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(54) Title: PRKC FUSIONS

(57) Abstract: The invention provides PRKC gene fusions, PRKC fusion proteins, and fragments of those genes and polypeptides. The invention further provides methods of diagnosing and treating diseases or disorders associated with PRKC fusions, such as conditions mediated by aberrant PRKC expression or activity, or overexpression of PRKC.

PRKC FUSIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/025,865, filed July 17, 2014, which is incorporated here by reference in its entirety to provide continuity of disclosure.

[0002] This invention relates to PRKC (protein kinase C family) gene fusions and PRKC fusion proteins. The invention further relates to methods of diagnosing and treating diseases or disorders associated with PRKC fusions, such as conditions mediated by aberrant PRKC expression or activity, or conditions associated with overexpression of PRKC.

[0003] Many forms of cancer are caused by genetic lesions that give rise to tumor initiation and growth. Genetic lesions may include chromosomal aberrations, such as translocations, inversions, deletions, copy number changes, gene expression level changes, and somatic and germline mutations. Indeed, the presence of such genomic aberrations is a hallmark feature of many cancers, including, for example, B cell cancer, lung cancer, breast cancer, ovarian cancer, pancreatic cancer, and colon cancer. In some models, cancer represents the phenotypic end-point of multiple genetic lesions that endow cells with a full range of biological properties required for tumorigenesis.

[0004] Recent efforts by The Cancer Genome Atlas (TCGA), the International Cancer Genome Consortium (ICGC), and dozens of other large-scale profiling efforts have generated an enormous amount of new sequencing data for dozens of cancer types – this includes whole-genome DNA, whole-exome DNA, and full-transcriptome RNA sequencing. These efforts have led to the identification of new driver genes and fusion genes within multiple cancer types. Fusions, particularly fusions involving kinases, are of particular interest, as such fusions have been shown to be oncogenic, and have been successfully targeted by new therapeutics. For example, anaplastic lymphoma kinase (ALK), one of the receptor tyrosine kinases, is known to become oncogenic when fused with various genes. See, e.g., M. Soda et al, "Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer," *Nature* 444:561-566 (2007).

[0005] A need exists for identifying novel genetic lesions associated with cancer. For example, the presence of fusions involving a kinase in samples collected from more than one source can indicate that the kinase is an oncogenic driver. The identification of such fusions can be an effective approach to diagnosis of cancers and development of compounds, compositions, methods, and assays for evaluating and treating cancer patients.

[0006] In one aspect, the invention provides a method for detecting the presence of a PRKC fusion in a biological sample. The method includes the steps of: (a) obtaining a biological sample from a mammal; and (b) contacting the sample with a reagent that detects a PRKC fusion, to determine whether a PRKC fusion is present in the biological sample. The sample can be from, e.g., a cancer patient. In some embodiments, the cancer can be a lung cancer, such as, e.g., lung squamous cell carcinoma or lung adenocarcinoma. In some embodiments, the fusion can be, e.g., an IGF2BP3:PRKCA fusion, a TANC2:PRKCA fusion, an ADCY9:PRKCB fusion, or an SPNS1:PRKCB fusion. In some embodiments, the IGF2BP3:PRKCA fusion has all or part of the nucleotide and/or amino acid sequence (such as, e.g., the fusion junction) set forth in SEQ ID NO:1 and SEQ ID NO:2, respectively. In some embodiments, the TANC2:PRKCA fusion has all or part of the nucleotide and/or amino acid sequence (such as, e.g., the fusion junction) set forth in SEQ ID NO:3 and SEQ ID NO:4, respectively. In some embodiments, the ADCY9:PRKCB fusion has all or part of the nucleotide and/or amino acid sequence (such as, e.g., the fusion junction) set forth in SEQ ID NO:5 and SEQ ID NO:6, respectively. In some embodiments, the SPNS1:PRKCB fusion has all or part of the nucleotide and/or amino acid sequence (such as, e.g., the fusion junction) set forth in SEQ ID NO:7 and SEQ ID NO:8, respectively.

[0007] In another aspect, the invention provides a method of diagnosing a patient having a disease or disorder associated with aberrant PRKC expression or activity, or overexpression of PRKC; the method includes: (a) obtaining a biological sample from the patient; and (b) contacting the sample with a reagent that detects a PRKC fusion to determine whether a PRKC fusion is present in the biological sample, whereby the detection of the PRKC fusion indicates the presence of a disorder associated with aberrant (e.g., constitutive) PRKC activity or expression, or overexpression of PRKC.

[0008] The invention also includes a method of determining a therapeutic regimen for treating a cancer in a human subject; a method of identifying a patient likely to respond to treatment with a PRKC inhibitor or a PRKC fusion inhibitor; a method of stratifying a patient population by detecting a PRKC fusion; a method of treating a patient; a method of inhibiting the proliferation of cells containing a PRKC fusion; a method of reducing an activity of a PRKC fusion; a method of treating a condition mediated by aberrant (e.g., constitutive) PRKC activity or expression; a method of treating a condition characterized by overexpression of PRKC; a method of identifying an agent that modulates the activity of a PRKC fusion; and a method of monitoring disease burden in a patient having a condition mediated by PRKC.

BRIEF DESCRIPTION OF THE FIGURES

[0009] FIG. 1 depicts the nucleotide sequence of an IGF2BP3:PRKCA gene fusion (SEQ ID NO:1) comprising a portion of the IGF2BP3 gene (NM_006547) up to and including exon 10 and a portion of the PRKCA gene (NM_002737) starting at exon 4. The underlined codons at nucleotides 1201-1203 and 1204-1206 encode the last amino acid of IGF2BP3 and the first amino acid of PRKCA, respectively. The slash after nucleotide 1203 indicates the breakpoint (fusion junction) where translocation and in-frame fusion has occurred.

[0010] FIG. 2 depicts the amino acid sequence of an IGF2BP3:PRKCA fusion protein (SEQ ID NO:2). The slash between amino acids 401 and 402 indicates the breakpoint or fusion junction between the IGF2BP3 and PRKCA proteins. Amino acids 401-402 correspond to the codons at nucleotides 1201-1203 and 1204-1206 in SEQ ID NO:1.

[0011] FIG. 3 depicts the nucleotide sequence of a TANC2:PRKCA gene fusion (SEQ ID NO:3) comprising a portion of the TANC2 gene (NM_025185) up to and including exon 2 and a portion of the PRKCA gene (NM_002737) starting at exon 6. The underlined codons at nucleotides 136-138 and 142-144 encode the last amino acid of TANC2 and the first amino acid of PRKCA, respectively. The slash after nucleotide 139 indicates the breakpoint (fusion junction) where translocation and in-frame fusion has occurred. The shading at nucleotides 139-141 indicates that nucleotides from both TANC2 and PRKCA are fused in frame to form a codon and encode an amino acid.

[0012] FIG. 4 depicts the amino acid sequence of a TANC2:PRKCA fusion protein (SEQ ID NO:4). The shaded amino acid at position 47 corresponds to nucleotides 139-141 in SEQ ID NO:3. This amino acid is encoded by nucleotides from both TANC2 and PRKCA.

[0013] FIG. 5A & 5B depict the nucleotide sequence of an ADCY9:PRKCB gene fusion (SEQ ID NO:5) comprising a ADCY9 gene (NM_001116) up to and including exon 2 and a portion of the PRKCB gene (NM_002738) starting at exon 3. The underlined codons at nucleotides 1690-1692 and 1696-1698 encode the last amino acid of ADCY9 and the first amino acid of PRKCB, respectively. The slash after nucleotide 1693 indicates the breakpoint (fusion junction) where translocation and in-frame fusion has occurred. The shading at nucleotides 1693-1695 indicates that nucleotides from both ADCY9 and PRKCB are fused in frame to form a codon and encode an amino acid.

[0014] FIG. 6 depicts the amino acid sequence of an ADCY9:PRKCB fusion protein (SEQ ID NO:6). The shaded amino acid at position 565 corresponds to the codon at nucleotides 1693-

1695 in SEQ ID NO:5. This amino acid is encoded by nucleotides from both ADCY9 and PRKCB.

[0015] FIG. 7 depicts the nucleotide sequence of a SPNS1:PRKCB gene fusion (SEQ ID NO:7) comprising a SPNS1 gene (NM_001142451) up to and including exon 2 and a portion of the PRKCB gene (NM_002738) starting at exon 3. The underlined codons at nucleotides 304-306 and 310-312 encode the last amino acid of SPNS1 and the first amino acid of PRKCB, respectively. The slash after nucleotide 307 indicates the breakpoint (fusion junction) where translocation and in-frame fusion has occurred. The shading at nucleotides 307-309 indicates that nucleotides from both SPNS1 and PRKCB are fused in frame to form a codon and encode an amino acid.

[0016] FIG. 8 depicts the amino acid sequence of an SPNS1:PRKCB fusion protein (SEQ ID NO:8). The shaded amino acid at position 103 corresponds to the codon at nucleotides 307-309 in SEQ ID NO:7. This amino acid is encoded by nucleotides from both SPNS1 and PRKCA.

EXEMPLARY EMBODIMENTS OF THE INVENTION

[0017] The invention is based, at least in part, on the discovery of novel recombination or translocation events in cancer patients that result in at least a fragment of a PRKC gene linked to a non-homologous promoter via a recombination or translocation event that may result in aberrant expression (e.g., in a location where the kinase is not typically expressed) or overexpression of the kinase domain of the PRKC gene and thus, an increase in kinase activity. In some embodiments, the recombination or translocation may result in constitutive activation of PRKC kinase activity. Thus, a new patient population is identified, which is characterized by the presence of a PRKC fusion, e.g., a PRKC gene fusion or fusion protein. This new patient population suffers from or is susceptible to disorders mediated by aberrant (e.g., constitutive) PRKC activity or expression, or overexpression of PRKC, such as, e.g., a cancer. In another aspect of the invention, a new subtype of cancer is identified, which is characterized by the presence of the PRKC fusions described herein. In some embodiments, the new patient population suffers from or is susceptible to a lung cancer (such as, e.g., lung squamous cell carcinoma or lung adenocarcinoma) characterized by the presence of a PKRC fusion. New methods of diagnosing and treating the patient population and the PRKC fusion cancer subtype are also provided.

[0018] The term "PRKC fusion" is used generically herein, and includes any fusion molecule (e.g., gene, gene product (e.g., cDNA, mRNA, or protein), and variants thereof) that includes a

fragment of PRKC, particularly the coding region for the kinase domain of PRKC, and a fragment of a second, non-homologous gene, such that the coding sequence for the kinase domain of PRKC is under control of the promoter of the non-homologous gene. In some embodiments, the PRKC fusion removes all or a portion of the coding region of the regulatory domain of PRKC, such as, e.g., the autoinhibitory pseudosubstrate segment. A PRKC fusion protein generally includes the kinase domain of PRKC.

PRKC Gene Fusions and Fusion proteins

[0019] PRKC is a family of protein kinase enzymes that are involved in controlling the function of other proteins through the phosphorylation of hydroxyl groups of serine and threonine amino acid residues on these proteins. PRKC in turn is activated by signals such as increases in the cellular concentration of diacylglycerol (DAG) or calcium ions (Ca^{2+}) and plays important roles in several signal transduction cascades. The activity of PRKC itself is also regulated by phosphorylation. The PRKC family contains fifteen isozymes in humans, which are divided into three subfamilies based on their second messenger requirements: conventional (or classical), novel, and atypical. Isoforms α (protein kinase C, alpha (PRKCA)) and β_I and β_{II} (protein kinase C, beta (PRKCB)) belong to the conventional subfamily. The structure of PRKC contains a regulatory domain and a catalytic domain tethered together by a hinge region. The regulatory domain at the N-teminus of PRKC includes the pseudosubstrate segment, which is a small sequence of amino acids that mimic a substrate and bind the substrate-binding cavity in the catalytic domain, keeping the enzyme inactive. When Ca^{2+} and DAG are present in sufficient concentrations, they bind to the regulatory domain and recruit PRKC to the membrane. This interaction with the membrane results in release of the pseudosubstrate segment from the catalytic site and activation of the enzyme. The catallytic region includes the kinase domain of PRKC. Upon activation, PRKC is translocated to the plasma membrane and carries out PRKC-mediated phosphorylation of substrate proteins. PRKC fusions have recently been described in papillary glioneuronal tumors (see Bridge, J. A. et al, *Brain Pathol. Zurich Switz.* 23: 121–128 (2013)) and benign fibrous histiocytoma (see Płaszczyca, A. et al, *Int. J. Biochem. Cell Biol.* doi:10.1016/j.biocel.2014.03.027 (2014)).

[0020] The invention provides novel PRKC fusions that are associated with different types of disorders. For example, the PRKC fusions disclosed herein can be associated with certain cancers, such as, e.g., a lung cancer. In some embodiments, the lung cancer associated with a PRKC fusion is lung squamous carcinoma or lung adenocarcinoma. In some embodiments, the

PRKC fusions disclosed herein can be associated with other disorders mediated by aberrant (e.g., constitutive) PRKC activity or expression, or overexpression of PRKC.

[0021] PRKC gene fusions are generated by a fusion between at least a part of the PRKC gene, such as, e.g., PRKCA or PRKCB, and a part of another gene as a result of a translocation (including inversion) within a chromosome or between chromosomes. As a result of a translocation, the PRKC gene may be placed under the transcriptional control of the partner gene promoter, resulting in aberrant PRKC activity or expression or overexpression of PRKC. Alternatively or additionally, the translocation may result in removal of all or a portion of the regulatory domain of PRKC, such as, e.g., the autoinhibitory pseudosubstrate segment, and cause PRKC to become constitutively activated. The overexpression and/or constitutive activation can lead to certain cancers. As used herein, the 5'-region is upstream of, and the 3'-region is downstream of, a fusion junction or breakpoint in one of the component genes. PRKC and the gene or protein that it is fused to is referred to as "fusion partners." Alternatively, they may be identified as a "PRKC gene fusion" or a "PRKC fusion protein," which are collectively termed "PRKC fusions." The PRKC fusions disclosed herein have a kinase activity. The phrase "having a kinase activity" as used in this application means having an activity as an enzyme phosphorylating the side chain of an amino acid, such as serine or threonine. In some embodiments, the PRKC fusion may include an in-frame fusion of the coding sequences of PRKC and the fusion partner that introduces amino acids into the fusion protein that are not part of PRKC or the fusion partner.

[0022] In some exemplary embodiments, the fusion partner is all or a portion of IGF2BP3 (insulin-like growth factor 2 mRNA binding protein 3). In other exemplary embodiments, the fusion partner is all or a portion of TANC2 (tetratricopeptide repeat, ankyrin repeat and coiled-coil containing 2). In other exemplary embodiments, the fusion partner is all or a portion of ADCY9 (adenylate cyclase type 9). In yet other exemplary embodiments, the fusion partner is all or a portion of SPNS1 (spinster homolog 1). In some embodiments, the PRKC gene is PRKCA. In other embodiments, the PRKC gene is PRKCB.

[0023] Reference to "all or a portion" or "all or part" of a PRKC gene fusion or SEQ ID NO:1, 3, 5, or 7, means that the nucleotide sequence comprises the entire PRKC gene fusion nucleotide sequence or a fragment of that sequence that comprises the fusion junction or breakpoint between PRKC and its fusion partner (such as, e.g., IGF2BP3, TANC2, ADCY9, or SPNS1). The fragment may comprise 7, 8, 9, 10, 12, 14, 16, 18, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 120, 150, 175, 200, 250, 300, or more

nucleotides spanning the fusion junction of the PRKC gene fusion. Reference to "all or a portion" or "all or part" of a PRKC fusion protein or SEQ ID NO:2, 4, 6, or 8, means an amino acid sequence that comprises the entire PRKC fusion protein amino acid sequence or a fragment of that sequence that comprises the fusion junction or breakpoint between PRKC and its fusion partner (such as, e.g., IGF2BP3, TANC2, ADCY9, or SPNS1). The fragment may comprise 8, 10, 12, 14, 15, 16, 18, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 75, or more amino acids spanning the fusion junction.

[0024] In certain embodiments of the invention, a fusion includes an in-frame fusion of all or a portion of the IGF2BP3 gene (e.g., an IGF2BP3 promotor or a functional fragment thereof, and one or more exons encoding IGF2BP3 or a fragment thereof) and an exon of the PRKCA gene (e.g., one or more exons encoding a PRKCA kinase domain or a functional fragment thereof). Such a fusion can be referred to as an IGF2BP3:PRKCA fusion. In one embodiment, the IGF2BP3:PRKCA fusion comprises sufficient PRKCA sequence to drive expression of a fusion protein that has kinase activity, e.g., has elevated activity as compared with wild type PRKCA in the same tissue or cell. In some embodiments, the IGF2BP3:PRKCA fusion removes all or a portion of the sequence encoding the regulatory domain of PRKCA, such as, e.g., the autoinhibitory psedosubstrate segment, and results in expression of a fusion protein that has constitutive kinase activity, e.g., has acitivty without upstream signaling or binding of a ligand or second messenger, such as, e.g., DAG or Ca^{2+}.

