

(19)



(11)

**EP 2 692 358 A1**

(12)

**EUROPEAN PATENT APPLICATION**  
published in accordance with Art. 153(4) EPC

(43) Date of publication:

**05.02.2014 Bulletin 2014/06**

(51) Int Cl.:

**A61K 39/395** (2006.01)     **A61P 1/00** (2006.01)  
**A61P 35/00** (2006.01)     **G01N 33/48** (2006.01)  
**G01N 33/53** (2006.01)     **G01N 33/574** (2006.01)  
**C07K 16/28** (2006.01)

(21) Application number: **12765858.1**

(22) Date of filing: **30.03.2012**

(86) International application number:

**PCT/JP2012/058668**

(87) International publication number:

**WO 2012/133814 (04.10.2012 Gazette 2012/40)**

(84) Designated Contracting States:

**AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**

- **SHIMIZU, Kazuharu**  
Kyoto-shi

**Kyoto 606-8501 (JP)**

- **SHIMADA, Yutaka**  
Toyama-shi

**Toyama 930-0194 (JP)**

- **TSUKADA, Kazuhiro**  
Toyama-shi

**Toyama 930-0194 (JP)**

(30) Priority: **31.03.2011 JP 2011080261**

(71) Applicant: **Kyoto University**

**Kyoto-shi, Kyoto 606-8501 (JP)**

(74) Representative: **Keirstead, Tanis Evelyne et al**

**Mewburn Ellis LLP**

**33 Gutter Lane**

**London EC2V 8AS (GB)**

(72) Inventors:

- **TSUCHIYA, Soken**

**Kyoto-shi**

**Kyoto 606-8501 (JP)**

- **TSUJIMOTO, Gozoh**

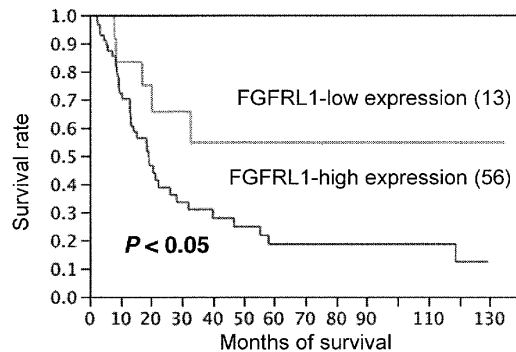
**Kyoto-shi**

**Kyoto 606-8501 (JP)**

(54) **THERAPEUTIC AGENT FOR CANCER, AND METHOD FOR DETERMINING PROGNOSIS OF CANCER**

(57) Disclosed are a novel therapeutic agent for cancer such as esophageal squamous cell carcinoma, a method for predicting the prognosis of cancer, and a method for detecting, or predicting the prognosis of, cancer such as esophageal squamous cell carcinoma using a sample that can be collected less invasively. The therapeutic agent for cancer comprises as an effective component an antibody that undergoes antigen-antibody reaction with FGFR1 to suppress the growth of cancer cells, or an antigen-binding fragment thereof. The method for predicting the prognosis of cancer comprises investigating the expression level of FGFR1 in a cancer tissue separated from a living body, and, in this method, a high expression level of FGFR1 indicates poor prognosis. The method for detecting cancer comprises measuring FGFR1 or a fragment thereof extracted from a body tissue, or FGFR1 or a fragment thereof in blood separated from a living body, and, in this method, a higher concentration of FGFR1 or the fragment thereof contained therein than the concentration of FGFR1 or the

fragment thereof in the tissue or blood of a healthy individual indicates the presence of cancer.



**Fig.2**

**EP 2 692 358 A1**

**Description**

## TECHNICAL FIELD

5 **[0001]** The present invention relates to a therapeutic agent for cancer, a method for predicting the prognosis of cancer, and a method for detecting cancer.

## BACKGROUND ART

10 **[0002]** Esophagus cancer is a cancer with the eighth highest incidence rate and the sixth highest number of deaths. In Japan, esophageal squamous cell carcinoma (ESCC) accounts for not less than 90% of esophagus cancer. ESCC is a highly malignant cancer that frequently causes distant metastasis and recurrence, and its prognosis is generally poor.

15 **[0003]** On the other hand, it has been reported that ovarian cancer shows abnormal expression of fibroblast growth factor receptor like-1 (hereinafter referred to as "FGFRL1") (Non-patent Document 1). However, in this report, no statistical analysis was carried out for the expression level of FGFRL1, and no analysis on the function of FGFRL1 was performed. Thus, this report never leads to inference of promotion of the cell growth by FGFRL1, utilization of its expression level for prediction of the prognosis, or its industrial applicability. Further, although it has been reported that microRNA (miRNA)-210 is involved in oncogenesis and that one of its target genes is FGFRL1 (Non-patent Document 2), this report does not clearly suggest utilization of FGFRL1 for prediction of the prognosis or for therapeutic agents. Further, the present inventors previously discovered that, in esophageal squamous cell carcinoma, microRNA-210 regulates the growth of cancer cells via FGFRL1 (Non-patent Document 3). However, this report only elucidated that a target gene of microRNA-210 is FGFRL1 and discussed about its downstream pathway, and no suggestion was made about possible use of an anti-FGFRL1 antibody for a therapeutic agent for cancer or a tool for prediction of the prognosis.

20 **[0004]** On the other hand, although FGFRL1 has been named a "molecule like a fibroblast growth factor receptor (FGFR)", it is clearly structurally different from other FGFRs since, unlike other FGFRs, FGFRL1 lacks the tyrosine kinase domain, which is a signaling region in the cell (Non-patent Document 4). Further, the functional aspect of FGFRL1 is also different from other FGFRs, and FGFRL1 has been considered to act to suppress the cell growth (Non-patent Document 5). Accordingly, FGFRL1 is a molecule clearly distinguishable from other FGFRs, and should be clearly distinguished from the FGFRs also in view of its biological role and the industrial applicability deduced therefrom.

