Title: ANTICANCER AGENT COMPRISING LK8 PROTEIN AS AN ACTIVE INGREDIENT

Abstract: The present invention relates to an anticancer agent comprising LK8 protein as an active ingredient. The anticancer agent of the present invention is effective to repress growth and metastasis of malignant tumor. Thus, the agent can be effectively used not only for repressor of cancer metastasis, but also for therapeutic agent of primary malignant tumor.


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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.
ANTICANCER AGENT COMPRISING LK8 PROTEIN AS
AN ACTIVE INGREDIENT

FIELD OF THE INVENTION

The present invention relates to an anticancer agent comprising a certain protein as an active ingredient, more precisely, to an anticancer agent comprising a protein corresponding to kringle KV38 of apolipoprotein(a) as an active ingredient.

BACKGROUND

A tumor is developed by uncontrollable disordered abnormal cell proliferation. If this tumor shows a destructive growth, invasiveness and metastasis, it is regarded as a malignant tumor. Invasiveness is a character to infiltrate or destroy surrounding tissues, and in particular, a basal layer forming a boundary of tissues is destroyed by the character, resulting in the local spread and sometimes inflow of a tumor through circulatory system. Metastasis means the spread of tumor cells from the original birthplace to other areas through lymphatic or blood vessels.
In a broad sense, metastasis also means the direct extension of tumor cells through peritoneal cavity or other space.

As of today, surgical operation, radiotherapy and chemotherapy have been used for the treatment of a cancer singly or jointly. The surgical operation is a way to remove diseased tissues. Thus, tumors in specific regions such as breast, colon and skin can be effectively removed by the surgical operation. However, a tumor in vertebra or dispersive tumor like leukemia cannot be properly treated by the surgical operation.

Chemotherapy blocks cell replication or metabolism, and has been used for the treatment of breast cancer, lung cancer and testis cancer. Though, patients with cancers who have been treated by chemotherapy have seriously suffered from the side effects of systemic chemotherapy. Motion sickness and vomiting are common but serious examples of all. The side effects of chemotherapy can even affect the life of a patient since they might drop the adaptability of a patient rapidly. Besides, Dose Limiting Toxicity (DLT) is also one of major side effects of
chemotherapy, which draws a careful attention in
the administration of a medicine. Mucositis is an
example of DLT against anticancer agents such as
5 fluorouracil which is an antimetabolic cytotoxic
agent, and methotrexate, and anticancer
antibiotics like doxorubicin. If a patient
suffers seriously from such side effects of
chemotherapy, he or she should be hospitalized and
given an anodyne for reducing pain. So, side
effects of chemotherapy and radiotherapy are the
biggest problem for the treatment of cancer
patients.

Therefore, it is an urgent need to develop an
anticancer agent originated from a living creature
in order to reduce side effects of chemotherapy.
In particular, among substances generated in a
living creature, a promising subject has been
found which does not attack cancer cells directly
but prevents cancer cells from growing by working
toward various endothelial cells helping cancer
cell growth. So, an anticancer agent containing
the subject can not only treat cancers but also
prevent metastasis.

Kringle is a kind of a protein structure

Apo(a) includes two types of kringle domains, KIV and KV, and an inactive protease-like region. The kringle domain KIV is divided into 10 subtypes (KIV-1 - KIV-10) according to the homology of amino acids, and 15 - 40 copy numbers of the domain are found in various human alleles of the apo(a) gene. Apo(a) forms a lipoprotein(a) (referred as 'Lp(a)' hereinafter) by covalent bond with apo B-100, a major protein component of low-density lipoprotein (LDL) (Fless, G.M., J. Biol. Chem., 261:8712-8717, 1986). The increase of Lp(a) content in cytoplasm itself is a major risk factor of artherosclerosis (Armstrong, V.W. et al.,

Based on the fact that artherosclerosis and cancer cell growth depend on angiogenesis, the present inventors have studied on an anticancer activity of KV38, one of human apo(a) kringle. As a result, the present inventors have completed this invention by confirming that the protein can be effectively used as an anticancer agent because it inhibits angiogenesis by an endogenous growth factor like bFGF which is necessary for cancer cell growth.

SUMMARY OF THE INVENTION

It is an object of this invention to provide an anticancer agent comprising a human apo(a) kringle KV38 (referred as 'LK8 protein' hereinafter) as an active ingredient.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

In order to achieve the above object of the present invention, the present invention provides
an anticancer agent comprising a LK8 protein as an active ingredient.