[0025] In some embodiments, the invention provides an IGF2BP3:PRKC gene fusion comprising the nucleotide sequence depicted in Figure 1 (SEQ ID NO:1), or a fragment thereof that includes the fusion junction. SEQ ID NO:1 comprises IGF2BP3 (NM_006547) up to exon 10 fused to PRKCA (NM_002737), beginning at exon 4. In some embodiments, the IGF2BP3:PRKCA gene fusion comprises a nucleotide sequence that is at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to all or part of SEQ ID NO:1. In some embodiments, the IGF2BP3:PRKCA gene fusion encodes a protein having the sequence depicted in Figure 2 (SEQ ID NO:2) or a sequence that is at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to all or part of SEQ ID NO:2.

[0026] In other embodiments of the invention, a fusion includes an in-frame fusion of all or a portion of the TANC2 gene (e.g., a TANC2 promotor or a functional fragment thereof, and one or more exons encoding a TANC2 or a fragment thereof) and an exon of the PRKCA gene (e.g., one or more exons encoding a PRKCA kinase domain or a functional fragment thereof). Such a fusion can be referred to as a TANC2:PRKCA fusion. In one embodiment, the

TANC2:PRKCA fusion comprises sufficient PRKCA sequence to drive expression of a fusion protein that has kinase activity, e.g., has elevated activity as compared with wild type PRKCA in the same tissue or cell. In some embodiments, the TANC2:PRKCA fusion removes all or a portion of the sequence encoding the regulatory domain of PRKCA, such as, e.g., the autoinhibitory pseudosubstrate segment, and results in expression of a fusion protein that has constitutive kinase activity, e.g., has acitivty without upstream signaling or binding of a ligand or second messenger, such as, e.g., DAG or Ca^{2+}.

[0027] In some embodiments, the invention provides an TANC2:PRKCA gene fusion comprising the nucleotide sequence depicted in Figure 3 (SEQ ID NO:3), or a fragment thereof that includes the fusion junction. SEQ ID NO:3 comprises TANC2 (NM_025185) up to exon 2 fused to PRKCA (NM_002737), beginning at exon 6. In some embodiments, the TANC2:PRKCA gene fusion comprises a nucleotide sequence that is at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to all or part of SEQ ID NO:3. In some embodiments, the TANC2:PRKCA gene fusion encodes a protein having the sequence depicted in Figure 4 (SEQ ID NO:4) or a sequence that is at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to all or part of SEQ ID NO:4.

[0028] In certain embodiments of the invention, a fusion includes an in-frame fusion of all or a portion of the ADCY9 gene (e.g., an ADCY9 promotor or a functional fragment thereof, and one or more exons encoding an ADCY9 or a fragment thereof) and an exon of the PRKCB gene (e.g., one or more exons encoding a PRKCB kinase domain or a functional fragment thereof). Such a fusion can be referred to as an ADCY9:PRKCB fusion. In some embodiments, the ADCY9:PRKCB fusion comprises sufficient PRKCB sequence to drive expression of a fusion protein that has kinase activity, e.g., has elevated activity as compared with wild type PRKCB in the same tissue or cell. In some embodiments, the ADCY9:PRKCB fusion removes all or a portion of the sequence encoding the regulatory domain of PRKCB, such as, e.g., the autoinhibitory psedosubstrate segment, and results in expression of a fusion protein that has constitutive kinase activity, e.g., has acitivty without upstream signaling or binding of a ligand or second messenger, such as, e.g., DAG or Ca^{2+}.

[0029] In some embodiments, the ADCY9:PRKCB fusion has the nucleotide sequence depicted in Figure 5 (SEQ ID NO:5), or a fragment thereof that includes the fusion junction. SEQ ID NO:5 comprises ADCY9 (NM_001116) up to and including exon 2 fused to PRKCB (NM_002738), beginning at exon 3. In some embodiments, the ADCY9:PRKCB gene fusion comprises a nucleotide sequence that is at least 85%, at least 90%, at least 95%, at least 97%, at

least 98%, or at leat 99% identical to all or part of SEQ ID NO:5. In some embodiments, the ADCY9:PRKCB fusion encodes a protein having the sequence depicted in Figure 6 (SEQ ID NO:6) or a sequence that is at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to all or part of SEQ ID NO:6.

[0030] In certain embodiments of the invention, a fusion includes an in-frame fusion of all or a portion of the SPNS1 gene (e.g., an SPNS1 promotor or a functional fragment thereof, and one or more exons encoding an SPNS1 or a fragment thereof) and an exon of the PRKCB gene (e.g., one or more exons encoding a PRKCB kinase domain or a functional fragment thereof). Such a fusion can be referred to as an SPNS1:PRKCB fusion. In one embodiment, the SPNS1:PRKCB fusion comprises sufficient PRKCB sequence to drive expression of a fusion protein that has kinase activity, e.g., has elevated activity as compared with wild type PRKCB in the same tissue or cell. In some embodiments, the SPNS1:PRKCB fusion removes all or a portion of the sequence encoding the regulatory domain of PRKCB, such as, e.g., the autoinhibitory pseudosubstrate segment, and results in expression of a fusion protein that has constitutive kinase activity, e.g., has acitivty without upstream signaling or binding of a ligand or second messenger, such as, e.g., DAG or Ca^{2+}.

[0031] In some embodiments, the invention provides an SPNS1:PRKCB gene fusion comprising the nucleotide sequence depicted in Figure 7 (SEQ ID NO:7), or a fragment of thereof that includes the fusion junction. SEQ ID NO:7 comprises SPNS1 (NM_001142451) up to exon 2 fused to PRKCB (NM_002738), beginning at exon 3. In some embodiments, the SPNS1:PRKCB gene fusion comprises a nucleotide sequence that is at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to all or part of SEQ ID NO:7. In some embodiments, the SPNS1:PRKCB gene fusion encodes a protein having the sequence depicted in Figure 8 (SEQ ID NO:8) or a sequence that is at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to all or part of SEQ ID NO:8.

[0032] The nucleic acid sequences of PRKC gene fusions may be used as probes, primers, or bait to identify nucleotides from a biological sample that include, flank, or hybridize to, PRKC fusions, such as IGF2BP3:PRKCA (e.g., all or part of SEQ ID NO: 1), TANC2:PRKCA (e.g., all or part of SEQ ID NO:3), ADCY9:PRKCB (e.g., all or part of SEQ ID NO:5), or SPNS1:PRKCB (e.g., all or part of SEQ ID NO:7), at, e.g., the fusion junctions. In certain embodiments, the probe, primer, or bait molecule is an oligonucleotide that allows capture, detection, and/or isolation of a PRKC gene fusion in a biological sample. In certain embodiments, the probes or primers derived from the nucleic acid sequences of PRKC gene

fusions (e.g., from the fusion junctions) may be used, for example, for polymerase chain reaction (PCR) amplification. The oligonucleotide can comprise a nucleotide sequence substantially complementary to a fragment of the PRKC gene fusion nucleic acid molecules described herein. The sequence identity between the nucleic acid fragment, e.g., the oligonucleotide and the target PRKC gene fusion sequence, need not be exact, so long as the sequences are sufficiently complementary to allow the capture, detection or isolation of the target sequence. In one embodiment, the nucleic acid fragment is a probe or primer that includes an oligonucleotide between about 5 and 25, e.g., between 10 and 20, or 10 and 15 nucleotides in length that includes the fusion junction of a PRKC fusion, such as, e.g., IGF2BP3:PRKCA (e.g., all or part of SEQ ID NO: 1), TANC2:PRKCA (e.g., all or part of SEQ ID NO:3), ADCY9:PRKCB (e.g., all or part of SEQ ID NO:5), or SPNS1:PRKCB (e.g., all or part of SEQ ID NO:7). In other embodiments, the nucleic acid fragment is a bait that includes an oligonucleotide between about 100 to 300 nucleotides, 130 and 230 nucleotides, or 150 and 200 nucleotides in length that includes the fusion junction of a PRKC fusion, such as, e.g., IGF2BP3:PRKCA (e.g., all or part of SEQ ID NO: 1), TANC2:PRKCA (e.g., all or part of SEQ ID NO: 3), ADCY9:PRKCB (e.g., all or part of SEQ ID NO:5), or SPNS1:PRKCB (e.g., all or part of SEQ ID NO:7).

[0033] In certain embodiments, the nucleic acid fragments hybridize to a nucleotide sequence that includes a breakpoint or fusion junction, e.g., a breakpoint or fusion junction as identified by a slash ("/") in FIGs. 1, 3, 5, or 7. For example, the nucleic acid fragment can hybridize to a nucleotide sequence that includes the fusion junction between the IGF2BP3 transcript and the PRKCA transcript (e.g., nucleotides 1201-1206 of SEQ ID NO:1), between the TANC2 transcript and the PRKCA transcript (e.g., nucleotides 136-144 of SEQ ID NO:3), between the ADCY9 transcript and the PRKCB transcript (e.g., nucleotides 1690-1698 of SEQ ID NO:5), or between the SPNS1 transcript and the PRKCB transcript (e.g., nucleotides 304-312 of SEQ ID NO:7), i.e., a nucleotide sequence that includes a portion of SEQ ID NO: 1, 3, 5, or 7.

[0034] In other embodiments, the nucleic acid fragment includes a bait that comprises a nucleotide sequence that hybridizes to a PRKC gene fusion nucleic acid molecule described herein, and thereby allows the detection, capture, and/or isolation of the nucleic acid molecule. In one embodiment, a bait is suitable for solution phase hybridization. In other embodiments, a bait includes a binding entity or detection entity, e.g., an affinity tag or fluorescent label, that allows detection, capture, and/or separation, e.g., by binding to a binding entity, of a hybrid formed by a bait and a nucleic acid hybridized to the bait.

[0035] In exemplary embodiments, the nucleic acid fragments hybridize to a nucleotide sequence that includes a fusion junction between the IGF2BP3 transcript and the PRKCA transcript, e.g., a nucleotide sequence within SEQ ID NO:1 comprising nucleotides 1201-1206 (such as, e.g., a sequence comprising nucleotides 1199-1208, 1194-1213, 1179-1228, or 1154-1253 of SEQ ID NO:1).

[0036] In other exemplary embodiments, the nucleic acid fragments hybridize to a nucleotide sequence that includes a fusion junction between the TANC2 transcript and the PRKCA transcript, e.g., a nucleotide sequence within SEQ ID NO:3 comprising nucleotides 136-144 (such as, e.g., a sequence comprising nucleotides 134-146, 129-151, 114-166, or 89-191 of SEQ ID NO:3).

[0037] In other exemplary embodiments, the nucleic acid fragments hybridize to a nucleotide sequence that includes a fusion junction between the ADCY9 transcript and the PRKCB transcript, e.g., a nucleotide sequence within SEQ ID NO:5 comprising nucleotides 1690-1698 (such as, e.g., a sequence comprising nucleotides 1688-1700, 1683-1705, 1668-1720, or 1643-1745 of SEQ ID NO:5).

[0038] In other exemplary embodiments, the nucleic acid fragments hybridize to a nucleotide sequences that includes a fusion junction between the SPNS1 transcript and the PRKCB transcript, e.g., a nucleotide sequence within SEQ ID NO:7 comprising nucleotides 304-312 (such as, e.g., a sequence comprising nucleotides 302-314, 297-319, 282-334, or 257-359 of SEQ ID NO:7).

[0039] Another aspect of the invention provides PRKC fusion proteins (such as, e.g., a purified or isolated IGF2BP3:PRKCA, TANC2:PRKCA, ADCY9:PRKCB, or SPNS1:PRKCB fusion protein), biologically active or antigenic fragments thereof, and use of those polypeptides for detecting and/or modulating the biological activity (such as tumorigenic activity) of a PRKC fusion protein. Exemplary embodiments of the PRKC fusion proteins comprise the amino acid sequence set forth in SEQ ID NO:2, 4, 6, or 8, and fragments of those sequences.

[0040] In some embodiments, the PRKC fusion protein of the invention can include a fragment of an IGF2BP3 protein, a TANC2 protein, an ADCY9 protein, or an SPNS1 protein, and a fragment of a PRKC protein, such as, e.g., a PRKCA protein or a PRKCB protein. In one embodiment, the PRKC fusion protein is an IGF2BP3:PRKCA fusion protein having the amino acid sequence of SEQ ID NO:2 or a fragment thereof, such as, e.g., amino acids 397-406, 392-411, or 377-426 of SEQ ID NO:2. In other embodiments, the PRKC fusion protein is a TANC2:PRKCA fusion protein having the amino acid sequence of SEQ ID NO:4 or a fragment

thereof, such as, e.g., amino acids 42-51, 37-56, or 22-71 of SEQ ID NO:4. In some embodiments, the PRKC fusion protein is an ADCY9:PRKCB fusion protein having the amino acid sequence of SEQ ID NO:6 or a fragment thereof, such as, e.g., amino acids 560-569, 555-574, or 540-589 of SEQ ID NO:6. In other embodiments, the PRKC fusion protein is an SPNS1:PRKCB fusion protein having the amino acid sequence of SEQ ID NO:8, or a fragment thereof, such as, e.g., amino acids 98-107, 93-112, or 78-127 of SEQ ID NO:8.

[0041] In some embodiments, the PRKC fusion protein is an IGF2BP3:PRKCA fusion protein comprising an amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:2 or a fragment thereof (e.g., amino acids 397-406, 392-411, or 377-426 of SEQ ID NO:2). In other embodiments, the PRKC fusion protein is a TANC2:PRKCA fusion protein comprising an amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:4 or a fragment thereof (e.g., amino acids 42-51, 37-56, or 22-71 of SEQ ID NO:4). In yet other embodiments, the PRKC fusion protein is an ADCY9:PRKCB fusion protein comprising an amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:6 or a fragment thereof (e.g., amino acids 560-569, 555-574, or 540-589 of SEQ ID NO:6). In some embodiments the PRKC fusion protein is an SPNS1:PRKCB fusion protein comprising an amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:8 or a fragment thereof (e.g., amino acids 98-107, 93-112, or 78-127 of SEQ ID NO:8).

[0042] In certain embodiments, the PRKC fusion protein includes a functional kinase domain. In some embodiments, the PRKC fusion protein comprises elevated PRKC activity as compared with wild type PRKC activity (e.g., in a cancer cell, a non-cancer cell adjacent to the cancer cell, or a non-cancer cell from a control sample, such as a cancer free subject). In some embodiments, the PRKC fusion protein removes all or a portion of the regulatory domain of PRKC, such as, e.g., the autoinhibitory pseudosubstrate segment. In such embodiments, the PRKC fusion protein comprises constitutive kinase activity, e.g., has acitivty without upstream signaling or binding of a ligand or second messenger, such as, e.g., DAG or Ca^{2+}. In one exemplary embodiment, the PRKC fusion protein is an IGF2BP3:PRKCA fusion and includes a PRKC serine/threonine kinase domain or a functional fragment thereof. In other exemplary embodiments, the PRKC fusion protein is a TANC2:PRKCA fusion and includes a PRKC serine/threonine kinase domain or a functional fragment thereof. In some exemplary

embodiments, the PRKC fusion protein is an ADCY9:PRKCB fusion and includes a PRKC serine/threonine kinase domain or a functional fragment thereof. In yet other exemplary embodiments, the PRKC fusion protein is an SPNS1:PRKCB fusion and includes a PRKC serine/threonine kinase domain or a functional fragment thereof.

[0043] In another embodiment, the PRKC fusion protein or fragment is a peptide, e.g., an immunogenic peptide or protein, that contains a fusion junction with a heterologous protein as described herein. Such immunogenic peptides or proteins can be used for vaccine preparation for use in the treatment or prevention of cancers caused by or exacerbated by PRKC gene fusions and PRKC fusion proteins. In other embodiments, such immunogenic peptides or proteins can be used to raise antibodies specific to the fusion protein. In some embodiments, the PRKC fusion protein is present in combination with or is further conjugated to one or more adjuvant(s) or immunogen(s), e.g., a protein capable of enhancing an immune response to the PRKC fusion protein (e.g., a hapten, a toxoid, etc.). In some embodiments, the PRKC fusion protein is an IGF2BP3:PRKCA, TANC2:PRKCA, ADCY9:PRKCB, or SPNS1:PRKCB fusion protein. In some embodiments, the PRKC fusion protein comprises the fusion junction of SEQ ID NO:2, 4, 6, or 8.

[0044] Thus, another aspect of the invention provides an antibody that binds to a PRKC fusion protein (such as, e.g., an IGF2BP3:PRKCA, a TANC2:PRKCA, an ADCY9:PRKCB, or an SPNS1:PRKCB fusion protein) or a fragment thereof. In certain embodiments, the antibody recognizes a PRKC fusion protein but does not recognize wild type PRKC or the wild type fusion partner (such as, e.g., IGF2BP3, TANC2, ADCY9, or SPNS1). In some embodiments, the antibody binds to an epitope comprising the junction between PRKC and the fusion partner (e.g., the junction of IGF2BP3:PRKCA, TANC2:PRKCA, ADCY9:PRKCB, or SPNS1:PRKCB). In one embodiment, the antibody binds to an IGF2BP3:PRKCA fusion protein having the amino acid sequence of SEQ ID NO:2 or a fragment thereof, such as, e.g., amino acids 397-406, 392-411, or 377-426 of SEQ ID NO:2. In other embodiments, the the antibody binds to a TANC2:PRKCA fusion protein having the amino acid sequence of SEQ ID NO:4 or a fragment thereof, such as, e.g., amino acids 42-51, 37-56, or 22-71 of SEQ ID NO:4. In some embodiments, the the antibody binds to an ADCY9:PRKCB fusion protein having the amino acid sequence of SEQ ID NO:6 or a fragment thereof, such as, e.g., amino acids 560-569, 555-574, or 540-589 of SEQ ID NO:6. In other embodiments, the the antibody binds to an SPNS1:PRKCB fusion protein having the amino acid sequence of SEQ ID NO:8 or a fragment thereof, such as, e.g., amino acids 98-107, 93-112, or 78-127 of SEQ ID NO:8.