30 **[0005]**

[Non-patent Document 1] International Journal of Molecular Medicine 16, 1169-1173, 2005

[Non-patent Document 2] Molecular Cell 35, 856-867, 2009.

[Non-patent Document 3] Journal of Biological Chemistry 286, 420-428, 2011

35 [Non-patent Document 4] Genomics 69, 275-279, 2000

[Non-patent Document 5] Journal of Biological Chemistry 278, 33857-33865, 2003

## SUMMARY OF THE INVENTION

40 PROBLEMS TO BE SOLVED BY THE INVENTION

**[0006]** An object of the present invention is to provide a novel therapeutic agent for cancer such as esophageal squamous cell carcinoma. Another object of the present invention is to provide a method for predicting the prognosis of cancer such as esophageal squamous cell carcinoma. Still another object of the present invention is to provide a method for detecting, or predicting the prognosis of, cancer such as esophageal squamous cell carcinoma using a sample that can be collected less invasively.

45 **[0007]** As described specifically in the Examples below, the present inventors collected esophageal squamous cell carcinoma tissues from a large number of esophageal squamous cell carcinoma patients to investigate expression of FGFRL1, and conducted a follow-up study on association of the expression level with the prognosis. As a result, it was found that high expression of FGFRL1 is associated with poor prognosis. Further, the present inventors discovered that allowing an anti-FGFRL1 antibody to act on esophageal squamous cell carcinoma cells enables suppression of the growth of the cancer cells.

50 **[0008]** That is, the present invention provides a therapeutic agent for cancer, comprising as an effective component an antibody that undergoes antigen-antibody reaction with FGFRL1 to suppress the growth of cancer cells, or an antigen-binding fragment thereof. Further, the present invention provides a therapeutic method for cancer, comprising administering to a cancer patient an effective amount of an antibody that undergoes antigen-antibody reaction with FGFRL1 to suppress the growth of cancer cells, or an antigen-binding fragment thereof. Further, the present invention provides a method for predicting the prognosis of cancer, comprising investigating the expression level of FGFRL1 in a cancer

tissue separated from a living body, wherein a high expression level of FGFR1 indicates poor prognosis. Further, the present invention provides a method for detecting cancer, comprising measuring FGFR1 or a fragment thereof extracted from a body tissue, or FGFR1 or a fragment thereof in blood separated from a living body, wherein a higher concentration of FGFR1 or the fragment thereof contained therein than the concentration of FGFR1 or the fragment thereof in the tissue or blood of a healthy individual indicates the presence of cancer. Further, the present invention provides a method for predicting the prognosis of cancer, comprising measuring FGFR1 or a fragment thereof in a tissue or blood separated from a cancer patient, wherein a high concentration of FGFR1 or the fragment thereof contained therein indicates poor prognosis.

## EFFECT OF THE INVENTION

**[0009]** By the present invention, a novel therapeutic agent for cancer such as esophageal squamous cell carcinoma was provided. Further, by the present invention, a novel method for predicting the prognosis of cancer such as esophageal squamous cell carcinoma was provided. Since this method enables prediction of the prognosis of a highly malignant cancer such as esophageal squamous cell carcinoma, and hence enables appropriate selection of a therapeutic method, the method contributes to treatment of cancer. Further, by the present invention, a method for detecting a cancer such as esophageal squamous cell carcinoma using a sample that can be collected less invasively was provided. Since this method is less invasive, the burden of the subject is light. Therefore, detection of cancer can be easily achieved also in medical examination and the like, and early detection and early treatment of cancer are possible.

## BRIEF DESCRIPTION OF THE DRAWINGS

### [0010]

Fig. 1 shows photographs for comparison of the results of immunohistochemistry of esophageal squamous cell carcinoma carried out in the Examples below with the results obtained for normal tissues.

Fig. 2 is a diagram showing the relationship between the expression level of FGFR1 in the esophageal squamous cell carcinoma tissue measured in the Examples below and the survival rate of patients at each month.

Fig. 3 is a diagram showing the results of measurement of the growth capacity of esophageal squamous cell carcinoma cells observed after allowing an anti-FGFR1 antibody to act on the cells in the Examples below, as compared to the results obtained by treatment with a control antibody.

## BEST MODE FOR CARRYING OUT THE INVENTION

**[0011]** As described above, the therapeutic agent for cancer of the present invention comprises as an effective component an antibody that undergoes antigen-antibody reaction with FGFR1 or an antigen-binding fragment thereof. Both the amino acid sequence and the gene base sequence are known for FGFR1. A base sequence of cDNA of FGFR1 and the amino acid sequence encoded thereby are described, for example, as NCBI Accession NO. NM\_001004356.2. The base sequence of cDNA of the FGFR1 gene and the amino acid sequence encoded thereby are shown in SEQ ID NO:1, and the amino acid sequence alone is shown in SEQ ID NO:2. FGFR1 is a single-transmembrane protein.

**[0012]** The antibody used herein is an antibody that suppresses the growth of cancer cells, and the antibody undergoes antigen-antibody reaction with the N-terminal region of FGFR1, that is, the epitope of the antibody is preferably present in the N-terminal region of FGFR1 or in a region comprising the whole or a part of the N-terminal region (that is, a region that extends from the N-terminal region to another region). The N-terminal region herein means the extracellular region of FGFR1, that is, the region between the N-terminus and the 378th amino acid in the amino acid sequence shown in SEQ ID NO:2. The antibody may be either a polyclonal antibody or a monoclonal antibody. A polyclonal antibody whose epitope is the N-terminal region of FGFR1 is also commercially available, and such a commercially available product may also be preferably used. Further, an antigen-binding fragment of the above-described antibody, such as the Fab fragment or the F(ab')<sub>2</sub> fragment, may also be used. Whether or not the antibody or fragment has an effect to suppress the growth of cancer cells can be investigated by, for example, using the well-known WST1 method as described in the Examples below.

