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Maeul Poongrim Apt., Jeongja-dong, Jangan-gu, Su-
won-si, Gyeonggi-do 440-300 (KR).

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(74) Agent: YANG, Boo-Hyun; 4th Fl. Oksan Bldg., 1627-10,
Bongcheon-7-dong, Gwanak-gu, Seoul 151-818 (KR).

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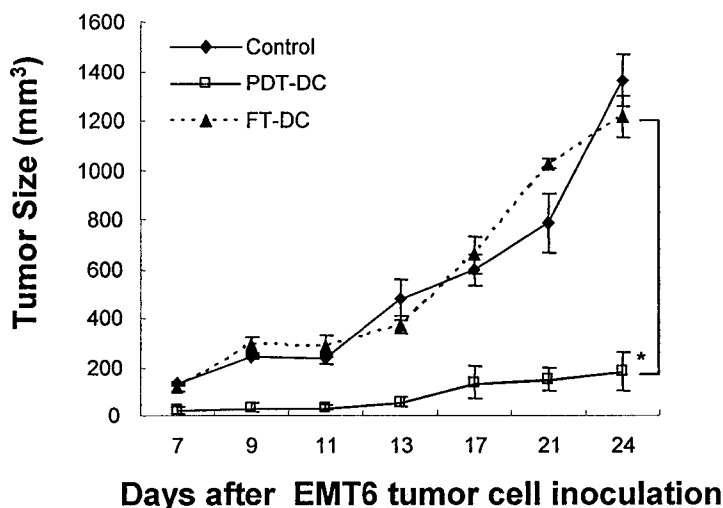
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(71) Applicants (for all designated States except US):
CREAGENE INC. [KR/KR]; 2F Joongang Induspia
V B/D, 138-6 Samgdaewon-dong, Joongwon-gu, Sung-
nam-Si, Kyungki-do 426-713 (KR). **KANG, Mi-Sun**
[KR/KR]; #501/ 65, Munjeong1-dong, Songpa-gu, Seoul
138-201 (KR).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **LIM, Dae-Seog**
[KR/KR]; 903-401 Byeolvit 9 Danji Byeoksan Apt.,
Hwajeong-dong, Deogyang-gu, Goyang-si, Gyeonggi-do
412-740 (KR). **JEONG, Ju-Ah** [KR/KR]; 404 Je-
iCastleresidence., 345-3 Sangsu-dong, Mapo-gu, Seoul
121-160 (KR). **LEE, Hyun-Soo** [KR/KR]; 110-205 Garam
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upon receipt of that report(54) Title: AUTOLOGOUS DENDRITIC CELL MEDIATED BY PHOTODYNAMIC THERAPY HAVING THE ABILITY TO
SUPPRESS THE GROWTH OF TUMOR**Fig. 3a**(57) Abstract: The present invention relates to
mature dendritic cells (DCs) having the enhanced
ability to induce anti-tumor immune responses,
and to a method for the preparation of the
above-mentioned DCs through the procedure of
pulsing immature DCs with lysates of cancer cells
on which photodynamic therapy (PDT) has been
conducted. The dendritic cell of this invention
acquires an activity to induce anti-tumor immune
responses so that it exerts a remarkable effect to
suppress the growth of tumor.

**AUTOLOGOUS DENDRITIC CELL MEDIATED BY PHOTODYNAMIC
THERAPY HAVING THE ABILITY TO SUPPRESS THE GROWTH OF TUMOR**

BACKGROUND OF THE INVENTION

5 **FIELD OF THE INVENTION**

The present invention relates to mature dendritic cells (DCs) having the ability to induce anti-cancer immune responses, and to a method for the preparation of the above-mentioned DCs through the procedure of pulsing immature DCs with lysates of cancer cells on which photodynamic therapy (PDT) has been conducted. In addition, the present invention relates to cancer cell lysates for the use of inducing the maturation of immature DCs to mature DCs having the ability to inhibit the growth of cancer cells, and to the preparation method thereof.

DESCRIPTION OF THE RELATED ART

15 Photodynamic therapy (PDT) is a treatment that is based upon the differential uptake by cancerous cells of photosensitizing agents, followed by irradiation of the cells to cause a photochemical reaction that is believed to generate chemically disruptive species, such as singlet oxygen. These disruptive species in turn injure the cells through reaction with cell parts, such as cellular and nuclear membranes (U.S. Pat. No. 5,211,938).

20 The exact therapeutic mechanisms of PDT have not been sufficiently elucidated. However, pre-clinical and early stage clinical studies have suggested that PDT may play a role in the induction of immune responses in the subject. It is also suggested that if cancer cells are treated with PDT, pro-inflammatory cytokines and heat shock proteins are produced in around cancer cells and change the environment of cancer cells to increase the immunogenicity.

According to the early studies on PDT, it is reported that PDT stimulates the expression of inflammatory factors such as TNF (tumor necrosis factor)- α , IL (interleukin)-6, IL-1 and of HSP (heat shock protein) 70.

The immune responses of T cell are initiated by the effect of mature dendritic cells on naïve T cell. Immature dendritic cells exert phagocytosis but cannot stimulate T cells. Accordingly, the maturation of dendritic cells is a key step in the induction of immune responses. The maturation of dendritic cells comprises the steps of modification of dendritic cells to more effective antigen presenting cells, migration of dendritic cells to the regional lymph node and activation of T cells. It is also known that certain heat shock proteins play important roles in the initiation of immune responses of T cells and are evaluated as an effective vaccine adjuvant in the cancer vaccination.

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Throughout this application, various patents and publications are referenced and citations are provided in parentheses. The disclosure of these patents and publications in their entities are hereby incorporated by references into this application in order to more fully describe this invention and the state of the art to which this invention pertains.

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DETAILED DESCRIPTION OF THIS INVENTION

The present inventors have discovered that where immature dendritic cells are matured by pulsing with lysates prepared from cancer cells on which photodynamic therapy has been conducted, the ability to induce anti-cancer immune responses of the matured dendritic cells has been significantly increased.

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Accordingly, it is an object of this invention to provide a method for preparing cancer cell lysates for pulsing immature dendritic cells.

It is another object of this invention to provide cancer cell lysates for pulsing immature dendritic cells prepared according to the above method.

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It is still another object of this invention to provide a composition for inducing the maturation of dendritic cells comprising cancer cell lysates as an active ingredient.

It is another object of this invention to provide a method for preparing mature dendritic cells that have a significantly improved ability to induce the anti-cancer immune responses.

It is still another object of this invention to provide a mature dendritic cell
5 that has a significantly improved ability to induce anti-cancer immune responses.

It is another object of this invention to provide a pharmaceutical composition for treating cancer comprising mature dendritic cells that have a significantly improved ability to induce anti-cancer immune responses.

It is still another object of this invention to provide a method for treating
10 cancer comprising administering to a subject suffering from cancer a pharmaceutical composition comprising mature dendritic cells that have a significantly improved ability to induce anti-cancer immune responses.

It is another object of this invention to provide a use of a composition comprising mature dendritic cells that have a significantly improved ability to
15 induce anti-cancer immune responses for preparing an anti-cancer medicine.

Other objects and advantages of the present invention will become apparent from the following detailed description together with the appended claims and drawings.

20 In one aspect of this invention, there is provided a method for preparing cancer cell lysates for pulsing dendritic cells, which comprises the steps of: (a) culturing cancer cells in the presence of a photosensitizing agent; (b) activating the photosensitizing agent bound to the cancer cells by irradiating the cultured cancer
25 cell with a light; and (c) obtaining cancer cell lysates from the cultured cancer cells.

The present inventors have discovered that where immature dendritic cells are matured by pulsing with lysates prepared from cancer cells on which photodynamic therapy has been conducted, the ability to induce anti-cancer

immune responses of the matured dendritic cells has been significantly enhanced.

The preparation method for cancer cell lysates for pulsing immature dendritic cells is described in detail below.

(a) culturing cancer cells in the presence of a photosensitizing agent

5 The first step of this invention is culturing cancer cells in the presence of a photosensitizing agent.

The term "cancer cell" used herein means a type of cell that has acquired an immortality to proliferate without any restriction. Cancer cell includes any type of cancer cell not limiting to the specific type of cancer cells.

10 According to a preferred embodiment of this invention, the cancer cell is a renal cancer cell or breast cancer cell.

The term "photosensitizing agent" used herein refers to a chemical compound that is able to bind to target cells, and when exposed to light of an appropriate wavelength, absorb the light, causing substance to be produced that
15 impair or destroy the target cells. A photosensitizing agent that may be used in the present invention may be found in Kreimer-Bimbaum, Seminars in Hematology (1989) 26(2):157-73[8]. Photosensitizing agents of this invention includes, but are not limited to, chlorines, bacteriochlorins, phthalocyanines, porphyrins, purpurins, merocyanines, psoralens, benzoporphyrin derivatives (BPD), and porfimer sodium
20 and pro-drugs such as delta-aminolevulinic acid, which can produce photosensitive agents such as protoporphyrin IX. Other suitable photosensitive compounds include ICG, methylene blue, toluidine blue, texaphyrins and any other agent that absorbs light in a range of 500 nm – 1100 nm.

25 According to a preferred embodiment of this invention, the photosensitizing agent is porphyrins.

A medium useful in the procedure of culturing cancer cells includes any conventional medium for culturing animal cells, preferably, a medium containing serum (e.g., fetal bovine serum, horse serum and human serum). The medium

used in this invention includes, for example, RPMI series (e.g., RPMI 1640), Eagles's MEM (Eagle's minimum essential medium, Eagle, H. Science 130:432(1959)), α -MEM (Stanner, C.P. et al., *Nat. New Biol.* 230:52(1971)), Iscove's MEM (Iscove, N. et al., *J. Exp. Med.* 147:923(1978)), 199 medium
5 (Morgan et al., *Proc. Soc. Exp. Bio. Med.* 73:1(1950)), CMRL 1066, RPMI 1640 (Moore et al., *J. Amer. Med. Assoc.* 199:519(1967)), F12 (Ham, *Proc. Natl. Acad. Sci. USA* 53:288(1965)), F10 (Ham, *R.G. Exp. Cell Res.* 29:515(1963)), DMEM (Dulbecco's modification of Eagle's medium, Dulbecco, R. et al., *Virology* 8:396(1959)), Mixture of DMEM and F12 (Barnes, D. et al., *Anal. Biochem.*
10 102:255(1980)), Way-mouth's MB752/1 (Waymouth, C. J. *Natl. Cancer Inst.* 22:1003(1959)), McCoy's 5A (McCoy, T.A., et al., *Proc. Soc. Exp. Biol. Med.* 100:115(1959)) and MCDB series (Ham, R.G. et al., *In Vitro* 14:11(1978)) but not limited to. The medium may contain other components, for example, antioxidant (e.g., β -mercaptoethanol). The detailed description of media is found in R. Ian
15 Freshney, *Culture of Animal Cells, A Manual of Basic Technique*, Alan R. Liss, Inc., New York, the teaching of which is incorporated herein by reference in its entity.

