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(54) **METHOD FOR DETERMINATION OF PROGNOSIS OF PROSTATE CANCER, AND DIAGNOSTIC AGENT FOR USE IN THE METHOD**

(52) **U.S. Cl.** **435/7.23; 435/7.1**
(58) **Field of Classification Search** None
See application file for complete search history.

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* cited by examiner

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G01N 33/574 (2006.01)

(57) **ABSTRACT**

A method for determining probability that prostate cancer will metastasize, as well as a diagnostic reagent used therefor is disclosed. It was discovered that the percentage that NF-κB-p65/RelA has the 254th amino acid threonine which is phosphorylated is significantly higher in the prostate cancer cells in the cases where the bone metastasis was observed than in the cases where bone metastasis was not observed. Thus, the method for determining probability that prostate cancer will metastasize comprises measuring human NF-κB-p65/RelA in which 254th amino acid threonine is phosphorylated, which human NF-κB-p65/RelA is contained in a prostate tissue separated from human.

4 Claims, 1 Drawing Sheet

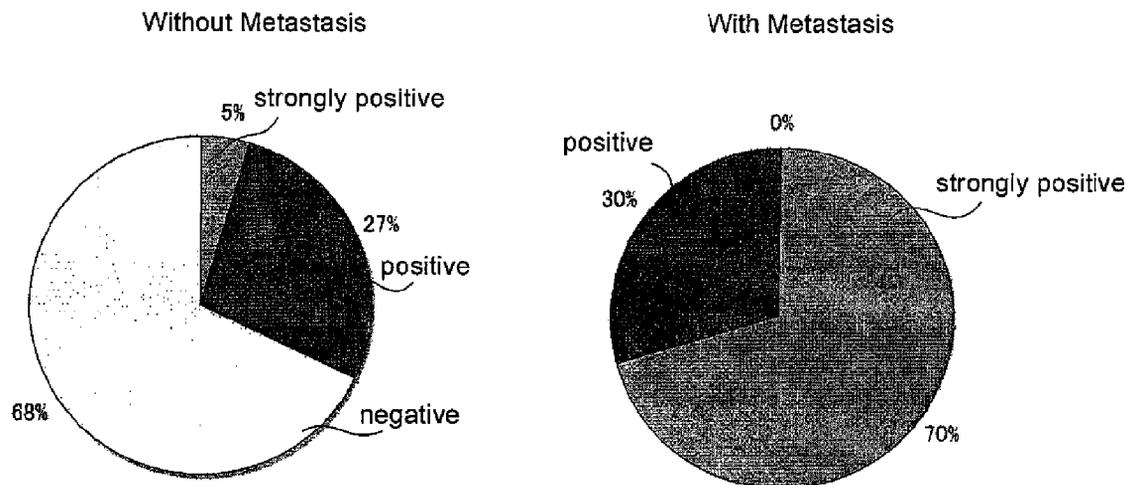


Fig.1

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**METHOD FOR DETERMINATION OF
PROGNOSIS OF PROSTATE CANCER, AND
DIAGNOSTIC AGENT FOR USE IN THE
METHOD**

TECHNICAL FIELD

The present invention relates to a method for determining probability that a prostate cancer will metastasize, and to a diagnostic reagent therefor.

BACKGROUND ART

In the U.S., prostate cancer has the highest prevalence among cancers in males. In Japan too, the prevalence of the prostate cancer is consistently increasing due to the change of lifestyle such as westernization of dietary life (Non-patent Literature 1). Since no specific symptoms are manifested in the early stage of prostate cancer, and since prostate cancer is often complicated with benign prostatic hyperplasia, the symptoms similar to those of benign prostatic hyperplasia, and dysuria, nocturia, urinary urgency and the like are found in some cases. Therefore, recently, even though no symptom is observed, most of prostate cancer is detected by measurement of PSA prostate-specific antigen in the blood in medical examination or health screening. Prostate cancer is characterized by high frequency of metastasis, and in some cases, advanced cancer is found because of low back pain due to bone metastasis (Non-patent Literature 2).

As the factor deciding the prognosis of prostate cancer, stage (in the following) thereof before surgery is important. Stage A: cases where prostate cancer was accidentally found in surgery of benign prostatic hyperplasia or bladder cancer Stage B: cases where the cancer is confined in the prostate Stage C: cases where the cancer is not metastasized to other organs (such as bone, lymph node, lung and liver), but the cancer is exposed to the outside of the coating membrane (the membrane coating the periphery of prostate)

Stage D: cases where the cancer is metastasized to other organs such as bone and lymph node

For the cases of Stage A to Stage C, basically, total extirpation of prostate is carried out. However, in a considerable number of cases, recurrence of the cancer within several years after the surgery is observed. Especially, refractoriness to hormone therapy and metastasis are important factors which aggravate the survival prognosis, so that to estimate these factors at an early stage has a great clinical significance in the selection of therapy or treatment of the patient. However, up to now, no molecular marker is known which enables to estimate the prognosis of prostate cancer, especially the metastasis thereof, at an early stage.