Hereinafter, the present invention is described in detail.

The anticancer agent of the present invention is characterized by including LK8 protein having an amino acid sequence represented by SEQ. ID. No 1 as an active ingredient. It is preferred to use the agent as a metastasis inhibitor, more preferably, it is used for the suppression of metastasis of colon carcinoma or rectal cancer to liver.

In addition, the anticancer agent is preferably used for the treatment of primary tumors. More preferably, the agent is used for the treatment of a cancer selected from a group consisting of prostate cancer, lung cancer, colon cancer and rectal cancer.

It is preferred for the anticancer agent of the present invention to contain LK8 protein by 0.1 ~ 100 mg/kg, more preferred to contain LK8 protein by 1 ~ 50 mg/kg, and administration times are 1 ~ 4 per day. But the composition is not
limited thereto, and is possibly changed according to the conditions of a patient, and types and progressing speed of a disease.

5 In this invention, "KV38" means an apo(a) kringle, and "LK8" means a recombinant protein of KV38. However, both KV38 and LK8 protein are called as LK8 protein in general unless mentioned specifically.

10 LK8 protein of the present invention is a domain corresponding to KV38 kringle of many apo(a) kringle domains and has an inhibiting effect on proliferation and differentiation of cancer cells and metastasis by suppressing the activity of endothelial cells in vitro and in vivo as well. As explained in the preferred embodiment of the present invention, the systemic administration of LK8 protein results in the inhibition of a primary tumor and its metastasis (see FIG. 2 ~ FIG. 6). Therefore, LK8 protein of the present invention can be effectively used as an anticancer agent especially for primary tumors and as a metastasis inhibitor owing to its functions of suppressing tumor growth and metastasis.
The treatment effect will be enhanced if LK8 protein of the present invention is used together with conventional chemotherapy or radiotherapy. Radiotherapy is performed to destroy a primary tumor, and if LK8 protein is administered during radiotherapy, metastasis can be prevented more effectively. As for chemotherapy, cytotoxicity caused by huge dosage of chemical anticancer agents is the biggest problem. If LK8 protein of the invention is administered during chemotherapy, the decreased amount of chemical anticancer agents will bring equivalent or even improved anticancer effects as well as lessen the cytotoxicity.

In conclusion, if the administration of LK8 protein is performed together with surgical operation, radiotherapy, chemotherapy or immunotherapy, the treatment effect will be maximized. And further, the continuous administration of LK8 protein extends dormancy of micrometastasis, suppresses the growth of a primary tumor and stabilizes conditions. Many conventional anticancer agents are designed for long term administration, causing troubles such as continuous production of a protein and high price.
of the product. The alternative to them is gene therapy. And LK8 protein is also expected to maximize the effect of an anticancer agent or a metastasis inhibitor if it is used in gene therapy.

The anticancer agent comprising LK8 protein of the present invention can be administered orally or parenterally and be used in general forms of pharmaceutical formulation.

The anticancer agent can be prepared for oral or parenteral administration by mixing with generally used fillers, extenders, binders, wetting agents, disintegrating agents, diluents such as surfactants, or excipients. Solid formulations for oral administration are tablets, pills, dusting powders, granules and capsules. These solid formulations are prepared by mixing one or more suitable excipients such as starch, calcium carbonate, sucrose or lactose, gelatin, etc. Except for the simple excipients, lubricants, for example magnesium stearate, talc, etc, can be used. Liquid formulations for oral administrations are suspensions, solutions, emulsions and syrups, and the above mentioned formulations can contain various excipients such
as wetting agents, sweeteners, aromatics and preservatives in addition to generally used simple diluents such as water and liquid paraffin. Formulations for parenteral administration are sterilized aqueous solutions, water-insoluble excipients, suspensions, emulsions, and suppositories. Water insoluble excipients and suspensions can contain, in addition to the active compound or compounds, propylene glycol, polyethylene glycol, vegetable oil like olive oil, injectable ester like ethylolate, etc. Suppositories can contain, in addition to the active compound or compounds, witepsol, macrogol, tween 61, cacao butter, laurin butter, glycerinated gelatin, etc.

LD₅₀ of the LK8 protein is about 1,000 mg/kg, suggesting that the anticancer agent of the present invention is very much safe (see Table 2).