[0045] In certain embodiments, the antibodies of the invention inhibit and/or neutralize the biological activity of the PRKC fusion protein, and more specifically, in some embodiments, the kinase activity of the PRKC fusion protein. In other embodiments, the antibodies may be used to detect a PRKC fusion protein or to diagnose a patient suffering from a disease or disorder associated with the expression of a PRKC fusion protein.

Detection and Diagnostic Methods

[0046] In another aspect, the invention provides a method of detecting the presence of a PRKC gene fusion or fusion protein, such as, e.g., an IGF2BP3:PRKCA, a TANC2:PRKCA, an ADCY9:PRKCB, or an SPNS1:PRKCB fusion as described herein. The presence of a PRKC fusion indicates that the mammal providing the biological sample suffers from or is at risk of developing a disorder mediated by aberrant (e.g., constitutive) PRKC activity or expression, or overexpression of PRKC, such as, e.g., a cancer. The presence of a PRKC fusion may also indicate that the disorder is treatable with a PRKC inhibitor, such as, e.g., a kinase inhibitor or an antibody specific to PRKC, or a PRKC fusion inhibitor. In some embodiments, the cancer is lung cancer. In some embodiments, the cancer is lung squamous cell carcinoma. In some embodiments, the cancer is lung adenocarcinoma. In some embodiments, the PRKC fusion present in the sample is IGF2BP3:PRKCA, TANC2:PRKCA, or ADCY9:PRKCB and the cancer to be treated is lung squamous cell carcinoma. In other embodiments, the PRKC fusion present in the sample is SPNS1:PRKCB and the cancer to be treated is lung adenocarcinoma.

[0047] In one embodiment, the PRKC fusion detected is a nucleic acid molecule or a polypeptide. The method includes detecting whether a PRKC fusion nucleic acid molecule or polypeptide is present in a cell (e.g., a circulating cell or a cancer cell), a tissue (e.g., a tumor), or a sample, e.g., a tumor sample, from a subject. In one embodiment, the sample is a nucleic acid sample. In one embodiment, the nucleic acid sample comprises DNA, e.g., genomic DNA or cDNA, or RNA, e.g., mRNA. In other embodiments, the sample is a protein sample.

[0048] The sample can be chosen from one or more sample types, such as, for example, tissue, e.g., cancerous tissue (e.g., a tissue biopsy), whole blood, serum, plasma, buccal scrape, sputum, saliva, cerebrospinal fluid, urine, stool, circulating tumor cells, circulating nucleic acids, or bone marrow.

I. Methods for Detecting Gene Fusions

[0049] In certain embodiments, the sample is acquired from a subject having or at risk of having a cancer (e.g., a patient), or alternatively, the method further includes acquiring a sample from the subject. In some embodiments, the PRKC fusion is detected in a nucleic acid molecule by a method chosen from one or more of: e.g., nucleic acid hybridization assay (e.g. in situ hybridization, comparative genomic hybridization, microarray, Southern blot, northern blot), amplification-based assays (e.g., PCR, PCR-RFLP assay, or real-time PCR), sequencing and genotyping (e.g. sequence-specific primers, high-performance liquid chromatography, or mass-spectrometric genotyping), and screening analysis (including metaphase cytogenetic analysis by karyotype methods.

(1) Hybridization methods

[0050] In some embodiments, the reagent hybridizes to a PRKC gene fusion, such as, e.g., nucleotides 1201-1206, 1199-1208, 1194-1213, 1179-1228, or 1154-1253 of SEQ ID NO:1. In alternate embodiments, the reagent detects the presence of nucleotides 136-144, 134-146, 129-151, 114-166, or 89-191 of SEQ ID NO:3, nucleotides 1690-1698, 1688-1700, 1683-1705, 1668-1720, or 1643-1745 of SEQ ID NO:5, or nucleotides 304-312, 302-314, 297-319, 282-334, or 257-359 of SEQ ID NO:7. In one embodiment, the method includes: contacting a nucleic acid sample, e.g., a genomic DNA sample (e.g., a chromosomal sample or a fractionated, enriched or otherwise pre-treated sample) or a gene product (e.g., mRNA, or cDNA), obtained from the subject, with a nucleic acid fragment e.g., a probe or primer as described herein (e.g., an exon-specific or a breakpoint-specific probe or primer), under conditions suitable for hybridization, and determining the presence or absence of the PRKC gene fusion, such as, e.g. IGF2BP3:PRKCA, TANC2:PRKCA, ADCY9:PRKCB, or SPNS1:PRKCB. In an alternate embodiment, the method includes the steps of obtaining a sample; exposing the sample to a nucleic acid probe which hybridizes to an mRNA or cDNA encoding a PRKC fusion protein that comprises amino acids 397-406, 392-411, or 377-426 of SEQ ID NO:2, amino acids 42-51, 37-56, or 22-71 of SEQ ID NO:4, amino acids 560-569, 555-574, or 540-589 of SEQ ID NO:6, or amino acids 98-107, 93-112, or 78-127 of SEQ ID NO:8.

[0051] Hybridization, as described throughout the specification, may be carried out under stringent conditions, e.g., medium or high stringency. See, e.g., J. Sambrook, E.F. Fritsch, and T. Maniatis, *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratory Pr; 2nd edition (1989); T. Brown, *Hybridization Analysis of DNA Blots. Current Protocols in*

Molecular Biology at 21:2.10.1–2.10.16 (2001). High stringency conditions for hybridization refer to conditions under which two nucleic acids must possess a high degree of base pair homology to each other in order to hybridize. Examples of highly stringent conditions for hybridization include hybridization in 4×sodium chloride/sodium citrate (SSC), at 65 or 70° C., or hybridization in 4×SSC plus 50% formamide at about 42 or 50° C., followed by at least one, at least two, or at least three washes in 1×SSC, at 65 or 70° C. Another example of highly stringent conditions includes hybridization in 2×SSC; 10×Denhardt solution (Fikoll 400+PEG+BSA; ratio 1:1:1); 0.1% SDS; 5 mM EDTA; 50 mM Na_2HPO_4; 250 µg/ml of herring sperm DNA; 50 µg/ml of tRNA; or 0.25 M of sodium phosphate buffer, pH 7.2; 1 mM EDTA7% SDS at 60°C; followed by washing 2×SSC, 0.1% SDS at 60° C.

[0052] The nucleic acid fragments can be detectably labeled with, e.g., a radiolabel, a fluorescent label, a bioluminescent label, a chemiluminescent label, an enzyme label, a binding pair label (e.g., biotin/streptavidin), an antigen label, or can include an affinity tag, or identifier (e.g., an adaptor, barcode or other sequence identifier). Labeled or unlabeled nucleic acids and/or nucleic acid fragments may be used in reagents for detecting, capturing, or isolating PRKC gene fusions. Labeled or unlabeled nucleic acids and/or nucleic acid fragments may be used in reagents for detecting, capturing, and/or isolating PRKC gene fusions, such as, e.g., IGF2BP3:PRKCA (e.g., all or part of SEQ ID NO: 1), TANC2:PRKCA (e.g., all or part of SEQ ID NO:3), ADCY9:PRKCB (e.g., all or part of SEQ ID NO:5), or SPNS1:PRKCB (e.g., all or part of SEQ ID NO:7). In some embodiments, the labeled reagent can be detected using, e.g., autoradiography, microscopy (e.g., brightfield, fluorescence, or electron microscopy), enzyme-linked immunosorbent assay (ELISA), or immunohistochemistry.

[0053] In some embodiments, the method comprises performing chromosome in situ hybridization with chromosomal DNA from a biological sample to detect the presence of a PRKC gene fusion (such as, e.g., IGF2BP3:PRKCA, TANC2:PRKCA, ADCY9:PRKCB, or SPNS1:PRKCB, as disclosed herein). In some embodiments, the chromosome in situ hybridization comprises the steps of: providing a chromosome (e.g., interphase or metaphase chromosome) preparation (e.g., by attaching the chromosomes to a substrate (e.g., glass)); denaturing the chromosomal DNA (e.g., by exposure to formamide) to separate the double strands of the polynucleotides from each other; exposing the nucleic acid probe to the chromosomes under conditions to allow hybridization of the probe to the target DNA; removing unhybridized or non-specifically hybridized probes by washing; and detecting the hybridization of the probe with the target DNA. In some embodiments, the chromosome in situ

hybridization is fluorescence in situ hybridization (FISH). In some embodiments, the probe is labeled directly by a fluorescent label, or indirectly by incorporation of a nucleotide containing a tag or reporter molecule (e.g., biotin, digoxigenin, or hapten) which after hybridization to the target DNA is then bound by fluorescently labeled affinity molecule (e.g., an antibody or streptavidin). In some embodiments, the hybridization of the probe with the target DNA in FISH can be visualized using a fluorescence microscope.

[0054] In other embodiments, the method comprises performing Southern blot with DNA polynucleotides from a biological sample to detect the presence of a PRKC gene fusion (such as, e.g., IGF2BP3:PRKCA, TANC2:PRKCA, ADCY9:PRKCB, or SPNS1:PRKCB, as disclosed herein). In some embodiments, the Southern blot comprises the steps of: optionally fragmenting the polynucleotides into smaller sizes by restriction endonucleases; separating the polynucleotides by gel electrophoresis; denaturing the polynucleotides (e.g., by heat or alkali treatment) to separate the double strands of the polynucleotides from each other; transferring the polynucleotides from the gel to a membrane (e.g., a nylon or nitrocellulose membrane); immobilizing the polynucleotides to the membrane (e.g., by UV light or heat); exposing the nucleic acid probe to the polynucleotides under conditions to allow hybridization of the probe to the target DNA; removing unhybridized or non-specifically hybridized probes by washing; and detecting the hybridization of the probe with the target DNA.

(2) Amplification-based assays

[0055] In certain embodiments, the method of detecting the presence of a PRKC gene fusion, comprises (a) performing a PCR amplification reaction with polynucleotides from a biological sample, wherein the amplification reaction utilizes a pair of primers which will amplify at least a fragment of the PRKC gene fusion, wherein the fragment comprises the fusion junction, wherein the first primer is in sense orientation and the second primer is in antisense orientation; and (b) detecting an amplification product, wherein the presence of the amplification product is indicative of the presence of a PRKC fusion polynucleotide in the sample. In specific exemplary embodiments, the PRKC gene fusion is IGF2BP3:PRKCA, such as, e.g., the gene fusion of SEQ ID NO: 1 or a fragment thereof comprising nucleotides 1201-1206, 1199-1208, 1194-1213, 1179-1228, or 1154-1253 of SEQ ID NO:1 In other exemplary embodiments, the gene fusion is TANC2:PRKCA such as, e.g. the gene fusion of SEQ ID NO:3 or a fragment thereof comprising nucleotides 136-144, 134-146, 129-151, 114-166, or 89-191 of SEQ ID NO:3. In other exemplary embodiments, the gene fusion is ADCY9:PRKCB such as, e.g. the gene fusion of SEQ ID NO:5 or a fragment thereof comprising nucleotides 1690-1698, 1688-

1700, 1683-1705, 1668-1720, or 1643-1745 of SEQ ID NO:5. In certain exemplary embodiments, the gene fusion is SPNS1:PRKCB such as, e.g. the gene fusion of SEQ ID NO:7 or a fragment thereof comprising nucleotides 304-312, 302-314, 297-319, 282-334, or 257-359 of SEQ ID NO:7.

[0056] In some embodiments, step (a) of performing a PCR amplification reaction comprises: (i) providing a reaction mixture comprising the polynucleotides (e.g., DNA or cDNA) from the biological sample, the pair of primers which will amplify at least a fragment of the PRKC gene fusion wherein the first primer is complementary to a sequence on the first strand of the polynucleotides and the second primer is complementary to a sequence on the second strand of the polynucleotides, a DNA polymerase, and a plurality of free nucleotides comprising adenine, thymine, cytosine, and guanine (dNTPs); (ii) heating the reaction mixture to a first predetermined temperature for a first predetermined time to separate the double strands of the polynucleotides from each other; (iii) cooling the reaction mixture to a second predetermined temperature for a second predetermined time under conditions to allow the first and second primers to hybridize with their complementary sequences on the first and second strands of the polynucleotides, and to allow the DNA polymerase to extend the primers; and (iv) repeating steps (ii) and (iii) for a predetermined number of cycles (e.g., 10, 15, 20, 25, 30, 35, 40, 45, or 50 cycles).

[0057] In some embodiments, the polynucleotides from the biological sample comprise RNA, and the method further comprises performing a RT-PCR amplification reaction with the RNA to synthesize cDNA as the template for subsequent or simultaneous PCR reactions. In some embodiments, the RT-PCR amplification reaction comprises providing a reaction mixture comprising the RNA, a primer which will amplify the RNA (e.g., a sequence-specific primer, a random primer, or oligo(dT)s), a reverse transcriptase, and dNTPs, and heating the reaction mixture to a third predetermined temperature for a third predetermined time under conditions to allow the reverse transcriptase to extend the primer.

(3) Sequencing and Genotyping

[0058] Another method for determining the presence of a PRKC gene fusion molecule (such as, e.g., IGF2BP3:PRKCA, TANC2:PRKCA, ADCY9:PRKCB, or SPNS1:PRKCB, as disclosed herein) includes: sequencing a portion of the nucleic acid molecule (e.g., sequencing the portion of the nucleic acid molecule that comprises the fusion junction of a PRKC gene fusion), thereby determining that the PRKC gene fusion is present in the nucleic acid molecule. Optionally, the sequence acquired is compared to a reference sequence, or a wild type reference

sequence. In one embodiment, the sequence is determined by a next generation sequencing method. In some embodiments, the sequencing is automated and/or high-throughput sequencing. The method can further include acquiring, e.g., directly or indirectly acquiring, a sample, e.g., a tumor or cancer sample, from a patient.

[0059] In some embodiments, the sequencing comprises chain terminator sequencing (Sanger sequencing), comprising: providing a reaction mixture comprising a nucleic acid molecule from a biological sample, a primer complementary to a region of the template nucleic acid molecule, a DNA polymerase, a plurality of free nucleotides comprising adenine, thymine, cytosine, and guanine (dNTPs), and at least one chain terminating nucleotide (e.g., at least one di-deoxynucleotide (ddNTPs) chosen from ddATP, ddTTP, ddCTP, and ddGTP), wherein the at least one chain terminating nucleotide is present in a low concentration so that chain termination occurs randomly at any one of the positions containing the corresponding base on the DNA strand; annealing the primer to a single strand of the nucleic acid molecule; extending the primer to allow incorporation of the chain terminating nucleotide by the DNA polymerase to produce a series of DNA fragments that are terminated at positions where that particular nucleotide is used; separating the polynucleotides by electrophoresis (e.g., gel or capillary electrophoresis); and determining the nucleotide order of the template nucleic acid molecule based on the positions of chain termination on the DNA fragments. In some embodiments, the sequencing is carried out with four separate base-specific reactions, wherein the primer or the chain terminating nucleotide in each reaction is labeled with a separate fluorescent label. In other embodiments, the sequencing is carried out in a single reaction, wherein the four chain terminating nucleotides mixed in the single reaction are each labeled with a separate fluorescent label.

[0060] In some embodiments, the sequencing comprises pyrosequencing (sequencing by synthesis), comprising: (i) providing a reaction mixture comprising a nucleic acid molecule from a biological sample, a primer complementary to a region of the template nucleic acid molecule, a DNA polymerase, a first enzyme capable of converting pyrophosphate into ATP, and a second enzyme capable using ATP to generates a detectable signal (e.g., a chemiluminescent signal, such as light) in an amount that is proportional to the amount of ATP; (ii) annealing the primer to a single strand of the nucleic acid molecule; (iii) adding one of the four free nucleotides (dNTPs) to allow incorporation of the correct, complementary dNTP onto the template by the DNA polymerase and release of pyrophosphate stoichiometrically; (iv) converting the released pyrophosphate to ATP by the first enzyme; (v) generating a detectable

signal by the second enzyme using the ATP; (vi) detecting the generated signal and analyzing the amount of signal generated in a pyrogram; (vii) removing the unincorporated nucleotides; and (viii) repeating steps (iii) to (vii). The method allows sequencing of a single strand of DNA, one base pair at a time, and detecting which base was actually added at each step. The solutions of each type of nucleotides are sequentially added and removed from the reaction. Light is produced only when the nucleotide solution complements the first unpaired base of the template. The order of solutions which produce detectable signals allows the determination of the sequence of the template.