On the other hand, it is known that blockade of NF- κ B (nuclear factor, NF)- κ B activity, which is one of the transcription factors, inhibits angiogenesis, infiltration and metastasis in human prostate cancer (Non-patent Literature 3). More particularly, Non-patent Literature 3 discloses that when a mutated I κ B α gene having an activity to inhibit NF- κ B activity was introduced into metastatic human prostate cancer cells PC-3M and the cells were transplanted to nude mice, angiogenesis, infiltration and metastasis were inhibited in the group wherein NF- κ B activity was reduced by the introduction of the mutated I κ B α gene when compared with the group wherein the human prostate cancer cells PC-3M into which the mutated I κ B α gene was not introduced were transplanted. Non-patent Literature 4 discloses that in prostate cancer cells, expression of NF- κ B significantly occurs in the nuclei. Non-patent Literature 5 discloses that by the action

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of Pin1 which is a type of peptidyl-prolyl isomerase that specifically acts on the sequence of phosphorylated threonine or serine and subsequent proline, which sequence exists in a protein, on p65/RelA which is a subunit of NF- κ B and in which the 254th amino acid threonine is phosphorylated, the binding of NF- κ B-p65/RelA to I κ B α that is an inhibition factor of NF- κ B is inhibited, so that nuclear accumulation and stability of NF- κ B-p65/RelA are increased and, in turn, the NF- κ B activity is increased.

Non-patent Literature 1: Wakai K. Descriptive epidemiology of prostate cancer in Japan and Western countries Nippon Rinsho. 2005 February; 63(2):207-12.

Non-patent Literature 2: Loberg R D, Gayed B A, Olson K B, Pienta K J. A paradigm for the treatment of prostate cancer bone metastases based on an understanding of tumor cell-microenvironment interactions. J Cell Biochem. 2005 Oct. 15; 96(3):439-46.

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DISCLOSURE OF THE INVENTION

Problems to be Solved by the Invention

An object of the present invention is to provide a method for determining probability that a prostate cancer will metastasize and a diagnostic reagent therefor.

Means for Solving the Problems

The present inventors intensively studied to discover that the percentage that NF- κ B-p65/RelA has the 254th amino acid threonine which is phosphorylated is significantly higher in the prostate cancer cells in the cases where the bone metastasis was observed than in the cases where bone metastasis was not observed, and that the probability that a prostate cancer will metastasize can be determined at an early stage by examining whether the 254th amino acid threonine in NF- κ B-p65/RelA is phosphorylated or not, thereby completing the present invention.

That is, the present invention provides a method for determining probability that a prostate cancer will metastasize, the method comprising measuring human NF- κ B-p65/RelA in which 254th amino acid threonine is phosphorylated, the human NF- κ B-p65/RelA being contained in a prostate tissue separated from human. The present invention also provides a diagnostic reagent for determining probability that a prostate cancer will metastasize, the reagent comprising an antibody or an antigen-binding fragment thereof, the antibody undergoing antigen-antibody reaction with human NF- κ B-p65/RelA in which 254th amino acid threonine is phosphorylated but not undergoing antigen-antibody reaction with human NF- κ B-p65/RelA in which 254th amino acid threonine is not phosphorylated.

Effects of the Invention

By the present invention, means by which the probability that a prostate cancer will metastasize can be determined at an early stage was first provided. By the present invention, since the probability that a prostate cancer will metastasize can be determined at an early stage, therapy or treatment of the patient can be properly selected, so that the present invention is expected to greatly contribute to the therapy of prostate cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 A FIGURE which comparatively shows the ratio of strongly positive, positive and negative results of immunohistochemical staining of prostate tissues in the cases where bone metastasis was observed and not observed, respectively, which immunohistochemical staining was carried in the Example of the present invention.

BEST MODE FOR CARRYING OUT THE INVENTION

NF- κ B is a transcription factor which is expressed widely in most cells. Since it is expressed in nuclei of cells, it is called nuclear factor. NF- κ B-p65/RelA is one of the subunits of NF- κ B. The amino acid sequence of human NF- κ B-p65/RelA and the cDNA sequence encoding it are known, and are shown in GenBank Accession No. M62399. The base sequence of the cDNA of human NF- κ B-p65/RelA and the amino acid sequence encoded thereby are shown in SEQ ID NO: 1 in SEQUENCE LISTING, and the amino acid sequence alone is shown in SEQ ID NO:2.

In the method of the present invention, the human NF- κ B-p65/RelA in which 254th amino acid threonine is phosphorylated (hereinafter also referred to as "254 phosphorylated NF- κ B-p65/RelA" for convenience). The term "threonine is phosphorylated" means that the hydroxyl group in the side chain of threonine is converted to a phosphoric acid ester. Phosphorylated threonine per se is well-known. In the present invention, the term "measure" includes any of detection, semi-quantification and quantification. The term "measure the 254 phosphorylated NF- κ B-p65/RelA" means to measure the 254 phosphorylated NF- κ B-p65/RelA distinctly from the NF- κ B-p65/RelA whose 254th threonine is not phosphorylated (hereinafter also referred to as "254 unphosphorylated NF- κ B-p65/RelA" for short).

An example of the preferred method for measuring the 254 phosphorylated NF- κ B-p65/RelA is an immunoassay using an antibody (hereinafter also referred to as "254 phosphorylated NF- κ B-p65/RelA-specific antibody") or an antigen-binding fragment thereof, which antibody undergoes antigen-antibody reaction with 254 phosphorylated NF- κ B-p65/RelA but does not undergo antigen-antibody reaction with 254 unphosphorylated NF- κ B-p65/RelA.

