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DESCRIPTION

ONCOLYTIC VIRUS REPLICATING SELECTIVELY IN TUMOR CELLS

TECHNICAL FIELD

The present invention relates to a virus showing antitumor effect by replicating in tumor cells; a polynucleotide contained in the virus; an anticancer agent comprising the virus; and a method of treating cancers using the virus.

BACKGROUND ART

At present, gene therapy is performed as one method for treating cancers. However, since a gene is introduced into diseased tissue or the like with a non-replication competent virus vector in gene therapy, the gene can be applied to only those regions around target cells taking into consideration the safety of the human body. Also, in the gene therapy currently practiced, satisfactory therapeutic effect cannot be achieved because of low efficiency in gene transfer.

It is known that telomerase activity is often enhanced in malignantly transformed cells or immortalized cell strains, whereas telomerase activity is hardly detected in normal somatic cells excluding such as germ line cells, blood lineage cells and epithelial stem cells.

Under circumstances, it is a major object of the present invention to let a virus grow in tumor cells by utilizing the telomerase activated therein to thereby bring death to the tumor cells efficiently.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows a schematic drawing of the structure of a oncolytic virus repliacting selectively in tumor cells. A replication cassette consisting of hTERT promoter, E1A gene, IRES sequence and E1B gene is inserted in the E1 gene region which non-replication competent virus vectors lack.

Fig. 2 shows comparison of telomerase activities in human cancer cells and normal cells.

Fig. 3 shows the expression of E1A and E1B mRNAs and proteins after TRAD infection in human cancer cells and normal cells.

Fig. 4 shows the intracellular replication of the virus after TRAD infection in human cancer cells and normal cells.

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Fig. 5 presents photographs showing, by staining with Coomassie brilliant blue, the cytotoxicity caused by TRAD in human cancer cells and normal cells.

Fig. 6 presents microscopic photographs showing the cytotoxicity caused by TRAD in human cancer cells and normal cells.

Fig. 7 presents graphs showing by means of XTT assay the cytotoxicity caused by TRAD in human cancer cells and normal cells.

Fig. 8 is a graph showing the antitumor effect produced by intratumoral, local administration of a non-replication competent, p53 gene-expressing adenovirus vector in an experiment using nude mice and human lung cancer cell H358.

Fig. 9 is a graph showing the antitumor effect produced by intratumoral, local administration of TRAD in an experiment using nude mice and human large bowel cancer cell SW620.

DISCLOSURE OF THE INVENTION

The present inventors have found for the first time that, by infecting cancer cells with a virus having a telomerase promoter and replication ability, it is possible to let the virus replicate in the cancer cells and bring death to them. Thus, the present invention has been achieved.

The present invention relates to the following items 1 to 10.

1. A polynucleotide comprising a promoter from human telomerase and at least one E1 gene.
2. The polynucleotide of item 1 above, wherein the E1 gene is an adenovirus-derived E1 gene.
3. The polynucleotide of item 1 or 2 above, wherein the promoter from human telomerase is hTERT.
4. The polynucleotide of any one of items 1 to 3 above, wherein the E1 gene comprises an E1A gene, an IRES sequence and an E1B gene in this order.
5. A virus comprising the polynucleotide of any one of items 1 to 4 above.
6. The virus of item 5 above, wherein the virus is an adenovirus.
7. An anticancer agent comprising the virus of item 5 or 6 above as an active ingredient and a pharmaceutically acceptable carrier, excipient or diluent.
8. A method of treating a cancer, comprising using the virus of item 5 or 6 above or using the anticancer agent of item 7 above.
9. The method of item 8 above, wherein the cancer is at least one cancer selected from the group consisting of stomach cancer, large bowel cancer, lung cancer, liver cancer, prostate

cancer, pancreas cancer, esophagus cancer, bladder cancer, gallbladder/bile duct cancer, breast cancer, uterine cancer, thyroid cancer and ovarian cancer.

10. The method of item 9 above, wherein the cancer is at least one selected from the group consisting of osteosarcoma and brain tumor.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is characterized by bringing death to cancer cells by infecting cancer cells with a virus having a telomerase promoter and replication ability and letting the virus grow in the cancer cells, based on the finding that a wide variety of cancer cells have telomerase activity.

