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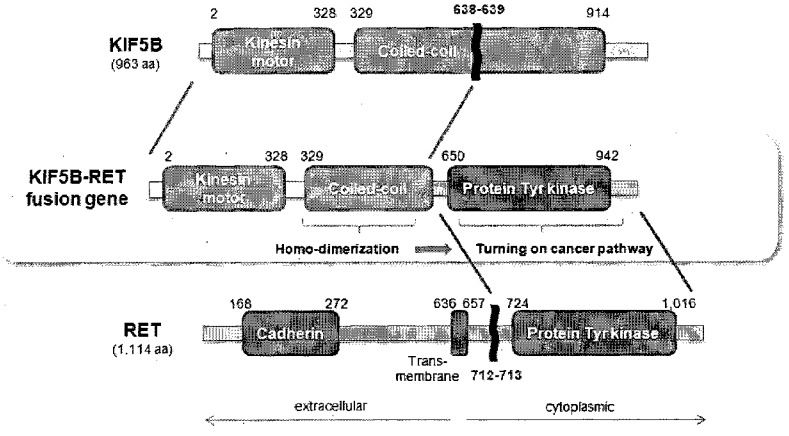
(54) Title: FUSION PROTEIN COMPRISING C-TERMINAL DOMAIN OF RET PROTEIN AND USE THEREOF AS A DIAGNOSING MARKER

FIG. 14



(57) Abstract: A fusion protein including N-terminal domain of a fusion partner at N-terminal and C-terminal domain of RET protein at C-terminal, a fusion gene encoding the fusion protein, and a use of the fusion protein or the fusion gene as a diagnosing marker for a cancer, are provided

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TITLE OF THE INVENTION

Fusion protein comprising C-terminal domain of RET protein and use thereof as a diagnosing marker

BACKGROUND OF THE INVENTION

(a) Field of the Invention

A fusion protein including N-terminal domain of a fusion partner at N-terminal and C-terminal domain of RET protein at C-terminal, a fusion gene encoding the fusion protein, and a use of the fusion protein or the fusion gene as a diagnosing marker for a cancer, are provided.

(b) Description of the Related Art

Lung cancer remains a leading cause of mortality in cancer, with around 1.38 million deaths worldwide annually. With conventional chemotherapeutic regimen, the median survival time for lung cancer patients in advanced stages is less than one year from diagnosis. Tobacco smoking is known to be the major risk factor of lung cancer in Western countries, where 85% to 90% of all lung cancers were attributed to smoking. However, approximately 25% of lung cancer patients worldwide are 'never-smokers'. Data from many Asian countries have shown that 'never-smokers' constitute 30-40% of non-small-cell lung cancer (NSCLC), which accounts for ~80% of lung cancer cases. In NSCLC, a dominant histological type is adenocarcinoma (~70%).

Lung cancer of never-smokers tends to be driven by single somatic mutation events, rather than global genetic and epigenetic changes. A subset of somatic mutations has been reported in NSCLC in the past few years, such as *EGFR*, *KRAS and ALK* genes (which are conventionally called as 'the triple-markers'). Mutations in the tyrosine kinase domain of *EGFR*, which are associated preferentially with NSCLC of non-smokers and Asians, are sensitive to *EGFR* targeted therapy, such as Gefitinib. Missense mutations in *KRAS* are common in the lung adenocarcinomas of smokers, and induce resistance to *EGFR* inhibitors.

Although several genetic mutations have been reported, a large proportion of lung cancer patients have been observed to have none of them in their cancer genome.

More than 40% of NSCLC appear to be driven by unknown genetic events. Therefore, it is needed to find more effective genetic markers for lung cancer.

SUMMARY OF THE INVENTION

An embodiment provides a fusion protein consisting essentially of N-terminal domain of a fusion partner and C-terminal domain of RET protein. The fusion protein may be KIF5B-RET fusion protein consisting essentially of N-terminal domain of KIF5B protein and C-terminal domain of RET protein.

Another embodiment provides a fusion gene encoding the fusion protein.

Another embodiment provides a recombinant vector including the fusion gene.

Another embodiment provided a method of diagnosing a lung cancer including: detecting at least one selected from the group consisting of an RET-involved chromosomal rearrangement including inversion or translocation in Chromosome 10; a fusion protein wherein RET protein is fused with other protein; a fusion gene encoding the fusion protein; and the overexpression of RET compared to a standard sample from an individual without a cancer, wherein when at least one selected from the above group is detected in the test sample, the subject from which the test sample taken is determined as a lung cancer patient.

Another embodiment provides a use of the KIF5B-RET fusion protein as a marker for diagnosing a lung cancer.

Another embodiment provides a composition for diagnosing a lung cancer comprising a material for detecting the fusion protein or the fusion gene.

Another embodiment provides a method of preventing or treating a lung cancer, comprising the step of administering a therapeutically effective amount of at least one inhibitor against the fusion protein, at least one inhibitor against the fusion gene encoding the fusion protein, at least one inhibitor against a RET coding gene, or a combination thereof, to a patient in need thereof.

Another embodiment provides a composition for preventing or treating a lung cancer, comprising at least one inhibitor against the fusion protein, at least one inhibitor against the fusion gene encoding the fusion protein, at least one inhibitor against a RET coding gene, or a combination thereof, as an active ingredient.

Another embodiment provides a use of at least one inhibitor against the fusion

protein, at least one inhibitor against the fusion gene encoding the fusion protein, at least one inhibitor against a RET coding gene, or a combination thereof for preventing or treating a lung cancer.

Still another embodiment provides a method of screening an anticancer drug against lung cancer including: treating a cell expressing the fusion protein with a sample compound; measuring the fusion protein expression level in the cell, wherein the fusion protein expression level in the cell treated with the sample compound is decreased compared with that before the treatment with the sample compound or that in a non-treated cell, the sample compound is determined as a candidate compound for the anticancer drug against lung cancer.

DETAILED DESCRIPTION OF THE EMBODIMENTS

The present inventors identified a fusion gene generated by a chromosomal inversion event in lung adenocarcinoma patients, to complete the present invention. It is found that the fusion gene is detected even in a young, never-smoker lung adenocarcinoma patient, whose cancer was negative for the previously known triple-markers (*EGFR, KRAS and ALK* genes). Therefore, the fusion gene is expected as an effective marker for a lung cancer, which can function as a marker even when the previously known triple-markers cannot function.

An embodiment provides a fusion gene specifically found at a cancer cell and a fusion protein encoded by the fusion gene.

In particular, a fusion protein including N-terminal domain of a fusion partner and C-terminal domain of RET protein is provided. The N-terminal domain of a fusion partner may be positioned at N-terminus of the fusion protein, and the C-terminal domain of RET protein may be positioned at C-terminus of the fusion protein. In the present invention, it is found that the existence of a fusion protein including RET protein is associated with the development of a cancer, such as a lung cancer.

The fusion partner may be a N-terminal domain of KIF5B protein, which is positioned at N-terminus of the fusion protein. In this case, the fusion protein may be represented as KIF5B-RET protein which includes N-terminal domain of KIF5B protein at N-terminus and C-terminal domain of RET protein at C-terminus.

Another embodiment provides a fusion gene encoding the fusion protein, where

a gene encoding the N-terminal domain of the fusion partner positions at 5' end and a gene encoding the C-terminal domain of the RET protein positions at 3' end. In a concrete embodiment, when the fusion protein is the KIF5B-RET protein, the fusion gene may be represented as KIF5B-RET gene, where a gene encoding the N-terminal domain of KIF5B positions at 5' end and a gene encoding the C-terminal domain of the RET protein positions at 3' end.

Another embodiment provides an expression vector including the fusion gene and optionally transcription elements (e.g., a promoter and the like) operably linked to the fusion gene. Another embodiment provides a transformant cell transformed with the expression vector.

The RET protein is a transmembrane receptor tyrosine kinase. The RET consists of extracellular region (which contains Cadherin-like domains), a trans-membrane domain and an intracellular region containing a tyrosine kinase domain. When the RET protein is dimerized by binding co-receptors and ligands, such as glial derived neurotrophic factor (GDNF), it is activated by auto-phosphorylation and then simulates downstream signaling pathways. The downstream signaling cascade of the RET is the mitogen-activated protein kinase (MAPK) pathway, which regulates cell survival/apoptosis, proliferation, differentiation, and migration. The normal expression of RET is important for neuronal development, but it is known not to be activated in differentiated tissues.

The RET protein may be derived from a mammal, such as a human. The human RET gene encoding the human RET protein is localized to chromosome 10 (10q11.2) and contains 19-21 exons depending on variants. The human RET protein may be encoded by a human RET gene represented by the NCBI accession number NM_020630 or NM_020975.

The C-terminal domain of RET protein may include an amino acid sequence encoded by a polynucleotide from 12th exon to the last exon (for example, 20th exon) of RET gene (e.g., NM_020630 or NM_020975). The C-terminal domain of RET protein may include consecutive at least about 300 amino acids from the start position of 12th exon (for example 713th position for the RET protein encoded byNM_020975) toward C-terminus of the RET protein encoded by NM_020630 or NM_020975. For example, the C-terminal domain of RET protein may include consecutive about 300 to about 450

amino acids, consecutive about 300 to about 420 amino acids, or consecutive about 300 to about 402 amino acids from the start position of 12th exon (e.g., 713th position) toward C-terminus of the RET protein encoded by NM_020630 (19 exons) or NM_020975 (20 exons).

The KIF5B protein, which is also called as Kinesin-1 heavy chain, is a protein encoded by KIF5B gene. The KIF5B protein may be derived from a mammal, such as a human. The human KIF5B gene encoding the human KIF5B protein is localized to chromosome 10 (10q11.22) and contains 26 exons. The human KIF5B protein may be encoded by a human KIF5B gene represented by the NCBI accession number NM_004521.

The N-terminal domain of KIF5B protein may include an amino acid sequence encoded by a polynucleotide from the first exon to 16th exon, or from the first exon to 15th exon, or from the first exon to 23th exon of KIF5B gene (e.g., NM_004521). The N-terminal domain of KIF5B protein may include consecutive at least about 329 amino acids from 1st position (that is, at least amino acid sequence from 1st to 329th positions) of the KIF5B protein encoded by NM_004521. The N-terminal domain of KIF5B protein may further include at least two coiled coil domain which starts from the amino acid of the 329th position of the KIF5B protein encoded by NM_004521. For example, the two coiled coil domain further included may have an amico acid sequence of 329th to 638th positions of the KIF5B protein encoded by NM_004521(SEQ ID NO: 21). The N-terminal domain of KIF5B protein may include consecutive about 329 to 900 amino acids, consecutive about 329 to 700 amino acids, consecutive about 329 to 650 amino acids, or consecutive about 329 to 638 amino acids from 1st position of the KIF5B protein encoded by NM_004521.

In the fusion protein, the fusion may occur between the 16th exon of KIF5B gene and 12th exon of RET gene, which is called as a fusion point or breakpoint. The term "a fusion region" may refer to a polynucleotide fragment (about ~30 nucleotides) or polypeptide (about ~30 amino acids) fragment around the fusion point.

As used herein, the exon number is numbered according to the exon number allocated by NCBI .

In an Embodiment, the fusion protein KIF5B-RET may have the amino acid

sequence of SEQ ID NO: 3, 7, 11 or 15, wherein a polypeptide fragment from 629th to 648th positions of SEQ ID NO: 3, from 629th to 648th positions of SEQ ID NO: 7, from 566th to 585th positions of SEQ ID NO: 11, and from 839th to 858th positions of SEQ ID NO: 15 may be a fusion region of the fusion protein KIF5B-RET. The fusion region of the fusion protein KIF5B-RET may have the amino acid sequence ofSEQ ID NO: 4, 8, 12 or 16. The fusion gene of KIF5B-RET encoding the fusion protein of KIF5B-RET may have the nucleotide sequence of SEQ ID NO: 1, 5, 9 or 13, wherein a polynucleotide from 1885th to 1944th positions of SEQ ID NO: 1, 1885th to 1944th positions of SEQ ID NO: 5, 1696th to 1755th positions of SEQ ID NO: 9, and 2515th to 2574th positions of SEQ ID NO: 13 may be a fusion region of the fusion gene KIF5B-RET. The fusion region of the fusion gene KIF5B-RET may have the nucleotide sequence of EQ ID NO: 2, 6, 10 or 14. The fusion genes, the fusion proteins, and the fusion regions thereof are shown in Figs. 27 to 34.

The nucleotide sequences of DNA molecules and the amino acid sequences of proteins encoded by the DNA molecules may be determined by an automated DNA sequencer or an automated peptide sequencer. The (nucleotide or amino acid) sequences determined by such automated sequencing means may include partial error compared with actual sequences. For Generally, the sequences determined by automated sequencing may have sequence identity of at least about 90%, at least about 95%, at least about 99%, or at least about 99.9% compared with actual sequences. Therefore, the fusion protein, the fusion gene or the fusion region may have an amino acid sequence or a nucleotide sequence having sequence identity of at least about 90%, at least about 95%, at least about 99%, or at least about 99.9% compared with the sequences of SEQ ID NOS: 1 to 17.

It is confirmed that the fusion protein and the fusion gene are specifically present in cancer region, and they are not present in other region around the cancer region in the same tissue, suggesting a use of the fusion protein and/or the fusion gene as a biomarker for a cancer, for example, a solid cancer, in particular a lung cancer. In addition, a RET-involved chromosomal rearrangement including inversion or translocation in Chromosome 10 or an overexpression of RET is also found in a cancer cell, in particular a lung cancer cell.

Therefore, another embodiment provides a method of diagnosing a cancer or a

method of providing information for diagnosing a cancer, including detecting, in a test sample obtained from a subject, at least one selected from the group consisting of:

a RET-involved chromosomal rearrangement including inversion or translocation in Chromosome 10;

a fusion protein including N-terminal domain of a fusion partner and C-terminal domain of RET protein;

a fusion gene encoding the fusion protein; and

an overexpression of RET compared to a standard sample from an individual without lung cancer,

wherein when at least one selected from the above group is detected in the test sample, the subject is determined as a patient suffered from a cancer.

The RET-involved chromosomal rearrangement may result in formation of the fusion protein or the fusion gene. For example, the RET-involved chromosomal rearrangement may be an inversion Chromosome 10. The inversion of Chromosome 10 may be detected by using a polynucleotide (a probe) capable of hybridizing with (complementarily binding to) the inversion region in Chromosome 10 and/or a primer pair capable of detecting the inversion of Chromosome 10, for example, capable of producing a polynucleotide fragment having consecutive 100 to 200 nucleotides including the inversion region in Chromosome 10. For example, the inversion of Chromosome 10 may be detected by using the primer pair may comprise 5'-CAGAATTTCACAAGGAGGGAAG-3' (SEQ ID NO: 18) and 5'-CAGGACCTCTGACTACAGTGGA-3' (SEQ ID NO: 19).

The fusion protein and the fusion gene are as described above.

In a concrete embodiment, the fusion protein may also be detected by detecting the presence of the fusion protein or the fusion gene or mRNA corresponding to the fusion gene.

The presence of the fusion protein may be detected be a general assay that measures the interaction between the fusion protein and a material (e.g., an antibody or an aptamer) specifically binding to the fusion protein. The general assay may be immunochromatography, immunohistochemical staining, enzyme liked immunosorbent assay (ELISA), radioimmunoassay (RIA), enzyme immunoassay (EIA), florescence immunoassay (FIA), luminescence immunoassay (LIA), western blotting, FACS, and the

like.

In addition, the presence of the fusion gene or the mRNA may be detected by a general assay such as PCR, FISH (fluorescent in situ hybridization), and the like, using a polynucleotide capable of hybridizing with (complementarily binding to) the fusion gene or the mRNA. The fusion gene may be detected and/or validated by using the integration techniques of whole-transcriptome (RNA) and/or whole-genome (DNA) sequencing through massively parallel sequencing technologies. The polynucleotide capable of hybridizing with the fusion gene or the mRNA may be a siRNA, an oligonucleotide, DNA probe, or DNA primer, which can detect the fusion gene or the mRNA by a direct hybridization with the fused or truncated gene or transcript in the test sample.

When the fusion gene is a fusion gene KIF5B-RET encoding the fusion protein of KIF5B-RET, the fusion gene KIF5B-RET may be detected by using a polynucleotide (a probe) capable of hybridizing with (complementarily binding to) the fusion region of SEQ ID NO: 2, 6, 10 or 14, and/or a primer pair capable of producing a polynucleotide fragment having consecutive 100 to 200 nucleotides including the fusion region of SEQ ID NO: 2, 6, 10 or 14 in SEQ ID NO: 1, 5, 9 or 13, respectively. For example, the fusion gene KIF5B-RET may be detected by using the primer pair of 5'-GTGAAACGTTGCAAGCAGTTAG-3' (KIF5B; SEQ ID NO: 20) and 5'-CCTTGACCACTTTTCCAAATTC-3' (RET; SEQ ID NO: 21) or 5'-TAAGGAAATGACCAACCACCAG-3' (KIF5B; SEQ ID NO: 22) and 5'-CCTTGACCACTTTTCCAAATTC-3' (RET; SEQ ID NO: 21). In addition, the fusion protein KIF5B-RET may be detected using an antibody or aptamer specifically binding to the fusion region of the fusion protein KIF5B-RET. For example, the fusion region of the fusion protein KIF5B-RET may have the amino acid sequence of SEQ ID NO: 4, 8, 12 or 16.

The term "capable of hybridizing with the fusion region (or the inversion region)" may refer to having a complementary sequence or a sequence having sequence identity of at least 90% with that of the fusion region (or the inversion region).

Another embodiment provides a composition for diagnosing a cancer, including one or more selected from the group consisting of a polynucleotide capable of hybridizing with the fusion region of SEQ ID NO: 2, 6, 10 or 14, a primer pair capable of

producing a polynucleotide fragment having consecutive 100 to 200 nucleotides including the fusion region of SEQ ID NO: 2, 6, 10 or 14 in SEQ ID NO: 1, 5, 9 or 13, respectively, a polynucleotide capable of hybridizing with the inversion region in Chromosome 10, a primer pair capable of producing a polynucleotide fragment having consecutive 100 to 200 nucleotides including the inversion region of Chromosome 10, and an antibody or aptamer binding to the fusion region of SEQ ID NO: 4, 8, 12 or 16. For example, the primer pair may be at least one selected from the group consisting of the primer pair of 5'-GTGAAACGTTGCAAGCAGTTAG-3' (KIF5B; SEQ ID NO: 20) and 5'-CCTTGACCACTTTTCCAAATTC-3' (RET; SEQ ID NO: 21) or 5'-TAAGGAAATGACCAACCACCAG-3' (KIF5B; SEQ ID NO: 22) and 5'-CCTTGACCACTTTTCCAAATTC-3' (RET; SEQ ID NO: 21), to detect the fusion gene of KIF5B-RET encoding the fusion protein, and the primer pair of 5'-CAGAATTTCACAAGGAGGGAAG-3' (SEQ ID NO: 18) and 5'-CAGGACCTCTGACTACAGTGGA-3' (SEQ ID NO: 19), to detect the inversion of Chromosome 10.

Another embodiment provides a use of the fusion protein and/or the fusion gene for diagnosing a cancer.

The patient may be any mammal, for example, a primate such as a human or monkey, a rodent such as a mouse or a rat, in particular a human.

The test sample may be a cell (e.g., a lung cell), a tissue (e.g., a lung tissue), or body fluid (e.g., blood) separated from the patient, for example a human. The patient may be being treated or planed to be treated with a kinase inhibitor. The test sample may include a cell derived from a human cancer cell or an extract thereof.

The fusion protein and/or the fusion gene may act as a target for treatment of a cancer.

Therefore, another embodiment provides a method of preventing and/or treating a cancer, comprising administering a pharmaceutically (therapeutically) effective amount of at least one inhibitor against the fusion protein, at least one inhibitor against the fusion gene encoding the fusion protein, at least one inhibitor against a RET coding gene, or a combination thereof, to a patient in need thereof. The method may further comprise the step of identifying the patient who needs the prevention and/or treatment of a cancer, prior to the step of administering.

Another embodiment provides a composition for preventing and/or treating a cancer, comprising at least one inhibitor against the fusion protein, at least one inhibitor against the fusion gene encoding the fusion protein, at least one inhibitor against a RET coding gene, or a combination thereof.

Another embodiment provides a use of an inhibitor against the fusion protein, an inhibitor against the fusion gene encoding the fusion protein, an inhibitor against a RET coding gene, or a combination thereof, for preventing and/or treating a cancer.

The inhibitor against the fusion protein of KIF5B-RET may be at least one selected from the group consisting of an aptamer specifically binding to the fusion protein; an antibody specifically binding to the fusion protein; and a kinase inhibitor such as sorafenib(4-[4-[[4-chloro-3-(trifluoromethyl)phenyl]carbamoylamino]phenoxy]-*N*-methyl-pyridine-2-carboxamide), cabozantinib(*N*-(4-((6,7-Dimethoxyquinolin-4-yl)oxy)phenyl)-*N*-(4-fluorophenyl)cyclopropane-1,1-dicarboxamide), and the like. The inhibitor against the fusion gene or the RET coding gene may be at least one selected from the group consisting of siRNA, shRNA, miRNA, and an aptamer, which are capable of specifically binding to the fusion gene or the RET coding gene.

In the present invention, the cancer may be any solid cancer, for example, a lung cancer, a liver cancer, a colon cancer, a pancreatic cancer, a gastric cancer, a breast cancer, an ovarian cancer, a renal cancer, a thyroid cancer, an esophageal cancer, a prostatic cancer, or a brain cancer. In concrete embodiment, the cancer may be a lung cancer, in particular a small cell lung cancer (SCLC) or a non-small cell lung cancer (NSCLC) such as a lung adenocarcinoma, a squamous cell lung carcinoma, or a large cell lung carcinoma.

Still another embodiment provides a method of screening an anticancer drug including:

contacting a sample compound to a cell expressing the fusion protein; and

measuring the fusion protein expression level in the cell,

wherein the fusion protein expression level in the cell treated with the sample compound is decreased compared with that before the treatment with the sample compound or that in a non-treated cell, the sample compound is determined as a candidate compound for the anticancer drug.

The method of screening an anticancer drug may further include the step of

measuring the fusion protein expression level in the cell before the treatment of the sample compound. In this case the sample compound may be determined as a candidate compound for the anticancer drug when the fusion protein expression level after treatment of the sample compound is decreased compared with that before the treatment with the sample compound in the same cell. Alternatively, the method of screening an anticancer drug may include providing cells expressing the fusion protein, and contacting a sample compound to a part of the provided cells. In this case the sample compound may be determined as a candidate compound for the anticancer drug when the fusion protein expression level in the cell contacted with the sample compound is decreased compared with that in the cells which are not contacted with the sample compound.