The 254 phosphorylated NF- κ B-p65/RelA-specific antibody may be a polyclonal antibody or a monoclonal antibody. The 254 phosphorylated NF- κ B-p65/RelA-specific polyclonal antibody can be obtained by preparing a polyclonal antibody by a conventional method using as an immunogen 254 phosphorylated NF- κ B-p65/RelA or a fragment thereof containing the 254th threonine; and removing, from the obtained polyclonal antibody, by adsorption the antibodies which also undergo antigen-antibody reaction with 254 unphosphorylated NF- κ B-p65/RelA (see the Example below). The monoclonal antibody can be obtained by preparing hybridomas each producing an anti-254 phosphorylated

NF- κ B-p65/RelA monoclonal antibody by a conventional method using as an immunogen 254 phosphorylated NF- κ B-p65/RelA or a fragment thereof containing the 254th threonine; selecting, from the obtained hybridomas, those producing the 254 phosphorylated NF- κ B-p65/RelA-specific antibody; culturing the selected hybridomas in vitro or in abdominal cavity of an animal, or the like; and recovering the 254 phosphorylated NF- κ B-p65/RelA-specific antibody produced by the hybridomas from the culture medium or ascites. In the immunoassay, the 254 phosphorylated NF- κ B-p65/RelA-specific antibody may be used, or a fragment thereof having a binding activity to the antigen, such as Fab fragment or F(ab')₂ fragment (in the present invention, referred to as "antigen-binding fragment") may also be used.

As the immunogen used for the preparation of the anti-254 phosphorylated NF- κ B-p65/RelA antibody, the whole molecule of 254 phosphorylated NF- κ B-p65/RelA or a fragment thereof containing the 254th threonine may be used, as mentioned above. In the latter case, the size of the peptide fragment is preferably not less than 10 amino acids, more preferably not less than 12 amino acids. Since a 254 phosphorylated NF- κ B-p65/RelA-specific antibody was obtained in the Example described below using a fragment having 12 amino acids ((VFRT(PO3H2)PPYADPSC) (SEQ ID NO: 3), the "(PO3H2)" after T means that the hydroxyl group in the side chain of this threonine (T) is phosphorylated), a size of 12 amino acids is sufficient. Needless to say, however, those having a longer size (the longest is the whole molecule of 254 phosphorylated NF- κ B-p65/RelA) can also be used as the immunogen. The peptide fragment of the 254 phosphorylated NF- κ B-p65/RelA which can be used as the immunogen can easily be chemically synthesized using a commercially available peptide synthesizer. Since the phosphorylated threonine per se is commercially available, a peptide fragment containing the phosphorylated threonine as the 254th threonine can be synthesized by using the commercially available threonine as a material in the step of binding the 254th threonine when the peptide is chemically synthesized. In cases where a peptide fragment is used as the immunogen, the peptide fragment may be used as the immunogen as it is, or the peptide fragment bound to a protein carrier such as bovine serum albumin, casein or keyhole limpet hemocyanin, may be used as the immunogen. Since a 254 phosphorylated NF- κ B-p65/RelA-specific antibody was obtained in the Example below by administering a peptide fragment consisting of 12 amino acids as it is, binding to the carrier protein is not indispensable.

The immunoassay can be carried out by immunohistochemical staining to a prostate tissue separated from the living body, or by a well-known immunoassay such as sandwich method, competition method or agglutination method using as a test sample a cell homogenate of a prostate tissue separated from the living body or the purified or partially purified product thereof containing NF- κ B-p65/RelA. Among these methods, immunohistochemical staining is preferred. The immunohistochemical staining can be carried out by a conventional method except that the 254 phosphorylated NF- κ B-p65/RelA-specific antibody is used as the antibody (see the Example below). Since a kit for immunohistochemical staining is commercially available, the immunohistochemical staining can be easily carried out using the commercially available kit and the above-described 254 phosphorylated NF- κ B-p65/RelA-specific antibody as the antibody.

As will be concretely described in the Example below, in the prostate cancer tissue metastasized to bone, the percentage that the reactivity with the 254 phosphorylated NF- κ B-

p65/RelA-specific antibody is positive (positive rate) was statistically significantly higher than in the cases where bone metastasis was not observed. Moreover, among the cases where the 254 phosphorylated NF- κ B-p65/RelA was positive, in the cases where the abundance of 254 phosphorylated NF- κ B-p65/RelA was especially high (in the cases where the immunohistochemical staining was strongly positive), the percentage of bone metastasis was especially high. Further, in most cases where bone metastasis occurred, the immunohistochemical staining was positive irrespective of the histological malignancy (Gleason grade). These results show that the 254 phosphorylated NF- κ B-p65/RelA can be utilized as a prostate cancer metastasis marker indicating the probability of metastasis of prostate cancer, and whether metastasis will occur in the future or not can be estimated irrespective of the histological malignancy.

The present invention will now be described more concretely by way of an example thereof. However, the present invention is not restricted to the Example below.