**[0013]** The antibody may be an antibody prepared by genetic engineering, or may be a humanized antibody prepared by replacing the Fc region with that of a human antibody for suppression of rejection reaction in human. Further, in antibody preparations, those prepared by binding a polyethylene glycol (PEG) chain or the like to an end of an antibody for making the antibody less likely to be degraded by protease in the living body are widely used. Also in the therapeutic agent for cancer of the present invention, a stabilizing structure such as a PEG chain may be attached to an end of the above-described antibody or the antigen-binding fragment thereof, and the resultant may be contained in its entirety in the agent as an effective component. In cases where the antibody or an antigen-binding fragment thereof is stabilized

by PEGylation, the size of the PEG is several thousand to 50,000, preferably about 10,000 to 50,000. The method for binding PEG to an end of a polypeptide is well known. Such a product prepared by attaching a stabilizing structure is also included in the "antibody or an antigen-binding fragment thereof" in the present invention.

5 **[0014]** Further, the present antibody may also be utilized as a complex antitumor agent by chemically binding a low molecular weight antitumor agent or a compound having cytotoxicity against cancer thereto, or may be utilized as a navigator in a drug delivery system (DDS) to cancer cells

10 **[0015]** In terms of the administration route of the therapeutic agent for cancer of the present invention, either parenteral administration or oral administration may be carried out. Parenteral administration such as injection to the cancer tissue, intravenous injection, or intramuscular injection is preferred. The dose may be appropriately set depending on the clinical condition and the severity of the disease to be treated. For example, the therapeutic agent is administered at a dose of 0.1 to 20 mg per administration, preferably 1 to 10 mg per administration, per kg body weight. Further, the therapeutic agent for cancer of the present invention may be formulated by a well-known method into, for example, a solution in which the agent is dissolved in a physiological buffer. Further, a known additive(s) may be added to the solution.

15 **[0016]** Examples of the cancer to be treated with the therapeutic agent for cancer of the present invention include, but are not limited to, epithelial solid cancers. Esophageal squamous cell carcinoma is especially preferred.

20 **[0017]** The present inventors discovered, as specifically described in the Examples below, that the expression level of FGFR1 in a cancer tissue can be used as an index for predicting the prognosis of the cancer, that is, the survival rate after initiation of cancer treatment. Thus, the present invention also provides a method for predicting the prognosis of cancer, which method comprises investigating the expression level of FGFR1 in a cancer tissue separated from a living body, wherein a high expression level of FGFR1 indicates poor prognosis. The expression level of FGFR1 can be measured by an immunoassay such as immunohistochemistry. For the immunoassay, the above-described anti-FGFR1 antibody or an antigen-binding fragment thereof may be used, and a polyclonal antibody or monoclonal antibody whose epitope is the extracellular region of FGFR1 may be preferably used. Since, as described above, such a polyclonal antibody is also commercially available, it is also possible to use a commercially available product. Since FGFR1 is expressed in a state where it is penetrating the membrane, the immunohistochemical staining as described in the Examples below is preferably carried out as the immunoassay, but the immunoassay is not limited thereto.

25 **[0018]** The higher the expression level of FGFR1, the poorer the prognosis may be. Thus, by preliminarily investigating the expression level of FGFR1 and the prognosis in a large number of patients with the same kind of cancer, it is possible to predict the prognosis based on how high the expression level of FGFR1 is. For example, as specifically described in the Examples below, in cases where the expression level is investigated by immunohistochemical staining, the prediction may be made based on evaluation of the stained area per cancer tissue (0%: -, 1-50%: +, 51-100%: ++), and the positivity per cell wherein strong positivity is evaluated as (+++) and negativity is evaluated as (-). In consideration of the extent of expression of FGFR1 in a normal tissue, a total value of not less than (++) can be judged as positive (that is, poor prognosis).

30 **[0019]** As described above, FGFR1 is a single-transmembrane protein, and has a structure that undergoes the action of protease in the extracellular region. Therefore, it is thought that a tissue fluid extracted from a tissue, or blood, may contain free FGFR1 or a free fragment of FGFR1. The present inventors inferred that, by quantifying free FGFR1 or a free fragment of FGFR1 in a tissue fluid extracted from a tissue, or blood, cancer can be detected. The present invention also provides a method for detecting cancer, which method comprises measuring FGFR1 or a fragment thereof extracted from a body tissue, or FGFR1 or a fragment thereof in blood separated from a living body, wherein a higher concentration of FGFR1 or the fragment thereof contained therein than the concentration of FGFR1 or the fragment thereof in the tissue or blood of a healthy individual indicates the presence of cancer. In such cases, the FGFR1 or a fragment thereof in the tissue extract or blood can be quantified by an immunoassay using an antibody that undergoes antigen-antibody reaction with the extracellular region of FGFR1. The immunoassay in such cases may be carried out by a well-known method such as ELISA, which is widely used for quantification of various proteins in body fluid; the sandwich method, in which a fluorescent label or chemiluminescent label is used; or the immunoagglutination method, in which sensitized particles prepared by immobilizing an antibody on latex particles are used. The cut-off value in such cases may be a value significantly different from the mean value in healthy individuals. The cut-off value may be, for example, 1.0 unit/mL, and the unit value in such cases is determined using as a standard the concentration in the tissue or blood of a healthy individual, although the unit value may vary depending on differential diagnosis from similar diseases and on background factors of the patient.

35 **[0020]** Further, based on the abundance of FGFR1 or a fragment thereof in a tissue fluid extracted from a tissue, or blood, prediction of the prognosis of cancer can be carried out similarly to the above-described cases where the prediction is carried out based on the expression level of FGFR1 in the cancer tissue. In such a case, the criteria for evaluation of the prognosis may vary depending on whether the survival rate or the recurrence rate is to be evaluated, and for what disease the evaluation is to be done.

40 **[0021]** The present invention is described below more specifically by way of Examples. However, the present invention is not limited to the Examples below.