The cell used in the screening method may be a cell derived from a cancer cell where the fusion gene or the fusion protein is expressed and/or activated, an extract of the cell, or a culture of the cell. The cancer cell may be a solid cancer cell, in particular a lung cancer, for example a non-small cell lung cancer such as a lung adenocarcinoma, as described above.

The fusion protein expression level may be detected be a general assay such as immunochromatography, immunohistochemical staining, enzyme liked immunosorbent assay (ELISA), radioimmunoassay (RIA), enzyme immunoassay (EIA), florescence immunoassay (FIA), luminescence immunoassay (LIA), western blotting, FACS, and the like.

The sample compound may be any natural or synthetic compound, for example at least one selected from the group consisting of a general compound, DNA, RNA, protein, and the like.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a microscopic image showing a paraffin section from a primary lung cancer tissue of a patient (AK55) obtained by CT-guided biopsy stained by hematoxylin and eosin, in magnification ratio of x100.

Fig. 2 is a microscopic image showing a paraffin section from a primary lung cancer tissue of a patient (AK55) obtained by CT-guided biopsy stained by hematoxylin and eosin, in magnification ratio of x400.

Fig. 3 is a microscopic image showing a result of immunohistochemical analysis of a primary lung cancer tissue for CK7.

Fig. 4 is a microscopic image showing a result of immunohistochemical analysis of a primary lung cancer tissue for TTF1.

Fig. 5 is a microscopic image showing a result of immunohistochemical analysis of a primary lung cancer tissue for CK20.

Fig. 6 shows a graphical representation of fusion genes identified in the lung cancer transcriptome sequencing.

Fig. 7 schematically shows *KIF5B-RET* fusion gene.

Fig. 8 is a graph showing RNA expression level of each *RET* exon.

Fig. 9 schematically shows a 10.6 Mb-long inversion event in chromosome 10 in the massively parallel sequencing of the cancer genome.

Fig. 10 shows a PCR amplification result for validation of *KIF5B-RET* fusion gene in RNA of AK55.

Fig. 11 shows a PCR amplification result for validation of *KIF5B-RET* fusion gene in DNA of AK55.

Fig. 12 shows a result of detection of the inversion breakpoint using Sanger sequencing for RNA validation.

Fig. 13 shows a result of detection of the inversion breakpoint using Sanger sequencing for DNA validation.

Fig. 14 schematically shows functional domains of KIF5B-RET fusion protein.

Fig. 15 shows a three-dimensional structure of KIF5B-RET fusion protein as predicted by the PHYRE2 algorithm.

Fig. 16 is a microscopic image showing a result of immunohistochemical analysis of *KIF5B-RET* expression in the lung cancer (bone metastasis) obtained from a patient (AK55) (x400).

Fig. 17 is a graph showing the results of analysis of RET expression in other lung adenocarcinomas.

Fig. 18 shows a result of network analysis of gene expression in the liver metastasis.

Fig. 19 shows results of FISH analysis for normal cell (A) and lung cancer cell (B).

Fig. 20 shows western blotting results of NIH3T3 cell line showing the expression of KIF5B-RET fusion protein in NIH3T3 cell line.

Fig. 21 shows the colony forming ability of NIH3T3 cell line transfected with KIF5B-RET fusion gene.

Fig. 22 shows the protein expression level in NIH3T3 cell line transfected with KIF5B-RET fusion gene under the treatment of a kinase inhibitor, Cabozantinib.

Fig. 23 is a graph showing the cell growth rate of KIF5B-RET fusion protein expressing cell under the treatment of a kinase inhibitor, Cabozantinib.

Fig. 24 is a gel electrophoresis image of liver metastatic lung cancer (AK55) and triple-negative lung adenocarcinoma (LC_S2).

Fig. 25A is a gel electrophoresis image of double-negative lung adenocarcinoma (LC_S6).

Fig. 25B is results of Identification of breakpoint of the KIF5B-RET fusion gene in LC_S6 using Sanger sequencing.

Fig. 26 schematically shows KIF5B-RET fusion transcripts of AK55, LC_S2, and LC_S6.

Fig 27 shows nucleotide sequence of KIF5B-RETa fusion gene and its fusion region, wherein the KIF5B domain is derived from NM_020975.

Fig 28 shows amino acid sequence of KIF5B-RETa fusion protein and its fusion region, wherein the KIF5B domain is derived from NM_020975.

Fig 29 shows nucleotide sequence of KIF5B-RETc fusion gene and its fusion region, wherein the KIF5B domain is derived from NM_020630.

Fig 30 shows amino acid sequence of KIF5B-RETc fusion protein and its fusion region, wherein the KIF5B domain is derived from NM_020630.

Fig 31 shows nucleotide sequence of KIF5B-RETa variant fusion gene and its fusion region, obtained from LC_S2.

Fig 32 shows amino acid sequence of KIF5B-RETa variant fusion protein and its fusion region, obtained from LC_S2.

Fig 33 shows nucleotide sequence of KIF5B-RETa variant fusion gene and its fusion region, obtained from LC_S6.

Fig 34 shows amino acid sequence of KIF5B-RETa variant fusion protein and its fusion region, obtained from LC_S6.

EXAMPLE

The present invention is further explained in more detail with reference to the following examples. These examples, however, should not be interpreted as limiting the scope of the present invention in any manner.

Example 1: Sample preparations

All protocols used in this example were approved by the Institutional Review Board of Seoul St. Mary's Hospital (Approval #KC11OISI0603). Paraffin-embedded tissues were obtained from primary lung cancer and bone metastasis of a patient AK55. A frozen tissue from biopsy of liver metastatic cancer from AK55 was also available to use. In addition, venous blood of AK55 was extracted. Genomic DNA was extracted from the lung cancer, bone metastasis, liver metastasis and blood of the patient AK55. Furthermore, RNA was extracted from the frozen liver metastasis of the patient AK55. Then cDNA was synthesized from total RNA as described in "Ju YS, Kim JI, Kim S, et al., Nat Genet 2011," which is incorporated herein by reference.

The patient AK55 (A 33-year-old man patient received a diagnosis of lung adenocarcinoma with multiple metastases) was healthy until 33 years of age, when a poorly differentiated adenocarcinoma developed in the right upper lobe of lung as shown in Figs. 1 and 2. Figs. 1 and 2 are microscopic images showing a paraffin section from a primary lung cancer tissue obtained by CT-guided biopsy (stained by hematoxylin and eosin) (Fig. 1: x100; Fig. 2: x400). In the cancer tissue, poorly differentiated tumor cell nests were present in the desmoplastic stroma. In addition, the cancer cells had plump cytoplasm and large pleomorphic nuclei.

The metastases in liver and multiple bones were also detected in positron emission tomography (PET) studies. For pathological diagnosis, he underwent CT-guided biopsy of primary lung cancer as well as ultrasound-guided biopsy of liver metastasis. The patient AK55 has no known family history of cancers from grandparents and he is a never-smoker. A week after diagnosis, he suffered from a neck fracture due to the metastasis in cervical bone, and underwent a C7 corpectomy. In pathologic studies, his lung adenocarcinoma was negative for known *EGFR*, *KRAS* and *ALK* mutations. The immunohistochemical analysis results for CK7, CK20 and TTF1 were

consistent with lung adenocarcinoma (Figs. 3-5; positive for CK7 (Fig. 3) and TTF1 (Fig. 4), negative for CK20 (Fig. 5)).

Figs. 3-5 are microscopic images showing results of immunohistochemical analyses of a primary lung cancer tissue (Fig. 3; CK7; Fig. 4: TTF1; Fig. 5: CK20). These analyses were done in the metastatic tumor in the cervical bone. CK7 and TTF1 were positive, but CK20 was negative. The results highly suggest that primary lung adenocarcinoma is the origin of this cancer.

Example 2: Whole-genome analysis

Genomic variants of each sample obtained from the patient AK55 as described in Example 1 was classified into single nucleotide variation (SNV), short insertion and deletion (indel) and large deletions, using modified criteria of whole-genome sequencing as described in "Ju YS, Kim JI, Kim S, et al., Nat Genet 2011" and "Kim JI, Ju YS, Park H, et al., Nature 2009;460:1011-5", which are incorporated herein by reference. Then, the genomic variants in cancer tissue were compared with those in blood to identify cancer-associated somatic mutations. DNA and RNA sequencing data was also analyzed as described in "Ju YS, Kim JI, Kim S, et al., Nat Genet 2011," which is incorporated herein by reference.

Because the DNA of primary lung cancer was extracted from a small amount of DNA in the paraffin-embedded tissue, the short-read redundancy was too high for analysis. Hence, the primary comparisons were done between the sequences from liver metastasis and blood. The sequencing experiments were performed using the standard methods of Illumina and described in "Ju YS, Kim JI, Kim S, et al., Nat Genet 2011" and "Kim JI, Ju YS, Park H, et al., Nature 2009;460:1011-5", which are incorporated herein by reference.

Sequencing libraries were generated according to the standard protocol of Illumina Inc. for high-throughput sequencing. Excluding the genomic DNA from paraffin-embedded bone metastasis (of which DNA concentration was too low and it did not qualify under the inventor's criteria for generating the sequencing library), samples were sequenced using Illumina HiSeq2000 and Genome Analyzer IIx. From whole-genome deep sequencing of cancer (liver metastasis) and normal tissue (blood) of the patient AK55, the inventors obtained 47.77x and 28.27x average read-depth, respectively. The

obtained results are shown in Table 1.

(Table 1) Summary statistics of sequencing analysis of the lung cancer patient AK55.

Analysis	Tissue	Source	Massively Parallel Sequencing				Validation
			Number of aligned reads	Read length (bp)	Throughput (Gbp)	Read depth (fold)	PCR and Sanger sequencing
Genome	Blood	Fresh	392,194,564	103	80.79	28.27	Yes
	Lung cancer	Paraffin-embedded	274,909,815	103	56.63	19.81	Yes
	Liver metastasis	Frozen	362,530,401	101	136.55	47.77	Yes
			293,140,533	108			
	Bone metastasis	Paraffin-embedded	-	-	-	-	Yes
Transcriptome	Liver metastasis	Frozen	89,682,934	101, 68	15.16	-	Yes

The whole-genome coverage was evenly distributed (excepting normal 'spikes' in the centromeric or telomeric regions) suggesting no evidence of aneuploidy in the cancer tissue (Fig. 6). Fig. 6 shows a graphical representation of fusion genes identified in the lung cancer transcriptome sequencing. Intra- and inter-chromosomal fusion genes are shown in the central layer. The thickness of lines shows the amount of evidence (number of spanning reads). The *KIF5B-RET* fusion gene is shown in red. Chromosome ideograms are shown in the outer layer. Coverage of cancer whole-genome sequencing is shown in the 1st middle layer. This suggests that the cancer genome has no large chromosomal aneuploidy. Expression level of genes is shown in the 2nd middle layer using heatmap.

In the cancer whole-genome sequence, we could not find any known cancer-related somatic point mutations, archived in OMIM (Online Mendelian Inheritance in Man) and SNPedia. The comparison of SNVs, indels and copy number variants (CNVs) between cancer and blood did not show any remarkable mutations in the cancer-related genes which could drive oncogenesis.

Example 3: Fusion gene analysis

For detection of fusion genes using transcriptome sequencing, discordant reads, where the ends of a read were aligned to different genes, and exon-spanning reads across the fusion breakpoint of chimeric transcripts, were used. For final fusion gene candidates, corresponding genomic rearrangements, such as inversions, translocations

and large deletions were assessed in the whole-genome sequencing data.

Transcriptome data were analyzed. The inventors have focused on detecting fusion genes since many cancers are known to be driven by fusion genes resulting from pathogenic chromosomal translocation or inversion.

To detect fusion genes, each end of about 300bp-long cDNA fragment was sequenced upto 101bp by next generation sequencing (Ju YS et al., Genome Res. 2012 22:436-445). From the sequence data, we examined the existence of a discordant read wherein the sequences of both ends are aligned on different chromosomes. In addition, exon-spanning reads, one of each end sequence is generated from a breakpoint of the fusion gene, was also examined. Discordant and exon-spanning reads indicate the existence of a fusion gene. Genes that have both discordant reads and exon-spanning reads were determined as lung cancer fusion genes.

The approaches identified 52 fusion genes (Table 2; Fig. 6).

(Table 2) Selected fusion genes (20 out of 52 in total) identified in AK55.

Category	Donor gene	Acceptor gene	Chr	Distance (Mb)	# of discordant reads	# of spanning reads	Evidence in whole-genome sequence
Intra-chromosomal							
	KIF5B	*RET*	10	10.580	34	60	YES (inversion)
	KIF5B	*KIAA1462*	10	1.970	4	4	-
	EEF1DP3	*FRY*	13	0.133	3	5	-
	RPS6KB1	*TMEM49*	17	0.097	4	31	-
	HACL1	*COLQ*	3	0.075	3	4	-
	TMEM56	*RWDD3*	1	0.073	4	11	-
	FAM18B2	*CDRT4*	17	0.065	4	29	-
	CTBS	*GNG5*	1	0.065	6	27	-
	METTL10	*FAM53B*	10	0.054	2	4	-
	AZGP1	*GJC3*	7	0.048	5	15	-
	NKX2-1	*SFTA3*	14	0.046	3	7	-
	ADSL	*SGSM3*	22	0.036	5	6	-
	ART4	*C12orf69*	12	0.034	3	4	-
	LOC100131434	*IDS*	X	0.031	2	11	-

LOC100130093	*SNAP47*	1	0.030	2	2	-
C15orf57	*MRPL42P5*	15	0.025	2	7	-
MIA2	*CTAGE5*	14	0.024	30	102	-
SH3D20	*ARHGAP27*	17	0.024	2	10	-
RBM14	*RBM4*	11	0.023	16	24	-
Inter-chromosomal						
RSPO1	*HP*	16; 1	-	2	3	-

Of these, 94.2% (n=49) were intrachromosomal fusions between adjacent genes (< 135 Kb), which may not have any functional roles in oncogenesis (Table 2). In addition, one (1.9%) were inter-chromosomal fusions, but these were generated by haptoglobin (*HP*), which is highly expressed genes in liver. Although the existence of this fusion gene is interesting biologically, given the molecular function of the gene, it is not believed to be tumorigenic. The remaining two (3.8%) were *KIF5B-RET* and *KIAA1462-KIF5B* fusion genes, which were intrachromosomal fusions between remote genes (>~2Mb). Of these, *KIAA1462-KIF5B* was excluded, since its expression level is low and *KIAA1462* is a hypothetical protein of which the molecular function is not known. Except *KIF5B-RET* fusion, we could not detect the corresponding chromosomal rearrangements (e.g. large deletion, inversion or translocation) in the fusion gene candidates.

The final fusion gene, *KIF5B-RET*, was interesting in particular, since *RET* is a well known tyrosine-kinase proto-oncogene. In addition, this fusion gene has not been reported in human cancer, hence it is considered to be novel. The characteristics of this gene fusion event were further confirmed using RNA sequencing data. The fusion gene was highly expressed, as evidenced by 34 discordant paired-end reads and 60 spanning reads across the fusion-junction (see Table 2 and Fig. 7). Fig. 7 schematically shows *KIF5B-RET* fusion gene. In the transcriptome sequencing, 34 discordant paired-end reads and 60 spanning reads across the exon-junction were identified. The existence of these reads is strong evidence of a fusion gene. A discordant paired-end read is defined as a read whose end-sequences are aligned to different genes. A spanning read is a read, one of whose end-sequences is aligned across the junction of

the predicted fusion transcripts. In this analysis, the fusion occurred between the 16th exon of *KIF5B* and 12th exon of *RET*.

These data showed that the end of the 16th exon of *KIF5B* and the start of the 12th exon of the *RET* proto-oncogene were integrated. The expression profile showed that the first to eleventh exons of *RET* were not expressed (Fig. 8) in the cancer tissue, suggesting most of the *RET* expression in the cancer took place from the fusion gene rather than from the intact *RET* gene. Fig. 8 is a graph showing RNA expression level of each *RET* exon. *RET* expression was observed from the 12th exon, downstream of the junction of the fusion gene. This suggests that all the RET expression originated from the *KIF5B-RET* fusion gene, rather than normal RET.

KIF5B and *RET* are 10.6 Mb away from each other, located at 10p11.22 and 10q11.21, respectively. Because the coding strands for the two genes are different, a 10.6 Mb-long inversion event is necessary for the fusion gene (see Fig. 9). Fig. 9 schematically shows a 10.6 Mb-long inversion event in chromosome 10 in the massively parallel sequencing of the cancer genome. This event is the cause of the KIF5B-RET fusion gene. KIF5B is generally expressed with its universal promoter. After the inversion event, this promoter activates global expression of the *KIF5B-RET* fusion gene.

This genomic inversion event was confirmed in the cancer by detecting reads supporting the inversion (8 reads in the liver metastasis; 1 reads in the primary lung cancer). In blood tissue, however, there was no corresponding chromosomal rearrangement in the whole-genome sequencing.

The above findings were further validated using PCR amplification and Sanger sequencing of genomic DNA and cDNA. The PCR reactions were at 95 ℃ for 10 min, 30 cycles of 95 ℃ for 30 s, 62 ℃ for 10s 72 ℃ for 10 s and, finally, 72 ℃ for 10 min. PCR and Sanger sequencing primers for genomic inversion were 5'-CAGAATTTCACAAGGAGGGAAG-3' (SEQ ID NO: 18) and 5'-CAGGACCTCTGACTACAGTGGA-3' (SEQ ID NO: 19). Primers for fusion transcripts are 5'-GTGAAACGTTGCAAGCAGTTAG-3' (SEQ ID NO: 20) and 5'-CCTTGACCACTTTTCCAAATTC-3' (SEQ ID NO: 21). All the Sanger sequencing experiments were performed at Macrogen Inc. (http://www.macrogen.com).

All three cancer-related tissues of the patient AK55 (lung cancer, bone and liver metastasis), excluding normal blood, showed PCR products resulting from the inversion event (Figs. 10 and 11). Figs. 10 and 11 show the obtained PCR amplification results for validation of *KIF5B-RET* fusion gene in RNA (Figs. 10) and DNA (Fig. 11) of the patient AK55. The validation of *KIF5B-RET* fusion gene in RNA and DNA was performed by PCR amplification using inversion-specific primers as described above and electrophoresis. The fusion gene is only detected in the RNA and DNA from the cancer tissue of the patient AK55.

Sanger sequencing of these products validated the fusion transcript again as well as finding the breakpoints of the inversion with nucleotide resolution (chr10:32,351,306-42,931,601 in human reference genome build 36.3). Figs. 12 and 13 show results of detection of the inversion breakpoint using Sanger sequencing for RNA (Fig. 12) and DNA (Fig. 13) validation. The fusion gene was successfully validated by Sanger sequencing. The inversion breakpoint in the genome was also identified to single-nucleotide resolution. The genomic breakpoints were located in the introns of KIF5B and RET. Two bases downstream from the breakpoint (chr10:42,931,604 in human reference genome build 36.3), a 1-bp deletion was generated, suggesting error-prone non-homologous end joining (NHEJ) might contribute to this inversion event after double-strand DNA breaks.

Interestingly, a single base-pair deletion was identified 2bp-adjacent to the breakpoint (chr10:42,931,604), suggesting an error-prone DNA repair mechanism, or non-homologous end joining (NHEJ), might have contributed to this inversion event after double-strand DNA breaks. Furthermore, the G-quadruplex (a non-B DNA) structure is predicted in the ~100 bp upstream of the breakpoint in *RET*, which is known to be fragile and a source of chromosomal translocations.

Example 4: Functional assessment of KIF5B-RET fusion kinase

The *RET* oncogene is a transmembrane receptor tyrosine kinase. *RET* consists of extracellular region (which contains Cadherin-like domains), a trans-membrane domain and an intracellular region containing a tyrosine kinase domain (see Fig. 14). Fig. 14 schematically shows functional domains of KIF5B-RET fusion protein. The fusion protein consists of 638 N-terminal residues of KIF5B and 402 C-terminal residues

of RET. The fusion gene has a protein tyrosine kinase domain together with a coiled-coil domain. The coiled-coil domain induces homo-dimerization which will activate the oncogenic protein tyrosine kinase domain by auto-phosphorylation.

When RET is dimerized by binding co-receptors and ligands, such as glial derived neurotrophic factor (GDNF), it is activated by auto-phosphorylation and then simulates downstream signaling pathways. The downstream signaling cascade of the *RET* proto-oncogene is the mitogen-activated protein kinase (MAPK) pathway, which regulates cell survival/apoptosis, proliferation, differentiation, and migration. The normal expression of *RET* is important for neuronal development, but it is known to not be activated in differentiated tissues.

KIF5B is a microtubule-based motor protein, ubiquitously expressed due to its active promoter and involved in the transport of organelles in eukaryotic cells. Its coiled-coil domain induces homo-dimerization, which is essential for its movement.

Fig. 15 shows a three-dimensional structure of KIF5B-RET fusion protein as predicted by the PHYRE2 algorithm. The N- and C-terminal of the fusion protein are colored in red and blue, respectively. Protein 3D modeling was performed using Phyre2 software using the protein sequence of the KIF5B-RET fusion gene (http://www.sbg.bio.ic.ac.uk/phyre2/).

Taken together, the *KIF5B-RET* fusion gene may be highly expressed and then dimerized after translation owing to KIF5B (Figs. 14 and 15). Then, the dimerized RET protein tyrosine kinase domain may be stimulated abnormally, thus facilitating the stimulation of an oncogenic pathway. Immunohistochemical analysis showed that the tyrosine kinase domain of RET was highly expressed in the lung cancer tissue (Fig. 16). Fig. 16 is a microscopic image showing a result of immunohistochemical analysis of *KIF5B-RET* expression in the lung cancer (bone metastasis) obtained from a patient (AK55) (x400). The protein is exclusively observed in tumor cells, suggesting the *KIF5B-RET* fusion protein has important roles in the cancer.

Example 5: Frequency assessment of RET overexpression in other lung cancer samples

The oncogenic effect of *RET* was first identified in papillary thyroid carcinoma (PTC) where diverse kinds of chromosomal translocations and inversions led to the

formation of PTC/RET fusion genes. Specific point mutations have also been reported as drivers in multiple endocrine neoplasia (MEN) types 2A and 2B. In addition, activated *RET* has been observed in prostate cancer, pancreatic cancer and melanoma. Its tumorigenecity is also supported by *RET* transgenic mice studies which generated a variety of malignancies. However, this gene has not been highlighted in lung cancer previously.

The frequency of *RET* overexpression in lung adenocarcinoma was evaluated using previous microarray data archived in databases. In particular, to investigate the RET overexpression in general lung adenocarcinoma, we analyzed the expression profile of lung adenocarcinoma archived in databases (Gene Expression Omnibus: http://www.ncbi.nlm.nih.gov/geo/ and The Cancer Genome Atlas (TCGA): http://cancergenome.nih.gov/).

