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(54) **Title**: FGR FUSIONS

(57) **Abstract**: FGR (feline Gardner-Rasheed sarcoma viral oncogene homolog) fusions are described herein. Further provided are methods of diagnosing and treating diseases or disorders associated with FGR fusions, such as conditions mediated by FGR aberrant activity, or overexpression.

FGR FUSIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/025,854, filed July 17, 2014, which is incorporated here by reference in its entirety to provide continuity of disclosure.

[0002] FGR (feline Gardner-Rasheed sarcoma viral oncogene homolog) fusions are described herein. Further provided are methods of diagnosing and treating diseases or disorders associated with FGR fusions, such as conditions mediated by aberrant FGR expression or activity, or conditions associated with overexpression of FGR.

[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 present disclosure provides a method for detecting the presence of an FGR gene 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 an FGR gene fusion, to determine whether an FGR gene fusion is present in the biological sample. In some embodiments, the sample can be from, e.g., a cancer patient. In some embodiments, the cancer can be selected from a lung, ovarian, skin, uterine and esophageal cancer. In some embodiments, the cancer is lung squamous carcinoma, ovarian serous cystadenocarcinoma, skin cutaneous melanoma, uterine carcinosarcoma or esophageal carcinoma. In some embodiments, the fusion can be, e.g., a WASF2:FGR fusion. In some embodiments, the WASF2:FGR fusion has all or a part of the nucleotide sequence (such as, e.g., the fusion junction) set forth in SEQ ID NO:1.

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

[0008] The present disclosure 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 an FGR inhibitor or an FGR fusion inhibitor; a method of stratifying a patient population by detecting an FGR fusion; a method of treating a patient; a method of inhibiting the proliferation of cells containing an FGR fusion; a method of reducing an activity of an FGR fusion; a method of treating a condition mediated by aberrant FGR expression or activity; a method of treating a condition characterized by overexpression of FGR; a method of identifying an agent that modulates the activity of an FGR fusion; and a method of monitoring disease burden in a patient having a condition mediated by FGR.

BRIEF DESCRIPTION OF THE FIGURES

[0009] Fig. 1 shows the nucleotide sequence of a WASF2:FGR gene fusion (SEQ ID NO:1) comprising exon number 1 of the WASF2 gene (Accession No. NM_006990) and exon numbers 2-13 of the FGR gene (Accession No. NM_005248). The slash after nucleotide 182 indicates the breakpoint (fusion junction) where the fusion has occurred. The underlined nucleotides are the start codon for FGR transcription.

[0010] Fig. 2 shows the amino acid sequence for FGR (SEQ ID NO:2).

[0011] **Fig. 3A** is a graph showing FGR DNA copy number versus the mRNA expression in lung squamous carcinoma (LUSC) samples; **Fig. 3B** is a graph showing FGR DNA copy number versus mRNA expression in uterine carcinosarcoma (UCS) samples; **Fig. 3C** is a graph showings FGR DNA copy number versus mRNA expression in skin cutaneous melanoma (SKCM) samples; **Fig. 3D** is a graph showings FGR DNA copy number versus mRNA expression in ovarian serous cystadenocarcinoma (OV) samples; and **Fig. 3E** is a graph showings FGR DNA copy number versus mRNA expression in esophageal carcinoma samples.

DETAILED DESCRIPTION

[0012] 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 an FGR 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 FGR. Thus, a new patient population is identified, which is characterized by the presence of an FGR fusion, such as, e.g., a WASF2:FGR gene fusion. This new patient population suffers from or is susceptible to disorders mediated by aberrant FGR expression or activity, or overexpression of FGR, 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 FGR fusions described herein. In some embodiments, the new patient population suffers from or is susceptible to a lung, ovarian, skin, uterine or esophageal cancer characterized by the presence of an FGR fusion. In some embodiments, the cancer is lung squamous carcinoma, ovarian serous cystadenocarcinoma, skin cutaneous melanoma, uterine carcinosarcoma or esophageal carcinoma. New methods of diagnosing and treating the patient population and the FGR fusion cancer subtype are also provided.

[0013] The feline Gardner-Rasheed sarcoma viral oncogene homolog (FGR) is a member of the Src family of protein tyrosine kinases (PTKs). FGR is a known proto-oncogene, and aberrant activity of FGR leads to a variety of disorders such as cancers. The encoded protein localizes to plasma membrane ruffles, and functions as a negative regulator of cell migration and adhesion triggered by the beta-2 integrin signal transduction pathway. Figs. 3A-3E show the DNA copy number versus the mRNA expression (from TCGA) for lung squamous carcinoma (LUSC), uterine carcinosarcoma (UCS), skin cutaneous melanoma (SKCM), ovarian serous cystadenocarcinoma (OV) and esophageal carcinoma; the arrow shows the

sample in which an FGR fusion has occurred. In each case, the fusions occur in samples with near-diploid FGR copy number, and FGR mRNA expression is among the highest of that cancer type. This is evidence that FGR fusions such as the ones described here lead to overexpression of FGR.

[0014] The term "FGR 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 from the FGR gene, particularly the coding region for the kinase domain of FGR, and a fragment from a second non-homologous gene, such that the coding sequence for the kinase domain of FGR is under control of the promoter of the non-homologous gene. FGR fusions can include, but not limited to, FGR gene fusions and FGR protein fusions. FGR and the gene that it is fused to are referred to as "fusion partners." Depending on where the fusion junction is, the fusion protein that is expressed may include at least the kinase domain of FGR. In some embodiments, the protein that is expressed includes the full FGR protein sequence.

FGR Gene Fusions

[0015] FGR gene fusions are generated by a fusion between at least a part of the FGR gene 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 FGR gene may be placed under the transcriptional control of the partner gene promoter, resulting in aberrant FGR expression or activity or overexpression of FGR. The FGR gene fusions disclosed herein can be associated with certain cancers, such as, e.g., lung cancer, ovarian cancer, skin cancer, uterine cancer or esophageal cancer. In some embodiments, the cancers associated with an FGR gene fusion is lung squamous carcinoma, ovarian serous cystadenocarcinoma, skin cutaneous melanoma, uterine carcinosarcoma or esophageal carcinoma. In some embodiments, the FGR gene fusions disclosed herein can be associated with other disorders mediated by aberrant FGR expression or activity, or overexpression of FGR.

[0016] Reference to "all or a portion" or "all or part" of an FGR gene fusion or SEQ ID NO:1, means that the nucleotide sequence comprises the entire FGR gene fusion nucleotide sequence or a fragment of that sequence that comprises the fusion junction or breakpoint between FGR and its fusion partner (such as, e.g., WASF2). 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 FGR gene fusion.

[0017] In some exemplary embodiments, the fusion partner is all or a portion of WASF2 (Wiskott-Aldrich syndrome protein family, member 2). In one embodiment, a fusion includes an exon of the gene WASF2 (e.g., one or more exons encoding a WASF2 promoter or a functional fragment thereof) and an exon of the FGR gene (e.g., one or more exons encoding a kinase domain of FGR or a functional fragment thereof). Such a fusion can be referred to as a WASF2:FGR fusion. As used herein, the 5'-region is upstream of, and the 3'-region is downstream of, a fusion point in one of the component genes. In one embodiment, the WASF2:FGR fusion causes the FGR protein (e.g., a kinase domain of FGR, or a functional fragment thereof) to be over-expressed. In one embodiment, the WASF2:FGR fusion causes the full-length (wild type) FGR protein to be over-expressed.