EXAMPLE

Materials and Methods

1. Preparation of 254 Phosphorylated NF- κ B-p65/RelA-Specific Antibody

A phosphorylated peptide (VFRT(PO3H2)PPYADPSC) (SEQ ID NO: 3) which was a fragment of p65/RelA, containing the 254th threonine (12 amino acids from the 251st valine to 262nd cysteine) wherein the 254th threonine had a phosphate group attached thereto was chemically synthesized using a peptide synthesizer. As the phosphorylated threonine to which a phosphate group was added, a commercially available product was used. The obtained peptide fragment was mixed with an equal amount of Freund complete adjuvant (first immunization) or Freund incomplete adjuvant (second and subsequent immunization), and an emulsion was prepared by sonication. The resulting viscous emulsion was subcutaneously administered to the back of a rabbit (New Zealand white rabbit). The immunization was conducted totally 4 times at 2-week intervals. At the time of third immunization, a small amount of the antigen protein was sampled, and antibody titer was measured by ELISA. Two weeks after the fourth immunization, the total blood was collected from the carotid artery under anesthesia with ketamine-xylazine. The collected blood was left to stand at room temperature for 1 hour, and then at 4° C. overnight, followed by centrifugation at 3000 rpm for 10 minutes to obtain a serum.

Using a commercially available antibody-purification kit (Amersham Bio., HiTrap Protein A HP), antibody was purified from the obtained antiserum. The buffers used were as follows:

Binding buffer	20 mM sodium phosphate, pH 7.0
Elution buffer	0.1 M sodium citrate, pH 3.0
Neutralization buffer	1.0 M Tris-HCl, pH 9.0

Concrete purification operation was as follows:

- (1) Preparation of column: A syringe was connected without introducing bubbles into the column, and 25 mL of ultrapure water was applied at a rate of 5 drops/second.
- (2) Equilibration of Column: 25 mL of binding buffer was applied at a rate of 5 drops/second.
- (3) Addition of sample: The prepared sample (dilution of antiserum) was applied at a rate of 5 drops/second, and the

adsorbed components were washed, followed by application of 25 mL of binding buffer at a rate of 5 drops/second.

- (4) Elution of antibody: 25 mL of elution buffer was applied at a rate of 5 drops/second, the eluted solution was collected in 3 mL fractions (300 μ L of neutralization buffer was added to each collection tube), the concentration of each of the eluted fractions was measured, the absorbance at 280 nm of each fraction was measured, the antibody fraction was recovered and dialyzed to PBS(-) overnight to exchange the buffer.
- From the obtained polyclonal antibody, the antibody which underwent antigen-antibody reaction also with 254 unphosphorylated NF- κ B-p65/RelA was removed by adsorption. More particularly, a biotinylated 254 unphosphorylated NF- κ B-p65/RelA (VFRTPPYADPSC) (SEQ ID NO: 3) chemically synthesized with a peptide synthesizer was bound to commercially available streptavidin-magnetic beads (amount of total bound antigen: 0.01 mg; amount of beads: 0.2 mg), and the beads were mixed with 10-fold diluted antibody solution (0.3 mL) at 4° C. for 2 hours.

Then the non-specific antibody adsorbed to the unphosphorylated peptide was removed using a magnet, and the supernatant was recovered to obtain a 254 phosphorylated NF- κ B-p65/RelA-specific antibody.

2. Immunohistochemical Staining

Using a commercially available immunohistochemical staining kit (VECTASTAIN ABC Kit), immunohistochemical staining with the 254 phosphorylated NF- κ B-p65/RelA-specific antibody obtained in the above-described 1 was performed on prostate tissues separated from patients. The concrete operation of the immunohistochemical staining was as follows:

- (1) Deparaffinization and hydrophilization of each tissue section with xylene and ethanol
- (2) Washing twice (each for 5 minutes) with 100 mL each of Tris buffered physiological saline (TBS).
- (3) Inactivation of endogenous peroxidase (1 hour) with 60 mL of TBS supplemented with 2 mL of 30% H₂O₂ (1%)
- (4) Washing twice (each for 5 minutes) with 100 mL each of TBS.
- (5) Blocking (1 hour) with goat serum in TBS (3 mL) (final concentration: 10%)
- (6) Washing once (for 5 minutes) with 100 mL of TBST (0.1% Tween 20 (trade name)-containing TBS)
- (7) Primary antibody (254 phosphorylated NF- κ B-p65/RelA-specific antibody prepared in the above-described 1) diluted (50-fold to 150-fold) with the blocking solution was added to the sample and the resultant was left to stand at room temperature for 2 hours or at 4° C. overnight.
- (8) Washing twice (each for 5 minutes) with 100 mL each of TBS
- (9) Secondary antibody (500 μ L of TBS supplemented with 2 μ L of biotinylated secondary antibody (goat anti-rabbit IgG antibody, included in the kit)) was added to the sample and the resultant was allowed to react for 2 hours.
- (10) Washing twice (each for 5 minutes) with 100 mL each of TBS
- (11) Preparation of AB solution (10 mL of TBS supplemented with 2 drops each of A: avidin solution (C solution) and B: biotinylated peroxidase (D solution). AB solution was prepared 30 minutes before use and allowed to react.
- (12) AB solution is added to the sample, and the resultant is allowed to react for 2 hours.
- (13) Washing twice (each for 5 minutes) with 100 mL each of TBS
- (14) DAB solution (TBS (5 mL) containing 50 μ g (10 μ g/mL) of diaminobenzidine (DAB) which is a substrate of peroxidase and 5 μ L (0.03%) of H₂O₂ (produced by VECTOR) is

added to the sample, and the resultant is allowed to react for about 5 to 10 minutes in the dark. Coloring is checked some-times.