[0061] In some embodiments, the method of determining the presence of a PRKC fusion (such as, e.g., IGF2BP3:PRKCA, TANC2:PRKCA, ADCY9:PRKCB, or SPNS1:PRKCB, as disclosed herein) comprises analyzing a nucleic acid sample (e.g., DNA, cDNA, or RNA, or an amplification product thereof) by HPLC. The method may comprise: passing a pressurized liquid solution containing the sample through a column filled with a sorbent, wherein the nucleic acid or protein components in the sample interact differently with the sorbent, causing different flow rates for the different components; separating the components as they flow out the column at different flow rates. In some embodiments, the HPLC is chosen from, e.g., reverse-phase HPLC, size exclusion HPLC, ion-exchange HPLC, and bioaffinity HPLC.

[0062] In some embodiments, the method of determining the presence of a PRKC fusion (such as, e.g., IGF2BP3:PRKCA, TANC2:PRKCA, ADCY9:PRKCB, or SPNS1:PRKCB, as disclosed herein) comprises analyzing a nucleic acid sample (e.g., DNA, cDNA, or RNA, or an amplification product thereof) by mass spectrometry. The method may comprise: ionizing the components in the sample (e.g., by chemical or electron ionization); accelerating and subjecting the ionized components to an electric or magnetic field; separating the ionized components based on their mass-to-charge ratios; and detecting the separated components by a detector capable of detecting charged particles (e.g., by an electron multiplier).

II. Methods for Detecting Fusion Proteins

[0063] Another aspect of the invention provides a method of detecting the presence of a PRKC fusion protein in a mammal. The method comprises the steps of obtaining a biological sample of a mammal (such as, e.g., a human cancer), and exposing that sample to at least one reagent that detects a PRKC fusion protein (e.g., an antibody that recognizes the PRKC fusion but does not recognize the wild type PRKC or the wild type fusion partner) to determine whether a PRKC fusion protein is present in the biological sample. The detection of a PRKC fusion protein indicates the presence of a mutant PRKC in the mammal (such as, e.g., in the human

cancer). In some embodiments, the PRKC fusion protein comprises an amino acid sequence having at least 85%, 90%, 95%, 97%, 98%, or 99% identity with an amino acid sequence of all or part of any one of SEQ ID NOs 2, 4, 6, or 8 or fragments thereof that include the fusion junction. In some embodiments the cancer is lung cancer. In some embodiments, the cancer is lung squamous cell carcinoma. In some embodiments, the cancer is lung adenocarcinoma.

[0064] In some embodiments, the reagent that detects a PRKC fusion protein can be detectably labeled with, e.g., a radiolabel, a fluorescent label, a bioluminescent label, a chemiluminescent label, an enzyme label, a binding pair label (e.g., biotin/streptavidin), an antigen label, or can include an affinity tag or identifier (e.g., an adaptor, barcode or other sequence identifier). In some embodiments, the labeled reagent can be detected using, e.g., autoradiography, microscopy (e.g., brightfield, fluorescence, or electron microscopy), ELISA, or immunohistochemistry. In some embodiments, the PRKC fusion protein is detected in a biological sample by a method chosen from one or more of: antibody-based detection (e.g., western blot, ELISA, immunohistochemistry), size-based detection methods (e.g., HPLC or mass spectrometry), or protein sequencing.

(1) Antibody-based detection

[0065] In some embodiments, the method comprises performing a western blot with polypeptides from a biological sample to detect the presence of a PRKC fusion protein (such as, e.g., IGF2BP3:PRKCA, TANC2:PRKCA, ADCY9:PRKCB, or SPNS1:PRKCB, as disclosed herein). In some embodiments, the western blot comprises the steps of: separating the polypeptides by gel electrophoresis; transferring the polypeptides from the gel to a membrane (e.g., a nitrocellulose or polyvinylidene difluoride (PVDF) membrane); blocking the membrane to prevent nonspecific binding by incubating the membrane in a dilute solution of protein (e.g., 3-5% bovine serum albumin (BSA) or non-fat dry milk in Tris-Buffered Saline (TBS) or I-Block, with a minute percentage (e.g., 0.1%) of detergent, such as, e.g., Tween 20 or Triton X-100); exposing the polypeptides to at least one reagent that detects a PRKC fusion protein (e.g., an antibody that recognizes the PRKC fusion but does not recognize the wild type PRKC or the wild type fusion partner); removing unbound or non-specifically bound reagent by washing; and detecting the binding of the reagent with the target protein. In some embodiments, the method comprises two-step detection: exposing the polypeptides to a primary antibody that specifically binds to a PRKC fusion protein; removing unbound or non-specifically bound primary antibody by washing; exposing the polypeptides to a secondary antibody that recognizes the primary antibody; removing unbound or non-specifically bound

secondary antibody by washing; and detecting the binding of the secondary antibody. In some embodiments, the reagent that detects a PRKC fusion protein (e.g., the fusion specific antibody, or the secondary antibody) is directly labeled for detection. In other embodiments, the reagent is linked to an enzyme, and the method further comprises adding a substrate of the enzyme to the membrane; and developing the membrane by detecting a detectable signal produced by the reaction between the enzyme and the substrate. For example, the reagent may be linked with horseradish peroxidase to cleave a chemiluminescent agent as a substrate, producing luminescence in proportion to the amount of the target protein for detection.

[0066] In some embodiments, the method comprises performing ELISA with polypeptides from a biological sample to detect the presence of a PRKC fusion protein (such as, e.g., IGF2BP3:PRKCA, TANC2:PRKCA, ADCY9:PRKCB, or SPNS1:PRKCB, as disclosed herein). In some embodiments, the ELISA is chosen from, e.g., direct ELISA, indirect ELISA, sandwich ELISA, and competitive ELISA.

[0067] In one embodiment, the direct ELISA comprises the steps of: attaching polypeptides from a biological sample to a surface; blocking the surface to prevent nonspecific binding by incubating the surface in a dilute solution of protein; exposing the polypeptides to an antibody that specifically binds to a PRKC fusion protein (e.g., an antibody that recognizes the PRKC fusion (such as, e.g., IGF2BP3:PRKCA, TANC2:PRKCA, ADCY9:PRKCB, or SPNS1:PRKCB, as disclosed herein) but does not recognize the wild type PRKC or the wild type fusion partner); removing unbound or non-specifically bound antibody by washing; and detecting the binding of the antibody with the target protein. In some embodiments, the antibody is directly labeled for detection. In other embodiments, the antibody is linked to an enzyme, and the method further comprises adding a substrate of the enzyme; and detecting a detectable signal produced by the reaction between the enzyme and the substrate.

[0068] In another embodiment, the indirect ELISA comprises the steps of: attaching polypeptides from a biological sample to a surface; blocking the surface to prevent nonspecific binding by incubating the surface in a dilute solution of protein; exposing the polypeptides to a primary antibody that specifically binds to a PRKC fusion protein (such as, e.g., IGF2BP3:PRKCA, TANC2:PRKCA, ADCY9:PRKCB, or SPNS1:PRKCB, as disclosed herein); removing unbound or non-specifically bound primary antibody by washing; exposing the polypeptides to a secondary antibody that recognizes the primary antibody; removing unbound or non-specifically bound secondary antibody by washing; and detecting the binding of the secondary antibody. In some embodiments, the secondary antibody is directly labeled

for detection. In other embodiments, the secondary antibody is linked to an enzyme, and the method further comprises adding a substrate of the enzyme; and detecting a detectable signal produced by the reaction between the enzyme and the substrate.

[0069] In some embodiments, the method comprises performing immunohistochemistry with polypeptides from a biological sample to detect the presence of a PRKC fusion protein (such as, e.g., IGF2BP3:PRKCA, TANC2:PRKCA, ADCY9:PRKCB, or SPNS1:PRKCB, as disclosed herein). In some embodiments, the immunohistochemistry comprises the steps of: fixing a cell or a tissue section (e.g., by paraformaldehyde or formalin treatment); permeabilizing the cell or tissue section to allow target accessibility; blocking the cell or tissue section to prevent nonspecific binding; exposing the cell or tissue section to at least one reagent that detects a PRKC fusion protein (e.g., an antibody that recognizes the PRKC fusion but does not recognize the wild type PRKC or the wild type fusion partner); removing unbound or non-specifically bound reagent by washing; and detecting the binding of the reagent with the target protein. In some embodiments, the reagent is directly labeled for detection. In other embodiments, the reagent is linked to an enzyme, and the method further comprises adding a substrate of the enzyme; and detecting a detectable signal produced by the reaction between the enzyme and the substrate. In some embodiments, the immunohistochemistry may comprise the two-step detection as in the indirect ELISA.

(2) Size-based detection methods

[0070] In some embodiments, the method of determining the presence of a PRKC fusion (such as, e.g., IGF2BP3:PRKCA, TANC2:PRKCA, ADCY9:PRKCB, or SPNS1:PRKCB, as disclosed herein) comprises analyzing a protein sample by HPLC. The method may comprise: passing a pressurized liquid solution containing the sample through a column filled with a sorbent, wherein the nucleic acid or protein components in the sample interact differently with the sorbent, causing different flow rates for the different components; separating the components as they flow out the column at different flow rates. In some embodiments, the HPLC is chosen from, e.g., reverse-phase HPLC, size exclusion HPLC, ion-exchange HPLC, and bioaffinity HPLC.

[0071] In some embodiments, the method of determining the presence of a PRKC fusion (such as, e.g., IGF2BP3:PRKCA, TANC2:PRKCA, ADCY9:PRKCB, or SPNS1:PRKCB, as disclosed herein) comprises analyzing a protein sample by mass spectrometry. The method may comprise: ionizing the components in the sample (e.g., by chemical or electron ionization); accelerating and subjecting the ionized components to an electric or magnetic field;

separating the ionized components based on their mass-to-charge ratios; and detecting the separated components by a detector capable of detecting charged particles (e.g., by an electron multiplier).

[0072] Detection of a PRKC gene fusion or a PRKC fusion protein in a patient can lead to assignment of the patient to the newly identified patient population that bears the PRKC fusion. Because this patient population can suffer from or be susceptible to a disorder associated with an aberrant (e.g., constitutive) PRKC activity or expression, or overexpression of PRKC, detection of the PRKC fusion can also lead to diagnosis of such disorder. Thus, a further aspect of the invention provides a method of stratifying a patient population (e.g., assigning a patient, to a group or class) and/or diagnosing a patient, comprising: obtaining a biological sample from the patient, contacting the sample with at least one reagent that detects a PRKC gene fusion or a PRKC fusion protein to determine whether a PRKC fusion is present in the biological sample. The detection of a PRKC fusion indicates that the patient belongs to the newly identified patient population that bears the PRKC fusion, and/or the presence of a disorder associated with aberrant (e.g., constitutive) PRKC activity or expression, or overexpression of PRKC, such as, e.g., a cancer. The detection of a PRKC fusion also identifies a new subtype of cancer, which is characterized by the presence of the PRKC fusion. In some embodiments, the cancer is lung cancer. In some embodiments, the caner is lung squamous cell carcinoma or lung adenocarcinoma. In certain embodiments, the PRKC fusion is IGF2BP3:PRKCA, TANC2:PRKCA, ADCY9:PRKCB, or SPNS1:PRKCB. In some embodiments, the IGF2BP3:PRKCA fusion has all or part of the nucleotide and/or amino acid sequence (such as, e.g., the fusion junction) set forth in SEQ ID NO:1 and SEQ ID NO:2, respectively. In some embodiments, the TANC2:PRKCA fusion has all or part of the nucleotide and/or amino acid sequence (such as, e.g., the fusion junction) set forth in SEQ ID NO:3 and SEQ ID NO:4, respectively. In some embodiments, the ADCY9:PRKCB fusion has all or part of the nucleotide and/or amino acid sequence (such as, e.g., the fusion junction) set forth in SEQ ID NO:5 and SEQ ID NO:6, respectively. In some embodiments, the SPNS1:PRKCB fusion has all or part of the nucleotide and/or amino acid sequence (such as, e.g., the fusion junction) set forth in SEQ ID NO:7 and SEQ ID NO:8, respectively.

[0073] In some embodiments, the PRKC gene fusion or PRKC fusion protein is detected prior to initiating, during, or after, a treatment of a patient with, e.g., a PRKC inhibitor or a PRKC fusion inhibitor. In one embodiment, the PRKC gene fusion or PRKC fusion protein is detected at the time the patient is diagnosed with a cancer. In other embodiment, the PRKC

fusion is detected at a pre-determined interval, e.g., a first point in time and at least at a subsequent point in time. In certain embodiments, in response to detection of a PRKC fusion, such as, e.g., IGF2BP3:PRKCA, TANC2:PRKCA, ADCY9:PRKCB, or SPNS1:PRKCB, the method further includes one or more of:

(1) stratifying a patient population (e.g., assigning a patient, to a group or class);

(2) identifying or selecting the patient as likely or unlikely to respond to a treatment, e.g., a PRKC inhibitor treatment (e.g., a kinase inhibitor treatment or an anti-PRKC antibody treatment), or a PRKC fusion inhibitor treatment as described herein;

(3) selecting a treatment regimen, e.g., administering or not administering a preselected therapeutic agent, such as, e.g., a PRKC inhibitor, or a PRKC fusion inhibitor;

(4) prognosticating the time course of the disease in the patient (e.g., evaluating the likelihood of increased or decreased patient survival); or

(5) monitoring the effectiveness of treatment (e.g., by detecting a reduction in the level of PRKC gene fusion or fusion protein in a patient sample).

[0074] In certain embodiments, upon detection of a PRKC gene fusion or PRKC fusion protein in a patient's biological sample, the patient is identified as likely to respond to a treatment that comprises a PRKC inhibitor, or a PRKC fusion inhibitor. In some embodiments, the PRKC fusion detected is an IGF2BP3:PRKCA fusion. In alternate embodiments, the PRKC fusion detected is a TANC2:PRKCA fusion. In some embodiments, the PRKC fusion detected is an ADCY9:PRKCB fusion. In some embodiments the PRKC fusion detected is an SPNS1:PRKCB fusion.

[0075] A further aspect of the invention provides a method of selecting a treatment option by detecting a PRKC fusion. The method comprises obtaining a biological sample from a patient and exposing the sample to at least one reagent that detects a PRKC gene fusion or fusion protein to determine whether a PRKC fusion is present in the biological sample. The detection of the PRKC fusion indicates the likelihood of the patient responding to treatment with a PRKC inhibitor, or a PRKC fusion inhibitor. The method may be augmented or personalized by evaluating the effect of a variety of PRKC, or PRKC fusion inhibitors on the biological sample shown to contain a PRKC fusion to determine the most appropriate inhibitor to administer. In certain embodiments, the PRKC fusion is IGF2BP3:PRKCA, TANC2:PRKCA, ADCY9:PRKCB, or SPNS1:PRKCB. In some embodiments, the IGF2BP3:PRKCA fusion has all or part of the nucleotide and/or amino acid sequence (such as, e.g., the fusion junction) set

forth in SEQ ID NO:1 and SEQ ID NO:2, respectively. In some embodiments, the TANC2:PRKCA fusion has all or part of the nucleotide and/or amino acid sequence (such as, e.g., the fusion junction) set forth in SEQ ID NO:3 and SEQ ID NO:4, respectively. In some embodiments, the ADCY9:PRKCB fusion has all or part of the nucleotide and/or amino acid sequence (such as, e.g., the fusion junction) set forth in SEQ ID NO:5 and SEQ ID NO:6, respectively. In some embodiments, the SPNS1:PRKCB fusion has all or part of the nucleotide and/or amino acid sequence (such as, e.g., the fusion junction) set forth in SEQ ID NO:7 and SEQ ID NO:8, respectively.

Methods of Treatment

[0076] Alternatively, or in combination with the detection and diagnostic methods described herein, the invention provides method for treating the newly identified patient population and the new PRKC fusion cancer subtype, which are characterized by the presence of a PRKC fusion. The patient population and cancer subtype can be associated with or predict the onset of a condition mediated by aberrant (e.g., constitutive) PRKC activity or expression, or overexpression of PRKC, such as, e.g., a cancer or a tumor harboring a PRKC fusion. The methods comprise administering a therapeutic agent, e.g., a PRKC inhibitor, such as e.g., a kinase inhibitor or an antibody specific to PRKC; or a PRKC fusion inhibitor, i.e., an inhibitor that blocks the activity of the PRKC fusion but not wild type PRKC or wild type fusion partner (such as, e.g., IGF2BP3, TANC2, ADCY9, or SPNS1), such as, e.g., an antibody specific to an IGF2BP3:PRKCA, a TANC2:PRKCA, an ADCY9:PRKCB, or an SPNS1:PRKCB fusion protein, e.g., any one of the antibodies described above; an agent that mimics the pseudosubstrate segment and binds the substrate-binding cavity in the catalytic domain of PRKC, keeping the enzyme inactive; or an RNA inhibitor that recognizes PRKC or the fusion junction of a PRKC gene fusion, including but not limited to siRNA, dsRNA, shRNA, or any other antisense nucleic acid inhibitor, alone or in combination with e.g., other chemotherapeutic agents or procedures, in an amount sufficient to treat a condition mediated by aberrant (e.g., constitutive) PRKC activity or expression, or overexpression of PRKC by one or more of the following: e.g., impeding growth of a cancer, causing a cancer to shrink by weight or volume, extending the expected survival time of the patient, inhibiting tumor growth, reducing tumor mass, reducing size or number of metastatic lesions, inhibiting the development of new metastatic lesions, prolonging survival, prolonging progression- free survival, prolonging time to progression, and/or enhancing quality of life.

