasone-induced 1
772	A6598	BM677885	RASL11B	RAS-like, family 11, member B
773	A2202	AJ001016	RAMP3	Receptor (calcitonin) activity modifying protein 3
774	B3911	BC038457	DKFZP586H2123	Regeneration associated muscle protease
775	A6666	BU728456	RIMS2	Regulating synaptic membrane exocytosis 2
776	A2739	AF073920	RGS6	Regulator of G-protein signalling 6
777	B6777	D31888	RCOR1	REST corepressor 1
778	B8016	AA528243	RTN4RL1	Reticulon 4 receptor-like 1
779	A3116	M38258	RARG	Retinoic acid receptor, gamma
780	B9056	AF433662	ARHGEF3	Rho guanine nucleotide exchange factor (GEF) 3
781	D5408	AA954092	RHBDL2	Rhomboid, veinlet-like 2 (Drosophila)
782	C6829	AB018260	RHOBTB2	Rho-related BTB domain containing 2
783	B2588	H84909	MGC34774	Ribosomal protein L13A-like
784	B3834	AB033040	RNF150	Ring finger protein 150
785	D3461	BC028373	RG9MTD2	RNA (guanine-9-) methyltransferase domain containing 2
786	B2780	CK822142	RBM5	RNA binding motif protein 5
787	A7828	NM_017495	RNPC1	RNA-binding region (RNP1, RRM) containing 1
788	F2307	AF010236	SGCD	Sarcoglycan, delta (35kDa dystrophin-associated glycoprotein)

789	B9057	AF361494	SOSTDC1	Sclerostin domain containing 1
790	A4424	NM_003966	SEMA5A	Sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5A
791	F1746	AF389426	SEMA6D	Sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6D
792	F1976	AB029496	LOC56920	Semaphorin sem2
793	C8190	BX248009	SERPINA4	Serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 4
794	A3024	NM_001756	SERPINA6	Serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 6
795	D2315	AY358700	SERPINA9	Serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 9
796	A7998	AA521405	SPAP1	SH2 domain containing phosphatase anchor protein 1
797	F3839	AF131754	SH3BGRL2	SH3 domain binding glutamic acid-rich protein like 2
798	A0917	AF036268	SH3GL2	SH3-domain GRB2-like 2
799	B1084	AA682533	SGOL2	Shugoshin-like 2 (S. pombe)
800	D0657	AB058780	SIAT2	Sialyltransferase 2 (monosialoganglioside sialyltransferase)
801	F3433	L13972	SIAT4A	Sialyltransferase 4A (beta-galactoside alpha-2,3-sialyltransferase)
802	C8638	BC059363	SIAT7C	Sialyltransferase 7 ((alpha-N-acetylneuraminyl-2,3-beta-galactosyl-1,3)-N-acetyl galactosaminide alpha-2,6-sialyltransferase) C
803	B3997	AY044437	SFXN5	Sideroflexin 5
804	A8433	NM_005843	STAM2	Signal transducing adaptor molecule (SH3 domain and ITAM motif) 2
805	B9455	BQ447358		Similar to B230208J24Rik protein
806	D5870	AA972840		Similar to hypothetical protein
807	B8401	BU743008		Similar to implantation-associated protein
808	C1732	AK091965	MGC15937	Similar to RIKEN cDNA 0610008P16 gene
809	B4760N	AA721101	LOC92162	Similar to RIKEN cDNA 2600017H02
810	D6250	BX108635		Similar to RIKEN cDNA C330003B14
811	B7600	BC007956	DKFZp727A071	Similar to tRNA synthetase class II
812	A9462	BM474898	SLIT2	Slit homolog 2 (Drosophila)
813	A9914	R61832	SNRPB2	Small nuclear ribonucleoprotein polypeptide B"

814	D3947	AI742927	SHPRH	SNF2 histone linker PHD RING helicase
815	A4875	NM_000336	SCNN1B	Sodium channel, nonvoltage-gated 1, beta (Liddle syndrome)
816	A1533	U38254	SCNN1D	Sodium channel, nonvoltage-gated 1, delta
817	A4701	U53347	SLC1A5	Solute carrier family 1 (neutral amino acid transporter), member 5
818	A1546	BX647484	SLC12A1	Solute carrier family 12 (sodium/potassium/chloride transporters), member 1
819	A3470	NM_003984	SLC13A2	Solute carrier family 13 (sodium-dependent dicarboxylate transporter), member 2
820	A7358N	NM_022829	SLC13A3	Solute carrier family 13 (sodium-dependent dicarboxylate transporter), member 3
821	A6918	BQ183489	SLC14A1	Solute carrier family 14 (urea transporter), member 1 (Kidd blood group)
822	A3628	U59299	SLC16A5	Solute carrier family 16 (monocarboxylic acid transporters), member 5
823	C0659	AK123243	SLC22A8	Solute carrier family 22 (organic anion transporter), member 8
824	A5748N	AB067483	SLC25A25	Solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 25
825	A2189	NM_000112	SLC26A2	Solute carrier family 26 (sulfate transporter), member 2
826	A3911	AF030880	SLC26A4	Solute carrier family 26, member 4
827	B1020	AF064255	SLC27A5	Solute carrier family 27 (fatty acid transporter), member 5
828	D6611	AI000771	SLC30A5	Solute carrier family 30 (zinc transporter), member 5
829	A7155	X77737	SLC4A1	Solute carrier family 4, anion exchanger, member 1 (erythrocyte membrane protein band 3, Diego blood group)
830	A4675	M95549	SLC5A2	Solute carrier family 5 (sodium/glucose cotransporter), member 2
831	A8796	AB020532	SLC7A7	Solute carrier family 7 (cationic amino acid transporter, y ⁺ system), member 7
832	B8005	NM_012244	SLC7A8	Solute carrier family 7 (cationic amino acid transporter, y ⁺ system), member 8
833	D8620	NM_003048	SLC9A2	Solute carrier family 9 (sodium/hydrogen exchanger), isoform 2
834	C1402	NM_198956	SP8	Sp8 transcription factor
835	A6689	BU741863	SPOCK	Sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican)

836	C8088	D87465	SPOCK2	Sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 2
837	D6561	AA994999	SYNE2	Spectrin repeat containing, nuclear envelope 2
838	B2953	BG182727	SPDY1	Speedy homolog 1 (<i>Drosophila</i>)
839	A1630N	NM_003117	SPAM1	Sperm adhesion molecule 1 (PH-20 hyaluronidase, zona pellucida binding)
840	D3872	AK095036	SPAG16	Sperm associated antigen 16
841	A6909	NM_018667	SMPD3	Sphingomyelin phosphodiesterase 3, neutral membrane (neutral sphingomyelinase II)
842	D3239	AA112743	SGPL1	Sphingosine-1-phosphate lyase 1
843	F6820	CR749297	SKIP	SPHK1 (sphingosine kinase type 1) interacting protein
844	B7559	AB073386	SGEF	Src homology 3 domain-containing guanine nucleotide exchange factor
845	D7386	BC071780	UNQ846	SRSR846
846	A5462	AF389338	SCD4	Stearoyl-CoA desaturase 4
847	B7313	AF059203	SOAT2	Sterol O-acyltransferase 2
848	B8432	AF352729	FLJ12541	Stimulated by retinoic acid gene 6 homolog (mouse)
849	A0042	AF029082	SFN	Stratifin
850	D7518	AA007591	SENP5	SUMO1/sentrin specific protease 5
851	D3685	AI911481	SUV420H2	Suppressor of variegation 4-20 homolog 2 (<i>Drosophila</i>)
852	A6670	AB018279	SV2A	Synaptic vesicle glycoprotein 2A
853	A4814	NM_004209	SYNGR3	Synaptogyrin 3
854	B3942	AA191573	SYNJ2	Synaptojanin 2
855	A5690	AB028952	SYNPO	Synaptopodin
856	B8366	AI342255	SYNPO2	Synaptopodin 2
857	C6207	AB188489	SYNPO2L	Synaptopodin 2-like
858	D3671	AA247642	SNAP23	Synaptosomal-associated protein, 23kDa
859	D0385	AK125106	SYTL5	Synaptotagmin-like 5
860	D3336	AA524150	SDC4	Syndecan 4 (amphiglycan, ryudocan)
861	A2780N	NM_003182	TAC1	Tachykinin, precursor 1 (substance K, substance P, neurokinin 1, neurokinin 2, neuromedin L, neurokinin alpha, neuropeptide K, neuropeptide gamma)
862	A3465	M74558	SIL	TAL1 (SCL) interrupting locus
863	B3047	AI361002	TBC1D17	TBC1 domain family, member 17
864	A1332	AF054910	TEKT2	Tektin 2 (testicular)
865	A1151N	M55618	TNC	Tenascin C (hexabrachion)
866	B3377	AA807166	TSGA10	Testis specific, 10
867	A4895	BC007290	TSPAN-1	Tetraspan 1
868	A2869	AF054839	TSPAN-2	Tetraspan 2
869	D5273	AA862436	THEA	Thioesterase, adipose associated
870	A4717	BC050383	TMPO	Thymopoietin
871	A4595	U96131	TRIP13	Thyroid hormone receptor interactor 13

872	B4649	BM996064	TJP3	Tight junction protein 3 (zona occludens 3)
873	A1583N	U91963	TLL1	Tolloid-like 1
874	A0461	NM_001068	TOP2B	Topoisomerase (DNA) II beta 180kDa
875	B6507	AI929792		Transcribed locus
876	C0437	H04828		Transcribed locus
877	C7770	AA165165		Transcribed locus
878	B9634	BX110180		Transcribed locus
879	A9694	AA719352		Transcribed locus
880	D0852	AA429665		Transcribed locus
881	B9979	CF595488		Transcribed locus
882	C6460	W96022		Transcribed locus
883	B7326	Z98489		Transcribed locus
884	B5115	T50062		Transcribed locus
885	B1454	AI820955		Transcribed locus
886	D5241	AA938971		Transcribed locus
887	A9673	AA609323		Transcribed locus
888	C7747	CA314541		Transcribed locus
889	D4261	AA877216		Transcribed locus
890	D3102	BX088780		Transcribed locus
891	D5026	AI822035		Transcribed locus
892	D7115	BX100620		Transcribed locus
893	D3291	AA766649		Transcribed locus
894	D3672	AA809349		Transcribed locus
895	D5414	BX114748		Transcribed locus
896	B2956	AA034053		Transcribed locus
897	C4551	N64389		Transcribed locus
898	D4230	BF513800		Transcribed locus
899	D3842	AA830448		Transcribed locus
900	B3515	AA741001		Transcribed locus
901	B2297	AW197616		Transcribed locus
902	B9513	H18456		Transcribed locus
903	B1956	AA663781		Transcribed locus
904	B2436	AI220328		Transcribed locus
905	D3092	BX088758		Transcribed locus
906	D5402	BX107944		Transcribed locus
907	A7973	BU738725		Transcribed locus
908	D5164	AA935766		Transcribed locus, moderately similar to XP_372039.2 similar to hypothetical protein (L1H 3 region) - human [Homo sapiens]
909	C4182	BU681491		Transcribed locus, moderately similar to XP_373053.2 similar to Microneme antigen [Homo sapiens]
910	B2547	BM725055		Transcribed locus, moderately similar to XP_498452.1 hypothetical gene supported by NM_173697 [Homo sapiens]

911	D6981	AW190289		Transcribed locus, moderately similar to XP_517655.1 similar to KIAA0825 protein [Pan troglodytes]
912	F7334	AW189299		Transcribed locus, strongly similar to NP_062555.1 carboxypeptidase X (M14 family) [Homo sapiens]
913	D5994	AA975173		Transcribed locus, strongly similar to XP_512017.1 hypothetical protein XP_512017 [Pan troglodytes]
914	D5731	AA970389		Transcribed locus, strongly similar to XP_529814.1 LOC454389 [Pan troglodytes]
915	B2994	BQ185194		Transcribed locus, weakly similar to NP_055301.1 neuronal thread protein AD7c-NTP [Homo sapiens]
916	D0634	AA417198		Transcribed locus, weakly similar to NP_060190.1 signal-transducing adaptor protein-2 [Homo sapiens]
917	B1722	AW188090		Transcribed locus, weakly similar to NP_080901.1 RIKEN cDNA 1700067P10 [Mus musculus]
918	A6940	AW135188		Transcribed locus, weakly similar to XP_509271.1 similar to RIKEN cDNA 4921537D05 [Pan troglodytes]
919	B1721	AA634326	TCF20	Transcription factor 20 (AR1)
920	A0038N	W73825	TCF21	Transcription factor 21
921	A7088	AB031046	TCF7L1	Transcription factor 7-like 1 (T-cell specific, HMG-box)
922	D2858	AA777755	TFCP2	Transcription factor CP2
923	F7399	AI928242	TFCP2L1	Transcription factor CP2-like 1
924	B7571N	BU619137	TGFBR3	Transforming growth factor, beta receptor III (betaglycan, 300kDa)
925	C4066	NM_013259	TAGLN3	Transgelin 3
926	D6474	NM_001007470	TRPM3	Transient receptor potential cation channel, subfamily M, member 3
927	A4792	NM_005723	TM4SF9	Transmembrane 4 superfamily member 9
928	A8963	N51413	TMAP1	Transmembrane anchor protein 1
929	C8474	N32157	TMC3	Transmembrane channel-like 3
930	C8660	AA196034	MGC3169	Transmembrane protein 38A
931	A6233	BM805669	TTR	Transthyretin (prealbumin, amyloidosis type I)
932	F6116	BC030244	TNNC1	Troponin C, slow
933	A7154	X74819	TNNT2	Troponin T2, cardiac
934	F0121	AF089854	TU3A	TU3A protein
935	B0708	BF673741	TUSC3	Tumor suppressor candidate 3
936	A7475	D17517	TYRO3	TYRO3 protein tyrosine kinase
937	A0930	X51420	TYRP1	Tyrosinase-related protein 1

938	A2978	X04741	UCHL1	Ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase)
939	B8223	BC041366	USP2	Ubiquitin specific protease 2
940	D5995	AA995921	UPP2	Uridine phosphorylase 2
941	A3089	AK091961	UMOD	Uromodulin (uromucoid, Tamm-Horsfall glycoprotein)
942	A0878	L13288	VIPR1	Vasoactive intestinal peptide receptor 1
943	A0114N	L07868	ERBB4	V-erb-a erythroblastic leukemia viral oncogene homolog 4 (avian)
944	A0141	BC002712	MYCN	V-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)
945	A0171	NM_002881	RALB	V-ral simian leukemia viral oncogene homolog B (ras related; GTP binding protein)
946	B0547	AA541526	WDFY3	WD repeat and FYVE domain containing 3
947	C6064	BX647751	WDR20	WD repeat domain 20
948	F0613	NM_005828	HAN11	WD-repeat protein
949	D6252	AA976712	LOC387914	WGAR9166
950	A6672	H27000	WBSCR17	Williams-Beuren syndrome chromosome region 17
951	A0342	X51630	WT1	Wilms tumor 1
952	B0955	AI339506	WAC	WW domain containing adaptor with coiled-coil
953	B4291	AK025198	XIST	X (inactive)-specific transcript
954	D6140	XM_033370	ZFHX2	Zinc finger homeobox 2
955	B7727	BC042636	ZNF134	Zinc finger protein 134 (clone pHZ-15)
956	A0881N	Z21707	ZNF197	Zinc finger protein 197
957	B6682N	AA180985	FLJ34222	Zinc finger protein 229
958	A9121	AB002388	ZNF536	Zinc finger protein 536
959	D3738	AA854756	ZYX	Zyxin
960	B2148	M61900		Homo sapiens prostaglandin D synthase precursor
961	C0556	H11294		ym14c11.s1 Soares infant brain 1NIB Homo sapiens cDNA clone IMAGE:48089 3'
962	B3371	AA831240		oc63h01.s1 NCI_CGAP_GCB1 Homo sapiens cDNA clone IMAGE:1354417 3'
963	F2351	AL162042		Homo sapiens mRNA; cDNA DKFZp761L1212 (from clone DKFZp761L1212)
964	C7105	R50993		yg63f02.s1 Soares infant brain 1NIB Homo sapiens cDNA clone IMAGE:37373 3'
965	E2104	AK000414		Homo sapiens cDNA FLJ20407 fis, clone KAT01658

966	B0267	R78436	yi82e09.s1 Soares placenta Nb2HP Homo sapiens cDNA clone IMAGE:145768 3'
967	A9975	AA621665	af54d01.s1 Soares_total_fetus_Nb2HF8_9w Homo sapiens cDNA clone IMAGE:1035457 3' similar to contains element MER7 repetitive element
968	D5032	AA931406	om98c07.s1 NCI_CGAP_Kid3 Homo sapiens cDNA clone IMAGE:1555212 3'
969	B1012	AW967446	EST379521 MAGE resequences, MAGJ Homo sapiens cDNA, mRNA sequence
970	B2883	AA179211	zp46d09.s1 Stratagene HeLa cell s3 937216 Homo sapiens cDNA clone IMAGE:612497 3'
971	A6779	AF191687	Homo sapiens alanine-glyoxylate aminotransferase homolog (TLH6)
972	D7380	AI016895	ou31c07.x1 Soares_NFL_T_GBC_S1 Homo sapiens cDNA clone IMAGE:1627884 3'

Verification of selected genes by semi-quantitative RT-PCR and Northern blots analyses

To confirm the reliability of the expression data obtained by cDNA microarray analysis, semi-quantitative RT-PCR experiments were performed for 21 genes (Accession No. NM_018092, AA632745, NM_007250, W86513, BC077726, AA156409, W57613, CR749811, AW972553, AL832896, AK021778, AK026403, AK025204, NM_000677, AF070609, AI290343, AA442590, NM_000552, BC000234, BC034014 and NM_004567) that were significantly up-regulated in informative cases with clear cell RCC. Among these genes, 13 genes (Accession No. NM_018092, AA632745, NM_007250, W86513, BC077726, AA156409, W57613, CR749811, AW972553, AL832896, AK021778, AK026403 or AK025204) were confirmed to be up-regulated in four RCC cell lines, Caki-1, Caki-2, 786-O and A498, as compared to renal proximal tubule epithelial cell (RPTEC) as a normal control. Among them, eight genes were also up-regulated in RCC cell lines (Figure 2a). These results were highly concordant with those of the microarray analysis in the great majority of the tested cases. In particular, C6700 was confirmed to be up-regulated in three, A704, OS-RC-2 and TUHR14TKB of more eight cell lines, ACHN, 769-P, RXF-631L, TUHR10TKB, Caki-1, Caki-2, 786-O and A-498 as compared with RPTEC (Figure 2b).

To further examine the expression pattern of these candidate genes, Northern blot analyses ere performed with multiple-human tissues and RCC cell lines using each cDNA fragment as probes (see Material and Method). Among the candidate genes, C6700, designed

to Semaphorin 5B, SEMA5B (Genbank Accession; BC077726) was exclusively expressed in normal human tissues except fetal brain and fetal kidney (Figure 3 left panel). In addition, northern blot analyses were performed with RCC cell lines using cDNA fragments of C6700 as a probe (see Material and Method). Expression of C6700 was up-regulated in three of 11 RCC cell lines (Figure 3, right panel). Next, USCS database revealed that F5749, designed to ABI gene family, member 3 (*NESH*) binding protein (*ABI3BP*) (Genbank Accession; AK025204), is located at chromosome 3q12 with transcripts, and has a 3545 bases-transcript consisting of 11 exons and a 4533 bases-transcript consisting of 35 exons. Multiple northern blot analysis revealed that the 3545 bases-transcript was no expression in any kinds of normal human tissues using a specific probe (Figure 4a), while approximately 2.0, 4.5 and 7.5 kb transcripts were observed to be ubiquitously expressed in normal human tissues using common sequence of 3545 bases- and 4533 bases-transcript as a probe (Figure 4b). C8919, designed to LOC339977 similar to hypothetical protein MGC38937 (Genbank Accession; CR749811), is located at chromosome 4q11 with transcripts, 3348 bases in length consisting of 4 exons. The approximately 3.4kb transcript was very weakly expressed only in skeletal muscle with multiple northern blot analysis (Figure 5). B7032N, designed to PFKFB4 (6-phosphofructo-2-kinase/fructose-2, 6-biphosphatase 4) gene (GenBank Accession No. NM_004567) whose approximately 3.5kb PFKFB4 transcript was up-regulated in 2 of 11 bladder cancer cell lines, but not expressed in normal organs except testis and pancreas (Figure 6). B9320, designed to *FBXL16* (F-box and leucine-rich repeat protein 16) gene (GenBank Accession No. BC034014 for V1 and NM_153350 for V2) whose approximately 4 kb (V1) and 2.4kb (V2) of two FBXL16 transcripts were significantly up-regulated in 2 of 4 bladder cancer cell lines. The 2.4kb transcript was expressed in kidney and thyroid, whereas 4 kb transcript was not expressed in normal organs except brain, testis, spinal cord and pancreas (Figure 7).

To further examine the characterization of B7032N, the expression of B7032N and the sub-cellular localization of the B7032N gene product were examined in mammalian cells. Firstly, when plasmids expressing B7032N protein (pCAGGS-B7032N-HA) were transiently transfected into COS7 cells, western blot analysis revealed that exogenous B7032N was expressed as an expected size band in both 24 and 48 hours after transfection (Figure 10a). Furthermore, immunocytochemical staining showed that exogenous localized to the cytoplasm in all of transfected-cells (Figure 10b).

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To further examine the characterization of B9320, the expression of B9320 and the sub-cellular localization of the B9320 gene product were examined in mammalian cells. Firstly, when plasmids expressing B9320 protein (pCAGGS-B9320-HA) were transiently transfected into COS7 cells, western blot analysis revealed that exogenous B9320 was expressed as a single band in both 24 and 48 hours after transfection (Figure 13a). Furthermore, immunocytochemical staining showed that exogenous localized to the cytoplasm in all of transfected-cells (Figure 13b).

Genomic structure of C6700

To obtain the entire cDNA sequences of C6700, RT-PCR was performed using RCC cell line, OS-RC-2 or A704 as a template. C6700, designed Semaphorin 5B, SEMA5B, located on the chromosome 3p21.1.

C6700 has two different transcriptional variants consisting of 23 exons, corresponding to C6700V1 and C6700V2, respectively (Figure 8a). There were alternative variations in exon 1, 16, and 20 of V2, and the other remaining exons were common to both variants, generating a novel stop codon within last exon of V2, but stop codon of V1 is within exon 22. A novel exon consisting of 32 bp as exon 1 of V2 variant was generated. Exon 16 of V2 was 3bp longer than that of the V1 at the 3' end, and exon 20 of V2 was also 3bp longer than that of V1 at 5' end. In addition, exon 21 was 131 bp shorter than that of V2 at the 3' end. The full-length cDNA sequences of C6700V1 and C6700V2 variants consist of 4725 and 4494 nucleotides, respectively. The ORF of these variants start at within each exon 1. Eventually, V1 and V2 transcripts encode 1093 and 1152 amino acids, respectively. To further confirm the expression pattern of each variant in RCC cell line, semi-quantitative RT-PCR was performed using the primer sets recognized to each variant (see Figure 8b). As a result, it was discovered that the V2 variant was specifically overexpressed in RCC cells (Figure 8b). Accordingly, further functional analysis was performed for C6700V2.

Growth-inhibitory effects of small-interfering RNA (siRNA) designed to reduce expression of C6700, B7032N and B9320

To assess the growth-promoting role of C6700, B7032N and B9320, the expression of endogenous C6700, B7032N and B9320 was knocked down in the RCC line OS-RC-2, including cells that have shown the overexpression of C6700, for C6700, RXF-631L and A498, that have shown the overexpression of B7032N, for B7032N, and Caki-2 and A498 that have shown the overexpression of B9320, for B9320, by means of the mammalian vector-

based RNA interference (RNAi) technique (see Materials and Methods). Expression levels of C6700, B7032N and B9320 were examined by semi-quantitative RT-PCR experiments.

As shown in Figure 9a, C6700 specific siRNAs (si1, si6 and si-#2) suppressed expression of C6700, compared with four control siRNA constructs (psiU6BX-Luciferase, Scramble, EGFP and Mock). Among the siRNA constructs examined, si-#2 effectively reduced expression of SEMA5B mRNA compared with control siRNAs (si-Scramble and si-Mock) (Fig. 9a). Significant decreases in the number of colonies (Fig. 9c) and in the numbers of viable cells measured by MTT assay were observed for the cells treated with si-#2 (Fig. 9b). To exclude a possibility of off-target effect by SEMA5B-siRNA (si-#2), two forms of siRNA with 3-bp replacement were generated. It was discovered that neither suppressed SEMA5B expression in OS-RC-2 cells (data not shown). These findings suggest that C6700 has a significant function in the cell survival and/or growth of the RCC.

As shown in Figure 11, B7032N (-si#4) suppressed expression of this gene as compared with a control siRNA-construct (si-Scramble) (Figure 11a, 11b). Colony-formation and MTT assays using these siRNA constructs indicated that introduction of PFKFB4-si#4 suppressed growth of RXF-631L (Figure 11a) and A498 (Figure 11b) cells. To further confirm the growth promoting effect of B7032N, NIH3T3-derivative cells were established that stably expressed exogenous B7032N (B7032N-A, -B, -C and -D cells). Western-blot analysis indicated high level of exogenous B7032N protein in four derivative clones (Figure 12a). Subsequent MTT assays showed that four derivative cell lines, B7032N-A, -B, -C and -D cells, grew much faster than cells transfected with mock plasmid (Mock-A, -B and -C cells) (Figure 12b), indicating B7032N expression was likely to enhance cell growth. These findings imply an oncogenic role of B7032N for development of RCC.