According to a specific example of this invention, the culture medium used for culturing cancer cells is RPMI-1640 or DMEM.

The time for culturing cancer cell in the presence of a photosensitizing agent
20 may be one sufficient for a photosensitizing agent to bind to cancer cell not being limited to the specific range.

(b) activating the photosensitizing agent bound to cancer cells by irradiating the cultured cancer cells with a light

After culturing cancer cells in the presence of a photosensitizing agent, the
25 photosensitizing agent bound to cancer cells is activated by proper irradiation of a light.

The amount of energy of activating irradiation used in this invention may be regulated by selecting the appropriate wavelength, power, power density, energy

density, and time of application. The wavelength of the radiation can be any wavelength absorbed by the photosensitizing agent, or any other wavelength that mediates the desired biological response in the target cancer cells. Some examples of type of photosensitizing agents and activating energy may be found in
5 U.S. Pat. Nos. 6,013,053; 6,011,563; 5,976,175; 5,971,918; 5,961,543; 5,944,748; 5,910,510; 5,849,027; 5,845,640; 5,835,648; 5,817,048; 5,798,523; 5,797,868; 5,793,781; 5,782,895; 5,707,401; 5,571,152; 5,533,508; 5,489,279; 5,441,531; 5,344,434; 5,219,346; 5,146,917; and 5,054,867.

The source of the light that can be used in the present method may be any
10 light source which is able to produce wavelength that can activate the photosensitizing agent. The source of light in this invention includes laser, lamp, optoelectric magnetic device, diode, and diode-laser, but not limited to.

According to a preferred embodiment of this invention, the source of the light energy is a laser or halogen lamp.

15 (c) obtaining cancer cell lysates from the cultured cancer cells

After activating the photosensitizing agent bound to the cancer cells by irradiation of a light, cancer cell lysates are prepared from the irradiated cultured cancer cells.

The term "cancer cell lysates" used herein means cancer cell lysis products
20 that comprise any proteins from cancer cells including cancer antigens.

According to a preferred embodiment of this invention, heat shock proteins may be contained in the cancer cell lysates.

According to a specific example of this invention, the cancer cell lysates are obtained through the steps of collecting the irradiated cultured media containing
25 cancer cells by using scrapper, centrifuging the collected media, and recovering the supernatant of them.

PDT (photodynamic therapy) is a therapeutic procedure to selectively destroy cancer cell by the differential uptake by cancerous cells of photosensitizing

agents, followed by irradiation of the cells to cause a photochemical reaction that is believed to generate chemically disruptive species, such as singlet oxygen.

The expression "cancer cell lysates prepared by PDT treatment" or "PDT-prepared cancer cell lysates" used herein refers to cancer cell lysates that are prepared through the procedure of the above described steps (a), (b) and (c).

In another aspect of this invention, there is provided a cancer cell lysates for pulsing dendritic cells prepared through the procedure of the steps (a), (b) and (c).

According to a preferred embodiment of this invention, the cancer cell lysates contains heat shock proteins.

The term "heat shock proteins (HSPs)" used herein means a protein whose intracellular concentration increases when a cell is exposed to a stressful stimulus such as heat. It is capable of binding other proteins or peptides, and releasing the bound proteins or peptides in the presence of adenosine triphosphate (ATP) of low pH. Three major families of HSPs have been identified based on molecular weight. The families have been called Hsp 60, Hsp 70 and Hsp 90, and where the numbers reflect the approximate molecular weight of the stress proteins in kilodaltons. Many members of these families were found subsequently to be induced in response to other stressful stimuli including, but not limited to, nutrient deprivation, metabolic disruption, oxygen radicals, and infection with intracellular pathogens (Welch, May 1993, *Scientific American* 56-64; Young, 1990, *Annu. Rev. Immunol.* 8:401-420; Craig, 1993, *Science* 260:1902-1903; Gething, et al., 1992, *Nature* 355:33-45; and Lindquist, et al., 1988, *Annu. Rev. Genetics* 22:631-677).

The major HSPs can accumulate to very high levels in stressed cells, but they occur at low to moderate levels in cells that have not been stressed. For example, the highly inducible mammalian hsp70 is hardly detectable at normal temperatures but becomes one of the most actively synthesized proteins in the cell upon heat shock (Welch, et al., 1985, *J. Cell. Biol.* 101:1198-1211).

HSPs appear to induce an inflammatory reaction at the tumor site and

ultimately cause a regression of the tumor burden in the cancer patients treated.

According to a preferred embodiment of this invention, the cancer cell lysates prepared through PDT treatment for pulsing dendritic cells comprises heat shock protein 70 (Hsp 70).

5 In another aspect of this invention, there is provided a composition for inducing the maturation of immature dendritic cells comprising the cancer cell lysates prepared by the method of this invention as an active ingredient.

The expression "inducing the maturation of immature dendritic cells" refers to an induction of the maturation of immature dendritic cells to mature dendritic
10 cells by contacting the immature dendritic cells with the specific antigens.

When immature dendritic cells are matured through a pulsing with cancer cell lysates that have been prepared by the method of this invention, the resulting matured dendritic cells have a remarkably increased activity to suppress the growth of tumor via the process of inducing immune responses against tumor.

15 In another aspect of this invention, there is provided a method for preparing mature dendritic cells having the ability to inhibit the growth of tumor, which comprises the steps of; (a) culturing cancer cells in the presence of a photosensitizing agent; (b) activating the photosensitizing agent bound to the cancer cells by irradiating the cultured cancer cells with a light; (c) obtaining
20 cancer cell lysates from the cultured cancer cells; and (d) inducing the maturation of immature dendritic cells by pulsing with the obtained cancer cell lysates.

Since the present method comprises, in principle, the steps of (a) to (c) described above, the common descriptions between them are omitted in order to avoid undue redundancy leading to the complexity of this specification.

25 In the step (d) of the method of this invention, immature dendritic cells are matured by pulsing with the antigen of PDT-prepared cancer cell lysates, which have been prepared through the procedure of the steps (a) to (c).

The expression "PDT-mediated dendritic cells" used herein refers to

dendritic cells matured by pulsing immature dendritic cells with PDT-prepared cancer cell antigens which have been prepared via the steps of (a) to (c).

The term "pulsing" used herein means contacting immature dendritic cells with the specific antigen.

5 When dendritic cells are contacted with antigens, they engulf and process the antigens, then present the processed antigens with MHC molecules on their surface to T cells. T cells that have been contacted with dendritic cells which present the processed specific antigens become to acquire the capability to induce immune responses to the specific antigens.

10 Where immature dendritic cells are matured by pulsing with the antigens in the PDT-prepared cell lysates of the specific cancer, the resulting matured dendritic cells have the increased ability to suppress the growth of tumor, since they have a remarkably enhanced potential to induce immune responses to the specific cancer cell.

15 The pulsing in the present invention may be carried out by contacting dendritic cells with the specific antigens for a sufficient time for the specific antigens in the cancer cell lysates to be presented on the surface of dendritic cells. Preferably, the contacting is co-culturing. According to a preferred embodiment of this invention, dendritic cells are pulsed with cancer cell lysates by co-culturing
20 immature dendritic cells with PDT-prepared cancer cell lysates.

 The term "dendritic cells (DCs)" used herein refers to professional antigen-presenting cells that can internalize antigen and process the antigen such that the antigen or peptide derived from the antigen is presented in the context of both MHC (major histocompatibility complex) class I complex and the MHC class II
25 complexes. Dendritic cells may be classified as immature or fully mature dendritic cells.

 As used herein, the term "immature dendritic cells (imDCs)" refers to dendritic cells that lack the cell surface markers found on mature DCs, such as

CD83 and CD14; express low levels of CCR7 and the cytosolic protein DC-LAMP, and low levels of the costimulatory molecules CD40, CD80 and CD 86, and usually express CD1a and CCR1, CCR2, CCR5 and CXCR1.

As used herein, the term "mature dendritic cells (mDCs)" refers to a population of dendritic cells which are matured from imDCs and have reduced expression of CD115, CD14 or CD68; and have increased expression of MHC class II, CD40, CD80, CD83 and CD86 as well as DC-LAMP; are characterized by their release of pro-inflammatory cytokines, and their ability to cause increased proliferation of naive allogeneic T cells and/or increased production of DC cytokines in a mixed lymphocyte reaction. Mature DCs typically express high levels of CCR7, and CXCR4 and low levels of CCR1 and CCR5.

The expression profiling of surface markers of DCs is able to be carried out by the flow cytometry analysis known to those skilled in the art. General procedures for isolating and culturing immature DCs are disclosed in U.S. Patent No. 5,994,126 and WO 97/29182, which are incorporated herein by references.

Suitable source for isolating immature dendritic cells is tissue that contains immature dendritic cells or their progenitors, and specifically include spleen, afferent lymph, bone marrow, blood, and cord blood, as well as blood cells elicited after administration of cytokines such as G-CSF or FLT-3 ligand.