Expression profiling of ten adenocarcinoma cell lines (Gemma A, Li C, Sugiyama Y, et al., BMC Cancer 2006;6:174) showed two samples highly expressing *RET*. On the other hand, *RET* was not activated in ten small cell cancer cell lines and nine squamous cell carcinoma cell lines in this dataset. We found a further 3 studies which profiled RET proto-oncogene expression in primary lung cancer. In the dataset of the first study (Ding L, Getz G, Wheeler DA, et al., Nature 2008;455:1069-75), 6 of the 75 tumors (8%) overexpressed *RET*. Another dataset (Kuner R, Muley T, Meister M, et al., Lung Cancer 2009;63:32-8) showed *RET* activation in 5 out of 40 samples (12.5%). Finally, The Cancer Genome Atlas (TCGA) dataset showed RET overexpression in 3 out of 32 samples (9.4%; Fig. 17). Fig. 17 is a graph showing the results of analysis of RET expression in other lung adenocarcinomas. The expression microarray data of 32 lung adenocarcinomas deposited in TCGA (The Cancer Genome Atlas) were analyzed. Of these, 3 samples showed clear overexpression of *RET*, suggesting the frequency of overexpression in lung adenocarcinoma is approximately 10%.

Taken together, these results suggest that the frequency of RET overexpression is ~10% in lung adenocarcinoma.

Fig. 18 shows a result of network analysis of gene expression in the liver metastasis. The network analysis was done using Cytoscape (http://www.cytoscape.org/) along with MiMI plugin (http://mimiplugin.ncibi.org/). Genes overexpressed in the cancer were mapped as a network, where the node size is

proportional to the relative expression. Major functional groups were labeled. Functionally important genes were colored in red.

Example 6: Identification of KIF5B-RET fusion gene by FISH analysis

To identify RET rearrangements, fluorescent in situ hybridization (FISH) was performed on AK55 cell lines and normal cell as a control by using a break-apart probe for RET. The slides were immersed in Citrisolve (Fisher Scientific, Pittsburgh, PA) for 15 minutes, jet air dried, immersed in Lugol solution for 5 minutes, and immersed in 2.5% sodium thiocyanate for 30 seconds. The slides were then placed in 10 mmol/L of citrate/citric acid solution (pH 6.0) and microwaved on the high setting for 5 minutes, followed by 15 to 45 minutes in 0.4% pepsin solution (pepsin A/0.9% sodium chloride at pH 1.5) at 37° C. Ten microliters of FISH reagent (7 μL LSI buffer [Vysis, Downers Grove, IL] and 3 μL probe) were placed on each slide, and the slides were coverslipped, denatured in a Hybrite (Vysis) set at a melt temperature of 80° C for 5 minutes, and incubated in a humidified chamber at 37° C for 12 hours. The slides were then washed in 2Xsaline sodium citrate/0.1% NP40 (US Biological, Swampscott, MA) at 70° C for 2 minutes and counterstained with 49,6-diamidino-2-phenyl indole dihydrochloride. The cells were analyzed by a microscopist (M.L.) using a fluorescent microscope equipped with appropriate filter sets. Chromosome inversion, a deduced chromosomal rearrangement is responsible for KIF5B-RET fusion. The obtained results of FISH are shown in Fig. 19, showing a split of red and green probes that flank the RET translocation site in a KIF5B-RET fusion positive tumor (arrows).

Example 7: Examination of cell growth rate and viability of a mammal cell transfected with KIF5B-RET fusion gene

By transfecting NIH 3T3 cells with a construct including cDNA encoding KIF5B-RET fusion protein and expressing the KIF5B-RET fusion protein, it was confirmed whether or not the expression of the KIF5B-RET fusion protein contributes to conversion from normal cell to tumor cell. NIH 3T3 cells (ATCC/ ATCC Number CRL-1658) were maintained in DMEM medium (Gibco BRL) supplemented with 10%(v/v) fetal bovine serum (FBS; Gibco BRL), penicillin, and streptomycin. Preparation of

supernatant of retrovirus and transfection were performed according to protocol provided by Platinum Retrovirus Expression System purchased from CELL BIOLABs. NIH3T3 cells were transducted with the supernatant of retrovirus including a pMXs-puro/fusion protein expression vector, and then the transducted cells were selected using puromycin(2ug/ml). Whole cell lysates from cell lines were subjected to SDS-PAGE followed by blotting onto a polyvinylidine difluoride(PVDF) membrane. The blot were blocked TBS containing 0.1% Tween 20 and 5% BSA, and probed with anti-RET(#3223, Cell signaling, USA), anti-phospho-RET(Tyr905) (#3221, Cell signaling, USA), and anti-actin (A5441, Sigma-Aldrich, USA). After washing with TBS containing 0.1% Tween 20, the membrane were incubated with horseradish peroxidase-conjugated anti-mouse or anti-rabbit secondary antibodies and treated with an enhanced chemiluminescence reagent (Pierce, #34080). The obtained results are shown in Fig. 20, indicating that the selected NIH3T3 cells are stably transformed with KIF5B-RET fusion gene through western blotting.

The growth rates of NIH3T3 parent cells and NIH3T3 stable cell lines expressing KIF5B-RETa, or KIF5B-RETc fusion gene (NIH3T3/KIF5B-RETa, NIH3T3/KIF5B-RETc) cells in FBS-containing or FBS-free medium were measured and compared with each other. The NIH3T3 cell and NIH3T3/KIF5B-RET cells were cultured with FBS containing media, or FBS-free media for 24 hour. And then, the obtained images are shown in Fig. 21. As shown in Fig. 21, the growth of non-transfected NIH3T3 cells is inhibited in FBS-free medium, but KIF5B-RET fusion gene transformed NIH3T3 cells grow and form colonies well even in FBS-free medium. These results indicate that the expression of KIF5B-RET fusion protein converts NIH3T3 cells properties and KIF5B-RET fusion gene transfected cells are capable of survival and growth even under the abnormal conditions such as FBS deficient medium owing to the KIF5B-RET fusion protein.

Example 8: Examination of inhibition of mammal solid tumor cell growth by the fusion protein inhibitor (Cabozantinib)

To confirm the effects of the fusion protein to stimulate growth and survival of cell lines (or tumor cells) expressing the fusion protein, the cell lines were treated with a inhibitor against a kinase or other domain in the fusion protein.

Specifically, KIF5B-RET transfected NIH3T3 cells (NIH3T3 /KIF5B-RET) (referring to Example 7) were treated with cabozantinib(4 Chem, Korea) in various concentrations for 2 days as shown in Fig. 22, and the expression levels of RET, phospho-RET, and actin (control) were measured by immunoblotting using corresponding antibodies. Anti-RET and anti-phospho-RET(Tyr905) antibodies were obtained from Cell Signaling Technology(#3223, #3221). Anti-actin antibody were obtained from Sigma Aldrich(#A5441).

The obtained results are shown in Fig. 22 showing that the expression of phospho-RET, which is an active form of RET, is decreased depending on the concentration of cabozantinib. These results indicate that the RET protein is abnormally activated in the fusion protein transfected cells, and the growth of the fusion protein transfected cells can be inhibited by treating a kinase inhibitor.

To quantitatively analyze such cell growth inhibition, the number of cells expressing the fusion protein is counted, and the cell growth inhibition was analyzed using WST-1 solution cell proliferation assay (Roche) according to protocol provided by the manufacturer. About 1000 to 5000 cells of the KIF5B-RET transfected NIH3T3 cells were seeded on 96-well plate, and grown in complete medium (DMEM, Gibco) supplemented with 10%(v/v) FBS. After 24 hours, the medium was replaced with 100 $\mu\ell$ of complete growth medium supplemented with 10%(v/v) FBS and cabozantinib in 100 nM concentrations as shown in Fig. 23, and then, the cells were further cultured for 72 hours. At end point of the cell culture, each well was added with 10 $\mu\ell$ of WST-1 solution and further cultured for 1 to 3 hours. Absorbance at 450nm was measured using a microplate reader. The growth inhibition was evaluated as mean ± SD value of the measured absorbance of cabozantinib treated cells compared with that of non-treated cells. The analyses were performed in triplicate. The obtained results are shown in Fig. 23. As shown in Fig. 23, the KIF5B-RET fusion protein contributes to increase of cell growth rate and cell survival of human tumor cells(such as NSCLC), and the inhibitor against the fusion protein is capable of leading to deceased cell survival and increased apoptosis.

Example 9: Detection of KIF5B-RET fusion gene in other patients

In order to show that the KIF5B-RET fusion gene also exists in other primary lung adenocarcinomas, a transcriptome of additional triple-negative (EGFR, KRAS, and EML4-ALK) primary lung adenocarcinoma was analyzed using massively parallel sequencing. The additional sample was called as LC_S2 (A 62-year-old man patient received a diagnosis of lung adenocarcinoma stage 3A). The sample of LC_S2 was prepared referring to the method described in Example 1. KIF5B-RET fusion transcripts were found in LC_S2. As in AK55, RET was highly expressed from 12th exon in LC_S2 as shown in Table 4.

(Table 4) Exon-by-exon RET expression

gene	accession	chrom	exon	start	end	length	strand	AK55	LC_S2
RET	NM_020630	10	exon1	43572516	43572779	263	+	0.03	0.10
RET	NM_020630	10	exon2	43595906	43596170	264	+	0.00	0.38
RET	NM_020630	10	exon3	43597789	43598077	288	+	0.18	0.68
RET	NM_020630	10	exon4	43600399	43600641	242	+	0.06	0.41
RET	NM_020630	10	exon5	43601823	43602019	196	+	0.07	0.32
RET	NM_020630	10	exon6	43604478	43604678	200	+	0.24	0.33
RET	NM_020630	10	exon7	43606654	43606913	259	+	0.14	0.43
RET	NM_020630	10	exon8	43607546	43607672	126	+	0.11	0.00
RET	NM_020630	10	exon9	43608300	43608411	111	+	0.26	0.27
RET	NM_020630	10	exon10	43609003	43609123	120	+	0.40	0.58
RET	NM_020630	10	exon11	43609927	43610184	257	+	0.24	0.66
RET	NM_020630	10	exon12	43612031	43612179	148	+	4.25	12.50
RET	NM_020630	10	exon13	43613820	43613928	108	+	5.82	7.74
RET	NM_020630	10	exon14	43614978	43615193	215	+	4.49	8.41
RET	NM_020630	10	exon15	43615528	43615651	123	+	7.13	14.60
RET	NM_020630	10	exon16	43617393	43617464	71	+	7.45	17.86
RET	NM_020630	10	exon17	43619118	43619256	138	+	8.94	18.15
RET	NM_020630	10	exon18	43620330	43620430	100	+	8.88	15.81
RET	NM_020630	10	exon19	43622022	43622952	930	+	8.21	8.37

Because KIF5B is generally expressed in differentiated tissue, the KIF5B-RET fusion gene could be expressed by the active promoter of KIF5B in those lung cancer tissues (AK55 and LC_S2). This fusion transcript in LC_S2 was validated using cDNA PCR.

The obtained validating data for AK55 and LC_S2 are shown in Fig. 24. Fig. 24 shows the results of analysis using cDNA PCR targeting KIF5B-RET fusion transcripts and gel electrophoresis in the liver metastatic lung cancer of AK55 and the additional triple-negative lung adenocarcinoma (LC_S2). cDNA from AK55(SEQ ID NO: 1) and LC_S2(SEQ ID NO: 9) shows clear evidence of the fusion transcript. Because the

fusion transcript in AK55 contains one more exon of KIF5B (exon 16) compared with that in LC_S2 (exon 15), the size of the PCR product in AK55 is longer than that in LC_S2.

In addition, the KIF5B-RET fusion gene was further assessed using cDNA PCR of a double-negative (EGFR and EML4-ALK were negative in pathologic studies; KRAS mutation status was unknown) primary lung adenocarcinoma (LC_S6) (A 58-year-old man patient received a diagnosis of lung adenocarcinoma stage 1A). The sample of LC_S2 was prepared referring to the method described in Example 1. The fusion transcript in LC_S2 was validated using cDNA PCR, confirming that LC_S6 showed the KIF5B-RET fusion gene (SEQ ID NO: 13) (Fig. 25). Fig. 25 shows the results of validation using cDNA PCR targeting KIF5B-RET fusion transcripts and gel electrophoresis in double-negative lung adenocarcinoma (LC_S6). LC_S6 shows clear evidence of the fusion transcript. The fusion transcript in LC_S6 contains seven more exons of KIF5B (exons 17–23) compared with that in AK55.

The breakpoint of the fusion gene in LC_S6 was identified using Sanger sequencing, and the obtained results are shown in Fig. 25B.

The validations relating to Figs. 24, 25A and 25B were performed using PCR amplification and Sanger sequencing of genomic DNA and cDNA. The PCR reactions were 10 min at 95℃; 30 cycles of 30 sec at 95℃, 10 sec at 62℃, and 10 sec at 72℃; and, finally, 10 min at 72℃. PCR and Sanger sequencing primers for genomic inversion of AK55 were 5'-CAGAATTTCACAAGGAGGGAAG-3' (KIF5B; SEQ ID NO: 18) and 5'-CAGGACCTCTGACTACAGTG GA-3' (RET; SEQ ID NO: 19). The primers for the fusion transcripts were 5'-GTGAAACGTTGCAAGCAGTTAG-3' (KIF5B; SEQ ID NO: 20; for AK55 and LC_S6) and 5'-CCTTGACCACTTTTCCAAATTC-3' (RET; SEQ ID NO: 21; or AK55, LC_S2 and LC_S6). For cDNA PCR in replication studies, a different KIF5B primer (5'-TAAGGAAATGACCAACCACCAG-3'; SEQ ID NO: 22) was used for LC_S2, since the KIF5B fusion breakpoint in LC_S2 was different to that in AK55. All the Sanger sequencing experiments were performed at Macrogen Inc. (http://www.macrogen.com).

Overall, we identified two more cases of the KIF5BRET fusion gene (LC_S2 and LC_S6) in primary lung adenocarcinomas in the replication study. These results clearly

show that KIF5B-RET fusion is not rare and that the fusion transcript generally exists in the primary lung adenocarcinomas. In addition, because it would be very unlikely to find identical nonfunctional fusion genes in different cancer tissues, these results also provide indirect evidence that the expression of the KIF5B-RET fusion gene has an important functional impact in lung cancer.

Interestingly in LC_S2 and LC_S6, exon 12 of RET was joined to exon 15 (LC_S2) and exon 23 (LC_S6) instead of to exon 16 of KIF5B as in AK55 (Fig. 26). Fig. 26 schematically shows KIF5B-RET fusion transcripts of AK55 (SEQ ID NO: 1), LC_S2(SEQ ID NO: 9), and LC_S6(SEQ ID NO: 13). Each rectangle indicates an exon of KIF5B (blue) and RET (red) gene.

These suggest that the double-strand breaks of DNAin KIF5B may not be consistent among primary lung cancers. However, because their coiled-coil domains are well preserved in the KIF5B-RET chimeric oncogene in both the samples (the length of coiled-coil domain in the fusion gene was 247 and 520 amino acids in LC_S2 and LC_S6, respectively), the dimerization activity is probably not very different compared with that of AK55 (310 amino acids).

The KIF5B-RET fusion genes and KIF5B-RET fusion proteins obtained from lung adenocarcinoma samples (AK55, LC_S2, and LC_S6) are summarized in the Table 4:

(Table 4)

		KIF5B (NM_004521)	**RET (NM_020975)**	**size**
AK55	nucleotide	1914 nt	1209 nt	3123 nt
	Amino acid	638 a.a	402 a.a	1040 a.a
	exon	1-16 exon	12-20 exon	25 exon
LC-S2	nucleotide	1725 nt	1209 nt	2934 nt
	Amino acid	575 a.a	402 a.a	977 a.a
	exon	1-15 exon	12-20 exon	24 exon
LC-S6	nucleotide	2544 nt	1209 nt	3753 nt
	Amino acid	848 a.a	402 a.a	1250 a.a

	exon	1-23 exon	12-20 exon	32 exon

WHAT IS CLAIMED IS:

1. A fusion protein consisting essentially of N-terminal domain of a fusion partner at N-terminal and C-terminal domain of RET protein at C-terminal.

2. The fusion protein according to Claim 1, wherein the fusion protein is KIF5B-RET protein consisting essentially of N-terminal domain of KIF5B protein at N-terminal and C-terminal domain of RET protein at C-terminal.

3. The fusion protein according to Claim 2, wherein the N-terminal domain of KIF5B protein consists essentially of consecutive at least about 329 amino acids from 1st position of the KIF5B protein encoded by the polynucleotide of NM_004521.

4. The fusion protein according to Claim 3, wherein the N-terminal domain of KIF5B protein comprises at least two KIF5B coiled coil domain which starts from the amino acid of the 329th position of the KIF5B protein.

5. The fusion protein according to Claim 3, wherein the N-terminal domain of KIF5B protein consists essentially of an amino acid sequence encoded by a polynucleotide from the first exon to 16th exon, or from the first exon to 15th exon, or from the first exon to 23th exon of the polynucleotide of NM_004521.

6. The fusion protein according to Claim 2, wherein the C-terminal domain of RET protein consists essentially of consecutive at least about 300 amino acids starting from an amino acid corresponding to the start position of 12th exon of NM_020630 or NM_020975 toward C-terminus of the RET protein encoded by the polynucleotide of NM_020630 or NM_020975.

7. The fusion protein according to Claim 7, wherein the C-terminal domain of RET protein consists essentially of consecutive about 300 to 450 amino acids starting

from an amino acid corresponding to the start position of 12th exon of NM_020630 or NM_020975 toward C-terminus of the RET protein encoded by the polynucleotide of NM_020630 or NM_020975.

8. The fusion protein according to Claim 2, having the amino acid sequence of SEQ ID NO: 3, 7, 11 or 15 or sequence identity of at least about 90% compared with the sequences of SEQ ID NO 3, 7, 11 or 15.

9. A fusion gene of KIF5B-RET encoding the fusion protein of KIF5B-RET of any one of Claims 1 to 8.

10. The fusion gene of KIF5B-RET according to Claim 9, having the nucleotide sequence of SEQ ID NO: 1, 5, 9 or 13 or sequence identity of at least about 90% compared with the sequences of SEQ ID NO 1, 5, 9 or 13.

11. A method of providing information for diagnosing a lung cancer, comprising the step of detecting, in a test sample obtained from a subject, at least one selected from the group consisting of:

a RET-involved chromosomal rearrangement including inversion or translocation in Chromosome 10;

a fusion protein including N-terminal domain of a fusion partner and C-terminal domain of RET protein according to any one of Claims 1 to 8;

a fusion gene encoding the fusion protein; and

an overexpression of RET compared to a standard sample from an individual without a cancer,

wherein when at least one selected from the above group is detected in the test sample, the subject is determined as a cancer patient.

12. The method according to Claim 11, wherein the RET-involved chromosomal rearrangement is the inversion Chromosome 10.

13. The method according to Claim 11, wherein the fusion gene encoding

the fusion protein is detected and validated by using the integration of whole-transcriptome (RNA) and whole-genome (DNA) sequencing through massively parallel sequencing technologies.

14. The method according to Claim 13, wherein the fusion gene encoding the fusion protein is detected by using one or more selected from the group consisting of a primer pair capable of producing a polynucleotide fragment having consecutive 100 to 200 nucleotides including the fusion region of SEQ ID NO: 2, 6, 10 or 14 in SEQ ID NO: 1, 5, 9 or 13, respectively, a polynucleotide capable of hybridizing with the fusion region of SEQ ID NO: 2, 6, 10 or 14, and an antibody or aptamer binding to the fusion region of SEQ ID NO: 4, 8, 12 or 16.

15. The method according to Claim 11, wherein the fusion gene encoding the fusion protein is detected by using a primer pair of 5'-GTGAAACGTTGCAAGCAGTTAG-3' (SEQ ID NO: 20) and 5'-CCTTGACCACTTTTCCAAATTC-3' (SEQ ID NO: 21) or 5'-TAAGGAAATGACCAACCACCAG-3' (SEQ ID NO: 22) and 5'-CCTTGACCACTTTTCCAAATTC-3' (SEQ ID NO: 21), and the inversion of Chromosome 10 is detected by using a primer pair of 5'-CAGAATTTCACAAGGAGGGAAG-3' (SEQ ID NO: 18) and 5'-CAGGACCTCTGACTACAGTGGA-3' (SEQ ID NO: 19).

16. A composition for diagnosing a lung cancer, comprising at least one selected from the group consisting of a polynucleotide capable of hybridizing with the fusion region of SEQ ID NO: 2, 6, 10 or 14, a primer pair capable of producing a polynucleotide fragment having consecutive 100 to 200 nucleotides comprising the fusion region of SEQ ID NO: 2, 6, 10 or 14 in SEQ ID NO: 1, 5, 9 or 13, respectively, a probe capable of hybridizing with the inversion region in Chromosome 10, a primer pair capable of producing a polynucleotide fragment having consecutive 100 to 200 nucleotides comprising the inversion region of Chromosome 10, and an antibody or aptamer binding to the fusion region of SEQ ID NO: 4, 8, 12 or 16.

17. The composition for diagnosing a lung cancer according to Claim 16, wherein the primer pair has the nucleotide sequence of 5'-GTGAAACGTTGCAAGCAGTTAG-3' (SEQ ID NO: 20) and 5'-CCTTGACCACTTTTCCAAATTC-3' (SEQ ID NO: 21), or 5'-TAAGGAAATGACCAACCACCAG-3' (SEQ ID NO: 22) and 5'-CCTTGACCACTTTTCCAAATTC-3' (SEQ ID NO: 21), to detect the fusion gene of KIF5B-RET encoding the fusion protein of Claim 1, or the nucleotide sequence of 5'-CAGAATTTCACAAGGAGGGAAG-3' (SEQ ID NO: 18) and 5'-CAGGACCTCTGACTACAGTGGA-3' (SEQ ID NO: 19), to detect the inversion of Chromosome 10.

18. A composition for preventing or treating a lung cancer, comprising at least one inhibitor against the fusion protein of any one of Claims 1 to 8, at least one inhibitor against the fusion gene encoding the fusion protein, at least one inhibitor against a RET coding gene, or a combination thereof, as an active ingredient.

19. The composition according to Claim 18, wherein

the inhibitor against the fusion protein is at least one selected from the group consisting of an aptamer specifically binding to the fusion protein, an antibody specifically binding to the fusion protein, sorafenib(4-[4-[[4-chloro-3-(trifluoromethyl)phenyl]carbamoylamino]phenoxy]-*N*-methyl-pyridine-2-carboxamide), and cabozantinib(*N*-(4-((6,7-Dimethoxyquinolin-4-yl)oxy)phenyl)-*N*-(4-fluorophenyl)cyclopropane-1,1-dicarboxamide), and

the inhibitor against the fusion gene or the RET coding gene is at least one selected from the group consisting of siRNA, shRNA, miRNA, and an aptamer, which are capable of specifically binding to the fusion gene or the RET coding gene.