As shown in Figure 14, B9320 (si#2) significantly suppressed expression of this gene as compared with a control siRNA-construct (si-Scramble) (Figure 14). Colony-formation and MTT assays using these siRNA constructs indicated that introduction of FBXL16-specific siRNA suppressed growth of Caki-2 (Figure 14a) and A498 (Figure 14b) cells. To further confirm the growth promoting effect of B9320, NIH3T3-derivative cells were established that stably expressed exogenous B9320 (FBXL16-1, -2, -3 and -4 cells). Western-blot analysis indicated high level of exogenous B9320 protein in four derivative clones (Figure 15a). Subsequent MTT assays showed that four derivative cell lines, FBXL16-1, -2, -3 and -4 cells, grew much faster than cells transfected with mock plasmid (Mock-1, -2 and -3 cells)

(Figure 15b), indicating B9320 expression was likely to enhance cell growth. These findings imply an oncogenic role of B9320 for development of RCC.

Table 6. Oligonucleotides sequences for small interfering RNA of *C6700*, *B7032N* and *9320*

	nucleotide sequence	SEQ ID NO
C6700-#1 sense	5'-CACCCCTACCTCTTCAGACTCAGCTTCAAGAGAGCTGAGTCTGAAGAGGTAG-3'	41
antisense	5'-AAAACCTACCTCTTCAGACTCAGCTCTCTTGAAGCTGAGTCTGAAGAGGTAG-3'	42
target	CTACCTCTTCAGACTCAGC (positions: V1;629-647, V2;502-520)	43
hairpin	CTACCTCTTCAGACTCAGCTTCAAGAGAGCTGAGTCTGAAGAGGTAG	44
C6700-#6 sense	5'-CACCCCATGTGAGCACTTGGATGTTCAAGAGACATCCAAGTGCTCACATGG-3'	45
antisense	5'-AAAACCATGTGAGCACTTGGATGTCTCTTGAACATCCAAGTGCTCACATGG-3'	46
target	CCATGTGAGCACTTGGATG (positions: V1;2055-2073, V2;1928-1946)	47
hairpin	CCATGTGAGCACTTGGATGTTCAAGAGACATCCAAGTGCTCATGG	48
C6700-#2 sense	5'-CACCGCAGCAACGTTGCAGCACATTCAAGAGATGTGCTGCAACGTTGCTGC-3'	79
antisense	5'-AAAAGCAGCAACGTTGCAGCACATCTCTTGAATGTGCTGCAACGTTGCTGC-3'	80
target	GCAGCAACGTTGCAGCACA (positions: V1;1931-1949, V2;1804-1822)	81
hairpin	GCAGCAACGTTGCAGCACATTCAAGAGATGTGCTGCAACGTTGCTGC	82
B7032N-#4 sense	5'-TCCCGAGGACAATCCAGACGGCTTTCAAGAGAAGCCGTCTGGATTGTCCTC-3'	104
antisense	5'-AAAAGAGGACAATCCAGACGGCTTCTCTTGAAGAGCCGTCTGGATTGTCCTC-3'	105
target	GAGGACAATCCAGACGGCT	106
hairpin	GAGGACAATCCAGACGGCTTTCAAGAGAAGCCGTCTGGATTGTCCTC	107
B7320-#2sense	5'-TCCCGGAGCTCTACAACGTGCTGTTCAAGAGACAGCACGTGTTAGAGCTCC-3'	108
antisense	5'-AAAAGGAGCTCTACAACGTGCTGTCTCTTGAACAGCACGTGTTAGAGCTCC-3'	109
target	GGAGCTCTACAACGTGCTG	110
hairpin	GGAGCTCTACAACGTGCTGTTCAAGAGACAGCACGTTGTTAGAGCTCC	111
SiEGFP sense	5'-CACCGAAGCAGCACGACTTCTTCTTCAAGAGAGAAGAAGTCGTGCTGCTTC-3'	49

antisense	5'-AAAAGAAGCAGCACGACTTCTTCTCTTGAAGAAGAAGT CGTGCTGCTTC-3'	50
target	GAAGCAGCACGACTTCTTC	51
hairpin	GAAGCAGCACGACTTCTTCTTCAAGAGAGAAGAAGTCGTGC TGCTTC	52
SiLUC sense	5'-CACCCGTACGCGGAATACTTCGATTCAAGAGATCGAAGTA TTCCGCGTACG-3'	53
SiLUC antisense	5'-AAAACGTACGCGGAATACTTCGATCTCTTGAATCGAAGTA TTCCGCGTACG-3'	54
target	CGTACGCGGAATACTTCGA	55
hairpin	CGTACGCGGAATACTTCGATTCAAGAGATCGAAGTATTCGCG GTACG	56
SiSCR sense	5'-CACCGCGCGCTTTGTAGGATTCGTTCAAGAGACGAATCCT ACAAAGCGCGC-3'	57
SiSCR antisense	5'-AAAAGCGCGCTTTGTAGGATTCGTCTCTTGAACGAATCCTA CAAAGCGCGC-3'	58
target	GCGCGCTTTGTAGGATTCG	59
hairpin	GCGCGCTTTGTAGGATTCGTTCAAGAGACGAATCCTACAAAG CGCGC	60
siMock sense	5'-CACCGTGTCTTCAAGCTTGAAGACTATTTT-3'	61
siMock antisense	5'-AAAATAGTCTTCAAGCTTGAAGACACGGTG-3'	62
The underline indicates specific siRNA sequence.		

Discussion

Recent progress in molecular biology has improved our understanding of the genesis of a wide range of human neoplasms. In RCC, several groups have reported microarray-based molecular profiling (Yao M, *et al.*, (2005) J Pathol.;205:377-87; Liou LS, *et al.*, (2004) BMC Urol.;4:9; Takahashi M, *et al.*, (2001) Proc Natl Acad Sci U S A.;98:9754-9; Boer JM, *et al.*, (2001) Genome Res.;11:1861-70; Young AN, *et al.*, (2001) Am J Pathol.;158:1639-51; Higgins JP, *et al.*, (2003) Am J Pathol.;162:925-32; Skubitz KM and Skubitz AP. (2002) J Lab Clin Med.;140:52-64.). Although those studies have highlighted candidate genes that might be useful as diagnostic markers, data based on expression of mRNAs isolated from tumor masses might not exactly reflect changes during the course of renal carcinogenesis since tumor masses are generally mixtures of various cell populations such as inflammatory cells, stroma cells, and fibroblasts in addition to cancer cells and the proportions of each cell type vary significantly from one individual to another. In addition, RCCs is considered to originate from renal proximal tubule epithelial cells in renal cortex. Therefore, microarray data published previously are likely to have been influenced significantly by cellular

heterogeneity among control preparations. In this invention, laser microbeam microdissection (LMM) was performed to obtain pure populations of renal cancer cells as well as control normal renal cortex cells. Compared with one representative expression profile data of clear cell RCC (ccRCC) using RNAs from bulk tumor tissues by means of micorarray (Takahashi M, *et al.*, (2003) *Oncogene*.;22:6810-8.), only 4 of the 85 (5%) genes which were commonly up-regulated in more than 75% of informative ccRCC cases in the data of the present invention (Table 4) were overlapped to bulk-expression profile data (Takahashi M, *et al.*, (2003) *Oncogene*.;22:6810-8.). These discrepancies were attributed to the fact that RCC cells are histopathologically heterogenous, and expression profiles from bulk tissues could be significantly influenced by contamination with non-cancerous cells. Surprisingly, none of top 24 genes that most significantly up-regulated in our gene list (Table 4) was included in the previous results of ccRCC-expression profiles from bulk tissues (Yao M, *et al.*, (2005) *J Pathol*.;205:377-87; Takahashi M, *et al.*, (2003) *Oncogene*.;22:6810-8.). This evidence suggests that the microarray data of the present invention is much reliable than that of others. This discrepancy may be attributed to the fact that previous expression profiles from bulk cells could be significantly influenced by contamination of renal medullas in normal kidney tissues used as a control. For example, genes that highly expressed in renal medulla, but not in renal proximal tubule epithelial cells in renal cortex, may be selected as down-regulated or unchanged genes in expression profiles from bulk tissues. Taking these points into consideration, it is crucial to perform LMM system to purify as much as possible the populations of cancerous and normal epithelial cells obtained from surgical specimens.

Genes with altered expression in most of ccRCCs may be serve as molecular diagnostic markers and candidates of RCC-therapeutic targets or may play causal role in renal carcinogenesis. Among up-regulated genes, NNMT (Nicotinamide N-methyltransferase), IGFBP3 (Insulin-like growth factor binding protein 3), ENPP3 (Ectonucleotide pyrophosphatase/phosphodiesterase 3), VEGF (Vascular endothelial growth factor), and VWF (Von Willebrand factor) were included in the present application as well as others. NNMT had been reported to be frequently increased in ccRCCs than in other types of RCCs and be correlated with good prognosis in RCCs (Yao M, *et al.*, (2005) *J Pathol*.;205:377-87.). IGFBP3 was shown by immunohistochemical staining that IGFBP-3 lead to the dysregulation of the IGF-axis within ccRCC (Takahashi M, *et al.*, (2005) *Int J Oncol*.;26:923-31; Cheung CW, *et al.*, (2004) *Kidney Int*.;65:1272-9.). IGFP-3 was also shown to play some roles in the regulation of RCC cell proliferation in an autocrine manner (Takahashi M, *et al.*, (2005) *Int J*

Oncol.;26:923-31; Cheung CW, *et al.*, (2004) Kidney Int.;65:1272-9.). Ecto-nucleotide pyrophosphatase /phosphodiesterase-I enzyme (E-NPP), cleaves phosphodiester and phosphosulfate bonds of a variety of substrates. Mammalian E-NPP is a family of three closely related proteins: E-NPP1, E-NPP2, and E-NPP3. The function of ENPP3 is unknown, but it was reported that the expression of ENPP3 was higher in tumor tissues than in surrounding tissues and the specific form of the ENPP3 protein was detected in the sera of patients with bile duct carcinoma (BDC) and that ENPP3 enhanced cell migration. Therefore, ENPP3 may be related to the invasiveness of neoplastic BDC and can be applicable as a tumor marker (Yano Y, *et al.*, (2004) Cancer Lett.;207:139-47.). Serum VEGF and vWF levels were indicated to be predictive markers for of immunomodulatory agents including interleukin 2 and IFN-alpha; the patients with higher levels of VEGF and vWF revealed poor response to these treatment comparing to those showing lower serum levels of these two proteins (Braybrooke JP, *et al.*, (2000) Clin Cancer Res.;6:4697-704.). Furthermore, ABCG1 (ATP-binding cassette, sub-family G (WHITE), member 1) and STC2 (Stanniocalcin 2) ABCG1, mediates the transport of cholesterol in general. Hernan et al. reported ABCG1 were known to be up-regulated in head and neck squamous cell carcinoma by microarray analysis and differential display (Yano Y, *et al.*, (2004) Cancer Lett.;207:139-47.).

On the other hand, among the down-regulated genes, WT1 (Wilms tumors 1), CDKN1C (Cyclin-dependent kinase inhibitor 1C), and GAS1 (Growth arrest-specific 1), which have been implicated their roles in growth suppression or apoptosis (Niu Z, *et al.*, (2005) J Urol.;174:1460-2; Kikuchi T, *et al.*, (2002) Oncogene.;21:2741-9; Watanabe H, *et al.*, (1998) Proc Natl Acad Sci U S A.;95:1392-7; Evdokiou A & Cowled PA. (1998) Int J Cancer.;75:568-77.). Niu Z, *et al.* have reported that expression of WT1 was significantly down-regulated in RCC tumors as compared with corresponding normal kidney tissues (Niu Z, *et al.*, (2005) J Urol.;174:1460-2.), although no significant correlation was noted between the mRNA level and the clinicopathological features of RCCs. CDKN1C has been known to induce cell cycle arrest by inhibiting the activity of cyclin dependent kinases. Epigenetic silencing of this gene has been reported in solid tumors such as colorectal, gastric, hepatocellular, and pancreatic cancers (Kikuchi T, *et al.*, (2002) Oncogene.;21:2741-9.), suggesting that CDKN1C functions as a tumor suppressor gene. GAS1 was also significantly down-regulated in 14 of 15 cases in this invention. Induction of GAS1 had been reported to suppress cell proliferation and/or leads to apoptosis in neuron cells, and expression of GAS1 decreased glial proliferation (Evdokiou A & Cowled PA. (1998) Int J Cancer.;75:568-77.).

Among the up-regulated genes in our list, the instant invention focused on Semaphorin 5B (SEMA5B) as a possible molecular target for RCC therapy due to frequent transactivation in RCCs and its undetectable level of expression in any of the normal human adult tissues the present inventors examined. In the context of the present invention, it was demonstrated that the knock-down of its expression level with siRNA resulted in the specifically significant suppression of RCC cell growth, suggesting that it is essential roll in enhancement of the cell growth. SEMA5B are a family of cell-surface and secreted glycoproteins that belong to a diverse group of genes encoding growth guidance cues (Kolodkin AL, *et al.*, (1993) Cell.;75:1389-99.). Semaphorins and their receptors, plexins, were originally identified in the nervous system, where they are required to establish the correct neuronal network. Their repertoire has been expanded to several non-neural processes, including cardiac and skeletal development, the immune response and epithelial morphogenesis. More recently, semaphorins have been implicated its role in tumour growth and metastasis (Tamagnone L & Comoglio PM. (2004) EMBO Rep.;5:356-61.). Herein, it was demonstrated that SEMA5B is frequently expressed in the clear cell type of RCC as well as RCC cell lines, implying that targeting to SEMA5B might be a promising approach for development of novel therapeutic drugs.

In this report, though the precise expression profiles of bladder cancer, B7032N and B9320 were identified as significantly over-expressed in RCC cells. Northern blot analysis with multiple normal human tissues and RCC cell lines showed that expression of B7032N was hardly detectable in normal human tissues except testis and pancreas and expression of B9320 was hardly detectable in normal human vital tissues examined. These results showed that these genes should serve as a valuable target for development of anti-cancer agents for RCC. Furthermore, it was demonstrated that knockdown of endogenous B7032N and B9320 resulted in suppression of cell growth in RCC cells. In conclusion, it was documented that B7032N and B9320 showed the growth promoting activity in mammalian cells, and that knockdown of its expression by siRNAs suppressed the growth of bladder cancers cells. The development of drugs to antagonize the function of B7032N and B9320 might be a rational strategy for cancer therapy. Although further analysis of the function of B7032N and B9320 will be necessary, the data provided should contribute to more profound understanding of RCC carcinogenesis and to development of novel therapies for RCCs.

Taken together, the up-regulated genes identified through the precise RCC-expression profiles of the present invention should shed light on a better understanding of renal carcinogenesis and provide the useful information to discover possible novel molecular targets for development of RCC treatment and also diagnostic tumor markers.

5 Industrial Applicability

The gene-expression analysis of renal cell carcinoma described herein, obtained through a combination of laser-capture dissection and genome-wide cDNA microarray, has identified specific genes as targets for cancer prevention and therapy. Based on the expression of a subset of these differentially expressed genes, the present invention provides
10 molecular diagnostic markers for identifying or detecting renal cell carcinoma.

The methods described herein are also useful in the identification of additional molecular targets for prevention, diagnosis and treatment of renal cell carcinoma. The data reported herein add to a comprehensive understanding of renal cell carcinoma, facilitate development of novel diagnostic strategies, and provide clues for identification of molecular
15 targets for therapeutic drugs and preventative agents. Such information contributes to a more profound understanding of renal cell carcinoma, and provides indicators for developing novel strategies for diagnosis, treatment, and ultimately prevention of renal cell carcinoma.

As demonstrated herein, cell growth can be suppressed through small interfering RNA (siRNA) that specifically target the C6700, B7032N or B9320 gene. Thus, novel siRNAs are
20 useful targets for the development of anti-cancer pharmaceuticals. For example, agents that block the expression of C6700, B7032N or B9320 or prevent its activity may find therapeutic utility as anti-cancer agents, particularly anti-cancer agents for the treatment of kidney cancer, such as renal cell carcinoma .

All patents, patent applications, and publications cited herein are incorporated by
25 reference in their entirety. Furthermore, while the invention has been described in detail and with reference to specific embodiments thereof, it is to be understood that the foregoing description is exemplary and explanatory in nature and is intended to illustrate the invention and its preferred embodiments. Through routine experimentation, one skilled in the art will readily recognize that various changes and modifications can be made therein without
30 departing from the spirit and scope of the invention. Thus, the invention is intended to be defined not by the above description, but by the following claims and their equivalents.

CLAIMS

1. A method of diagnosing RCC or a predisposition for developing RCC in a subject, comprising determining a level of expression of a RCC-associated gene in a patient derived biological sample, wherein an increase or decrease in said sample expression
5 level as compared to a normal control level of said gene indicates that said subject suffers from or is at risk of developing RCC.
2. The method of claim 1, wherein said RCC-associated gene is selected from the group consisting of RCC 1-251, wherein an increase in said sample expression level as
10 compared to a normal control level indicates said subject suffers from or is at risk of developing RCC.
3. The method of claim 1, wherein said sample expression level is at least 10% greater than said normal control level.
4. The method of claim 1, wherein said RCC-associated gene is selected from the group consisting of RCC 252-972, wherein a decrease in said sample expression level as
15 compared to a normal control level indicates said subject suffers from or is at risk of developing RCC.
5. The method of claim 4, wherein said sample expression level is at least 10% lower than said normal control level.
6. The method of claim 1, wherein said method further comprises determining said level of
20 expression of a plurality of RCC-associated genes.
7. The method of claim 1, wherein the gene expression level is determined by a method selected from the group consisting of:
 - (a) detecting mRNA of the an RCC-associated gene,
 - (b) detecting a protein encoded by the RCC-associated gene, and
 - 25 (c) detecting a biological activity of a protein encoded by the RCC-associated gene.
8. The method of claim 1, wherein said hybridization step is carried out on a DNA array.
9. The method of claim 1, wherein said patient-derived biological sample comprises an epithelial cell.
10. The method of claim 1, wherein said patient-derived biological sample comprises a renal

cell carcinoma cell.

11. The method of claim 7 wherein said patient-derived biological sample comprises an epithelial cell from a renal cell carcinoma.
12. An RCC reference expression profile, comprising a pattern of gene expression of two or more RCC-associated genes selected from the group consisting of RCC 1-972.
13. An RCC reference expression profile, comprising a pattern of gene expression of two or more RCC-associated genes selected from the group consisting of RCC 1-251.
14. An RCC reference expression profile, comprising a pattern of gene expression of two or more RCC-associated genes selected from the group consisting of RCC 252-972.
15. A method of screening for a compound for treating or preventing renal cell carcinoma, said method comprising the steps of:
 - (a) contacting a test compound with a polypeptide encoded by a polynucleotide selected from the group consisting of RCC 1-972;
 - (b) detecting the binding activity between the polypeptide and the test compound; and
 - (c) selecting the test compound that binds to the polypeptide.
16. A method of screening for a compound for treating or preventing renal cell carcinoma, said method comprising the steps of:
 - (a) contacting a candidate compound with a cell expressing one or more marker genes, wherein the one or more marker genes is selected from the group consisting of RCC 1-972; and
 - (b) selecting the candidate compound that reduces the expression level of one or more marker genes selected from the group consisting of RCC 1-251, or elevates the expression level of one or more marker genes selected from the group consisting of RCC 252-972 as compared to a control.
17. The method of claim 16, wherein said cell comprises a renal cell carcinoma cell.
18. A method of screening for a compound for treating or preventing renal cell carcinoma, said method comprising the steps of:
 - (a) contacting a test compound with a polypeptide encoded by a polynucleotide selected from the group consisting of RCC 1-972;

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

(c) selecting the test compound that suppresses the biological activity of the polypeptide encoded by the polynucleotide selected from the group consisting of RCC 1-251 in comparison with the biological activity of said polypeptide detected in the absence of the test compound, or enhances the biological activity of the polypeptide encoded by the polynucleotide selected from the group consisting of RCC 252-972 in comparison with the biological activity of said polypeptide detected in the absence of the test compound.

19. A method of screening for compound for treating or preventing renal cell carcinoma, said method comprising the steps of:

(a) contacting a candidate compound with a cell into which a vector comprising the transcriptional regulatory region of one or more marker genes and a reporter gene that is expressed under the control of the transcriptional regulatory region has been introduced, wherein the one or more marker genes are selected from the group consisting of RCC 1-972

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

(c) selecting the candidate compound that reduces the expression or activity level of said reporter gene when said marker gene is an up-regulated marker gene selected from the group consisting of RCC 1-251 or that enhances the expression or activity level of said reporter gene when said marker gene is a down-regulated marker gene selected from the group consisting of RCC 252-972, as compared to the level detected in the absence of the test compound.

20. A kit comprising a detection reagent which binds to (a) two or more nucleic acid sequences selected from the group consisting of RCC 1-972 or (b) polypeptides encoded thereby.

21. An array comprising two or more nucleic acids which bind to one or more nucleic acid sequences selected from the group consisting of RCC 1-972.

22. A method of treating or preventing renal cell carcinoma in a subject comprising administering to said subject an antisense composition, said antisense composition comprising a nucleotide sequence complementary to a coding sequence selected from the group consisting of RCC 1-251.

23. A method of treating or preventing renal cell carcinoma in a subject comprising administering to said subject a siRNA composition, wherein said siRNA composition reduces the expression of a nucleic acid sequence selected from the group consisting of RCC 1-251.
- 5 24. A method of treating or preventing renal cell carcinoma in a subject comprising the step of administering to said subject a pharmaceutically effective amount of an antibody or an immunologically active fragment thereof that binds to a protein encoded by any one gene selected from the group consisting of RCC 1-251.
- 10 25. A method of treating or preventing renal cell carcinoma in a subject comprising administering to said subject a vaccine comprising (a) a polypeptide encoded by a nucleic acid selected from the group consisting of RCC 1-251, (b) an immunologically active fragment of said polypeptide, or (c) a polynucleotide encoding the polypeptide.
- 15 26. A method of treating or preventing renal cell carcinoma in a subject comprising administering to said subject a compound that increases the expression of a polynucleotide selected from the group consisting of RCC 252-972 or the activity of a polypeptide encoded thereby.
27. A method of treating or preventing renal cell carcinoma in a subject, said method comprising the step of administering a compound obtained by a method according to any one of claims 15-19.
- 20 28. A method of treating or preventing renal cell carcinoma in a subject comprising administering to said subject a pharmaceutically effective amount of an agent comprising (a) a polynucleotide selected from the group consisting of RCC 252-972, or (b) polypeptide encoded thereby.
- 25 29. A composition for treating or preventing renal cell carcinoma, said composition comprising a pharmaceutically effective amount of an antisense polynucleotide or small interfering RNA against a polynucleotide selected from the group consisting of RCC 1-251.
30. A composition for treating or preventing renal cell carcinoma, said composition comprising a pharmaceutically effective amount of an antibody or fragment thereof that binds to a protein encoded by a gene selected from the group consisting of RCC 1-251.
- 30 31. A composition for treating or preventing renal cell carcinoma, said composition

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comprising a pharmaceutically effective amount of a compound selected by a method of any one of claims 15-19 as an active ingredient, and a pharmaceutically acceptable carrier.

32. A method of treating or preventing renal cell carcinoma in a subject comprising administering to said subject a composition comprising a small interfering RNA (siRNA) that inhibits expression of *C6700V2*, *B7032N* or *B9320 V2*.

33. The method of claim 32, wherein said siRNA comprises a sense nucleic acid sequence and an anti-sense nucleic acid sequence that specifically hybridizes to a sequence from *C6700V2*, *B7032N* or *B9320 V2*.

34. The method of claim 32, wherein the kidney cancer is an renal cell carcinoma (RCC).

35. The method of claim 33, wherein said siRNA comprises a ribonucleotide sequence corresponding to a sequence selected from the group consisting of SEQ ID NOs: 43, 47, 81, 106 or 110 as the target sequence.

36. The method of claim 35, 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: 43, 47, 81, 106 or 110.

[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].

37. The method of claim 32, wherein said composition comprises a transfection-enhancing agent.

38. 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: 43, 47, 81, 106 or 110, 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 *C6700V2*, *B7032N* or *B9320 V2* gene, inhibits expression of said gene.

39. The double-stranded molecule of claim 38, wherein said target sequence comprises at least about 10 contiguous nucleotides from the nucleotide sequences selected from the

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group of SEQ ID NOs: 65, 112 or 118.

40. The double-stranded molecule of claim 39, 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: 65, 112 or 118.

5 41. The double-stranded molecule of claim 40, 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.

42. The double-stranded molecule of claim 39, wherein the double-stranded molecule is an oligonucleotide of less than about 100 nucleotides in length.

10 43. The double-stranded molecule of claim 42, wherein the double-stranded molecule is an oligonucleotide of less than about 75 nucleotides in length.

44. The double-stranded molecule of claim 43, wherein the double-stranded molecule is an oligonucleotide of less than about 50 nucleotides in length.

15 45. The double-stranded molecule of claim 44, wherein the double-stranded molecule is an oligonucleotide of less than about 25 nucleotides in length.

46. The double-stranded molecule of claim 45, wherein the double stranded molecule is an oligonucleotide of between about 19 and about 25 nucleotides in length.

47. A vector encoding the double-stranded molecule of claim 39.

20 48. The vector of claim 47, wherein the vector encodes a transcript having a secondary structure and comprises the sense strand and the antisense strand.

49. The vector of claim 48, wherein the transcript further comprises a single-stranded ribonucleotide sequence linking said sense strand and said antisense strand.

25 50. 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: 43, 47, 81, 106 or 110, and said antisense strand nucleic acid consists of a sequence complementary to the sense strand.

51. The vector of claim 49, wherein said polynucleotide has the general formula

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

wherein [A] is a nucleotide sequence of SEQ ID NOs: 43, 47, 81, 106 or 110; [B] is a

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nucleotide sequence consisting of 3 to 23 nucleotides; and [A'] is a nucleotide sequence complementary to [A].

52. A pharmaceutical composition for treating or preventing renal cell carcinoma comprising a pharmaceutically effective amount of a small interfering RNA (siRNA) that inhibits
5 expression of *C6700V2*, *B7032N* or *B9320 V2* as an active ingredient, and a pharmaceutically acceptable carrier.

53. The pharmaceutical composition of claim 51, wherein the siRNA comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 43, 47, 81, 106 or 110 as the target sequence.