According to a specific embodiment of this invention, a tissue source may be treated prior to culturing with substances that stimulate hematopoiesis, such as, for example, G-CSF, FLT-3, GM-CSF, M-CSF, TGF- β , and thrombopoietin in order to increase the proportion of dendritic cell precursors relative to other cell types. Such pretreatment may also remove cells which may compete with the proliferation of the dendritic cell precursors or inhibit their survival. Pretreatment may also be used to make the tissue source more suitable for in vitro culture. Those skilled in the art would recognize that the method of treatment will likely depend on the particular tissue source. For example, spleen or bone marrow would first be treated so as to

obtain single cells followed by suitable cell separation techniques to separate leukocytes from other cell types as described in U.S. Pat. Nos. 5,851,756 and 5,994,126 which are herein incorporated by references. Treatment of blood would preferably involve cell separation techniques to separate leukocytes from other cell types including red blood cells (RBCs) which are toxic. Removal of RBCs may be accomplished by standard methods known in the art. According to a preferred embodiment of the invention, the tissue source is blood or bone marrow.

According to a further embodiment, immature dendritic cells are derived from multipotent blood monocyte precursors (see WO 97/29182). These multipotent cells typically express CD14, CD32, CD68 and CD115 monocyte markers with little or no expression of CD83, or p55 or accessory molecules such as CD40 and CD86. When cultured in the presence of cytokines such as a combination of GM-CSF and IL-4 or IL-13 as described below, the multipotent cells give rise to the immature dendritic cells. The immature dendritic cells can be modified, for example using vectors expressing IL-10 to keep them in an immature state *in vitro* or *in vivo*. Those skilled in the art would recognize that any number of modifications may be introduced to the disclosed methods for isolating immature dendritic cells and maintaining them in an immature state *in vitro* and *in vivo* having regard to the objects of the several embodiments of the invention here disclosed.

Cells obtained from the appropriate tissue source are cultured to form a primary culture, preferably, on an appropriate substrate in a culture medium supplemented with granulocyte/macrophage colony-stimulating factor (GM-CSF), a substance which promotes the differentiation of pluripotent cells to immature dendritic cells as described in U.S. Pat. Nos. 5,851,756 and 5,994,126 which are herein incorporated by references. In a preferred embodiment, the substrate would include any tissue compatible surface to which cells may adhere. Preferably, the substrate is commercial plastic treated for use in tissue culture.

To further increase the yield of immature dendritic cells, other factors, in addition to GM-CSF, may be added to the culture medium which block or inhibit proliferation of non-dendritic cell types. Examples of factors which inhibit non-dendritic cell proliferation include interleukin-4 (IL-4) and/or interleukin-13 (IL-13), which are known to inhibit macrophage proliferation. The combination of these substances increases the number of immature dendritic cells present in the culture by preferentially stimulating proliferation of the dendritic cell precursors, while at the same time inhibiting growth of non-dendritic cell types.

According to a specific example of the invention, an enriched population of immature dendritic cells can be generated from blood monocyte precursors by plating mononuclear cells on plastic tissue culture plates and allowing them to adhere. The plastic adherent cells are then cultured in the presence of GM-CSF and IL-4 in order to expand the population of immature dendritic cells. Other cytokines such as IL-13 may be employed instead of using IL-4.

A medium useful in the procedure of obtaining immature dendritic cells includes any conventional medium for culturing animal cells, preferably, a medium containing serum (e.g., fetal bovine serum, horse serum and human serum). The medium used in this invention includes, for example, RPMI series (e.g., RPMI 1640), Eagles's MEM (Eagle's minimum essential medium, Eagle, H. Science 130:432(1959)), α -MEM (Stanner, C.P. et al., Nat. New Biol. 230:52(1971)), Iscove's MEM (Iscove, N. et al., J. Exp. Med. 147:923(1978)), 199 medium (Morgan et al., Proc. Soc. Exp. Bio. Med. 73:1(1950)), CMRL 1066, RPMI 1640 (Moore et al., J. Amer. Med. Assoc. 199:519(1967)), F12 (Ham, Proc. Natl. Acad. Sci. USA 53:288(1965)), F10 (Ham, R.G. Exp. Cell Res. 29:515(1963)), DMEM (Dulbecco's modification of Eagle's medium, Dulbecco, R. et al., Virology 8:396(1959)), Mixture of DMEM and F12 (Barnes, D. et al., Anal. Biochem. 102:255(1980)), Way-mouth's MB752/1 (Waymouth, C. J. Natl. Cancer Inst. 22:1003(1959)), McCoy's 5A (McCoy, T.A., et al., Proc. Soc. Exp. Biol. Med.

100:115(1959)) and MCDB series (Ham, R.G. et al., In Vitro 14:11(1978)) but not limited to. The medium may contain other components, for example, antioxidant (e.g., β -mercaptoethanol). The detailed description of media is found in R. Ian Freshney, Culture of Animal Cells, A Manual of Basic Technique, Alan R. Liss, Inc.,
5 New York (1984), the teaching of which is incorporated herein by reference in its entity.

According to a preferred embodiment of this invention, immature dendritic cells are cultured in the presence of TNF (tumor necrosis factor)- α , IFN- γ and poly(I) · poly(C) in order to induce the maturation of immature dendritic cells.

10 Mature dendritic cells exhibit expression of surface markers of CD83, DC-LAMP, p55, CCR-7, and have high expression level of MHC class II and costimulatory molecule of CD86. Immature dendritic cells are identified based on typical morphologies, low expression level of MHC class II and costimulatory molecules, and non expression level of markers of mature dendritic cells (e.g.,
15 CD83, CD-LAMP). In addition, positive markers for immature dendritic cells, for example, DC-SIGN, Langerin and CD1a can be used.

By utilizing standard antibody staining techniques known in the art, it is possible to assess the proportion of immature dendritic cells in any given culture. Antibodies may also be used to isolate or purify immature dendritic cells from
20 mixed cell cultures by flow cytometry or other cell sorting techniques well known in the art.

According to a preferred embodiment of this invention, in the step (a) of the procedure for preparing mature dendritic cells, the cancer cell is a renal cancer cell or breast cancer cell.

25 According to a preferred embodiment of this invention, in the step (a) of the procedure for preparing mature dendritic cells, the photosensitizing agent is selected from the group consisting of chlorins, bacteriochlorins, phthalocyanines, porphyrins, purpurins, merocyanines, psoralens, benzoporphyrin derivatives (BPD),

and porfimer sodium.

According to another preferred embodiment of this invention, the photosensitizing agent is porphyrins.

According to a preferred embodiment of this invention, in the step (b) of the procedure for preparing mature dendritic cells, the source of a light is selected from the group consisting of laser, lamp, optoelectric magnetic device, diode, and diode-laser.

According to another preferred embodiment of this invention, the source of a light is a laser or halogen lamp.

In another aspect of this invention, there is provided a mature dendritic cell which has been prepared according to the method of this invention such that the mature dendritic cell has the ability to induce anti-cancer immune responses.

The present mature dendritic cell, which has been prepared by pulsing with PDT-prepared cancer cell lysates as an antigen, has an enhanced ability to induce immune responses to the cancer cell such that it is able to effectively suppress the growth of the cancer cell.

According to a preferred embodiment of this invention, the mature dendritic cell of this invention has increased expression level of MHC class II.

According to another preferred embodiment of this invention, the mature dendritic cell of the present invention has the capability to induce the proliferation of CD8 T cells and activates the cancer cell specific cytotoxic T lymphocytes, and thus exerts the ability to suppress the growth of cancer cells.

According to another preferred embodiment of this invention, the dendritic cell of the instant invention has the activity to stimulate Th1 immune responses and suppress Th2 immune responses. The dendritic cell of this invention promotes the secretion of IFN- γ (Th1 cytokine) and reduces the secretion of IL-4 (Th2 cytokine) so that they increase the ratio of Th1/Th2. As a result, the dendritic cell prepared by the method of the present invention induces immune responses in

vivo.

In another aspect of this invention, there is provided a pharmaceutical composition for treating a cancer, which comprises (a) a pharmaceutically effective amount of the mature dendritic cell of claim 16; and (b) a pharmaceutically acceptable carrier.

Since the dendritic cell of this invention prepared by pulsing with PDT-prepared cancer cell lysates has acquired a capability to induce the cancer cell specific immune responses such that it is able to suppress the growth of cancer cell, and it can be used as an anti-cancer agent.

According to a preferred embodiment of this invention, the dendritic cell in the pharmaceutical composition has the increased expression level of MHC class II.

According to a preferred embodiment of this invention, the dendritic cell in the pharmaceutical composition has the capability to induce the proliferation of CD8 T cells and stimulated the activity of the cancer cell specific CTL (cytotoxic T lymphocyte).

According to a preferred embodiment of the instant invention, the dendritic cell in the pharmaceutical composition stimulates Th 1 immune responses and suppresses Th2 immune responses.

According to another preferred embodiment of this invention, the mature dendritic cell used in the pharmaceutical composition is an autologous or syngeneic dendritic cell. Most preferably autologous dendritic cells are employed in the present invention. Since the pharmaceutical composition of this invention contains an autologous dendritic cell which has been derived from a subject, it has advantages of little elicitation of immune responses to the injected dendritic cells.

According to a preferred embodiment of this invention, the cancer that is a therapeutic target of the pharmaceutical composition of this invention is renal cancer or breast cancer.

In the pharmaceutical compositions of this invention, the pharmaceutically

acceptable carrier may be conventional one for formulation, including lactose, dextrose, sucrose, sorbitol, mannitol, starch, rubber arable, potassium phosphate, arginate, gelatin, potassium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrups, methyl cellulose, methylhydroxy benzoate, propylhydroxy benzoate, talc, magnesium stearate, and mineral oils, but not limited to. The pharmaceutical composition according to the present invention may further include a lubricant, a humectant, a sweetener, a flavoring agent, an emulsifier, a suspending agent, and a preservative. Details of suitable pharmaceutically acceptable carriers and formulations can be found in *Remington's Pharmaceutical Sciences* (19th ed., 1995), which is incorporated herein by reference.

The pharmaceutical composition according to the present invention may be administered via the oral or parenterally. It is preferably administered via intravenous (percutaneous) or subcutaneous route.

A suitable dose of the pharmaceutical composition of the present invention may vary depending on pharmaceutical formulation methods, administration methods, the patient's age, body weight, sex, severity of diseases, diet, administration time, administration route, an excretion rate and sensitivity for a used pharmaceutical composition. Preferably, the pharmaceutical composition of the present invention is administered with a daily dose of 0.001-100 mg/kg (body weight).