20. Use of at least one inhibitor against the fusion protein of any one of Claims 1 to 8, at least one inhibitor against the fusion gene encoding the fusion protein, at least one inhibitor against a RET coding gene, or a combination thereof, in preventing or treating a lung cancer.

21. The use according to Claim 20, wherein

the inhibitor against the fusion protein is at least one selected from the group consisting of an aptamer specifically binding to the fusion protein, an antibody specifically binding to the fusion protein, sorafenib(4-[4-[[4-chloro-3-(trifluoromethyl)phenyl]carbamoylamino]phenoxy]-*N*-methyl-pyridine-2-carboxamide), and cabozantinib(*N*-(4-((6,7-Dimethoxyquinolin-4-yl)oxy)phenyl)-*N*-(4-fluorophenyl)cyclopropane-1,1-dicarboxamide), and

the inhibitor against the fusion gene or the RET coding gene is at least one selected from the group consisting of siRNA, shRNA, miRNA, and an aptamer, which are capable of specifically binding to the fusion gene or the RET coding gene.

22. A method of screening an anticancer drug including:

contacting a sample compound to a cell expressing the fusion protein; and

measuring the fusion protein expression level in the cell,

wherein the fusion protein expression level in the cell treated with the sample compound is decreased compared with that before the treatment with the sample compound or that in a non-treated cell, the sample compound is determined as a candidate compound for the anticancer drug.

FIG. 1

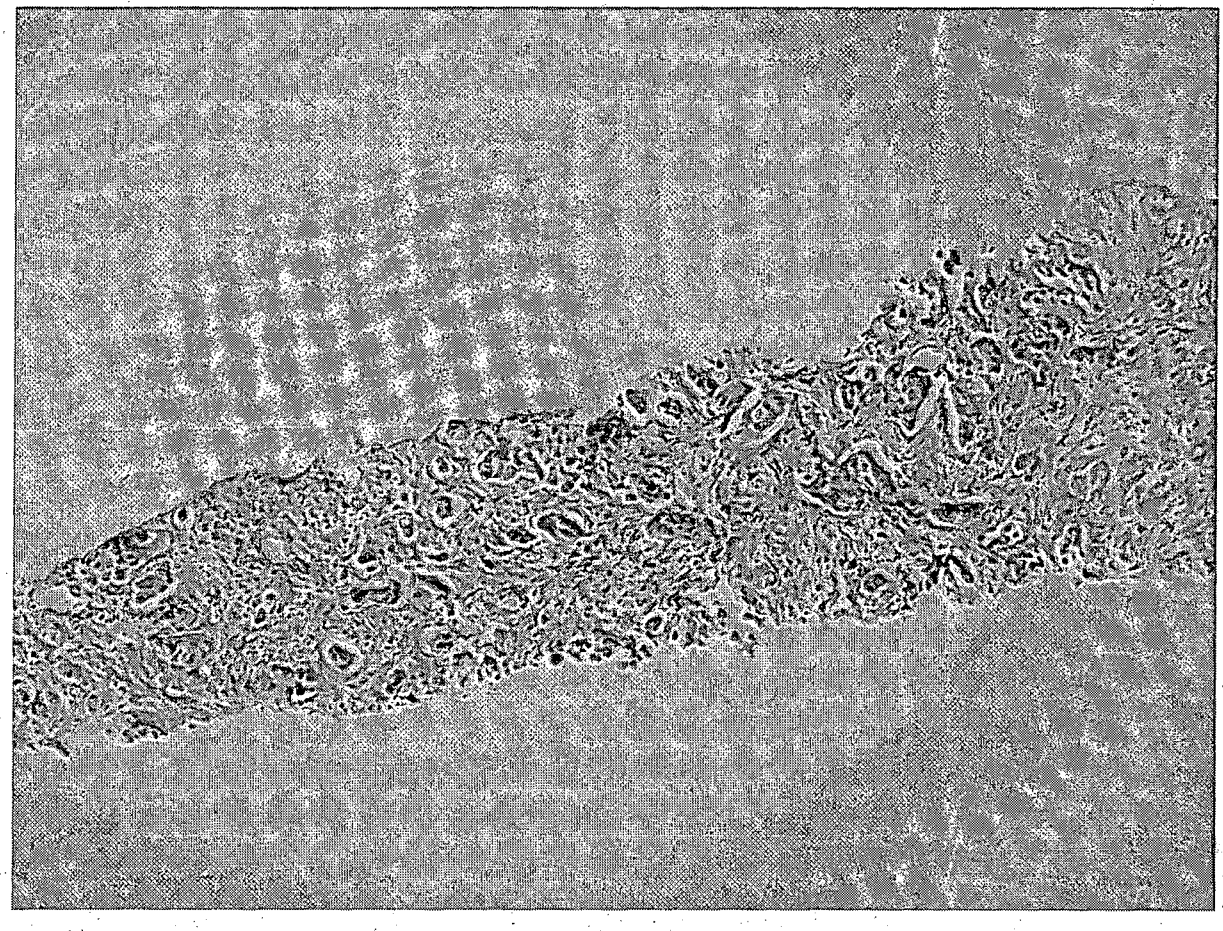


FIG. 2

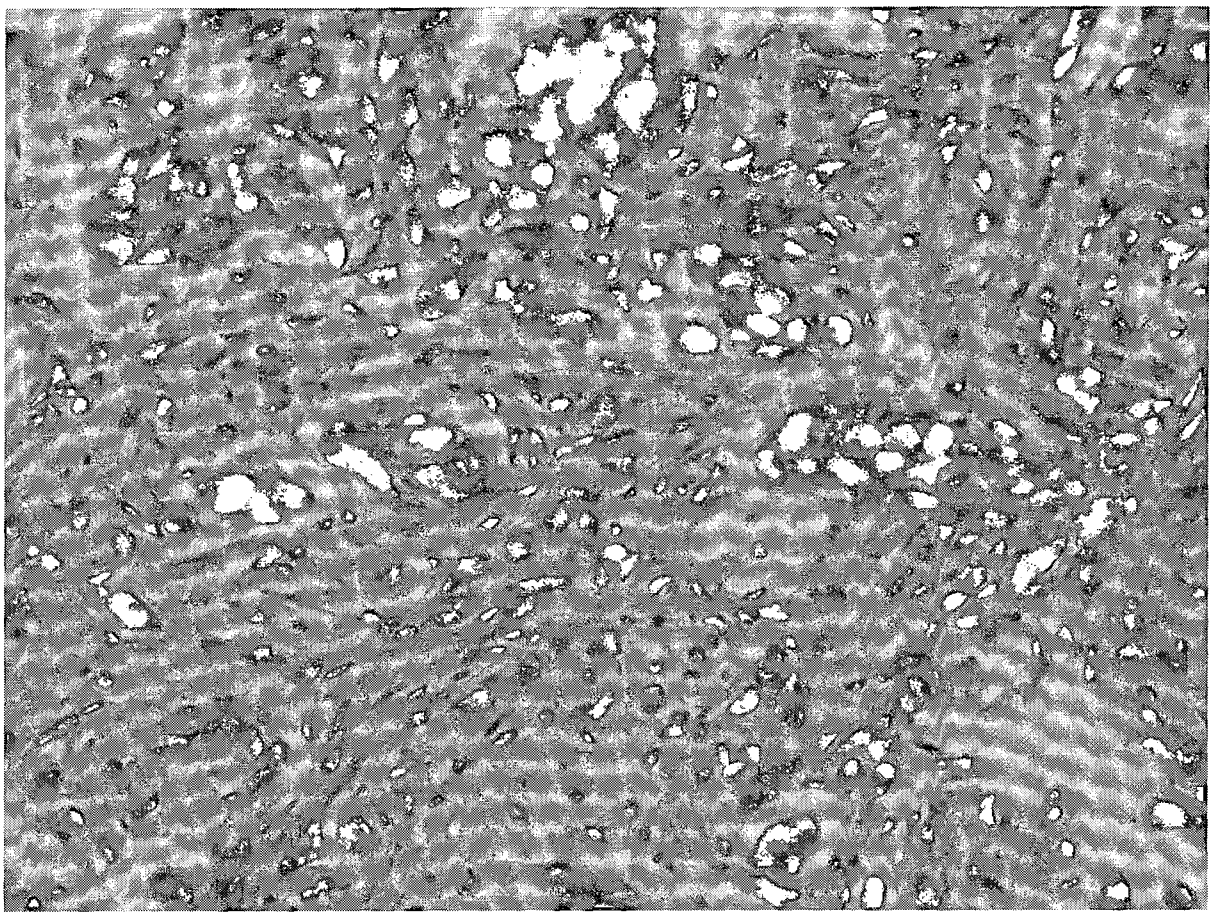


FIG. 3

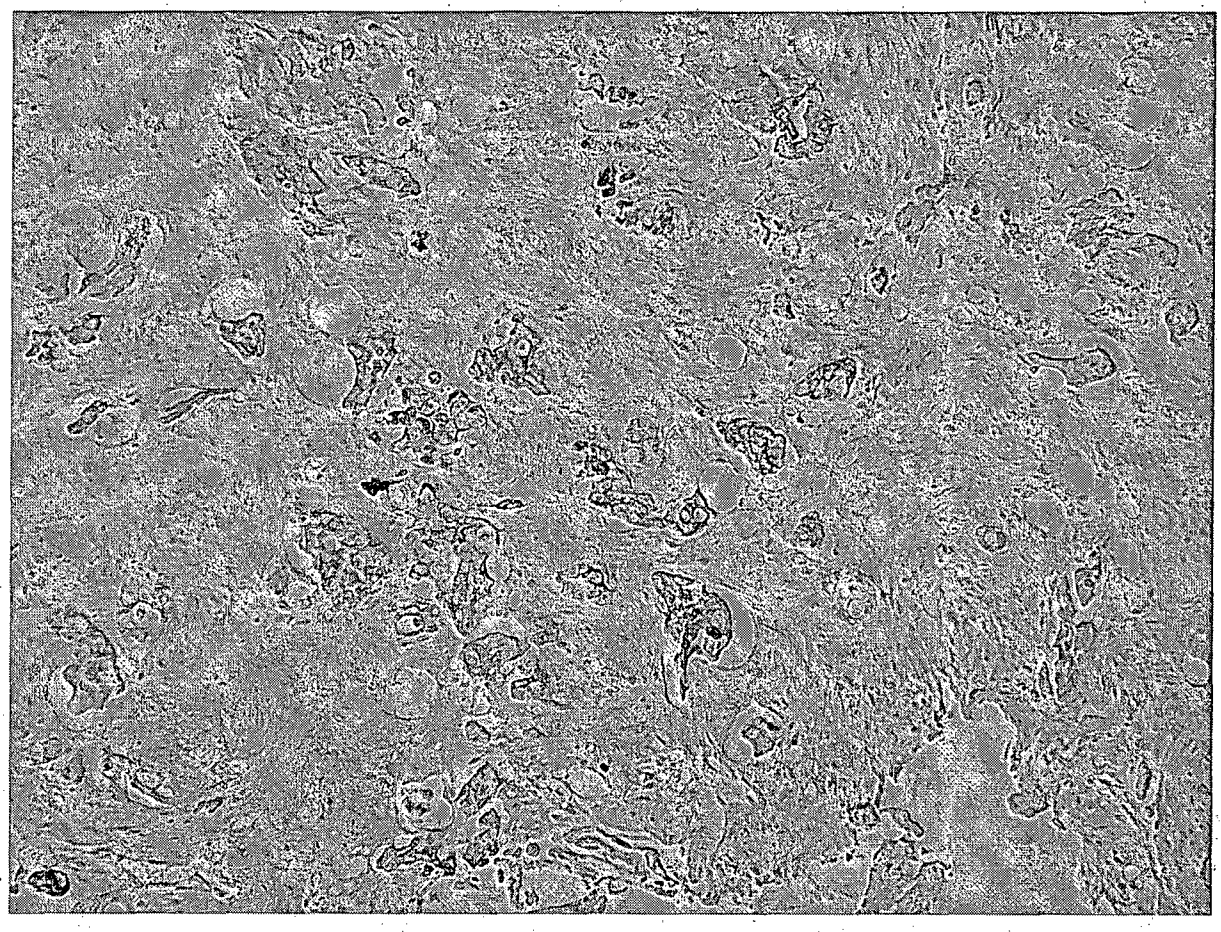


FIG. 4

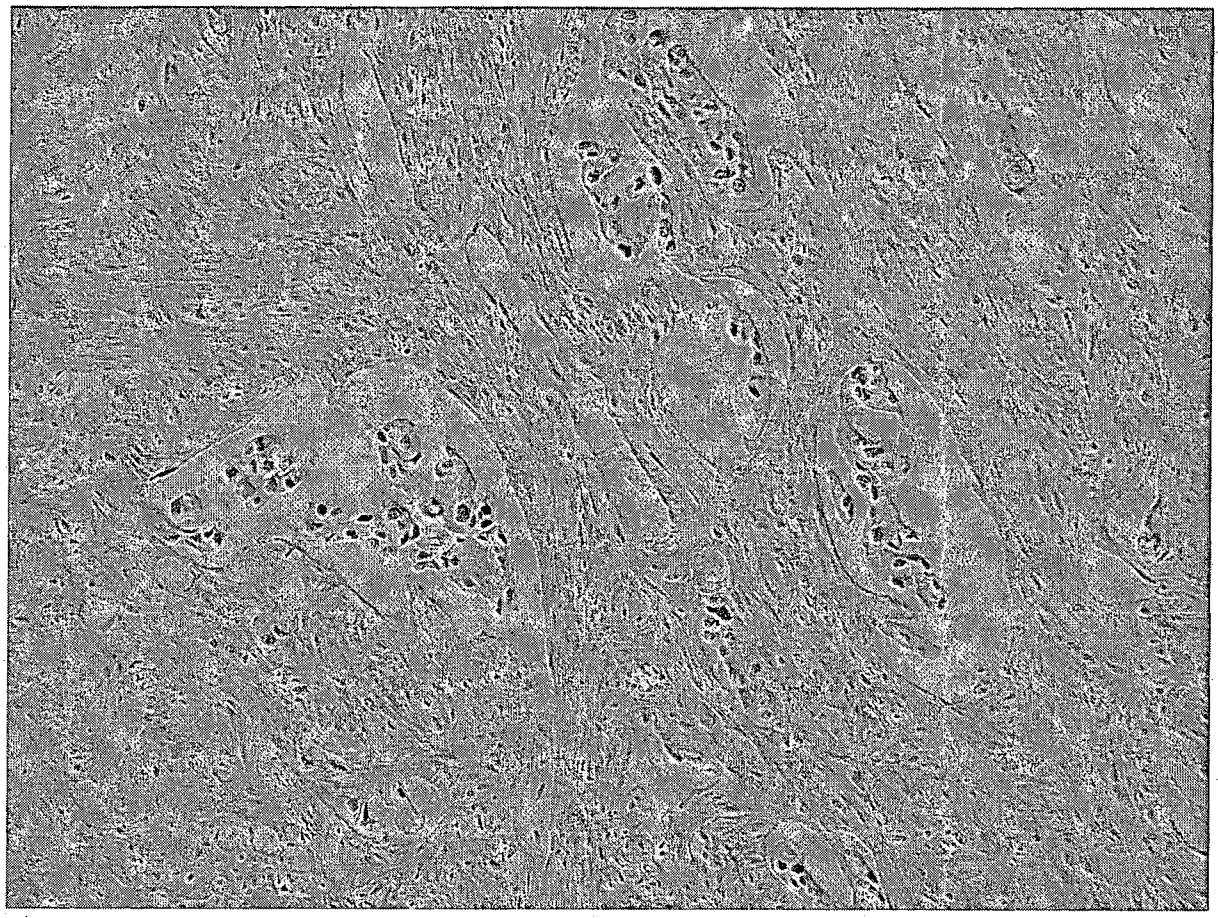


FIG. 5

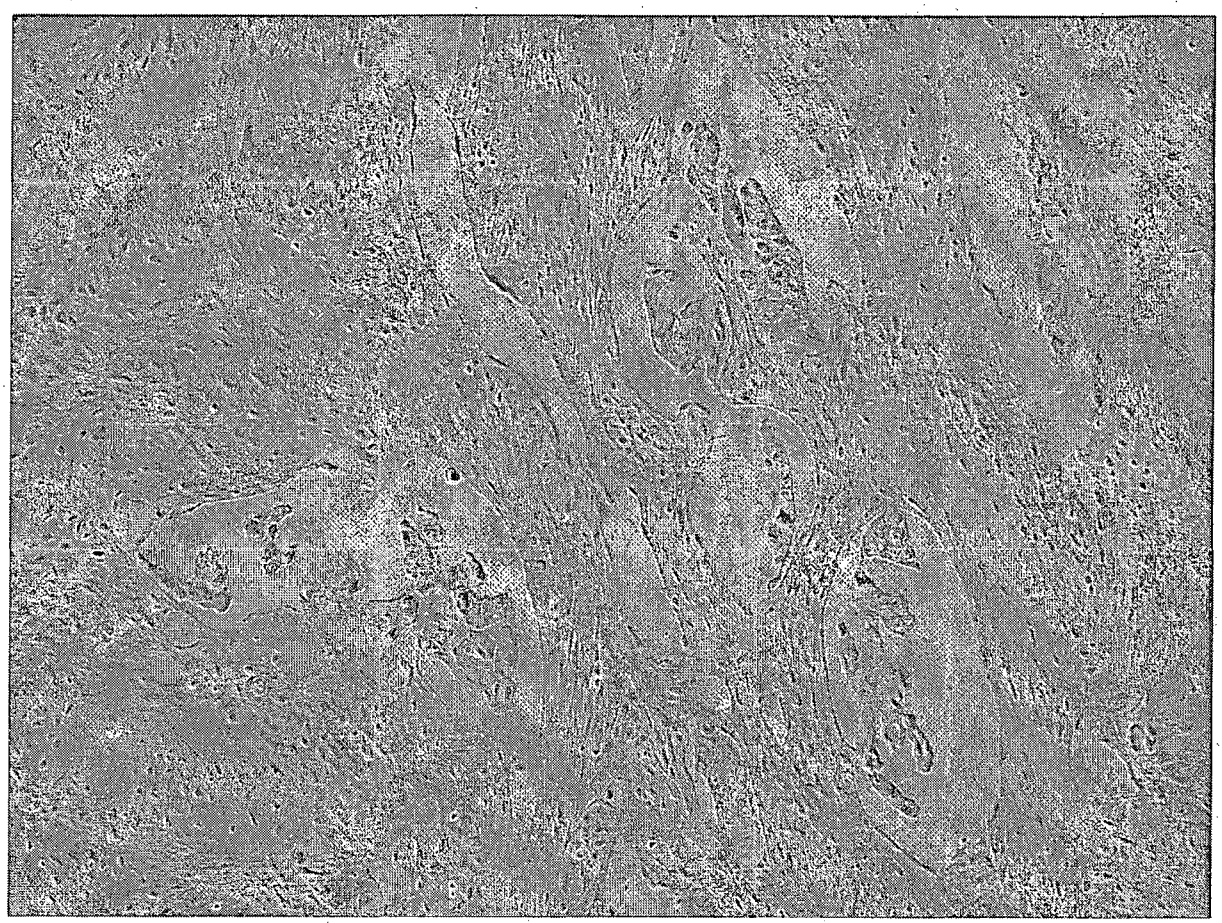


FIG. 6

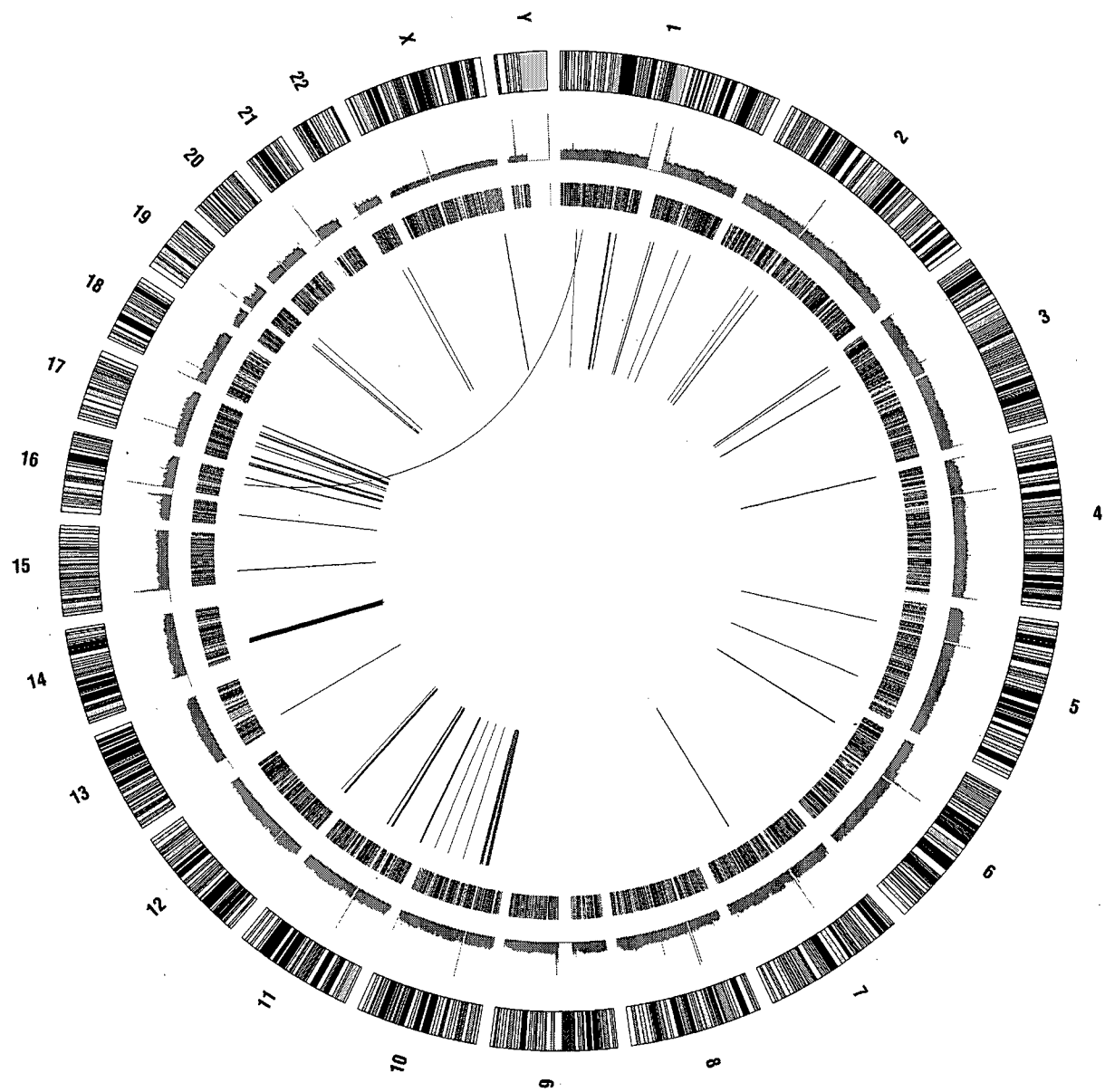


FIG. 7

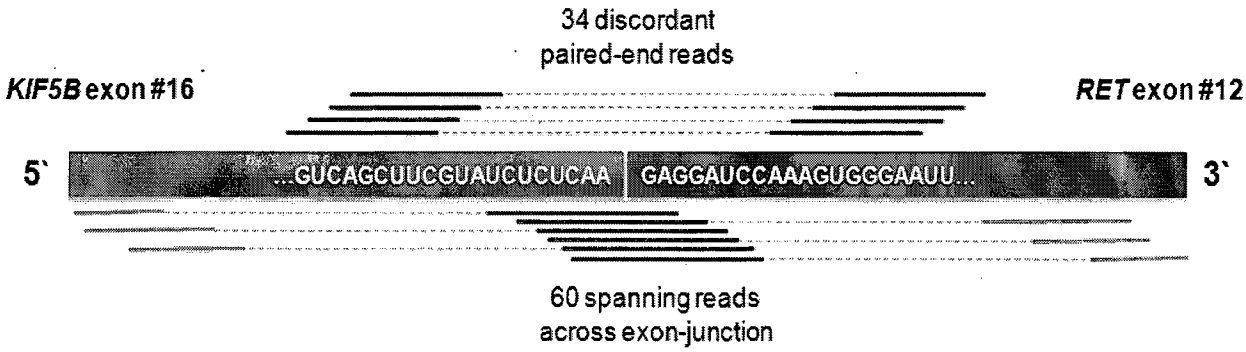

34 discordant
paired-end reads
KIF5B exon #16
RET exon #12
5`
...GUCAGCUUCGUAUCUCUCAA
GAGGAUCCAAAGUGGGAAUU...
3`
60 spanning reads
across exon-junction