[0018] In a particular embodiment, the present disclosure provides a WASF2:FGR 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 WASF2 up to exon number 1, in the untranslated region of the WASF2 gene, fused to exon number 2 of FGR, in the untranslated region of the FGR gene. In some embodiments, the WASF2:FGR gene fusion codes for wild-type FGR protein (SEQ ID NO:2). In some embodiments, the WASF2:FGR 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.

[0019] The nucleic acid sequences of FGR gene fusions may be used as probes, primers, or bait to identify nucleotides from a biological sample that include, flank, or hybridize to FGR fusions, such as WASF2:FGR (e.g., all or part of SEQ ID NO: 1), at, e.g., the fusion junction. In certain embodiments, the probe, primer, or bait molecule is an oligonucleotide that allows capture, detection, and/or isolation of an FGR gene fusion in a sample. In certain embodiments, the probes or primers derived from the nucleic acid sequences of FGR 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 FGR gene fusion nucleic acid molecules described herein. The sequence identity between the nucleic acid fragment, e.g., the oligonucleotide and the target FGR gene fusion sequence, need not be exact, so long as the sequences are sufficiently complementary to allow the capture, detection, and/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 between 10 and 15, nucleotides in length that includes the fusion junction of an FGR fusion, such as e.g.,

WASF2:FGR (e.g., all or part of SEQ ID NO: 1). 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 an FGR fusion, such as e.g., WASF2:FGR (e.g., all or part of SEQ ID NO: 1).

[0020] 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 FIG. 1. For example, the nucleic acid fragment can hybridize to a nucleotide sequence that includes the fusion junction between the WASF2 transcript and the FGR transcript, i.e., a nucleotide sequence that includes a portion of SEQ ID NO: 1 (e.g., nucleotides 181-184 of SEQ ID NO:1). In some embodiments, the nucleic acid fragment includes a nucleotide sequence within exon 1 of a WASF2 gene and exons 2-13 of an FGR gene (e.g., a portion of SEQ ID NO: 1 comprising nucleotides 181-184, such as, e.g., a sequence comprising nucleotides 178-187, 173-192, 158-207, 133-232, 108-257, or 83-282 of SEQ ID NO: 1).

[0021] In other embodiments, the nucleic acid fragment includes a bait that comprises a nucleotide sequence that hybridizes to an FGR 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. In exemplary embodiments, the nucleic acid fragments used as bait comprise a nucleotide sequence within SEQ ID NO:1 comprising nucleotides 181-184 (such as, e.g., a sequence comprising nucleotides 178-187, 173-192, 158-207, 133-232, 108-257, or 83-282 of SEQ ID NO:1).

Detection and Diagnostic Methods

[0022] In another aspect, the present disclosure provides a method of detecting the presence of an FGR gene fusion, such as, e.g., a WASF2:FGR fusion as described herein. The presence of an FGR fusion can indicate that the mammal providing the biological sample suffers from or is at risk of developing a disorder mediated by aberrant FGR expression or activity, or overexpression of FGR, such as, e.g., a cancer. The presence of an FGR gene fusion may also indicate that the cancer is treatable with an FGR inhibitor (such as, e.g., a kinase inhibitor or an antibody specific to FGR) or an FGR fusion inhibitor. In some embodiments, the FGR fusion present in the sample is WASF2:FGR and the cancer to be treated is selected from a lung,

ovarian, skin, uterine cancer and esophageal cancer. In some embodiments, the cancer is lung squamous carcinoma, ovarian serous cystadenocarcinoma, skin cutaneous melanoma, uterine carcinosarcoma or esophageal carcinoma.

[0023] In one embodiment, the FGR fusion detected is a nucleic acid molecule. The method includes detecting whether an FGR fusion nucleic acid molecule 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 some embodiments, the sample is from a mammalian (e.g., human) cancer. In some embodiments, the human cancer is selected from a lung, ovarian, skin, uterine cancer and esophageal cancer. In some embodiments, the human cancer is lung squamous carcinoma, ovarian serous cystadenocarcinoma, skin cutaneous melanoma, uterine carcinosarcoma or esophageal carcinoma.

[0024] The methods described herein may be employed to detect the presence of an FGR fusion polynucleotide in a biological sample of a mammal. One such method comprises the steps of obtaining a biological sample from the mammal and contacting that sample with at least one reagent that detects an FGR fusion, to determine whether an FGR fusion is present in the biological sample. The sample can be chosen from one or more of 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.

[0025] In some embodiments, the FGR fusion is detected in a nucleic acid molecule by one or more methods chosen from: nucleic acid hybridization assays (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

[0026] In some embodiments, the reagent hybridizes to an FGR gene fusion. In some embodiments, the reagent hybridizes to a nucleotide sequence that includes the fusion junction of an FGR gene fusion, such as, e.g., nucleotides 178-187, 173-192, 173-192, 158-207, 133-232, 108-257, or 83-282 of SEQ ID NO:1.

[0027] 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 FGR gene fusion, such as, e.g., WASF2:FGR as described herein.

[0028] 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.

[0029] 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 FGR gene fusions. Labeled or unlabeled nucleic acids and/or nucleic acid fragments may be used in reagents for detecting, capturing, and/or isolating FGR gene fusions, such as, e.g., WASF2:FGR (e.g., all or part of SEQ ID NO: 1). 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.

[0030] In some embodiments, the method comprises performing chromosome in situ hybridization with chromosomal DNA from a biological sample to detect the presence of an FGR gene fusion (such as, e.g., WASF2:FGR, 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.

[0031] In other embodiments, the method comprises performing Southern blot with DNA polynucleotides from a biological sample to detect the presence of an FGR gene fusion (such as, e.g., WASF2:FGR, 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

[0032] In certain embodiments, the method of determining the presence of an FGR 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 FGR 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 an FGR fusion polynucleotide in the sample. In specific exemplary embodiments, the FGR gene fusion is WASF2:FGR, such as, e.g., the gene fusion of SEQ ID NO: 1, or a fragment thereof comprising nucleotides 181-184, 178-187, 173-192, 158-207, 133-232, 108-257, or 83-282 of SEQ ID NO:1.

[0033] 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 FGR 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).

[0034] 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

[0035] Another method for determining the presence of an FGR gene fusion molecule (such as, e.g., WASF2:FGR, 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 an FGR gene fusion), thereby determining that the FGR 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.

[0036] 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.

[0037] 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.

[0038] In some embodiments, the method of determining the presence of an FGR fusion (such as, e.g., WASF2:FGR, 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.

[0039] In some embodiments, the method of determining the presence of an FGR fusion (such as, e.g., WASF2:FGR, 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).

[0040] Detection of an FGR gene fusion in a patient can lead to assignment of the patient to the newly identified patient population that bears the FGR gene fusion. Because this patient population can suffer from or be susceptible to a disorder associated with aberrant FGR expression or activity, or overexpression of FGR, detection of an FGR gene fusion can also lead to diagnosis of such disorder. Thus, a further aspect of the present disclosure 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 an FGR gene fusion to determine whether an FGR fusion is present in the biological sample. The detection of an FGR fusion indicates that the patient belongs to the newly identified patient population that bears the FGR gene fusion, and/or the presence of a disorder associated with aberrant FGR expression or activity or overexpression of FGR, such as e.g., a cancer. The detection of an FGR fusion also identifies a new subtype of cancer, which is characterized by the presence of the FGR fusion. In some embodiments, the cancer is lung cancer, ovarian cancer, skin cancer, uterine cancer or esophageal cancer. In some embodiments, the cancer is chosen from, e.g., lung squamous carcinoma, ovarian serous cystadenocarcinoma, skin cutaneous melanoma, uterine carcinosarcoma and esophageal carcinoma. In certain embodiments, the FGR fusion is WASF2:FGR. In some embodiments, the WASF2:FGR fusion has all or a part of the nucleotide sequence (such as, e.g., the fusion junction) set forth in SEQ ID NO:1.