According to the conventional techniques known to those skilled in the art, the pharmaceutical composition may be formulated with pharmaceutically acceptable carrier and/or vehicle as described above, finally providing several forms including a unit dose form and a multi-dose form.

In another aspect of this invention, there is provided a method for treating or preventing cancer, which comprises administering to a subject suffering from cancer a pharmaceutical composition comprising (a) a pharmaceutically effective amount of the mature dendritic cell of claim 16; and (b) a pharmaceutically acceptable carrier.

According to a preferred embodiment of this invention, the mature dendritic

cell used in the method of this invention is autologous.

According to a preferred embodiment of this invention, the cancer that can be treated or prevented by the method of this invention is renal cancer or breast cancer.

5 In another aspect of this invention, there is provided a use of the composition comprising the mature dendritic cell of this invention for the preparation of anti-cancer medicine.

According to a preferred embodiment of this invention, the mature dendritic cell is autologous.

10 According to a preferred embodiment of this invention, the cancer that is a target of the medicine is renal cancer or breast cancer.

The features and advantages of this invention can be summarized as follows:

15 (i) The present invention provides cancer cell lysates for pulsing dendritic cells in order to prepare mature dendritic cells that have the ability to induce anti-cancer immune responses, and also provides the method for preparing thereof.

(ii) The instant invention provides PDT-mediated dendritic cells that have a remarkably enhanced activity to induce anti-cancer immune responses, and also
20 provides the method for preparing thereof.

(iii) This invention provides a pharmaceutical composition comprising the dendritic cell that has a remarkable activity to suppress the growth of cancer cells.

(iv) PDT-prepared cancer cell lysates of the present invention can be used to manufacture dendritic cells whose potential to induce cancer cell specific
25 immune responses has been significantly enhanced.

(v) The dendritic cells, whose potential to induce cancer cell specific immune responses has been remarkably enhanced, can be used as an anti-cancer agent.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1a shows immunological phenotypes of immature dendritic cells (imDC), mature dendritic cells (mDC), dendritic cells (PDT DC) pulsed with photodynamic therapy (PDT)-prepared cancer cell lysates, dendritic cells (FT DC) pulsed with freezing-thawing method (FT)-prepared cancer cell lysates.

Fig. 1b shows survival rates of PDT-DC pulsed with PDT-prepared cancer cell lysates, F/T-DC pulsed with FT-prepared cancer cell lysates, and mDC having no pulsing.

Fig. 1c shows amounts of secreted IL-12 from PDT-DC pulsed with PDT-prepared cancer cell lysates and F/T-DC pulsed with FT-prepared cancer cell lysates, and mDC having no pulsing.

Fig. 2 shows the result of western blotting exhibiting the expression levels of heat shock proteins, HSP70, HSP-27, and HO-1 in EMT6 breast cancer cell that have been irradiated after being treated with the photosensitizing agent of porphyrins.

Fig. 3a shows tumor growth rate (-□-) in the breast cancer (EMT6) mouse model in which PDT-DCs that had been pulsed with PDT-prepared cancer cell lysates were administered 3 days after transplantation of cancer cells; tumor growth rate (-▲-) in the breast cancer mouse model in which FT-DCs that had been pulsed with cancer cell lysates prepared by freeze-thawing method were administered 3 days after transplantation of cancer cells; and tumor growth rate of control (-◆-).

Fig. 3b shows tumor growth rate (-□-) in the renal cancer (RENCA) mouse model in which PDT-DCs that had been pulsed with PDT-prepared cancer cell lysates were administered 3 days after transplantation of cancer cell; tumor growth rate (-▲-) in the renal cancer mouse model in which FT-DCs that had been pulsed with cancer cell lysates prepared by freeze-thawing method were administered 3 days after transplantation of cancer cells; and tumor growth rate of control (-◆-).

From the results of Figs. 3a and 3b, it can be understood that dendritic cells of this invention that have been prepared by pulsing with PDT-prepared cancer cell lysates significantly suppress the growth of breast cancer (EMT6) and renal cancer

(RENCA) in the mouse model.

Fig. 3c shows survival rates (-□-) of the individual animal of breast cancer (EMT6) mouse model which was vaccinated with PDT-DCs that had been pulsed with PDT-prepared cancer cell lysates; survival rates (-▲-) of individual animal of breast cancer mouse model which was vaccinated with FT-DCs that had been pulsed with cancer cell lysates prepared by freeze-thawing method; and survival rates of control (-◆-).

Fig. 3d shows survival rates (-□-) of individual animal of renal cancer (RENCA) mouse model which was vaccinated with PDT-DCs that had been pulsed with PDT-prepared cancer cell lysates; survival rates (-▲-) of individual animal of renal cancer mouse model which was vaccinated with FT-DCs that had been pulsed with cancer cell lysates prepared by freeze-thawing method; and survival rates of control (-◆-).

Fig. 3e is a photograph that shows the size of tumors in control, breast cancer mouse model (PDT-DC) that was administered with DCs pulsed with PDT-prepared cancer cell lysates, and breast cancer mouse model (FT-DC) that was administered with DCs pulsed with cancer cell lysates prepared by freeze-thawing method.

Fig. 4a shows that the proliferation of spleen CD8 T cells was significantly increased where the mouse was administered with PDT-DCs prepared by pulsing with PDT-prepared cancer cell lysates. However, the proliferation of spleen CD8 T cell was not greatly increased where the mouse treated with FT-DCs prepared by pulsing with cancer cell lysates prepared by freeze-thawing method. There is 10.2 folds increase in PDT-DC group compared to non-treating group, and 5.3 folds increase in FT-DC group compared to non-treating group.

Fig. 4b shows the measurement of EMT6 cancer cell specific CTL (cytotoxic T lymphocyte) activity in the group treated with PDT-DCs (-□-) pulsed with PDT-prepared cancer cell lysates; the measurement of EMT6 cancer cell specific CTL activity in the treating group treated with FT-DCs (-●-) pulsed with cancer cell

lysates prepared by freeze-thawing method, and control (-■-). From the results shown in Fig. 4b, it can be understood that CTL is more effectively activated by PDT-DCs than FT-DCs.

Fig. 4c shows that the level of Th1 immune responses is remarkably increased after treatment with dendritic cells. From the result of Fig. 4c, it can be understood that the level of Th1 immune responses is effectively increased and the level of Th2 immune response is effectively decreased by PDT-DCs pulsed with PDT-prepared cancer cell lysates. In addition, the effectiveness of FT-DCs pulsed with FT-prepared cancer cell lysates to stimulate Th1 immune responses and to suppress Th2 immune response is not as strong as PDT-DCs.

Fig. 5a and Fig. 5b show suppression effect of tumor growth of PDT-DCs of this invention. In the breast cancer mouse model (DC injection on 3 days after transplantation of cancer cells of EMT6 or 4T.1 cell), the tumor growth suppression effect of the treatment with PDT-DCs pulsed with PDT-prepared cancer cell lysates was superior to the treatment with FT-DCs pulsed with cancer cell lysates prepared freeze-thawing method.

Fig. 5c and Fig. 5d show survival rates of individual animal of mouse model. In the breast cancer mouse model (DC injection on 7 days after transplantation of cancer cells of EMT6 or 4T.1 cell), the survival rates in the treatment with PDT-DCs pulsed with PDT-prepared cancer cell lysates was superior to the treatment with FT-DCs pulsed with cancer cell lysates prepared freeze-thawing method.

The present invention will now be described in further detail by examples. It would be obvious to those skilled in the art that these examples are intended to be more concretely illustrative and the scope of the present invention as set forth in the appended claims is not limited to or by the examples.

EXAMPLES

Materials and Methods

Animal

Specific pathogen-free female Balb/c mice, 6 to 7 weeks of age, were
5 purchased from Orient Bio (Gyeonggi-do, SungNam-Si, Republic of Korea). The mice
were maintained under standard conditions of temperature and light, and were fed
standard laboratory chow and water. Every mouse had adaptation period before
being used in the experimentation.

10 Reagents

The culture medium used was DMEM and RPMI 1640 (GIBCO Laboratories,
Grand Island, NY, USA) supplemented with 10% FBS (GIBCO Laboratories, Grand
Island, NY, USA), 50 μ M 2-mercaptoethanol (Life technologies, Gaithersburg, MD,
USA), 100 μ g/ml streptomycin, 100 U/ml penicillin, and 25 μ g/ml amphotericin B
15 (GIBCO Laboratories; Grand Island, NY, USA).

Recombinant mouse (rm) TNF- α was purchased from BD Bioscience
(Mountain View, CA, USA) and polyribonucleosinic polyribocytidylic acid,
(Poly(I) · Poly(C)) was purchased from Amersham Biosciences (Piscataway, NJ, USA).
rmGM-CSF and IL-4 were purchased from CreaGene (Gyeonggi-do, SungNam-Si,
20 Republic of Korea).

For flow cytometric phenotyping, fluorescein isothiocyanate (FITC)- or
phycoerythrin (PE)- conjugated monoclonal antibodies to MHC class II, CD80, CD86,
CD54, CD40, CD14, CD4, CD8, IFN- γ and CD11c were purchased from BD
PharMingen (San Diego, CA, USA). For western blotting, mouse antibody to HSP70
25 (heat shock protein 70) was purchased from BD Bioscience (Mountain View, CA,
USA).

Cell Lines

Breast cancer cell line 4T-1 (ATCC CRL-2539) obtained from Balb/C was purchased from ATCC (Rockville, Md, USA), RENCA (renal cell adenocarcinoma line) (80022) was provided from Korean Cell Line Bank (Seoul, South Korea). Cell lines were maintained in RPMI-1640 media (complete media supplemented with 10% heat inactivated FBS, 100 U/ml penicillin, 100 µg/ml streptomycin). Breast cancer cell line EMT6 (ATCC CRL-2755) obtained from Balb/C was purchased from ATCC (Rockville, Md, USA) and maintained in DMEM media (complete media supplemented with 10% heat inactivated FBS, 100 U/ml penicillin, 100 µg/ml streptomycin).