(15) Washing once (for 5 minutes) with 100 mL of TBS

(16) Staining of nuclei of cells in blue with hematoxylin reagent

(17) Ethanol substitution, dropping xylene and a sealant, and seal with a cover glass

3. Materials

Prostate cancer operation samples extirpated in Yokohama City University Hospital and related hospitals and pre-operation needle biopsies (total 49 cases, breakdown was as follows:)

Cases where bone metastasis occurred within 3 to 5 years (27 cases)

Cases where bone metastasis did not occur in the period mentioned above (22 cases)

Bone metastatic site samples of prostate cancer (6 cases)

4. Results

Immunostaining was performed on the prostate cancer tissue samples by immunohistochemistry with the antibody. The samples used were the needle biopsy tissue samples or the extirpated prostate samples of the cases (27 cases) where bone metastasis occurred during the follow-up period of 3 to 5 years (including the cases where bone metastasis was found at the first visit), and the cases (22 cases) where bone metastasis did not occur during the above-mentioned period. Bone metastatic site samples of prostate cancer (6 cases) were also stained, and evaluation at the metastatic site was also conducted.

As a result of immunohistochemical staining, in prostate cancer cases where bone metastasis occurred, positive image (brown) was observed in the primary focus and in the metastatic focus in the prostate cancer cells. In the staining pattern, strong staining was observed chiefly in the nuclei. Further, irrespective of histological malignancy (Gleason grade), in most of the bone metastasis cases, the results were positive and in most of the cases where bone metastasis did not occur, the results were negative.

The results of staining were classified into 3 grades, namely, negative, positive, and strongly positive by two or more pathologists. The negative grade was the cases where

staining with the antibody was not observed; the strongly positive grade was the cases where strong staining with the antibody was observed; and the positive grade was the cases where staining with the antibody was observed, but the degree of staining was not so strong as the strongly positive grade.

The results are shown in Table 1 below and FIG. 1. As shown in Table 1 and FIG. 1, irrespective of the histological malignancy, in the cases where bone metastasis occurred, 19 cases were strongly positive grade, 8 cases were positive grade and 0 case was negative grade, so that the antibody reaction was positive in 100% of the cases. On the other hand, in the cases where bone metastasis did not occur, 1 case was strongly positive grade, 6 cases were positive grade and 15 cases were negative grade, so that most of them was negative grade. Since the follow-up period is short, it is thought that there is a possibility that in the strongly positive case and the positive cases in the cases where bone metastasis did not occur, bone metastasis may occur in the future. Further, in the site of bone metastasis, among the 6 cases, 3 cases were strongly positive grade and 3 cases were positive grade, so that the positive rate was 100% too. To determine whether there is a significant difference between the groups with bone metastasis and without bone metastasis in the results of staining with the antibody, a statistical test (chi square test) was carried out. As a result, in the bone metastasis-positive group, the staining with the antibody was significantly strongly positive or positive with a 99% significant difference ($P < 0.01$ ($\chi^2_{0.01} = 31.2 > \chi^2(2; 0.01) = 9.21$). By these results, it was proved that the antibody can be used as a molecular marker (diagnostic reagent) for determining the probability that the prostate cancer will metastasized.

TABLE 1

Immunohistochemical Staining	Number of Cases	
	without metastasis	with metastasis
Strongly Positive	1	19
Positive	6	8
Negative	15	0
Total	22	27

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 3

<210> SEQ ID NO 1

<211> LENGTH: 1767

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (80) .. (1735)

<400> SEQUENCE: 1

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cgccccggg accccggcc atg gac gaa ctg ttc ccc ctc atc ttc ccg gca 112
Met Asp Glu Leu Phe Pro Leu Ile Phe Pro Ala
1 5 10

gag cca gcc cag gcc tct ggc ccc tat gtg gag atc att gag cag ccc 160
Glu Pro Ala Gln Ala Ser Gly Pro Tyr Val Glu Ile Ile Glu Gln Pro
15 20 25