[0041] In some embodiments, the FGR gene fusion is detected prior to initiating, during, or after, a treatment of a patient with, e.g., an FGR inhibitor or an FGR fusion inhibitor. In one embodiment, the FGR gene fusion is detected at the time the patient is diagnosed with a disorder, e.g., a cancer. In other embodiment, the FGR 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 an FGR fusion, such as, e.g., WASF2:FGR, 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., an FGR inhibitor treatment (e.g., a kinase inhibitor treatment) or an FGR 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., an FGR inhibitor or an FGR 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 FGR fusion in a patient sample).

[0042] In certain embodiments, upon detection of an FGR gene fusion in a patient's biological sample, the patient is identified as likely to respond to a treatment that comprises an FGR inhibitor or an FGR fusion inhibitor. In some embodiments, the FGR fusion detected is a WASF2:FGR fusion.

[0043] A further aspect of the present disclosure provides a method of selecting a treatment option by detecting an FGR fusion. The method comprises obtaining a biological sample from a patient and exposing the sample to at least one reagent that detects an FGR gene fusion to determine whether an FGR fusion is present in the biological sample. The detection of the FGR gene fusion indicates the likelihood of the patient responding to treatment with an FGR inhibitor or an FGR fusion inhibitor. The method may be augmented or personalized by evaluating the effect of a variety of FGR inhibitors or FGR fusion inhibitors on the biological sample shown to contain an FGR fusion to determine the most appropriate inhibitor to administer. In certain embodiments, the FGR fusion is WASF2:FGR. In some embodiments, the WASF2:FGR fusion has all or a part of the nucleotide sequence (such as, e.g., the fusion junction) set forth in SEQ ID NO:1.

Methods of Treatment

[0044] Alternatively, or in combination with the detection and diagnostic methods described herein, the present disclosure provides methods for treating the newly identified patient population and the new FGR fusion cancer subtype, which are characterized by the presence of an FGR fusion. The patient population and cancer subtype can be associated with or predict the onset of a condition mediated by aberrant FGR expression or activity, or overexpression of FGR, such as, e.g., a cancer or a tumor harboring an FGR fusion. The methods comprise administering a therapeutic agent, e.g., an FGR inhibitor, such as e.g., a kinase inhibitor or an antibody specific to FGR, or an FGR fusion inhibitor, such as, e.g., an RNA inhibitor that recognizes FGR or the fusion junction of an FGR gene fusion, including but not limited to siRNA, dsRNA, shRNA, or any other antisense nucleic acid inhibitor, as described herein, alone or in combination with e.g., other chemotherapeutic agents or procedures, in an amount sufficient to treat a condition mediated by aberrant FGR expression or activity, or overexpression of FGR, 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.

[0045] In certain embodiments, the FGR fusion of the invention may be inhibited by an FGR inhibitor or an FGR fusion inhibitor. In some embodiments, the therapeutic agent is an FGR inhibitor, such as, e.g., a compound, biological or chemical, which inhibits, directly or indirectly, the expression and/or activity of FGR. For example, the FGR inhibitors may be an

antibody (such as, e.g., an anti-FGR antibody) or a small molecule inhibitor (such as, e.g., a kinase inhibitor). In some embodiments, the inhibitors may act directly on FGR itself, modify the activity of FGR, or inhibit the expression of FGR. In other embodiments, the inhibitors may indirectly inhibit FGR activity by inhibiting the activity of proteins or molecules other than FGR itself. For example, the inhibitors may modulate the activity of regulatory kinases that phosphorylate or dephosphorylate FGR, interfere with binding of ligands, or inhibit the activity of interacting or downstream proteins or molecules.

[0046] In other embodiments, the therapeutic agent is an FGR fusion inhibitor that inhibits the expression of nucleic acid encoding an FGR fusion. Examples of FGR fusion inhibitors include nucleic acid molecules, for example, antisense molecules, ribozymes, RNAi, or triple helix molecules, that hybridize to a nucleic acid encoding an FGR 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 an FGR fusion. In some embodiments, the antisense molecule recognizes the WASF2:FGR fusion junction, or an RNA inhibitor, including but not limited to small interfering RNA (siRNA), double stranded RNA (dsRNA), and short-hairpin RNA (shRNA), or any other antisense nucleic acid inhibitor, specific to the WASF2:FGR fusion junction.

[0047] 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 FGR expression or activity, or overexpression of FGR, such as, delaying or minimizing one or more symptoms associated with a cancer or a tumor harboring an FGR fusion (such as, e.g., WASF2:FGR, 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 condition. 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 FGR expression or activity or overexpression of FGR, or enhances the therapeutic efficacy of another therapeutic agent.

[0048] In certain embodiments, the cancer or tumor harboring an FGR fusion is lung cancer, ovarian cancer, skin cancer, uterine cancer or esophageal cancer. In some embodiments, the cancer or tumor harboring an FGR fusion is chosen from, e.g., lung squamous carcinoma, ovarian serous cystadenocarcinoma, skin cutaneous melanoma, uterine carcinosarcoma, and esophageal carcinoma.

[0049] In some embodiments, the patient to be treated is suffering from a condition mediated by aberrant FGR expression or activity, or overexpression of FGR, e.g., cancer or a tumor harboring an FGR fusion (such as, e.g., lung squamous carcinoma, ovarian serous cystadenocarcinoma, skin cutaneous melanoma, uterine carcinosarcoma or esophageal carcinoma). The method for treating the condition comprises administering to the patient a therapeutically effective amount of an FGR inhibitor or an FGR fusion inhibitor as described above.

Screening Methods

[0050] Therapeutic agents, such as e.g., FGR inhibitors or FGR fusion inhibitors, used in the therapeutic methods of the present disclosure can be evaluated using the screening assays described herein. Thus, the present disclosure provides a method of identifying an agent useful for treating a condition mediated by aberrant FGR expression or activity, overexpression of FGR, e.g., cancer or a tumor harboring an FGR fusion, which in some embodiments is selected from a lung, ovarian, skin, uterine cancer and esophageal cancer (such as e.g., lung squamous carcinoma, ovarian serous cystadenocarcinoma, skin cutaneous melanoma, uterine carcinosarcoma or esophageal carcinoma), comprising contacting a cell expressing an FGR gene fusion with a candidate agent and using the methods described herein 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 an FGR 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., an FGR fusion-expressing cell present in a subject, e.g., an animal subject (e.g., an in vivo animal model).

[0051] Exemplary parameters to evaluate in determining the efficacy of a therapeutic agent for treating a condition mediated by aberrant FGR expression or activity, or overexpression of FGR, such as, e.g., a cancer or a tumor harboring an FGR fusion, include one or more of:

(i) a change in an activity of a cell containing an FGR fusion (e.g., a tumor cell or a recombinant cell), e.g., a change in proliferation, morphology, migration, adhesion, or tumorigenicity of the cell;

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

(iii) a change in the level, e.g., expression level (transcription and/or translation) of an FGR gene fusion; or

(iv) a change in an activity of a signaling pathway involving FGR (e.g., the beta-2 integrin signaling pathway), e.g., phosphorylation or activity of an interacting or downstream protein, or expression of a downstream gene.