10 **Mouse Bone Marrow Cell Preparation**

Bone marrow prepared from Balb/c mice was flushed out of femurs with RPMI-1640 by use of a syringe and separated as a single cell in the media by using a screen mesh. RBCs (red blood cells) were lysed by washing with ACK lysis buffer (Tris-buffered 0.15 M ammonium chloride solution) and remaining cells having nuclear were counted with hemocytometer. The survival rate of cells was confirmed by Trypan Blue Exclusion (routinely > 90%).

Preparation of Cancer Cell Lysates

F/T prepared cancer cell lysates were prepared from cancer cell lines of EMT6, 4T-1, or RENCA through the procedure of freezing-thawing method using liquid nitrogen (-180°C) and incubator (37°C) and removing cell debris by centrifugation at 600 rpm.

PDT prepared cancer cell lysates were manufactured as follows: Cancer cell lines of EMT6, 4T-1, or RENCA (supplied by Yonsei University Medical School, Seoul, Republic of Korea) were incubated in the complete medium for 4 hr in the presence of 1 µg/ml porphyrins. The incubated cells were washed with serum-free media, transferred to 1X PBS, and irradiated with a laser or halogen lamp (6 J/cm², 20 mW/cm²). After that, the PDT-treated cells were transferred to serum-free media

and cultured in the incubator. After culturing, cells and supernatants were collected using scrapper and removed cell debris by centrifugation at 600 rpm for 20 min. The protein contents in the supernatant were measured by Bradford method. The F/T or PDT prepared cancer cell lysates were used as antigens for the induction of the maturation of dendritic cells.

Western Blotting of Heat Shock Proteins

In order to compare expression levels of heat shock proteins in F/T prepared cancer cell lysates that used as a conventional cancer antigen to that of heat shock proteins in PDT prepared cancer cell lysates, western blotting to the heat shock proteins was carried out. Samples of cell lysates were prepared by the same procedures as described in the preparation of cancer cell lysates. After the amount of proteins was analyzed, equivalent amount of proteins was loaded and electrophoresis was done. Proteins separated on SDS-PAGE were transferred to nitrocellulose membrane and incubated in the blocking solution (5% skim milk in PBST) for 10 min at room temperature in order to prevent non-specific bindings of antibody. Primary antibodies of anti-mHSP70, anti-mHSP27, anti-mHO-1 were added with the ratio of 1:10,000 and incubated for 1 hr at room temperature. After incubation, unbound antibodies were removed by washing with PBST (0.01% Tween20 in PBS) for three times for 5 min. After blocking with blocking solution (5% skim milk in PBST) for 10 min at room temperature, AP (alkaline phosphatase) conjugated mouse secondary antibodies were added with the ratio of 1:10,000 and incubated for 1 hr at room temperature.

After incubation, unbound antibodies were removed by washing with PBST (0.01% Tween20 in PBS) for 5 min for three times and substrate solution of NBT (p-nitro-blue tetrazolium chloride) and BCIP (5-bromo-4-chloro-3-indolyl-phosphate; Sigma) were added for color reaction.

Ex Vivo Culture of Myeloid DCs

Culturing myeloid dendritic cells isolated from mouse bone marrow monocytes was carried out by the method modified from Inaba et al [1]. Myeloid cells were separated from the isolated bone marrow monocytes, and incubated for 6 days in the presence of 10 ng/ml rmGM-CSF and 10 ng/ml rmIL-4 at the concentration of 1×10^6 cells/ml. On sixth day of incubation, cancer cell lysates 50 ug/ml were added and incubated for additional 8 hr. In order to induce the maturation of dendritic cells, 20 ng/ml TNF- α , 100 U/ml IFN- γ , 10 ug/ml Poly(I) · Poly(C) were added and incubated for additional 18 hr. Incubated dendritic cells were collected on seventh day of incubation and their phenotypes were analyzed. Dendritic cells for therapeutic injection were recovered, suspended in physiological saline solution and administered it to mouse subcutaneously within 2 hr (1×10^6 cells / 100 ul / mouse).

Transplantation of Cancer Cells and Injection of Therapeutic Dendritic Cells

Cultured breast cancer cells, EMT6 and 4T-1 were collected and transplanted into subcutaneous tissue with the concentration of 10^5 cells/ mouse, or RENCA cells were collected and transplanted into subcutaneous tissues with the concentration of 1×10^6 cells/mouse. The administration of therapeutic dendritic cells (1×10^6 cells/ mouse) was done on seventh day after injection of cancer cells (7 day model) or on third day after injection of cancer cells (3 day model). The dendritic cells were injected into subcutaneous tissues twice at the interval of one week. The size of tumor growing in the subcutaneous tissues was measured twice per a week using caliper. The change of systemic immune function was detected with spleen cells.

25

Flow Cytometric Aanalysis

The phenotype determination of dendritic cells or spleen cells was performed in accordance with the conventional method of Yoon et al [2]. Spleen monocytes

(1×10^5 cells/100 μ l) were suspended in FACS buffer (PBS mixed with 0.1% sodium azide and 1% FBS) containing fluorescence labeled antibodies and cultured at 4 °C for 20 min. Cells were washed after culturing, suspended in 500 μ l FACS buffer, and analyzed with flow cytometer (BD Bioscience, Mountain View, CA, USA).

5

Spleen Lymphocyte Proliferation Reaction

In order to confirm the effective function of cultured dendritic cells or enhancement in tumor specific immune responses after therapeutic treatment with dendritic cells, the changes in proliferation rate of spleen monocytes were measured.

10 Spleen lymphocytes separated from experimental mouse group were incubated in the presence of 20 μ g/ml cancer cell lysates and 20 U/ml IL-2 for 5 days. The extent of proliferation of spleen lymphocytes was analyzed with FACS using antibodies of FITC-conjugated anti-mouse CD8 and PE-conjugated anti-mouse IFN- γ .

15 **Analysis of the Function of Cytotoxic T Cell**

The activity of tumor specific cytotoxic T cell (CTL) induced after dendritic cell injection therapy was analyzed in accordance with the method described by Liu SQ et al [3]. The activity of lymphocytes, the effector cells (E), to kill EMT6 cell, the target cells of CTL reaction, was measured. Target cells (1×10^4 cells/100 μ l) were
20 mixed with effector cells in various concentrations, incubated in a 96 well plate for 24 hr, and then the number of viable target cells was determined by crystal violet assay. Crystal violet assay that measures the activity of CD8⁺ T cell to kill adherent target cells has a credible sensitivity. The cells in the well were washed with PBS and
25 fixed with 10% Formalin (100 μ l/ well) for 1 hr at room temperature. The cell was stained with Crystal violet solution (0.4% in water, 100 μ l/ well) for 30 min at room temperature. Plate was washed with water and dried at room temperature. After 200 μ l of 80% methanol was added into the respective wells, the survival rates of cells were determined by measuring an absorbance at 570 nm. The measurement of cell

survival rates of control group was done by measuring an absorbance at 570 nm in 100% target cells containing no effector cell.

Enzyme-Linked Immuno-Sorbent Assay: ELISA

5 In order to measure the expression level of Th1 cytokine IFN- γ and Th2 cytokine IL-4, ELISA was performed on the supernatant of culture media of mouse spleen cells. In addition, in order to characterize the nature of dendritic cells, ELISA was carried out on the supernatant of dendritic cell culture media so as to measure the expression level of IL-2, Th2 cytokine that can induce the migration of dendritic
10 cells. The extent of secretion of cytokines was determined by ELISA (R&D system, Abington, OX, UK) in accordance with instructions of manufacturer.

Statistical Analysis

 The in vivo experiment carried out with 4 mice for one group. The statistical
15 significance was confirmed by ANOVA (analyses of variance) using Fisher protected least significant difference test. A p -value of less than 0.05 was considered significant.

RESULTS

20 Analysis of phenotypes of dendritic cells pulsed with PDT-prepared cancer cell lysates and IL-12 cytokine secretion analysis

 The immune responses of T cells initiate by the reaction of mature DCs with naïve T cells. Although immature dendritic cells have a phagocytic function, they are weak T cell stimulator. The maturation of dendritic cells is an important process in
25 the inducing mechanism of immune responses. PDT-prepared cancer cell lysates have more effectiveness than F/T-prepared cancer cell lysates in the induction of the maturation of dendritic cells, and thus make it possible to manufacture more effective tumor vaccines. In order to support this, immature dendritic cells were

obtained from bone marrow cells that have been isolated from Balb/C and induced to be matured by culturing in the presence of PDT-prepared lysates or FT-prepared lysates. The phenotype of DCs was examined with FACS analysis by use of surface markers of dendritic cells, CD80, CD86, CD54, D40, MHC class II and surface marker of monocytes, CD14 as a control. Although there was little difference in the expression level of phenotypic markers between dendritic cells that have been cultured with F/T prepared lysates and PDT prepared lysates, the expression level of MHC class II in DCs incubated with PDT prepared lysates was increased compared to DCs pulsed with F/T prepared lysates. Accordingly, it is shown that PDT-prepared lysates more effectively induce the maturation of phenotypes of dendritic cells (Fig. 1a).

In order to characterize other properties of dendritic cells pulsed with PDT-prepared lysates, the survival rate of DCs and amount of IL-12 secreted from DCs were determined. The survival rate was measured by use of FACS analysis with PI (propidium iodide) staining after culturing dendritic cells in serum free media for one week. The results depicted in figure 1b show slight increase in the survival rate of DCs pulsed with PDT-prepared lysates (Fig. 1b).

IL-2 cytokine induces Th1 response by activating T cells and NK cells, and stimulates migration of dendritic cells so as to increase the immune responses by T cells at the secondary immune organs. By the results of ELISA as to IL-12 cytokine, it was confirmed that there was little difference in the secretion level of IL-12 in the respective mDCs, F/T DCs and PDT DCs (Fig. 1c).