FIG. 8

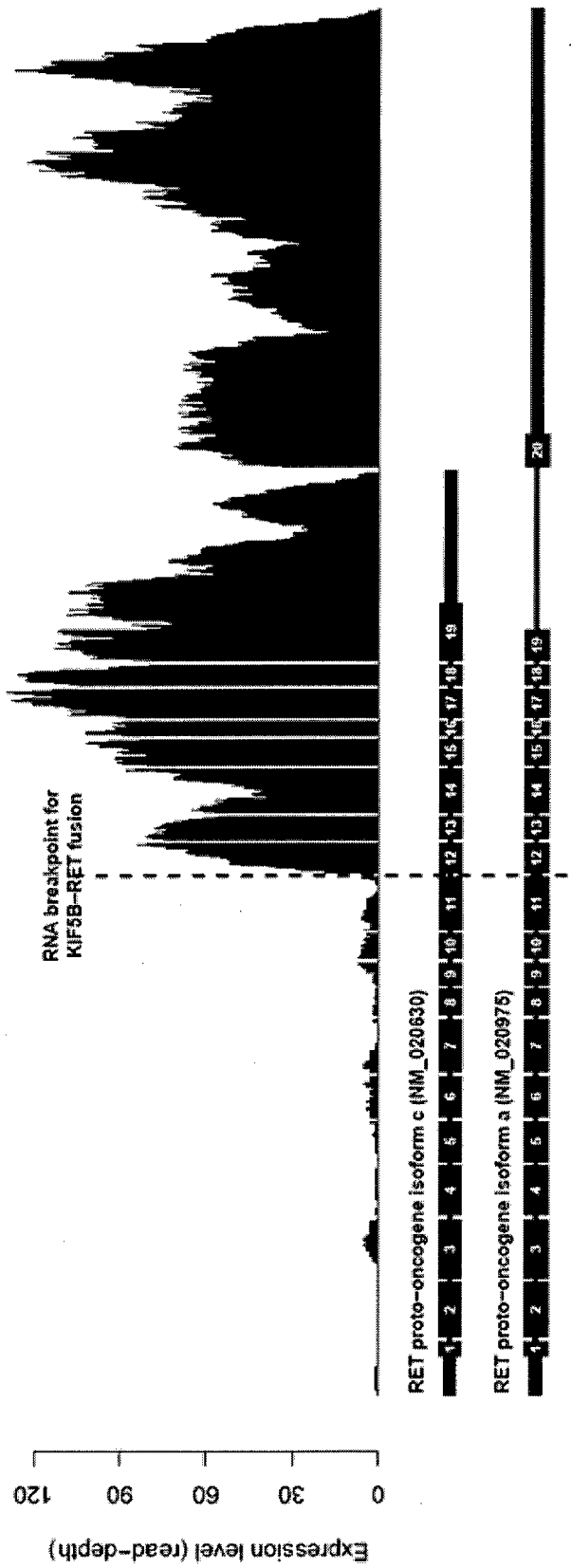

RNA breakpoint for
KIF5B–RET fusion
RET proto-oncogene isoform c (NM_020630)
RET proto-oncogene isoform a (NM_020975)
Expression level (read-depth)
0
30
60
90
120

FIG. 9

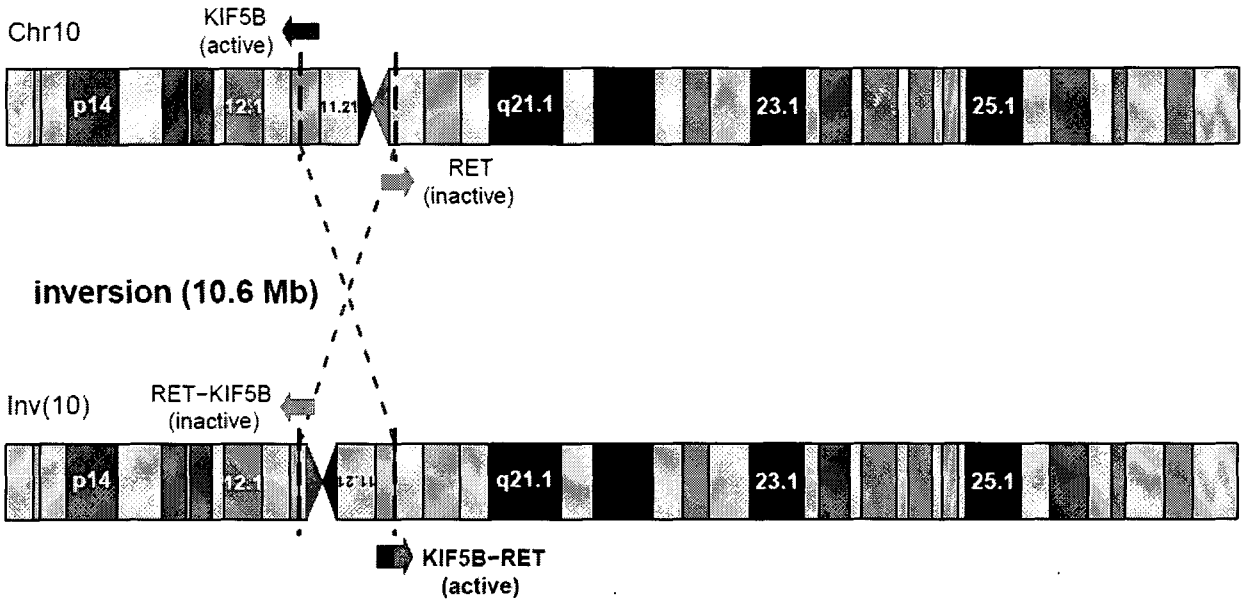


FIG. 10

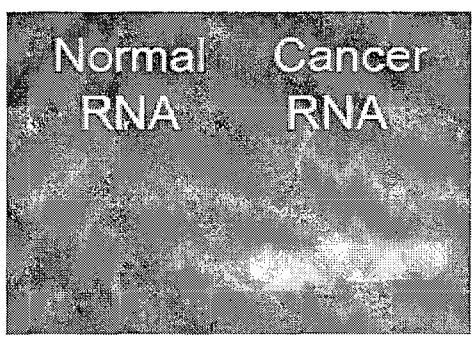


FIG. 11

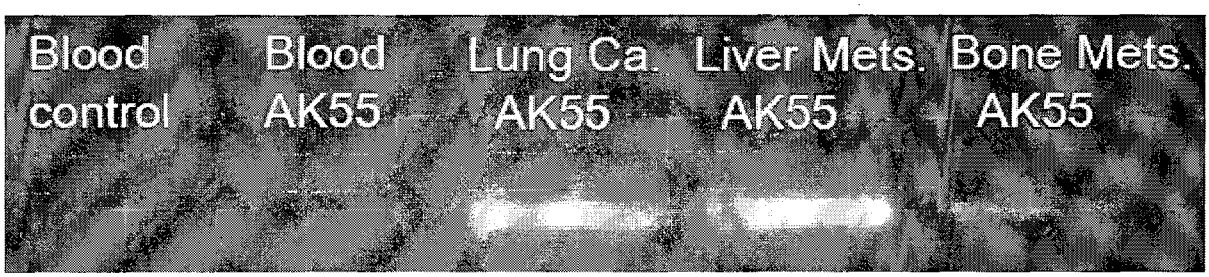


FIG. 12

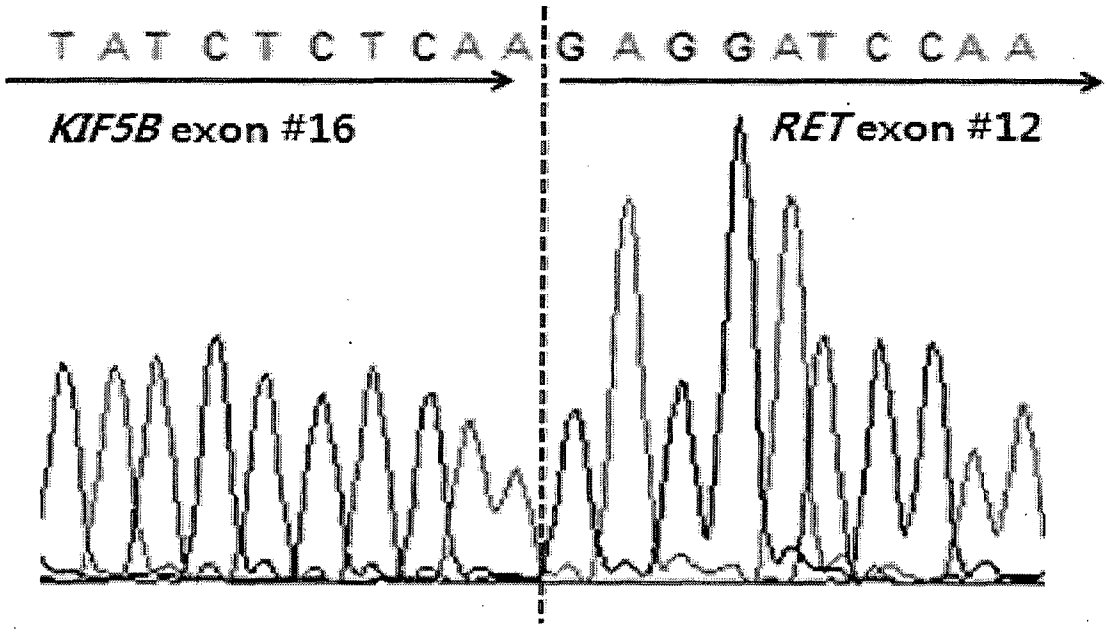

T A T C T C T C A A G A G G A T C C A A
KIF5B exon #16
RET exon #12

FIG. 13

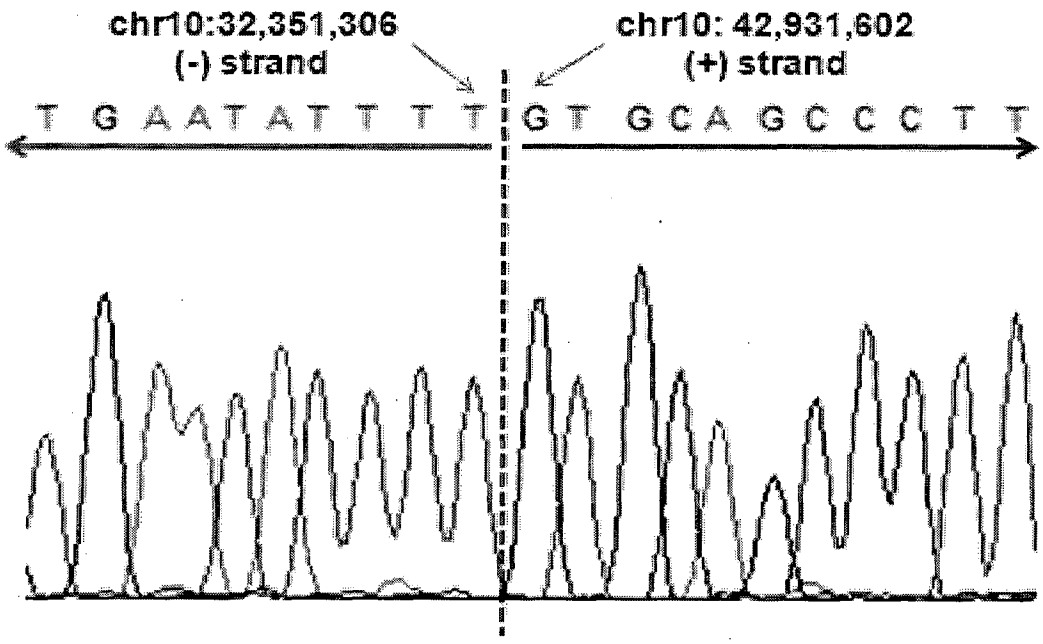

chr10:32,351,306
(-) strand
chr10: 42,931,602
(+) strand
T G A A T A T T T T G T G C A G C C C T T

FIG. 14

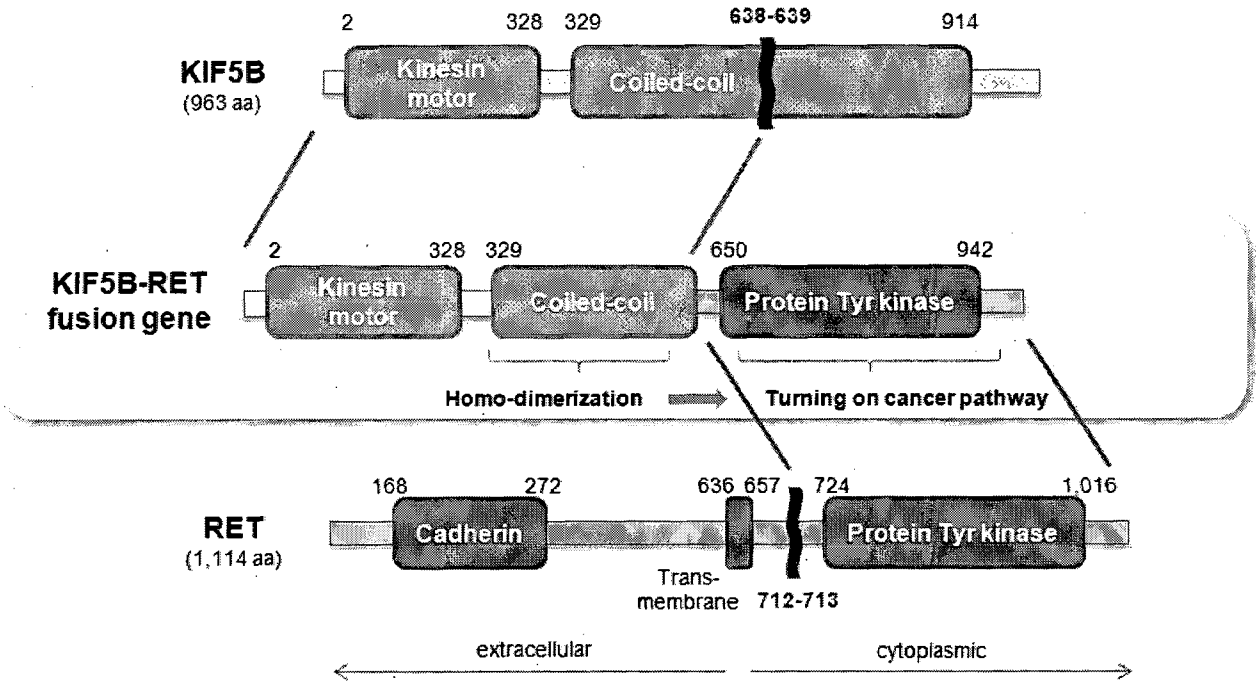

KIF5B
(963 aa)
2
328
329
638-639
914
Kinesin motor
Coiled-coil
KIF5B-RET fusion gene
2
328
329
650
942
Kinesin motor
Coiled-coil
Protein Tyr kinase
Homo-dimerization
Turning on cancer pathway
RET
(1,114 aa)
168
272
636
657
724
1,016
Cadherin
Protein Tyr kinase
Trans-membrane
712-713
extracellular
cytoplasmic

FIG. 15

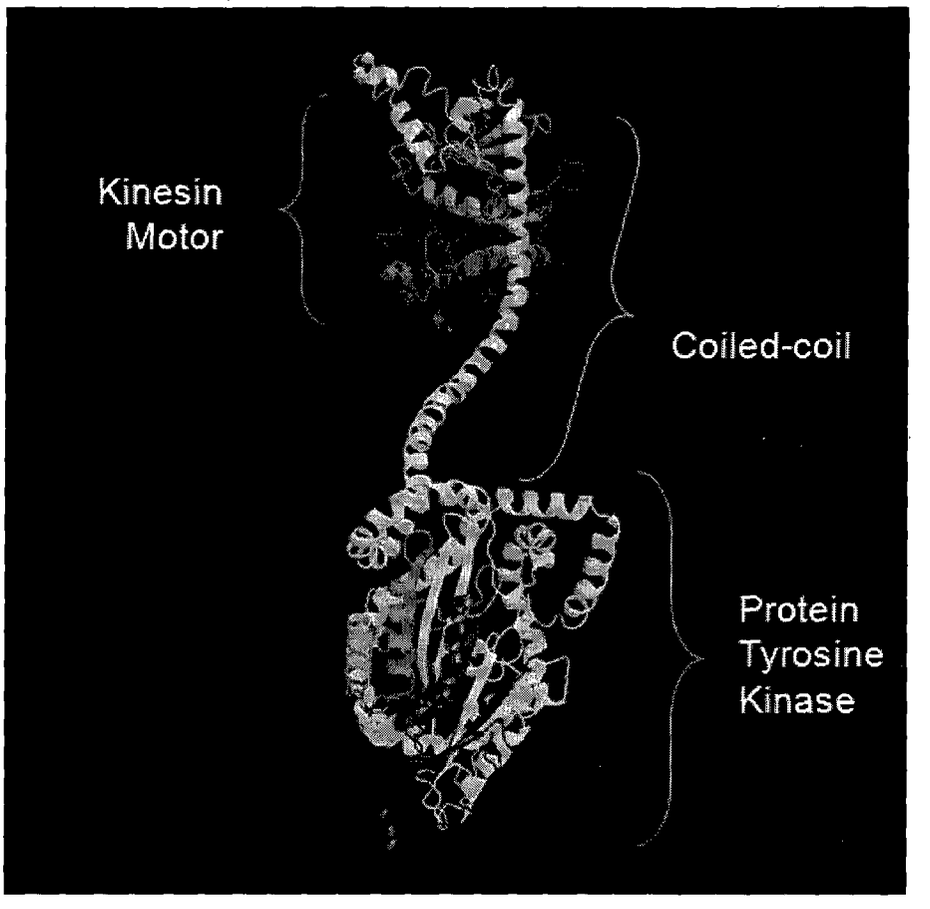


FIG. 16

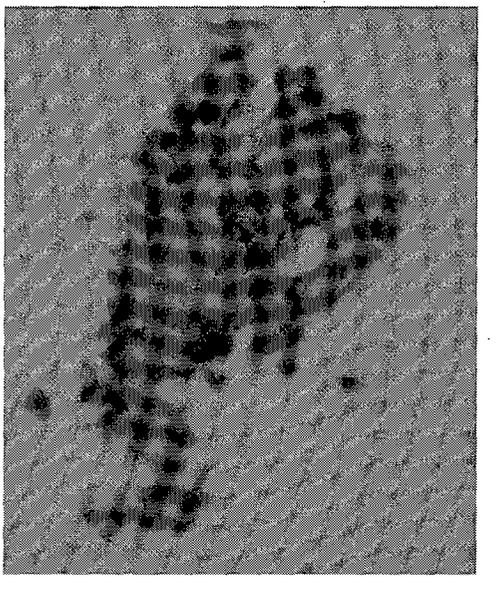


FIG. 17

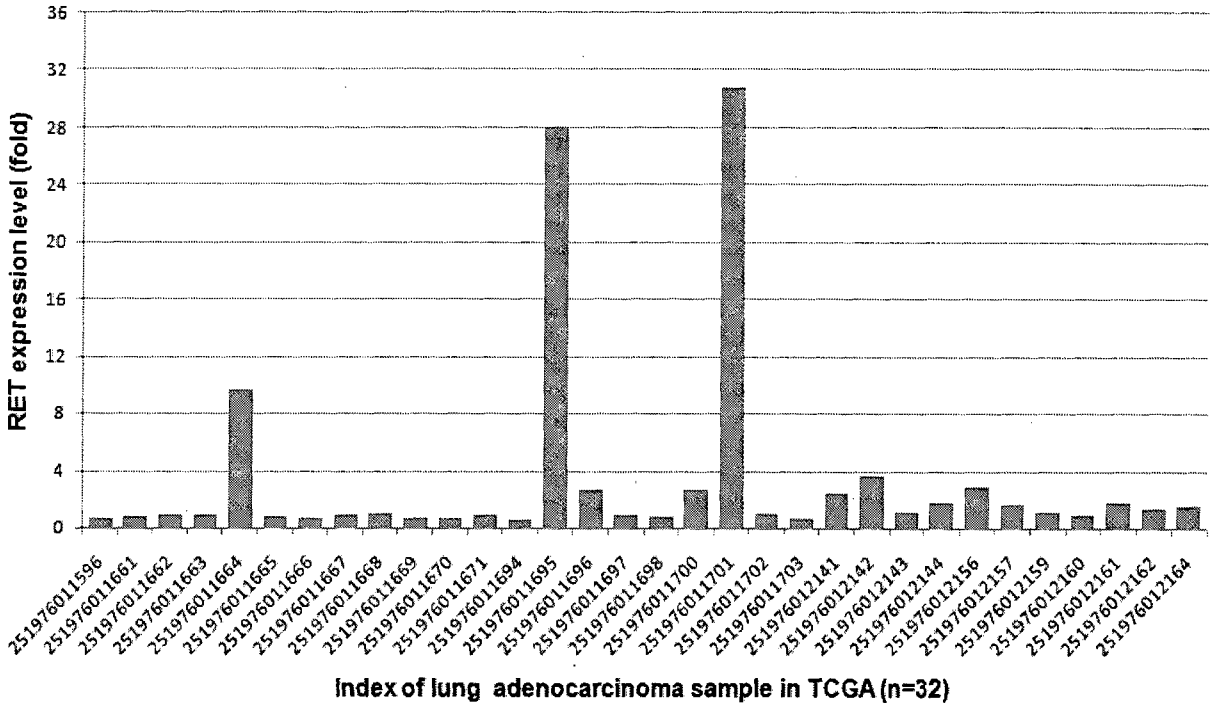

RET expression level (fold)
36
32
28
24
20
16
12
8
4
0
Index of lung adenocarcinoma sample in TCGA (n=32)