BRIEF DESCRIPTION OF THE DRAWINGS

The application of the preferred embodiments of the present invention is best understood with reference to the accompanying drawings, wherein:
FIG. 1 is a schematic representation of the expression vector ‘pMBRI-LK8 (8.25 kb)’ of LK8 gene, in which LK8 cDNA (261 bp) is inserted between AOX1 promoter and AOX1 terminator,

FIG. 2 is a set of photographs and a graph showing that the pulmonary metastasis of a murine melanoma cell line, B16F10, which was injected into mouse (C57BL/6) tail vein, is inhibited by the treatment of LK8 protein,

(a) Lung of the mouse treated with PBS only,

(b) Lung of the mouse treated with LK8 protein by 1 mg/kg,

(c) A graph showing that metastasis of B16F10 cells to lung of the mouse is inhibited by the treatment of LK8 protein,

FIG. 3 is a set of photographs and a graph showing that the metastasis of liver by a mouse colorectal cancer cell line CT-26 transplanted into mouse spleen is inhibited by the treatment of LK8 protein,

(a) Liver of the mouse treated with PBS (control) and LK8 protein (10 mg/kg/day),

(b) A graph showing the colony number of CT-
26 cells metastasized into liver of the mouse treated with PBS (control) and LK8 protein (10 mg/kg/day),

(c) photographs showing the distribution of CT-26 cancer cells metastasized into liver of the mouse treated with PBS (control) and LK8 protein (10 mg/kg/day), which is observed by hematoxylin & eosin staining,

FIG. 4 is a set of graphs showing the changes of the size of a tumor in a mouse transplanted with human prostate carcinoma PC-3 cells, according to the administration of LK8 protein,

(a) A graph showing the changes of the size of a tumor after treating LK8 protein by 100 mg/kg/day,

(b) A graph showing the changes of the size of a tumor after treating LK8 protein by 50 mg/kg/day,

FIG. 5 is a graph showing the changes of the size of a tumor in a mouse transplanted with human lung carcinoma A549 cells, according to the administration of LK8 protein,
FIG. 6 is a set of graphs showing the changes of the size of a tumor (a) and the weight of a tumor (b) in mice transplanted with human rectal and colon carcinoma LS 174T cells, according to the administration of LK8 protein.

EXAMPLES

Practical and presently preferred embodiments of the present invention are illustrative as shown in the following Examples.

However, it will be appreciated that those skilled in the art, on consideration of this disclosure, may make modifications and improvements within the spirit and scope of the present invention.

Example 1: Preparation of LK8 protein

1-1 Construction of LK8 expression vector pMBRI-LK8

In order to prepare LK8 protein effectively, the present inventors constructed LK8 expression vector first.
For the expression of LK8 gene, pPIC9 vector (8.0 kb, Invitrogen, Netherland) was used as a basic vector. As seen in the representative map, pPIC9 expression vector includes promoter AOX1 which provides high expression by methanol, α-factor secretion signal which enables the secretion of an expressed protein, 3’AOX1(TT), AOX1 polyadenylation signal, which enables effective termination of transcription and polyadenylation, and a DNA fragment coding histidinol dehydrogenase of wild type Pichia pastoris used as a selectable marker for a transformant of the above strain, in order.

At first, LK8 gene was amplified by PCR with primers ‘LK8N-Xhol’ represented by SEQ. ID. No 2 and ‘LK8C-EcoRI’ represented by SEQ. ID. No 3, using pET15b/LK8 (see PCT/KR99/00554) as a template. The PCR product was digested with restriction enzymes XhoI and EcoRI, followed by subcloning by inserting the product into pPIC9 vector which was digested with the same restriction enzymes. Finally, the LK8 gene expression vector ‘pMBRI-LK8 (8.25 kb) was constructed (FIG. 1).
Preparation of a transformant including pMBRI-LK8

*Pichia pastoris*, a methylotrophic yeast, was used as a host for the preparation of a recombinant transformant.

Particularly, LK8 gene expression vector 'pMBRI-LK8' was treated with a restriction enzyme SacI, leading to linearization. The vector was inserted into AOX1 gene of the above host strain chromosome by homologous recombination. At that time, electroporation was performed for transformation. A recombinant yeast transformant was selected from histidine-deficient medium by examining colony forming. PCR was performed to confirm if LK8 cDNA was inserted into AOX1 region of chromosome of the selected recombinant transformant. And then, the recombinant transformant was cultured and the expression of LK8 gene was induced by methanol. As a result, the expression of LK8 gene was confirmed, suggesting that the protein was mass-secreted in the culture medium.