Secretion of heat shock proteins induced by PDT prepared cancer cell lysates

Heat shock proteins (HSPs) play an important role as a regulator in inflammatory reactions and immune responses. The expression level of HSPs is increased by external stimulations induced from physiological causes (growth or

differentiation of cells or tissues), pathological causes (infections, inflammatory reactions, tumors and autoimmunity), or environmental causes (heat shock, heavy metals, oxygen free radical) [4, 5]. It is reported that chemotherapy, radiotherapy, and hyperthermia in cancer cells can increase intracellular expression levels of HSP70 [6, 7]. After EMT6 cancer cells were treated with the photosensitizing agent of porphyrins and irradiation of a laser or halogen lamp, the expression level of HSP was measured by western blotting. EMT6 cancer cells were treated with 1 ug/ml of the photosensitizing agent of porphyrins, incubated for 4 hr, transferred to serum-free medium and cultured for 1, 2, 4, 6 and 12 hr respectively. The protein content in the supernatant was measured in accordance with Bradford method and expression levels of HSP70, HO-1, HSP27 were detected from supernatants that have the same protein content. The expression level of HSP70 reached the maximum at 2 hr after treatment of PDT on EMT6 cancer cell and decreased after 2 hr. The expression level of the other heat shock protein of HO-1 (Hemeoxygenase-1) in the PDT prepared group was higher than in the F/T prepared group. However, the expression level of HSP 27 in the F/T prepared group was higher than in the PDT treated group (Fig. 2).

Therapeutic effectiveness of dendritic cells pulsed with PDT-prepared cancer cell lysates in the cancer animal model (DC injection after 3 days of transplantation)

The therapeutic effectiveness of dendritic cells pulsed with cancer cell lysates was determined in the mouse model in which EMT6 cancer cells (5×10^5 cells/mouse) were administered into subcutaneous tissues. Two types of cancer cell lysates of PDT-prepared cancer cell lysates and F/T-prepared cancer cell lysates were used as cancer antigens in the procedure to induce the maturation of dendritic cells. Primary injection of dendritic cells pulsed with PDT-prepared or F/T-prepared cancer cell lysates was done on third day after injection of EMT6 cancer cells, secondary

injection of dendritic cells was done on one week after injection. The growth of cancer cells was inhibited where dendritic cells pulsed with cancer cell lysates were injected. In addition, more significant effectiveness of cancer cell growth inhibition was observed where dendritic cells pulsed with PDT-prepared cancer cell lysates were administered compared to the administration of DCs pulsed with F/T-prepared cancer cell lysates (Figs. 3a and 3e).

The same experiment as to EMT6 cells was performed in the mouse model of Renal cell carcinoma (RENCA). It is confirmed that the RENCA growth inhibition effect induced by administration of dendritic cells pulsed with PDT-prepared cancer cell lysates was greater than the inhibition effect induced by dendritic cells pulsed with F/T-prepared cancer cell lysates (Figs 3b). The survival rate of individual mouse was more increased when the mouse was vaccinated with dendritic cells pulsed with PDT-prepared cancer cell lysates (Figs 3c and 3d). The general symptoms observed in non-therapeutic groups such as death, an extreme reduction of body weight, and blunting in motility were improved in therapeutic groups of dendritic cells. There was no toxicity visible with naked eyes, which might be induced by the administration of dendritic cells.

Observation of changes in immune responses in cancer animal model after administration of dendritic cells pulsed with PDT-prepared cancer cell lysates (administration of dendritic cells after 3 days of cancer cell transplantation)

The changes in systemic immune responses were detected from mouse spleen cells on second weeks after the last administration of dendritic cells. Tumor specific responses of lymphocytes that are expected to be induced by dendritic cells pulsed with cancer cell lysates were examined. The proliferation of spleen CD8 T cells was remarkably increased after administration of dendritic cells (PDT-DCs) matured by the process of the present invention (Fig 4a). About 10.2 folds of

proliferation increase in the treated group with DCs pulsed with PDT-prepared cancer cell lysates was measured compared to the untreated group. In addition, about 5.3 folds of proliferation increase in the treated group with DCs pulsed with F/T prepared cancer cell lysates was detected. The increase in the CTL activity
5 specific to EMT6 cancer cells was confirmed in dendritic cell treated group (Fig 4b).

When the CTL activity in the cultured cells isolated from spleen was measured, the increase having a statistical significance was detected in the therapy group (PDT-DC) treated with DCs pulsed with PDT-prepared cancer cell lysates compared to the therapy group (F/T-DC) treated with DCs pulsed with F/T-prepared cancer cell
10 lysates.

Th1 immune responses were effectively increased after treatment with dendritic cells. There was more increase in Th1 cell immune responses and more decrease in Th2 cell immune responses in the group (PDT-DC) treated with DCs pulsed with PDT-prepared cancer cell lysates than the group (F/T-DC) treated with
15 DCs pulsed with FT-prepared cancer cell lysates (Fig 4c).

Therapeutic effects of DCs pulsed with PDT-prepared cancer cell lysates in the breast cancer animal model

The therapeutic effects of DCs pulsed with PDT-prepared cancer cell lysates
20 were examined in the breast cancer mouse model having tumor developed from transplanted EMT6 or 4T-1 cells. The size of tumor was measured after injection of 1×10^6 DCs with two times a week after one week of tumor generation. The more remarkable inhibition effect of tumor growth in the group (PDT-DC) treated with DCs pulsed with PDT-prepared cancer cell lysates was detected compared to the group
25 (FT-DC) treated with FT-prepared cancer cell lysates (Figs. 5a and 5b). When the survival rate of individual animal was measured, it was confirmed that the survival rate was increased in the group (PDT-DC) treated with DCs pulsed with PDT-prepared cancer cell lysates (Figs 5c and 5d).

Having described a preferred embodiment of the present invention, it is to be understood that variants and modifications thereof falling within the spirit of the invention may become apparent to those skilled in the art, and the scope of this invention is to be determined by appended claims and their equivalents.

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What is claimed is:

1. A method for preparing cancer cell lysates for pulsing dendritic cells, which comprises the steps of:
 - (a) culturing cancer cells in the presence of a photosensitizing agent;
 - 5 (b) activating the photosensitizing agent bound to cancer cells by irradiating the cultured cancer cells with a light; and
 - (c) obtaining cancer cell lysates from the cultured cancer cells.
2. The method according to claim 1, wherein the cancer cell is a renal cancer cell
10 or breast cancer cell.
3. The method according to claim 1, wherein the photosensitizing agent is selected from the group consisting of chlorins, bacteriochlorins, phthalocyanines, porphyrins, purpurins, merocyanines, psoralens, benzoporphyrin derivatives, and
15 porfimer sodium.
4. The method according to claim 3, wherein the photosensitizing agent is porphyrins.
- 20 5. The method according to claim 1, wherein the source of the light is selected from the group consisting of laser, lamp, optoelectric magnetic device, diode, and diode-laser.
6. The method according to claim 5, wherein the source of the light is a laser or
25 halogen lamp.
7. A cancer cell lysate for pulsing dendritic cells, which is prepared in accordance with the method of any one of claims 1 to 6.

8. The cancer cell lysate according to claim 7, wherein the lysate comprises heat shock proteins.
- 5 9. A composition for inducing the maturation of immature dendritic cells, which comprises the cancer cell lysate of claim 7 as an active ingredient.
10. A method for preparing mature dendritic cells having the ability to inhibit the growth of tumor, which comprises the steps of;
- 10 (a) culturing cancer cells in the presence of a photosensitizing agent;
- (b) activating the photosensitizing agent bound to the cancer cells by irradiating the cultured cancer cells with a light;
- (c) obtaining cancer cell lysates from the cultured cancer cells; and
- 15 (d) inducing the maturation of dendritic cells by pulsing immature dendritic cells with the obtained cancer cell lysates.
11. The method according to claim 10, wherein the cancer cell is a renal cancer cell or breast cancer cell.
- 20 12. The method according to claim 10, wherein the photosensitizing agent is selected from the group consisting of chlorins, bacteriochlorins, phthalocyanines, porphyrins, purpurins, merocyanines, psoralens, benzoporphyrin derivatives, and porfimer sodium.
- 25 13. The method according to claim 12, wherein the photosensitizing agent is porphyrins.
14. The method according to claim 10, wherein the source of the light is selected

from the group consisting of laser, lamp, optoelectric magnetic device, diode, and diode-laser.

15 15. The method according to claim 14, wherein the source of the light is a laser or halogen lamp.

10 16. A mature dendritic cell which is prepared according to the method of any one of claims 10 to 15 such that the mature dendritic cell has the enhanced ability to induce anti-tumor immune responses.

17. A pharmaceutical composition for treating cancer, which comprises (a) a pharmaceutically effective amount of the mature dendritic cell of claim 16; and (b) a pharmaceutically acceptable carrier.

15 18. The pharmaceutical composition according to claim 17, wherein the mature dendritic cell is autologous.

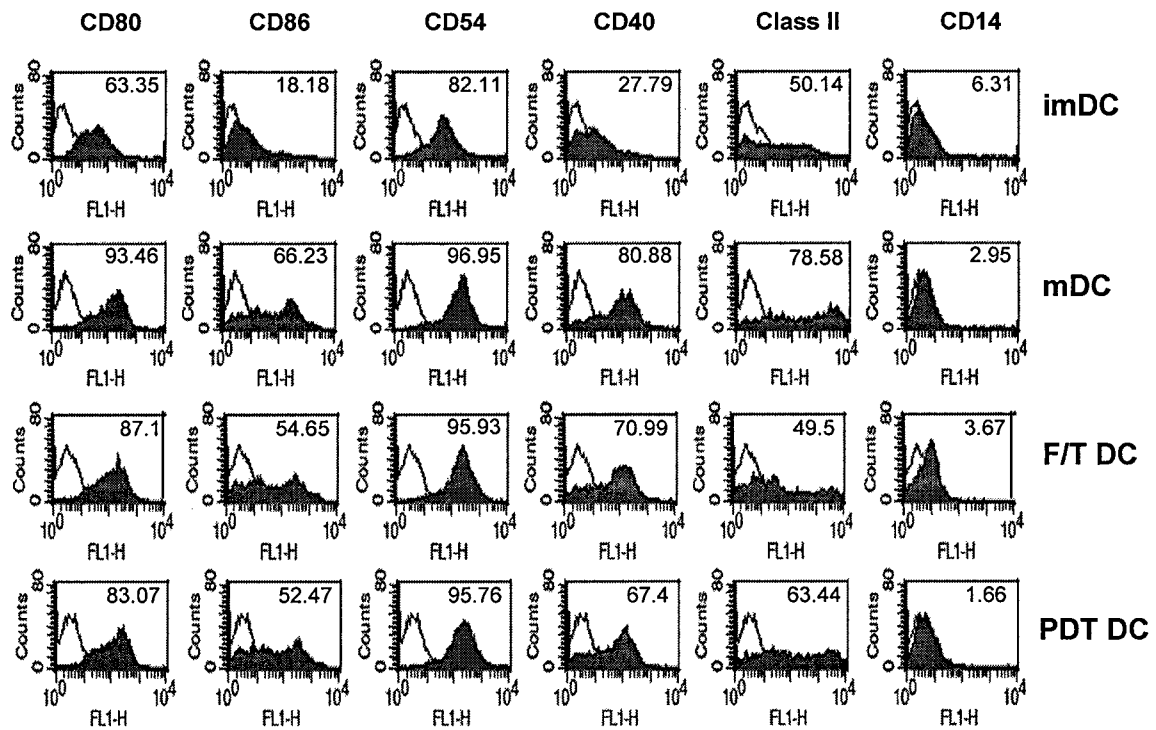
19. The pharmaceutical composition according to claim 17, wherein the cancer is renal cancer or breast cancer.