The virus used in the present invention is not particularly limited. From the viewpoint of safety, adenovirus is preferable. Among adenovirus species, type 5 adenovirus is particularly preferable from the viewpoint of, for example, easiness in use.

E1 gene contained in viral polynucleotide refers to one of early genes of viruses. Viruses have early (E) genes and late (L) genes involved in their DNA replication. E1 gene encodes a protein involved in the regulation of transcription of viral genome.

The E1 gene used in the present invention may be derived from any virus. Preferably, an adenovirus-derived E1 gene is used.

It is known that E1 gene is composed of E1A, E1B and other elements. E1A protein encoded by E1A gene activates the transcription of a group of genes (E1B, E2, E4, etc.) necessary for the production of infectious virus.

E1B protein encoded by E1B gene assists the accumulation of late gene (L gene) mRNA in the cytoplasm of the infected host cell to thereby inhibit the protein synthesis in the host cell. Thus, E1B protein promotes viral replication. The sequences of adenovirus E1A gene and E1B gene are shown in SEQ ID NO: 1 and SEQ ID NO: 2, respectively.

In the present invention, a known E1 gene may be used as it is. Preferably, an E1 gene having an E1A gene, an IRES sequence and an E1B gene in this order (i.e., an E1 gene in which an IRES sequence is inserted between its E1A gene and E1B gene) is used. With the use of such an E1 gene, the replication ability of the virus of the invention will be high when a host cell has been infected with the virus.

As long as the effect of the invention can be achieved, at least one nucleotide may be inserted into at least one site selected from the group consisting of (a) between IRES sequence and E1A gene, (b) between IRES sequence and E1B gene, (c) upstream of E1A gene, and (d) downstream of E1B gene. As long as the effect of the invention can be achieved, at least one, preferably several nucleotides may be substituted, deleted, inserted or

added in the E1A gene, IRES sequence, E1B gene or E1 gene.

"IRES sequence" is a protein synthesis initiation signal specific to picornavirus. It is believed that this sequence serves as a ribosome-binding site because it contains a complementary sequence to the 3' terminal sequence of 18S ribosomal RNA. It is known that picornavirus-derived mRNA is translated via this sequence.

Translation efficiency from IRES sequence is high. Even from the middle of mRNA, protein synthesis is performed in a cap structure non-dependent manner. Therefore, in the virus of the present invention, both E1A gene and E1B gene located downstream of the IRES sequence are translated independently by a promoter from human telomerase. IRES sequence is shown in SEQ ID NO: 3.

In the present invention, it is preferable that E1 gene has a promoter from human telomerase upstream thereof, because such a promoter is capable of promoting the replication of the virus of the invention in cancer cells having telomerase activity. The promoter from human telomerase is not particularly limited as long as the promoter is derived from human. Among all, hTERT is preferable.

hTERT is a gene encoding human telomerase reverse transcriptase. A number of transcription factor-binding sequences are confirmed in a 1.4 kbp region upstream of the 5' end of this gene. This region is believed to be hTERT promoter. In particular, a 181 bp sequence located upstream of the translation initiation site is a core region important for the expression of the downstream gene.

In the present invention, any sequence comprising this core region may be used as a promoter from human telomerase. Preferably, an upstream sequence of approximately 378 bp containing the core region completely is used. It has been confirmed that this sequence of approximately 378 bp is equivalent to the 181 bp core region alone in gene expression efficiency. The sequence of hTERT is shown in SEQ ID NO: 4.

A gene having the telomerase promoter of the invention and the E1 gene of the invention (a gene comprising E1A gene, IRES gene and E1B gene) may be obtained by conventional genetic engineering techniques.

As the E1 gene, an E1 gene from a known virus having that gene may be used. Preferably, an E1 gene derived from adenovirus is used.

Alternatively, E1A gene and E1B gene may be amplified from E1 gene-expressing cells (preferably, E1 gene-expressing 293 cells or the like) by RT-PCR and/or DNA-PCR using primers such as E1A-S, E1A-AS, E1B-S and E1B-AS. If necessary, their sequences are confirmed using a conventional method such as TA cloning. Then, E1A and E1B DNA fragments may be cut out using a known restriction enzyme such as EcoRI.

E1A and E1B may be inserted into a known vector such as pIRES by conventional genetic engineering techniques to thereby prepare E1A-IRES-E1B sequence within the vector. Subsequently, hTERT promoter sequence which was cut out with restriction enzymes such as MluI and BglII may be inserted into the XhoI site or the like located upstream of E1A.