[0077] As used herein, and unless otherwise specified, a "therapeutically effective amount" of a compound is an amount sufficient to provide a therapeutic benefit in the treatment or management of a condition mediated by aberrant PRKC expression or activity, or overexpression of PRKC, such as, delaying or minimizing one or more symptoms associated with a cancer or a tumor harboring a PRKC fusion (such as, e.g., IGF2BP3:PRKCA, TANC2:PRKCA, ADCY9:PRKCB, or SPNS1:PRKCB, as described herein). A therapeutically effective amount of a compound means an amount of therapeutic agent, alone or in combination with other therapeutic agents, which provides a therapeutic benefit in the treatment or management of the cancer. The term "therapeutically effective amount" can encompass an amount that improves overall therapy, reduces or avoids symptoms or causes of the condition mediated by aberrant PRKC expression or activity or overexpression of PRKC, or enhances the therapeutic efficacy of another therapeutic agent.

[0078] In certain embodiments, the therapeutic agent is a PRKC inhibitor, or a PRKC fusion inhibitor. In some embodiments, the therapeutic agent is a PRKC inhibitor, e.g., a compound, biological or chemical, which inhibits, directly or indirectly, the expression and/or activity of PRKC. For example, the PRKC inhibitors may be an antibody (such as, e.g., antibodies specific to PRKC, e.g., PRKCA or PRKCB) or a small molecule inhibitor. In some embodiments, the inhibitors may act directly on PRKC itself, modify the activity of PRKC, or inhibit the expression of PRKC. In other embodiments, the inhibitors may indirectly inhibit PRKC activity by inhibiting the activity of proteins or molecules other than PRKC itself. For example, the inhibitors may modulate the activity of regulatory kinases that phosphorylate or dephosphorylate PRKC, interfere with binding of ligands, or inhibit the activity of interacting or downstream proteins or molecules. Exemplary PRKC inhibitors also include kinase inhibitors, which may be a pan-kinase inhibitor with activity against several different kinases or a kinase-specific inhibitor (e.g., a serine/threonine kinase inhibitor, or an inhibitor specific to the kinase activity of PRKC). In some embodiments, the PRKC inhibitor is a PRKC-specific inhibitor.

[0079] In other embodiments, the therapeutic agent is a PRKC fusion inhibitor, e.g., a PRKC gene fusion inhibitor or a PRKC protein fusion inhibitor. In some embodiments, the PRKC fusion inhibitor inhibits the expression of nucleic acid encoding a PRKC fusion. Examples of PRKC fusion inhibitors include nucleic acid molecules, for example, antisense molecules, ribozymes, RNAi, or triple helix molecules, that hybridize to a nucleic acid encoding a PRKC fusion (e.g., genomic DNA, or mRNA), or a transcription and/or translation regulatory region,

and blocks or reduces mRNA and/or protein expression of a PRKC fusion. In some embodiments, the antisense molecule recognizes the PRKC fusion junction, or an RNA inhibitor, including but not limited to siRNA, dsRNA, and short-hairpin RNA specific to the PRKC fusion junction. In some embodiments, the PRKC fusion is IGF2BP3:PRKCA, TANC2:PRKCA, ADCY9:PRKCB, or SPNS1:PRKCB. Exemplary PRKC fusion inhibitors also include an antibody that recognizes the fusion protein, and/or does not recognize the wild type fusion partners. In some embodiments, the PRKC fusion inhibitors include an antibody that recognizes IGF2BP3:PRKCA, TANC2:PRKCA, ADCY9:PRKCB, or SPNS1:PRKCB fusion protein, and/or does not recognize wild type PRKC, e.g., PRKCA or PRKCB, IGF2BP3, TANC2, ADCY9, or SPNS1. In some embodiments, the PRKC fusion protein (such as, e.g., an IGF2BP3:PRKCA fusion protein, a TANC2:PRKCA fusion protein, an ADCY9:PRKCB fusion protein, or an SPNS1:PRKCB fusion protein) is inhibited by an agent that mimics the pseudosubstrate segment and binds the substrate-binding cavity in the catalytic domain of PRKC, keeping the enzyme inactive.

[0080] In some embodiments, the patient to be treated is suffering from lung squamous cell carcinoma or lung adenocarcinoma and the method for treating the condition comprises administering to the patient a therapeutically effective amount of a PRKC inhibitor or a PRKC fusion inhibitor. In some embodiments, the patient to be treated is suffering from lung squamous cell carcinoma, and the method for treating the condition comprises administering to the patient a therapeutically effective amount of a PRKC inhibitor, or a PRKC gene fusion inhibitor or a PRKC fusion protein inhibitor as described above. In some embodiments, the patient to be treated is suffering from lung adenocarcinoma, and the method for treating the condition comprises administering to the patient a therapeutically effective amount of a PRKC inhibitor, or a PRKC gene fusion inhibitor or a PRKC fusion protein inhibitor.

Screening Methods

[0081] Therapeutic agents, such as, e.g., PRKC inhibitors, and PRKC fusion inhibitors (gene fusion and fusion protein), used in the therapeutic methods of the invention can be evaluated using the screening assays described herein. Thus, the invention provides a method of identifying an agent useful for treating a condition mediated by aberrant (e.g., constitutive) PRKC activity or expression, or overexpression of PRKC, such as, e.g., a cancer or a tumor harboring a PRKC fusion, such as, e.g., lung squamous cell carcinoma or lung adenocarcinoma, comprising contacting a cell expressing a PRKC gene fusion or PRKC fusion protein with a candidate agent and using one of the detection methods referenced above to

determine whether the expression level of the fusion is decreased or a biological function associated with the fusion is altered. In one embodiment, therapeutic agents can be evaluated in a cell-free system, e.g., a cell lysate or in a reconstituted system. In other embodiments, the therapeutic agents are evaluated in a cell in culture, e.g., a cell expressing a PRKC fusion (e.g., a mammalian cell, a tumor cell or cell line, a recombinant cell). In yet other embodiments, the therapeutic agents are evaluated in a cell in vivo (e.g., a PRKC fusion-expressing cell present in a subject, e.g., an animal subject (e.g., an in vivo animal model).

[0082] Exemplary parameters to evaluate in determining the efficacy of a therapeutic agent for treating a condition mediated by aberrant (e.g., constitutive) PRKC activity or expression, or overexpression of PRKC, such as, e.g., a cancer or a tumor harboring a PRKC fusion include one or more of:

(i) a change in binding activity, e.g., direct binding of the candidate agent to a PRKC fusion protein or a binding competition between a known ligand and the candidate agent to a PRKC fusion protein;

(ii) a change in kinase activity, e.g., phosphorylation levels of a PRKC fusion protein (e.g., an increased or decreased phosphorylateion or autophosphorylation) or a change in phosphorylation of a target of a PRKC kinase -- in certain embodiments, a change in kinase activity, e.g., phosphorylation, is detected by any of Western blot (e.g., using an anti-PRKC antibody; or a phosphor-specific antibody, detecting a shift in the molecular weight of a PRKC fusion protein), mass spectrometry, immunoprecipitation, immunohistochemistry, immunomagnetic beads, among others;

(iii) a change in an activity of a cell containing a PRKC fusion (e.g., a tumor cell or a recombinant cell), e.g., a change in proliferation, morphology, or tumorigenicity of the cell;

(iv) a change in tumor present in an animal subject, e.g., size, appearance, or proliferation of the tumor;

(v) a change in the level, e.g., expression level (transcription and/or translation), of a PRKC fusion protein or nucleic acid molecule; or

(vi) a change in an activity of a signaling pathway involving PRKC, e.g., phosphorylation or activity of an interacting or downstream target, or expression level of a target gene.

[0083] In some embodiments, the PRKC fusion is an IGF2BP3:PRKCA fusion, a TANC2:PRKCA fusion, an ADCY9:PRKCB fusion, or an SPNS1:PRKCB fusion.

[0084] In one embodiment, a change in the activity of a PRKC fusion, or interaction of a PRKC fusion with a downstream ligand detected in a cell free assay in the presence of a candidate agent indicates that the candidate agent will be effective as a therapeutic agent for treatment of a condition mediated by aberrant (e.g., constitutive) PRKC activity or expression, or overexpression of PRKC, such as, e.g., a cancer or a tumor harboring a PRKC fusion.

[0085] In other embodiments, a change in an activity of a cell expressing a PRKC fusion, such as, e.g., IGF2BP3:PRKCA, TANC2:PRKCA, ADCY9:PRKCB, or SPNS1:PRKCB, as described herein, (e.g., a mammalian cell, a tumor cell or cell line, a recombinant cell) is detected in a cell in culture. In one embodiment, the cell is a recombinant cell that is modified to express a PRKC fusion nucleic acid, e.g., is a recombinant cell transfected with a PRKC fusion nucleic acid. The transfected cell can show a change in response to the expressed PRKC fusion, e.g., increased proliferation, changes in morphology, increased tumorigenicity, and/or acquired a transformed phenotype. A change in any of the activities of the cell, e.g., the recombinant cell, in the presence of the candidate agent can be detected. For example, a decrease in one or more of: proliferation, tumorigenicity, or transformed morphology, in the presence of the candidate agent can be indicative of an inhibitor of a PRKC fusion. In other embodiments, a change in binding activity or phosphorylation of PRKC or its interacting or downstream proteins or molecules as described herein is detected.

[0086] In yet other embodiment, a change in a tumor present in an animal subject (e.g., an in vivo animal model) is detected. In one embodiment, a tumor containing animal or a xenograft comprising cells expressing a PRKC fusion (e.g., tumorigenic cells expressing a PRKC fusion) is employed. The therapeutic agents can be administered to the animal subject and a change in the tumor is evaluated. In one embodiment, the change in the tumor includes one or more of a tumor growth, tumor size, tumor burden, or survival is evaluated. A decrease in one or more of tumor growth, tumor size, tumor burden, or an increased survival is indicative that the candidate agent is an inhibitor or modulator.

[0087] In another aspect of the invention provides a method or assay for screening for agents that modulate (e.g., inhibit) the expression or activity of a PRKC fusion as described herein. The method includes contacting e.g., a PRKC fusion, or a cell expressing a PRKC fusion, with a candidate agent; and detecting a change in a parameter associated with a PRKC fusion, e.g., a change in the expression or an activity of the PRKC fusion. The method can, optionally, include comparing the treated parameter to a reference value, e.g., a control sample (e.g., comparing a parameter obtained from a sample with the candidate agent to a parameter

obtained from a sample without the candidate agent). In one embodiment, if a decrease in expression or activity of the PRKC fusion is detected, the candidate agent is identified as an inhibitor. In another embodiment, if an increase in expression or activity of the PRKC fusion is detected, the candidate agent is identified as an activator. In certain embodiments, the PRKC fusion is a PRKC gene fusion or PRKC fusion protein, such as, e.g., an IGF2BP3:PRKCA fusion, a TANC2:PRKCA fusion, an ADCY9:PRKCB fusion, or an SPNS1:PRKCB fusion.

[0088] In one embodiment, the contacting step is detected in a cell-free system, e.g., a cell lysate or in a reconstituted system. In other embodiments, the contacting step is detected in a cell in culture, e.g., a cell expressing a PRKC fusion (e.g., a mammalian cell, a tumor cell or cell line, a recombinant cell). In yet other embodiments, the contacting step is detected in a cell in vivo (e.g., a PRKC expressing cell present in a subject, e.g., an animal subject (e.g., an in vivo animal model)).

[0089] Exemplary parameters evaluated in identifying an agent that modulates the activity of a PRKC fusion, e.g., a PRKC fusion (e.g., an IGF2BP3:PRKCA fusion, a TANC2:PRKCA fusion, an ADCY9:PRKCB fusion, or an SPNS1:PRKCB fusion) include one or more of:

(i) a change in binding activity, e.g., direct binding of the candidate agent to a PRKC fusion protein; a binding competition between a known ligand and the candidate agent to a PRKC fusion protein;

(ii) a change in kinase activity, e.g., phosphorylation levels of a PRKC fusion protein (e.g., an increased or decreased phosphorylation or autophosphorylation) or a change in phosphorylation of a target of a PRKC kinase -- in certain embodiments, a change in kinase activity, e.g., phosphorylation, is detected by any of Western blot (e.g., using an anti-PRKC antibody; or a phosphor-specific antibody, detecting a shift in the molecular weight of a PRKC fusion protein), mass spectrometry, immunoprecipitation, immunohistochemistry, immunomagnetic beads, among others;

(iii) a change in an activity of a cell containing a PRKC fusion (e.g., a tumor cell or a recombinant cell), e.g., a change in proliferation, morphology or tumorigenicity of the cell;

(iv) a change in tumor present in an animal subject, e.g., size, appearance, proliferation, of the tumor;

(v) a change in the level, e.g., expression level (transcription and/or translation), of a PRKC fusion protein or nucleic acid molecule; or

(vi) a change in an activity of a signaling pathway involving PRKC, e.g., phosphorylation or activity of an interacting or downstream target, or expression level of a target gene.

Methods for Validating PRKC Fusions

[0090] PRKC gene fusions, such as, e.g., PRKC gene fusions (e.g., IGF2BP3:PRKCA gene fusion, TANC2:PRKCA gene fusion, ADCY9:PRKCB gene fusion, or SPNS1:PRKCB gene fusion) may be evaluated to ensure that the breakpoints are in-frame and can produce a protein product containing the full kinase domain, i.e., that the breakpoint occurs such that complete triplet codons are intact, and that the RNA sequence will produce a viable protein. The PRKC gene fusion can be transfected into cells to confirm that the protein is functionally active with respect to kinase activity and oncogenic activity. cDNA encoding the PRKC fusion protein can be produced by standard solid-phase DNA synthesis. Alternatively the PRKC fusion cDNA can be produced by RT-PCR using tumor mRNA extracted from samples containing the gene fusion. The DNA amplified can be subcloned into an appropriate vector and characterized by DNA sequence analysis or in vitro/in vivo expression analyses.

[0091] Expression vectors containing the PRKC gene fusion (such as, e.g., a PRKC gene fusion, e.g., an IGF2BP3:PRKCA gene fusion, a TANC2:PRKCA gene fusion, an ADCY9:PRKCB gene fusion, or an SPNS1:PRKCB gene fusion) can be introduced into host cells to thereby produce a PRKC fusion protein (such as, e.g., a PRKC fusion protein, e.g., an IGF2BP3:PRKCA fusion protein, a TANC2:PRKCA fusion protein, an ADCY9:PRKCB fusion protein, or an SPNS1:PRKCB fusion protein). The PRKC fusion protein expression vector can be a yeast expression vector, a vector for expression in insect cells, e.g., a baculovirus expression vector, or a vector suitable for expression in mammalian cells. Vector DNA can be introduced into host cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell.

[0092] Cells harboring the expression vector carrying the recombinant PRKC gene fusion can then be tested for production of the unique fusion protein via standard Western blotting using either an antibody probe that detects the gene product itself or that recognizes a tag peptide (e.g., FLAG tag) that can be added to the gene product via the expression vector (using standard, commercially available reagents). Western blotting can be used to confirm the ectopic expression of the encoded PRKC fusion protein by comparing the samples from cells

transfected with the vector containing the PRKC gene fusion cDNA to cells transfected with the empty expression vector. The functional activity can be assessed by measuring the level of phosphorylation on the kinase or substrate. Comparison of the level of phosphorylation activity between the wild type (normal) form of PRKC and the PRKC fusion protein can indicate if the PRKC fusion protein has elevated activity that could drive oncogenic activity. Whether the PRKC gene fusion is oncogenic can be assessed by measuring capacity of the expressed PRKC fusion protein to transform cells, that is, to enable cells to grow and proliferate under conditions which are not permissive for growth of normal cells. One commonly used method of measuring the transforming activity of a kinase is by assessing if expression of the gene product can allow BaF3 cells to grow in the absence of the growth factor IL3, which is required for the survival and growth of BaF3 cells. Another assay for measuring transforming activity is a soft agar growth assay. This is another standard method which tests the capacity of an introduced gene product to confer the ability to grow in a soft agar matrix, or anchorage-independent conditions. These methods and others can be used to test the oncogenic activity of a PRKC gene fusion (such as, e.g., an IGF2BP3:PRKCA gene fusion, a TANC2:PRKCA gene fusion, an ADCY9:PRKCB gene fusion, or an SPNS1:PRKCB gene fusion) and provide a level of validation of a PRKC fusion protein (such as, e.g., an IGF2BP3:PRKCA fusion protein, a TANC2:PRKCA fusion protein, an ADCY9:PRKCB fusion protein, or an SPNS1:PRKCB fusion protein) as a potential target for treating patients that harbor these fusions.