20 20. A method for treating or preventing cancer, which comprises administering to a subject suffering from cancer a pharmaceutical composition comprising (a) a pharmaceutically effective amount of the mature dendritic cell of claim 16; and (b) a pharmaceutically acceptable carrier.

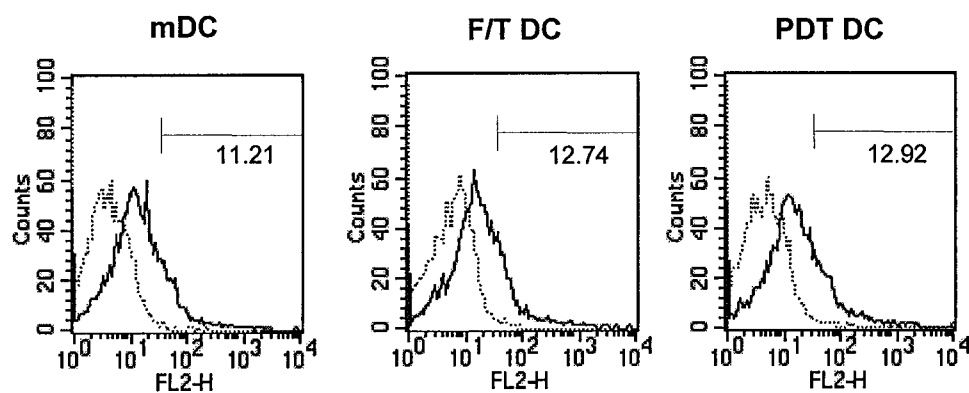
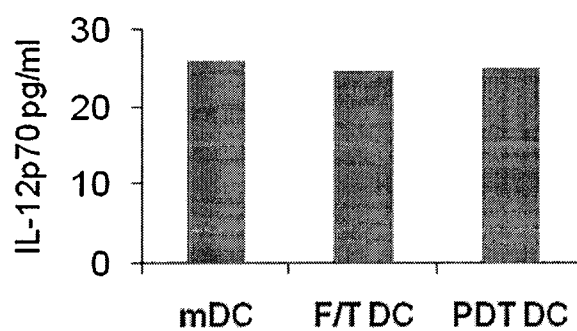
25 21. The method according to claim 20, wherein the mature dendritic cell is autologous.

22. The method according to claim 20, wherein the cancer is renal cancer or breast cancer.
23. A use of the composition comprising the mature dendritic cell of claim 16 for
5 the preparation of anti-cancer medicine.
24. The use according to claim 23, wherein the mature dendritic cell is autologous.
- 10 25. The use according to claim 23, wherein the cancer is renal cancer or breast cancer.

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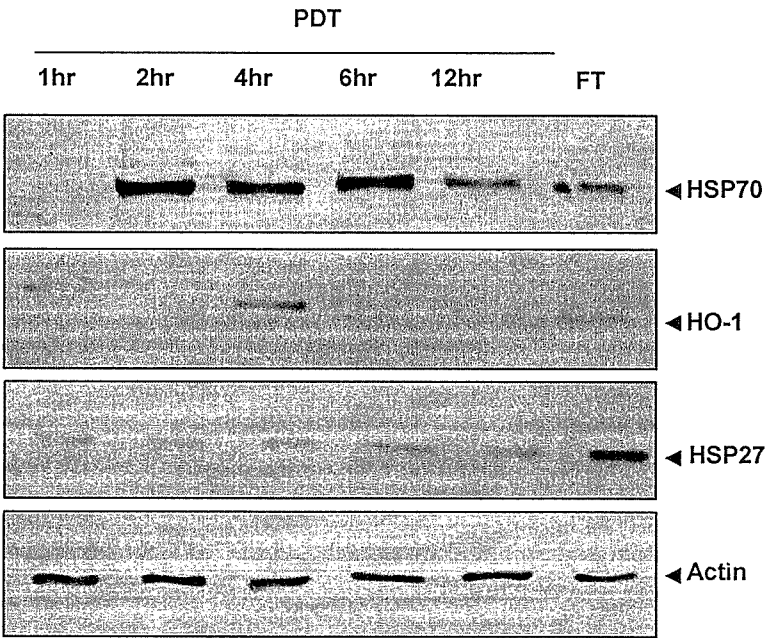
Fig. 1a

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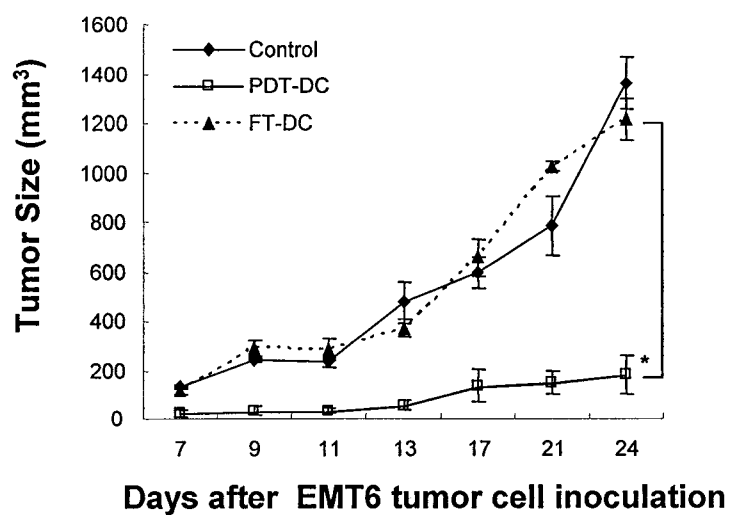
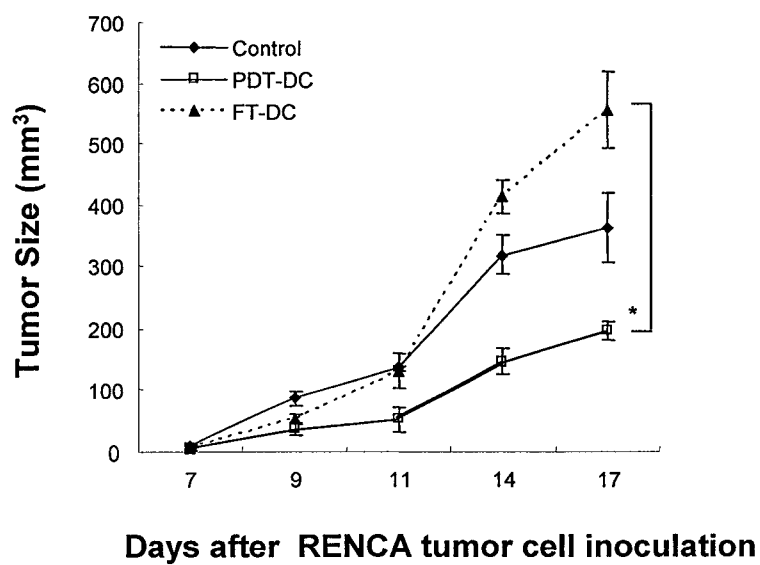
Fig. 1b**Fig. 1c**

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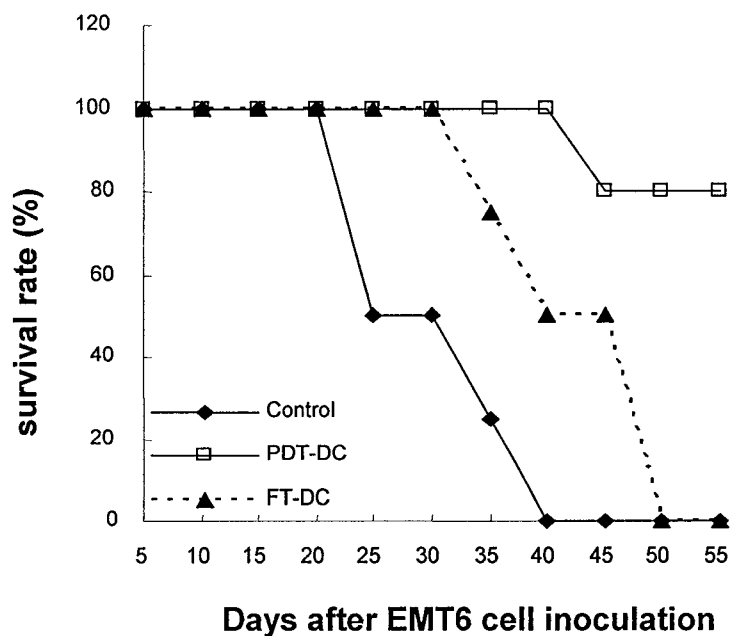
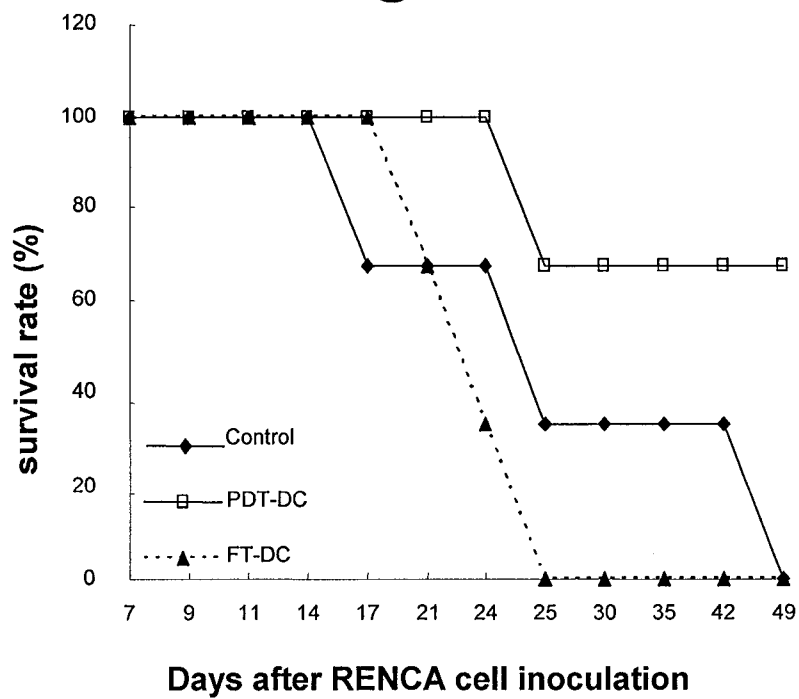
Fig. 2