If necessary, cytomegalovirus (CMV) promoter contained in a known vector such as pShuttle may be removed with restriction enzymes such as MfeI and NheI; then, a sequence cut out from phTERT-E1A-IRES-E1B with restriction enzymes NheI and NotI may be inserted into the site (resultant vector is designated "pSh-hAIB").

From the resultant pSh-hAIB, a sequence comprising necessary portions (including hTERT promoter, E1A gene, IRES sequence and E1B gene) may be cut out with restriction enzymes such as I-CeuI and PI-SceI, and then inserted into a viral DNA such as Adeno-X Viral DNA using a commercial kit such as Adeno-X Expression System (Clontech) (the resultant DNA is designated "AdenoX-hAIB").

The above-described sequence comprising hTERT promoter, E1A gene, IRES sequence and E1B gene may be inserted into any site of a viral gene as long as the effect of the present invention can be achieved. For example, in adenovirus for gene therapy from which E1 gene has been deleted, the above-described sequence is preferably inserted into the deleted site.

It is possible to linearize AdenoX-hAIB with a known restriction enzyme such as PacI and then transfect into cultured cells such as 293 cells, to thereby prepare a infectious recombinant adenovirus (the resultant virus is sometimes called the "virus of the present invention" or "TRAD"). The method of transfection is not particularly limited. From the viewpoint of efficiency, such methods as the calcium phosphate method or electroporation may be preferable.

The thus obtained virus of the present invention can be replicated by conventional methods for viral replication, e.g. infecting host cells such as 293 cells with the virus.

The virus of the present invention may be used as an anticancer agent. This anticancer agent may be used not only for treating cancers but also for preventing postoperative relapse of cancers, preventing cancer metastasis and/or for prophylaxis of cancers.

The kinds of cancers to which the anticancer agent of the invention is applied are not particularly limited. The anticancer agent is applicable to any kind of cancer. For example, the anticancer agent is effective for cancers in the stomach, large bowel, lung, liver, prostate, pancreas, esophagus, bladder, gallbladder/bile duct, breast, uterus, thyroid, ovary,

etc. as well as brain tumor and osteosarcoma. Among all, the anticancer agent is especially effective for solid tumor.

The anticancer agent of the invention may be applied to diseased sites as it is. Alternatively, the anticancer agent may be introduced into humans (target cells or organs) by any known method, e.g. intravenous, intramuscular, intraperitoneal or subcutaneous injection; inhalation through the nasal cavity, oral cavity or lung; oral administration; administration in the form of suppository; and administration in the form of external medicine.

The virus of the invention may be treated, for example, by the lyophilization method to enable easy handling and then used alone, or prepared into pharmaceutical compositions by mixing with known pharmaceutically acceptable carriers such as excipients, fillers, binders, lubricants; or known additives (including such as buffers, isotonic agents, chelating agents, coloring agents, preservatives, flagrances, flavoring agents, and sweetening agents).

The anticancer agent of the present invention may be administered orally or parenterally depending on the form of the agent, e.g. oral administration agents such as tablets, capsules, powders, granules, pills, liquids, syrups, etc. and parenteral administration agents such as injections, external medicines, suppositories, eye drops, etc. Preferably, local injection into muscle or abdominal cavity, or intravenous injection may be enumerated.

Dose levels are selected appropriately depending on the kind of active ingredient, the administration route, the target of administration, and the age, body weight, sex, symptoms and other conditions of the patient. Usually, dose levels may be selected so that the virus of the invention (the active ingredient) is administered at a daily dose of about 10^6-10^{11} PFU, preferably about 10^9-10^{11} PFU. This amount may be administered once a day, or may be divided into several portions and administered at several times a day.

When the virus of the invention is administered, it is also possible to use a known immunosuppressant or the like to suppress the immunity of the living body to thereby make the viral infection easy.

Further, the virus of the invention may be used jointly with at least one anticancer agent selected from the group consisting of non-replication competent viruses (such as virus comprising p53 gene) used in conventional gene therapy, known anticancer agents and radiation.