FIG. 18

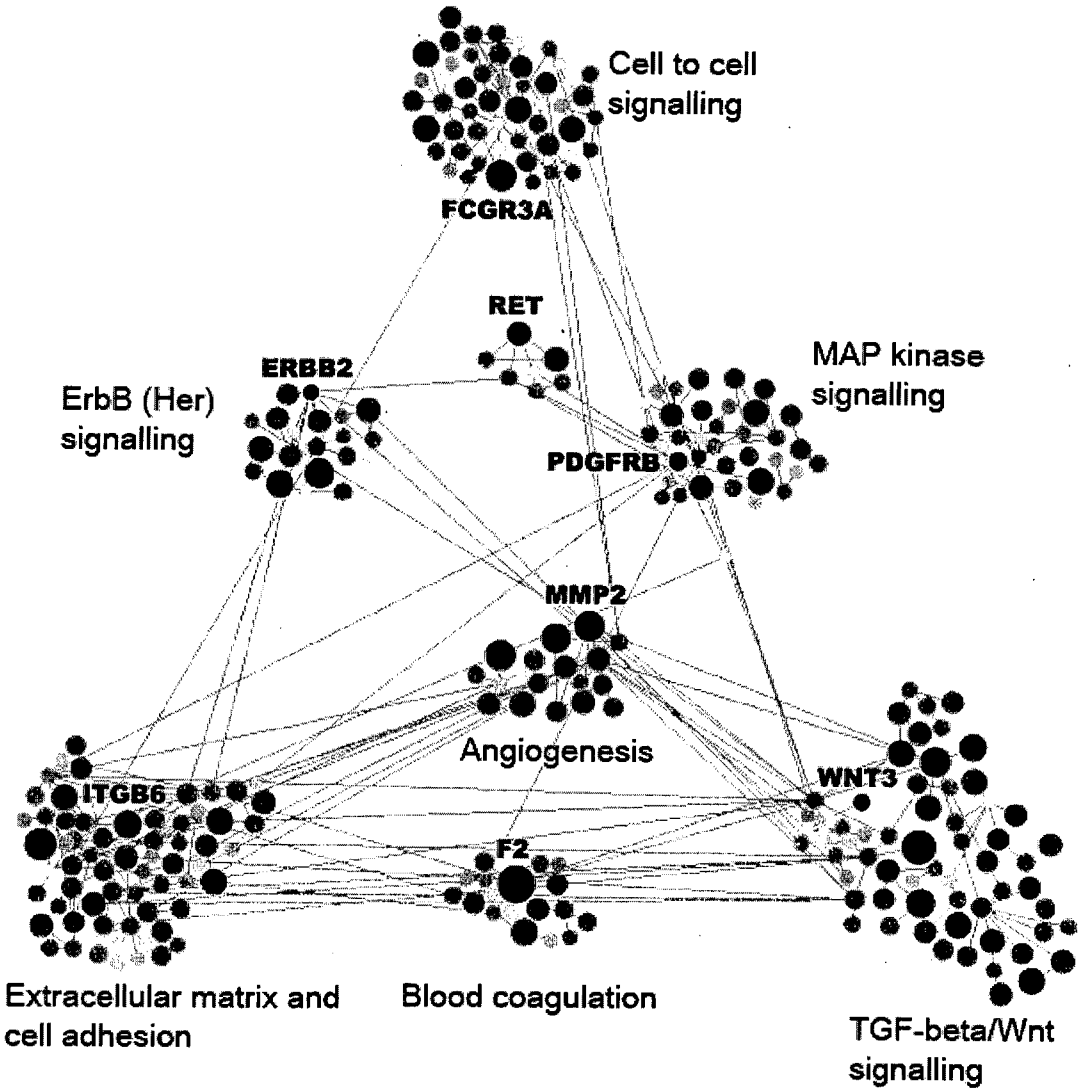
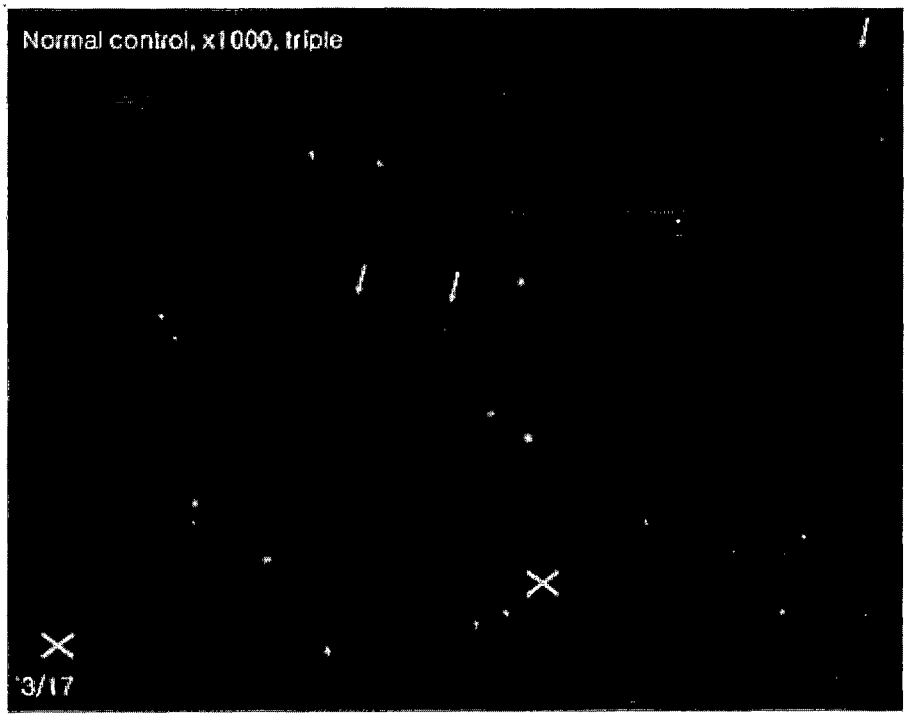
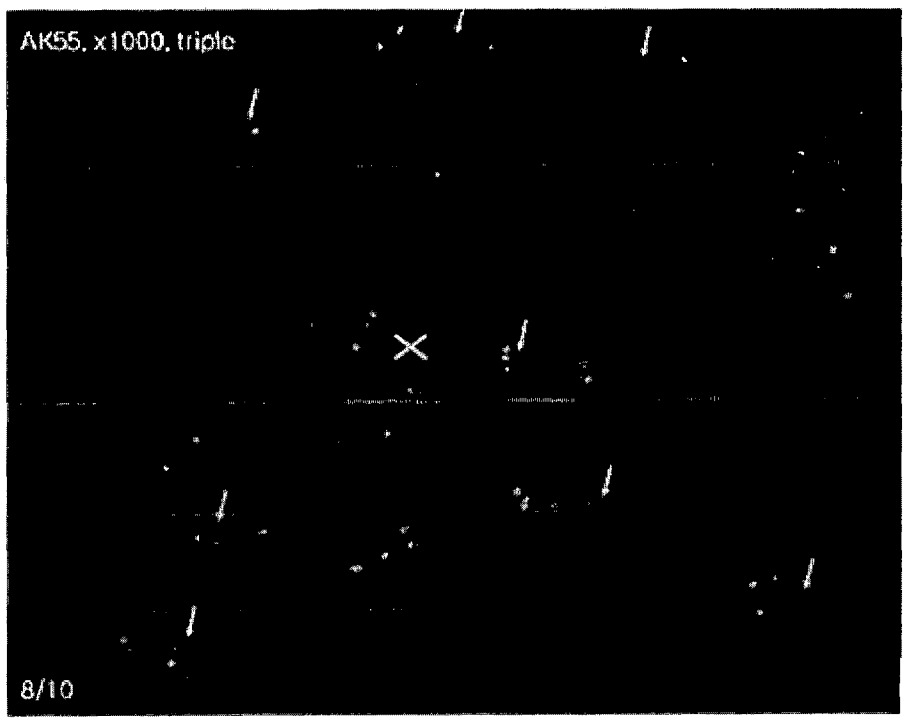

Cell to cell signalling
FCGR3A
RET
ERBB2
ErbB (Her) signalling
MAP kinase signalling
PDGFRB
MMP2
Angiogenesis
ITGB6
WNT3
F2
Extracellular matrix and cell adhesion
Blood coagulation
TGF-beta/Wnt signalling

FIG. 19



(A)



(B)

FIG. 20

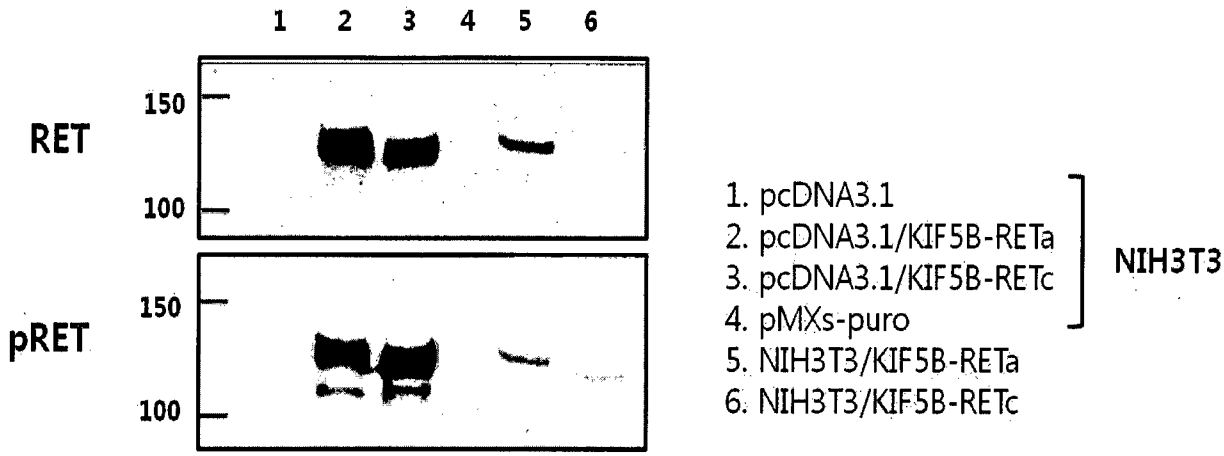

1 2 3 4 5 6
RET
pRET
150
100
150
100
1. pcDNA3.1
2. pcDNA3.1/KIF5B-RETa
3. pcDNA3.1/KIF5B-RETc
4. pMXs-puro
5. NIH3T3/KIF5B-RETa
6. NIH3T3/KIF5B-RETc
NIH3T3

FIG. 21

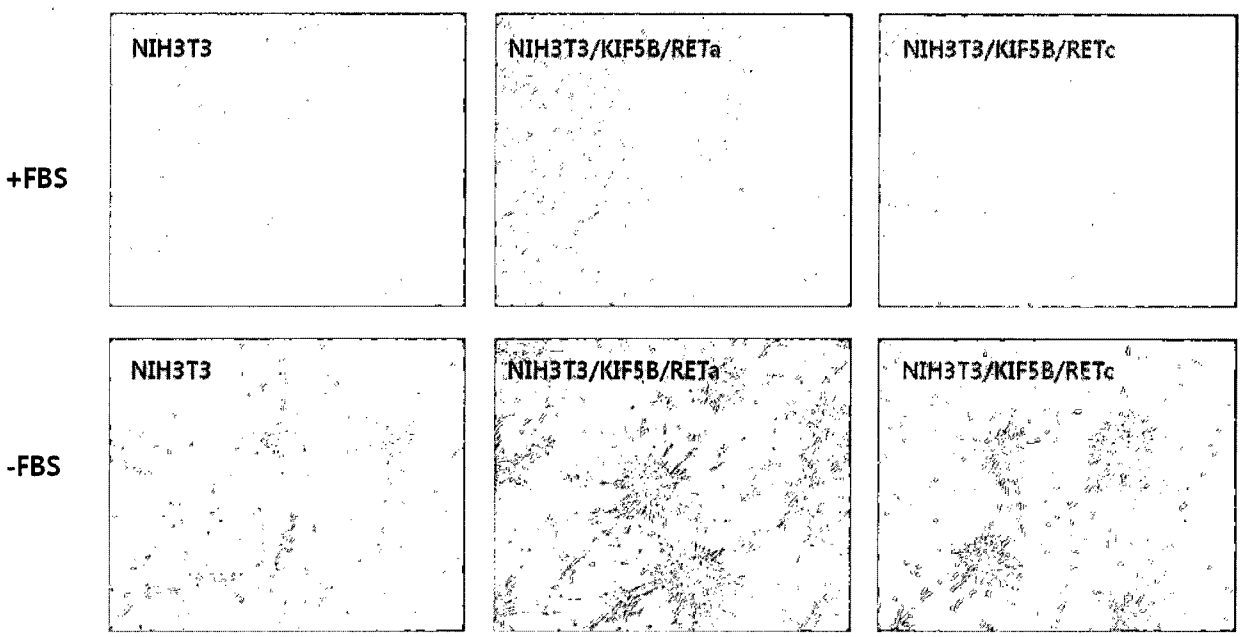

+FBS
-FBS
NIH3T3
NIH3T3/KIF5B/RETa
NIH3T3/KIF5B/RETc
NIH3T3
NIH3T3/KIF5B/RETa
NIH3T3/KIF5B/RETc

FIG. 22

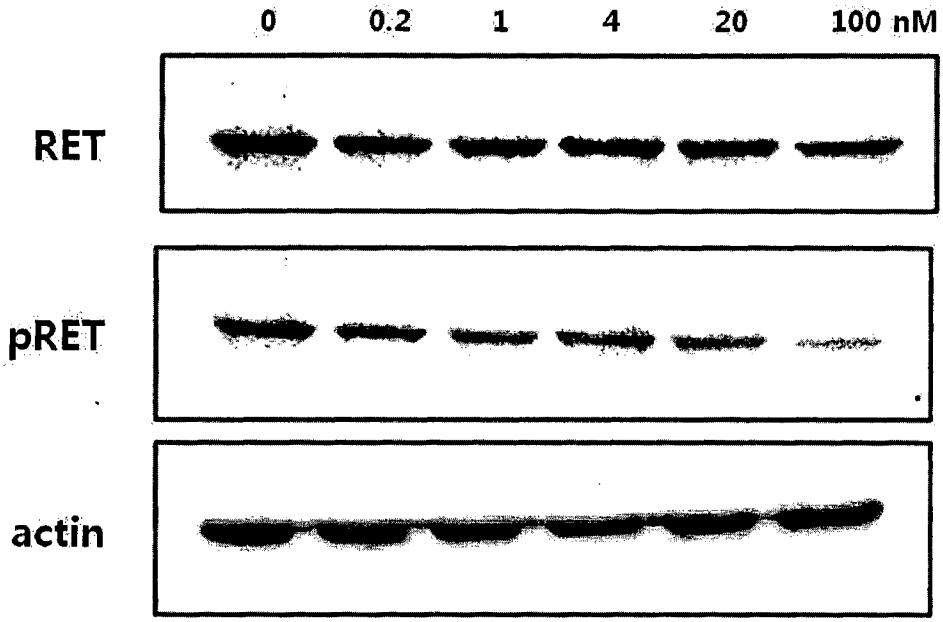

0
0.2
1
4
20
100 nM
RET
pRET
actin

FIG. 23

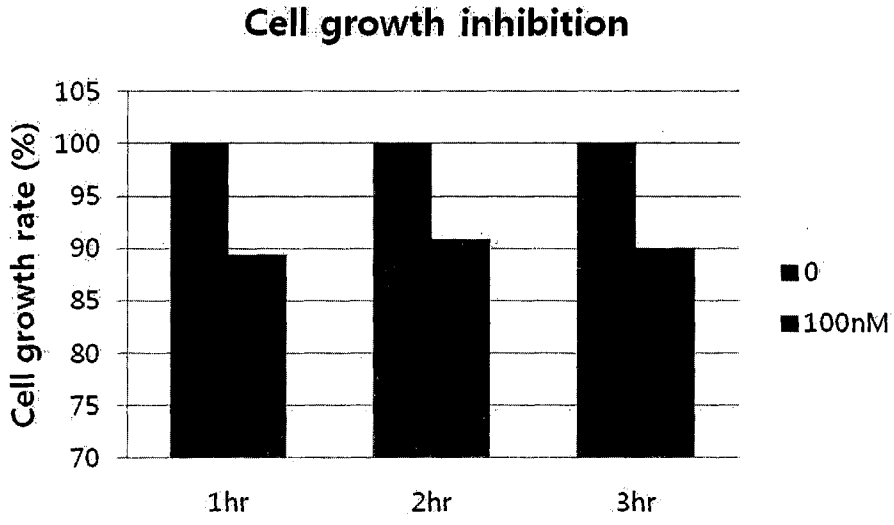

Cell growth inhibition
Cell growth rate (%)
105
100
95
90
85
80
75
70
1hr
2hr
3hr
0
100nM

FIG. 24

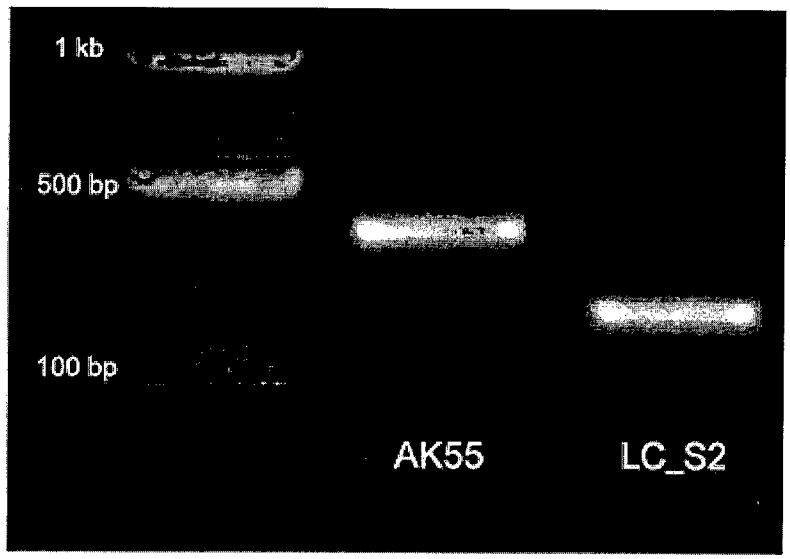


FIG. 25A

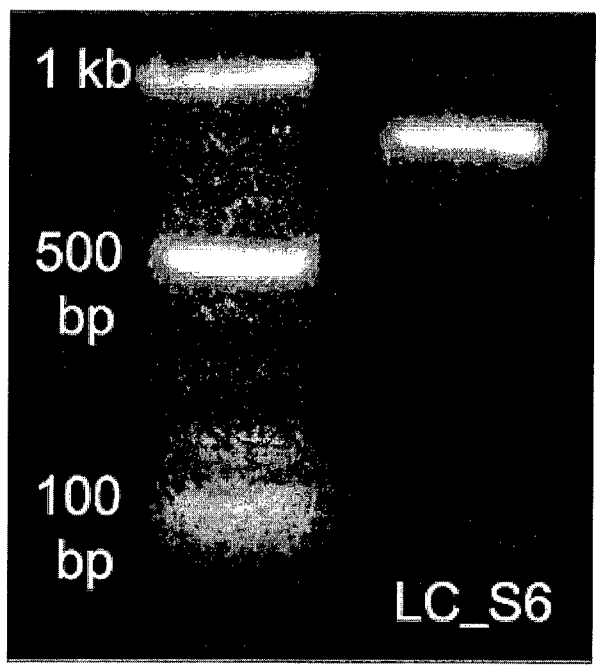


FIG. 25B

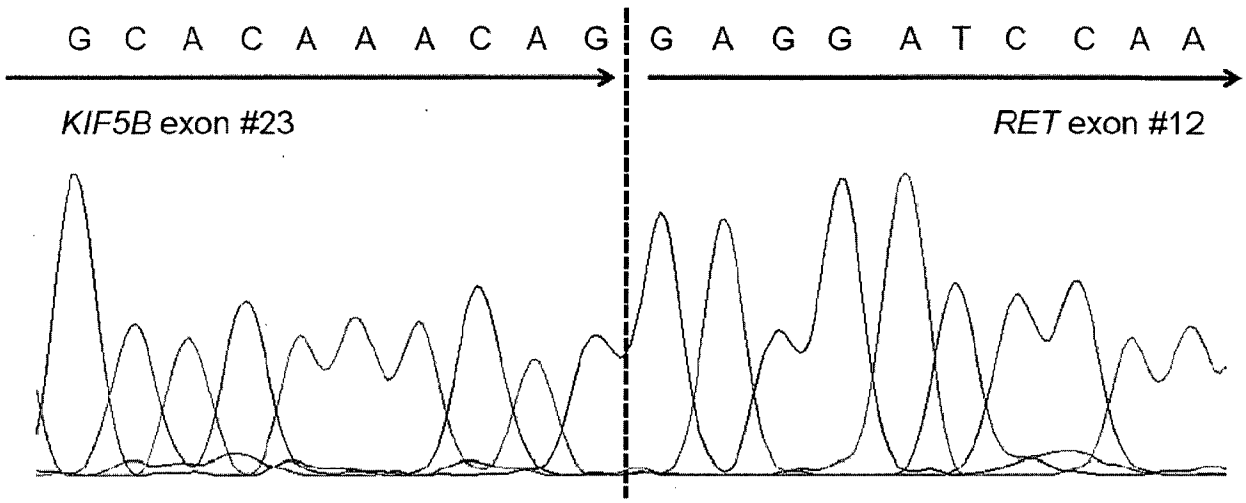


FIG. 26

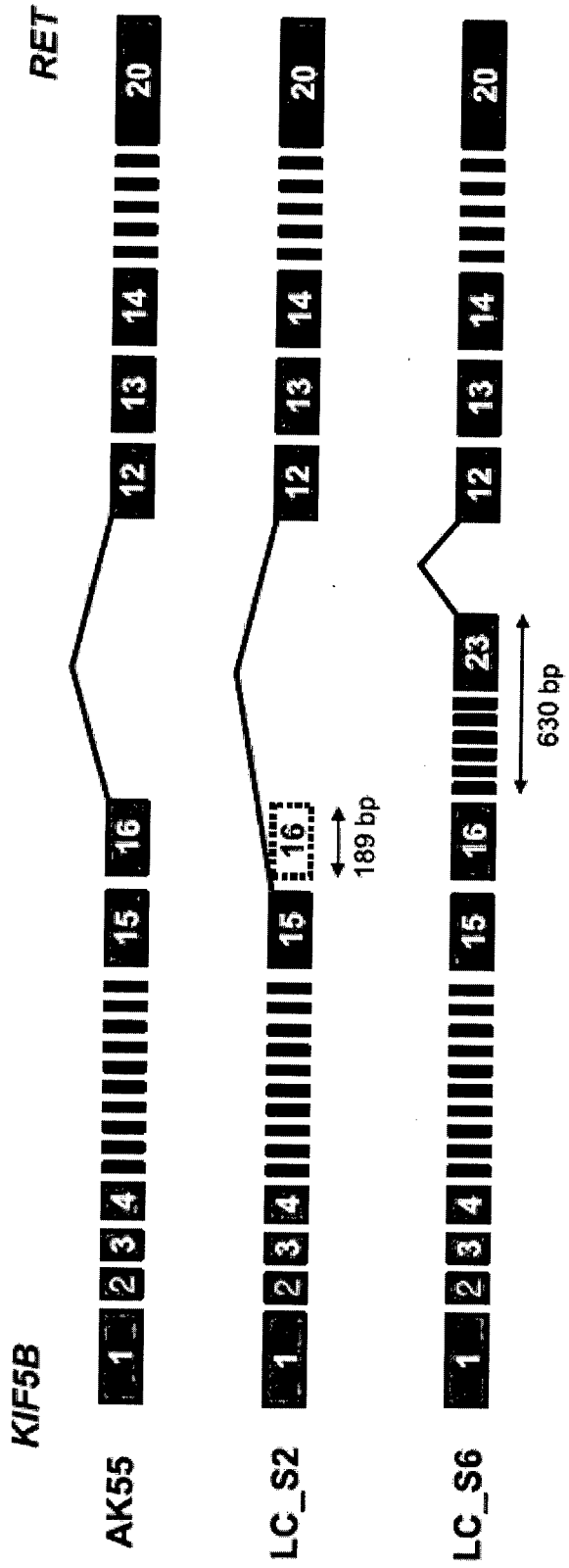


FIG. 27

KIF5B-RETa fusion gene (3123bp; SEQ ID NO: 1; 5'-terminal domain of KIF5B: italic type; 3'-terminal of RET: boldface)
Fusion region (SEQ ID NO: 2; underlined)