## EXAMPLES

## Example 1

## 5 Immunohistochemical Staining

**[0022]** Tissue sections were prepared from esophageal squamous cell carcinoma tissues collected from 69 esophageal squamous cell carcinoma patients, and subjected to deparaffinization (3 times of 5 minutes of immersion in xylene, 2 times of 3 minutes of immersion in 100% ethanol, 3 minutes of immersion in 95% ethanol, 3 minutes of immersion in 90% ethanol, 3 minutes of immersion in 85% ethanol, 5 minutes of washing with running water, and then 5 minutes of immersion in distilled water) and then antigen retrieval by heat treatment (treatment in 1 mM Tris buffer pH 9.0) supplemented with 0.1 mM EDTA at 95°C for 40 minutes, followed by allowing the resultant to cool at room temperature for 20 minutes, washing with running water and then immersion in distilled water). Subsequently, endogenous peroxidase was blocked with 3% hydrogen peroxide solution (at room temperature for 10 minutes), and the sections were then washed with distilled water 3 times, followed by immersion in 5 mM Tris buffer (pH 7.2) supplemented with 0.005% Tween 20 and 15 mM NaCl at room temperature for 5 minutes for achieving equilibration. Anti-FGFRL1 antibody H-300 (Santa cruse) was 50-fold diluted with Dako REAL antibody diluent (Dako), and treatment was carried out with the resulting dilution for 30 minutes. After washing the sections with 5 mM Tris buffer (pH 7.2) supplemented with 0.005% Tween 20 (trade name) and 15 mM NaCl 3 times, coloring with DAB (diaminobenzidine) was performed using Dako ChemMate ENVISION kit (Dako), followed by washing with distilled water and then performing counter staining with Dako REAL Hematoxylin (prepared by 4-fold dilution with distilled water and then addition of Tween 20 (trade name) to adjust the Tween 20 concentration to 0.01 %) at room temperature for 3 minutes. Thereafter, washing with water, dehydration, clearing and embedding were carried out (5 minutes of washing with water, 5 minutes of immersion in 80% ethanol, 5 minutes of immersion in 90% ethanol, 5 minutes of immersion in 95% ethanol, 2 times of 5 minutes of immersion in 100% ethanol, and 3 times of 5 minutes of immersion in xylene, followed by embedding with Leica CV5030). The stained area per cancer tissue (0%: 0, 1-50%: 1, 51-100%: 2) and the staining intensity (no signal: 0, weak: 1, moderate: 2, marked: 3) were scored, and a total score of not less than 4 was defined as high expression of the FGFRL1 protein, and a total score of less than 4 was defined as low expression of the FGFRL1 protein. The survival rate was compared between both groups of patients by the Kaplan-Meier method and the log-rank test.

**[0023]** The results of immunohistochemical staining are shown in Fig. 1, and the relationship between the expression level of FGFRL1 and the survival rate of patients at each month is shown in Fig. 2. As is evident from Fig. 2, the prognosis was poor in the cases where the expression level of FGFRL1 was high, and the survival rate at Month 60 in these cases was a little more than one third of the survival rate observed in the cases where the expression level of FGFRL1 was low.

## 35 Example 2

## Pharmacological Effect

**[0024]** An esophageal squamous cell carcinoma-derived cell line KYSE-170 was plated in Ham F12 (Nissui)/RPMI1640 (Gibco) medium (pH 6.8) supplemented with fetal bovine serum (5%, Equitech-Bio) filtered through a 0.22- $\mu$ m PVDF membrane filter (Millipore), penicillin (100 unit/ml, Meiji), gentacin (4.44  $\mu$ mg/l, Schering) and sodium hydrogen carbonate (0.2%) on a 96-well dish ( $5 \times 10^3$  cells/100  $\mu$ L/well), and cultured under the conditions of 5% CO<sub>2</sub>, a humidity of 100% and a temperature of 37°C. Twenty four hours later, the cells were treated with anti-FGFRL1 antibody H-300 (recognition site: N-terminus/extracellular region) or C-20 (recognition site: C-terminus/intracellular region) (Santa cruse) and a control IgG (Santa cruse) of the animal from which it was derived, which were diluted with the above-described Ham F12/RPMI1640 medium (final concentration, 20  $\mu$ g/ml). After 24 hours of culture, the cell growth was evaluated by the well-known WST1 method using a commercially available reagent.

**[0025]** The results are shown in Fig. 3. As shown in Fig. 3, in the case where the monoclonal antibody whose epitope is the N-terminal region, that is, the extracellular region, of FGFRL1 was used, the growth of esophageal squamous cell carcinoma cells was significantly suppressed as compared to the case where the treatment was carried out with the control antibody. Thus, such an antibody is useful as a therapeutic agent for esophageal squamous cell carcinoma.

## [SEQUENCE LISTING]

55 **[0026]**

EP 2 692 358 A1

SEQUENCE LISTING

<110> Kyoto University

5 <120> Therapeutic agent of cancers and method of predicting prognosis of cancers

<130> PF454-PCT

<160> 2

10 <170> PatentIn version 3.1

<210> 1

<211> 3215

15 <212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (150)..(1664)

20 <223>

<400> 1

gccgccccgc cccgagaccg ggccccggggg cgcggggcgg cgggatgcgg cgccccggggc 60

ggcgatgacc gcggagcgca cgccgcggggc ccggccctga ccccgcgcgc cgcccgtga 120

gccccccgcc gaggtccgga caggccgag atg acg ccg agc ccc ctg ttg ctg 173  
Met Thr Pro Ser Pro Leu Leu Leu  
1 5

30 ctc ctg ctg ccg ccg ctg ctg ctg ggg gcc ttc ccg ccg gcc gcc gcc 221  
Leu Leu Leu Pro Pro Leu Leu Leu Gly Ala Phe Pro Pro Ala Ala Ala  
10 15 20

gcc cga gcc ccc cca aag atg gcg gac aag gtg gtc cca cgg cag gtg 269  
Ala Arg Gly Pro Pro Lys Met Ala Asp Lys Val Val Pro Arg Gln Val  
25 30 35 40

gcc cgg ctg ggc cgc act gtg cgg ctg cag tgc cca gtg gag ggg gac 317  
Ala Arg Leu Gly Arg Thr Val Arg Leu Gln Cys Pro Val Glu Gly Asp  
45 50 55