10 54. The composition of claim 52, wherein the siRNA has the general formula

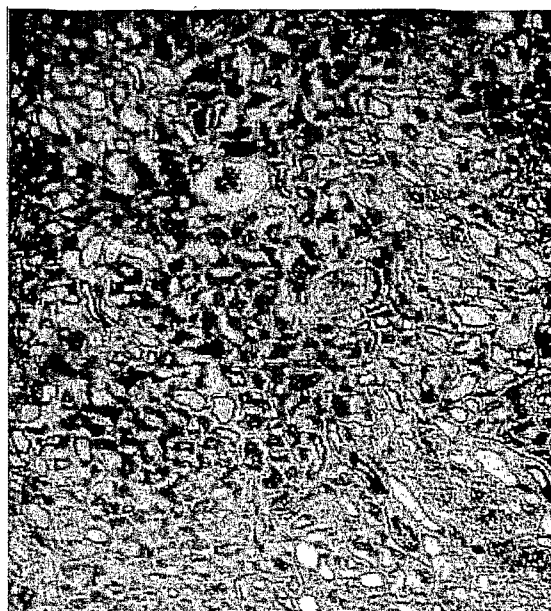
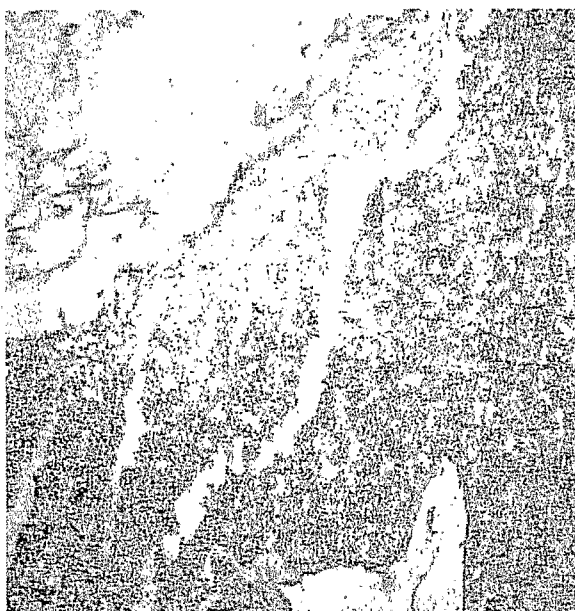
5'-[A]-[B]-[A']-3'

wherein [A] is a ribonucleotide sequence corresponding to a nucleotide sequence of SEQ ID NOs: 43, 47, 81, 106 or 110; [B] is a ribonucleotide sequence consisting of 3 to 23 nucleotides; and [A'] is a ribonucleotide sequence complementary to [A].

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Fig. 1

a



b



c

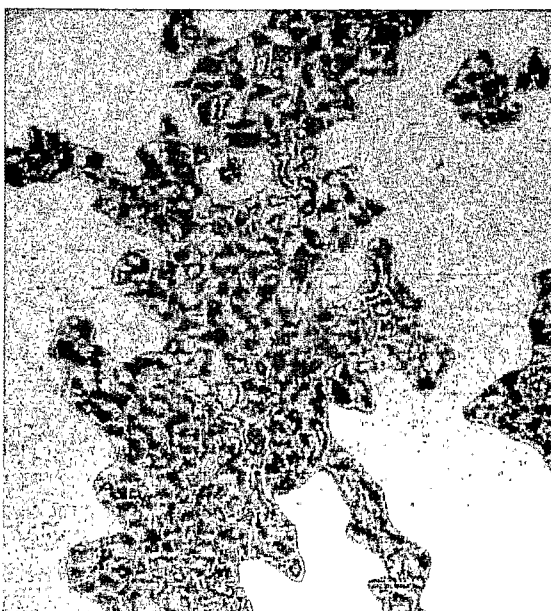
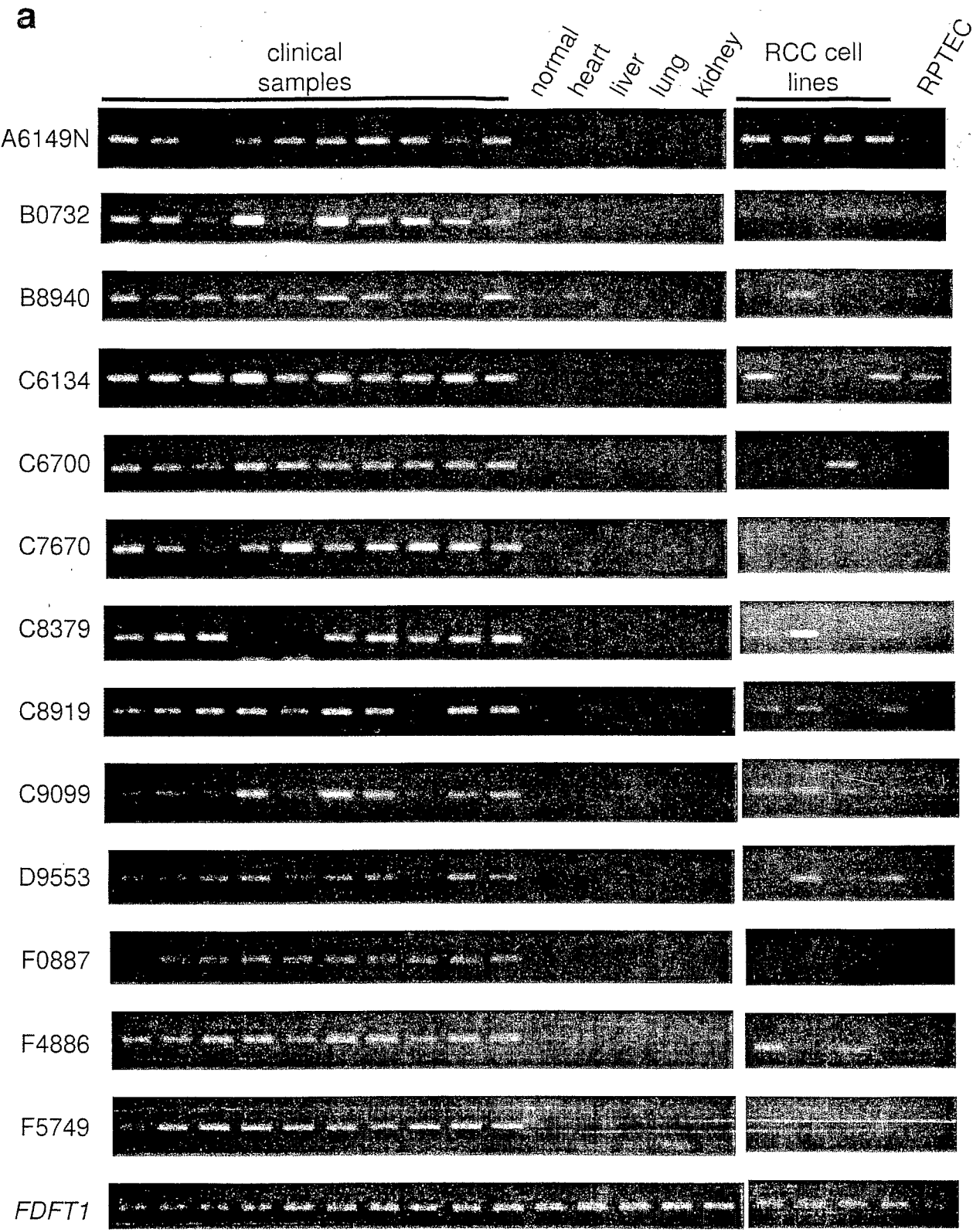
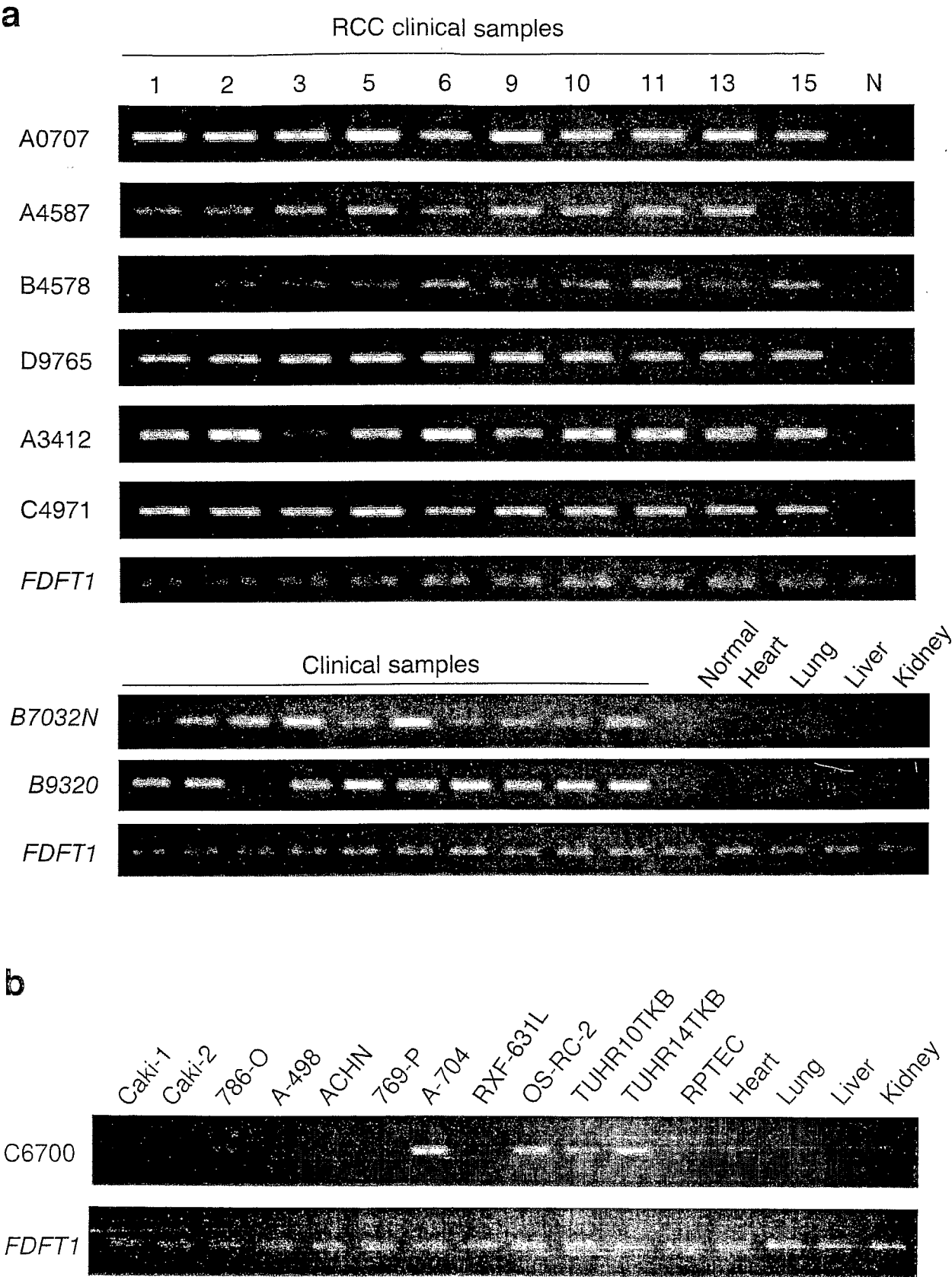


Fig. 2



Continuation of Fig. 2



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Fig. 3

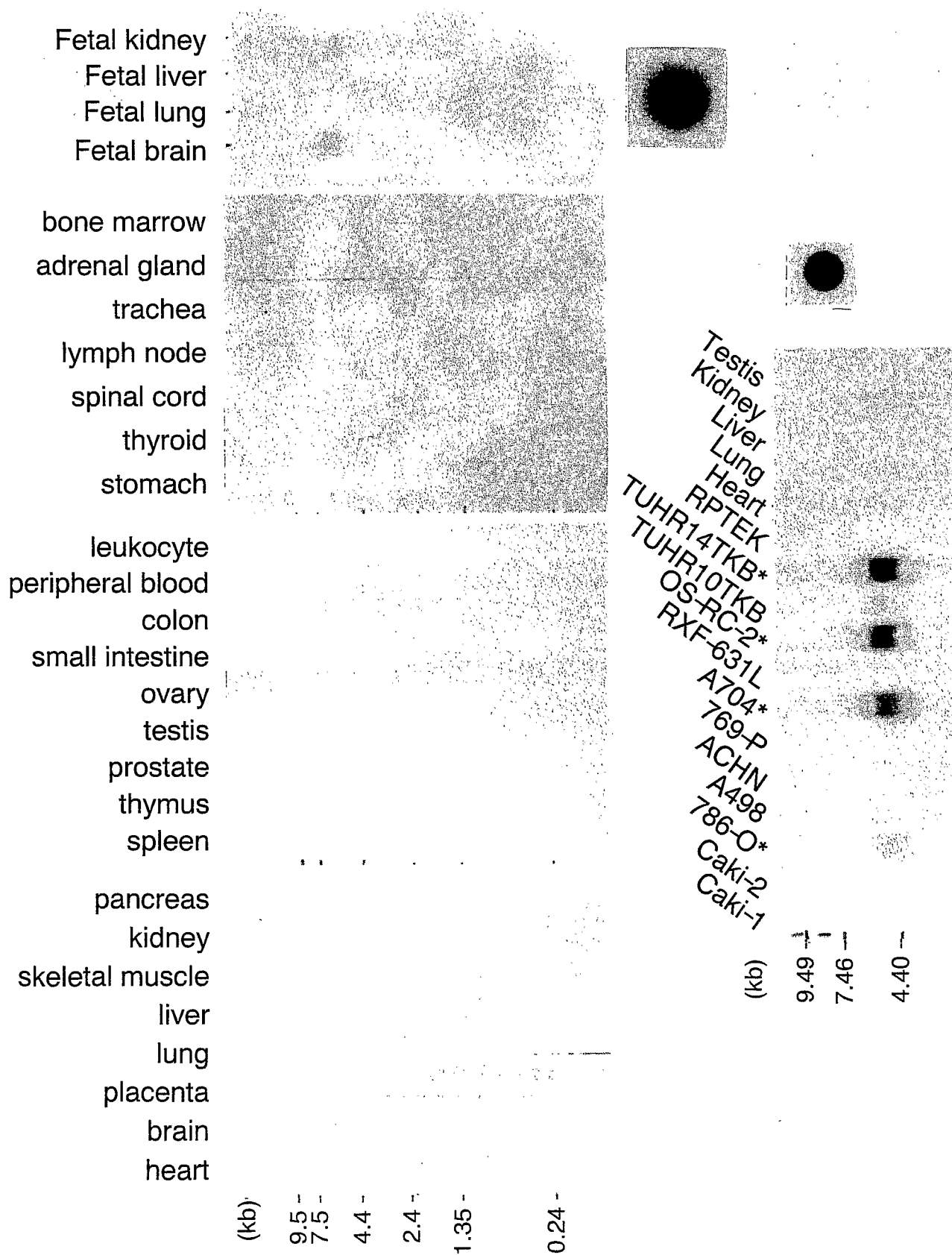
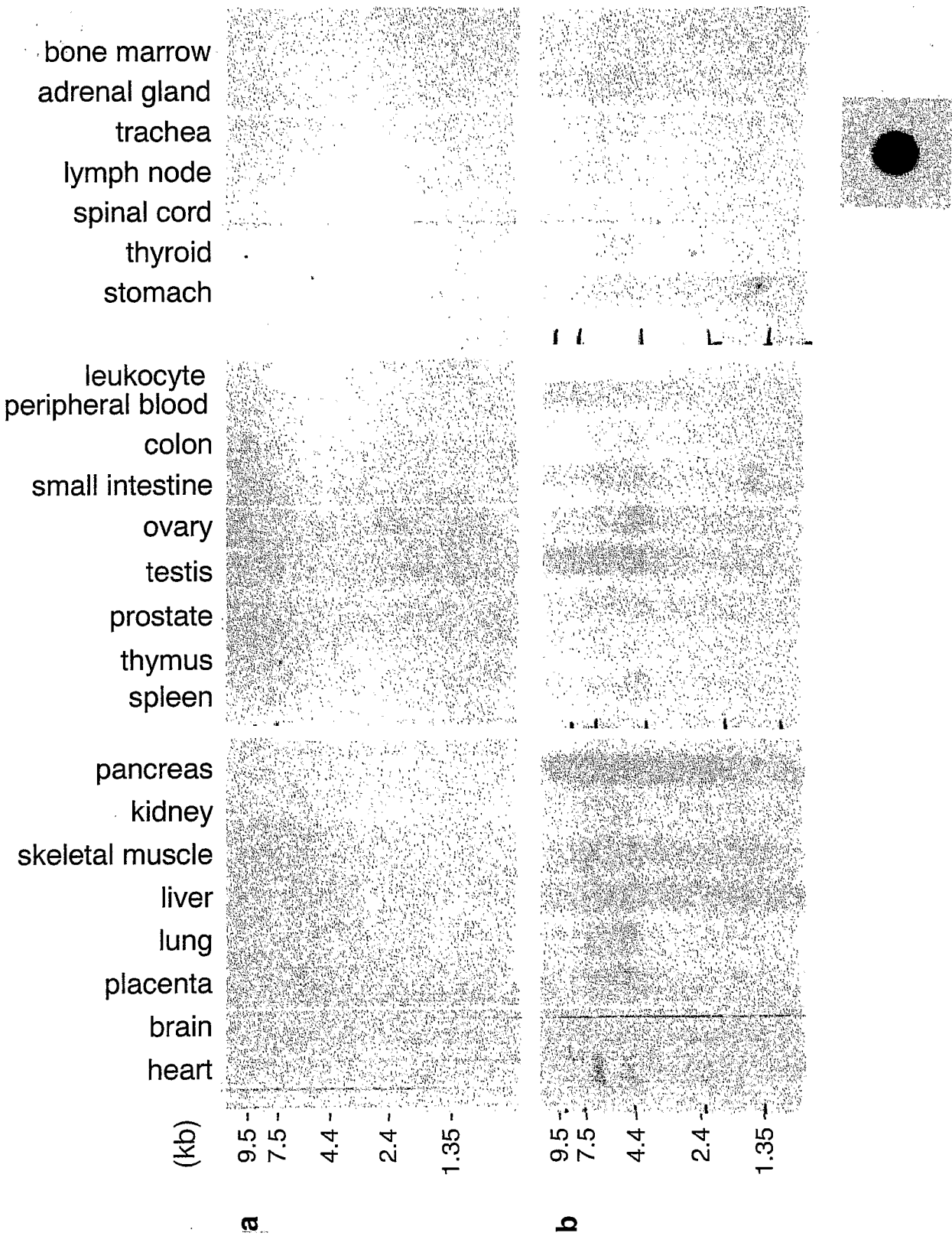