[0093] A change in an activity of a cell can be detected in a cell in culture, e.g., a cell expressing a fusion (e.g., a mammalian cell, a tumor cell or cell line, a recombinant cell). The transfected cell can show a change in response to the expressed fusion, e.g., increased proliferation, changes in morphology, increased tumorigenicity, and/or an acquired transformed phenotype.

[0094] To further validate the biological implication of the gene fusion, a change in any of the activities of the cell, e.g., the recombinant cell, in the presence of a known inhibitor of one of the fusion partners, e.g., a PRKC inhibitor, can be detected. For example, a decrease in one or more of: proliferation, tumorigenicity, and transformed morphology, in the presence of the PRKC inhibitor can be indicative of an inhibitor of a fusion. In other embodiments, a change in binding activity or phosphorylation of PRKC or its interacting or downstream proteins or molecules is detected.

[0095] All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification will supersede any contradictory material. Unless otherwise required by context, singular terms shall include the plural and plural terms shall include the singular. The use of "or" means "and/or" unless stated otherwise. The use of the term "including," as well as other forms, such as "includes" and "included," is not limiting. All ranges given in the application encompass the endpoints unless stated otherwise.

[0096] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

We claim:

1. A method for detecting the presence of a PRKC fusion in a biological sample, comprising the steps of:

(a) obtaining a biological sample from a mammal; and

(b) contacting the sample with a reagent that detects a PRKC fusion to determine whether a PRKC fusion is present in the biological sample,

wherein the detection of the PRKC fusion indicates the presence of a PRKC fusion in the biological sample.

2. A method of diagnosing a patient, the method comprising:

(a) obtaining a biological sample from a patient; and

(b) contacting the sample with a reagent that detects a PRKC fusion to determine whether a PRKC fusion is present in the biological sample,

whereby the detection of the PRKC fusion indicates the presence of or susceptibility to a disorder associated with aberrant PRKC expression or activity, or overexpression of PRKC.

3. A method of determining a therapeutic regimen for treating a cancer in a human subject, the method comprising:

(a) obtaining a biological sample from a tumor in the subject;

(b) contacting the sample with a reagent that detects a PRKC fusion to determine whether a PRKC fusion is present in the biological sample; and

(c) determining the therapeutic regimen based on the presence or absence of a PRKC fusion in the sample.

4. A method of identifying a patient likely to respond to treatment with a PRKC inhibitor or a PRKC fusion inhibitor, the method comprising:

(a) obtaining a biological sample from the patient

(b) contacting the sample with a reagent that detects a PRKC fusion to determine whether a PRKC fusion is present in the biological sample,

wherein the detection of the PRKC fusion indicates the likelihood of the patient responding to treatment with a PRKC inhibitor or a PRKC fusion inhibitor.

5. A method of stratifying a patient population by detecting a PRKC fusion, the method comprising:

(a) obtaining a biological sample from each patient in the population;

(b) contacting each of the samples with a reagent that detects a PRKC fusion to determine the presence or absence of a PRKC fusion; and

(c) selecting patients having PRKC fusion for treatment with a PRKC inhibitor or a PRKC fusion inhibitor.

6. The method of any one of claims 1-5, wherein the method of detecting a PRKC fusion in the biological sample comprises one or more assays selected from: in situ hybridization, comparative genomic hybridization, microarray, Southern blot, northern blot, PCR, PCR-RFLP assay, real-time PCR, sequence-specific primers, high-performance liquid chromatography, or mass-spectrometric genotyping, and metaphase cytogenetic analysis by karyotype methods.

7. The method of any one of claims 1-6, wherein the method comprises:

(a) performing a PCR amplification reaction with polynucleotides from the biological sample,

wherein the amplification reaction utilizes a pair of primers which will amplify at least a fragment of a PRKC fusion comprising a fusion junction, and

wherein the first primer is in sense orientation and the second primer is in antisense orientation; and

(b) detecting an amplification product, wherein the presence of the amplification product is indicative of the presence of a PRKC fusion polynucleotide in the sample.

8. The method of claim 7, wherein the step of performing a PCR amplification reaction comprises:

(i) providing a reaction mixture comprising polynucleotides from the biological sample, the pair of primers which will amplify at least a fragment of a PRKC gene fusion comprising a fusion junction, wherein the first primer is complementary to a sequence on the first strand of the polynucleotides and the second primer is complementary to a sequence on the second strand of the polynucleotides, a DNA polymerase, and a plurality of free nucleotides comprising adenine, thymine, cytosine, and guanine (dNTPs);

(ii) heating the reaction mixture to a first predetermined temperature for a first predetermined time to separate the double strands of the polynucleotides from each other;

(iii) cooling the reaction mixture to a second predetermined temperature for a second predetermined time under conditions to allow the first and second primers to hybridize with their complementary sequences on the first and second strands of the polynucleotides, and to allow the DNA polymerase to extend the primers; and

(iv) repeating steps (ii) and (iii) for a predetermined number of cycles.

9. The method of any one of claims 1-8, where in the method employs a probe or primer that is detectably labeled with a radiolabel, a fluorescent label, a bioluminescent label, a chemiluminescent label, an enzyme label, a binding pair label, an antigen-label, a reporter molecule, an affinity tag, an adaptor, a barcode, or a sequence identifier.

10. The method of claim 9, wherein the labeled probe or primer is detected using autoradiography, microscopy, brightfield microscopy, fluorescence microscopy, electron microscopy, enzyme-linked immunosorbent assay (ELISA), or immunohistochemistry.

11. The method of any one of claims 1-10, wherein the biological sample is selected from tissue, whole blood, serum, plasma, buccal scrape, sputum, saliva, cerebrospinal fluid, urine, stool, circulating tumor cells, circulating nucleic acids, or bone marrow.

12. The method of any one of claims 1-11, wherein the biological sample is from a human cancer.

13. The method of claim 12, wherein the human cancer is lung cancer.

14. The method of claim 12, wherein the human cancer is lung squamous cell carcinoma.

15. The method of claim 12, wherein the human cancer is lung adenocarcinoma.

16. The method of any of claims 1-15, wherein the PRKC gene fusion is an IGF2BP3:PRKCA gene fusion.

17. The method of claim 16, wherein the IGF2BP3:PRKCA gene fusion comprises all or part of the nucleotide sequence set forth in SEQ ID NO:1 comprising the fusion junction.

18. The method of any of claims 1-15, wherein the PRKC gene fusion is a

TANC2:PRKCA gene fusion.

19. The method of claim 18, wherein the TANC2:PRKCA gene fusion comprises all or part of the nucleotide sequence set forth in SEQ ID NO:3 comprising the fusion junction.

20. The method of any of claims 1-15, wherein the PRKC gene fusion is an ADCY9:PRKCB gene fusion.

21. The method of claim 20, wherein the ADCY9:PRKCB gene fusion comprises all or part of the nucleotide sequence set forth in SEQ ID NO:5 comprising the fusion junction.

22. The method of any of claims 1-15, wherein the PRKC gene fusion is an SPNS1:PRKCB gene fusion.

23. The method of claim 22, wherein the SPNS1:PRKCB gene fusion comprises all or part of the nucleotide sequence set forth in SEQ ID NO:7 comprising the fusion junction.

24. The method of any of claims 1-23, wherein the reagent is a probe or primer that hybridizes to the fusion junction of a PRKC fusion.

25. The method of any of claims 1-24, wherein the reagent hybridizes to

(a) nucleotides 1201-1206, 1199-1208, 1194-1213, 1179-1228, or 1154-1253 of SEQ ID NO:1;

(b) nucleotides 136-144, 134-146, 129-151, 114-166, or 89-191 of SEQ ID NO:3;

(c) nucleotides 1690-1698, 1688-1700, 1683-1705, 1668-1720, or 1643-1745 of SEQ ID NO:5; or

(d) nucleotides 304-312, 302-314, 297-319, 282-334, or 257-359 of SEQ ID NO:7.

26. The method of any of claims 1-25, wherein the reagent detects an mRNA or cDNA encoding a PRKC fusion protein.

27. The method of any one of claims 1-5, wherein the reagent detects a PRKC fusion protein but does not detect wild type PRKC or wild type fusion partner.

28. The method of any one of claims 1-5 and 27, where in the reagent is detectably labeled with a radiolabel, a fluorescent label, a bioluminescent label, a chemiluminescent label, an enzyme label, a binding pair label, an antigen-label, a reporter molecule, an affinity tag, an

adaptor, barcode, or a sequence identifier.

29. The method of claim 28, wherein the labeled reagent is detected using one or more of autoradiography, microscopy, brightfield microscopy, fluorescence microscopy, electron microscopy, enzyme-linked immunosorbent assay (ELISA), direct ELISA, indirect ELISA, sandwich ELISA, competitive ELISA, immunohistochemistry, western blot, HPLC, and mass spectrometry.

30. The method of any one of claims 1-5 and 27-29, wherein the reagent comprises an antibody specific to a PRKC fusion protein.

31. The method of any one of claims 27-30, wherein the PRKC fusion protein is an IGF2BP3:PRKCA fusion protein.

32. The method of claim 31, wherein the IGF2BP3:PRKCA fusion protein comprises all or part of the amino acid sequence set forth in SEQ ID NO:2 comprising the fusion junction.

33. The method of any one of claims 27-30, wherein the PRKC fusion protein is a TANC2:PRKCA fusion protein.

34. The method of claim 33, wherein the TANC2:PRKCA fusion protein comprises all or part of the amino acid sequence set forth in of SEQ ID NO:4 comprising the fusion junction.

35. The method of any one of claims 27-30, wherein the PRKC fusion protein is an ADCY9:PRKCB fusion protein.

36. The method of claim 35, wherein the ADCY9:PRKCB fusion protein comprises all or part of the amino acid sequence set forth in of SEQ ID NO:6 comprising the fusion junction.

37. The method of any one of claims 27-30, wherein the PRKC fusion protein is an SPNS1:PRKCB fusion protein.

38. The method of claim 37, wherein the SPNS1:PRKCB fusion protein comprises all or part of the amino acid sequence set forth in of SEQ ID NO:8 comprising the fusion junction.

39. The method of any of claims 1-5 and 27-38, wherein the reagent reacts with

(a) amino acids 397-406, 392-411, or 377-426 of SEQ ID NO:2;

(b) amino acids 42-51, 37-56, or 22-71 of SEQ ID NO:4;

(c) amino acids 560-569, 555-574, or 540-589 of SEQ ID NO:6; or

(d) amino acids 98-107, 93-112, or 78-127 of SEQ ID NO:8.

40. The method of any of claims 27-39, wherein the biological sample is from a human cancer.

41. The method of claim 40, wherein the human cancer is lung cancer.

42. The method of claim 40, wherein the human cancer is lung squamous cell carcinoma.

43. The method of claim 40, wherein the human cancer is lung adenocarcinoma.

44. A method of treating a patient, the method comprising acquiring knowledge about the presence of a PRKC fusion, and then administering to the patient a therapeutically effective amount of a PRKC inhibitor or a PRKC fusion inhibitor.

45. The method of claim 44, wherein the knowledge about the presence of a PRKC fusion is acquired by the method of any one of claims 1-43.

46. The method of claim 44 or 45, wherein the patient is suffering from a cancer.

47. The method of claim 46, wherein the cancer is lung cancer.

48. The method of claim 46, wherein the cancer is lung squamous cell carcinoma.

49. The method of claim 46, wherein the cancer is lung adenocarcinoma.

50. A method of inhibiting the proliferation of cells containing a PRKC fusion, the method comprising contacting the cells with a PRKC inhibitor or a PRKC fusion inhibitor.

51. A method of reducing an activity of a PRKC fusion comprising contacting the PRKC fusion with an agent that inhibits an activity or expression of PRKC.

52. The method of claim 50 or 51, wherein the contacting step is effected in vitro.

53. The method of claim 50 or 51, wherein the contacting step is effected in cells in culture.

54. The method of claim 50 or 51, wherein the contacting step is effected in vivo.

55. The method of any one of claims 51-54, wherein the agent that inhibits an activity or expression of PRKC is a PRKC inhibitor or a PRKC fusion inhibitor.

56. A method of treating a condition mediated by aberrant PRKC expression or activity, the method comprising administering to a subject a therapeutically effective amount of a PRKC inhibitor or a PRKC fusion inhibitor.

57. A method of treating a condition characterized by overexpression of PRKC, the method comprising administering to a subject a therapeutically effective amount of a PRKC inhibitor or a PRKC fusion inhibitor.

58. A method of treating a cancer, the method comprising administering to a subject a therapeutically effective amount of a PRKC inhibitor or a PRKC fusion inhibitor.

59. The method of claim 58, wherein the cancer is lung cancer.

60. The method of claim 58, wherein the cancer is lung squamous cell carcinoma.

61. The method of claim 58, wherein the cancer is lung adenocarcinoma.

62. The method of any of claims 44-61, wherein the agent that inhibits an activity or expression of PRKC or the PRKC inhibitor is a kinase inhibitor.

63. The method of any of claims 44-61, wherein the agent that inhibits an activity or expression of PRKC or the PRKC inhibitor is an anti-PRKC antibody.

64. The method of any one of claims 44-61, wherein the agent that inhibits an activity or expression of PRKC or the PRKC inhibitor is a PRKC fusion inhibitor.

65. The method of claim 64, wherein the PRKC fusion inhibitor is an antibody that recognizes the fusion junction of a PRKC fusion.

66. The method of any one of claims 44-65, wherein the PRKC fusion is an IGF2BP3:PRKCA, TANC2:PRKCA, ADCY9:PRKCB, or SPNS1:PRKCB fusion.

67. The method of any one of claims 44-66, wherein the PRKC fusion inhibitor is an antibody that binds to

(a) a fragment of an IGF2BP3:PRKCA fusion comprising amino acids 397-406, 392-411, or 377-426 of SEQ ID NO:2;

(b) a fragment of a TANC2:PRKCA fusion comprising amino acids 42-51, 37-56, or 22-71 of SEQ ID NO:4;

(c) a fragment of an ADCY9:PRKCB fusion comprising amino acids 560-569, 555-574, or 540-589 of SEQ ID NO:6; or

(d) a fragment of an SPNS1:PRKCB fusion comprising amino acids 98-107, 93-112, or 78-127 of SEQ ID NO:8.

68. A method of identifying an agent useful for treating lung squamous cell carcinoma, the method comprising contacting a cell expressing a PRKC fusion with a candidate agent and determining whether the expression level of the fusion is decreased or a biological function associated with the fusion is altered.

69. A method of identifying an agent useful for treating lung adenocarcinoma, the method comprising contacting a cell expressing a PRKC fusion with a candidate agent and determining whether the expression level of the fusion is decreased or a biological function associated with the fusion is altered.

70. A method of identifying an agent that modulates the activity of a PRKC fusion, comprising contacting a PRKC fusion with a candidate agent; and detecting a change in a parameter associated with a PRKC fusion.

71. The method of any of claims 68-70, wherein the PRKC fusion is an IGF2BP3:PRKCA, TANC2:PRKCA, ADCY9:PRKCB, or SPNS1:PRKCB fusion.

72. The method of any one of claims 68-71, wherein the PRKC fusion comprises all of part of

(a) the nucleotide sequence set forth in SEQ ID NO:1, 3, 5, or 7 comprising the fusion junction; or

(b) the amino acid sequence set forth in SEQ ID NO:2, 4, 6, or 8 comprising the fusion junction.