40 ccg ccg ccg ctg acc atg tgg acc aag gat ggc cgc acc atc cac agc 365  
Pro Pro Pro Leu Thr Met Trp Thr Lys Asp Gly Arg Thr Ile His Ser  
60 65 70

ggc tgg agc cgc ttc cgc gtg ctg ccg cag ggg ctg aag gtg aag cag 413  
Gly Trp Ser Arg Phe Arg Val Leu Pro Gln Gly Leu Lys Val Lys Gln  
75 80 85

gtg gag cgg gag gat gcc ggc gtg tac gtg tgc aag gcc acc aac ggc 461  
Val Glu Arg Glu Asp Ala Gly Val Tyr Val Val Cys Lys Ala Thr Asn Gly  
90 95 100

50 ttc gcc agc ctg agc gtc aac tac acc ctc gtc gtg ctg gat gac att 509  
Phe Gly Ser Leu Ser Val Asn Tyr Thr Leu Val Val Leu Asp Asp Ile  
105 110 115 120

55 agc cca ggg aag gag agc ctg ggg ccc gac agc tcc tct ggg ggt caa 557  
Ser Pro Gly Lys Glu Ser Leu Gly Pro Asp Ser Ser Ser Gly Gly Gln  
125 130 135

EP 2 692 358 A1

	gag gac ccc gcc agc cag cag tgg gca cga ccg cgc ttc aca cag ccc	605
	Glu Asp Pro Ala Ser Gln Gln Trp Ala Arg Pro Arg Phe Thr Gln Pro	
	140 145 150	
5	tcc aag atg agg cgc cgg gtg atc gca cgg ccc gtg ggt agc tcc gtg	653
	Ser Lys Met Arg Arg Arg Val Ile Ala Arg Pro Val Gly Ser Ser Val	
	155 160 165	
10	cgg ctc aag tgc gtg gcc agc ggg cac cct cgg ccc gac atc acg tgg	701
	Arg Leu Lys Cys Val Ala Ser Gly His Pro Arg Pro Asp Ile Thr Trp	
	170 175 180	
15	atg aag gac gac cag gcc ttg acg cgc cca gag gcc gct gag ccc agg	749
	Met Lys Asp Asp Gln Ala Leu Thr Arg Pro Glu Ala Ala Glu Pro Arg	
	185 190 195 200	
20	aag aag aag tgg aca ctg agc ctg aag aac ctg cgg ccg gag gac agc	797
	Lys Lys Lys Trp Thr Leu Ser Leu Lys Asn Leu Arg Pro Glu Asp Ser	
	205 210 215	
25	ggc aaa tac acc tgc cgc gtg tcg aac cgc gcg ggc gcc atc aac gcc	845
	Gly Lys Tyr Thr Cys Arg Val Ser Asn Arg Ala Gly Ala Ile Asn Ala	
	220 225 230	
30	acc tac aag gtg gat gtg atc cag cgg acc cgt tcc aag ccc gtg ctc	893
	Thr Tyr Lys Val Asp Val Ile Gln Arg Thr Arg Ser Lys Pro Val Leu	
	235 240 245	
35	aca ggc acg cac ccc gtg aac acg acg gtg gac ttc ggg ggg acc acg	941
	Thr Gly Thr His Pro Val Asn Thr Thr Val Asp Phe Gly Gly Thr Thr	
	250 255 260	
40	tcc ttc cag tgc aag gtg cgc agc gac gtg aag ccg gtg atc cag tgg	989
	Ser Phe Gln Cys Lys Val Arg Ser Asp Val Lys Pro Val Ile Gln Trp	
	265 270 275 280	
45	ctg aag cgc gtg gag tac ggc gcc gag ggc cgc cac aac tcc acc atc	1037
	Leu Lys Arg Val Glu Tyr Gly Ala Glu Gly Arg His Asn Ser Thr Ile	
	285 290 295	
50	gat gtg ggc ggc cag aag ttt gtg gtg ctg ccc acg ggt gac gtg tgg	1085
	Asp Val Gly Gly Gln Lys Phe Val Val Leu Pro Thr Gly Asp Val Trp	
	300 305 310	
55	tcg cgg ccc gac ggc tcc tac ctc aat aag ctg ctc atc acc cgt gcc	1133
	Ser Arg Pro Asp Gly Ser Tyr Leu Asn Lys Leu Leu Ile Thr Arg Ala	
	315 320 325	
60	cgc cag gac gat gcg ggc atg tac atc tgc ctt ggc gcc aac acc atg	1181
	Arg Gln Asp Asp Ala Gly Met Tyr Ile Cys Leu Gly Ala Asn Thr Met	
	330 335 340	
65	ggc tac agc ttc cgc agc gcc ttc ctc acc gtg ctg cca gac cca aaa	1229
	Gly Tyr Ser Phe Arg Ser Ala Phe Leu Thr Val Leu Pro Asp Pro Lys	
	345 350 355 360	
70	ccg cca ggg cca cct gtg gcc tcc tcg tcc tcg gcc act agc ctg ccg	1277
	Pro Pro Gly Pro Pro Val Ala Ser Ser Ser Ser Ala Thr Ser Leu Pro	
	365 370 375	
75	tgg ccc gtg gtc atc ggc atc cca gcc ggc gct gtc ttc atc ctg ggc	1325
	Trp Pro Val Val Ile Gly Ile Pro Ala Gly Ala Val Phe Ile Leu Gly	