[0052] In some embodiments, the FGR fusion is a WASF2:FGR fusion.

[0053] In other embodiments, a change in an activity of a cell expressing an FGR fusion, such as, e.g., WASF2:FGR, 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 an FGR fusion nucleic acid, e.g., is a recombinant cell transfected with an FGR fusion nucleic acid. The transfected cell can show a change in response to the expressed FGR fusion, e.g., increased proliferation, increased migration, decreased adhesion, 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: e.g., proliferation, migration, tumorigenicity, transformed morphology, in the presence of the candidate agent can be indicative of an inhibitor of an FGR fusion. In other embodiments, a change in binding activity or phosphorylation of FGR or its interacting or downstream proteins or molecules as described herein is detected.

[0054] 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 an FGR fusion (e.g., tumorigenic cells expressing an FGR 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, e.g., tumor growth, tumor size, tumor burden, tumor metastasis, or survival is evaluated. A decrease in one or more of tumor growth, tumor size, tumor burden, or tumor metastasis, or an increased survival is indicative that the candidate agent is an inhibitor.

[0055] Another aspect of the present disclosure provides a method or assay for screening for agents that modulate (e.g., inhibit) the expression or activity of an FGR fusion as described herein. The method includes contacting e.g., an FGR fusion, or a cell expressing an FGR fusion, with a candidate agent; and detecting a change in a parameter associated with an FGR fusion, e.g., a change in the expression or an activity of the FGR 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 FGR fusion is detected, the candidate agent is identified as an inhibitor. In another embodiment, if an increase in expression or activity of the FGR fusion is detected, the candidate agent is identified as an activator. In certain embodiments, the fusion is an FGR gene fusion, e.g., a WASF2:FGR fusion.

[0056] 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 an FGR 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., an FGR expressing cell present in a subject, e.g., an animal subject (e.g., an in vivo animal model).

[0057] Exemplary parameters evaluated in identifying an agent that modulates the activity of an FGR fusion (e.g., a WASF2:FGR fusion) include one or more of:

(i) a change in an activity of a cell containing an FGR fusion (e.g., a tumor cell or a recombinant cell), e.g., a change in proliferation, morphology, migration, adhesion, or tumorigenicity of the cell;

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

(iii) a change in the level, e.g., expression level (transcription and/or translation) of an FGR fusion; or

(iv) a change in an activity of a signaling pathway involving FGR (e.g., the beta-2 integrin signaling pathway), e.g., phosphorylation or activity of an interacting downstream protein, or expression of a downstream gene.

Methods for Validating FGR Fusions

[0058] FGR gene fusions (such as, e.g., WASF2:FGR 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 FGR 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 FGR protein can be produced by standard solid-phase DNA synthesis. Alternatively the FGR 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.

[0059] Expression vectors containing the FGR gene fusion (such as, e.g., WASF2:FGR gene fusion) can be introduced into host cells to thereby produce an FGR protein. The FGR 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.

[0060] Cells harboring the expression vector carrying the recombinant FGR 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 FGR protein by comparing the samples from cells transfected with the vector containing the FGR 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. Whether the FGR gene fusion is oncogenic can be assessed by measuring capacity of the expressed FGR 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 an FGR gene fusion (such as, e.g., WASF2:FGR gene fusion) as a potential target for treating patients that harbor these fusions.

[0061] 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.

[0062] 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., an FGR inhibitor, can be detected. For example, a decrease in one or more of: proliferation, tumorigenicity, and transformed morphology, in the presence of the BETA inhibitor can be indicative of an inhibitor of a fusion. In other embodiments, a change in binding activity or phosphorylation of FGR or its interacting or downstream proteins or molecules is detected.

[0063] 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," "having", and "containing," as well as other forms, such as "includes" and "included," "has" and "had," and "contains" and "contained," is not limiting. All ranges given in the application encompass the endpoints unless stated otherwise.

[0064] 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 an FGR 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 an FGR fusion to determine whether an FGR fusion is present in the biological sample,

wherein the detection of the FGR fusion indicates the presence of an FGR 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 an FGR fusion to determine whether an FGR fusion is present in the biological sample,

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

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 an FGR fusion to determine whether an FGR fusion is present in the biological sample; and

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

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

(a) obtaining a biological sample from the patient

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

wherein the detection of the FGR fusion indicates the likelihood of the patient responding to treatment with an FGR inhibitor or an FGR fusion inhibitor.

5. A method of stratifying a patient population by detecting an FGR 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 an FGR fusion to determine the presence or absence of an FGR fusion; and

(c) selecting patients having FGR fusion for treatment with an FGR inhibitor or an FGR fusion inhibitor.

6. The method of any one of claims 1-5, wherein the method of detecting an FGR 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 an FGR 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 an FGR 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 an FGR 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 squamous carcinoma.

14. The method of claim 12, wherein the human cancer is ovarian serous cystadenocarcinoma.

15. The method of claim 12, wherein the human cancer is skin cutaneous melanoma.

16. The method of claim 12, wherein the human cancer is uterine carcinosarcoma.

17. The method of claim 12, wherein the human cancer is esophageal carcinoma.

18. The method of any of claims 1-17, wherein the FGR gene fusion is a WASF2:FGR gene fusion.

19. The method of claim 18, wherein the WASF2:FGR gene fusion comprises all or part of the nucleotide sequence set forth in SEQ ID NO:1 comprising the fusion junction.

20. The method of any of claims 1-19, wherein the reagent is a probe or primer that hybridizes to the fusion junction of an FGR fusion.

21. The method of any of claims 1-20, wherein the reagent hybridizes to nucleotides 178-187, 173-192, 158-207, 133-232, 108-257, or 83-282 of SEQ ID NO:1.

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

23. The method of claim 22, wherein the knowledge about the presence of an FGR fusion is acquired by the method of any one of claims 1-21.

24. The method of claim 21 or 22, wherein the patient is suffering from a cancer.

25. The method of claim 24, wherein the cancer is lung squamous carcinoma.

26. The method of claim 24, wherein the cancer is ovarian serous cystadenocarcinoma.

27. The method of claim 24, wherein the cancer is skin cutaneous melanoma.

28. The method of claim 24, wherein the cancer is uterine carcinosarcoma.

29. The method of claim 24, wherein the human cancer is esophageal carcinoma.

30. A method of inhibiting the proliferation of cells containing an FGR fusion, the method comprising contacting the cells with an FGR inhibitor or an FGR fusion inhibitor.

31. A method of reducing an activity of an FGR fusion comprising contacting the FGR fusion with an agent that inhibits an activity or expression of FGR.

32. The method of claim 30 or 31, wherein the contacting step is effected in vitro.

33. The method of claim 30 or 31, wherein the contacting step is effected in cells in culture.

34. The method of claim 30 or 31, wherein the contacting step is effected in vivo.

35. The method of any one of claims 32-34, wherein the agent that inhibits an activity or expression of FGR is an FGR inhibitor or an FGR fusion inhibitor.

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

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

38. A method of treating a cancer, the method comprising administering to a subject a therapeutically effective amount of an FGR inhibitor or an FGR fusion inhibitor.