FIG. 1

```
ATGAACAAAC TGTATATCGG AAACCTCAGC GAGAACGCCG CCCCCTCGGA CCTAGAAAGT   60
ATCTTCAAGG ACGCCAAGAT CCCGGTGTCG GGACCCTTCC TGGTGAAGAC TGGCTACGCG  120
TTCGTGGACT GCCCGGACGA GAGCTGGGCC CTCAAGGCCA TCGAGGCGCT TTCAGGTAAA  180
ATAGAACTGC ACGGGAAACC CATAGAAGTT GAGCACTCGG TCCCAAAAAG GCAAAGGATT  240
CGGAAACTTC AGATACGAAA TATCCCGCCT CATTTACAGT GGGAGGTGCT GGATAGTTTA  300
CTAGTCCAGT ATGGAGTGGT GGAGAGCTGT GAGCAAGTGA ACACTGACTC GGAAACTGCA  360
GTTGTAAATG TAACCTATTC CAGTAAGGAC CAAGCTAGAC AAGCACTAGA CAAACTGAAT  420
GGATTTCAGT TAGAGAATTT CACCTTGAAA GTAGCCTATA TCCCTGATGA AATGGCCGCC  480
CAGCAAAACC CCTTGCAGCA GCCCCGAGGT CGCCGGGGGC TTGGGCAGAG GGGCTCCTCA  540
AGGCAGGGGT CTCCAGGATC CGTATCCAAG CAGAAACCAT GTGATTTGCC TCTGCGCCTG  600
CTGGTTCCCA CCCAATTTGT TGGAGCCATC ATAGGAAAAG AAGGTGCCAC CATTCGGAAC  660
ATCACCAAAC AGACCCAGTC TAAAATCGAT GTCCACCGTA AAGAAAATGC GGGGGCTGCT  720
GAGAAGTCGA TTACTATCCT CTCTACTCCT GAAGGCACCT CTGCGGCTTG TAAGTCTATT  780
CTGGAGATTA TGCATAAGGA AGCTCAAGAT ATAAAATTCA CAGAAGAGAT CCCCTTGAAG  840
ATTTTAGCTC ATAATAACTT TGTTGGACGT CTTATTGGTA AAGAAGGAAG AAATCTTAAA  900
AAAATTGAGC AAGACACAGA CACTAAAATC ACGATATCTC CATTGCAGGA ATTGACGCTG  960
TATAATCCAG AACGCACTAT TACAGTTAAA GGCAATGTTG AGACATGTGC CAAAGCTGAG   1020
GAGGAGATCA TGAAGAAAAT CAGGGAGTCT TATGAAAATG ATATTGCTTC TATGAATCTT   1080
CAAGCACATT TAATTCCTGG ATTAAATCTG AACGCCTTGG GTCTGTTCCC ACCCACTTCA   1140
GGGATGCCAC CTCCCACCTC AGGGCCCCCT TCAGCCATGA CTCCTCCCTA CCCGCAGTTT   1200
GAG/GACCCCA GGAGCAAGCA CAAGTTCAAA ATCCACACTT ACGGAAGCCC CACCTTCTGC  1260
GATCACTGTG GGTCACTGCT CTATGGACTT ATCCATCAAG GGATGAAATG TGACACCTGC   1320
GATATGAACG TTCACAAGCA ATGCGTCATC AATGTCCCCA GCCTCTGCGG AATGGATCAC   1380
ACTGAGAAGA GGGGGCGGAT TTACCTAAAG GCTGAGGTTG CTGATGAAAA GCTCCATGTC   1440
ACAGTACGAG ATGCAAAAAA TCTAATCCCT ATGGATCCAA ACGGGCTTTC AGATCCTTAT   1500
GTGAAGCTGA AACTTATTCC TGATCCCAAG AATGAAAGCA AGCAAAAAAC CAAAACCATC   1560
CGCTCCACAC TAAATCCGCA GTGGAATGAG TCCTTTACAT TCAAATTGAA ACCTTCAGAC   1620
AAAGACCGAC GACTGTCTGT AGAAATCTGG GACTGGGATC GAACAACAAG GAATGACTTC   1680
ATGGGATCCC TTTCCTTTGG AGTTTCGGAG CTGATGAAGA TGCCGGCCAG TGGATGGTAC   1740
AAGTTGCTTA ACCAAGAAGA AGGTGAGTAC TACAACGTAC CCATTCCGGA AGGGGACGAG   1800
GAAGGAAACA TGGAACTCAG GCAGAAATTC GAGAAAGCCA AACTTGGCCC TGCTGGCAAC   1860
AAAGTCATCA GTCCCTCTGA AGACAGGAAA CAACCTTCCA ACAACCTTGA CCGAGTGAAA   1920
CTCACGGACT TCAATTTCCT CATGGTGTTG GGAAAGGGGA GTTTTGGAAA GGTGATGCTT   1980
GCCGACAGGA AGGGCACAGA AGAACTGTAT GCAATCAAAA TCCTGAAGAA GGATGTGGTG   2040
ATTCAGGATG ATGACGTGGA GTGCACCATG GTAGAAAAGC GAGTCTTGGC CCTGCTTGAC   2100
AAACCCCCGT TCTTGACGCA GCTGCACTCC TGCTTCCAGA CAGTGGATCG GCTGTACTTC   2160
GTCATGGAAT ATGTCAACGG TGGGGACCTC ATGTACCACA TTCAGCAAGT AGGAAAATTT   2220
AAGGAACCAC AAGCAGTATT CTATGCGGCA GAGATTTCCA TCGGATTGTT CTTTCTTCAT   2280
AAAAGAGGAA TCATTTATAG GGATCTGAAG TTAGATAACG TCATGTTGGA TTCAGAAGGA   2340
CATATCAAAA TTGCTGACTT TGGGATGTGC AAGGAACACA TGATGGATGG AGTCACGACC   2400
AGGACCTTCT GTGGGACTCC AGATTATATC GCCCCAGAGA TAATCGCTTA TCAGCCGTAT   2460
GGAAAATCTG TGGACTGGTG GGCCTATGGC GTCCTGTTGT ATGAAATGCT TGCCGGGCAG   2520
CCTCCATTTG ATGGTGAAGA TGAAGACGAG CTATTTCAGT CTATCATGGA GCACAACGTT   2580
TCCTATCCAA AATCCTTGTC CAAGGAGGCT GTTTCTATCT GCAAAGGACT GATGACCAAA   2640
CACCCAGCCA AGCGGCTGGG CTGTGGGCCT GAGGGGGAGA GGGACGTGAG AGAGCATGCC   2700
TTCTTCCGGA GGATCGACTG GGAAAAACTG GAGAACAGGG AGATCCAGCC ACCATTCAAG   2760
CCCAAAGTGT GTGGCAAAGG AGCAGAGAAC TTTGACAAGT TCTTCACACG AGGACAGCCC   2820
GTCTTAACAC CACCTGATCA GCTGGTTATT GCTAACATAG ACCAGTCTGA TTTTGAAGGG   2880
TTCTCGTATG TCAACCCCCA GTTTGTGCAC CCCATCTTAC AGAGTGCAGT ATGA         2934
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(SEQ ID NO:1)

FIG. 2

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MNKLYIGNLS ENAAPSDLES IFKDAKIPVS GPFLVKTGYA FVDCPDESWA LKAIEALSGK  60
IELHGKPIEV EHSVPKRQRI RKLQIRNIPP HLQWEVLDSL LVQYGVVESC EQVNTDSETA 120
VVNVTYSSKD QARQALDKLN GFQLENFTLK VAYIPDEMAA QQNPLQQPRG RRGLGQRGSS 180
RQGSPGSVSK QKPCDLPLRL LVPTQFVGAI IGKEGATIRN ITKQTQSKID VHRKENAGAA 240
EKSITILSTP EGTSAACKSI LEIMHKEAQD IKFTEEIPLK ILAHNNFVGR LIGKEGRNLK 300
KIEQDTDTKI TISPLQELTL YNPERTITVK GNVETCAKAE EEIMKKIRES YENDIASMNL 360
QAHLIPGLNL NALGLFPPTS GMPPPTSGPP SAMTPPYPQF E/DPRSKHKFK IHTYGSPTFC  420
DHCGSLLYGL IHQGMKCDTC DMNVHKQCVI NVPSLCGMDH TEKRGRIYLK AEVADEKLHV 480
TVRDAKNLIP MDPNGLSDPY VKLKLIPDPK NESKQKTKTI RSTLNPQWNE SFTFKLKPSD 540
KDRRLSVEIW DWDRTTRNDF MGSLSFGVSE LMKMPASGWY KLLNQEEGEY YNVPIPEGDE 600
EGNMELRQKF EKAKLGPAGN KVISPSEDRK QPSNNLDRVK LTDFNFLMVL GKGSFGKVML 660
ADRKGTEELY AIKILKKDVV IQDDDVECTM VEKRVLALLD KPPFLTQLHS CFQTVDRLYF 720
VMEYVNGGDL MYHIQQVGKF KEPQAVFYAA EISIGLFFLH KRGIIYRDLK LDNVMLDSEG 780
HIKIADFGMC KEHMMDGVTT RTFCGTPDYI APEIIAYQPY GKSVDWWAYG VLLYEMLAGQ 840
PPFDGEDEDE LFQSIMEHNV SYPKSLSKEA VSICKGLMTK HPAKRLGCGP EGERDVREHA 900
FFRRIDWEKL ENREIQPPFK PKVCGKGAEN FDKFFTRGQP VLTPPDQLVI ANIDQSDFEG 960
FSYVNPQFVH PILQSAV                                              977
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(SEQ ID NO:2)

FIG. 3

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ATGTTTCGGA ATAGTCTCAA GATGCTGCTT ACTGGTGGGA AATCAAGTCG TAAAAACAGG   60
TCAAGTGATG GAGGGAGCGA GGAACCACCG GATCGAAGAC AGTCAAGTGT AGACTCTCGC  120
CAAAGCCGCT CTGGGCAAG/T ACGAGATGCA AAAAATCTAA TCCCTATGGA TCCAAACGGG  180
CTTTCAGATC CTTATGTGAA GCTGAAACTT ATTCCTGATC CCAAGAATGA AAGCAAGCAA  240
AAAACCAAAA CCATCCGCTC CACACTAAAT CCGCAGTGGA ATGAGTCCTT TACATTCAAA  300
TTGAAACCTT CAGACAAAGA CCGACGACTG TCTGTAGAAA TCTGGGACTG GGATCGAACA  360
ACAAGGAATG ACTTCATGGG ATCCCTTTCC TTTGGAGTTT CGGAGCTGAT GAAGATGCCG  420
GCCAGTGGAT GGTACAAGTT GCTTAACCAA GAAGAAGGTG AGTACTACAA CGTACCCATT  480
CCGGAAGGGG ACGAGGAAGG AAACATGGAA CTCAGGCAGA AATTCGAGAA AGCCAAACTT  540
GGCCCTGCTG GCAACAAAGT CATCAGTCCC TCTGAAGACA GGAAACAACC TTCCAACAAC  600
CTTGACCGAG TGAAACTCAC GGACTTCAAT TTCCTCATGG TGTTGGGAAA GGGGAGTTTT  660
GGAAAGGTGA TGCTTGCCGA CAGGAAGGGC ACAGAAGAAC TGTATGCAAT CAAAATCCTG  720
AAGAAGGATG TGGTGATTCA GGATGATGAC GTGGAGTGCA CCATGGTAGA AAAGCGAGTC  780
TTGGCCCTGC TTGACAAACC CCCGTTCTTG ACGCAGCTGC ACTCCTGCTT CCAGACAGTG  840
GATCGGCTGT ACTTCGTCAT GGAATATGTC AACGGTGGGG ACCTCATGTA CCACATTCAG  900
CAAGTAGGAA AATTTAAGGA ACCACAAGCA GTATTCTATG CGGCAGAGAT TTCCATCGGA  960
TTGTTCTTTC TTCATAAAAG AGGAATCATT TATAGGGATC TGAAGTTAGA TAACGTCATG  1020
TTGGATTCAG AAGGACATAT CAAAATTGCT GACTTTGGGA TGTGCAAGGA ACACATGATG  1080
GATGGAGTCA CGACCAGGAC CTTCTGTGGG ACTCCAGATT ATATCGCCCC AGAGATAATC  1140
GCTTATCAGC CGTATGGAAA ATCTGTGGAC TGGTGGGCCT ATGGCGTCCT GTTGTATGAA  1200
ATGCTTGCCG GGCAGCCTCC ATTTGATGGT GAAGATGAAG ACGAGCTATT TCAGTCTATC  1260
ATGGAGCACA ACGTTTCCTA TCCAAAATCC TTGTCCAAGG AGGCTGTTTC TATCTGCAAA  1320
GGACTGATGA CCAAACACCC AGCCAAGCGG CTGGGCTGTG GGCCTGAGGG GGAGAGGGAC  1380
GTGAGAGAGC ATGCCTTCTT CCGGAGGATC GACTGGGAAA AACTGGAGAA CAGGGAGATC  1440
CAGCCACCAT TCAAGCCCAA AGTGTGTGGC AAAGGAGCAG AGAACTTTGA CAAGTTCTTC  1500
ACACGAGGAC AGCCCGTCTT AACACCACCT GATCAGCTGG TTATTGCTAA CATAGACCAG  1560
TCTGATTTTG AAGGGTTCTC GTATGTCAAC CCCCAGTTTG TGCACCCCAT CTTACAGAGT  1620
GCAGTATGA                                                          1629
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(SEQ ID NO:3)

FIG. 4

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MFRNSLKMLL TGGKSSRKNR SSDGGSEEPP DRRQSSVDSR QSRSGQVRDA KNLIPMDPNG   60
LSDPYVKLKL IPDPKNESKQ KTKTIRSTLN PQWNESFTFK LKPSDKDRRL SVEIWDWDRT  120
TRNDFMGSLS FGVSELMKMP ASGWYKLLNQ EEGEYYNVPI PEGDEEGNME LRQKFEKAKL  180
GPAGNKVISP SEDRKQPSNN LDRVKLTDFN FLMVLGKGSF GKVMLADRKG TEELYAIKIL  240
KKDVVIQDDD VECTMVEKRV LALLDKPPFL TQLHSCFQTV DRLYFVMEYV NGGDLMYHIQ  300
QVGKFKEPQA VFYAAEISIG LFFLHKRGII YRDLKLDNVM LDSEGHIKIA DFGMCKEHMM  360
DGVTTRTFCG TPDYIAPEII AYQPYGKSVD WWAYGVLLYE MLAGQPPFDG EDEDELFQSI  420
MEHNVSYPKS LSKEAVSICK GLMTKHPAKR LGCGPEGERD VREHAFFRRI DWEKLENREI  480
QPPFKPKVCG KGAENFDKFF TRGQPVLTPP DQLVIANIDQ SDFEGFSYVN PQFVHPILQS  540
AV                                                                  542
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(SEQ ID NO:4)

FIG. 5A

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ATGGCTTCCC CACCCCACCA GCAGCTGCTG CATCACCACA GCACCGAGGT GAGCTGCGAC   60
TCCAGCGGGG ACAGCAACAG CGTGCGCGTC AAGATCAACC CCAAGCAGCT GTCCTCCAAC  120
AGCCACCCCA AGCACTGCAA ATACAGCATC TCCTCTAGCT GCAGCAGCTC TGGGGACTCC  180
GGGGGCGTCC CCCGGCGAGT GGGCGGCGGA GGCCGGCTGC GCAGGCAGAA GAAGCTGCCC  240
CAGCTGTTCG AGAGGGCCTC CAGCCGCTGG TGGGACCCCA AGTTCGACTC GGTGAACCTG  300
GAGGAGGCCT GCCTGGAGCG CTGCTTCCCG CAGACCCAGC GCCGGTTCCG GTATGCGCTC  360
TTCTACATCG GCTTCGCCTG CCTTCTGTGG AGCATCTATT TTGCGGTCCA CATGAGATCC  420
AGACTGATCG TCATGGTCGC CCCCGCGCTG TGCTTCCTCC TGGTGTGTGT GGGCTTCTTT  480
CTGTTTACCT TCACCAAGCT GTACGCCCGG CATTACGCGT GGACCTCGCT GGCTCTCACC  540
CTGCTGGTGT TCGCCCTGAC CCTGGCTGCG CAGTTCCAGG TCTTGACGCC TGTCTCAGGA  600
CGCGGCGACA GCTCCAACCT TACGGCCACA GCCCGGCCCA CAGATACTTG CTTATCTCAA  660
GTGGGGAGCT TCTCCATGTG CATCGAAGTG CTCTTTTTGC TCTATACCGT CATGCACTTA  720
CCTTTGTACC TGAGTTTGTG TCTGGGGGTG GCCTACTCTG TCCTTTTCGA GACCTTTGGC  780
TACCATTTCC GGGATGAAGC CTGCTTCCCC TCGCCCGGAG CCGGGGCCCT GCACTGGGAG  840
CTGCTGAGCA GGGGGCTGCT CCACGGCTGC ATCCACGCCA TCGGGGTCCA CCTGTTCGTC  900
ATGTCCCAGG TGAGGTCCAG GAGCACCTTC CTCAAGGTGG GGCAATCCAT TATGCACGGG  960
AAGGACCTGG AAGTGGAAAA AGCCCTCAAA GAGAGGATGA TTCATTCCGT GATGCCAAGA  1020
ATCATAGCCG ATGACTTAAT GAAGCAGGGA GATGAGGAGA GTGAGAATTC TGTCAAGAGG  1080
CATGCCACCT CGAGCCCCAA GAACAGGAAG AAAAAGTCTT CCATCCAAAA AGCTCCTATA  1140
GCCTTCCGCC CTTTTAAGAT GCAGCAGATC GAAGAAGTCA GTATTTTATT TGCAGATATC  1200
GTGGGCTTCA CCAAGATGAG TGCCAACAAG TCTGCCCACG CCCTGGTGGG TCTCCTGAAC  1260
GATCTGTTCG GTCGCTTCGA CCGCCTGTGT GAGGAGACCA AGTGTGAGAA AATCAGCACC  1320
CTGGGAGACT GTTACTACTG CGTGGCGGGC TGTCCCGAGC CCCGGGCCGA CCATGCCTAC  1380
TGCTGCATCG AGATGGGCCT GGGCATGATC AAGGCCATCG AGCAGTTCTG CCAGGAGAAG  1440
AAGGAGATGG TGAACATGAG AGTCGGGGTG CACACGGGCA CCGTCCTTTG CGGCATCCTG  1500
GGCATGAGGA GGTTTAAATT TGACGTGTGG TCCAACGATG TGAACCTGGC CAATCTCATG  1560
GAGCAGCTGG GAGTGGCCGG CAAAGTTCAC ATTTCTGAGG CCACCGCAAA ATACTTAGAT  1620
GACCGGTACG AAATGGAAGA TGGGAAAGTT ATTGAACGGC TGGGCCAGAG CGTGGTTGCT  1680
GACCAGTTGA AAG/TTTGCTG CTTTGTGGTG CACAAGCGGT GCCATGAATT TGTCACATTC  1740
TCCTGCCCTG GCGCTGACAA GGGTCCAGCC TCCGATGACC CCCGCAGCAA ACACAAGTTT  1800
AAGATCCACA CGTACTCCAG CCCCACGTTT TGTGACCACT GTGGGTCACT GCTGTATGGA  1860
CTCATCCACC AGGGGATGAA ATGTGACACC TGCATGATGA ATGTGCACAA GCGCTGCGTG  1920
ATGAATGTTC CCAGCCTGTG TGGCACGGAC CACACGGAGC GCCGCGGCCG CATCTACATC  1980
CAGGCCCACA TCGACAGGGA CGTCCTCATT GTCCTCGTAA GAGATGCTAA AAACCTTGTA  2040
CCTATGGACC CCAATGGCCT GTCAGATCCC TACGTAAAAC TGAAACTGAT TCCCGATCCC  2100
AAAAGTGAGA GCAAACAGAA GACCAAAACC ATCAAATGCT CCCTCAACCC TGAGTGGAAT  2160
GAGACATTTA GATTTCAGCT GAAAGAATCG GACAAAGACA GAAGACTGTC AGTAGAGATT  2220
TGGGATTGGG ATTTGACCAG CAGGAATGAC TTCATGGGAT CTTTGTCCTT TGGGATTTCT  2280
GAACTTCAGA AAGCCAGTGT TGATGGCTGG TTTAAGTTAC TGAGCCAGGA GGAAGGCGAG  2340
TACTTCAATG TGCCTGTGCC ACCAGAAGGA AGTGAGGCCA ATGAAGAACT GCGGCAGAAA  2400
TTTGAGAGGG CCAAGATCAG TCAGGGAACC AAGGTCCCGG AAGAAAAGAC GACCAACACT  2460
GTCTCCAAAT TTGACAACAA TGGCAACAGA GACCGGATGA AACTGACCGA TTTTAACTTC  2520
CTAATGGTGC TGGGGAAAGG CAGCTTTGGC AAGGTCATGC TTTCAGAACG AAAAGGCACA  2580
GATGAGCTCT ATGCTGTGAA GATCCTGAAG AAGGACGTTG TGATCCAAGA TGATGACGTG  2640
GAGTGCACTA TGGTGGAGAA GCGGGTGTTG GCCCTGCCTG GGAAGCCGCC CTTCCTGACC  2700
CAGCTCCACT CCTGCTTCCA GACCATGGAC CGCCTGTACT TTGTGATGGA GTACGTGAAT  2760
GGGGGCGACC TCATGTATCA CATCCAGCAA GTCGGCCGGT TCAAGGAGCC CCATGCTGTA  2820
TTTTACGCTG CAGAAATTGC CATCGGTCTG TTCTTCTTAC AGAGTAAGGG CATCATTTAC  2880
CGTGACCTAA AACTTGACAA CGTGATGCTC GATTCTGAGG GACACATCAA GATTGCCGAT  2940
TTTGGCATGT GTAAGGAAAA CATCTGGGAT GGGGTGACAA CCAAGACATT CTGTGGCACT  3000
CCAGACTACA TCGCCCCCGA GATAATTGCT TATCAGCCCT ATGGGAAGTC CGTGGATTGG  3060
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FIG. 5B

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TGGGCATTTG GAGTCCTGCT GTATGAAATG TTGGCTGGGC AGGCACCCTT TGAAGGGGAG  3120
GATGAAGATG AACTCTTCCA ATCCATCATG GAACACAACG TAGCCTATCC CAAGTCTATG  3180
TCCAAGGAAG CTGTGGCCAT CTGCAAAGGG CTGATGACCA AACACCCAGG CAAACGTCTG  3240
GGTTGTGGAC CTGAAGGCGA ACGTGATATC AAAGAGCATG CATTTTTCCG GTATATTGAT  3300
TGGGAGAAAC TTGAACGCAA AGAGATCCAG CCCCCTTATA AGCCAAAAGC TTGTGGGCGA  3360
AATGCTGAAA ACTTCGACCG ATTTTTCACC CGCCATCCAC CAGTCCTAAC ACCTCCCGAC  3420
CAGGAAGTCA TCAGGAATAT TGACCAATCA GAATTCGAAG GATTTTCCTT TGTTAACTCT  3480
GAATTTTTAA AACCCGAAGT CAAGAGCTAA                                   3510
```