EP 2 692 358 A1

	380	385	390	
	acc ctg ctc ctg tgg ctt tgc	cag gcc cag aag aag ccg tgc acc ccc		1373
5	Thr Leu Leu Leu Trp Leu Cys	Gln Ala Gln Lys Lys Pro Cys Thr Pro		
	395	400	405	
	gcg cct gcc cct ccc ctg cct ggg cac cgc ccg ccg ggg acg gcc cgc			1421
	Ala Pro Ala Pro Pro Leu Pro Gly His Arg Pro Pro Gly Thr Ala Arg			
	410	415	420	
10	gac cgc agc gga gac aag gac ctt ccc tcg ttg gcc gcc ctc agc gct			1469
	Asp Arg Ser Gly Asp Lys Asp Leu Pro Ser Leu Ala Ala Leu Ser Ala			
	425	430	435	440
	ggc cct ggt gtg ggg ctg tgt gag gag cat ggg tct ccg gca gcc ccc			1517
15	Gly Pro Gly Val Gly Leu Cys Glu Glu His Gly Ser Pro Ala Ala Pro			
	445	450	455	
	cag cac tta ctg ggc cca ggc cca gtt gct ggc cct aag ttg tac ccc			1565
	Gln His Leu Leu Gly Pro Gly Pro Val Ala Gly Pro Lys Leu Tyr Pro			
	460	465	470	
20	aaa ctc tac aca gac atc cac aca cac aca cac aca cac tct cac aca			1613
	Lys Leu Tyr Thr Asp Ile His Thr His Thr His Thr His Ser His Thr			
	475	480	485	
	cac tca cac gtg gag ggc aag gtc cac cag cac atc cac tat cag tgc			1661
25	His Ser His Val Glu Gly Lys Val His Gln His Ile His Tyr Gln Cys			
	490	495	500	
	tag acggcaccgt atctgcagtg ggcacggggg ggccggccag acaggcagac			1714
30	tgggaggatg gaggacggag ctgcagacga aggcagggga cccatggcga ggaggaatgg			1774
	ccagcaccctc aggcagtctg tgtgtgaggc atagcccctg gacacacaca cacagacaca			1834
	cacactgcct ggatgcatgt atgcacacac atgcgcgcac acgtgctccc tgaaggcaca			1894
35	cgtagcaca cacgcacatg cacagatatg ccgcctgggc acacagataa gctgccc aaa			1954
	tgcacgcaca cgcacagaga catgccagaa catacaagga catgctgcct gaacatacac			2014
	acgcacacc atgcgcagat gtgctgcctg gacacacaca cacacacgga tatgctgtct			2074
40	ggacgcacac acgtgcagat atggtatccg gacacacacg tgcacagata tgctgcctgg			2134
	acacacagat aatgctgcct tgacacacac atgcacggat attgcctgga cacacacaca			2194
	cacacgtgtg cacagatatg ctgtctggac acgcacacac atgcagatat gctgcctgga			2254
45	cacacacttc cagacacacg tgcacaggcg cagatatgct gcctggacac acgcagatat			2314
	gctgtctagt cacacacaca cgcagacatg ctgtccggac acacacacgc atgcacagat			2374
	atgctgtccg gacacacaca cgcacgcaga tatgctgcct ggacacacac acagataatg			2434
50	ctgcctcaac actcacacac gtgcagatat tgcctggaca cacacatgtg cacagatatg			2494
	ctgtctggac atgcacacac gtgcagatat gctgtccgga tacacacgca cgcacacatg			2554
	cagatatgct gcctgggcac aacttccgg acacacatgc acacacaggt gcagatatgc			2614
55	tgcttgaca cacgcagact gacgtgcttt tgggagggtg tgccgtgaag cctgcagtac			2674

EP 2 692 358 A1

gtgtgccgtg aggctcatag ttgatgaggg actttccctg ctccaccgtc actcccccaa 2734  
ctctgcccgc ctctgtcccc gcctcagtc ccgcctccat ccccgctct gtcccctggc 2794  
5 cttggcggct atttttgcca cctgccttgg gtgccagga gtcccctact gctgtgggct 2854  
ggggttgggg gcacagcagc cccaagcctg agaggctgga gcccatggct agtggctcat 2914  
ccccactgca ttctccccct gacacagaga aggggccttg gtatttatat ttaagaaatg 2974  
10 aagataatat taataatgat ggaaggaaga ctgggttgca gggactgtgg tctctcctgg 3034  
ggcccgggac ccgcctggtc tttcagccat gctgatgacc acaccccgtc caggccagac 3094  
accaccccc accccactgt cgtggtggcc ccagatctct gtaattttat gtagagtttg 3154  
15 agctgaagcc ccgtatattt aatttatattt gttaaacatg aaagtgcac ctttccctcc 3214  
a 3215

20 <210> 2  
<211> 504  
<212> PRT  
<213> Homo sapiens

25 <400> 2

Met Thr Pro Ser Pro Leu Leu Leu Leu Leu Leu Pro Pro Leu Leu Leu  
1 5 10 15

30 Gly Ala Phe Pro Pro Ala Ala Ala Ala Arg Gly Pro Pro Lys Met Ala  
20 25 30

35 Asp Lys Val Val Pro Arg Gln Val Ala Arg Leu Gly Arg Thr Val Arg  
35 40 45

Leu Gln Cys Pro Val Glu Gly Asp Pro Pro Pro Leu Thr Met Trp Thr  
50 55 60

40 Lys Asp Gly Arg Thr Ile His Ser Gly Trp Ser Arg Phe Arg Val Leu  
65 70 75 80

45 Pro Gln Gly Leu Lys Val Lys Gln Val Glu Arg Glu Asp Ala Gly Val  
85 90 95

50 Tyr Val Cys Lys Ala Thr Asn Gly Phe Gly Ser Leu Ser Val Asn Tyr  
100 105 110

Thr Leu Val Val Leu Asp Asp Ile Ser Pro Gly Lys Glu Ser Leu Gly  
115 120 125

55 Pro Asp Ser Ser Ser Gly Gly Gln Glu Asp Pro Ala Ser Gln Gln Trp  
130 135 140

EP 2 692 358 A1

Ala Arg Pro Arg Phe Thr Gln Pro Ser Lys Met Arg Arg Arg Val Ile  
 145 150 155 160