Fig. 4



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Fig. 5

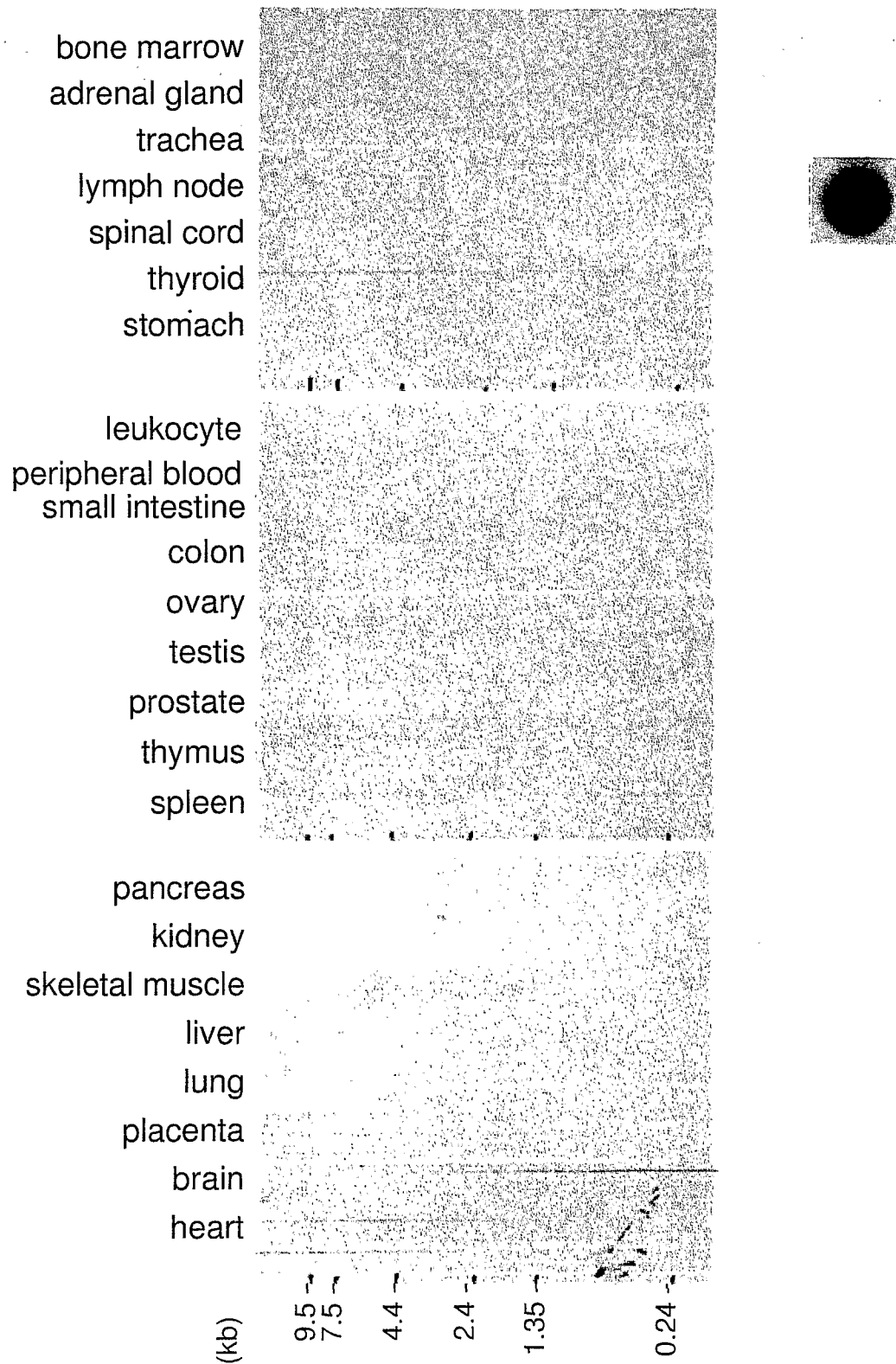


Fig. 6

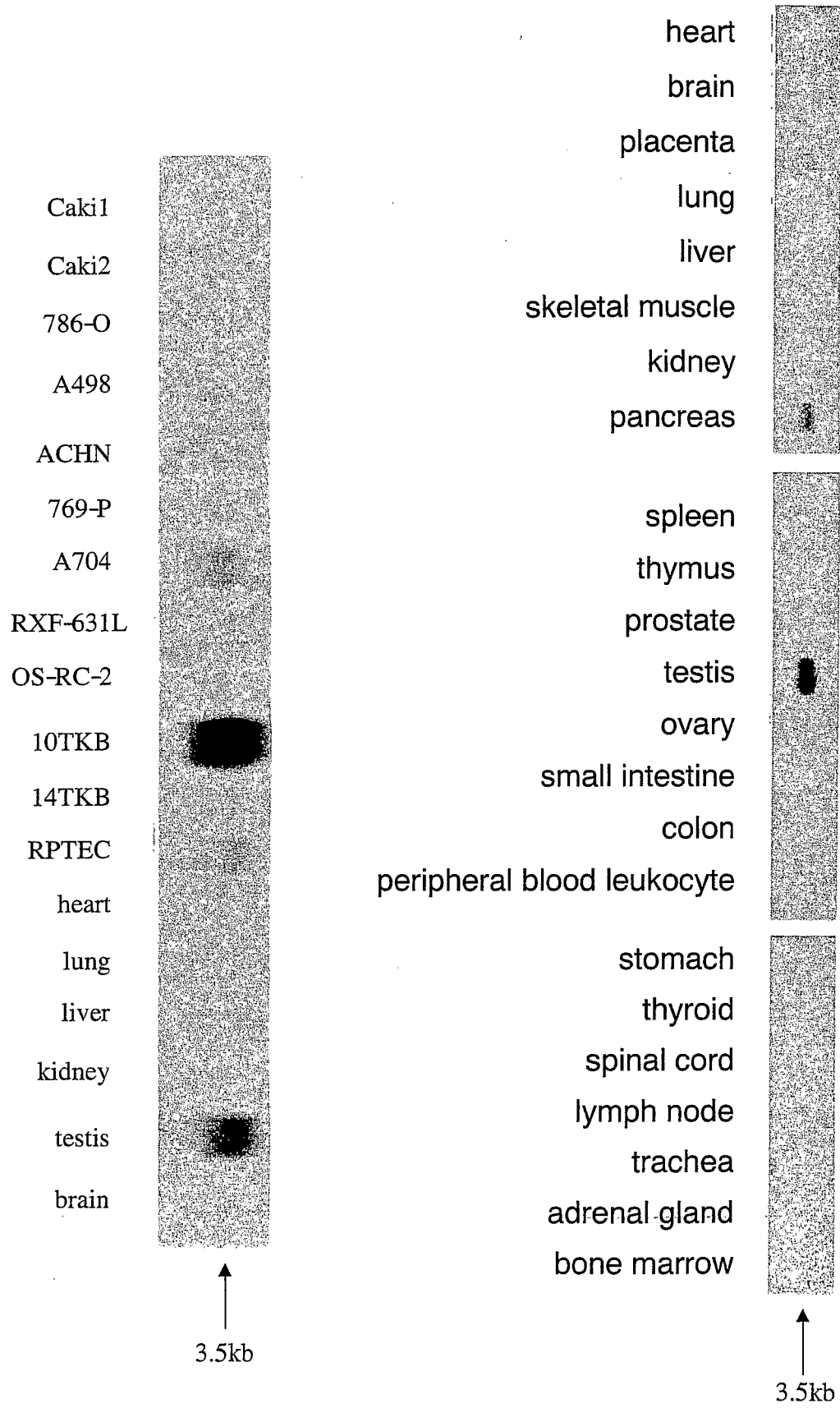


Fig. 7

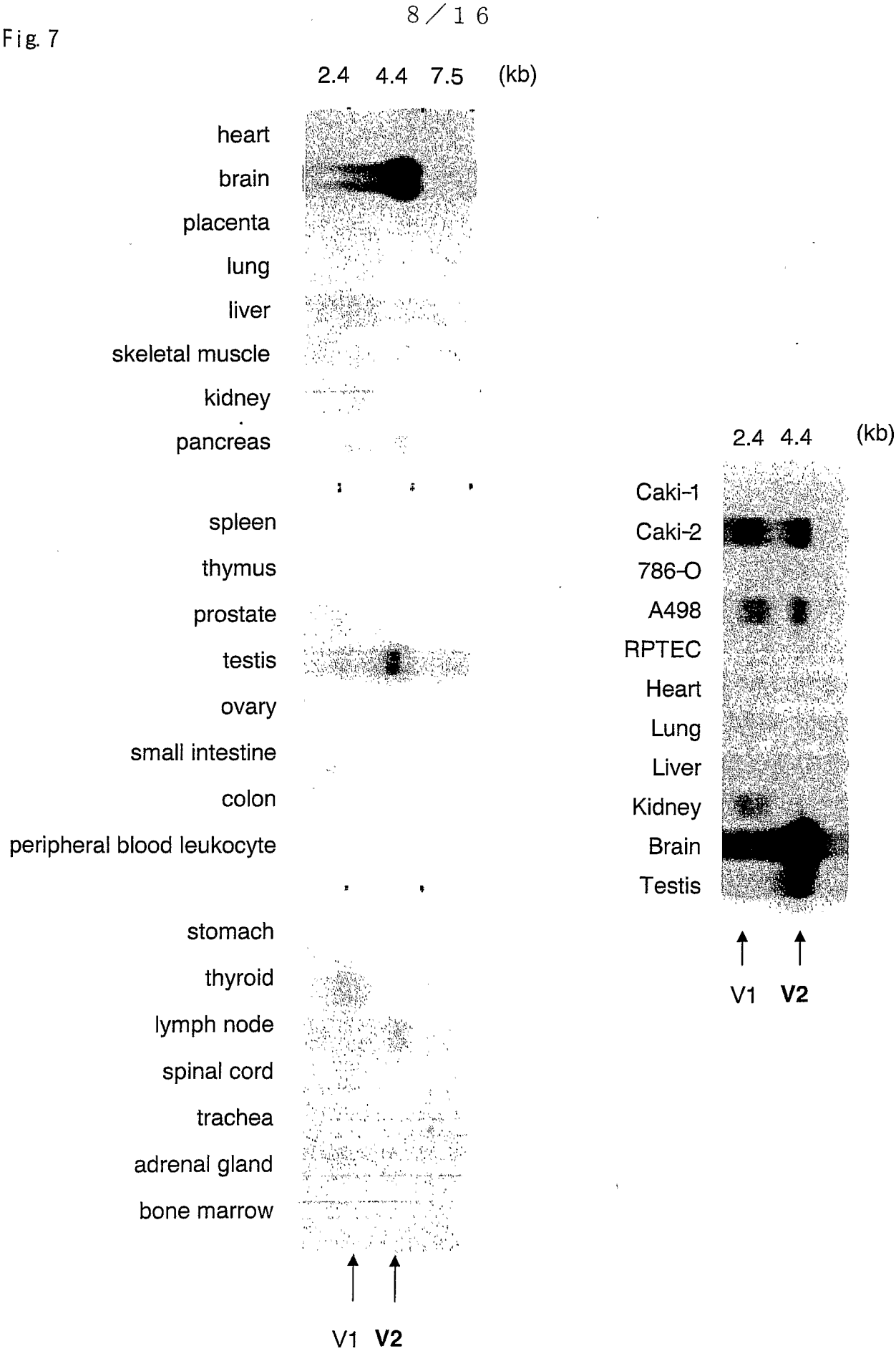


Fig. 8

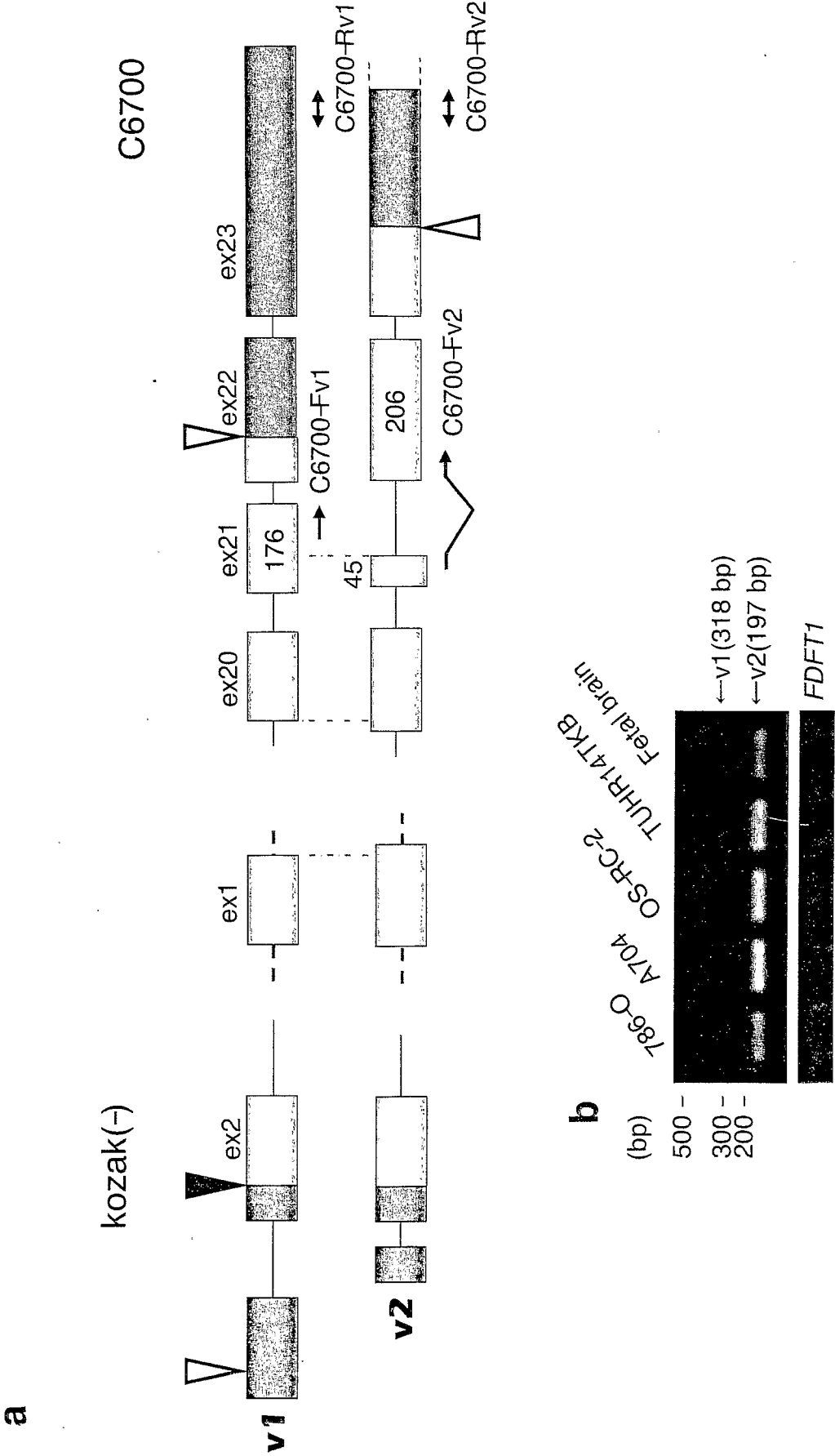


Fig. 9

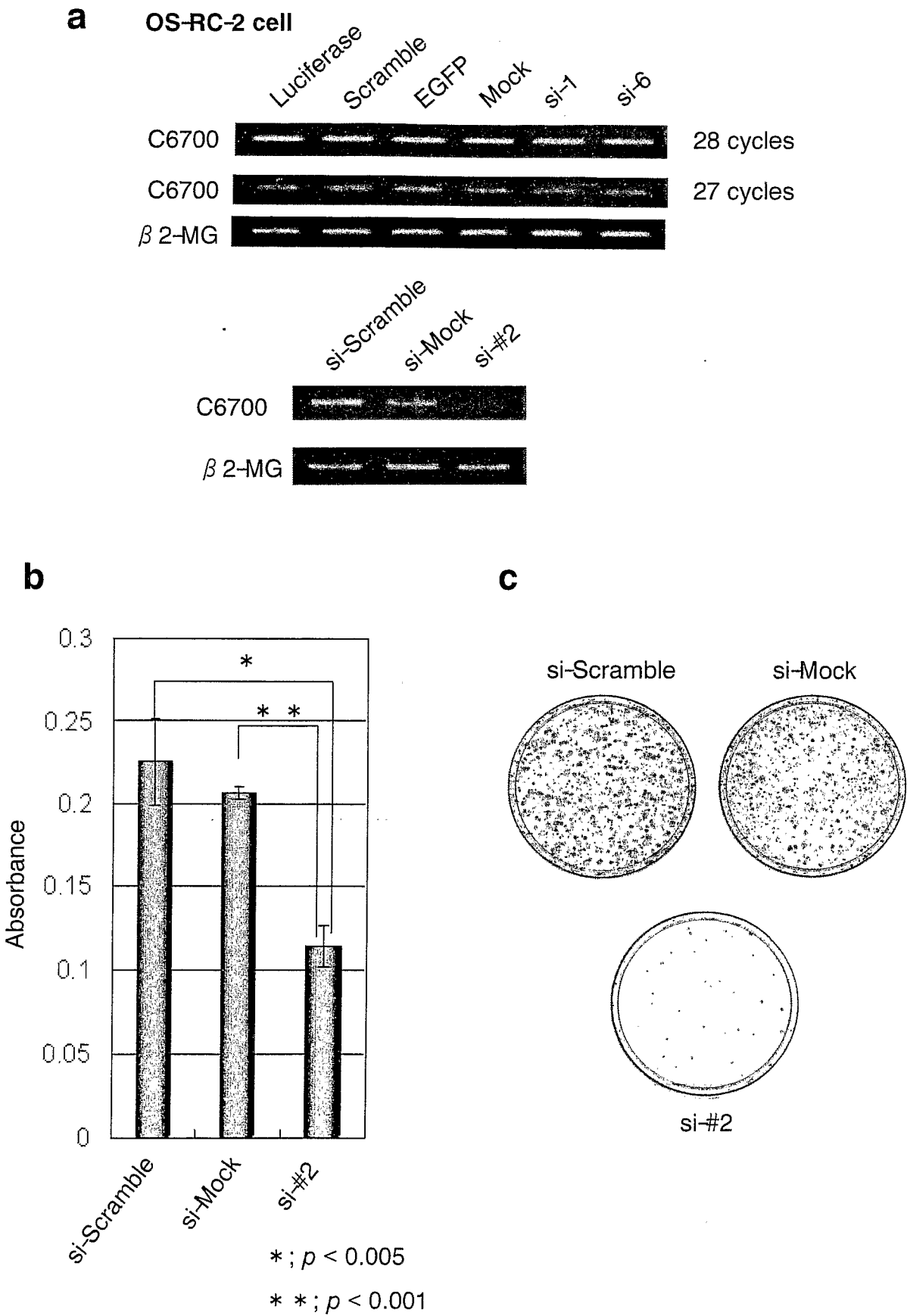


Fig. 10

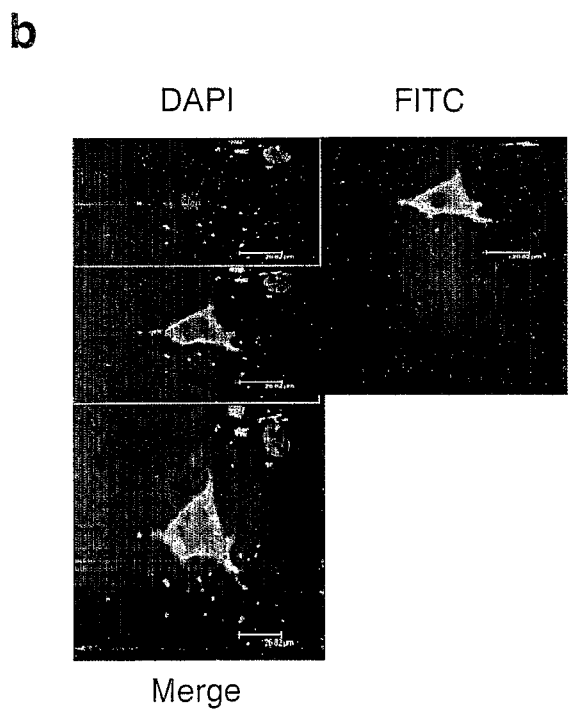
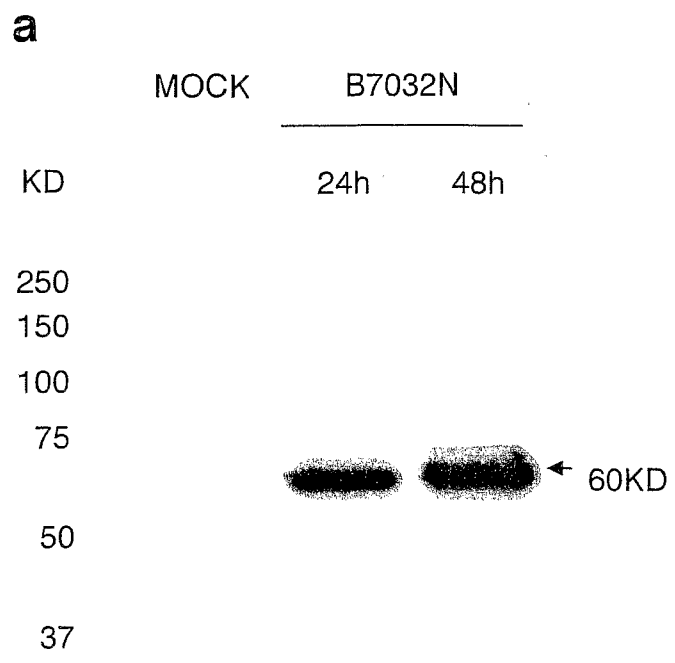
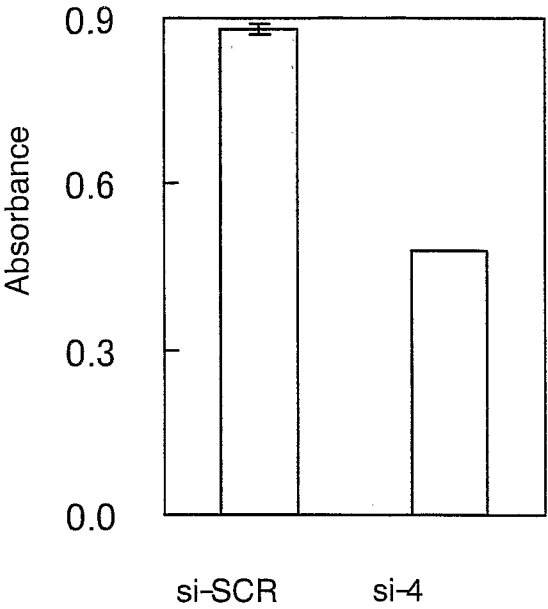
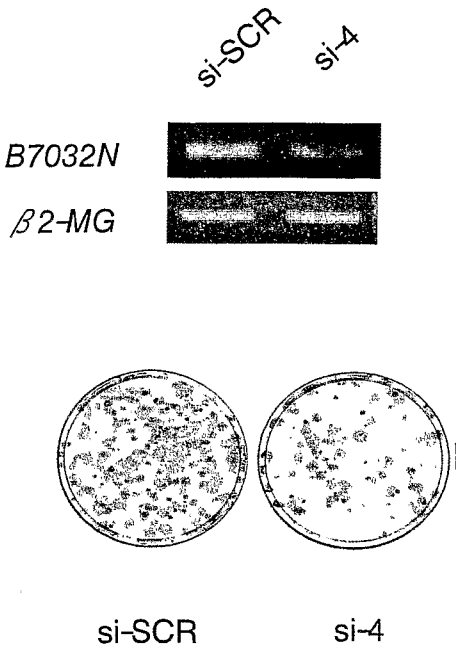
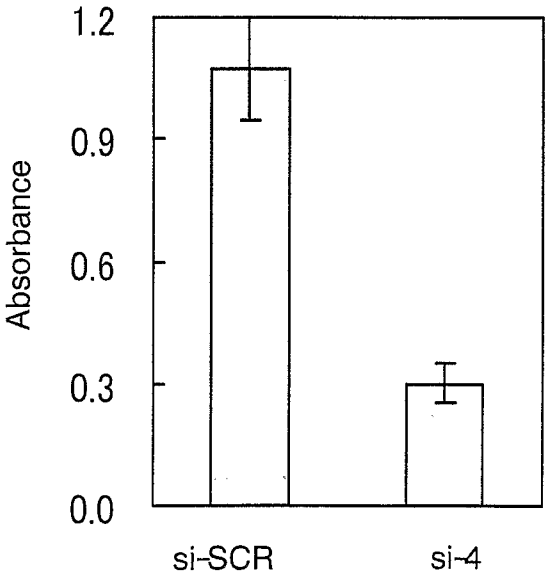
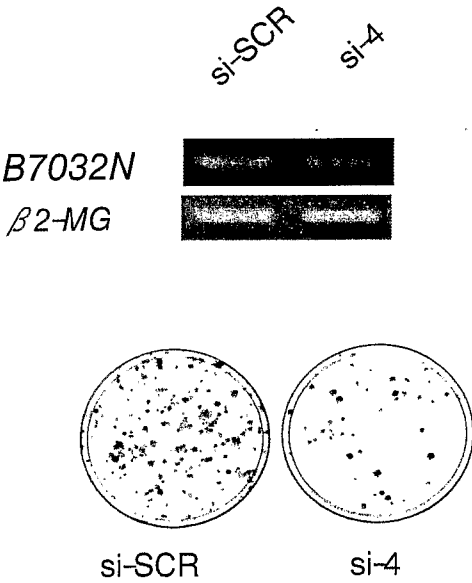


Fig. 11

a RXF-631L



b A498



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Fig. 12

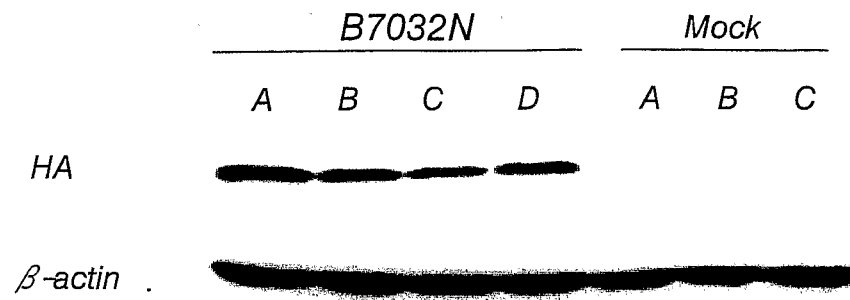
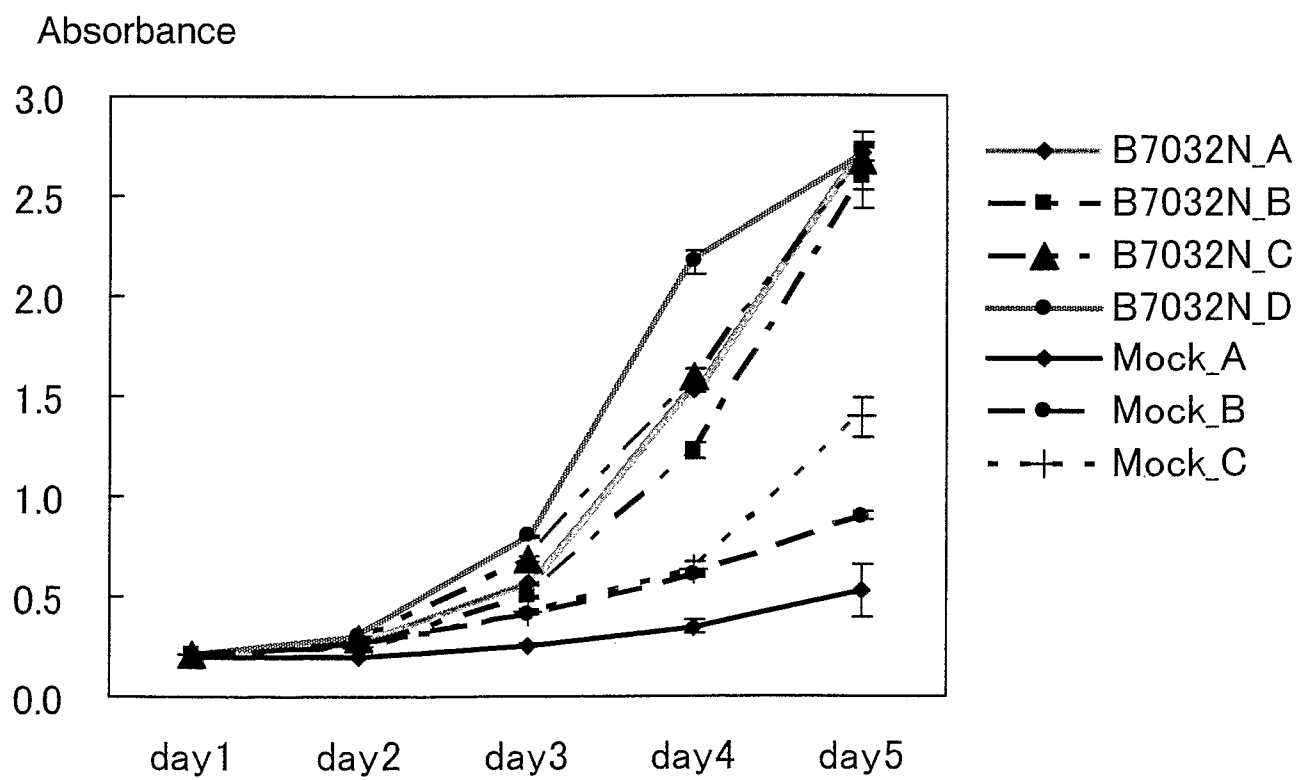
a**b**