aag cag cgg ggc atg cgc ttc cgc tac aag tgc gag ggg cgc tcc gcg 208

-continued

Lys	Gln	Arg	Gly	Met	Arg	Phe	Arg	Tyr	Lys	Cys	Glu	Gly	Arg	Ser	Ala		
		30						35					40				
ggc	agc	atc	cca	ggc	gag	agg	agc	aca	gat	acc	acc	aag	acc	cac	ccc		256
Gly	Ser	Ile	Pro	Gly	Glu	Arg	Ser	Thr	Asp	Thr	Thr	Lys	Thr	His	Pro		
	45					50					55						
acc	atc	aag	atc	aat	ggc	tac	aca	gga	cca	ggg	aca	gtg	cgc	atc	tcc		304
Thr	Ile	Lys	Ile	Asn	Gly	Tyr	Thr	Gly	Pro	Gly	Thr	Val	Arg	Ile	Ser		
	60				65					70					75		
ctg	gtc	acc	aag	gac	cct	cct	cac	cgg	cct	cac	ccc	cac	gag	ctt	gta		352
Leu	Val	Thr	Lys	Asp	Pro	Pro	His	Arg	Pro	His	Pro	His	Glu	Leu	Val		
				80					85					90			
gga	aag	gac	tgc	cgg	gat	ggc	ttc	tat	gag	gct	gag	ctc	tgc	ccg	gac		400
Gly	Lys	Asp	Cys	Arg	Asp	Gly	Phe	Tyr	Glu	Ala	Glu	Leu	Cys	Pro	Asp		
		95						100					105				
cgc	tgc	atc	cac	agt	ttc	cag	aac	ctg	gga	atc	cag	tgt	gtg	aag	aag		448
Arg	Cys	Ile	His	Ser	Phe	Gln	Asn	Leu	Gly	Ile	Gln	Cys	Val	Lys	Lys		
		110					115					120					
cgg	gac	ctg	gag	cag	gct	atc	agt	cag	cgc	atc	cag	acc	aac	aac	aac		496
Arg	Asp	Leu	Glu	Gln	Ala	Ile	Ser	Gln	Arg	Ile	Gln	Thr	Asn	Asn	Asn		
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ccc	ttc	caa	ggt	cct	ata	gaa	gag	cag	cgt	ggg	gac	tac	gac	ctg	aat		544
Pro	Phe	Gln	Val	Pro	Ile	Glu	Glu	Gln	Arg	Gly	Asp	Tyr	Asp	Leu	Asn		
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Ala	Val	Arg	Leu	Cys	Phe	Gln	Val	Thr	Val	Arg	Asp	Pro	Ser	Gly	Arg		
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ccc	ctc	cgc	ctg	ccg	cct	gtc	ctt	cct	cat	ccc	atc	ttt	gac	aat	cgt		640
Pro	Leu	Arg	Leu	Pro	Pro	Val	Leu	Pro	His	Pro	Ile	Phe	Asp	Asn	Arg		
			175					180					185				
gcc	ccc	aac	act	gcc	gag	ctc	aag	atc	tgc	cga	gtg	aac	cga	aac	tct		688
Ala	Pro	Asn	Thr	Ala	Glu	Leu	Lys	Ile	Cys	Arg	Val	Asn	Arg	Asn	Ser		
		190					195					200					
ggc	agc	tgc	ctc	ggt	ggg	gat	gag	atc	ttc	cta	ctg	tgt	gac	aag	gtg		736
Gly	Ser	Cys	Leu	Gly	Gly	Asp	Glu	Ile	Phe	Leu	Leu	Cys	Asp	Lys	Val		
	205				210						215						
cag	aaa	gag	gac	att	gag	gtg	tat	ttc	acg	gga	cca	ggc	tgg	gag	gcc		784
Gln	Lys	Glu	Asp	Ile	Glu	Val	Tyr	Phe	Thr	Gly	Pro	Gly	Trp	Glu	Ala		
	220				225					230					235		
cga	ggc	tcc	ttt	tgc	caa	gct	gat	gtg	cac	cga	caa	gtg	gcc	att	gtg		832
Arg	Gly	Ser	Phe	Ser	Gln	Ala	Asp	Val	His	Arg	Gln	Val	Ala	Ile	Val		
				240					245					250			
ttc	cgg	acc	cct	ccc	tac	gca	gac	ccc	agc	ctg	cag	gct	cct	gtg	cgt		880
Phe	Arg	Thr	Pro	Pro	Tyr	Ala	Asp	Pro	Ser	Leu	Gln	Ala	Pro	Val	Arg		
			255					260					265				
gtc	tcc	atg	cag	ctg	cgg	cgg	cct	tcc	gac	cgg	gag	ctc	agt	gag	ccc		928
Val	Ser	Met	Gln	Leu	Arg	Arg	Pro	Ser	Asp	Arg	Glu	Leu	Ser	Glu	Pro		
		270					275						280				
atg	gaa	ttc	cag	tac	ctg	cca	gat	aca	gac	gat	cgt	cac	cgg	att	gag		976
Met	Glu	Phe	Gln	Tyr	Leu	Pro	Asp	Thr	Asp	Asp	Arg	His	Arg	Ile	Glu		
	285					290					295						
gag	aaa	cgt	aaa	agg	aca	tat	gag	acc	ttc	aag	agc	atc	atg	aag	aag		1024
Glu	Lys	Arg	Lys	Arg	Thr	Tyr	Glu	Thr	Phe	Lys	Ser	Ile	Met	Lys	Lys		
	300				305					310					315		
agt	cct	ttc	agc	gga	ccc	acc	gac	ccc	cgg	cct	cca	cct	cga	cgc	att		1072
Ser	Pro	Phe	Ser	Gly	Pro	Thr	Asp	Pro	Arg	Pro	Pro	Pro	Arg	Arg	Ile		
				320					325					330			
gct	gtg	cct	tcc	cgc	agc	tca	gct	tct	gtc	ccc	aag	cca	gca	ccc	cag		1120
Ala	Val	Pro	Ser	Arg	Ser	Ser	Ala	Ser	Val	Pro	Lys	Pro	Ala	Pro	Gln		
			335					340					345				
ccc	tat	ccc	ttt	acg	tca	tcc	ctg	agc	acc	atc	aac	tat	gat	gag	ttt		1168