The secreted LK8 protein of the present invention was composed of amino acid sequence
represented by SEQ. ID. No 1.

<1-3> Cultivation of a recombinant strain

<1-3-1> Seed culture

5 In this invention, a recombinant strain was obtained by inserting LK8 gene into *Pichia pastoris*. The established strain was seed-cultured for 24 hours to obtain appropriate biomass and activity (when it was diluted by 20 times, OD_{600} was 0.8 - 1.2).

Seed culture was performed in YDP medium (1% yeast extract, 2% peptone, 2% dextrose) for 24 hours by shaking culture. 75 L fermenter was used. The volume of a beginning medium was 20 L and the final volume of culture solution was adjusted to 40 L by fed-batch culture method.

<1-3-2> Main culture

After completing seed culture in YPD medium, main culture was performed with seed culture solution as much as 30% out of the primary medium. Main culture was performed in a fermenter containing ingredients seen in the below table 1. When the ferment was saturated by the supply of
methanol, the ferment was recovered more than 10% for the production of LK8 protein. In the meantime, methanol was being supplied continuously to induce the continuous expression of the protein. The procedure was repeated to produce LK8 protein. Consumption speed of a carbon source was in proportion to the amount of cells. So, when some of ferment was recovered, the speed of methanol supply was automatically regulated not to be out of ±20%. From the repetition of the above culture process, during which fermentation was continued for over 200 hours, the secreted LK8 protein was obtained by 250 mg per 1 L of culture solution.

<Table 1>

Medium composition for main culture

<table>
<thead>
<tr>
<th>Kinds of medium</th>
<th>Component</th>
<th>Concentration</th>
</tr>
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<tbody>
<tr>
<td>Medium for main culture</td>
<td>Methanol</td>
<td>500 g/L</td>
</tr>
<tr>
<td></td>
<td>Trace metal solution</td>
<td>8 ml/L</td>
</tr>
<tr>
<td>Medium composition of trace metal solution</td>
<td>CuSO$_4$·5H$_2$O</td>
<td>4 g/L</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>-----------------</td>
<td>-------</td>
</tr>
<tr>
<td></td>
<td>KI</td>
<td>0.3 g/L</td>
</tr>
<tr>
<td></td>
<td>MnSO$_4$·H$_2$O</td>
<td>4 g/L</td>
</tr>
<tr>
<td></td>
<td>NaMo·H$_2$O</td>
<td>0.1 g/L</td>
</tr>
<tr>
<td></td>
<td>H$_3$BO$_4$</td>
<td>0.01 g/L</td>
</tr>
<tr>
<td></td>
<td>CoCl$_2$</td>
<td>0.1 g/L</td>
</tr>
<tr>
<td></td>
<td>ZnCl$_2$</td>
<td>3 g/L</td>
</tr>
<tr>
<td></td>
<td>FeSO$_4$·H$_2$O</td>
<td>10 g/L</td>
</tr>
<tr>
<td></td>
<td>Biotin</td>
<td>0.1 g/L</td>
</tr>
<tr>
<td></td>
<td>H$_2$SO$_4$</td>
<td>5 mL/L</td>
</tr>
</tbody>
</table>

Example 2: Experimental lung metastasis by intravenous administration of B16F10 murine melanoma cells

B16F10 cells (1.8 x 10$^5$), mouse melanoma (referred as 'melanoma cells' hereinafter) (American Type Culture Collection), were administered in tail vein of a C57BL/6 mouse (Charles River Japan, Inc.). From the next day, LK8 protein, prepared in the above <Example 1>, was injected subcutaneously twice (1 mg/kg/day, 0.2 mg/kg/day) a day for 14 days. A control group was injected with PBS instead of LK8 protein. On the 13$^{th}$ day from cell transplantation, the mouse was dissected, and lung was taken to count the number of colony of the metastasized cancer cells (melanoma cells).
As a result, huge colonies were formed in lungs of the control group mice injected with PBS, suggesting the transfer of melanoma cells (FIG. 2a). On the contrary, the number and the size of colonies generated by the transfer of melanoma cells were much less and smaller in the experimental group mice injected with LK8 protein (FIG. 2b). In conclusion, the group treated with LK8 protein by 1 mg/kg showed 53% metastasis inhibition, comparing to the control group (FIG. 2c).