Fig. 13

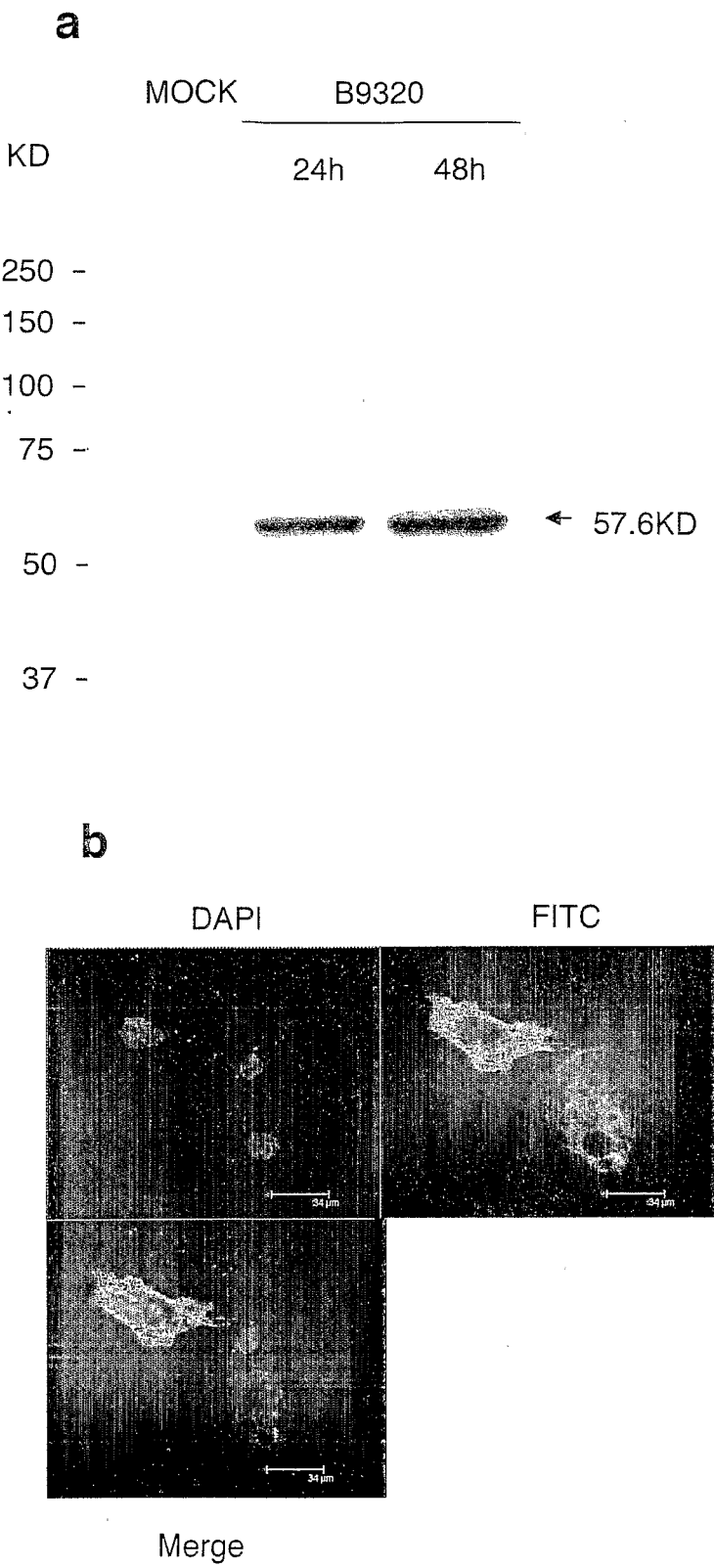
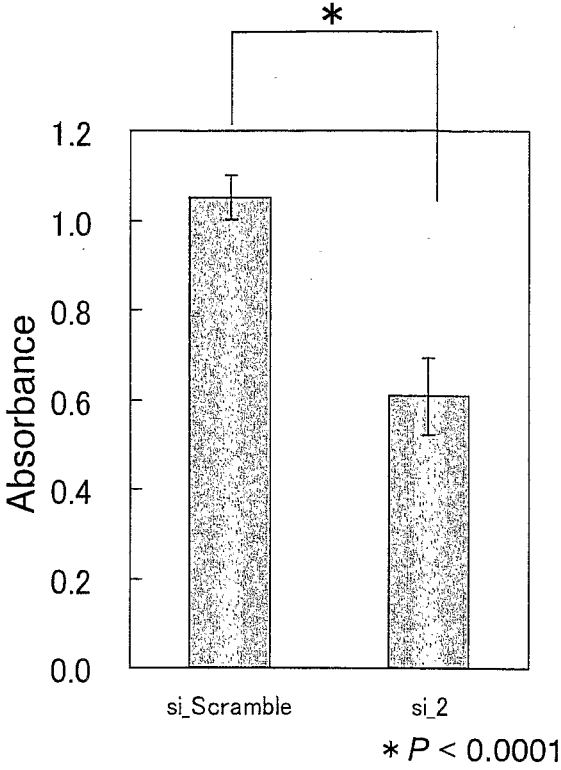
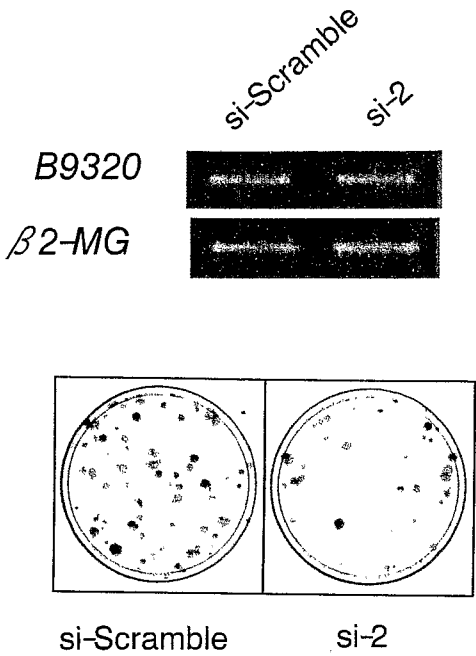
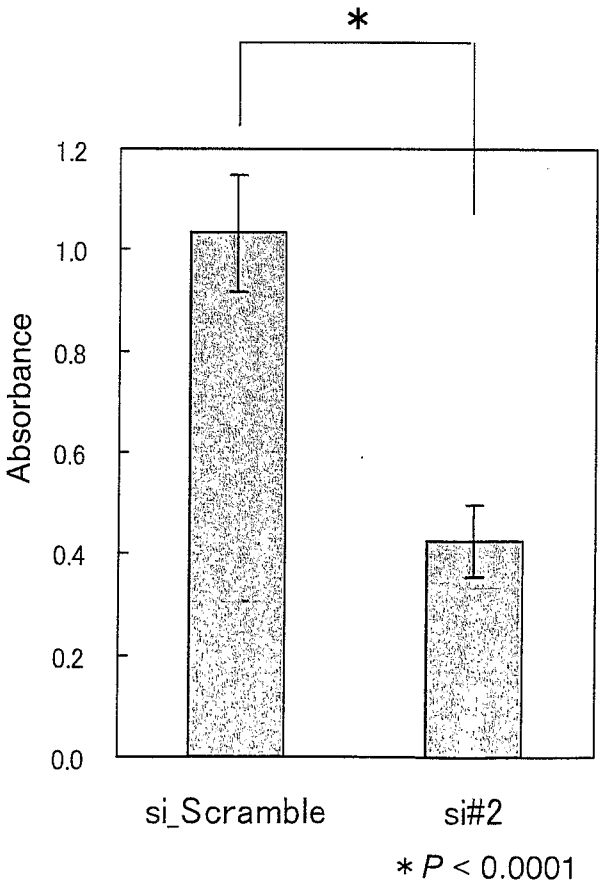
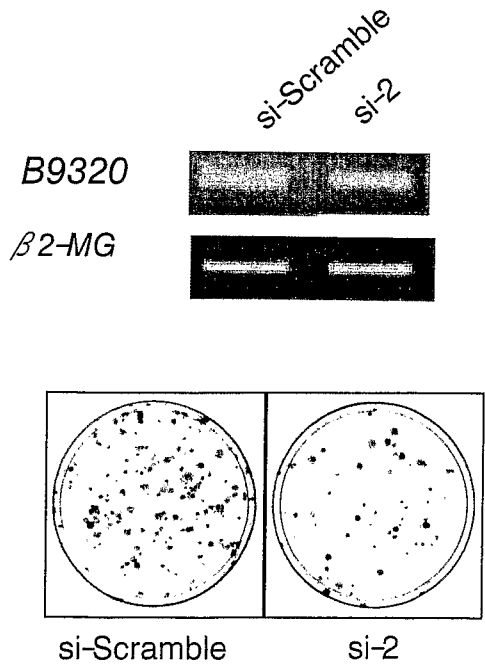


Fig. 14

a Caki-2

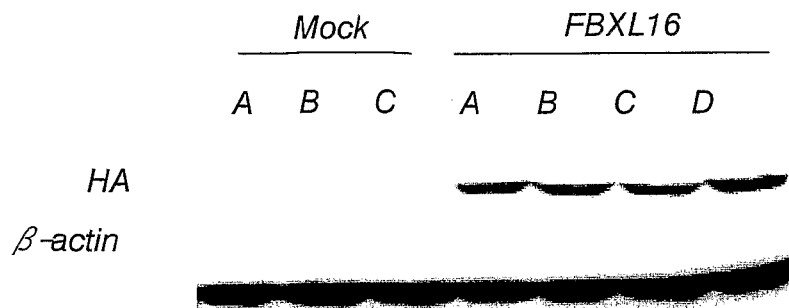
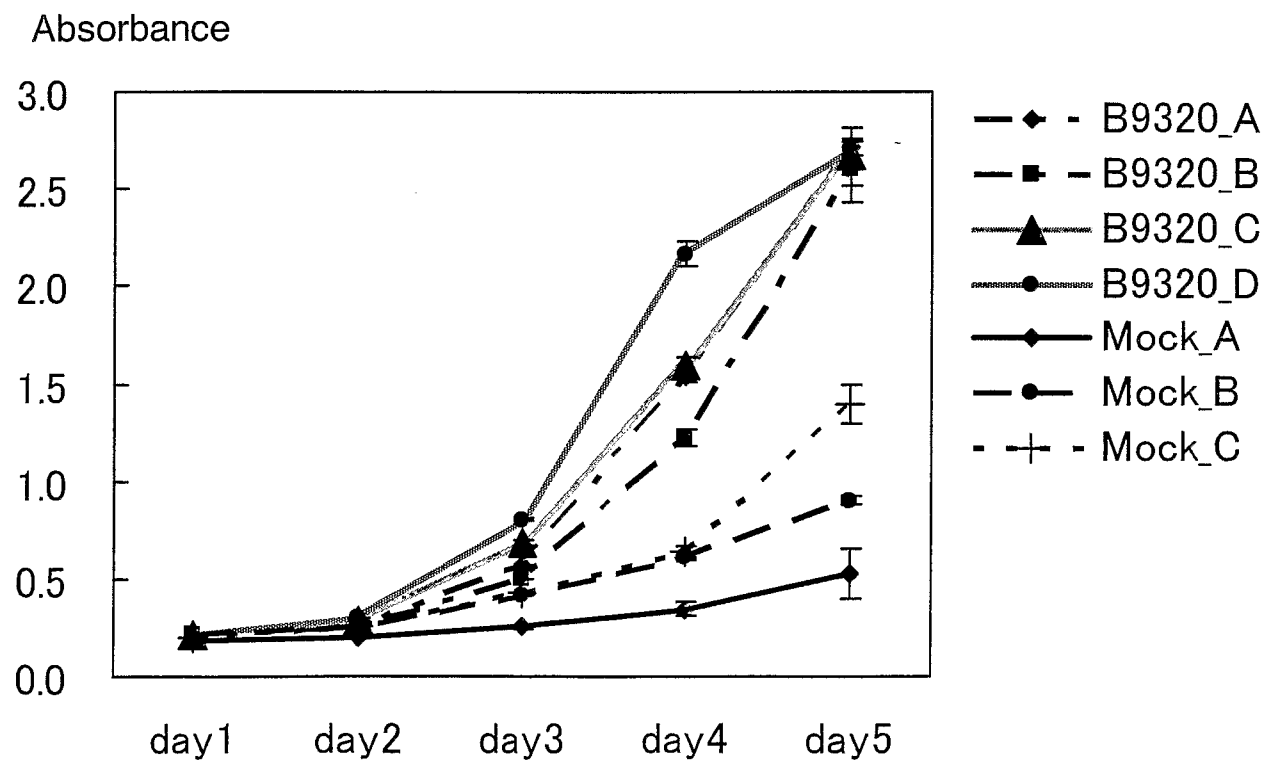


b A498



16 / 16

Fig. 15

a**b**

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SEQUENCE LISTING

<110> ONCOTHERAPY SCIENCE, INC.
THE UNIVERSITY OF TOKYO

<120> METHOD FOR DIAGNOSING RENAL CELL CARCINOMA

<130> ONC-A0505P

<150> US 60/703,640

<151> 2005-07-28

<150> US 60/799,960

<151> 2006-05-11

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<170> PatentIn version 3.3

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16/102

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17/102

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19/102

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24/102

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ctccccgtat cccctactcg ttcttcacaa ttctgtaggt gagtggttcc agctggtgcc 180

tggcctgtgt ctcttgg atg ccc tgt ggc ttc agt ccg tct cct gtt gcc 230

Met Pro Cys Gly Phe Ser Pro Ser Pro Val Ala

1 5 10

cac cac ctc gtc cct ggg ccg cct gat acc cca gcc caa cag cta agg 278

His His Leu Val Pro Gly Pro Pro Asp Thr Pro Ala Gln Gln Leu Arg

15 20 25

tgt gga tgg aca gta ggg ggc tgg ctt ctc tca ctg gtc agg ggt ctt 326

Cys Gly Trp Thr Val Gly Gly Trp Leu Leu Ser Leu Val Arg Gly Leu

30 35 40

ctc ccc tgt ctg cct ccc gga gct agg act gca gag ggg cct atc atg 374

Leu Pro Cys Leu Pro Pro Gly Ala Arg Thr Ala Glu Gly Pro Ile Met

45 50 55

gtg ctt gca ggc ccc ctg gct gtc tcg ctg ttg ctg ccc agc ctc aca 422

Val Leu Ala Gly Pro Leu Ala Val Ser Leu Leu Leu Pro Ser Leu Thr

60 65 70 75

ctg ctg gtg tcc cac ctc tcc agc tcc cag gat gtc tcc agt gag ccc 470

Leu Leu Val Ser His Leu Ser Ser Ser Gln Asp Val Ser Ser Glu Pro

80 85 90

agc agt gag cag cag ctg tgc gcc ctt agc aag cac ccc acc gtg gcc 518

25 / 102

Ser Ser Glu Gln Gln Leu Cys Ala Leu Ser Lys His Pro Thr Val Ala	
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ttt gaa gac ctg cag ccg tgg gtc tct aac ttc acc tac cct gga gcc	566
Phe Glu Asp Leu Gln Pro Trp Val Ser Asn Phe Thr Tyr Pro Gly Ala	
110 115 120	
cgg gat ttc tcc cag ctg gct ttg gac ccc tcc ggg aac cag ctc atc	614
Arg Asp Phe Ser Gln Leu Ala Leu Asp Pro Ser Gly Asn Gln Leu Ile	
125 130 135	
gtg gga gcc agg aac tac ctc ttc aga ctc agc ctt gcc aat gtc tct	662
Val Gly Ala Arg Asn Tyr Leu Phe Arg Leu Ser Leu Ala Asn Val Ser	
140 145 150 155	
ctt ctt cag gcc aca gag tgg gcc tcc agt gag gac acg cgc cgc tcc	710
Leu Leu Gln Ala Thr Glu Trp Ala Ser Ser Glu Asp Thr Arg Arg Ser	
160 165 170	
tgc caa agc aaa ggg aag act gag gag gag tgt cag aac tac gtg cga	758
Cys Gln Ser Lys Gly Lys Thr Glu Glu Glu Cys Gln Asn Tyr Val Arg	
175 180 185	
gtc ctg atc gtc gcc ggc cgg aag gtg ttc atg tgt gga acc aat gcc	806
Val Leu Ile Val Ala Gly Arg Lys Val Phe Met Cys Gly Thr Asn Ala	
190 195 200	
ttt tcc ccc atg tgc acc agc aga cag gtg ggg aac ctc agc cgg act	854
Phe Ser Pro Met Cys Thr Ser Arg Gln Val Gly Asn Leu Ser Arg Thr	
205 210 215	
att gag aag atc aat ggt gtg gcc cgc tgc ccc tat gac cca cgc cac	902
Ile Glu Lys Ile Asn Gly Val Ala Arg Cys Pro Tyr Asp Pro Arg His	

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220	225	230	235	
aac tcc aca gct gtc atc tcc tcc cag ggg gag ctc tat gca gcc acg				950
Asn Ser Thr Ala Val Ile Ser Ser Gln Gly Glu Leu Tyr Ala Ala Thr				
	240	245	250	
gtc atc gac ttc tca ggt cgg gac cct gcc atc tac cgc agc ctg ggc				998
Val Ile Asp Phe Ser Gly Arg Asp Pro Ala Ile Tyr Arg Ser Leu Gly				
	255	260	265	
agt ggg cca ccg ctt cgc act gcc caa tat aac tcc aag tgg ctt aat				1046
Ser Gly Pro Pro Leu Arg Thr Ala Gln Tyr Asn Ser Lys Trp Leu Asn				
	270	275	280	
gag cca aac ttc gtg gca gcc tat gat att ggg ctg ttt gca tac ttc				1094
Glu Pro Asn Phe Val Ala Ala Tyr Asp Ile Gly Leu Phe Ala Tyr Phe				
	285	290	295	
ttc ctg cgg gag aac gca gtg gag cac gac tgt gga cgc acc gtg tac				1142
Phe Leu Arg Glu Asn Ala Val Glu His Asp Cys Gly Arg Thr Val Tyr				
300	305	310	315	
tct cgc gtg gcc cgc gtg tgc aag aat gac gtg ggg ggc cga ttc ctg				1190
Ser Arg Val Ala Arg Val Cys Lys Asn Asp Val Gly Gly Arg Phe Leu				
	320	325	330	
ctg gag gac aca tgg acc aca ttc atg aag gcc cgg ctc aac tgc tcc				1238
Leu Glu Asp Thr Trp Thr Thr Phe Met Lys Ala Arg Leu Asn Cys Ser				
	335	340	345	
cgc ccg ggc gag gtc ccc ttc tac tat aac gag ctg cag agt gcc ttc				1286
Arg Pro Gly Glu Val Pro Phe Tyr Tyr Asn Glu Leu Gln Ser Ala Phe				
	350	355	360	

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cac ttg ccg gag cag gac ctc atc tat gga gtt ttc aca acc aac gta 1334
 His Leu Pro Glu Gln Asp Leu Ile Tyr Gly Val Phe Thr Thr Asn Val
 365 370 375

aac agc atc gcg gct tct gct gtc tgc gcc ttc aac ctc agt gct atc 1382
 Asn Ser Ile Ala Ala Ser Ala Val Cys Ala Phe Asn Leu Ser Ala Ile
 380 385 390 395

tcc cag gct ttc aat ggc cca ttt cgc tac cag gag aac ccc agg gct 1430
 Ser Gln Ala Phe Asn Gly Pro Phe Arg Tyr Gln Glu Asn Pro Arg Ala
 400 405 410

gcc tgg ctc ccc ata gcc aac ccc atc ccc aat ttc cag tgt ggc acc 1478
 Ala Trp Leu Pro Ile Ala Asn Pro Ile Pro Asn Phe Gln Cys Gly Thr
 415 420 425

ctg cct gag acc ggt ccc aac gag aac ctg acg gag cgc agc ctg cag 1526
 Leu Pro Glu Thr Gly Pro Asn Glu Asn Leu Thr Glu Arg Ser Leu Gln
 430 435 440

gac gcg cag cgc ctc ttc ctg atg agc gag gcc gtg cag ccg gtg aca 1574
 Asp Ala Gln Arg Leu Phe Leu Met Ser Glu Ala Val Gln Pro Val Thr
 445 450 455

ccc gag ccc tgt gtc acc cag gac agc gtg cgc ttc tca cac ctc gtg 1622
 Pro Glu Pro Cys Val Thr Gln Asp Ser Val Arg Phe Ser His Leu Val
 460 465 470 475

gtg gac ctg gtg cag gct aaa gac acg ctc tac cat gta ctc tac att 1670
 Val Asp Leu Val Gln Ala Lys Asp Thr Leu Tyr His Val Leu Tyr Ile
 480 485 490

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ggc acc gag tgc ggc acc atc ctg aag gcg ctg tcc acg gcg agc cgc	1718
Gly Thr Glu Ser Gly Thr Ile Leu Lys Ala Leu Ser Thr Ala Ser Arg	
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agc ctc cac ggc tgc tac ctg gag gag ctg cac gtg ctg ccc ccc ggg	1766
Ser Leu His Gly Cys Tyr Leu Glu Glu Leu His Val Leu Pro Pro Gly	
510 515 520	
cgc cgc gag ccc ctg cgc agc ctg cgc atc ctg cac agc gcc cgc gcg	1814
Arg Arg Glu Pro Leu Arg Ser Leu Arg Ile Leu His Ser Ala Arg Ala	
525 530 535	
ctc ttc gtg ggg ctg aga gac ggc gtc ctg cgg gtc cca ctg gag agg	1862
Leu Phe Val Gly Leu Arg Asp Gly Val Leu Arg Val Pro Leu Glu Arg	
540 545 550 555	
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Cys Ala Ala Tyr Arg Ser Gln Gly Ala Cys Leu Gly Ala Arg Asp Pro	
560 565 570	
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Tyr Cys Gly Trp Asp Gly Lys Gln Gln Arg Cys Ser Thr Leu Glu Asp	
575 580 585	
agc tcc aac atg agc ctc tgg acc cag aac atc acc gcc tgt cct gtg	2006
Ser Ser Asn Met Ser Leu Trp Thr Gln Asn Ile Thr Ala Cys Pro Val	
590 595 600	
cgg aat gtg aca cgg gat ggg ggc ttc ggc cca tgg tca cca tgg caa	2054
Arg Asn Val Thr Arg Asp Gly Gly Phe Gly Pro Trp Ser Pro Trp Gln	
605 610 615	
cca tgt gag cac ttg gat ggg gac aac tca ggc tct tgc ctg tgt cga	2102

Pro Cys Glu His Leu Asp Gly Asp Asn Ser Gly Ser Cys Leu Cys Arg	
620 625 630 635	
gct cga tcc tgt gat tcc cct cga ccc cgc tgt ggg ggc ctt gac tgc	2150
Ala Arg Ser Cys Asp Ser Pro Arg Pro Arg Cys Gly Gly Leu Asp Cys	
640 645 650	
ctg ggg cca gcc atc cac atc gcc aac tgc tcc agg aat ggg gcg tgg	2198
Leu Gly Pro Ala Ile His Ile Ala Asn Cys Ser Arg Asn Gly Ala Trp	
655 660 665	
acc ccg tgg tca tcg tgg gcg ctg tgc agc acg tcc tgt ggc atc ggc	2246
Thr Pro Trp Ser Ser Trp Ala Leu Cys Ser Thr Ser Cys Gly Ile Gly	
670 675 680	
ttc cag gtc cgc cag cga agt tgc agc aac cct gct ccc cgc cac ggg	2294
Phe Gln Val Arg Gln Arg Ser Cys Ser Asn Pro Ala Pro Arg His Gly	
685 690 695	
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Gly Arg Ile Phe Val Gly Lys Ser Arg Glu Glu Arg Phe Cys Asn Glu	
700 705 710 715	
aac acg cct tgc ccg gtg ccc atc ttc tgg gct tcc tgg ggc tcc tgg	2390
Asn Thr Pro Cys Pro Val Pro Ile Phe Trp Ala Ser Trp Gly Ser Trp	
720 725 730	
agc aag tgc agc agc aac tgt gga ggg ggc atg cag tcg cgg cgt cgg	2438
Ser Lys Cys Ser Ser Asn Cys Gly Gly Gly Met Gln Ser Arg Arg Arg	
735 740 745	
gcc tgc gag aac ggc aac tcc tgc ctg ggc tgc ggc gag ttc aag acg	2486
Ala Cys Glu Asn Gly Asn Ser Cys Leu Gly Cys Gly Glu Phe Lys Thr	

30 / 102

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Cys Asn Pro Glu Gly Cys Pro Glu Val Arg Arg Asn Thr Pro Trp Thr			
765	770	775	
ccg tgg ctg ccc gtg aac gtg acg cag ggc ggg gca cgg cag gag cag			2582
Pro Trp Leu Pro Val Asn Val Thr Gln Gly Gly Ala Arg Gln Glu Gln			
780	785	790	795
cggttc cgc ttc acc tgc cgc gcg ccc ctt gca gac ccg cac ggc ctg			2630
Arg Phe Arg Phe Thr Cys Arg Ala Pro Leu Ala Asp Pro His Gly Leu			
800	805	810	
cag ttc ggc agg aga agg acc gag acg agg acc tgt ccc gcg gac ggc			2678
Gln Phe Gly Arg Arg Arg Thr Glu Thr Arg Thr Cys Pro Ala Asp Gly			
815	820	825	
tcc ggc tcc tgc gac acc gac gcc ctg gtg gag gtc ctc ctg cgc agc			2726
Ser Gly Ser Cys Asp Thr Asp Ala Leu Val Glu Val Leu Leu Arg Ser			
830	835	840	
ggg agc acc tcc ccg cac acg gtg agc ggg ggc tgg gcc gcc tgg ggc			2774
Gly Ser Thr Ser Pro His Thr Val Ser Gly Gly Trp Ala Ala Trp Gly			
845	850	855	
ccg tgg tcg tcc tgc tcc cgg gac tgc gag ctg ggc ttc cgc gtc cgc			2822
Pro Trp Ser Ser Cys Ser Arg Asp Cys Glu Leu Gly Phe Arg Val Arg			
860	865	870	875
aag aga acg tgc act aac ccg gag ccc cgc aac ggg ggc ctg ccc tgc			2870
Lys Arg Thr Cys Thr Asn Pro Glu Pro Arg Asn Gly Gly Leu Pro Cys			
880	885	890	

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gtg ggc gat gct gcc gag tac cag gac tgc aac ccc cag gct tgc cca 2918
 Val Gly Asp Ala Ala Glu Tyr Gln Asp Cys Asn Pro Gln Ala Cys Pro
 895 900 905

gtt cgg ggt gct tgg tcc tgc tgg acc tca tgg tct cca tgc tca gct 2966
 Val Arg Gly Ala Trp Ser Cys Trp Thr Ser Trp Ser Pro Cys Ser Ala
 910 915 920

tcc tgt ggt ggg ggt cac tat caa cgc acc cgt tcc tgc acc agc ccc 3014
 Ser Cys Gly Gly Gly His Tyr Gln Arg Thr Arg Ser Cys Thr Ser Pro
 925 930 935

gca ccc tcc cca ggt gag gac atc tgt ctc ggg ctg cac acg gag gag 3062
 Ala Pro Ser Pro Gly Glu Asp Ile Cys Leu Gly Leu His Thr Glu Glu
 940 945 950 955

gca cta tgt gcc aca cag gcc tgc cca ggc tgg tgc ccc tgg tct gag 3110
 Ala Leu Cys Ala Thr Gln Ala Cys Pro Gly Trp Ser Pro Trp Ser Glu
 960 965 970

tgg agt aag tgc act gac gac gga gcc cag agc cga agc cgg cac tgt 3158
 Trp Ser Lys Cys Thr Asp Asp Gly Ala Gln Ser Arg Ser Arg His Cys
 975 980 985

gag gag ctc ctc cca ggg tcc agc gcc tgt gct gga aac agc agc cag 3206
 Glu Glu Leu Leu Pro Gly Ser Ser Ala Cys Ala Gly Asn Ser Ser Gln
 990 995 1000

agc cgc ccc tgc ccc tac agc gag att ccc gtc atc ctg cca gcc 3251
 Ser Arg Pro Cys Pro Tyr Ser Glu Ile Pro Val Ile Leu Pro Ala
 1005 1010 1015