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Pro Tyr Pro Phe Thr Ser Ser Leu Ser Thr Ile Asn Tyr Asp Glu Phe
   350                               355                 360
ccc acc atg gtg ttt cct tct ggg cag atc agc cag gcc tcg gcc ttg   1216
Pro Thr Met Val Phe Pro Ser Gly Gln Ile Ser Gln Ala Ser Ala Leu
   365                               370                 375

gcc ccg gcc cct ccc caa gtc ctg ccc cag gct cca gcc cct gcc cct   1264
Ala Pro Ala Pro Pro Gln Val Leu Pro Gln Ala Pro Ala Pro Ala Pro
   380                               385                 390                 395

gct cca gcc atg gta tca gct ctg gcc cag gcc cca gcc cct gtc cca   1312
Ala Pro Ala Met Val Ser Ala Leu Ala Gln Ala Pro Ala Pro Val Pro
   400                               405                 410

gtc cta gcc cca ggc cct cct cag gct gtg gcc cca cct gcc ccc aag   1360
Val Leu Ala Pro Gly Pro Pro Gln Ala Val Ala Pro Pro Ala Pro Lys
   415                               420                 425

ccc acc cag gct ggg gaa gga acg ctg tca gag gcc ctg ctg cag ctg   1408
Pro Thr Asp Ala Gly Glu Gly Thr Leu Ser Glu Ala Leu Leu Gln Leu
   430                               435                 440

cag ttt gat gat gaa gac ctg ggg gcc ttg ctt ggc aac agc aca gac   1456
Gln Phe Asp Asp Glu Asp Leu Gly Ala Leu Leu Gly Asn Ser Thr Asp
   445                               450                 455

cca gct gtg ttc aca gac ctg gca tcc gtc gac aac tcc gag ttt cag   1504
Pro Ala Val Phe Thr Asp Leu Ala Ser Val Asp Asn Ser Glu Phe Gln
   460                               465                 470                 475

cag ctg ctg aac cag ggc ata cct gtg gcc ccc cac aca act gag ccc   1552
Gln Leu Leu Asn Gln Gly Ile Pro Val Ala Pro His Thr Thr Glu Pro
   480                               485                 490

atg ctg atg gag tac cct gag gct ata act cgc cta gtg aca ggg gcc   1600
Met Leu Met Glu Tyr Pro Glu Ala Ile Thr Arg Leu Val Thr Gly Ala
   495                               500                 505

cag agg ccc ccc gac cca gct cct gct cca ctg ggg gcc ccg ggg etc   1648
Gln Arg Pro Pro Asp Pro Ala Pro Ala Pro Leu Gly Ala Pro Gly Leu
   510                               515                 520

ccc aat ggc ctc ctt tca gga gat gaa gac ttc tcc tcc att gcg gac   1696
Pro Asn Gly Leu Leu Ser Gly Asp Glu Asp Phe Ser Ser Ile Ala Asp
   525                               530                 535

atg gac ttc tca gcc ctg ctg agt cag atc agc tcc taa gggggtgacg   1745
Met Asp Phe Ser Ala Leu Leu Ser Gln Ile Ser Ser
   540                               545                 550

cctgccctcc ccagagcact gg   1767

<210> SEQ ID NO 2
<211> LENGTH: 551
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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Ser Gly Pro Tyr Val Glu Ile Ile Glu Gln Pro Lys Gln Arg Gly Met
20                               25                               30
Arg Phe Arg Tyr Lys Cys Glu Gly Arg Ser Ala Gly Ser Ile Pro Gly
35                               40                               45
Glu Arg Ser Thr Asp Thr Thr Lys Thr His Pro Thr Ile Lys Ile Asn
50                               55                               60
Gly Tyr Thr Gly Pro Gly Thr Val Arg Ile Ser Leu Val Thr Lys Asp
65                               70                               75                               80
Pro Pro His Arg Pro His Pro His Glu Leu Val Gly Lys Asp Cys Arg
85                               90                               95
Asp Gly Phe Tyr Glu Ala Glu Leu Cys Pro Asp Arg Cys Ile His Ser