39. The method of claim 38, wherein the cancer is lung squamous carcinoma.

40. The method of claim 38, wherein the cancer is ovarian serous cystadenocarcinoma.

41. The method of claim 38, wherein the cancer is skin cutaneous melanoma.

42. The method of claim 38, wherein the cancer is uterine carcinosarcoma.

43. The method of claim 38, wherein the human cancer is lung squamous carcinoma.

44. The method of any of claims 21-40, wherein the agent that inhibits an activity or expression of FGR or the FGR inhibitor is a kinase inhibitor.

45. The method of any of claims 22-42, wherein the agent that inhibits an activity or expression of FGR or the FGR inhibitor is an anti-FGR antibody.

46. The method of any one of claims 22-42, wherein the agent that inhibits an activity or expression of FGR or the FGR inhibitor is an FGR fusion inhibitor.

47. A method of identifying an agent useful for treating lung squamous carcinoma, the method comprising contacting a cell expressing an FGR 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.

48. A method of identifying an agent useful for treating ovarian serous

cystadenocarcinoma, the method comprising contacting a cell expressing an FGR 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.

49. A method of identifying an agent useful for treating skin cutaneous melanoma, the method comprising contacting a cell expressing an FGR 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.

50. A method of identifying an agent useful for treating uterine carcinosarcoma, the method comprising contacting a cell expressing an FGR 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.

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

52. The method of any of claims 22-50, wherein the FGR fusion is a WASF2:FGR fusion.

53. The method of any one of claims 22-51, wherein the FGR fusion comprises all of part of the nucleotide sequence set forth in SEQ ID NO:1 comprising the fusion junction.

FIG. 1

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   1  GCCCAGGGCG GGGAATCGCG TAATGGCGGA CACAGGCAGG GCGAGCGCGG    50
  51  CTGGGGGCGT AGCGCGCTGA GGGGGTCCGG CCGTTTGGCA GCCCGCGAGG   100
 101  CGGTCCGCGG GAGCACACTC TGTGCGGAGA CTGGGCGGCC GGCCGACCCT   150
 151  TCCTGTCGCT GACGGCGACT GCGGGAGGCC AG/GTCTCTGA CCCCTCCCAA  200
 201  GGATCATGCC GCAGCCCCAC TGACCCAGGA GTAGGGGCCT AAGGGCAGGG   250
 251  AACCTGGAAT GGGCTGTGTG TTCTGCAAGA AATTGGAGCC GGTGGCCACG   300
 301  GCCAAGGAGG ATGCTGGCCT GGAAGGGGAC TTCAGAAGCT ACGGGGCAGC   350
 351  AGACCACTAT GGGCCTGACC CCACTAAGGC CCGGCCTGCA TCCTCATTTG   400
 401  CCCACATCCC CAACTACAGC AACTTCTCCT CTCAGGCCAT CAACCCTGGC   450
 451  TTCCTTGATA GTGGCACCAT CAGGGGTGTG TCAGGGATTG GGGTGACCCT   500
 501  GTTCATTGCC CTGTATGACT ATGAGGCTCG AACTGAGGAT GACCTCACCT   550
 551  TCACCAAGGG CGAGAAGTTC CACATCCTGA ACAATACTGA AGGTGACTGG   600
 601  TGGGAGGCTC GGTCTCTCAG CTCCGGAAAA ACTGGCTGCA TTCCCAGCAA   650
 651  CTACGTGGCC CCTGTTGACT CAATCCAAGC TGAAGAGTGG TACTTTGGAA   700
 701  AGATTGGGAG AAAGGATGCA GAGAGGCAGC TGCTTTCACC AGGCAACCCC   750
 751  CAGGGGGCCT TTCTCATTCG GGAAAGCGAG ACCACCAAAG GTGCCTACTC   800
 801  CCTGTCCATC CGGGACTGGG ATCAGACCAG AGGCGATCAT GTGAAGCATT   850
 851  ACAAGATCCG CAAACTGGAC ATGGGCGGCT ACTACATCAC CACACGGGTT   900
 901  CAGTTCAACT CGGTGCAGGA GCTGGTGCAG CACTACATGG AGGTGAATGA   950
 951  CGGGCTGTGC AACCTGCTCA TCGCGCCCTG CACCATCATG AAGCCGCAGA  1000
1001  CGCTGGGCCT GGCCAAGGAC GCCTGGGAGA TCAGCCGCAG CTCCATCACG  1050
1051  CTGGAGCGCC GGCTGGGCAC CGGCTGCTTC GGGGATGTGT GGCTGGGCAC  1100
1101  GTGGAACGGC AGCACTAAGG TGGCGGTGAA GACGCTGAAG CCGGGCACCA  1150
1151  TGTCCCCGAA GGCCTTCCTG GAGGAGGCGC AGGTCATGAA GCTGCTGCGG  1200
1201  CACGACAAGC TGGTGCAGCT GTACGCCGTG GTGTCGGAGG AGCCCATCTA  1250
1251  CATCGTGACC GAGTTCATGT GTCACGGCAG CTTGCTGGAT TTTCTCAAGA  1300
1301  ACCCAGAGGG CCAGGATTTG AGGCTGCCCC AATTGGTGGA CATGGCAGCC  1350
1351  CAGGTAGCTG AGGGCATGGC CTACATGGAA CGCATGAACT ACATTCACCG  1400
1401  CGACCTGAGG GCAGCCAACA TCCTGGTTGG GGAGCGGCTG GCGTGCAAGA  1450
1451  TCGCAGACTT TGGCTTGGCG CGTCTCATCA AGGACGATGA GTACAACCCC  1500
1501  TGCCAAGGTT CCAAGTTCCC CATCAAGTGG ACAGCCCCAG AAGCTGCCCT  1550
1551  CTTTGGCAGA TTCACCATCA AGTCAGACGT GTGGTCCTTT GGGATCCTGC  1600
1601  TCACTGAGCT CATCACCAAG GGCCGAATCC CCTACCCAGG CATGAATAAA  1650
1651  CGGGAAGTGT TGGAACAGGT GGAGCAGGGC TACCACATGC CGTGCCCTCC  1700
1701  AGGCTGCCCA GCATCCCTGT ACGAGGCCAT GGAACAGACC TGGCGTCTGG  1750
1751  ACCCGGAGGA GAGGCCTACC TTCGAGTACC TGCAGTCCTT CCTGGAGGAC  1800
1801  TACTTCACCT CCGCTGAACC ACAGTACCAG CCCGGGGATC AGACATAGCC  1850
1851  TGTCCGGGCA TCAACCCTCT CTGGCGGTGG CCACCAGTCC TTGCCAATCC  1900
1901  CCAGAGCTGT TCTTCCAAAG CCCCCAGGCT GGCTTAGAAC CCCATAGAGT  1950
1951  CCTAGCATCA CCGAGGACGT GGCTGCTCTG ACACCACCTA GGGCAACCTA  2000
2001  CTTGTTTTAC AGATGGGGCA AAAGGAGGCC CAGAGCTGAT CTCTCATCCG  2050
2051  CTCTGGCCCC AAGCACTATT TCTTCCTTTT CCACTTAGGC CCCTACATGC  2100
2101  CTGTAGCCTT TCTCACTCCA TCCCCACCCA AAGTGCTCAG ACCTTGTCTA  2150
2151  GTTATTTATA AAACTGTATG TACCTCCCTC ACTTCTCTCC TATCACTGCT  2200
2201  TTCCTACTCT CCTTTTATCT CACTCTAGTC CAGGTGCCAA GAATTTCCCT  2250
2251  TCTACCCTCT ATTCTCTTGT GTCTGTAAGT TACAAAGTCA GGAAAAGTCT  2300
2301  TGGCTGGACC CCTTTCCTGC TGGGTGGATG CAGTGGTCCA GGACTGGGGT  2350
2351  CTGGGCCCAG GTTTGAGGGA GAAGGTTGCA GAGCACTTCC CACCTCTCTG  2400
2401  AATAGTGTGT ATGTGTTGGT TTATTGATTC TGTAAATAAG TAAAATGACA  2450
2451  ATATGAATCC TCAAACCATG AAAAAAAAAA AAAAAAAA               2488
```

(SEQ ID NO:1)

FIG. 2