3 2 / 1 0 2

tcc agc	atg gag gag gcc acc	gac tgt gca ggt aaa	aga aac cgg	3296		
Ser Ser	Met Glu Glu Ala Thr	Asp Cys Ala Gly Lys	Arg Asn Arg			
1020	1025	1030				
acc tac	ctc atg ctg cgg tcc	tcc cag ccc tcc age	acc cca ctc	3341		
Thr Tyr	Leu Met Leu Arg Ser	Ser Gln Pro Ser Ser	Thr Pro Leu			
1035	1040	1045				
caa agt	ctg gac tct ttc cac	atc ctg ctc cag aca	gcc aag ctt	3386		
Gln Ser	Leu Asp Ser Phe His	Ile Leu Leu Gln Thr	Ala Lys Leu			
1050	1055	1060				
tgt tgg	ggt ccc cac tgc ttt	gag atg ggt tca atc	tca tcc act	3431		
Cys Trp	Gly Pro His Cys Phe	Glu Met Gly Ser Ile	Ser Ser Thr			
1065	1070	1075				
tgg tgg	cca cgg gca tct cct	gct tct tgg gct ctg	ggc tcc tga	3476		
Trp Trp	Pro Arg Ala Ser Pro	Ala Ser Trp Ala Leu	Gly Ser			
1080	1085	1090				
ccctagcagt	gtacctgtct	tgccagcact	gccagcgtca	gtcccaggag	tccacactgg	3536
tccatcctgc	cacccccaac	catttgcact	acaagggcgg	aggcaccccg	aagaatgaaa	3596
agtacacacc	catggaattc	aagaccctga	acaagaataa	cttgatccct	gatgacagag	3656
ccaacttcta	cccattgcag	cagaccaatg	tgtacacgac	tacttactac	ccaagccccc	3716
tgaacaaaca	cagcttcggg	cccgaggcct	cacctggaca	acggtgcttc	cccaacagct	3776
gataccgccg	tcttggggac	ttgggcttct	tgccttcata	aggcacagag	cagatggaga	3836
tgggacagtg	gagccagttt	ggttttctcc	ctctgcacta	ggccaagaac	ttgctgcctt	3896

3 3 / 1 0 2

gcctgtgggg ggtcccatcc ggcttcagag agctctggct ggcatigacc atgggggaaa 3956
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 gctggtacag gcctcagcct tgccctcaat gcacgaaagg tggcccagga gagaggatca 4496
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 gctcaacaga gttgttggct gagacctgct tgggagtctc tgctggccct tcatctgttc 4616
 aggaacacac acacacacac actcacacac gcacacacaa tcacaatttg ctacagcaac 4676
 aaaaaagaca ttgggctgtg gcattattaa ttaaagatga tatccagtc 4725

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<211> 1092

<212> PRT

3 4 / 1 0 2

<213> Homo sapiens

<400> 64

Met Pro Cys Gly Phe Ser Pro Ser Pro Val Ala His His Leu Val Pro
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Gly Pro Pro Asp Thr Pro Ala Gln Gln Leu Arg Cys Gly Trp Thr Val
 20 25 30

Gly Gly Trp Leu Leu Ser Leu Val Arg Gly Leu Leu Pro Cys Leu Pro
 35 40 45

Pro Gly Ala Arg Thr Ala Glu Gly Pro Ile Met Val Leu Ala Gly Pro
 50 55 60

Leu Ala Val Ser Leu Leu Leu Pro Ser Leu Thr Leu Leu Val Ser His
 65 70 75 80

Leu Ser Ser Ser Gln Asp Val Ser Ser Glu Pro Ser Ser Glu Gln Gln
 85 90 95

Leu Cys Ala Leu Ser Lys His Pro Thr Val Ala Phe Glu Asp Leu Gln
 100 105 110

Pro Trp Val Ser Asn Phe Thr Tyr Pro Gly Ala Arg Asp Phe Ser Gln

3 5 / 1 0 2

115

120

125

Leu Ala Leu Asp Pro Ser Gly Asn Gln Leu Ile Val Gly Ala Arg Asn
130 135 140

Tyr Leu Phe Arg Leu Ser Leu Ala Asn Val Ser Leu Leu Gln Ala Thr
145 150 155 160

Glu Trp Ala Ser Ser Glu Asp Thr Arg Arg Ser Cys Gln Ser Lys Gly
165 170 175

Lys Thr Glu Glu Glu Cys Gln Asn Tyr Val Arg Val Leu Ile Val Ala
180 185 190

Gly Arg Lys Val Phe Met Cys Gly Thr Asn Ala Phe Ser Pro Met Cys
195 200 205

Thr Ser Arg Gln Val Gly Asn Leu Ser Arg Thr Ile Glu Lys Ile Asn
210 215 220

Gly Val Ala Arg Cys Pro Tyr Asp Pro Arg His Asn Ser Thr Ala Val
225 230 235 240

Ile Ser Ser Gln Gly Glu Leu Tyr Ala Ala Thr Val Ile Asp Phe Ser
245 250 255

3 6 / 1 0 2

Gly Arg Asp Pro Ala Ile Tyr Arg Ser Leu Gly Ser Gly Pro Pro Leu
260 265 270

Arg Thr Ala Gln Tyr Asn Ser Lys Trp Leu Asn Glu Pro Asn Phe Val
275 280 285

Ala Ala Tyr Asp Ile Gly Leu Phe Ala Tyr Phe Phe Leu Arg Glu Asn
290 295 300

Ala Val Glu His Asp Cys Gly Arg Thr Val Tyr Ser Arg Val Ala Arg
305 310 315 320

Val Cys Lys Asn Asp Val Gly Gly Arg Phe Leu Leu Glu Asp Thr Trp
325 330 335

Thr Thr Phe Met Lys Ala Arg Leu Asn Cys Ser Arg Pro Gly Glu Val
340 345 350

Pro Phe Tyr Tyr Asn Glu Leu Gln Ser Ala Phe His Leu Pro Glu Gln
355 360 365

Asp Leu Ile Tyr Gly Val Phe Thr Thr Asn Val Asn Ser Ile Ala Ala
370 375 380

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Ser Ala Val Cys Ala Phe Asn Leu Ser Ala Ile Ser Gln Ala Phe Asn
385 390 395 400

Gly Pro Phe Arg Tyr Gln Glu Asn Pro Arg Ala Ala Trp Leu Pro Ile
405 410 415

Ala Asn Pro Ile Pro Asn Phe Gln Cys Gly Thr Leu Pro Glu Thr Gly
420 425 430

Pro Asn Glu Asn Leu Thr Glu Arg Ser Leu Gln Asp Ala Gln Arg Leu
435 440 445

Phe Leu Met Ser Glu Ala Val Gln Pro Val Thr Pro Glu Pro Cys Val
450 455 460

Thr Gln Asp Ser Val Arg Phe Ser His Leu Val Val Asp Leu Val Gln
465 470 475 480

Ala Lys Asp Thr Leu Tyr His Val Leu Tyr Ile Gly Thr Glu Ser Gly
485 490 495

Thr Ile Leu Lys Ala Leu Ser Thr Ala Ser Arg Ser Leu His Gly Cys
500 505 510

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Tyr Leu Glu Glu Leu His Val Leu Pro Pro Gly Arg Arg Glu Pro Leu
515 520 525

Arg Ser Leu Arg Ile Leu His Ser Ala Arg Ala Leu Phe Val Gly Leu
530 535 540

Arg Asp Gly Val Leu Arg Val Pro Leu Glu Arg Cys Ala Ala Tyr Arg
545 550 555 560

Ser Gln Gly Ala Cys Leu Gly Ala Arg Asp Pro Tyr Cys Gly Trp Asp
565 570 575

Gly Lys Gln Gln Arg Cys Ser Thr Leu Glu Asp Ser Ser Asn Met Ser
580 585 590

Leu Trp Thr Gln Asn Ile Thr Ala Cys Pro Val Arg Asn Val Thr Arg
595 600 605

Asp Gly Gly Phe Gly Pro Trp Ser Pro Trp Gln Pro Cys Glu His Leu
610 615 620

Asp Gly Asp Asn Ser Gly Ser Cys Leu Cys Arg Ala Arg Ser Cys Asp
625 630 635 640

Ser Pro Arg Pro Arg Cys Gly Gly Leu Asp Cys Leu Gly Pro Ala Ile

3 9 / 1 0 2

645

650

655

His Ile Ala Asn Cys Ser Arg Asn Gly Ala Trp Thr Pro Trp Ser Ser
660 665 670

Trp Ala Leu Cys Ser Thr Ser Cys Gly Ile Gly Phe Gln Val Arg Gln
675 680 685

Arg Ser Cys Ser Asn Pro Ala Pro Arg His Gly Gly Arg Ile Phe Val
690 695 700

Gly Lys Ser Arg Glu Glu Arg Phe Cys Asn Glu Asn Thr Pro Cys Pro
705 710 715 720

Val Pro Ile Phe Trp Ala Ser Trp Gly Ser Trp Ser Lys Cys Ser Ser
725 730 735

Asn Cys Gly Gly Gly Met Gln Ser Arg Arg Arg Ala Cys Glu Asn Gly
740 745 750

Asn Ser Cys Leu Gly Cys Gly Glu Phe Lys Thr Cys Asn Pro Glu Gly
755 760 765

Cys Pro Glu Val Arg Arg Asn Thr Pro Trp Thr Pro Trp Leu Pro Val
770 775 780

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Asn Val Thr Gln Gly Gly Ala Arg Gln Glu Gln Arg Phe Arg Phe Thr
785 790 795 800

Cys Arg Ala Pro Leu Ala Asp Pro His Gly Leu Gln Phe Gly Arg Arg
805 810 815

Arg Thr Glu Thr Arg Thr Cys Pro Ala Asp Gly Ser Gly Ser Cys Asp
820 825 830

Thr Asp Ala Leu Val Glu Val Leu Leu Arg Ser Gly Ser Thr Ser Pro
835 840 845

His Thr Val Ser Gly Gly Trp Ala Ala Trp Gly Pro Trp Ser Ser Cys
850 855 860

Ser Arg Asp Cys Glu Leu Gly Phe Arg Val Arg Lys Arg Thr Cys Thr
865 870 875 880

Asn Pro Glu Pro Arg Asn Gly Gly Leu Pro Cys Val Gly Asp Ala Ala
885 890 895

Glu Tyr Gln Asp Cys Asn Pro Gln Ala Cys Pro Val Arg Gly Ala Trp
900 905 910

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Ser Cys Trp Thr Ser Trp Ser Pro Cys Ser Ala Ser Cys Gly Gly Gly
915 920 925

His Tyr Gln Arg Thr Arg Ser Cys Thr Ser Pro Ala Pro Ser Pro Gly
930 935 940

Glu Asp Ile Cys Leu Gly Leu His Thr Glu Glu Ala Leu Cys Ala Thr
945 950 955 960

Gln Ala Cys Pro Gly Trp Ser Pro Trp Ser Glu Trp Ser Lys Cys Thr
965 970 975

Asp Asp Gly Ala Gln Ser Arg Ser Arg His Cys Glu Glu Leu Leu Pro
980 985 990

Gly Ser Ser Ala Cys Ala Gly Asn Ser Ser Gln Ser Arg Pro Cys Pro
995 1000 1005

Tyr Ser Glu Ile Pro Val Ile Leu Pro Ala Ser Ser Met Glu Glu
1010 1015 1020

Ala Thr Asp Cys Ala Gly Lys Arg Asn Arg Thr Tyr Leu Met Leu
1025 1030 1035

4 2 / 1 0 2

Arg Ser Ser Gln Pro Ser Ser Thr Pro Leu Gln Ser Leu Asp Ser
 1040 1045 1050

Phe His Ile Leu Leu Gln Thr Ala Lys Leu Cys Trp Gly Pro His
 1055 1060 1065

Cys Phe Glu Met Gly Ser Ile Ser Ser Thr Trp Trp Pro Arg Ala
 1070 1075 1080

Ser Pro Ala Ser Trp Ala Leu Gly Ser
 1085 1090

<210> 65

<211> 4494

<212> DNA

<213> Homo sapiens

<220>

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<222> (71).. (3526)

<400> 65

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tgtctcttgg atg ccc tgt ggc ttc agt ccg tct cct gtt gcc cac cac 109

Met Pro Cys Gly Phe Ser Pro Ser Pro Val Ala His His

1

5

10

4 3 / 1 0 2

ctc gtc cct ggg ccg cct gat acc cca gcc caa cag cta agg tgt gga	157
Leu Val Pro Gly Pro Pro Asp Thr Pro Ala Gln Gln Leu Arg Cys Gly	
15 20 25	
tggtg aca gta ggg ggc tgg ctt ctc tca ctg gtc agg ggt ctt ctc ccc	205
Trp Thr Val Gly Gly Trp Leu Leu Ser Leu Val Arg Gly Leu Leu Pro	
30 35 40 45	
tgt ctg cct ccc gga gct agg act gca gag ggg cct atc atg gtg ctt	253
Cys Leu Pro Pro Gly Ala Arg Thr Ala Glu Gly Pro Ile Met Val Leu	
50 55 60	
gca ggc ccc ctg gct gtc tcg ctg ttg ctg ccc agc ctc aca ctg ctg	301
Ala Gly Pro Leu Ala Val Ser Leu Leu Leu Pro Ser Leu Thr Leu Leu	
65 70 75	
gtg tcc cac ctc tcc agc tcc cag gat gtc tcc agt gag ccc agc agt	349
Val Ser His Leu Ser Ser Ser Gln Asp Val Ser Ser Glu Pro Ser Ser	
80 85 90	
gag cag cag ctg tgc gcc ctt agc aag cac ccc acc gtg gcc ttt gaa	397
Glu Gln Gln Leu Cys Ala Leu Ser Lys His Pro Thr Val Ala Phe Glu	
95 100 105	
gac ctg cag ccg tgg gtc tct aac ttc acc tac cct gga gcc cgg gat	445
Asp Leu Gln Pro Trp Val Ser Asn Phe Thr Tyr Pro Gly Ala Arg Asp	
110 115 120 125	
ttc tcc cag ctg gct ttg gac ccc tcc ggg aac cag ctc atc gtg gga	493
Phe Ser Gln Leu Ala Leu Asp Pro Ser Gly Asn Gln Leu Ile Val Gly	
130 135 140	
gcc agg aac tac ctc ttc aga ctc agc ctt gcc aat gtc tct ctt ctt	541

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Ala Arg Asn Tyr Leu Phe Arg Leu Ser Leu Ala Asn Val Ser Leu Leu	
145 150 155	
cag gcc aca gag tgg gcc tcc agt gag gac acg cgc cgc tcc tgc caa	589
Gln Ala Thr Glu Trp Ala Ser Ser Glu Asp Thr Arg Arg Ser Cys Gln	
160 165 170	
agc aaa ggg aag act gag gag gag tgt cag aac tac gtg cga gtc ctg	637
Ser Lys Gly Lys Thr Glu Glu Glu Cys Gln Asn Tyr Val Arg Val Leu	
175 180 185	
atc gtc gcc ggc cgg aag gtg ttc atg tgt gga acc aat gcc ttt tcc	685
Ile Val Ala Gly Arg Lys Val Phe Met Cys Gly Thr Asn Ala Phe Ser	
190 195 200 205	
ccc atg tgc acc agc aga cag gtg ggg aac ctc agc cgg act att gag	733
Pro Met Cys Thr Ser Arg Gln Val Gly Asn Leu Ser Arg Thr Ile Glu	
210 215 220	
aag atc aat ggt gtg gcc cgc tgc ccc tat gac cca cgc cac aac tcc	781
Lys Ile Asn Gly Val Ala Arg Cys Pro Tyr Asp Pro Arg His Asn Ser	
225 230 235	
aca gct gtc atc tcc tcc cag ggg gag ctc tat gca gcc acg gtc atc	829
Thr Ala Val Ile Ser Ser Gln Gly Glu Leu Tyr Ala Ala Thr Val Ile	
240 245 250	
gac ttc tca ggt cgg gac cct gcc atc tac cgc agc ctg ggc agt ggg	877
Asp Phe Ser Gly Arg Asp Pro Ala Ile Tyr Arg Ser Leu Gly Ser Gly	
255 260 265	
cca ccg ctt cgc act gcc caa tat aac tcc aag tgg ctt aat gag cca	925
Pro Pro Leu Arg Thr Ala Gln Tyr Asn Ser Lys Trp Leu Asn Glu Pro	

4 5 / 1 0 2

270	275	280	285	
aac ttc gtg gca gcc tat gat att ggg ctg ttt gca tac ttc ttc ctg				973
Asn Phe Val Ala Ala Tyr Asp Ile Gly Leu Phe Ala Tyr Phe Phe Leu				
	290	295	300	
cgg gag aac gca gtg gag cac gac tgt gga cgc acc gtg tac tct cgc				1021
Arg Glu Asn Ala Val Glu His Asp Cys Gly Arg Thr Val Tyr Ser Arg				
	305	310	315	
gtg gcc cgc gtg tgc aag aat gac gtg ggg ggc cga ttc ctg ctg gag				1069
Val Ala Arg Val Cys Lys Asn Asp Val Gly Gly Arg Phe Leu Leu Glu				
	320	325	330	
gac aca tgg acc aca ttc atg aag gcc cgg ctc aac tgc tcc cgc ccg				1117
Asp Thr Trp Thr Thr Phe Met Lys Ala Arg Leu Asn Cys Ser Arg Pro				
	335	340	345	
ggc gag gtc ccc ttc tac tat aac gag ctg cag agt gcc ttc cac ttg				1165
Gly Glu Val Pro Phe Tyr Tyr Asn Glu Leu Gln Ser Ala Phe His Leu				
	350	355	360	365
ccg gag cag gac ctc atc tat gga gtt ttc aca acc aac gta aac agc				1213
Pro Glu Gln Asp Leu Ile Tyr Gly Val Phe Thr Thr Asn Val Asn Ser				
	370	375	380	
att gcg gct tct gct gtc tgc gcc ttc aac ctc agt gct atc tcc cag				1261
Ile Ala Ala Ser Ala Val Cys Ala Phe Asn Leu Ser Ala Ile Ser Gln				
	385	390	395	
gct ttc aat ggc cca ttt cgc tac cag gag aac ccc agg gct gcc tgg				1309
Ala Phe Asn Gly Pro Phe Arg Tyr Gln Glu Asn Pro Arg Ala Ala Trp				
	400	405	410	

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ctc ccc ata gcc aac ccc atc ccc aat ttc cag tgt ggc acc ctg cct	1357
Leu Pro Ile Ala Asn Pro Ile Pro Asn Phe Gln Cys Gly Thr Leu Pro	
415 420 425	
gag acc ggt ccc aac gag aac ctg acg gag cgc agc ctg cag gac gcg	1405
Glu Thr Gly Pro Asn Glu Asn Leu Thr Glu Arg Ser Leu Gln Asp Ala	
430 435 440 445	
cag cgc ctc ttc ctg atg agc gag gcc gtg cag ccg gtg aca ccc gag	1453
Gln Arg Leu Phe Leu Met Ser Glu Ala Val Gln Pro Val Thr Pro Glu	
450 455 460	
ccc tgt gtc acc cag gac agc gtg cgc ttc tca cac ctc gtg gtg gac	1501
Pro Cys Val Thr Gln Asp Ser Val Arg Phe Ser His Leu Val Val Asp	
465 470 475	
ctg gtg cag gct aaa gac acg ctc tac cat gta ctc tac att ggc acc	1549
Leu Val Gln Ala Lys Asp Thr Leu Tyr His Val Leu Tyr Ile Gly Thr	
480 485 490	
gag tcg ggc acc atc ctg aag gcg ctg tcc acg gcg agc cgc agc ctc	1597
Glu Ser Gly Thr Ile Leu Lys Ala Leu Ser Thr Ala Ser Arg Ser Leu	
495 500 505	
cac ggc tgc tac ctg gag gag ctg cac gtg ctg ccc ccc ggg cgc cgc	1645
His Gly Cys Tyr Leu Glu Glu Leu His Val Leu Pro Pro Gly Arg Arg	
510 515 520 525	
gag ccc ctg cgc agc ctg cgc atc ctg cac agc gcc cgc gcg ctc ttc	1693
Glu Pro Leu Arg Ser Leu Arg Ile Leu His Ser Ala Arg Ala Leu Phe	
530 535 540	

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gtg ggg ctg aga gac ggc gtc ctg cgg gtc cca ctg gag agg tgc gcc	1741
Val Gly Leu Arg Asp Gly Val Leu Arg Val Pro Leu Glu Arg Cys Ala	
545 550 555	
gcc tac cgc agc cag ggg gca tgc ctg ggg gcc cgg gac ccg tac tgt	1789
Ala Tyr Arg Ser Gln Gly Ala Cys Leu Gly Ala Arg Asp Pro Tyr Cys	
560 565 570	
ggc tgg gac ggg aag cag caa cgt tgc agc aca ctc gag gac agc tcc	1837
Gly Trp Asp Gly Lys Gln Gln Arg Cys Ser Thr Leu Glu Asp Ser Ser	
575 580 585	
aac atg agc ctc tgg acc cag aac atc acc gcc tgt cct gtg cgg aat	1885
Asn Met Ser Leu Trp Thr Gln Asn Ile Thr Ala Cys Pro Val Arg Asn	
590 595 600 605	
gtg aca cgg gat ggg ggc ttc ggc cca tgg tca cca tgg caa cca tgt	1933
Val Thr Arg Asp Gly Gly Phe Gly Pro Trp Ser Pro Trp Gln Pro Cys	
610 615 620	
gag cac ttg gat ggg gac aac tca ggc tct tgc ctg tgt cga gct cga	1981
Glu His Leu Asp Gly Asp Asn Ser Gly Ser Cys Leu Cys Arg Ala Arg	
625 630 635	
tcc tgt gat tcc cct cga ccc cgc tgt ggg ggc ctt gac tgc ctg ggg	2029
Ser Cys Asp Ser Pro Arg Pro Arg Cys Gly Gly Leu Asp Cys Leu Gly	
640 645 650	
cca gcc atc cac atc gcc aac tgc tcc agg aat ggg gcg tgg acc ccg	2077
Pro Ala Ile His Ile Ala Asn Cys Ser Arg Asn Gly Ala Trp Thr Pro	
655 660 665	
tgg tca tcg tgg gcg ctg tgc agc acg tcc tgt ggc atc ggc ttc cag	2125

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Trp Ser Ser Trp Ala Leu Cys Ser Thr Ser Cys Gly Ile Gly Phe Gln	
670 675 680 685	
gtc cgc cag cga agt tgc agc aac cct gct ccc cgc cac ggg ggc cgc	2173
Val Arg Gln Arg Ser Cys Ser Asn Pro Ala Pro Arg His Gly Gly Arg	
690 695 700	
atc tgc gtg ggc aag agc cgg gag gaa cgg ttc tgt aat gag aac acg	2221
Ile Cys Val Gly Lys Ser Arg Glu Glu Arg Phe Cys Asn Glu Asn Thr	
705 710 715	
cct tgc ccg gtg ccc atc ttc tgg gct tcc tgg ggc tcc tgg agc aag	2269
Pro Cys Pro Val Pro Ile Phe Trp Ala Ser Trp Gly Ser Trp Ser Lys	
720 725 730	
tgc agc agc aac tgt gga ggg ggc atg cag tcg cgg cgt cgg gcc tgc	2317
Cys Ser Ser Asn Cys Gly Gly Gly Met Gln Ser Arg Arg Arg Ala Cys	
735 740 745	
gag aac ggc aac tcc tgc ctg ggc tgc ggc gtg gag ttc aag acg tgc	2365
Glu Asn Gly Asn Ser Cys Leu Gly Cys Gly Val Glu Phe Lys Thr Cys	
750 755 760 765	
aac ccc gag ggc tgc ccc gaa gtg cgg cgc aac acc ccc tgg acg ccg	2413
Asn Pro Glu Gly Cys Pro Glu Val Arg Arg Asn Thr Pro Trp Thr Pro	
770 775 780	
tgg ctg ccc gtg aac gtg acg cag ggc ggg gca cgg cag gag cag cgg	2461
Trp Leu Pro Val Asn Val Thr Gln Gly Gly Ala Arg Gln Glu Gln Arg	
785 790 795	
ttc cgc ttc acc tgc cgc gcg ccc ctt gca gac ccg cac ggc ctg cag	2509
Phe Arg Phe Thr Cys Arg Ala Pro Leu Ala Asp Pro His Gly Leu Gln	

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800	805	810	
ttc ggc agg aga agg acc gag acg agg acc tgt ccc gcg gac ggc tcc Phe Gly Arg Arg Arg Thr Glu Thr Arg Thr Cys Pro Ala Asp Gly Ser			2557
815	820	825	
ggc tcc tgc gac acc gac gcc ctg gtg gag gac ctc ctg cgc agc ggg Gly Ser Cys Asp Thr Asp Ala Leu Val Glu Asp Leu Leu Arg Ser Gly			2605
830	835	840	845
agc acc tcc ccg cac acg gtg agc ggg ggc tgg gcc gcc tgg ggc ccg Ser Thr Ser Pro His Thr Val Ser Gly Gly Trp Ala Ala Trp Gly Pro			2653
850	855	860	
tgg tcg tcc tgc tcc cgg gac tgc gag ctg ggc ttc cgc gtc cgc aag Trp Ser Ser Cys Ser Arg Asp Cys Glu Leu Gly Phe Arg Val Arg Lys			2701
865	870	875	
aga acg tgc act aac ccg gag ccc cgc aac ggg ggc ctg ccc tgc gtg Arg Thr Cys Thr Asn Pro Glu Pro Arg Asn Gly Gly Leu Pro Cys Val			2749
880	885	890	
ggc gat gct gcc gag tac cag gac tgc aac ccc cag gct tgc cca gtt Gly Asp Ala Ala Glu Tyr Gln Asp Cys Asn Pro Gln Ala Cys Pro Val			2797
895	900	905	
cgg ggt gct tgg tcc tgc tgg acc tca tgg tct cca tgc tca gct tcc Arg Gly Ala Trp Ser Cys Trp Thr Ser Trp Ser Pro Cys Ser Ala Ser			2845
910	915	920	925
tgt ggt ggg ggt cac tat caa cgc acc cgt tcc tgc acc agc ccc gca Cys Gly Gly Gly His Tyr Gln Arg Thr Arg Ser Cys Thr Ser Pro Ala			2893
930	935	940	