Example 3: Experimental liver metastasis by intra-splenic administration of murine colorectal cancer cell CT-26

CT-26 cells (American Type Culture Collection), mouse colorectal cancer cells, were injected in spleen to induce metastasis into liver. Then, metastasis-inhibiting effect of LK8 protein was investigated. Particularly, CT-26 cells, which were 80% grown up on a plate, were washed with PBS, followed by singularizing with 0.02%
EDTA. The single cells were washed with PBS again, then resuspended in PBS carefully. The suspension was stained with Trypan blue to count the number of cells. Cell density was adjusted to $5 \times 10^5/\text{ml}$, which was injected into each mouse by 100 $\mu\text{l}$. 6~8 week old BALB/c mice (Charles River Japan, Inc.) were used. After the right side of abdomen was surgically excised, cancer cell suspension was carefully injected to the spleen using a 30-gauge needle. LK8 protein was subcutaneously injected to a experimental group twice a day by 10 $\text{mg/kg/day}$, and likewise, saline was injected as a control group. 14 days later, the mice were sacrificed to examine the liver (FIG. 3a), and the number of colonies seen on surface of the liver was counted, which was fixed in 10% formalin solution. There was no big difference in weight among groups. However, the number of colonies which were transferred to liver was much less in an experimental group which was administered with LK8 protein by 10 $\text{mg/kg/day}$ than in a control group. The decrease of the number of colonies in an experimental group injected with LK8 protein by 10 $\text{mg/kg/day}$ was about 60%, comparing to a control group (FIG. 3b). Besides, liver tissue section
which was fixed in formalin solution was stained with H&E, and observed. As a result, a tumor region was much limited in an experimental group treated with LK8 protein comparing to a control group (FIG. 3c).

Example 4: Growth inhibition of a primary tumor

In order to investigate the effect of LK8 protein on the anti-angiogenesis in vivo, a xenografted tumor model was used. Every relevant experiment was performed with 4 week old randomly crossed female Balb/c nu/nu nude mice (Charles River Japan, Inc.), which were raised under sterilized condition.

<4-1> Human prostatic carcinoma (PC-3)

Human prostatic carcinoma PC-3 cells (American Type Culture Collection) were cultured in RPMI 1640 medium (GIBCO\textsuperscript{TM}, Invitrogen Corporation) supplemented with 10% FBS, and about $5 \times 10^6$ PC-3 cells were subcutaneously injected in central muscular region of the back of the nude mouse. Exactly 10 days after the transplantation, LK8 protein was injected by 100 mg/kg/day.
Meanwhile, a control group was injected PBS only instead of LK8 protein. The treatment was continued for 30 days, then, the size of a tumor was measured every 3 or 4 days. As a result, tumor growth was inhibited by the treatment of LK8 protein, which was about 60% inhibition comparing to a control group (FIG. 4a). When the LK8 protein was injected by 50 mg/kg/day, tumor growth was inhibited in similar fashion over 60% comparing to a control group (FIG. 4b).

<4-2> Human lung carcinoma (A549)

Human lung carcinoma A549 cells (American Type Culture Collection) were cultured in DMEM medium (GIBCO™, Invitrogen Corporation) supplemented with 10% FBS. Then, 1 X 10^7 tumor cells were subcutaneously injected in central muscular region of the back of the nude mouse. Exactly 5 days after the transplantation, LK8 protein was injected by 50 mg/kg/day. A control group was administered PBS only instead of LK8 protein. The treatment was continued for 46 days, then, the size of a tumor was measured every 3 or 4 days. As a result, tumor growth was inhibited by the treatment of LK8 protein, which was 61%
inhibition comparing to a control (FIG. 5).

\textbf{<4-3> Human colon and rectal carcinoma (LS 174T)}

Human colon and rectal carcinoma LS 174T cells (American Type Culture Collection) were cultured in RPMI medium (GIBCO\textsuperscript{TM}, Invitrogen Corporation) supplemented with 10% FBS. Then, 5 \times 10^6 tumor cells were subcutaneously injected in central muscular region of the back of the nude mouse. Exactly 5 days after the transplantation, LK8 protein was injected by 50 mg/kg/day. A control group was administered PBS only instead of LK8 protein. The treatment was continued for 34 days, then, the size of a tumor was measured every 3 or 4 days. As a result, tumor growth was inhibited by the treatment of LK8 protein, which was 64% inhibition comparing to a control (FIG. 6a). And, the weight of the tumor was decreased by the treatment of LK8 protein about 68.7%, which was measured on the final day of the experiment (FIG. 6b).