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100					105					110					
Phe	Gln	Asn	Leu	Gly	Ile	Gln	Cys	Val	Lys	Lys	Arg	Asp	Leu	Glu	Gln
		115					120					125			
Ala	Ile	Ser	Gln	Arg	Ile	Gln	Thr	Asn	Asn	Asn	Pro	Phe	Gln	Val	Pro
		130					135					140			
Ile	Glu	Glu	Gln	Arg	Gly	Asp	Tyr	Asp	Leu	Asn	Ala	Val	Arg	Leu	Cys
		145					150					155			
Phe	Gln	Val	Thr	Val	Arg	Asp	Pro	Ser	Gly	Arg	Pro	Leu	Arg	Leu	Pro
				165								170			175
Pro	Val	Leu	Pro	His	Pro	Ile	Phe	Asp	Asn	Arg	Ala	Pro	Asn	Thr	Ala
				180					185					190	
Glu	Leu	Lys	Ile	Cys	Arg	Val	Asn	Arg	Asn	Ser	Gly	Ser	Cys	Leu	Gly
		195					200					205			
Gly	Asp	Glu	Ile	Phe	Leu	Leu	Cys	Asp	Lys	Val	Gln	Lys	Glu	Asp	Ile
		210					215					220			
Glu	Val	Tyr	Phe	Thr	Gly	Pro	Gly	Trp	Glu	Ala	Arg	Gly	Ser	Phe	Ser
		225					230					235			240
Gln	Ala	Asp	Val	His	Arg	Gln	Val	Ala	Ile	Val	Phe	Arg	Thr	Pro	Pro
				245					250					255	
Tyr	Ala	Asp	Pro	Ser	Leu	Gln	Ala	Pro	Val	Arg	Val	Ser	Met	Gln	Leu
			260					265					270		
Arg	Arg	Pro	Ser	Asp	Arg	Glu	Leu	Ser	Glu	Pro	Met	Glu	Phe	Gln	Tyr
			275				280					285			
Leu	Pro	Asp	Thr	Asp	Asp	Arg	His	Arg	Ile	Glu	Glu	Lys	Arg	Lys	Arg
			290				295					300			
Thr	Tyr	Glu	Thr	Phe	Lys	Ser	Ile	Met	Lys	Lys	Ser	Pro	Phe	Ser	Gly
		305					310					315			320
Pro	Thr	Asp	Pro	Arg	Pro	Pro	Pro	Arg	Arg	Ile	Ala	Val	Pro	Ser	Arg
				325					330					335	
Ser	Ser	Ala	Ser	Val	Pro	Lys	Pro	Ala	Pro	Gln	Pro	Tyr	Pro	Phe	Thr
			340					345					350		
Ser	Ser	Leu	Ser	Thr	Ile	Asn	Tyr	Asp	Glu	Phe	Pro	Thr	Met	Val	Phe
			355				360					365			
Pro	Ser	Gly	Gln	Ile	Ser	Gln	Ala	Ser	Ala	Leu	Ala	Pro	Ala	Pro	Pro
			370				375					380			
Gln	Val	Leu	Pro	Gln	Ala	Pro	Ala	Pro	Ala	Pro	Ala	Pro	Ala	Met	Val
			385				390					395			400
Ser	Ala	Leu	Ala	Gln	Ala	Pro	Ala	Pro	Val	Pro	Val	Leu	Ala	Pro	Gly
				405					410					415	
Pro	Pro	Gln	Ala	Val	Ala	Pro	Pro	Ala	Pro	Lys	Pro	Thr	Gln	Ala	Gly
			420					425					430		
Glu	Gly	Thr	Leu	Ser	Glu	Ala	Leu	Leu	Gln	Leu	Gln	Phe	Asp	Asp	Glu
			435				440					445			
Asp	Leu	Gly	Ala	Leu	Leu	Gly	Asn	Ser	Thr	Asp	Pro	Ala	Val	Phe	Thr
			450				455					460			
Asp	Leu	Ala	Ser	Val	Asp	Asn	Ser	Glu	Phe	Gln	Gln	Leu	Leu	Asn	Gln
			465				470					475			480
Gly	Ile	Pro	Val	Ala	Pro	His	Thr	Thr	Glu	Pro	Met	Leu	Met	Glu	Tyr
				485					490					495	
Pro	Glu	Ala	Ile	Thr	Arg	Leu	Val	Thr	Gly	Ala	Gln	Arg	Pro	Pro	Asp
			500					505					510		
Pro	Ala	Pro	Ala	Pro	Leu	Gly	Ala	Pro	Gly	Leu	Pro	Asn	Gly	Leu	Leu
			515				520					525			

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Ser Gly Asp Glu Asp Phe Ser Ser Ile Ala Asp Met Asp Phe Ser Ala
 530 535 540

Leu Leu Ser Gln Ile Ser Ser
 545 550

<210> SEQ ID NO 3

<211> LENGTH: 12

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic peptide fragment used as an immunogen

<400> SEQUENCE: 3

Val Phe Arg Thr Pro Pro Tyr Ala Asp Pro Ser Cys
 1 5 10

The invention claimed is:

1. A method for determining probability that a prostate cancer will metastasize, said method comprising measuring human NF- κ B-p65/RelA in which 254th amino acid threonine is phosphorylated, said human NF- κ B-p65/RelA being contained in a prostate tissue separated from human, wherein an increase in the levels of human NF- κ B-p65/RelA in which the 254th amino acid threonine is phosphorylated in a patient compared to the levels of human NF- κ B-p65/RelA in which the 254th amino acid threonine is phosphorylated in patients not having metastasized prostate cancer indicates an increase in the probability that a prostate cancer will metastasize.

20 2. The method according to claim 1, carried out by an immunoassay using an antibody or an antigen-binding fragment thereof, said antibody undergoing antigen-antibody reaction with human NF- κ B-p65/RelA in which 254th amino acid threonine is phosphorylated but not undergoing antigen-antibody reaction with human NF- κ B-p65/RelA in which 254th amino acid threonine is not phosphorylated.

25 3. The method according to claim 2, wherein said immunoassay is immunohistochemical staining.

30 4. The method according to according to any one of claims 1 to 3, wherein the metastasis is bone metastasis.

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