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ccc tcc cca ggt gag gac atc tgt ctc ggg ctg cac acg gag gag gca	2941
Pro Ser Pro Gly Glu Asp Ile Cys Leu Gly Leu His Thr Glu Glu Ala	
945 950 955	
cta tgt gcc aca cag gcc tgc cca gaa ggc tgg tcg ccc tgg tct gag	2989
Leu Cys Ala Thr Gln Ala Cys Pro Glu Gly Trp Ser Pro Trp Ser Glu	
960 965 970	
tgg agt aag tgc act gac gac gga gcc cag agc cga agc cgg cac tgt	3037
Trp Ser Lys Cys Thr Asp Asp Gly Ala Gln Ser Arg Ser Arg His Cys	
975 980 985	
gag gag ctc ctc cca ggg tcc agc gcc tgt gct gga aac agc agc cag	3085
Glu Glu Leu Leu Pro Gly Ser Ser Ala Cys Ala Gly Asn Ser Ser Gln	
990 995 1000 1005	
agc cgc ccc tgc ccc tac agc gag att ccc gtc atc ctg cca gcc	3130
Ser Arg Pro Cys Pro Tyr Ser Glu Ile Pro Val Ile Leu Pro Ala	
1010 1015 1020	
tcc agc atg gag gag gcc acc ggc tgt gca ggg ttc aat ctc atc	3175
Ser Ser Met Glu Glu Ala Thr Gly Cys Ala Gly Phe Asn Leu Ile	
1025 1030 1035	
cac ttg gtg gcc acg ggc atc tcc tgc ttc ttg ggc tct ggg ctc	3220
His Leu Val Ala Thr Gly Ile Ser Cys Phe Leu Gly Ser Gly Leu	
1040 1045 1050	
ctg acc cta gca gtg tac ctg tct tgc cag cac tgc cag cgt cag	3265
Leu Thr Leu Ala Val Tyr Leu Ser Cys Gln His Cys Gln Arg Gln	
1055 1060 1065	

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tcc cag gag tcc aca	ctg gtc cat cct gcc	acc ccc aac cat ttg	3310
Ser Gln Glu Ser Thr	Leu Val His Pro Ala	Thr Pro Asn His Leu	
1070	1075	1080	
cac tac aag ggc gga	ggc acc ccg aag aat	gaa aag tac aca ccc	3355
His Tyr Lys Gly Gly	Gly Thr Pro Lys Asn	Glu Lys Tyr Thr Pro	
1085	1090	1095	
atg gaa ttc aag acc	ctg aac aag aat aac	ttg atc cct gat gac	3400
Met Glu Phe Lys Thr	Leu Asn Lys Asn Asn	Leu Ile Pro Asp Asp	
1100	1105	1110	
aga gcc aac ttc tac	cca ttg cag cag acc	aat gtg tac acg act	3445
Arg Ala Asn Phe Tyr	Pro Leu Gln Gln Thr	Asn Val Tyr Thr Thr	
1115	1120	1125	
act tac tac cca agc	ccc ctg aac aaa cac	agc ttc cgg ccc gag	3490
Thr Tyr Tyr Pro Ser	Pro Leu Asn Lys His	Ser Phe Arg Pro Glu	
1130	1135	1140	
gcc tca cct gga caa	cgg tgc ttc ccc aac	agc tga taccgccgtc	3536
Ala Ser Pro Gly Gln	Arg Cys Phe Pro Asn	Ser	
1145	1150		
ctggggactt gggcttcttg	ccttcataag gcacagagca	gatggagatg ggacagtgga	3596
gccagtttgg ttttctccct	ctgcactagg ccaagaactt	gctgccttgc ctgtgggggg	3656
tcccatccgg cttcagagag	ctctggctgg cattgaccat	gggggaaagg gctggtttca	3716
ggctgacata tggccgcagg	tccagttcag cccaggtctc	tcatggttat cttccaaccc	3776
actgtcacgc tgacactatg	ctgccatgcc tgggctgtgg	acctactggg catttgagga	3836

5 2 / 1 0 2

attggagaat ggagatggca agagggcagg cttttaagtt tgggttggag acaacttcct 3896
 gtggccccc caagctgagt ctggccttct ccagctggcc ccaaaaaagg cctttgctac 3956
 atcctgatta tctctgaaag taatcaatca agtggctcca gtagctctgg attttctgcc 4016
 agggctgggc cattgtggtg ctgcccagat atgacatggg accaaggcca gcgcaggtta 4076
 ttcacctctg cctggaagtc tatactctac ccagggcatc cctctggtca gaggcagtga 4136
 gtactgggaa ctggaggctg acctgtgctt agaagtcctt taatctgggc tggtacaggc 4196
 ctcagccttg ccctcaatgc acgaaagggtg gcccaggaga gaggatcaat gccataggag 4256
 gcagaagtct ggctctctg cctctatgga gactatcttc cagttgctgc tcaacagagt 4316
 tgttggctga gacctgcttg ggagtctctg ctggcccttc atctgttcag gaacacacac 4376
 acacacacac tcacacacgc acacacaatc acaatttgct acagcaacaa aaaagacatt 4436
 gggctgtggc attattaatt aaagatgata tccagtctca aaaaaaaaaa aaaaaaaaaa 4494

<210> 66

<211> 1151

<212> PRT

<213> Homo sapiens

<400> 66

Met Pro Cys Gly Phe Ser Pro Ser Pro Val Ala His His Leu Val Pro

1

5

10

15

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Gly Pro Pro Asp Thr Pro Ala Gln Gln Leu Arg Cys Gly Trp Thr Val
20 25 30

Gly Gly Trp Leu Leu Ser Leu Val Arg Gly Leu Leu Pro Cys Leu Pro
35 40 45

Pro Gly Ala Arg Thr Ala Glu Gly Pro Ile Met Val Leu Ala Gly Pro
50 55 60

Leu Ala Val Ser Leu Leu Leu Pro Ser Leu Thr Leu Leu Val Ser His
65 70 75 80

Leu Ser Ser Ser Gln Asp Val Ser Ser Glu Pro Ser Ser Glu Gln Gln
85 90 95

Leu Cys Ala Leu Ser Lys His Pro Thr Val Ala Phe Glu Asp Leu Gln
100 105 110

Pro Trp Val Ser Asn Phe Thr Tyr Pro Gly Ala Arg Asp Phe Ser Gln
115 120 125

Leu Ala Leu Asp Pro Ser Gly Asn Gln Leu Ile Val Gly Ala Arg Asn
130 135 140

5 4 / 1 0 2

Tyr Leu Phe Arg Leu Ser Leu Ala Asn Val Ser Leu Leu Gln Ala Thr
145 150 155 160

Glu Trp Ala Ser Ser Glu Asp Thr Arg Arg Ser Cys Gln Ser Lys Gly
165 170 175

Lys Thr Glu Glu Glu Cys Gln Asn Tyr Val Arg Val Leu Ile Val Ala
180 185 190

Gly Arg Lys Val Phe Met Cys Gly Thr Asn Ala Phe Ser Pro Met Cys
195 200 205

Thr Ser Arg Gln Val Gly Asn Leu Ser Arg Thr Ile Glu Lys Ile Asn
210 215 220

Gly Val Ala Arg Cys Pro Tyr Asp Pro Arg His Asn Ser Thr Ala Val
225 230 235 240

Ile Ser Ser Gln Gly Glu Leu Tyr Ala Ala Thr Val Ile Asp Phe Ser
245 250 255

Gly Arg Asp Pro Ala Ile Tyr Arg Ser Leu Gly Ser Gly Pro Pro Leu
260 265 270

5 5 / 1 0 2

Arg Thr Ala Gln Tyr Asn Ser Lys Trp Leu Asn Glu Pro Asn Phe Val
275 280 285

Ala Ala Tyr Asp Ile Gly Leu Phe Ala Tyr Phe Phe Leu Arg Glu Asn
290 295 300

Ala Val Glu His Asp Cys Gly Arg Thr Val Tyr Ser Arg Val Ala Arg
305 310 315 320

Val Cys Lys Asn Asp Val Gly Gly Arg Phe Leu Leu Glu Asp Thr Trp
325 330 335

Thr Thr Phe Met Lys Ala Arg Leu Asn Cys Ser Arg Pro Gly Glu Val
340 345 350

Pro Phe Tyr Tyr Asn Glu Leu Gln Ser Ala Phe His Leu Pro Glu Gln
355 360 365

Asp Leu Ile Tyr Gly Val Phe Thr Thr Asn Val Asn Ser Ile Ala Ala
370 375 380

Ser Ala Val Cys Ala Phe Asn Leu Ser Ala Ile Ser Gln Ala Phe Asn
385 390 395 400

Gly Pro Phe Arg Tyr Gln Glu Asn Pro Arg Ala Ala Trp Leu Pro Ile

5 6 / 1 0 2

405

410

415

Ala Asn Pro Ile Pro Asn Phe Gln Cys Gly Thr Leu Pro Glu Thr Gly

420

425

430

Pro Asn Glu Asn Leu Thr Glu Arg Ser Leu Gln Asp Ala Gln Arg Leu

435

440

445

Phe Leu Met Ser Glu Ala Val Gln Pro Val Thr Pro Glu Pro Cys Val

450

455

460

Thr Gln Asp Ser Val Arg Phe Ser His Leu Val Val Asp Leu Val Gln

465

470

475

480

Ala Lys Asp Thr Leu Tyr His Val Leu Tyr Ile Gly Thr Glu Ser Gly

485

490

495

Thr Ile Leu Lys Ala Leu Ser Thr Ala Ser Arg Ser Leu His Gly Cys

500

505

510

Tyr Leu Glu Glu Leu His Val Leu Pro Pro Gly Arg Arg Glu Pro Leu

515

520

525

Arg Ser Leu Arg Ile Leu His Ser Ala Arg Ala Leu Phe Val Gly Leu

530

535

540

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Arg Asp Gly Val Leu Arg Val Pro Leu Glu Arg Cys Ala Ala Tyr Arg
545 550 555 560

Ser Gln Gly Ala Cys Leu Gly Ala Arg Asp Pro Tyr Cys Gly Trp Asp
565 570 575

Gly Lys Gln Gln Arg Cys Ser Thr Leu Glu Asp Ser Ser Asn Met Ser
580 585 590

Leu Trp Thr Gln Asn Ile Thr Ala Cys Pro Val Arg Asn Val Thr Arg
595 600 605

Asp Gly Gly Phe Gly Pro Trp Ser Pro Trp Gln Pro Cys Glu His Leu
610 615 620

Asp Gly Asp Asn Ser Gly Ser Cys Leu Cys Arg Ala Arg Ser Cys Asp
625 630 635 640

Ser Pro Arg Pro Arg Cys Gly Gly Leu Asp Cys Leu Gly Pro Ala Ile
645 650 655

His Ile Ala Asn Cys Ser Arg Asn Gly Ala Trp Thr Pro Trp Ser Ser
660 665 670

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Trp Ala Leu Cys Ser Thr Ser Cys Gly Ile Gly Phe Gln Val Arg Gln
675 680 685

Arg Ser Cys Ser Asn Pro Ala Pro Arg His Gly Gly Arg Ile Cys Val
690 695 700

Gly Lys Ser Arg Glu Glu Arg Phe Cys Asn Glu Asn Thr Pro Cys Pro
705 710 715 720

Val Pro Ile Phe Trp Ala Ser Trp Gly Ser Trp Ser Lys Cys Ser Ser
725 730 735

Asn Cys Gly Gly Gly Met Gln Ser Arg Arg Arg Ala Cys Glu Asn Gly
740 745 750

Asn Ser Cys Leu Gly Cys Gly Val Glu Phe Lys Thr Cys Asn Pro Glu
755 760 765

Gly Cys Pro Glu Val Arg Arg Asn Thr Pro Trp Thr Pro Trp Leu Pro
770 775 780

Val Asn Val Thr Gln Gly Gly Ala Arg Gln Glu Gln Arg Phe Arg Phe
785 790 795 800

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Thr Cys Arg Ala Pro Leu Ala Asp Pro His Gly Leu Gln Phe Gly Arg
805 810 815

Arg Arg Thr Glu Thr Arg Thr Cys Pro Ala Asp Gly Ser Gly Ser Cys
820 825 830

Asp Thr Asp Ala Leu Val Glu Asp Leu Leu Arg Ser Gly Ser Thr Ser
835 840 845

Pro His Thr Val Ser Gly Gly Trp Ala Ala Trp Gly Pro Trp Ser Ser
850 855 860

Cys Ser Arg Asp Cys Glu Leu Gly Phe Arg Val Arg Lys Arg Thr Cys
865 870 875 880

Thr Asn Pro Glu Pro Arg Asn Gly Gly Leu Pro Cys Val Gly Asp Ala
885 890 895

Ala Glu Tyr Gln Asp Cys Asn Pro Gln Ala Cys Pro Val Arg Gly Ala
900 905 910

Trp Ser Cys Trp Thr Ser Trp Ser Pro Cys Ser Ala Ser Cys Gly Gly
915 920 925

Gly His Tyr Gln Arg Thr Arg Ser Cys Thr Ser Pro Ala Pro Ser Pro

6 0 / 1 0 2

930

935

940

Gly Glu Asp Ile Cys Leu Gly Leu His Thr Glu Glu Ala Leu Cys Ala
945 950 955 960

Thr Gln Ala Cys Pro Glu Gly Trp Ser Pro Trp Ser Glu Trp Ser Lys
965 970 975

Cys Thr Asp Asp Gly Ala Gln Ser Arg Ser Arg His Cys Glu Glu Leu
980 985 990

Leu Pro Gly Ser Ser Ala Cys Ala Gly Asn Ser Ser Gln Ser Arg Pro
995 1000 1005

Cys Pro Tyr Ser Glu Ile Pro Val Ile Leu Pro Ala Ser Ser Met
1010 1015 1020

Glu Glu Ala Thr Gly Cys Ala Gly Phe Asn Leu Ile His Leu Val
1025 1030 1035

Ala Thr Gly Ile Ser Cys Phe Leu Gly Ser Gly Leu Leu Thr Leu
1040 1045 1050

Ala Val Tyr Leu Ser Cys Gln His Cys Gln Arg Gln Ser Gln Glu
1055 1060 1065

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Ser Thr Leu Val His Pro Ala Thr Pro Asn His Leu His Tyr Lys
1070 1075 1080

Gly Gly Gly Thr Pro Lys Asn Glu Lys Tyr Thr Pro Met Glu Phe
1085 1090 1095

Lys Thr Leu Asn Lys Asn Asn Leu Ile Pro Asp Asp Arg Ala Asn
1100 1105 1110

Phe Tyr Pro Leu Gln Gln Thr Asn Val Tyr Thr Thr Thr Tyr Tyr
1115 1120 1125

Pro Ser Pro Leu Asn Lys His Ser Phe Arg Pro Glu Ala Ser Pro
1130 1135 1140

Gly Gln Arg Cys Phe Pro Asn Ser
1145 1150

<210> 67

<211> 20

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer sequence for RT-PCR

6 2 / 1 0 2

<400> 67

cctgaagggt gcctagttga

20

<210> 68

<211> 22

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer sequence for RT-PCR

<400> 68

actcaaaaac atccacaggt ga

22

<210> 69

<211> 20

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer sequence for RT-PCR

<400> 69

ggctgtggtg cagtaaccat

20

<210> 70

<211> 25

<212> DNA

<213> Artificial

6 3 / 1 0 2

<220>

<223> An artificially synthesized primer sequence for RT-PCR

<400> 70

catctacaaa gtaatgettc ccagt

25

<210> 71

<211> 20

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer sequence for RT-PCR

<400> 71

tccctcacgt tattggaagc

20

<210> 72

<211> 20

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer sequence for RT-PCR

<400> 72

tccacatcct tcctcaaagg

20

<210> 73

6 4 / 1 0 2

<211> 20

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer sequence for RT-PCR

<400> 73

atgatggcca ttttgatgct

20

<210> 74

<211> 20

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer sequence for RT-PCR

<400> 74

gctctccacg ttggtaggtc

20

<210> 75

<211> 20

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer sequence for RT-PCR

<400> 75

caccaatggc tctgttgtgt

20

6 5 / 1 0 2

<210> 76

<211> 23

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer sequence for RT-PCR

<400> 76

taagagctca gcctttattg tgg

23

<210> 77

<211> 23

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer sequence for RT-PCR

<400> 77

gctactacat gattggtgag cag

23

<210> 78

<211> 23

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer sequence for RT-PCR

6 6 / 1 0 2

<400> 78

ctttaattga ggtcacaggc atc

23

<210> 79

<211> 51

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized oligonucleotide for siRNA

<400> 79

caccgcagca acgttgcagc acattcaaga gatgtgctgc aacgttgctg c

51

<210> 80

<211> 51

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized oligonucleotide for siRNA

<400> 80

aaaagcagca acgttgcagc acatctcttg aatgtgctgc aacgttgctg c

51

<210> 81

<211> 19

<212> DNA

<213> Artificial

6 7 / 1 0 2

<220>

<223> A target sequence for siRNA

<400> 81

gcagcaacgt tgcagcaca

19

<210> 82

<211> 47

<212> DNA

<213> Artificial

<220>

<223> A hairpin structure of siRNA

<400> 82

gcagcaacgt tgcagcacat tcaagagatg tgctgcaacg ttgctgc

47

<210> 83

<211> 19

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer sequence for RT-PCR

<400> 83

ctggaaacag cagccagag

19

<210> 84

6 8 / 1 0 2

<211> 20

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer sequence for RT-PCR

<400> 84

cagtgcctggc aagacaggta

20

<210> 85

<211> 20

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer sequence for RT-PCR

<400> 85

aacttagagg tggggagcag

20

<210> 86

<211> 22

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer sequence for RT-PCR

<400> 86

cacaaccatg ccttacttta tc

22

69/102

<210> 87

<211> 23

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer sequence for RT-PCR

<400> 87

gcaactccta actccccctct gta

23

<210> 88

<211> 23

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer sequence for RT-PCR

<400> 88

cagagagcca gagagtgaga gag

23

<210> 89

<211> 23

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer sequence for RT-PCR

7 0 / 1 0 2

<400> 89

gcatatacag gagaatgagg tcg

23

<210> 90

<211> 19

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer sequence for RT-PCR

<400> 90

ggagtgggaa ccgcagaac

19

<210> 91

<211> 20

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer sequence for RT-PCR

<400> 91

gacccccacc ttcaaatac

20

<210> 92

<211> 22

<212> DNA

<213> Artificial

7 1 / 1 0 2

<220>

<223> An artificially synthesized primer sequence for RT-PCR

<400> 92

gctcgtgctc gacaggtgtg ta

22

<210> 93

<211> 18

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer sequence for RT-PCR

<400> 93

caggtcatgg ccgggttc

18

<210> 94

<211> 22

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer sequence for RT-PCR

<400> 94

gggtcagaaa gtgtctggac tt

22

<210> 95

7 2 / 1 0 2

<211> 20

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer sequence for RT-PCR

<400> 95

caacagccca aagattttcc

20

<210> 96

<211> 23

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer sequence for RT-PCR

<400> 96

ggtcacgtga taaaatagca caa

23

<210> 97

<211> 26

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer for PCR

<400> 97

aacgaattca tggcgtcccc acggga

26

7 3 / 1 0 2

<210> 98

<211> 28

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer for PCR

<400> 98

caactcgagc tggtagcag gcaccgtg

28

<210> 99

<211> 27

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer for PCR

<400> 99

caagaattca tgtcgagccc gggcac

27

<210> 100

<211> 30

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer for PCR

7 4 / 1 0 2
,

<400> 100

gctgaattca cctcaatgac gaggcagcgg

30

<210> 101

<211> 20

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer sequence for RT-PCR

<400> 101

cggtggtctc tcctccttgt

20

<210> 102

<211> 20

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer sequence for RT-PCR

<400> 102

gcctcgaggt tatgcttgaa

20

<210> 103

<211> 20

<212> DNA

<213> Artificial

7 5 / 1 0 2

<220>

<223> An artificially synthesized primer sequence for RT-PCR

<400> 103

atgcagtcac tcacgctcag

20

<210> 104

<211> 51

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized oligonucleotide for siRNA

<400> 104

tcccaggac aatccagacg gctttcaaga gaagccgtct ggattgtcct c

51

<210> 105

<211> 51

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized oligonucleotide for siRNA

<400> 105

aaaaggagac aatccagacg gcttctcttg aaagccgtct ggattgtcct c

51

<210> 106

7 6 / 1 0 2

<211> 19

<212> DNA

<213> Artificial

<220>

<223> A target sequence for siRNA

<400> 106

gaggacaatc cagacggct

19

<210> 107

<211> 47

<212> DNA

<213> Artificial

<220>

<223> siRNA hairpin design

<400> 107

gaggacaatc cagacggctt tcaagagaag ccgtctggat tgcctc

47

<210> 108

<211> 51

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized oligonucleotide for siRNA

<400> 108

tcccggagct ctacaacgtg ctgttcaaga gacagcacgt tgtagagctc c

51

77/102

<210> 109

<211> 51

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized oligonucleotide for siRNA

<400> 109

aaaaggagct ctacaacgtg ctgtctcttg aacagcacgt ttagagctc c 51

<210> 110

<211> 19

<212> DNA

<213> Artificial

<220>

<223> A target sequence for siRNA

<400> 110

ggagctctac aacgtgctg 19

<210> 111

<211> 47

<212> DNA

<213> Artificial

<220>

<223> siRNA hairpin design

78 / 102

<400> 111

ggagctctac aacgtgctgt tcaagagaca gcacgttgta gagctcc

47

<210> 112

<211> 3503

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (18)..(1427)

<400> 112

ctcatccccgg ccccgagg atg gcg tcc cca cgg gaa ttg aca cag aac ccc 50

Met Ala Ser Pro Arg Glu Leu Thr Gln Asn Pro

1

5

10

ctg aag aag atc tgg atg cca tac agc aat ggg cgg ccc gct ctg cac 98

Leu Lys Lys Ile Trp Met Pro Tyr Ser Asn Gly Arg Pro Ala Leu His

15

20

25

gct tgc cag cgc ggt gtg tgc atg acc aac tgc cca act ctc att gtc 146

Ala Cys Gln Arg Gly Val Cys Met Thr Asn Cys Pro Thr Leu Ile Val

30

35

40

atg gtg ggc ctg ccc gcc agg ggc aag acc tac atc tcc aag aag ctg 194

Met Val Gly Leu Pro Ala Arg Gly Lys Thr Tyr Ile Ser Lys Lys Leu

45

50

55

act cga tac ctg aac tgg att ggt gtg ccc act cgg gag ttc aat gtt 242

79/102

Thr	Arg	Tyr	Leu	Asn	Trp	Ile	Gly	Val	Pro	Thr	Arg	Glu	Phe	Asn	Val		
60					65					70					75		
ggc cag tat cgc cgg gac gtg gtc aag acc tac aaa tct ttt gaa ttt																	290
Gly	Gln	Tyr	Arg	Arg	Asp	Val	Val	Lys	Thr	Tyr	Lys	Ser	Phe	Glu	Phe		
				80					85					90			
ttt ctc ccc gac aat gaa gag ggc ctg aaa atc agg aag cag tgt gcc																	338
Phe	Leu	Pro	Asp	Asn	Glu	Glu	Gly	Leu	Lys	Ile	Arg	Lys	Gln	Cys	Ala		
				95					100					105			
ctg gca gcc ctc cgt gac gtc cgg cgg ttc ctt agt gag gag ggg gga																	386
Leu	Ala	Ala	Leu	Arg	Asp	Val	Arg	Arg	Phe	Leu	Ser	Glu	Glu	Gly	Gly		
				110					115					120			
cat gtg gcg gtt ttt gat gcc aca aac acc acc cga gaa cgg aga gcg																	434
His	Val	Ala	Val	Phe	Asp	Ala	Thr	Asn	Thr	Thr	Arg	Glu	Arg	Arg	Ala		
				125					130					135			
acc atc ttt aat ttt gga gaa cag aat ggc tac aag acc ttt ttt gtc																	482
Thr	Ile	Phe	Asn	Phe	Gly	Glu	Gln	Asn	Gly	Tyr	Lys	Thr	Phe	Phe	Val		
140						145					150				155		
gag tcc atc tgt gtg gat cct gag gtc ata gct gcc aac atc gtg caa																	530
Glu	Ser	Ile	Cys	Val	Asp	Pro	Glu	Val	Ile	Ala	Ala	Asn	Ile	Val	Gln		
				160						165				170			
gtg aaa ctg ggc agc cct gac tat gtc aac cgc gac agt gat gag gct																	578
Val	Lys	Leu	Gly	Ser	Pro	Asp	Tyr	Val	Asn	Arg	Asp	Ser	Asp	Glu	Ala		
				175						180				185			
acg gag gac ttc atg agg cgc att gag tgc tat gag aac tcc tac gag																	626
Thr	Glu	Asp	Phe	Met	Arg	Arg	Ile	Glu	Cys	Tyr	Glu	Asn	Ser	Tyr	Glu		

80/102

190	195	200	
tcg cta gat gag gac ctg gat agg gac ctg tcc tat atc aag atc atg			674
Ser Leu Asp Glu Asp Leu Asp Arg Asp Leu Ser Tyr Ile Lys Ile Met			
205	210	215	
gat gtg ggc cag agc tac gtg gtg aac cgt gtg gct gac cac atc cag			722
Asp Val Gly Gln Ser Tyr Val Val Asn Arg Val Ala Asp His Ile Gln			
220	225	230	235
agc cgc atc gta tat tac ctc atg aac atc cac gtg acc ccc cgc tcc			770
Ser Arg Ile Val Tyr Tyr Leu Met Asn Ile His Val Thr Pro Arg Ser			
240	245	250	
atc tac ctc tgc cgg cac ggg gag agc gag ctc aac ctc aag ggc cgg			818
Ile Tyr Leu Cys Arg His Gly Glu Ser Glu Leu Asn Leu Lys Gly Arg			
255	260	265	
att ggc ggg gac cca gga ctg tcc cct cgg ggc agg gag ttt gcc aag			866
Ile Gly Gly Asp Pro Gly Leu Ser Pro Arg Gly Arg Glu Phe Ala Lys			
270	275	280	
agt cta gcc cag ttc atc agt gac caa aat atc aag gat ctg aag gtc			914
Ser Leu Ala Gln Phe Ile Ser Asp Gln Asn Ile Lys Asp Leu Lys Val			
285	290	295	
tgg aca agc cag atg aag agg aca atc cag acg gct gag gca ctg ggt			962
Trp Thr Ser Gln Met Lys Arg Thr Ile Gln Thr Ala Glu Ala Leu Gly			
300	305	310	315
gtg ccc tat gaa cag tgg aag gtc ctc aac gag atc gat gcg ggc gtc			1010
Val Pro Tyr Glu Gln Trp Lys Val Leu Asn Glu Ile Asp Ala Gly Val			
320	325	330	