The virus of the invention infected to the living body (cancer cells or cancer tissues) is capable of replicating in the cancer cells and bringing death to those cells. By thus bringing death to cancer cells, the virus of the invention can treat cancers, inhibit the growth

of tumor cells, and prevent metastasis of cancer cells.

It is believed that there is an extremely low possibility that the anticancer agent of the invention will produce side effects for the reasons described below. Thus, the anticancer agent of the invention can be said a very safe preparation.

(1) There is little telomerase activity in normal somatic cells, and yet adenovirus itself is hard to be infected to suspending cells such as hematopoietic cells. Therefore, when adenovirus is used in the present invention, still higher selectivity for tumor kinds is obtained.

(2) Since the virus of the invention has replication ability, it is possible to use this virus at a lower concentration than that of conventional non-replication competent virus used in conventional gene therapy.

(3) Even when the virus of the invention has been administered in excess, antiviral action works through ordinary immune reaction in the living body.

EXAMPLES

Hereinbelow, examples will be provided in order to illustrate the present invention in more detail. Needless to say, the present invention is not limited to these examples.

Example 1

<Preparation of TRAD>

An E1A gene of 899 bp was amplified from RNA extracted from 293 cells by RT-PCR using specific primers (E1A-S: SEQ ID NO: 5; E1A-AS: SEQ ID NO: 6). An E1B gene of 1823 bp was amplified from DNA extracted from 293 cells by DNA-PCR using primers (E1B-S: SEQ ID NO: 7; E1B-AS: SEQ ID NO: 8).

These PCR products were subjected to TA cloning (TA Cloning Kit Dual Promoter; Invitrogen) to thereby confirm their sequences. Then, DNA fragments of 899 bp (E1A) and 1823 bp (E1B) were cut out, respectively, with restriction enzyme EcoRI.

E1A and E1B were inserted into the MluI site and the SalI site of pIRES vector (Clontech), respectively, in the normal orientation (E1A-IRES-E1B).

A 455 bp hTERT promoter sequence which had been cut out with restriction enzymes MluI and BglII was inserted into the XhoI site located upstream of the E1A of E1A-IRES-E1B (phTERT-E1A-IRES-E1B).

The cytomegalovisus (CMV) promoter contained in pShuttle vector was removed by treatment with restriction enzymes MfeI and NheI. Then, a 3828 bp sequence cut out

from phTERT-E1A-IRES-E1B using restriction enzymes NheI and NotI was inserted into that site (pSh-hAIB).

A 4381 bp sequence was cut out from pSh-hAIB using restriction enzymes I-CeuI and P1-SceI, and inserted into the Adeno-X Viral DNA of Adeno-X Expression System (Clontech) (AdenoX-hAIB). This AdenoX-hAIB was treated with restriction enzyme PacI for linearization and then transfected into 293 cells by the phosphate calcium method. Thus. a infectious recombinant adenovirus (TRAD) was prepared. A schematic drawing of TRAD is shown in Fig. 1.

Example 2

<Comparison of Telomerase Activities in Human Cancer Cells and Normal Cells>

RNA was extracted from the following 10 kinds of cells using RNAzol (Cinna/Biotecx): human lung cancer cells (A549, H226Br and H1299); human large bowel cancer cells (SW620, DLD-1 and LoVo); human embryonic kidney cell 293; human umbilical vascular endothelial cell HUVEC immortalized by the introduction of SV40 gene; and human normal fibroblast cells (WI38 and NHLF). The resultant RNA was subjected to real time quantitative reverse transcription (RT)-PCR using Light Cycler DNA TeloTAGGG Kit (Roche Molecular Biochemicals), followed by comparison of expression levels of hTERT gene in respective cells. The results are shown in Fig. 2.

When expression levels were compared taking the level in A549 cells (which showed the highest expression) as 1.0, hTERT gene expression from 0.18 to 1.00 was observed in cancer cells (such as A549, H226Br, H1299, SW620, DLD-1, Lovo) and 293 cells, whereas no expression was detected in immortalized cell HuVEC and normal cells (such as WI38, NHLF).

Example 3

<Expression of E1A and E1B mRNAs and Proteins after TRAD Infection in Human Cancer Cells and Normal Cells>

Human large bowel cancer cell SW620 and human normal fibroblast cell WI38 were cultured *in vitro*. Then, each cell was infected with TRAD at concentrations of MOI (multiplicity of infection) 0.1 and 1, followed by recovery of RNA after 36 hours. As a positive control, 293 cells were used.