\textbf{Example 5: Acute toxicity test of LK8 protein}

5-week old SPF SD (Sprague Dawley) line rats
were used in the test for acute toxicity. Rats were divided into 5 groups, and 5 rats per each group were administered once with LK8 protein by the dosage of 260 mg/kg, 364 mg/kg, 510 mg/kg, 714 mg/kg and 1000 mg/kg, respectively, by intravenous injection (Table 2). For 14 days after test material, LK8 protein, administration, death, clinical symptoms and weight change in rats were observed. The hematological tests and biochemical tests of blood were performed, and any abnormal signs in the gastrointestinal organs of chest and abdomen were examined visually during autopsy. As a result, a weak toxicity was detected in the group administered with 1000 mg/kg of LK8 protein, but neither toxicity nor death was found in other groups in most tests including weight changes, blood test, hematological tests and biochemical tests of blood, autopsy, etc. Therefore, the LK8 protein used in this experiment was evaluated to be safe substance since it did not cause any toxic change in rats up to the level of 1,000 mg/kg in rats and its estimated LD₅₀ values were much greater than 1,000 mg/kg in rats (Table 2).

(Table 2)
The number of deaths according to days after LK8 protein administration

<table>
<thead>
<tr>
<th>Amount of administration (mg/kg)</th>
<th>The number of deaths /Total test rats</th>
<th>Days after LK 8 protein administration</th>
</tr>
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<tr>
<td>260</td>
<td>0/5</td>
<td>0 0 0 0 0 0 0 0 0 0 1 1 1 1 1 1</td>
</tr>
<tr>
<td>364</td>
<td>0/5</td>
<td>0 0 0 0 0 0 0 0 0 0 1 1 1 1 1 1</td>
</tr>
<tr>
<td>510</td>
<td>0/5</td>
<td>0 0 0 0 0 0 0 0 0 0 1 1 1 1 1 1</td>
</tr>
<tr>
<td>714</td>
<td>0/5</td>
<td>0 0 0 0 0 0 0 0 0 0 1 1 1 1 1 1</td>
</tr>
<tr>
<td>1000</td>
<td>3/5</td>
<td>0 0 3 0 0 0 0 0 0 0 1 1 1 1 1 1</td>
</tr>
</tbody>
</table>

**INDUSTRIAL APPLICABILITY**

As explained hereinbefore, LK8 protein has an inhibiting effect on metastasis, in particular, on the growth of human prostatic cancer, lung cancer, colon cancer and rectal cancer as being systemically administered. Thus, an anticancer agent containing LK8 protein of the present invention can be effectively used as a treatment agent for a primary tumor or a metastasis
inhibitor.

Those skilled in the art will appreciate that the conceptions and specific embodiments disclosed in the foregoing description may be readily utilized as a basis for modifying or designing other embodiments for carrying out the same purposes of the present invention. Those skilled in the art will also appreciate that such equivalent embodiments do not depart from the spirit and scope of the invention as set forth in the appended claims.
What is claimed is

1. An anticancer agent containing LK8 protein represented by SEQ. ID. No 1 as an effective ingredient.

2. The anticancer agent as set forth in claim 1, wherein the effective dose of LK8 protein is 0.1 ~ 100 mg/kg.

3. The anticancer agent as set forth in claim 2, wherein the effective dose of LK8 protein is 1 ~ 50 mg/kg.

4. The anticancer agent as set forth in claim 1, wherein the agent is used for the inhibition of metastasis of cancer.

5. The anticancer agent as set forth in claim 1, wherein the metastasis of cancer is the metastasis of colon cancer or rectal cancer.

6. The anticancer agent as set forth in claim 1, wherein the agent is used for the treatment of a primary tumor.
7. The anticancer agent as set forth in claim 6, wherein the primary tumor is selected from a group consisting of prostate cancer, colon cancer, rectal cancer and lung cancer.
FIG. 2

(a)  
(b)  
(c)  

metastasis (%)  

PBS  LK8 1 mg/Kg  LK8 0.2 mg/Kg
FIG. 3

(a) Control  LK8

(b) metastasized colony No.