(SEQ ID NO:5)

FIG. 6

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MASPPHQQLL HHHSTEVSCD SSGDSNSVRV KINPKQLSSN SHPKHCKYSI SSSCSSSGDS   60
GGVPRRVGGG GRLRRQKKLP QLFERASSRW WDPKFDSVNL EEACLERCFP QTQRRFRYAL  120
FYIGFACLLW SIYFAVHMRS RLIVMVAPAL CFLLVCVGFF LFTFTKLYAR HYAWTSLALT  180
LLVFALTLAA QFQVLTPVSG RGDSSNLTAT ARPTDTCLSQ VGSFSMCIEV LFLLYTVMHL  240
PLYLSLCLGV AYSVLFETFG YHFRDEACFP SPGAGALHWE LLSRGLLHGC IHAIGVHLFV  300
MSQVRSRSTF LKVGQSIMHG KDLEVEKALK ERMIHSVMPR IIADDLMKQG DEESENSVKR  360
HATSSPKNRK KKSSIQKAPI AFRPFKMQQI EEVSILFADI VGFTKMSANK SAHALVGLLN  420
DLFGRFDRLC EETKCEKIST LGDCYYCVAG CPEPRADHAY CCIEMGLGMI KAIEQFCQEK  480
KEMVNMRVGV HTGTVLCGIL GMRRFKFDVW SNDVNLANLM EQLGVAGKVH ISEATAKYLD  540
DRYEMEDGKV IERLGQSVVA DQLKVCCFVV HKRCHEFVTF SCPGADKGPA SDDPRSKHKF  600
KIHTYSSPTF CDHCGSLLYG LIHQGMKCDT CMMNVHKRCV MNVPSLCGTD HTERRGRIYI  660
QAHIDRDVLI VLVRDAKNLV PMDPNGLSDP YVKLKLIPDP KSESKQKTKT IKCSLNPEWN  720
ETFRFQLKES DKDRRLSVEI WDWDLTSRND FMGSLSFGIS ELQKASVDGW FKLLSQEEGE  780
YFNVPVPPEG SEANEELRQK FERAKISQGT KVPEEKTTNT VSKFDNNGNR DRMKLTDFNF  840
LMVLGKGSFG KVMLSERKGT DELYAVKILK KDVVIQDDDV ECTMVEKRVL ALPGKPPFLT  900
QLHSCFQTMD RLYFVMEYVN GGDLMYHIQQ VGRFKEPHAV FYAAEIAIGL FFLQSKGIIY  960
RDLKLDNVML DSEGHIKIAD FGMCKENIWD GVTTKTFCGT PDYIAPEIIA YQPYGKSVDW  1020
WAFGVLLYEM LAGQAPFEGE DEDELFQSIM EHNVAYPKSM SKEAVAICKG LMTKHPGKRL  1080
GCGPEGERDI KEHAFFRYID WEKLERKEIQ PPYKPKACGR NAENFDRFFT RHPPVLTPPD  1140
QEVIRNIDQS EFEGFSFVNS EFLKPEVKS                                    1169
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(SEQ ID NO:6)

FIG. 7

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ATGGCCGGGT CCGACACCGC GCCCTTCCTC AGCCAGGCGG ATGACCCGGA CGACGGGCCA   60
GTGCCTGGCA CCCCGGGGTT GCCAGGGTCC ACGGGGAACC CGAAGTCCGA GGAGCCCGAG  120
GTCCCGGACC AGGAGGGGCT GCAGCGCATC ACCGGCCTGT CTCCCGGCCG TTCGGCTCTC  180
ATAGTGGCGG TGCTGTGCTA CATCAATCTC CTGAACTACA TGGACCGCTT CACCGTGGCT  240
GGCGTCCTTC CCGACATCGA GCAGTTCTTC AACATCGGGG ACAGTAGCTC TGGGCTCATC  300
CAGACCG/TTT GCTGCTTTGT GGTGCACAAG CGGTGCCATG AATTTGTCAC ATTCTCCTGC   360
CCTGGCGCTG ACAAGGGTCC AGCCTCCGAT GACCCCCGCA GCAAACACAA GTTTAAGATC  420
CACACGTACT CCAGCCCCAC GTTTTGTGAC CACTGTGGGT CACTGCTGTA TGGACTCATC  480
CACCAGGGGA TGAAATGTGA CACCTGCATG ATGAATGTGC ACAAGCGCTG CGTGATGAAT  540
GTTCCCAGCC TGTGTGGCAC GGACCACACG GAGCGCCGCG GCCGCATCTA CATCCAGGCC  600
CACATCGACA GGGACGTCCT CATTGTCCTC GTAAGAGATG CTAAAAACCT TGTACCTATG  660
GACCCCAATG GCCTGTCAGA TCCCTACGTA AAACTGAAAC TGATTCCCGA TCCCAAAAGT  720
GAGAGCAAAC AGAAGACCAA AACCATCAAA TGCTCCCTCA ACCCTGAGTG GAATGAGACA  780
TTTAGATTTC AGCTGAAAGA ATCGGACAAA GACAGAAGAC TGTCAGTAGA GATTTGGGAT  840
TGGGATTTGA CCAGCAGGAA TGACTTCATG GGATCTTTGT CCTTTGGGAT TTCTGAACTT  900
CAGAAAGCCA GTGTTGATGG CTGGTTTAAG TTACTGAGCC AGGAGGAAGG CGAGTACTTC  960
AATGTGCCTG TGCCACCAGA AGGAAGTGAG GCCAATGAAG AACTGCGGCA GAAATTTGAG   1020
AGGGCCAAGA TCAGTCAGGG AACCAAGGTC CCGGAAGAAA AGACGACCAA CACTGTCTCC   1080
AAATTTGACA ACAATGGCAA CAGAGACCGG ATGAAACTGA CCGATTTTAA CTTCCTAATG   1140
GTGCTGGGGA AAGGCAGCTT TGGCAAGGTC ATGCTTTCAG AACGAAAAGG CACAGATGAG   1200
CTCTATGCTG TGAAGATCCT GAAGAAGGAC GTTGTGATCC AAGATGATGA CGTGGAGTGC   1260
ACTATGGTGG AGAAGCGGGT GTTGGCCCTG CCTGGGAAGC CGCCCTTCCT GACCCAGCTC   1320
CACTCCTGCT TCCAGACCAT GGACCGCCTG TACTTTGTGA TGGAGTACGT GAATGGGGGC   1380
GACCTCATGT ATCACATCCA GCAAGTCGGC CGGTTCAAGG AGCCCCATGC TGTATTTTAC   1440
GCTGCAGAAA TTGCCATCGG TCTGTTCTTC TTACAGAGTA AGGGCATCAT TTACCGTGAC   1500
CTAAAACTTG ACAACGTGAT GCTCGATTCT GAGGGACACA TCAAGATTGC CGATTTTGGC   1560
ATGTGTAAGG AAAACATCTG GGATGGGGTG ACAACCAAGA CATTCTGTGG CACTCCAGAC   1620
TACATCGCCC CCGAGATAAT TGCTTATCAG CCCTATGGGA AGTCCGTGGA TTGGTGGGCA   1680
TTTGGAGTCC TGCTGTATGA AATGTTGGCT GGGCAGGCAC CCTTTGAAGG GGAGGATGAA   1740
GATGAACTCT TCCAATCCAT CATGGAACAC AACGTAGCCT ATCCCAAGTC TATGTCCAAG   1800
GAAGCTGTGG CCATCTGCAA AGGGCTGATG ACCAAACACC CAGGCAAACG TCTGGGTTGT   1860
GGACCTGAAG GCGAACGTGA TATCAAAGAG CATGCATTTT TCCGGTATAT TGATTGGGAG   1920
AAACTTGAAC GCAAAGAGAT CCAGCCCCCT TATAAGCCAA AAGCTTGTGG GCGAAATGCT   1980
GAAAACTTCG ACCGATTTTT CACCCGCCAT CCACCAGTCC TAACACCTCC CGACCAGGAA   2040
GTCATCAGGA ATATTGACCA ATCAGAATTC GAAGGATTTT CCTTTGTTAA CTCTGAATTT   2100
TTAAAACCCG AAGTCAAGAG CTAA                                          2124
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(SEQ ID NO:7)

FIG. 8

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MAGSDTAPFL SQADDPDDGP VPGTPGLPGS TGNPKSEEPE VPDQEGLQRI TGLSPGRSAL  60
IVAVLCYINL LNYMDRFTVA GVLPDIEQFF NIGDSSSGLI QTVCCFVVHK RCHEFVTFSC 120
PGADKGPASD DPRSKHKFKI HTYSSPTFCD HCGSLLYGLI HQGMKCDTCM MNVHKRCVMN 180
VPSLCGTDHT ERRGRIYIQA HIDRDVLIVL VRDAKNLVPM DPNGLSDPYV KLKLIPDPKS 240
ESKQKTKTIK CSLNPEWNET FRFQLKESDK DRRLSVEIWD WDLTSRNDFM GSLSFGISEL 300
QKASVDGWFK LLSQEEGEYF NVPVPPEGSE ANEELRQKFE RAKISQGTKV PEEKTTNTVS 360
KFDNNGNRDR MKLTDFNFLM VLGKGSFGKV MLSERKGTDE LYAVKILKKD VVIQDDDVEC 420
TMVEKRVLAL PGKPPFLTQL HSCFQTMDRL YFVMEYVNGG DLMYHIQQVG RFKEPHAVFY 480
AAEIAIGLFF LQSKGIIYRD LKLDNVMLDS EGHIKIADFG MCKENIWDGV TTKTFCGTPD 540
YIAPEIIAYQ PYGKSVDWWA FGVLLYEMLA GQAPFEGEDE DELFQSIMEH NVAYPKSMSK 600
EAVAICKGLM TKHPGKRLGC GPEGERDIKE HAFFRYIDWE KLERKEIQPP YKPKACGRNA 660
ENFDRFFTRH PPVLTPPDQE VIRNIDQSEF EGFSFVNSEF LKPEVKS               707
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(SEQ ID NO:8)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/040560

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68
ADD.

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)
C12Q

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, Sequence Search, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/038887 A1 (SNU R & DB FOUND) 13 March 2014 (2014-03-13) paragraph [0176] - paragraph [0180]; claims 14, 18, 19; figures 6, 41, 42; example 5; table 50 paragraph [0159] - paragraph [0165] paragraph [0147] - paragraph [0149] ----- -/--	1-55, 58-62, 64-72

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
6 November 2015	17/11/2015

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/040560

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PLASZCZYCA ANNA ET AL: "Fusions involving protein kinase C and membrane-associated proteins in benign fibrous histiocytoma", INTERNATIONAL JOURNAL OF BIOCHEMISTRY AND CELL BIOLOGY, PERGAMON, GB, vol. 53, 8 April 2014 (2014-04-08), pages 475-481, XP029040270, ISSN: 1357-2725, DOI: 10.1016/J.BIOCEL.2014.03.027 the whole document	1,6-8, 11,26
X	JULIA A. BRIDGE ET AL: "Identification of a Novel, Recurrent SLC44A1-PRKCA Fusion in Papillary Glioneuronal Tumor", BRAIN PATHOLOGY., vol. 23, no. 2, 23 July 2012 (2012-07-23), pages 121-128, XP055221005, CH ISSN: 1015-6305, DOI: 10.1111/j.1750-3639.2012.00612.x the whole document	1,2,6-8, 11,12,26
X	PHILIP J. STEPHENS ET AL: "Complex landscapes of somatic rearrangement in human breast cancer genomes", NATURE, vol. 462, no. 7276, 24 December 2009 (2009-12-24), pages 1005-1010, XP055064910, ISSN: 0028-0836, DOI: 10.1038/nature08645 page 1009, right-hand column, last paragraph; table 3	1,6-8, 11,12,26
X	US 2012/171219 A1 (ACEVEDO-DUNCAN MILDRED [US] ET AL) 5 July 2012 (2012-07-05) claims 1, 12, 13	56-58,63
X,P	NICOLAS STRANSKY ET AL: "The landscape of kinase fusions in cancer", NATURE COMMUNICATIONS, vol. 5, 10 September 2014 (2014-09-10), page 4846, XP055223951, DOI: 10.1038/ncomms5846 page 4, left-hand column, paragraph 2; figures 1, S9	1-72
A	WO 2013/113942 A2 (MAX PLANCK GESELLSCHAFT [DE]) 8 August 2013 (2013-08-08) claims 1, 2, 21, 42, 52	1-72

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2015/040560

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2014038887 A1	13-03-2014	KR 20140033283 A	18-03-2014
		WO 2014038887 A1	13-03-2014
US 2012171219 A1	05-07-2012	NONE	
WO 2013113942 A2	08-08-2013	NONE	