The recovered RNA was reverse-transcribed using GeneAmp RNA PCR Core Kit. The resultant DNA was amplified 30 cycles in GeneAmp PCR System 9700 Thermal Cycler (PE Applied Biosystems) using primers for E1A gene and E1B gene. The PCR products

were electrophoresed on 1.2% agarose gel and stained with ethidium bromide to thereby visualize bands (upper two panels in Fig. 3A). The intensities of the bands were measured with an image analyzer, quantitatively determined using GAPDH as an internal control and then shown in graphs (the bottom panel in Fig. 3A).

Human large bowel cancer cell SW620 and human normal fibroblast cell WI38 were cultured *in vitro*. Then, each cell was infected with TRAD at concentrations of MOI 0.1 and 1. After 48 hours, adherent cells were recovered and reacted in a lysis solution for 30 minutes, followed by centrifugation. The protein concentration in the resultant supernatant was measured. Briefly, the supernatant was electrophoresed on 12% polyacrylamide gel and transferred onto a membrane. Then, Western blot analysis was performed with anti-adenovirus 5 E1A antibody (PharMingen International). The results are shown in Fig. 3B.

While strong expression of E1A gene (502 bp) and E1B gene (543 bp) was clearly observed as a result of TRAD infection in cancer cell SW620, only weak expression of these genes was observed in normal cell WI38 (Fig. 3A). In the positive control 293 cells, medium expression of these genes was observed.

The results of Western blot analysis revealed that expression of E1A protein increased in SW620 as the concentration of TRAD increased from MOI 0.1 to 1 (Fig. 3B). On the other hand, expression of E1A protein was detected little in WI38 even when TRAD was used at MOI 1.

Example 4

<Examination of Intracellular Viral Replication after TRAD Infection in Human Cancer Cells and Normal Cells >

Human cancer cells (SW620 and H1299) and human normal cells (WI38 and NHLF) were infected with TRAD at MOI 1 for 2 hours at 37 °C. Then, the TRAD-containing culture broth was discarded. After cells were washed with a fresh culture broth once, a fresh culture broth was added further. Immediately thereafter (*i.e.*, on day 0), cells were recovered with a scraper and subjected to repetition of freezing and thawing. Then, they were suspended in 1 ml of a culture broth. Further, virus was recovered on day 1, 2, 3, 5 and 7 in the same manner, followed by measurement of viral titer. The results are shown in Fig. 4.

In normal cells WI38 and NHLF, TRAD increased from 10^2 PFU on day 1 to about 10^5 PFU on day 3 showing 100- to 1000-fold growth. On the other hand, in cancer cells SW620 and H1299, TRAD increased to 10^7-10^8 PFU showing 10^5- to 10^6-fold growth.

Thus. viral growth specific to cancer cells was confirmed.

Example 5

<Cytotoxic Activity of TRAD in Human Cancer Cells and Normal Cells>

Five kinds of human cancer cells (SW620, H1299, A549, DLD-1 and H226Br) were plated on 24-well plates at 6-8 x 10^4 cells/well, and two kinds of human normal cells (WI38 and NHLF) were plated on 24-well plates at 2-4 x 10^4 cells/well. After 24 hours, they were infected with TRAD at MOI 0.01, 0.1, 1, 2 and 5. Ninety-six hours after the infection, morphological changes in SW620, DLD-1 and NHLF cells were observed under microscopy. Further, culture broth was discarded from all of the cells. Then, viable cells were stained with Coomassie brilliant blue, and macroscopic images were taken into with a scanner.

SW620 and H1299 were plated at 10^4 cells/well and NHLF was plated at 5 x 10^3 cells/well, respectively, on 96-well plates. Cells were infected with TRAD at MOI 0 (non-infected cells), 0.01, 0.1 and 1. Then, the numbers of viable cells were measured by XTT assay on day 1, 2, 3, 5 and 7. The viable cell count was determined for each four wells. Taking the count in the non-infected cells as 1.0, counts in other cells were represented in graphs in means +/- SDs. Respective results are shown in Figs. 5, 6 and 7.

In cancer cells SW620, H1299, A549, DLD-1 and H226Br, cell counts decrease and areas stained with blue reduce in a TRAD concentration-dependant manner. On the other hand, in normal cells WI38 and NHLF, no remarkable decrease in the number of viable cells stained with blue was recognized (Fig. 5).