5 Ala Arg Pro Val Gly Ser Ser Val Arg Leu Lys Cys Val Ala Ser Gly  
 165 170 175

10 His Pro Arg Pro Asp Ile Thr Trp Met Lys Asp Asp Gln Ala Leu Thr  
 180 185 190

Arg Pro Glu Ala Ala Glu Pro Arg Lys Lys Lys Trp Thr Leu Ser Leu  
 195 200 205

15 Lys Asn Leu Arg Pro Glu Asp Ser Gly Lys Tyr Thr Cys Arg Val Ser  
 210 215 220

20 Asn Arg Ala Gly Ala Ile Asn Ala Thr Tyr Lys Val Asp Val Ile Gln  
 225 230 235 240

Arg Thr Arg Ser Lys Pro Val Leu Thr Gly Thr His Pro Val Asn Thr  
 245 250 255

25 Thr Val Asp Phe Gly Gly Thr Thr Ser Phe Gln Cys Lys Val Arg Ser  
 260 265 270

30 Asp Val Lys Pro Val Ile Gln Trp Leu Lys Arg Val Glu Tyr Gly Ala  
 275 280 285

35 Glu Gly Arg His Asn Ser Thr Ile Asp Val Gly Gly Gln Lys Phe Val  
 290 295 300

Val Leu Pro Thr Gly Asp Val Trp Ser Arg Pro Asp Gly Ser Tyr Leu  
 305 310 315 320

40 Asn Lys Leu Leu Ile Thr Arg Ala Arg Gln Asp Asp Ala Gly Met Tyr  
 325 330 335

45 Ile Cys Leu Gly Ala Asn Thr Met Gly Tyr Ser Phe Arg Ser Ala Phe  
 340 345 350

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

50 Ser Ser Ser Ala Thr Ser Leu Pro Trp Pro Val Val Ile Gly Ile Pro  
 370 375 380

55 Ala Gly Ala Val Phe Ile Leu Gly Thr Leu Leu Leu Trp Leu Cys Gln



chemistry

- 5
10. A method for detecting cancer, said method comprising measuring FGFR1 or a fragment thereof extracted from a body tissue, or FGFR1 or a fragment thereof in blood separated from a living body, wherein a higher concentration of FGFR1 or the fragment thereof contained therein than the concentration of FGFR1 or the fragment thereof in the tissue or blood of a healthy individual indicates the presence of cancer.
11. The method according to claim 10, wherein said cancer is esophageal squamous cell carcinoma.
- 10
12. A method for predicting the prognosis of cancer, said method comprising measuring FGFR1 or a fragment thereof in a tissue or blood separated from a cancer patient, wherein a high concentration of FGFR1 or the fragment thereof contained therein indicates poor prognosis.
- 15
13. The method according to claim 12, wherein said cancer is esophageal squamous cell carcinoma.