81 / 102

tgt gag gaa atg acc tac gag gaa att cag gat aat tat cca ctg gag	1058
Cys Glu Glu Met Thr Tyr Glu Glu Ile Gln Asp Asn Tyr Pro Leu Glu	
335 340 345	
ttc gcc ctg cgg gac cag gac aag tac cgg tac cgg tac cct aaa ggg	1106
Phe Ala Leu Arg Asp Gln Asp Lys Tyr Arg Tyr Arg Tyr Pro Lys Gly	
350 355 360	
gag tcc tac gag gac ctg gtc cag aga ctg gag cct gtc atc atg gag	1154
Glu Ser Tyr Glu Asp Leu Val Gln Arg Leu Glu Pro Val Ile Met Glu	
365 370 375	
ctg gag agg caa gag aat gtg ctg gtc atc tgc cac cag gct gtg atg	1202
Leu Glu Arg Gln Glu Asn Val Leu Val Ile Cys His Gln Ala Val Met	
380 385 390 395	
cgc tgc ctg ctg gcc tac ttc ctc gac aag gca gca gaa cag ctg ccc	1250
Arg Cys Leu Leu Ala Tyr Phe Leu Asp Lys Ala Ala Glu Gln Leu Pro	
400 405 410	
tac ctc aag tgt ccg ctg cac aca gtc ctg aag ctg act cct gtg gca	1298
Tyr Leu Lys Cys Pro Leu His Thr Val Leu Lys Leu Thr Pro Val Ala	
415 420 425	
tat ggt tgt aaa gtg gag tcc ata ttc ctg aac gtg gct gct gtg aac	1346
Tyr Gly Cys Lys Val Glu Ser Ile Phe Leu Asn Val Ala Ala Val Asn	
430 435 440	
acg cac cgg gac agg cct cag aac gtg gac atc tca aga cct cca gag	1394
Thr His Arg Asp Arg Pro Gln Asn Val Asp Ile Ser Arg Pro Pro Glu	
445 450 455	

8 2 / 1 0 2

gaa gcc ctt gtc acg gtg cct gct cac cag tga ccatgttcat ccactgtgac 1447
Glu Ala Leu Val Thr Val Pro Ala His Gln
460 465

cactaggcag gcactgctct ctgcagaggg ggtcattcca ggcctccag tgttgtgat 1507

agtcaccatg ccatgcaggg atattcttga agccacacat ggctggcgga acccagagcc 1567

cccaccccag cccacctggc tctttgttga cagtggcgga caaggttgtg cgtggctcct 1627

gacctgctgc taagagtcac ttgaccagac tgcattctga tgggctgcgc ggaggttgcc 1687

cagccccagt ttcttcgggc gcagctctta ggtgttcact ctgccagct cagttggctt 1747

tgtgaagtgt gaaaccctac aatgtgaaag gaaagtgcct gctgtgatgt tcctactgtg 1807

gccagctgc ccagcatgga cctggtgact ctccacaggg cctctaccat cctctctgtg 1867

gccacttcct gagccagagg ccaggtcttc atggggccct gagcttctgc tgcctctggt 1927

gagagggaga gcccttccca tccttaccba ccaggaaacta gagccccaac cacagcagat 1987

gcttctctggg cagccactgg ccaggccgtt gtatccatgt cacccttagt tgtgggcatt 2047

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cctctcccct tcctggctg tggacactgg cagccaatgg ggaggcggcc tctcccctgt 2347

83 / 102

tgctgcccgt gtccacacag gaatgtacac tgggtggcaga aacatgcatt tgccataaat 2407

aattcagaaa cacttgtatg gtccgtgttaa acttctcttg tgtttgttga cttctgcctg 2467

tgggtctgag gaagggagaa ggcagaaccc ataggcagtc cctgggcaag ggaggatttt 2527

cccgcccgca gcacagcctt ccattcatgt gcacgtgagt ctggtgaaaa tggccttgtg 2587

aagtccagtg tctgtcatgc ccctgagttc ttttgggtccc agagaggcag cctgggcagt 2647

ctgtttacag ctggcaaaca gactggctgg caagttgtgg ggtgggtca gaaagtgtct 2707

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gacagggtag agggacatcc agggatcagg gcatgagtgg gggcaacccc ccggcctctg 3307

8 4 / 1 0 2

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gctggctggc gtgcccattg tgcagatatt ttcccagatt cccagaatg gatggtattg 3427

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taaaaaaaaa aaaaaa 3503

<210> 113

<211> 469

<212> PRT

<213> Homo sapiens

<400> 113

Met Ala Ser Pro Arg Glu Leu Thr Gln Asn Pro Leu Lys Lys Ile Trp

1 5 10 15

Met Pro Tyr Ser Asn Gly Arg Pro Ala Leu His Ala Cys Gln Arg Gly

20 25 30

Val Cys Met Thr Asn Cys Pro Thr Leu Ile Val Met Val Gly Leu Pro

35 40 45

Ala Arg Gly Lys Thr Tyr Ile Ser Lys Lys Leu Thr Arg Tyr Leu Asn

50 55 60

Trp Ile Gly Val Pro Thr Arg Glu Phe Asn Val Gly Gln Tyr Arg Arg

65					70					75						80
Asp	Val	Val	Lys	Thr	Tyr	Lys	Ser	Phe	Glu	Phe	Phe	Leu	Pro	Asp	Asn	
				85					90					95		
Glu	Glu	Gly	Leu	Lys	Ile	Arg	Lys	Gln	Cys	Ala	Leu	Ala	Ala	Leu	Arg	
			100					105						110		
Asp	Val	Arg	Arg	Phe	Leu	Ser	Glu	Glu	Gly	Gly	His	Val	Ala	Val	Phe	
		115					120					125				
Asp	Ala	Thr	Asn	Thr	Thr	Arg	Glu	Arg	Arg	Ala	Thr	Ile	Phe	Asn	Phe	
	130					135					140					
Gly	Glu	Gln	Asn	Gly	Tyr	Lys	Thr	Phe	Phe	Val	Glu	Ser	Ile	Cys	Val	
145					150					155					160	
Asp	Pro	Glu	Val	Ile	Ala	Ala	Asn	Ile	Val	Gln	Val	Lys	Leu	Gly	Ser	
			165						170					175		
Pro	Asp	Tyr	Val	Asn	Arg	Asp	Ser	Asp	Glu	Ala	Thr	Glu	Asp	Phe	Met	
		180						185					190			
Arg	Arg	Ile	Glu	Cys	Tyr	Glu	Asn	Ser	Tyr	Glu	Ser	Leu	Asp	Glu	Asp	
	195						200						205			

86 / 102

Leu Asp Arg Asp Leu Ser Tyr Ile Lys Ile Met Asp Val Gly Gln Ser
210 215 220

Tyr Val Val Asn Arg Val Ala Asp His Ile Gln Ser Arg Ile Val Tyr
225 230 235 240

Tyr Leu Met Asn Ile His Val Thr Pro Arg Ser Ile Tyr Leu Cys Arg
245 250 255

His Gly Glu Ser Glu Leu Asn Leu Lys Gly Arg Ile Gly Gly Asp Pro
260 265 270

Gly Leu Ser Pro Arg Gly Arg Glu Phe Ala Lys Ser Leu Ala Gln Phe
275 280 285

Ile Ser Asp Gln Asn Ile Lys Asp Leu Lys Val Trp Thr Ser Gln Met
290 295 300

Lys Arg Thr Ile Gln Thr Ala Glu Ala Leu Gly Val Pro Tyr Glu Gln
305 310 315 320

Trp Lys Val Leu Asn Glu Ile Asp Ala Gly Val Cys Glu Glu Met Thr
325 330 335

87/102

Tyr Glu Glu Ile Gln Asp Asn Tyr Pro Leu Glu Phe Ala Leu Arg Asp
340 345 350

Gln Asp Lys Tyr Arg Tyr Arg Tyr Pro Lys Gly Glu Ser Tyr Glu Asp
355 360 365

Leu Val Gln Arg Leu Glu Pro Val Ile Met Glu Leu Glu Arg Gln Glu
370 375 380

Asn Val Leu Val Ile Cys His Gln Ala Val Met Arg Cys Leu Leu Ala
385 390 395 400

Tyr Phe Leu Asp Lys Ala Ala Glu Gln Leu Pro Tyr Leu Lys Cys Pro
405 410 415

Leu His Thr Val Leu Lys Leu Thr Pro Val Ala Tyr Gly Cys Lys Val
420 425 430

Glu Ser Ile Phe Leu Asn Val Ala Ala Val Asn Thr His Arg Asp Arg
435 440 445

Pro Gln Asn Val Asp Ile Ser Arg Pro Pro Glu Glu Ala Leu Val Thr
450 455 460

88/102

Val Pro Ala His Gln

465

<210> 114

<211> 2539

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (500)..(754)

<400> 114

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tggcgctgga gtacgtggcc tgcgacctgc accgcctaga ggagctcgtg ctcgacaggt 120

gcgcgcccc gggccgcgcc gggcggggct gggccgggcg gggctgcacg gggcggggcg 180

gggcggggct gcacggggcc gggggcgggg gcttccggcg gggctggggc tgtggcgcg 240

cgggcagagc cctcggagcc tggggagtgg gaaccgcaga accgcagcac gggttggtg 300

gacccccgtc cttcccgtg ggtgggagcg aggaggggcc agcggctgct ccgggtgggg 360

caggaaggga ttacgccag gggcactccc aggaagacga gggatggccg gggccaggtg 420

atttgaaggt gggggtcccc tctggtgcaa ccacaggtgt gtacgcatca cggacactgg 480

cctcagctat ctgtccacc atg tcg tcc ctc cgc agc ctc tac ctg cga tgg 532

Met Ser Ser Leu Arg Ser Leu Tyr Leu Arg Trp

89/102

1	5	10	
tgc tgc cag gtg caa gac ttc ggg ctg aag cac ctc ctg gcc ctg ggg			580
Cys Cys Gln Val Gln Asp Phe Gly Leu Lys His Leu Leu Ala Leu Gly			
15	20	25	
agt ttg cgc ctc ctg tct ctg gca ggc tgc ccg ctg ctc acc acc acc			628
Ser Leu Arg Leu Leu Ser Leu Ala Gly Cys Pro Leu Leu Thr Thr Thr			
30	35	40	
ggg ctg tgc ggc ctg gtg cag ctg cag gag ctg gag gag ctg gag ctg			676
Gly Leu Ser Gly Leu Val Gln Leu Gln Glu Leu Glu Glu Leu Glu Leu			
45	50	55	
acc aac tgc ccc ggg gcc acc ccc gag ctc ttc aag tat ttc tgc cag			724
Thr Asn Cys Pro Gly Ala Thr Pro Glu Leu Phe Lys Tyr Phe Ser Gln			
60	65	70	75
cac ctg ccc cgc tgc ctc gtc att gag tag cgcgaggccc ccgccccggt			774
His Leu Pro Arg Cys Leu Val Ile Glu			
80			
cgcgggaacc cggccatgac ctgggcgggg gcgcggggcg ccgccgagcc ccctcttccc			834
gccttgcgct cgggggagcc cccgcgcccc cggcccagcg cgggaggcgg ggcgagccga			894
gggaaagccc ctccccgacc ttcggtccct ccgccctccc agccccgccc cgggcagggg			954
ggcggcgggt gggcccgccc cacgcacgca cgcacactcg gggactttgt goatgccct			1014
ogtgcccgca ctgcacgccg ccctccgcca cgcccacagc cacagccgcc gccatcactc			1074
gctcgccctc ccgcttgggg ggcggggctc ggtccttggg ggggctttga gctctccaga			1134

90 / 102

ctgtgccctt accgccttcc ccgccacacc cgctctgtct tcccactgtc cccccatcc 1194

cgggcagggc ccagtgggat tgagggggct ggggtcccca ggacacgggc ccagaagagc 1254

cccacgggct tcctgcatct tccaccgcac catacctgga gccctccgag ggggtgtcagg 1314

ggaaacaggc caccgcaaaa gccatggccc gccgccgaga gccaagccc caccgcacc 1374

tcctcaccca tccagcctga cccacgggc ctctcctcct ccttgccgct gtgtggggca 1434

gtcccctgtc cgccccaaaa cccggccttg gtcccctggc aggctgagag aattgggcag 1494

ggagagggcg gaagggtgg cgatcgcttg gagtcattaa cgtgatcca gctgactccg 1554

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tggacccagc caagggtcag ggtcagcact gggtcagcga ctccaatctt ccagtggcca 1794

gcacacccta gacacccga ggaggagggt ctcttttcta gctgcccc ccacccccac 1854

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tcggcccag acatttgaa gtcttgggg atgctggcaa atctcagctg ttgctgagga 1974

gggggctggg accccttccc atcccaacct tgagccccag gagataccgc gccacaccc 2034

aatcttggga cactccctat ctggttgaa gagagtaacc agtttccaga gagccagaga 2094

91 / 102

gtgagagaga gaaagagagt gagagagaga gagaaagaga gagagagatg ctgttgaatc 2154
 agaaacagat caacagccca aagattttcc tgtccctgga gtgccagccc caggaagctc 2214
 cagggctgag tggtcaggag ccagttttcc cagccccctcc tccccacaac ccctagtggg 2274
 gaggggcagc tgtccatttg cccaaagtat taatgcaact gaagctgtga tatttccaac 2334
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 tttggtaagt gtttcttgtg ctattttatc acgtgacctg tttataaaaa tatatattaa 2514
 aaaagttgta aaaaaaaaaa aaaaa 2539

<210> 115

<211> 84

<212> PRT

<213> Homo sapiens

<400> 115

Met Ser Ser Leu Arg Ser Leu Tyr Leu Arg Trp Cys Cys Gln Val Gln
 1 5 10 15

Asp Phe Gly Leu Lys His Leu Leu Ala Leu Gly Ser Leu Arg Leu Leu
 20 25 30

Ser Leu Ala Gly Cys Pro Leu Leu Thr Thr Thr Gly Leu Ser Gly Leu

9 2 / 1 0 2

35

40

45

Val Gln Leu Gln Glu Leu Glu Glu Leu Glu Leu Thr Asn Cys Pro Gly

50

55

60

Ala Thr Pro Glu Leu Phe Lys Tyr Phe Ser Gln His Leu Pro Arg Cys

65

70

75

80

Leu Val Ile Glu

<210> 116

<211> 23

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer sequence for RT-PCR

<400> 116

cttcagact catctgtcag agc

23

<210> 117

<211> 3427

<212> DNA

<213> Homo sapiens

9 3 / 1 0 2

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tccgtgcgct ccccatgacg ggaggtcgcc cggcggacag cgtggcatga gccaggagcc 180

cgggccgaga gcgtgccagg aag atg tcg agc ccg ggc atc gac ggc gac ccc 233

Met Ser Ser Pro Gly Ile Asp Gly Asp Pro

1

5

10

aag cct cca tgc ttg cct cga aac ggt ctg gtg aag ctg ccg ggc cag 281

Lys Pro Pro Cys Leu Pro Arg Asn Gly Leu Val Lys Leu Pro Gly Gln

15

20

25

ccc aac ggc ctg ggt gcg gcc agc atc acc aag ggc acg cca gcc acc 329

Pro Asn Gly Leu Gly Ala Ala Ser Ile Thr Lys Gly Thr Pro Ala Thr

30

35

40

aag aac cgc ccc tgc cag cca cca ccc cca ccc acc ctc cca cca cca 377

Lys Asn Arg Pro Cys Gln Pro Pro Pro Pro Pro Thr Leu Pro Pro Pro

45

50

55

agc ctg gct gct cca ctg tcc cgg gct gcc ctg gct ggg ggc ccg tgc 425

Ser Leu Ala Ala Pro Leu Ser Arg Ala Ala Leu Ala Gly Gly Pro Cys

60

65

70

acc ccg gca ggt gga cca gcc tca gcc ttg gca cct ggg cac cca ggc 473

Thr Pro Ala Gly Gly Pro Ala Ser Ala Leu Ala Pro Gly His Pro Ala

94/102

75	80	85	90	
gag cgg ccg ccg ctg gcc acg gac gag aag atc ctc aat ggg ctc ttc				521
Glu Arg Pro Pro Leu Ala Thr Asp Glu Lys Ile Leu Asn Gly Leu Phe				
	95	100	105	
tgg tat ttc tcg gcc tgc gag aag tgt gtg ctg gcc cag gtg tgc aag				569
Trp Tyr Phe Ser Ala Cys Glu Lys Cys Val Leu Ala Gln Val Cys Lys				
	110	115	120	
gcc tgg cgg cgc gtg ctg tac cag ccc aag ttc tgg gca ggc ctc acg				617
Ala Trp Arg Arg Val Leu Tyr Gln Pro Lys Phe Trp Ala Gly Leu Thr				
	125	130	135	
ccg gtg ctg cat gcc aag gag ctc tac aac gtg ctg cct ggt ggc gag				665
Pro Val Leu His Ala Lys Glu Leu Tyr Asn Val Leu Pro Gly Gly Glu				
	140	145	150	
aag gag ttc gtg aac ctg cag ggt ttt gcc gcc aga ggc ttc gag ggc				713
Lys Glu Phe Val Asn Leu Gln Gly Phe Ala Ala Arg Gly Phe Glu Gly				
	155	160	165	170
ttc tgc ctg gtt ggc gtc tcc gac ctg gac atc tgt gag ttc att gac				761
Phe Cys Leu Val Gly Val Ser Asp Leu Asp Ile Cys Glu Phe Ile Asp				
	175	180	185	
aac tat gcg ctc tcc aag aag ggt gtc aaa gcc atg agc ctc aag cgc				809
Asn Tyr Ala Leu Ser Lys Lys Gly Val Lys Ala Met Ser Leu Lys Arg				
	190	195	200	
tcc acc atc acg gac gca ggc ctc gag gtt atg ctt gaa cag atg cag				857
Ser Thr Ile Thr Asp Ala Gly Leu Glu Val Met Leu Glu Gln Met Gln				
	205	210	215	

9 5 / 1 0 2

ggc gtg gtg cgt ctg gag ctg tgc ggc tgc aac gac ttc acc gag gcc 905
 Gly Val Val Arg Leu Glu Leu Ser Gly Cys Asn Asp Phe Thr Glu Ala
 220 225 230

ggg ctg tgg tcc agc ctg agc gcg cgc atc acc tgc ctg agc gtg agt 953
 Gly Leu Trp Ser Ser Leu Ser Ala Arg Ile Thr Ser Leu Ser Val Ser
 235 240 245 250

gac tgc atc aac gtg gcc gac gac gcc atc gcg gcc atc tgc cag ctg 1001
 Asp Cys Ile Asn Val Ala Asp Asp Ala Ile Ala Ala Ile Ser Gln Leu
 255 260 265

ctg ccc aac ctg gcg gag ctg agc ctg cag gcc tac cac gtg acg gac 1049
 Leu Pro Asn Leu Ala Glu Leu Ser Leu Gln Ala Tyr His Val Thr Asp
 270 275 280

acg gcg ctg gcc tac ttc acg gcg cgc cag ggc cac agc acg cac acg 1097
 Thr Ala Leu Ala Tyr Phe Thr Ala Arg Gln Gly His Ser Thr His Thr
 285 290 295

ctg cgc ctg ctc tcc tgc tgg gag atc acc aac cac ggc gtg gtc aac 1145
 Leu Arg Leu Leu Ser Cys Trp Glu Ile Thr Asn His Gly Val Val Asn
 300 305 310

gtg gtg cac agc ctg ccc aac ctc acc gcg ctc agc ctc tgc ggc tgc 1193
 Val Val His Ser Leu Pro Asn Leu Thr Ala Leu Ser Leu Ser Gly Cys
 315 320 325 330

tcc aag gtc acc gac gac ggc gtg gag ctc gtg gcc gag aac ctg cgc 1241
 Ser Lys Val Thr Asp Asp Gly Val Glu Leu Val Ala Glu Asn Leu Arg
 335 340 345

9 6 / 1 0 2

aag ctg cgc agc ctt gac ctc tcg tgg tgc cca cgc atc acc gac atg	1289
Lys Leu Arg Ser Leu Asp Leu Ser Trp Cys Pro Arg Ile Thr Asp Met	
350 355 360	
gcg ctg gag tac gtg gcc tgc gac ctg cac cgc cta gag gag ctc gtg	1337
Ala Leu Glu Tyr Val Ala Cys Asp Leu His Arg Leu Glu Glu Leu Val	
365 370 375	
ctc gac agg tgt gta cgc atc acg gac act ggc ctc agc tat ctg tcc	1385
Leu Asp Arg Cys Val Arg Ile Thr Asp Thr Gly Leu Ser Tyr Leu Ser	
380 385 390	
acc atg tcg tcc ctc cgc agc ctc tac ctg cga tgg tgc tgc cag gtg	1433
Thr Met Ser Ser Leu Arg Ser Leu Tyr Leu Arg Trp Cys Cys Gln Val	
395 400 405 410	
caa gac ttc ggg ctg aag cac ctc ctg gcc ctg ggg agt ttg cgc ctc	1481
Gln Asp Phe Gly Leu Lys His Leu Leu Ala Leu Gly Ser Leu Arg Leu	
415 420 425	
ctg tct ccg gca ggc tgc ccg ctg ctc acc acc acc ggg ctg tcg ggc	1529
Leu Ser Pro Ala Gly Cys Pro Leu Leu Thr Thr Thr Gly Leu Ser Gly	
430 435 440	
ctg gtg cag ctg cag gag ctg gag gag ctg gag ctg acc aac tgc ccc	1577
Leu Val Gln Leu Gln Glu Leu Glu Glu Leu Glu Leu Thr Asn Cys Pro	
445 450 455	
ggg gcc acc ccc gag ctc ttc aag tat ttc tcg cag cac ctg ccc cgc	1625
Gly Ala Thr Pro Glu Leu Phe Lys Tyr Phe Ser Gln His Leu Pro Arg	
460 465 470	
tgc ctc gtc att gag tag cgcgaggccc ccgccccggt cgcgggaacc	1673

97/102

Cys Leu Val Ile Glu

475

cgcccatgac ctgggcgggg gcgcggggcg ccgccgagcc ccctcttccc gccttgcgct 1733

cgggggagacc cccgcgcccc cgccccagcg cgggagacgg ggcgagccga gggaaagccc 1793

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ctgcacgccc ccctccgcca cgccacagcc acagccgccc ccatcactcg ctgcacctcc 1973

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ggcaccgagc ttcaccctgg gtgggtctcc tcaggtggag tctgcagagt ggaccagcc 2633
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99/102

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35 40 45

Pro Pro Pro Pro Pro Thr Leu Pro Pro Pro Ser Leu Ala Ala Pro Leu
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Ser Arg Ala Ala Leu Ala Gly Gly Pro Cys Thr Pro Ala Gly Gly Pro
65 70 75 80

Ala Ser Ala Leu Ala Pro Gly His Pro Ala Glu Arg Pro Pro Leu Ala
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Thr Asp Glu Lys Ile Leu Asn Gly Leu Phe Trp Tyr Phe Ser Ala Cys
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Tyr Gln Pro Lys Phe Trp Ala Gly Leu Thr Pro Val Leu His Ala Lys
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Glu Leu Tyr Asn Val Leu Pro Gly Gly Glu Lys Glu Phe Val Asn Leu
145 150 155 160

Gln Gly Phe Ala Ala Arg Gly Phe Glu Gly Phe Cys Leu Val Gly Val
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Ser Asp Leu Asp Ile Cys Glu Phe Ile Asp Asn Tyr Ala Leu Ser Lys
180 185 190

Lys Gly Val Lys Ala Met Ser Leu Lys Arg Ser Thr Ile Thr Asp Ala
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Gly Leu Glu Val Met Leu Glu Gln Met Gln Gly Val Val Arg Leu Glu
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Leu Ser Gly Cys Asn Asp Phe Thr Glu Ala Gly Leu Trp Ser Ser Leu
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Ser Ala Arg Ile Thr Ser Leu Ser Val Ser Asp Cys Ile Asn Val Ala

1 0 1 / 1 0 2

245

250

255

Asp Asp Ala Ile Ala Ala Ile Ser Gln Leu Leu Pro Asn Leu Ala Glu

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Leu Ser Leu Gln Ala Tyr His Val Thr Asp Thr Ala Leu Ala Tyr Phe

275

280

285

Thr Ala Arg Gln Gly His Ser Thr His Thr Leu Arg Leu Leu Ser Cys

290

295

300

Trp Glu Ile Thr Asn His Gly Val Val Asn Val Val His Ser Leu Pro

305

310

315

320

Asn Leu Thr Ala Leu Ser Leu Ser Gly Cys Ser Lys Val Thr Asp Asp

325

330

335

Gly Val Glu Leu Val Ala Glu Asn Leu Arg Lys Leu Arg Ser Leu Asp

340

345

350

Leu Ser Trp Cys Pro Arg Ile Thr Asp Met Ala Leu Glu Tyr Val Ala

355

360

365

Cys Asp Leu His Arg Leu Glu Glu Leu Val Leu Asp Arg Cys Val Arg

370

375

380

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Ile Thr Asp Thr Gly Leu Ser Tyr Leu Ser Thr Met Ser Ser Leu Arg
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Ser Leu Tyr Leu Arg Trp Cys Cys Gln Val Gln Asp Phe Gly Leu Lys
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His Leu Leu Ala Leu Gly Ser Leu Arg Leu Leu Ser Pro Ala Gly Cys
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Pro Leu Leu Thr Thr Thr Gly Leu Ser Gly Leu Val Gln Leu Gln Glu
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Leu Glu Glu Leu Glu Leu Thr Asn Cys Pro Gly Ala Thr Pro Glu Leu
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Phe Lys Tyr Phe Ser Gln His Leu Pro Arg Cys Leu Val Ile Glu
465 470 475