0  50  100  150  200  250
Control  LK8

(c) Control  LK8 (10mg/kg)

(X40)
FIG. 4

(a) Tumor size (mm$^3$) vs. Days after treatment

- Control
- LK8 100mg/kg/day

(b) Tumor size (mm$^3$) vs. Days after treatment

- Control
- LK8 50mg/kg/day
FIG. 6

(a) Tumor size (mm³) vs. Days after treatment

(b) Tumor weight (g) for Control vs. LK8 (50 mg/kg/day) samples
SEQUENCE LISTING

1 MOGAM BIOTECHNOLOGY INSTITUTE

2 Anticancer agent comprising LK8 protein as an active ingredient

3 2p-11-19

4 KopatentIn 1.71

5 1

6 75

7 PRT

8 Homo sapiens

9 Cys Met Phe Gly Asn Gly Tyr Arg Gly Lys Ala Thr Thr Val Thr Gly Thr Pro Cys Gln Glu Trp Ala Ala Gln Glu Pro His Arg 20 25 30

10 His Ser Thr Phe Ile Pro Gly Thr Asn Lys Trp Ala Gly Leu Glu Lys 35 40 45

11 Asn Tyr Cys Arg Asn Pro Asp Gly Asp Ile Asn Gly Pro Trp Cys Tyr 50 55 60

12 Thr Met Asn Pro Arg Lys Leu Phe Asp Tyr Cys 65 70 75

13 2

14 34

15 DNA

16 Artificial Sequence

17 LK8N-XhoI primer

18 tccgctcag aaagagaac aagactgtat gttt 34

19 3

20 31

21 DNA

22 Artificial Sequence

23 LK8C-EcoRI primer
cgaaatttta agaggatgca cagagagga t
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 A61K 38/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7 A61K 38/16

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

Korean patents and applications for inventions since 1975

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

NCBI protein blast program

STN [Caslink, (KV38 orLK8) and (tumor or cancer or antitumor or anticancer or angiogenesis)]

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tr>
<td>X</td>
<td>WO 01/19868 A1 (MOGAM BIOTECHNOLOGY RESEARCH INSTITUTE) 22 March 2001 See page 4 lines 30-34, page 9 lines 9-11, and claim 3</td>
<td>1 - 7</td>
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<tr>
<td>A</td>
<td>SCANU et al., 'Apolipoprotein(a): structural and functional consequence of mutations in kringle type 10 (or kringle 4-37)', Clinical Genetics, July 1994, Vol.46, pp.42-45 See the whole document.</td>
<td>1 - 7</td>
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<tr>
<td>A</td>
<td>SCANU et al., 'A single point mutation (Trp72-&gt;Arg) in human apo(a) kringle 4-37 associated with a lysine binding defect in Lp(a)', Biochimica et Biophysica Acta, Oct 1994, Vol. 1227, pp.41-45 See the whole document.</td>
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<tr>
<td>A</td>
<td>GABEL et al., 'Sequences within apolipoprotein(a) kringle IV types 6-8 bind directly to low-density lipoprotein and mediate noncovalent association of apolipoprotein (a) with apolipoprotein B-100', Biochemistry, May 1998, Vol. 37, pp.7892-7898 See the whole document.</td>
<td>1 - 7</td>
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Further documents are listed in the continuation of Box C. See patent family annex.

Date of the actual completion of the international search
13 MAY 2004 (13.05.2004)

Date of mailing of the international search report
14 MAY 2004 (14.05.2004)

Name and mailing address of the ISA/KR

Korean Intellectual Property Office
920 Dunsan-dong, Seo-gu, Daejeon 302-701, Republic of Korea

Authorized officer
LEE, Mi Jeong

Facsimile No. 82-42-472-7140

Telephone No. 82-42-481-5601

Form PCT/ISA/210 (second sheet) (January 2004)
1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

a. type of material
   - [X] a sequence listing
   - [ ] table(s) related to the sequence listing

b. format of material
   - [X] in written format
   - [X] in computer readable form

c. time of filing/furnishing
   - [X] contained in the international application as filed
   - [X] filed together with the international application in computer readable form
   - [ ] furnished subsequently to this Authority for the purposes of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:
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