20

25

30

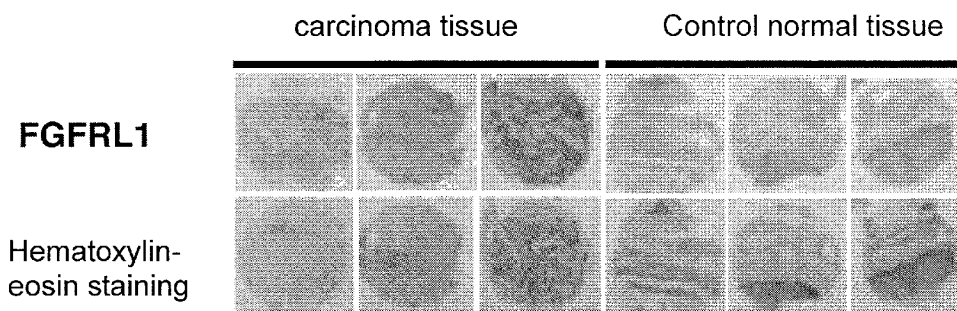
35

40

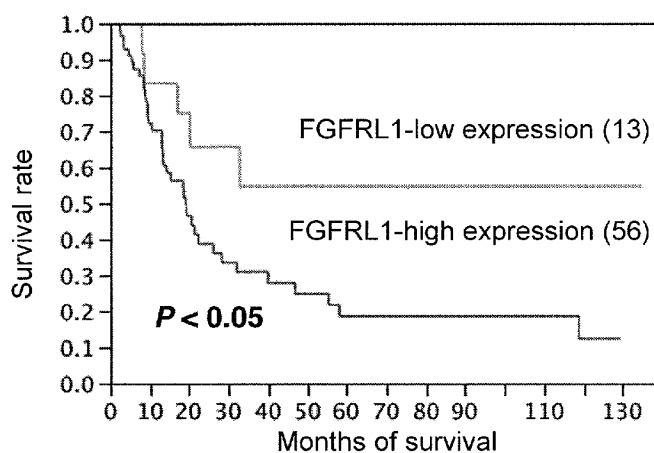
45

50

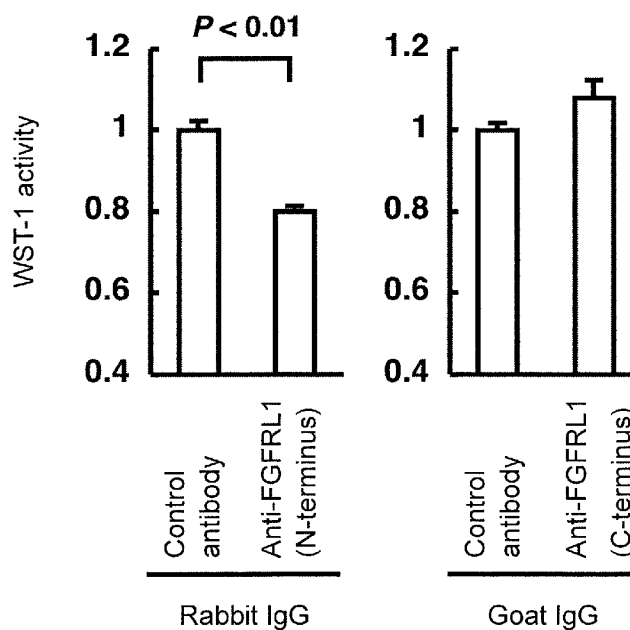
55



**Fig.1**



**Fig.2**



**Fig.3**

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP2012/058668

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> A61K39/395(2006.01)i, A61P1/00(2006.01)i, A61P35/00(2006.01)i, G01N33/48 (2006.01)i, G01N33/53(2006.01)i, G01N33/574(2006.01)i, C07K16/28 (2006.01)n According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) A61K39/395, A61P1/00, A61P35/00, G01N33/48, G01N33/53, G01N33/574, C07K16/28 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Jitsuyo Shinan Koho 1922-1996 Jitsuyo Shinan Toroku Koho 1996-2012 Kokai Jitsuyo Shinan Koho 1971-2012 Toroku Jitsuyo Shinan Koho 1994-2012 Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) CA/MEDLINE/EMBASE/BIOSIS (STN), JSTPlus/JMEDPlus/JST7580 (JDreamII)		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	JP 2003-527858 A (Amgen Inc.), 24 September 2003 (24.09.2003), claims 19 to 33; paragraphs [0151] to [0166], [0252], [0289], [0290]; fig. 2 & US 2008/0044859 A1 & EP 1268793 A1 & WO 2001/070977 A2	1, 2 3
X Y	JP 2005-537786 A (Genesis Research & Development Corp.), 15 December 2005 (15.12.2005), claims 25 to 27; paragraphs [0193] to [0197] & US 2002/0058335 A1 & EP 1511759 A1 & WO 2000/058463 A1 & CN 1358228 A	1, 2 3
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed		"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family
Date of the actual completion of the international search 01 May, 2012 (01.05.12)		Date of mailing of the international search report 15 May, 2012 (15.05.12)
Name and mailing address of the ISA/ Japanese Patent Office		Authorized officer
Facsimile No.		Telephone No.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP2012/058668

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	TSUCHIYA, S. et al, MicroRNA-210 regulates cancer cell proliferation through targeting fibroblast growth factor receptor-like 1 (FGFRL1), The Journal of biological chemistry, 2011.01, Vol.286, No.1, p.420-428	7-13 3
X	SCHILD, C. et al, Aberrant expression of FGFRL1, a novel FGF receptor, in ovarian tumors, International journal of molecular medicine, 2005, Vol.16, No.6, p.1169-1173	7, 9, 10, 12
A	TRUEB, B. et al, Biology of FGFRL1, the fifth fibroblast growth factor receptor, Cellular and Molecular Life Sciences, 2010.11, Vol.68, No.6, p.951-964	1-13

Form PCT/ISA/210 (continuation of second sheet) (July 2009)

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP2012/058668

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

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

1.  Claims Nos.: 4-6  
because they relate to subject matter not required to be searched by this Authority, namely:  
"Methods for treatment of cancers" set forth in claims 4 to 6 pertain to "methods for treatment of the human body by therapy" and thus relate to a subject matter which this International Searching Authority is not required, under the provisions of PCT Rule 39.1(iv), to search.
2.  Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (July 2009)

**REFERENCES CITED IN THE DESCRIPTION**

*This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.*

**Non-patent literature cited in the description**

- *International Journal of Molecular Medicine*, 2005, vol. 16, 1169-1173 **[0005]**
- *Molecular Cell*, 2009, vol. 35, 856-867 **[0005]**
- *Journal of Biological Chemistry*, 2011, vol. 286, 420-428 **[0005]**
- *Genomics*, 2000, vol. 69, 275-279 **[0005]**
- *Journal of Biological Chemistry*, 2003, vol. 278, 33857-33865 **[0005]**

专利名称(译)	用于癌症的治疗剂和用于确定癌症预后的方法		
公开(公告)号	<a href="#">EP2692358A4</a>	公开(公告)日	2014-12-10
申请号	EP2012765858	申请日	2012-03-30
[标]申请(专利权)人(译)	国立大学法人京都大学		
申请(专利权)人(译)	京都大学		
当前申请(专利权)人(译)	京都大学		
[标]发明人	TSUCHIYA SOKEN TSUJIMOTO GOZOH SHIMIZU KAZUHARU SHIMADA YUTAKA TSUKADA KAZUHIRO		
发明人	TSUCHIYA, SOKEN TSUJIMOTO, GOZOH SHIMIZU, KAZUHARU SHIMADA, YUTAKA TSUKADA, KAZUHIRO		
IPC分类号	A61K39/395 A61P1/00 A61P35/00 G01N33/48 G01N33/53 G01N33/574 C07K16/28		
CPC分类号	C07K16/2863 C07K16/30 C07K2317/24 C07K2317/34 C07K2317/73 G01N33/57407 G01N33/57446 G01N2333/71 G01N2800/52		
优先权	2011080261 2011-03-31 JP		
其他公开文献	EP2692358A1		
外部链接	<a href="#">Espacenet</a>		

#### 摘要(译)

本发明公开了一种用于癌症的新型治疗剂，例如食道鳞状细胞癌，用于预测癌症预后的方法，以及使用可收集的样品检测或预测诸如食道鳞状细胞癌的癌症的预后的方法。更少侵略性。癌症治疗剂包含作为有效成分的抗体，其与FGFRL1进行抗原 - 抗体反应以抑制癌细胞的生长，或其抗原结合片段。预测癌症预后的方法包括研究从活体分离的癌组织中FGFRL1的表达水平，并且在该方法中，高表达水平的FGFRL1表明预后不良。检测癌症的方法包括测量从体组织中提取的FGFRL1或其片段，或从活体分离的血液中的FGFRL1或其片段，并且在该方法中，其中包含的FGFRL1或其片段的浓度高于其中的FGFRL1或其片段。FGFRL1或其片段在健康个体的组织或血液中的浓度表明存在癌症。