ATGGCGGACCTGGCCGAGTGCAACATCAAAGTGATGTGTCGCTTCAGACCTCTCAACGAGTC
TGAAGTGAACCGCGGCGACAAGTACATCGCCAAGTTTCAGGGAGAAGACACGGTCGTGATCG
CGTCCAAGCCTTATGCATTTGATCGGGTGTTCCAGTCAAGCACATCTCAAGAGCAAGTGTATAA
TGACTGTGCAAAGAAGATTGTTAAAGATGTACTTGAAGGATATAATGGAACAATATTTGCATATG
GACAAACATCCTCTGGGAAGACACACACAATGGAGGGTAAACTTCATGATCCAGAAGGCATGG
GAATTATTCCAAGAATAGTGCAAGATATTTTTAATTATATTTACTCCATGGATGAAAATTTGGAATT
TCATATTAAGGTTTCATATTTTGAAATATATTTGGATAAGATAAGGGACCTGTTAGATGTTTCAAAG
ACCAACCTTTCAGTTCATGAAGACAAAAACCGAGTTCCCTATGTAAAGGGGTGCACAGAGCGT
TTTGTATGTAGTCCAGATGAAGTTATGGATACCATAGATGAAGGAAAATCCAACAGACATGTAGC
AGTTACAAATATGAATGAACATAGCTCTAGGAGTCACAGTATATTTCTTATTAATGTCAAACAAGA
GAACACACAAACGGAACAAAAGCTGAGTGGAAAACTTTATCTGGTTGATTTAGCTGGTAGTGAA
AAGGTTAGTAAAACTGGAGCTGAAGGTGCTGTGCTGGATGAAGCTAAAAACATCAACAAGTCA
CTTTCTGCTCTTGGAAATGTTATTTCTGCTTTGGCTGAGGGTAGTACATATGTTCCATATCGAGAT
AGTAAAATGACAAGAATCCTTCAAGATTCATTAGGTGGCAACTGTAGAACCACTATTGTAATTTG
CTGCTCTCCaTCATCATACAATGAGTCTGAAACAAAaTCTACACTCTTATTTGGCCAAaGGGCCa
AAACAATTAAGAAcACAGTTTGTGTCAATGTGGAGTTAACTGCAGAACAGTGGAAAAAGAAGTA
TGAAAAAGAAAAAGAAAAAAATAAGATCCTGCGGAACACTATTCAGTGGCTTGAAAATGAGCTC
AACAGATGGCGTAATGGGGAGACGGTGCCTATTGATGAACAGTTTGACAAAGAGAAAGCCAAC
TTGGAAGCTTTCACAGTGGATAAAGATATTACTCTTACCAATGATAAACCAGCAACCGCAATTGG
AGTTATAGGAAATTTTACTGATGCTGAAAGAAGAAAGTGTGAAGAAGAAATTGCTAAATTATACA
AACAGCTTGATGACAAGGATGAAGAAATTAACCAGCAAAGTCAACTGGTAGAGAAACTGAAGA
CGCAAATGTTGGATCAGGAGGAGCTTTTGGCATCTACCAGAAGGGATCAAGACAATATGCAAG
CTGAGCTGAATCGCCTTCAAGCAGAAAATGATGCCTCTAAAGAAGAAGTGAAAGAAGTTTTACA
GGCCCTAGAAGAACTTGCTGTCAATTATGATCAGAAGTCTCAGGAAGTTGAAGACAAAACTAAG
GAATATGAATTGCTTAGTGATGAATTGAATCAGAAATCGGCAACTTTAGCGAGTATAGATGCTGA
GCTTCAGAAACTTAAGGAAATGACCAACCACCAGAAAAAACGAGCAGCTGAGATGATGGCATC
TTTACTAAAAGACCTTGCAGAAATAGGAATTGCTGTGGGAAATAATGATGTAAAGCAGCCTGAG
GGAACTGGCATGATAGATGAAGAGTTCACTGTTGCAAGACTCTACATTAGCAAAATGAAGTCAG
AAGTAAAAACCATGGTGAAACGTTGCAAGCAGTTAGAAAGCACACAAACTGAGAGCAACAAAA
AAATGGAAGAAAATGAAAAGGAGTTAGCAGCA<u>*TGTCAGCTTCGTATCTCTCAAGAGGATCCAA*</u>
<u>AGTGGGAATTC</u>CCTCGGAAGAACTTGGTTCTTGGAAAAACTCTAGGAGAAGGCGAATTTGG
AAAAGTGGTCAAGGCAACGGCCTTCCATCTGAAAGGCAGAGCAGGGTACACCACGGTGGC
CGTGAAGATGCTGAAAGAGAACGCCTCCCCGAGTGAGCTGCGAGACCTGCTGTCAGAGTTC
AACGTCCTGAAGCAGGTCAACCACCCACATGTCATCAAATTGTATGGGGCCTGCAGCCAGG
ATGGCCCGCTCCTCCTCATCGTGGAGTACGCCAAATACGGCTCCCTGCGGGGCTTCCTCCG
CGAGAGCCGCAAAGTGGGGCCTGGCTACCTGGGCAGTGGAGGCAGCCGCAACTCCAGCTC
CCTGGACCACCCGGATGAGCGGGCCCTCACCATGGGCGACCTCATCTCATTTGCCTGGCAG
ATCTCACAGGGGATGCAGTATCTGGCCGAGATGAAGCTCGTTCATCGGGACTTGGCAGCCA
GAAACATCCTGGTAGCTGAGGGGCGGAAGATGAAGATTTCGGATTTCGGCTTGTCCCGAGAT
GTTTATGAAGAGGATTCCTACGTGAAGAGGAGCCAGGGTCGGATTCCAGTTAAATGGATGGC
AATTGAATCCCTTTTTGATCATATCTACACCACGCAAAGTGATGTATGGTCTTTTGGTGTCCTG
CTGTGGGAGATCGTGACCCTAGGGGGAAACCCCTATCCTGGGATTCCTCCTGAGCGGCTCTT
CAACCTTCTGAAGACCGGCCACCGGATGGAGAGGCCAGACAACTGCAGCGAGGAGATGTA
CCGCCTGATGCTGCAATGCTGGAAGCAGGAGCCGGACAAAAGGCCGGTGTTTGCGGACAT
CAGCAAAGACCTGGAGAAGATGATGGTTAAGAGGAGAGACTACTTGGACCTTGCGGCGTCC
ACTCCATCTGACTCCCTGATTTATGACGACGGCCTCTCAGAGGAGGAGACACCGCTGGTGG
ACTGTAATAATGCCCCCCTCCCTCGAGCCCTCCCTTCCACATGGATTGAAAACAAACTCTATG
GCATGTCAGACCCGAACTGGCCTGGAGAGAGTCCTGTACCACTCACGAGAGCTGATGGCAC
TAACACTGGGTTTCCAAGATATCCAAATGATAGTGTATATGCTAACTGGATGCTTTCACCCTCA
GCGGCAAAATTAATGGACACGTTTGATAGTTAA

FIG. 28

KIF5B-RETa fusion protein (1040aa; SEQ ID NO: 3; N-terminal domain of KIF5B: italic type; C-terminal of RET: boldface)
Fusion region (SEQ ID NO: 4; underlined)

MADLAECNIKVMCRFRPLNESEVNRGDKYIAKFQGEDTVVIASKPYAFDRVFQSSTSQEQVYNDC
AKKIVKDVLEGYNGTIFAYGQTSSGKTHTMEGKLHDPEGMGIIPRIVQDIFNYIYSMDENLEFHIKVS
YFEIYLDKIRDLLDVSKTNLSVHEDKNRVPYVKGCTERFVCSPDEVMDTIDEGKSNRHVAVTNMNE
HSSRSHSIFLINVKQENTQTEQKLSGKLYLVDLAGSEKVSKTGAEGAVLDEAKNINKSLSALGNVIS
ALAEGSTYVPYRDSKMTRILQDSLGGNCRTTIVICCSPSSYNESETKSTLLFGQRAKTIKNTVCVNV
ELTAEQWKKKYEKEKEKNKILRNTIQWLENELNRWRNGETVPIDEQFDKEKANLEAFTVDKDITLT
NDKPATAIGVIGNFTDAERRKCEEEIAKLYKQLDDKDEEINQQSQLVEKLKTQMLDQEELLASTRR
DQDNMQAELNRLQAENDASKEEVKEVLQALEELAVNYDQKSQEVEDKTKEYELLSDELNQKSAT
LASIDAELQKLKEMTNHQKKRAAEMMASLLKDLAEIGIAVGNNDVKQPEGTGMIDEEFTVARLYISK
MKSEVKTMVKRCKQLESTQTESNKKMEENEKELAA<u>CQLRISQEDPKWEF</u>**PRKNLVLGKTLGEGE**
FGKVVKATAFHLKGRAGYTTVAVKMLKENASPSELRDLLSEFNVLKQVNHPHVIKLYGACSQDG
PLLLIVEYAKYGSLRGFLRESRKVGPGYLGSGGSRNSSSLDHPDERALTMGDLISFAWQISQGM
QYLAEMKLVHRDLAARNILVAEGRKMKISDFGLSRDVYEEDSYVKRSQGRIPVKWMAIESLFDHI
YTTQSDVWSFGVLLWEIVTLGGNPYPGIPPERLFNLLKTGHRMERPDNCSEEMYRLMLQCWKQ
EPDKRPVFADISKDLEKMMVKRRDYLDLAASTPSDSLIYDDGLSEEETPLVDCNNAPLPRALPST
WIENKLYGMSDPNWPGESPVPLTRADGTNTGFPRYPNDSVYANWMLSPSAAKLMDTFDS

FIG. 29

KIF5B-RETc fusion gene (2997bp: SEQ ID NO:5; 5'-terminal domain of KIF5B: italic type; 3'-terminal of RET: boldface)
Fusion region (SEQ ID NO: 6; underlined)

ATGGCGGACCTGGCCGAGTGCAACATCAAAGTGATGTGTCGCTTCAGACCTCTCAACGAGTC
TGAAGTGAACCGCGGCGACAAGTACATCGCCAAGTTTCAGGGAGAAGACACGGTCGTGATCG
CGTCCAAGCCTTATGCATTTGATCGGGTGTTCCAGTCAAGCACATCTCAAGAGCAAGTGTATAA
TGACTGTGCAAAGAAGATTGTTAAAGATGTACTTGAAGGATATAATGGAACAATATTTGCATATG
GACAAACATCCTCTGGGAAGACACACACAATGGAGGGTAAACTTCATGATCCAGAAGGCATGG
GAATTATTCCAAGAATAGTGCAAGATATTTTTAATTATATTTACTCCATGGATGAAAATTTGGAATT
TCATATTAAGGTTTCATATTTTGAAATATATTTGGATAAGATAAGGGACCTGTTAGATGTTTCAAAG
ACCAACCTTTCAGTTCATGAAGACAAAAACCGAGTTCCCTATGTAAAGGGGTGCACAGAGCGT
TTTGTATGTAGTCCAGATGAAGTTATGGATACCATAGATGAAGGAAAATCCAACAGACATGTAGC
AGTTACAAATATGAATGAACATAGCTCTAGGAGTCACAGTATATTTCTTATTAATGTCAAACAAGA
GAACACACAAACGGAACAAAAGCTGAGTGGAAAACTTTATCTGGTTGATTTAGCTGGTAGTGAA
AAGGTTAGTAAAACTGGAGCTGAAGGTGCTGTGCTGGATGAAGCTAAAAACATCAACAAGTCA
CTTTCTGCTCTTGGAAATGTTATTTCTGCTTTGGCTGAGGGTAGTACATATGTTCCATATCGAGAT
AGTAAAATGACAAGAATCCTTCAAGATTCATTAGGTGGCAACTGTAGAACCACTATTGTAATTTG
CTGCTCTCCaTCATCATACAATGAGTCTGAAACAAAaTCTACACTCTTATTTGGCCAAaGGGCCa
AAACAATTAAGAAcACAGTTTGTGTCAATGTGGAGTTAACTGCAGAACAGTGGAAAAAGAAGTA
TGAAAAAGAAAAAGAAAAAAATAAGATCCTGCGGAACACTATTCAGTGGCTTGAAAATGAGCTC
AACAGATGGCGTAATGGGGAGACGGTGCCTATTGATGAACAGTTTGACAAAGAGAAAGCCAAC
TTGGAAGCTTTCACAGTGGATAAAGATATTACTCTTACCAATGATAAACCAGCAACCGCAATTGG
AGTTATAGGAAATTTTACTGATGCTGAAAGAAGAAAGTGTGAAGAAGAAATTGCTAAATTATACA
AACAGCTTGATGACAAGGATGAAGAAATTAACCAGCAAAGTCAACTGGTAGAGAAACTGAAGA
CGCAAATGTTGGATCAGGAGGAGCTTTTGGCATCTACCAGAAGGGATCAAGACAATATGCAAG
CTGAGCTGAATCGCCTTCAAGCAGAAAATGATGCCTCTAAAGAAGAAGTGAAAGAAGTTTTACA
GGCCCTAGAAGAACTTGCTGTCAATTATGATCAGAAGTCTCAGGAAGTTGAAGACAAAACTAAG
GAATATGAATTGCTTAGTGATGAATTGAATCAGAAATCGGCAACTTTAGCGAGTATAGATGCTGA
GCTTCAGAAACTTAAGGAAATGACCAACCACCAGAAAAAACGAGCAGCTGAGATGATGGCATC
TTTACTAAAAGACCTTGCAGAAATAGGAATTGCTGTGGGAAATAATGATGTAAAGCAGCCTGAG
GGAACTGGCATGATAGATGAAGAGTTCACTGTTGCAAGACTCTACATTAGCAAAATGAAGTCAG
AAGTAAAAACCATGGTGAAACGTTGCAAGCAGTTAGAAAGCACACAAACTGAGAGCAACAAAA
AAATGGAAGAAAATGAAAAGGAGTTAGCAGCA<u>*TGTCAGCTTCGTATCTCTCAA*</u>GAGGATCCAA
<u>AGTGGGAATTC</u>CCTCGGAAGAACTTGGTTCTTGGAAAAACTCTAGGAGAAGGCGAATTTGG
AAAAGTGGTCAAGGCAACGGCCTTCCATCTGAAAGGCAGAGCAGGGTACACCACGGTGGC
CGTGAAGATGCTGAAAGAGAACGCCTCCCCGAGTGAGCTTCGAGACCTGCTGTCAGAGTTC
AACGTCCTGAAGCAGGTCAACCACCCACATGTCATCAAATTGTATGGGGCCTGCAGCCAGG
ATGGCCCGCTCCTCCTCATCGTGGAGTACGCCAAATACGGCTCCCTGCGGGGCTTCCTCCG
CGAGAGCCGCAAAGTGGGGCCTGGCTACCTGGGCAGTGGAGGCAGCCGCAACTCCAGCTC
CCTGGACCACCCGGATGAGCGGGCCCTCACCATGGGCGACCTCATCTCATTTGCCTGGCAG
ATCTCACAGGGGATGCAGTATCTGGCCGAGATGAAGCTCGTTCATCGGGACTTGGCAGCCA
GAAACATCCTGGTAGCTGAGGGGCGGAAGATGAAGATTTCGGATTTCGGCTTGTCCCGAGA
TGTTTATGAAGAGGATTCCTACGTGAAGAGGAGCCAGGGTCGGATTCCAGTTAAATGGATG
GCAATTGAATCCCTTTTTGATCATATCTACACCACGCAAAGTGATGTATGGTCTTTTGGTGTC
CTGCTGTGGGAGATCGTGACCCTAGGGGGAAACCCCTATCCTGGGATTCCTCCTGAGCGGC
TCTTCAACCTTCTGAAGACCGGCCACCGGATGGAGAGGCCAGACAACTGCAGCGAGGAGA
TGTACCGCCTGATGCTGCAATGCTGGAAGCAGGAGCCGGACAAAAGGCCGGTGTTTGCGG
ACATCAGCAAAGACCTGGAGAAGATGATGGTTAAGAGGAGAGACTACTTGGACCTTGCGG
CGTCCACTCCATCTGACTCCCTGATTTATGACGACGGCCTCTCAGAGGAGGAGACACCGCT
GGTGGACTGTAATAATGCCCCCCTCCCTCGAGCCCTCCCTTCCACATGGATTGAAAACAAA
CTCTATGGTAGAATTTCCCATGCATTTACTAGATTCTAG

FIG. 30

KIF5B-RETc fusion protein (998aa; SEQ ID NO: 7; N-terminal domain of KIF5B: italic type; C-terminal of RET: boldface)
Fusion region (SEQ ID NO:8; underlined)

MADLAECNIKVMCRFRPLNESEVNRGDKYIAKFQGEDTVVIASKPYAFDRVFQSSTSQEQVYNDC
AKKIVKDVLEGYNGTIFAYGQTSSGKTHTMEGKLHDPEGMGIIPRIVQDIFNYIYSMDENLEFHIKVS
YFEIYLDKIRDLLDVSKTNLSVHEDKNRVPYVKGCTERFVCSPDEVMDTIDEGKSNRHVAVTNMNE
HSSRSHSIFLINVKQENTQTEQKLSGKLYLVDLAGSEKVSKTGAEGAVLDEAKNINKSLSALGNVIS
ALAEGSTYVPYRDSKMTRILQDSLGGNCRTTIVICCSPSSYNESETKSTLLFGQRAKTIKNTVCVNV
ELTAEQWKKKYEKEKEKNKILRNTIQWLENELNRWRNGETVPIDEQFDKEKANLEAFTVDKDITLT
NDKPATAIGVIGNFTDAERRKCEEEIAKLYKQLDDKDEEINQQSQLVEKLKTQMLDQEELLASTRR
DQDNMQAELNRLQAENDASKEEVKEVLQALEELAVNYDQKSQEVEDKTKEYELLSDELNQKSAT
LASIDAELQKLKEMTNHQKKRAAEMMASLLKDLAEIGIAVGNNDVKQPEGTGMIDEEFTVARLYISK
MKSEVKTMVKRCKQLESTQTESNKKMEENEKELAA<u>*CQLRISQEDPKWEF*</u>**PRKNLVLGKTLGEGE**
FGKVVKATAFHLKGRAGYTTVAVKMLKENASPSELRDLLSEFNVLKQVNHPHVIKLYGACSQDG
PLLLIVEYAKYGSLRGFLRESRKVGPGYLGSGGSRNSSSLDHPDERALTMGDLISFAWQISQGM
QYLAEMKLVHRDLAARNILVAEGRKMKISDFGLSRDVYEEDSYVKRSQGRIPVKWMAIESLFDHI
YTTQSDVWSFGVLLWEIVTLGGNPYPGIPPERLFNLLKTGHRMERPDNCSEEMYRLMLQCWKQ
EPDKRPVFADISKDLEKMMVKRRDYLDLAASTPSDSLIYDDGLSEEETPLVDCNNAPLPRALPST
WIENKLYGMSDPNWPGESPVPLTRADGTNTGFPRYPNDSVYANWMLSPSAAKLMDTFDS

FIG. 31

KIF5B-RETa variant (LC-S2) fusion gene (2934bp; SEQ ID NO 9; 5'-terminal domain of KIF5B: italic type; 3'-terminal of RET: boldface)
Fusion region (SEQ ID NO:10; underlined)