```
  1  MGCVFCKKLE PVATAKEDAG LEGDFRSYGA ADHYGPDPTK ARPASSFAHI    50
 51  PNYSNFSSQA INPGFLDSGT IRGVSGIGVT LFIALYDYEA RTEDDLTFTK   100
101  GEKFHILNNT EGDWWEARSL SSGKTGCIPS NYVAPVDSIQ AEEWYFGKIG   150
151  RKDAERQLLS PGNPQGAFLI RESETTKGAY SLSIRDWDQT RGDHVKHYKI   200
201  RKLDMGGYYI TTRVQFNSVQ ELVQHYMEVN DGLCNLLIAP CTIMKPQTLG   250
251  LAKDAWEISR SSITLERRLG TGCFGDVWLG TWNGSTKVAV KTLKPGTMSP   300
301  KAFLEEAQVM KLLRHDKLVQ LYAVVSEEPI YIVTEFMCHG SLLDFLKNPE   350
351  GQDLRLPQLV DMAAQVAEGM AYMERMNYIH RDLRAANILV GERLACKIAD   400
401  FGLARLIKDD EYNPCQGSKF PIKWTAPEAA LFGRFTIKSD VWSFGILLTE   450
451  LITKGRIPYP GMNKREVLEQ VEQGYHMPCP PGCPASLYEA MEQTWRLDPE   500
501  ERPTFEYLQS FLEDYFTSAE PQYQPGDQT                         529
```

(SEQ ID NO:2)

FIG. 3A

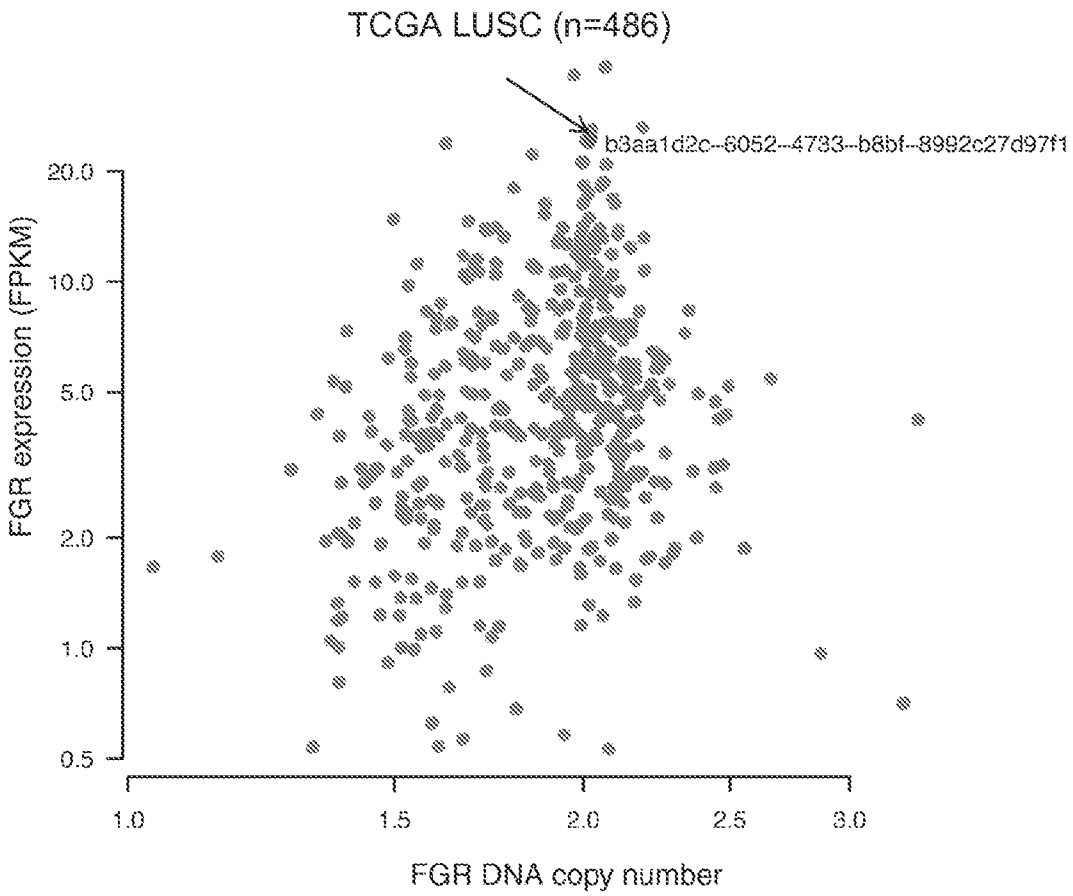

TCGA LUSC (n=486)
b3aa1d2c--6052--4733--b8bf--8992c27d97f1
FGR expression (FPKM)
20.0
10.0
5.0
2.0
1.0
0.5
1.0
1.5
2.0
2.5
3.0
FGR DNA copy number

FIG. 3B

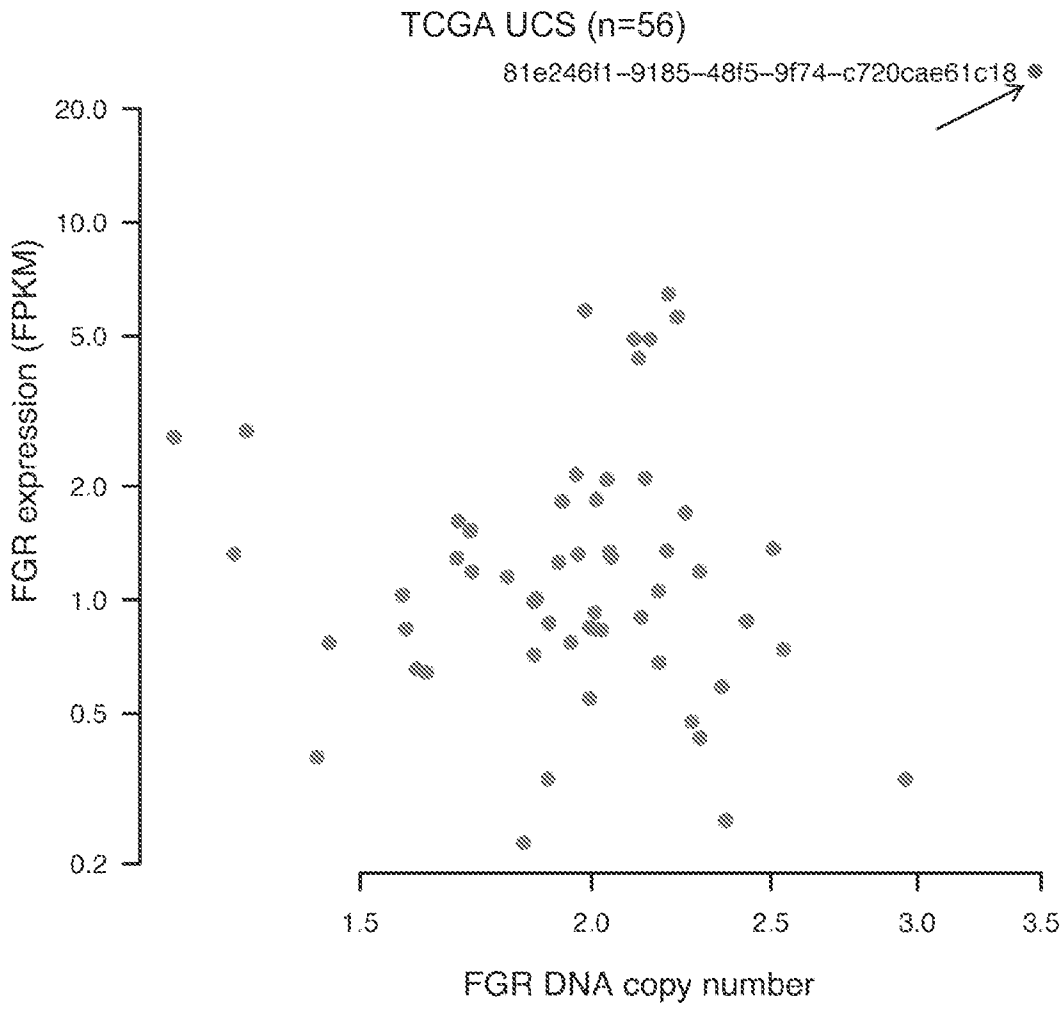

TCGA UCS (n=56)
81e246f1-9185-48f5-9f74-c720cae61c18
20.0
10.0
5.0
2.0
1.0
0.5
0.2