In the microscopic observation, SW620 and DLD-1 cells were peeled off from the plate bottom, became round-shaped and showed decrease in cell density; on the other hand, NHLF cells showed little morphological change and no decrease in cell count (Fig. 6).

In SW620 and H1299 cells, almost 100% cell death was observed by day 3 as a result of TRAD infection at MOI 1. More than 80% decrease in cell count was recognized even at MOI 0.1. On the other hand, NHLF showed almost no decrease in cell count even on day 3. Although NHLF showed about 60% decrease in cell count on day 7 when TRAD was used at MOI 1, it indicated no viral influence at MOI 0.01 (Fig. 7).

Example 6

<Examination of the Antitumor Activity of TRAD in Animal Models>

Human lung cancer cell H358 was transplanted subcutaneously into the back of 5-6 week-old nude mice at 5 x 10^6 cells/mouse. When the tumor became approximately 5-6

mm in diameter, a non-replication competent adenovirus vector (Ad-p53) was injected intratumorally and locally for consecutive two days at 1 x 10^8 PFU, 3 x 10^8 PFU and 1 x 10^9 PFU per day. Then, two axes of each tumor crossing at right angles were measured at regular intervals. The estimated tumor weight was calculated by the following formula: (major axis) x (minor axis)^2/2. As a control, a non-replication competent adenovirus vector dl312 containing no inserted gene was used.

Human large bowel cancer cell SW620 was transplanted subcutaneously into the back of 5-6 week-old nude mice at 5 x 10^6 cells/mouse. When the tumor became approximately 5-6 mm in diameter, 2 x 10^7 PFU of dl312/day and 4 x 10^3 PFU of TRAD/day were injected intratumorally and locally for consecutive three days. The axes of each tumor were measured in the same manner as described above, followed by calculation of the estimated tumor weight. The results are shown in Figs. 8 and 9 (the term "Mock" appearing in these Figures represents control to which PBS (phosphate buffered saline) was administered).

Administration of Ad-p53 at 3 x 10^8 PFU and 1 x 10^9 PFU inhibited the growth of H358 tumor significantly ($p<0.05$). However, administration of Ad-p53 at 1 x 10^8 PFU revealed no significant growth inhibition (Fig. 8). Administration of dl312 (control) indicated no influence upon tumor growth.

Intratumoral administration of TRAD at 4 x 10^3 PFU, which is extremely lower than the concentration of Ad-p53 that showed antitumor effect, inhibited the growth of SW620 tumor significantly ($p<0.05$). Administration of dl312 (control) indicated no influence upon tumor growth.

From what have been described above, it is understood that the virus of the present invention grows efficiently in cancer cells and brings death to them. Further, since the virus of the invention has the ability to grow, it is capable of manifesting potent anti-cancer effect even at a low concentration. Thus, it is also possible to reduce side effect by administering the virus at a low concentration.

CLAIMS

1. A polynucleotide comprising a promoter from human telomerase and at least one E1 gene.

2. The polynucleotide according to claim 1, wherein the E1 gene is an adenovirus-derived E1 gene.

3. The polynucleotide according to claim 1 or 2, wherein the promoter from human telomerase is hTERT.

4. The polynucleotide according to any one of claims 1 to 3, wherein the E1 gene comprises an E1A gene, an IRES sequence and an E1B gene in this order.

5. A virus comprising the polynucleotide according to any one of claims 1 to 4.

6. The virus according to claim 5, wherein the virus is an adenovirus.

7. An anticancer agent comprising the virus according to claim 5 or 6 as an active ingredient and a pharmaceutically acceptable carrier, excipient or diluent.

8. A method of treating a cancer, comprising using the virus according to claim 5 or 6 or using the anticancer agent according to claim 7.

9. The method according to claim 8, wherein the cancer is at least one cancer selected from the group consisting of stomach cancer, large bowel cancer, lung cancer, liver cancer, prostate cancer, pancreas cancer, esophagus cancer, bladder cancer, gallbladder/bile duct cancer, breast cancer, uterine cancer, thyroid cancer and ovarian cancer.

10. The method according to claim 9, wherein the cancer is at least one selected from the group consisting of osteosarcoma and brain tumor.