ATGGCGGACCTGGCCGAGTGCAACATCAAAGTGATGTGTCGCTTCAGACCTCTCAACGAGTC
TGAAGTGAACCGCGGCGACAAGTACATCGCCAAGTTTCAGGGAGAAGACACGGTCGTGATCG
CGTCCAAGCCTTATGCATTTGATCGGGTGTTCCAGTCAAGCACATCTCAAGAGCAAGTGTATA
ATGACTGTGCAAAGAAGATTGTTAAAGATGTACTTGAAGGATATAATGGAACAATATTTGCATA
TGGACAAACATCCTCTGGGAAGACACACACAATGGAGGGTAAACTTCATGATCCAGAAGGCA
TGGGAATTATTCCAAGAATAGTGCAAGATATTTTTAATTATATTTACTCCATGGATGAAAATTTG
GAATTTCATATTAAGGTTTCATATTTTGAAATATATTTGGATAAGATAAGGGACCTGTTAGATGT
TTCAAAGACCAACCTTTCAGTTCATGAAGACAAAAACCGAGTTCCCTATGTAAAGGGGTGCAC
AGAGCGTTTTGTATGTAGTCCAGATGAAGTTATGGATACCATAGATGAAGGAAAATCCAACAG
ACATGTAGCAGTTACAAATATGAATGAACATAGCTCTAGGAGTCACAGTATATTTCTTATTAAT
GTCAAACAAGAGAACACACAAACGGAACAAAAGCTGAGTGGAAAACTTTATCTGGTTGATTTA
GCTGGTAGTGAAAAGGTTAGTAAAACTGGAGCTGAAGGTGCTGTGCTGGATGAAGCTAAAAA
CATCAACAAGTCACTTTCTGCTCTTGGAAATGTTATTTCTGCTTTGGCTGAGGGTAGTACATAT
GTTCCATATCGAGATAGTAAAATGACAAGAATCCTTCAAGATTCATTAGGTGGCAACTGTAGAA
CCACTATTGTAATTTGCTGCTCTCCATCATCATACAATGAGTCTGAAACAAAATCTACACTCTTA
TTTGGCCAAAGGGCCAAAACAATTAAGAACACAGTTTGTGTCAATGTGGAGTTAACTGCAGAA
CAGTGGAAAAAGAAGTATGAAAAAGAAAAAGAAAAAAATAAGATCCTGCGGAACACTATTCAG
TGGCTTGAAAATGAGCTCAACAGATGGCGTAATGGGGAGACGGTGCCTATTGATGAACAGTT
TGACAAAGAGAAAGCCAACTTGGAAGCTTTCACAGTGGATAAAGATATTACTCTTACCAATGAT
AAACCAGCAACCGCAATTGGAGTTATAGGAAATTTTACTGATGCTGAAAGAAGAAAGTGTGAA
GAAGAAATTGCTAAATTATACAAACAGCTTGATGACAAGGATGAAGAAATTAACCAGCAAAGTC
AACTGGTAGAGAAACTGAAGACGCAAATGTTGGATCAGGAGGAGCTTTTGGCATCTACCAGA
AGGGATCAAGACAATATGCAAGCTGAGCTGAATCGCCTTCAAGCAGAAAATGATGCCTCTAAA
GAAGAAGTGAAAGAAGTTTTACAGGCCCTAGAAGAACTTGCTGTCAATTATGATCAGAAGTCT
CAGGAAGTTGAAGACAAAACTAAGGAATATGAATTGCTTAGTGATGAATTGAATCAGAAATCG
GCAACTTTAGCGAGTATAGATGCTGAGCTTCAGAAACTTAAGGAAATGACCAACCACCAGAAA
AAACGAGCAGCTGAGATGATGGCATCTTTACTAAAAGACCTTGCAGAAATAGGAATTGCT<u>*GTG*</u>
<u>*GGAAATAATGATGTAAAGGAGGATCCAAAGTGGGAATTC*</u>CCTCGGAAGAACTTGGTTCTTGG
AAAAACTCTAGGAGAAGGCGAATTTGGAAAAGTGGTCAAGGCAACGGCCTTCCATCTGAAA
GGCAGAGCAGGGTACACCACGGTGGCCGTGAAGATGCTGAAAGAGAACGCCTCCCCGAGT
GAGCTGCGAGACCTGCTGTCAGAGTTCAACGTCCTGAAGCAGGTCAACCACCCACATGTCA
TCAAATTGTATGGGGCCTGCAGCCAGGATGGCCCGCTCCTCCTCATCGTGGAGTACGCCAAA
TACGGCTCCCTGCGGGGCTTCCTCCGCGAGAGCCGCAAAGTGGGGCCTGGCTACCTGGGC
AGTGGAGGCAGCCGCAACTCCAGCTCCCTGGACCACCCGGATGAGCGGGCCCTCACCATG
GGCGACCTCATCTCATTTGCCTGGCAGATCTCACAGGGGATGCAGTATCTGGCCGAGATGAA
GCTCGTTCATCGGGACTTGGCAGCCAGAAACATCCTGGTAGCTGAGGGGCGGAAGATGAAG
ATTTCGGATTTCGGCTTGTCCCGAGATGTTTATGAAGAGGATTCCTACGTGAAGAGGAGCCA
GGGTCGGATTCCAGTTAAATGGATGGCAATTGAATCCCTTTTTGATCATATCTACACCACGCA
AAGTGATGTATGGTCTTTTGGTGTCCTGCTGTGGGAGATCGTGACCCTAGGGGGAAACCCCT
ATCCTGGGATTCCTCCTGAGCGGCTCTTCAACCTTCTGAAGACCGGCCACCGGATGGAGAG
GCCAGACAACTGCAGCGAGGAGATGTACCGCCTGATGCTGCAATGCTGGAAGCAGGAGCC
GGACAAAAGGCCGGTGTTTGCGGACATCAGCAAAGACCTGGAGAAGATGATGGTTAAGAG
GAGAGACTACTTGGACCTTGCGGCGTCCACTCCATCTGACTCCCTGATTTATGACGACGGCC
TCTCAGAGGAGGAGACACCGCTGGTGGACTGTAATAATGCCCCCCTCCCTCGAGCCCTCCC
TTCCACATGGATTGAAAACAAACTCTATGGCATGTCAGACCCGAACTGGCCTGGAGAGAGTC
CTGTACCACTCACGAGAGCTGATGGCACTAACACTGGGTTTCCAAGATATCCAAATGATAGT
GTATATGCTAACTGGATGCTTTCACCCTCAGCGGCAAAATTAATGGACACGTTTGATAGTTAA

FIG. 32

KIF5B-RETa variant (LC-S2) fusion protein (977aa; SEQ ID NO 11; N-terminal domain of KIF5B: italic type; C-terminal of RET: boldface)
Fusion region (SEQ ID NO:12; underlined)

MADLAECNIKVMCRFRPLNESEVNRGDKYIAKFQGEDTVVIASKPYAFDRVFQSSTSQEQVYNDC
AKKIVKDVLEGYNGTIFAYGQTSSGKTHTMEGKLHDPEGMGIIPRIVQDIFNYIYSMDENLEFHIKVS
YFEIYLDKIRDLLDVSKTNLSVHEDKNRVPYVKGCTERFVCSPDEVMDTIDEGKSNRHVAVTNMNE
HSSRSHSIFLINVKQENTQTEQKLSGKLYLVDLAGSEKVSKTGAEGAVLDEAKNINKSLSALGNVIS
ALAEGSTYVPYRDSKMTRILQDSLGGNCRTTIVICCSPSSYNESETKSTLLFGQRAKTIKNTVCVNV
ELTAEQWKKKYEKEKEKNKILRNTIQWLENELNRWRNGETVPIDEQFDKEKANLEAFTVDKDITLT
NDKPATAIGVIGNFTDAERRKCEEEIAKLYKQLDDKDEEINQQSQLVEKLKTQMLDQEELLASTRR
DQDNMQAELNRLQAENDASKEEVKEVLQALEELAVNYDQKSQEVEDKTKEYELLSDELNQKSAT
LASIDAELQKLKEMTNHQKKRAAEMMASLLKDLAEIGIAVGNNDVKEDPKWEFPRKNLVLGKTLG
EGEFGKVVKATAFHLKGRAGYTTVAVKMLKENASPSELRDLLSEFNVLKQVNHPHVIKLYGACS
QDGPLLLIVEYAKYGSLRGFLRESRKVGPGYLGSGGSRNSSSLDHPDERALTMGDLISFAWQIS
QGMQYLAEMKLVHRDLAARNILVAEGRKMKISDFGLSRDVYEEDSYVKRSQGRIPVKWMAIESL
FDHIYTTQSDVWSFGVLLWEIVTLGGNPYPGIPPERLFNLLKTGHRMERPDNCSEEMYRLMLQC
WKQEPDKRPVFADISKDLEKMMVKRRDYLDLAASTPSDSLIYDDGLSEEETPLVDCNNAPLPRA
LPSTWIENKLYGMSDPNWPGESPVPLTRADGTNTGFPRYPNDSVYANWMLSPSAAKLMDTFDS

FIG. 33

KIF5B-RETa variant (LC-S6) fusion gene (3753bp; SEQ ID NO 13; 5'-terminal domain of KIF5B: italic type; 3'-terminal of RET: boldface) Fusion region (SEQ ID NO:14; underlined)

ATGGCGGACCTGGCCGAGTGCAACATCAAAGTGATGTGTCGCTTCAGACCTCTCAACGAGTCTGAAGTG
AACCGCGGCGACAAGTACATCGCCAAGTTTCAGGGAGAAGACACGGTCGTGATCGCGTCCAAGCCTTAT
GCATTTGATCGGGTGTTCCAGTCAAGCACATCTCAAGAGCAAGTGTATAATGACTGTGCAAAGAAGATTG
TTAAAGATGTACTTGAAGGATATAATGGAACAATATTTGCATATGGACAAACATCCTCTGGGAAGACACAC
ACAATGGAGGGTAAACTTCATGATCCAGAAGGCATGGGAATTATTCCAAGAATAGTGCAAGATATTTTTAA
TTATATTTACTCCATGGATGAAAATTTGGAATTTCATATTAAGGTTTCATATTTTGAAATATATTTGGATAAG
ATAAGGGACCTGTTAGATGTTTCAAAGACCAACCTTTCAGTTCATGAAGACAAAAACCGAGTTCCCTATGT
AAAGGGGTGCACAGAGCGTTTTGTATGTAGTCCAGATGAAGTTATGGATACCATAGATGAAGGAAAATCC
AACAGACATGTAGCAGTTACAAATATGAATGAACATAGCTCTAGGAGTCACAGTATATTTCTTATTAATGT
CAAACAAGAGAACACACAAACGGAACAAAAGCTGAGTGGAAAACTTTATCTGGTTGATTTAGCTGGTAGT
GAAAAGGTTAGTAAAACTGGAGCTGAAGGTGCTGTGCTGGATGAAGCTAAAAACATCAACAAGTCACTTT
CTGCTCTTGGAAATGTTATTTCTGCTTTGGCTGAGGGTAGTACATATGTTCCATATCGAGATAGTAAAATG
ACAAGAATCCTTCAAGATTCATTAGGTGGCAACTGTAGAACCACTATTGTAATTTGCTGCTCTCCATCATC
ATACAATGAGTCTGAAACAAAATCTACACTCTTATTTGGCCAAAGGGCCAAAACAATTAAGAACACAGTTT
GTGTCAATGTGGAGTTAACTGCAGAACAGTGGAAAAAGAAGTATGAAAAAGAAAAAGAAAAAAATAAGAT
CCTGCGGAACACTATTCAGTGGCTTGAAAATGAGCTCAACAGATGGCGTAATGGGGAGACGGTGCCTAT
TGATGAACAGTTTGACAAAGAGAAAGCCAACTTGGAAGCTTTCACAGTGGATAAAGATATTACTCTTACCA
ATGATAAACCAGCAACCGCAATTGGAGTTATAGGAAATTTTACTGATGCTGAAAGAAGAAAGTGTGAAGA
AGAAATTGCTAAATTATACAAACAGCTTGATGACAAGGATGAAGAAATTAACCAGCAAAGTCAACTGGTAG
AGAAACTGAAGACGCAAATGTTGGATCAGGAGGAGCTTTTGGCATCTACCAGAAGGGATCAAGACAATAT
GCAAGCTGAGCTGAATCGCCTTCAAGCAGAAAATGATGCCTCTAAAGAAGAAGTGAAAGAAGTTTACAG
GCCCTAGAAGAACTTGCTGTCAATTATGATCAGAAGTCTCAGGAAGTTGAAGACAAAACTAAGGAATATG
AATTGCTTAGTGATGAATTGAATCAGAAATCGGCAACTTTAGCGAGTATAGATGCTGAGCTTCAGAAACTT
AAGGAAATGACCAACCACCAGAAAAAACGAGCAGCTGAGATGATGGCATCTTTACTAAAAGACCTTGCAG
AAATAGGAATTGCTGTGGGAAATAATGATGTAAAGCAGCCTGAGGGAACTGGCATGATAGATGAAGAGTT
CACTGTTGCAAGACTCTACATTAGCAAAATGAAGTCAGAAGTAAAAACCATGGTGAAACGTTGCAAGCAG
TTAGAAAGCACACAAACTGAGAGCAACAAAAAAATGGAAGAAAATGAAAAGGAGTTAGCAGCATGTCAGC
TTCGTATCTCTCAACATGAAGCCAAAATCAAGTCATTGACTGAATACCTTCAAAATGTGGAACAAAAGAAA
AGACAGTTGGAGGAATCTGTCGATGCCCTCAGTGAAGAACTAGTCCAGCTTCGAGCACAAGAGAAAGTC
CATGAAATGGAAAAGGAGCACTTAAATAAGGTTCAGACTGCAAATGAAGTTAAGCAAGCTGTTGAACAGC
AGATCCAGAGCCATAGAGAAACTCATCAAAAACAGATCAGTAGTTTGAGAGATGAAGTAGAAGCAAAAGC
AAAACTTATTACTGATCTTCAAGACCAAAACCAGAAAATGATGTTAGAGCAGGAACGTCTAAGAGTAGAAC
ATGAGAAGTTGAAAGCCACAGATCAGGAAAAGAGCAGAAAACTACATGAACTTACGGTTATGCAAGATAG
ACGAGAACAAGCAAGACAAGACTTGAAGGGTTTGGAAGAGACAGTGGCAAAAGAACTTCAGACTTTACA
CAACCTGCGCAAACTCTTTGTTCAGGACCTGGCTACAAGAGTTAAAAAGAGTGCTGAGATTGATTCTGAT
GACACCGGAGGCAGCGCTGCTCAGAAGCAAAAAATCTCCTTTCTTGAAAATAATCTTGAACAG<u>*CTCACTA*</u>
<u>*AAGTGCACAAACAG*</u>**<u>GAGGATCCAAAGTGGGAATTC</u>**CCTCGGAAGAACTTGGTTCTTGGAAAAACTCTAG
GAGAAGGCGAATTTGGAAAAGTGGTCAAGGCAACGGCCTTCCATCTGAAAGGCAGAGCAGGGTACAC
CACGGTGGCCGTGAAGATGCTGAAAGAGAACGCCTCCCCGAGTGAGCTGCGAGACCTGCTGTCAGAG
TTCAACGTCCTGAAGCAGGTCAACCACCCACATGTCATCAAATTGTATGGGGCCTGCAGCCAGGATGG
CCCGCTCCTCCTCATCGTGGAGTACGCCAAATACGGCTCCCTGCGGGGCTTCCTCCGCGAGAGCCGCA
AAGTGGGGCCTGGCTACCTGGGCAGTGGAGGCAGCCGCAACTCCAGCTCCCTGGACCACCCGGATGA
GCGGGCCCTCACCATGGGCGACCTCATCTCATTTGCCTGGCAGATCTCACAGGGGATGCAGTATCTGG
CCGAGATGAAGCTCGTTCATCGGGACTTGGCAGCCAGAAACATCCTGGTAGCTGAGGGGCGGAAGAT
GAAGATTTCGGATTTCGGCTTGTCCCGAGATGTTTATGAAGAGGATTCCTACGTGAAGAGGAGCCAGGG
TCGGATTCCAGTTAAATGGATGGCAATTGAATCCCTTTTTGATCATATCTACACCACGCAAAGTGATGTAT
GGTCTTTTGGTGTCCTGCTGTGGGAGATCGTGACCCTAGGGGGAAACCCCTATCCTGGGATTCCTCCTG
AGCGGCTCTTCAACCTTCTGAAGACCGGCCACCGGATGGAGAGGCCAGACAACTGCAGCGAGGAGAT
GTACCGCCTGATGCTGCAATGCTGGAAGCAGGAGCCGGACAAAAGGCCGGTGTTTGCGGACATCAGC
AAAGACCTGGAGAAGATGATGGTTAAGAGGAGAGACTACTTGGACCTTGCGGCGTCCACTCCATCTGA
CTCCCTGATTTATGACGACGGCCTCTCAGAGGAGGAGACACCGCTGGTGGACTGTAATAATGCCCCCCT
CCCTCGAGCCCTCCCTTCCACATGGATTGAAAACAAACTCTATGGCATGTCAGACCCGAACTGGCCTGG
AGAGAGTCCTGTACCACTCACGAGAGCTGATGGCACTAACACTGGGTTTCCAAGATATCCAAATGATAG
TGTATATGCTAACTGGATGCTTTCACCCTCAGCGGCAAAATTAATGGACACGTTTGATAGTTAA

FIG. 34

KIF5B-RETa variant (LC-S6) fusion protein (1250aa; SEQ ID NO:15; N-terminal domain of KIF5B: italic type; C-terminal of RET: boldface) Fusion region (SEQ ID NO:16; underlined)

MADLAECNIKVMCRFRPLNESEVNRGDKYIAKFQGEDTVVIASKPYAFDRVFQSSTSQEQVYNDC
AKKIVKDVLEGYNGTIFAYGQTSSGKTHTMEGKLHDPEGMGIIPRIVQDIFNYIYSMDENLEFHIKVS
YFEIYLDKIRDLLDVSKTNLSVHEDKNRVPYVKGCTERFVCSPDEVMDTIDEGKSNRHVAVTNMNE
HSSRSHSIFLINVKQENTQTEQKLSGKLYLVDLAGSEKVSKTGAEGAVLDEAKNINKSLSALGNVIS
ALAEGSTYVPYRDSKMTRILQDSLGGNCRTTIVICCSPSSYNESETKSTLLFGQRAKTIKNTVCVNV
ELTAEQWKKKYEKEKEKNKILRNTIQWLENELNRWRNGETVPIDEQFDKEKANLEAFTVDKDITLT
NDKPATAIGVIGNFTDAERRKCEEEIAKLYKQLDDKDEEINQQSQLVEKLKTQMLDQEELLASTRR
DQDNMQAELNRLQAENDASKEEVKEVLQALEELAVNYDQKSQEVEDKTKEYELLSDELNQKSAT
LASIDAELQKLKEMTNHQKKRAAEMMASLLKDLAEIGIAVGNNDVKQPEGTGMIDEEFTVARLYISK
MKSEVKTMVKRCKQLESTQTESNKKMEENEKELAACQLRISQHEAKIKSLTEYLQNVEQKKRQLE
ESVDALSEELVQLRAQEKVHEMEKEHLNKVQTANEVKQAVEQQIQSHRETHQKQISSLRDEVEAK
AKLITDLQDQNQKMMLEQERLRVEHEKLKATDQEKSRKLHELTVMQDRREQARQDLKGLEETVA
KELQTLHNLRKLFVQDLATRVKKSAEIDSDDTGGSAAQKQKISFLENNLEQLTKVHKQEDPKWEF
PRKNLVLGKTLGEGEFGKVVKATAFHLKGRAGYTTVAVKMLKENASPSELRDLLSEFNVLKQVN
HPHVIKLYGACSQDGPLLLIVEYAKYGSLRGFLRESRKVGPGYLGSGGSRNSSSLDHPDERALT
MGDLISFAWQISQGMQYLAEMKLVHRDLAARNILVAEGRKMKISDFGLSRDVYEEDSYVKRSQG
RIPVKWMAIESLFDHIYTTQSDVWSFGVLLWEIVTLGGNPYPGIPPERLFNLLKTGHRMERPDNC
SEEMYRLMLQCWKQEPDKRPVFADISKDLEKMMVKRRDYLDLAASTPSDSLIYDDGLSEEETPL
VDCNNAPLPRALPSTWIENKLYGMSDPNWPGESPVPLTRADGTNTGFPRYPNDSVYANWMLSP
SAAKLMDTFDS

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2012/009056

A. CLASSIFICATION OF SUBJECT MATTER

C07K 19/00(2006.01)i, C12N 15/62(2006.01)i, G01N 33/68(2006.01)i, G01N 33/15(2006.01)i, A61K 38/00(2006.01)i, A61P 35/00(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K 19/00; A61K 39/395; C07H 21/04; C07K 14/47; G01N 15/33; G01N 33/53; C12Q 1/68

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

Korean utility models and applications for utility models
Japanese utility models and applications for utility models

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

eKOMPASS(KIPO internal) & Keywords: fusion protein, RET, KIF5B-RET, lung cancer, RET-involved chromosomal rearrangement, inversion, translocation, chromosome 10

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2008-031551 A2 (NOVARTIS FORSCHUNGSSTIFTUNG, ZWEIGNIEDERLASSUNG et al.) 20 March 2008 See abstract; claims 1-15.	1-10,19-25
X A	US 2009-0136502 A1 (ARAI, TOSHIMITSU et al.) 28 May 2009 See abstract; paragraph [0146].	21,23 1-10,19,20,22,24,25
A	WO 97-44356 A2 (BIOGEN, INC.) 27 November 1997 See abstract; claims 31-34.	1-10,19-25
A	US 2007-0212689 A1 (BIANCHI, DIANA W. et al.) 13 September 2007 See abstract; claims 1-43.	1-10,19-25
A	US 7741034 B2 (KATZ, RUTH et al.) 22 June 2010 See abstract and claim 1.	1-10,19-25
PX	JU, YOUNG SEOK et al., `A transforming KIF5B and RET gene fusion in lung adenocarcinoma revealed from whole-genome and transcriptome sequencing`, Genome Research, 22 December 2011, Vol.22, pp.436-445, ISSN 1088-9051. See abstract.	1,2

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 March 2013 (28.03.2013)	**29 March 2013 (29.03.2013)**

Name and mailing address of the ISA/KR
Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon Metropolitan City, 302-701, Republic of Korea
Facsimile No. 82-42-472-7140

Authorized officer
HEO, Joo Hyung
Telephone No. 82-42-481-8150

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2012/009056

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

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

1. ☒ Claims Nos.: 11-15
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 11-15 pertain to methods for treatment of the human body by surgery as well as diagnostic methods, and thus relate to a subject matter which this International Searching Authority is not required, under Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.

2. ☒ Claims Nos.: 16-18
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claims of the present application are not numbered consecutively in Arabic numbers, since claims 16-18 are found missing (PCT Rule 6.1(b)).

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

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

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

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

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

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

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

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR2012/009056

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR2012/009056

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