FGR expression (FPKM)
1.5
2.0
2.5
3.0
3.5
FGR DNA copy number

FIG. 3C

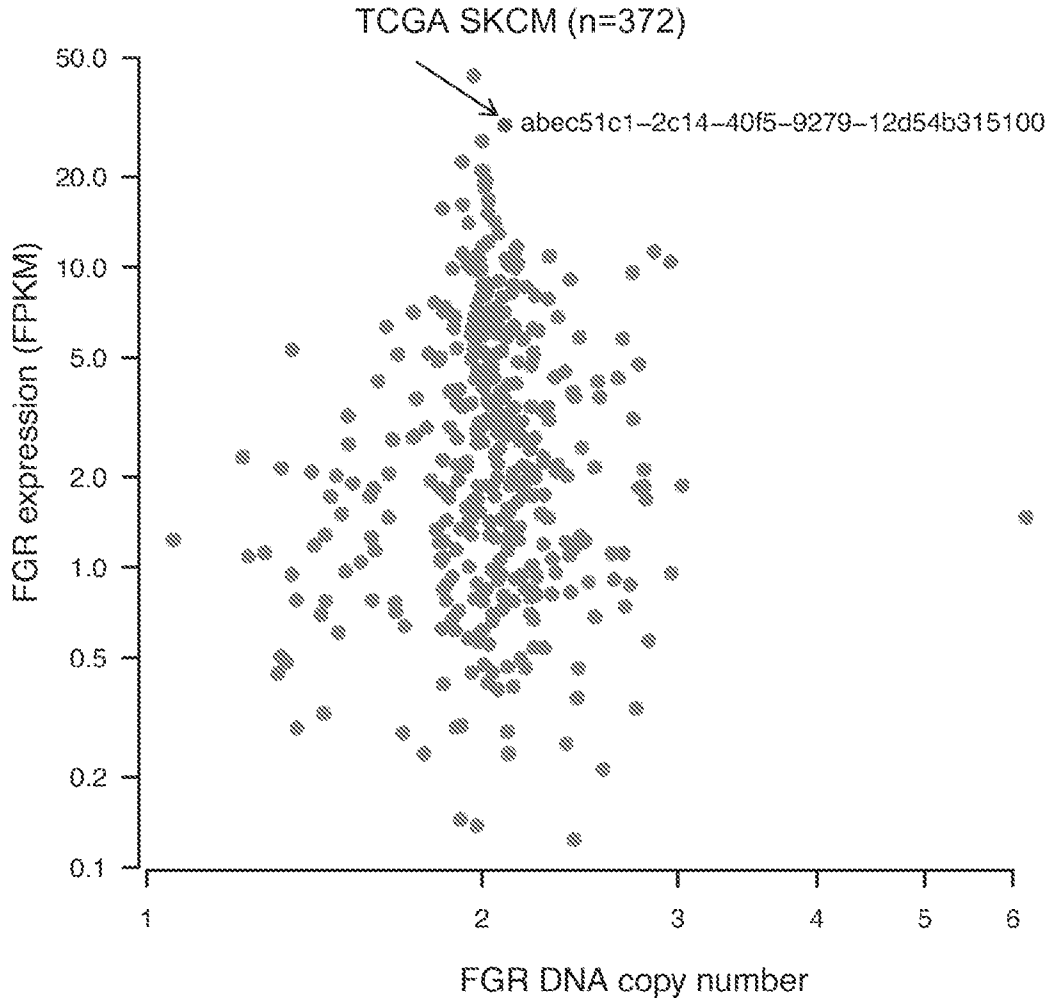

TCGA SKCM (n=372)
abec51c1-2c14-40f5-9279-12d54b315100
50.0
20.0
10.0
5.0
2.0
1.0
0.5
0.2
0.1
FGR expression (FPKM)
1
2
3
4
5
6
FGR DNA copy number

FIG. 3D

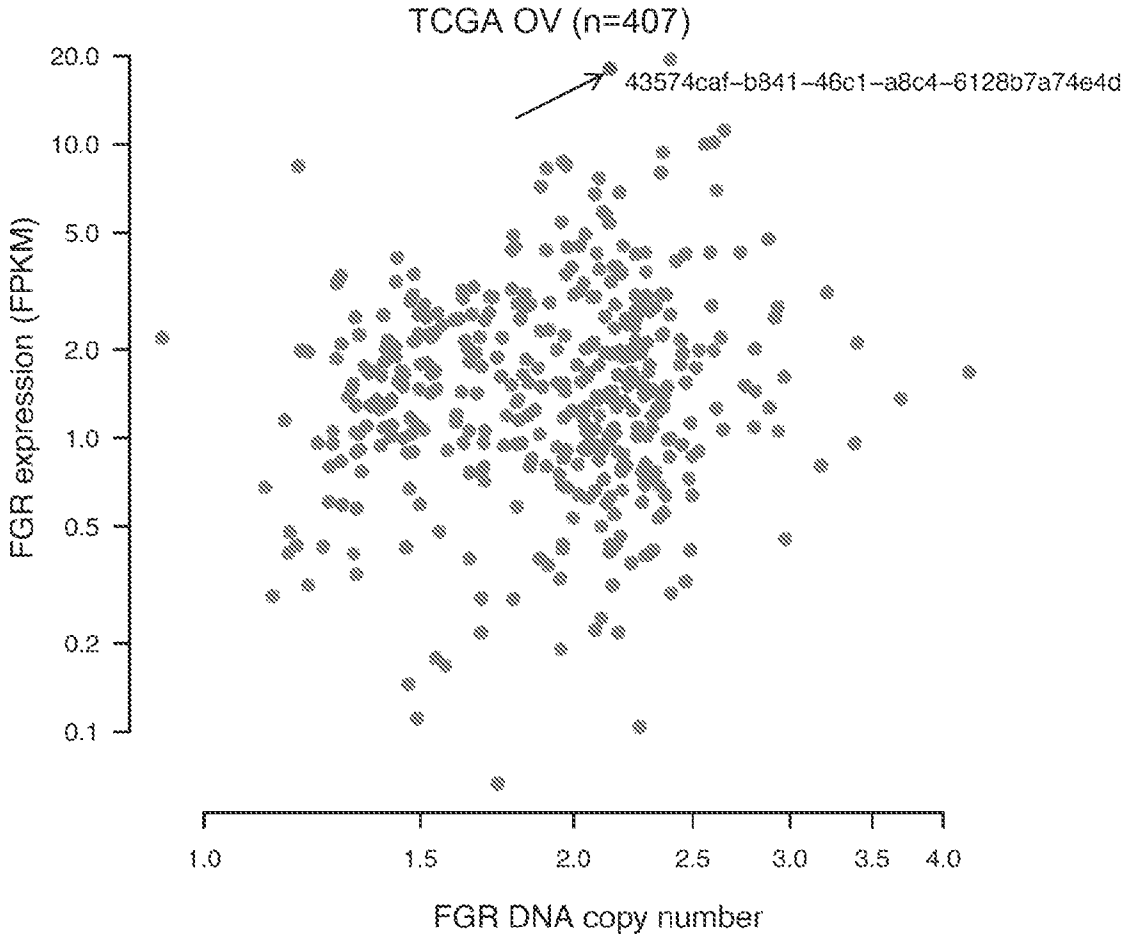
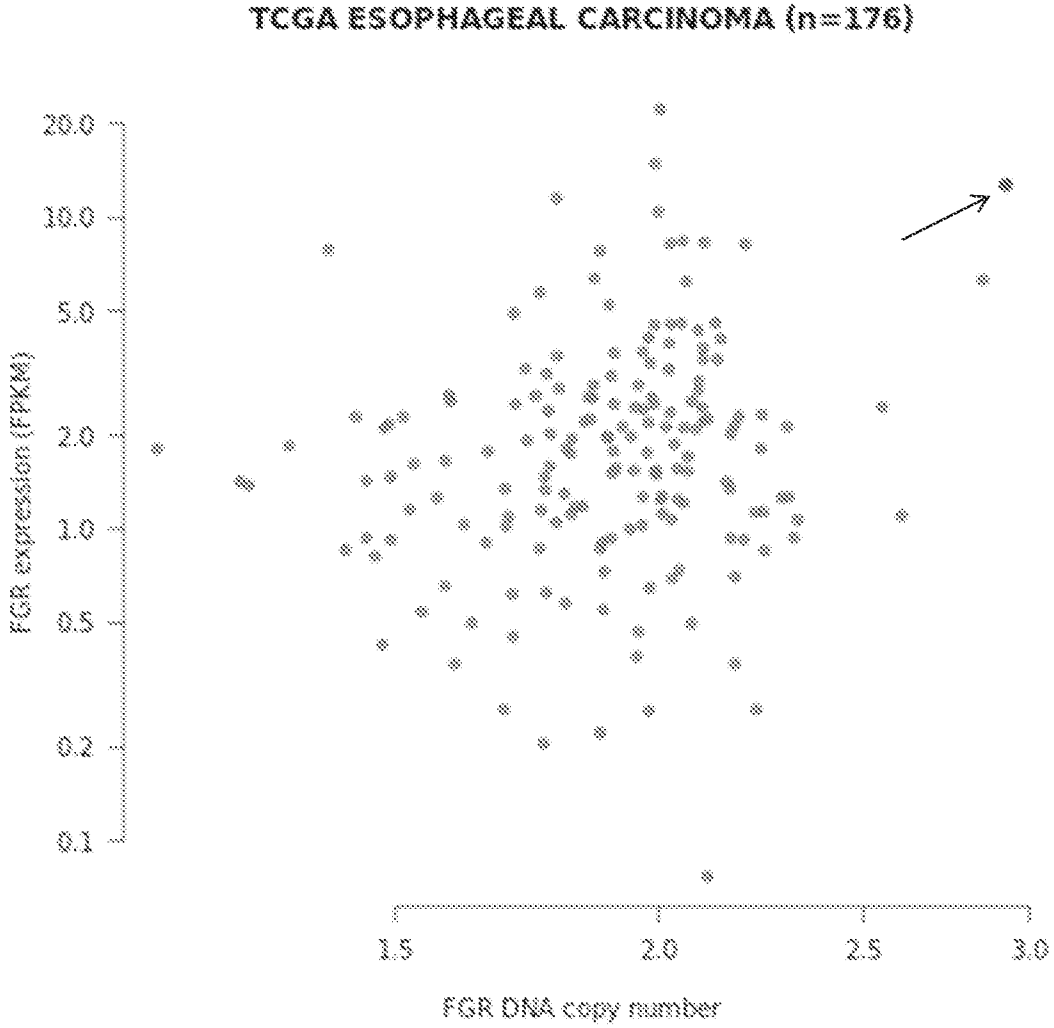

TCGA OV (n=407)
43574caf-b841-46c1-a8c4-6128b7a74e4d
FGR expression (FPKM)
20.0
10.0
5.0
2.0
1.0
0.5
0.2
0.1
1.0
1.5
2.0
2.5
3.0
3.5
4.0
FGR DNA copy number

FIG. 3E


TCGA ESOPHAGEAL CARCINOMA (n=176)
FGR expression (FPKM)
20.0
10.0
5.0
2.0
1.0
0.5
0.2
0.1
1.5
2.0
2.5
3.0
FGR DNA copy number

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/040553

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP 2005 052018 A (KYOWA HAKKO KOGYO KK) 3 March 2005 (2005-03-03) figure 2 -----	1
X	J. C. MONTERO ET AL: "Inhibition of Src Family Kinases and Receptor Tyrosine Kinases by Dasatinib: Possible Combinations in Solid Tumors", CLINICAL CANCER RESEARCH, vol. 17, no. 17, 1 September 2011 (2011-09-01), pages 5546-5552, XP055217710, ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-10-2616 abstract ----- -/--	36-46

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

[X] See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 October 2015	13/10/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Aslund, Fredrik

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	H.-S. KIM ET AL: "Functional Roles of Src and Fgr in Ovarian Carcinoma", CLINICAL CANCER RESEARCH, vol. 17, no. 7, 7 February 2011 (2011-02-07), pages 1713-1721, XP055217711, ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-10-2081 page 6	36-46
X	E. ZUCCATO ET AL: "Sorting of Fas ligand to secretory lysosomes is regulated by mono-ubiquitylation and phosphorylation", JOURNAL OF CELL SCIENCE, vol. 120, no. 1, 12 December 2006 (2006-12-12), pages 191-199, XP055217743, ISSN: 0021-9533, DOI: 10.1242/jcs.03315 page 194; figure 1	51

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2015/040553

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 2005052018 A	03-03-2005	JP 4401702 B2	20-01-2010
		JP 2005052018 A	03-03-2005