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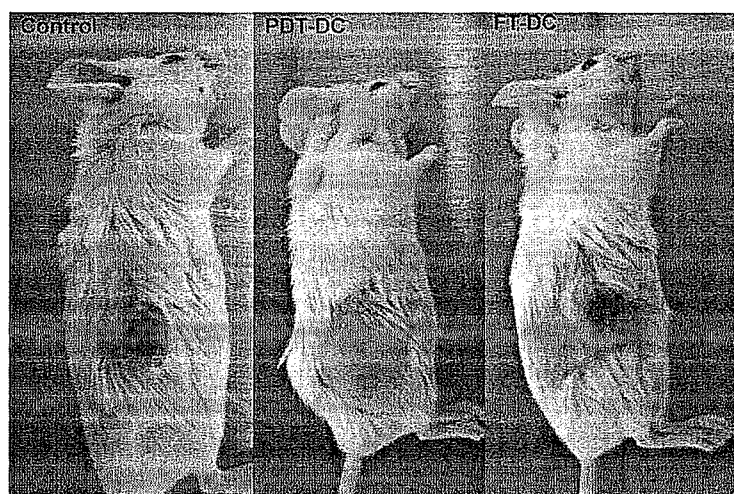
Fig. 3a**Fig. 3b**

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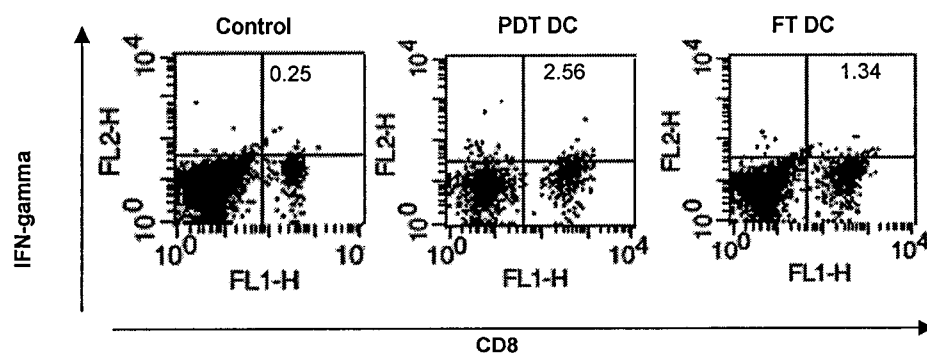
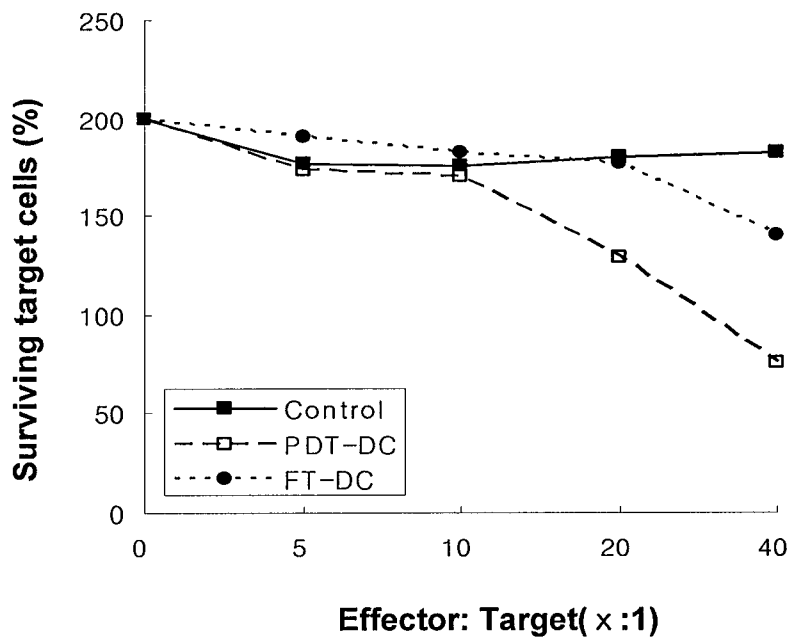
Fig. 3c**Fig. 3d**

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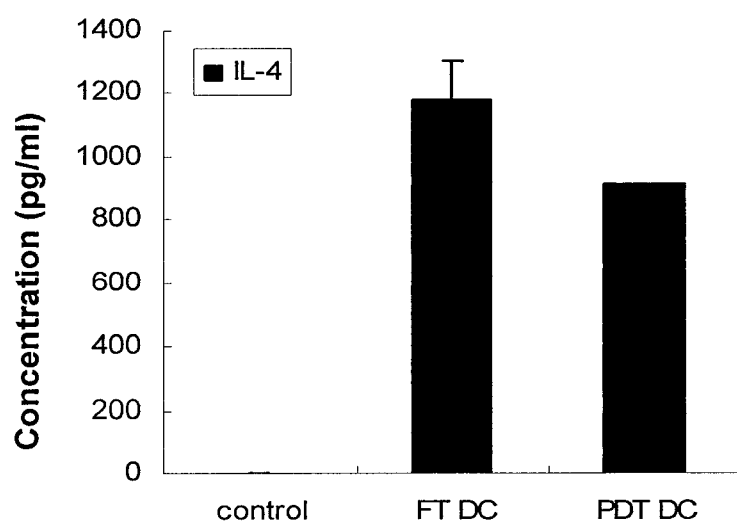
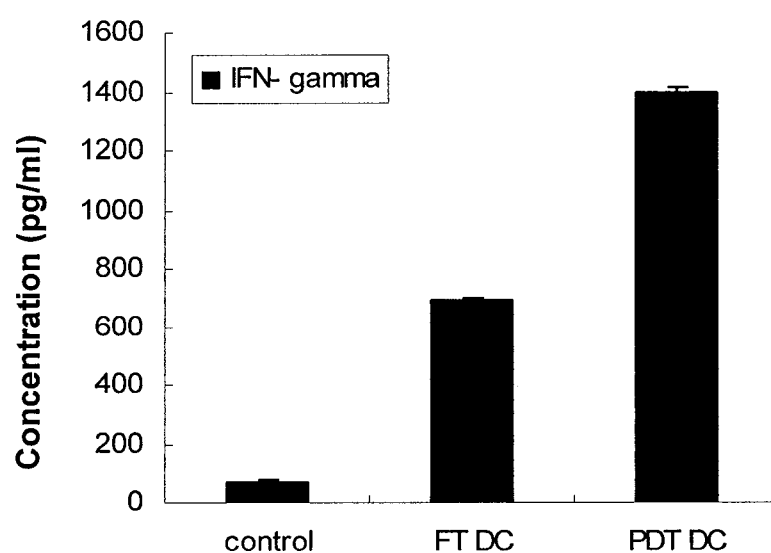
Fig. 3e



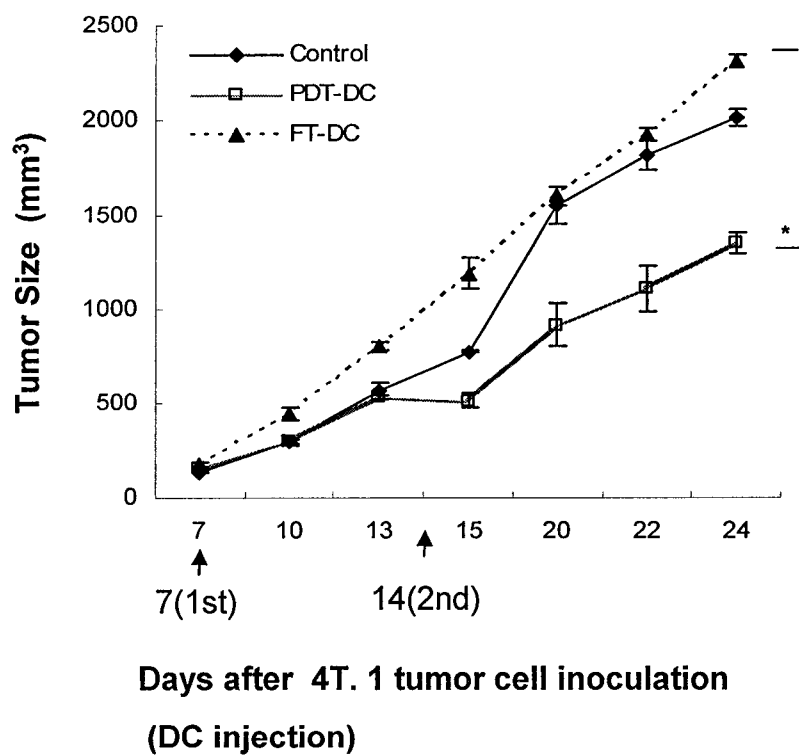
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Fig. 4a**Fig. 4b**

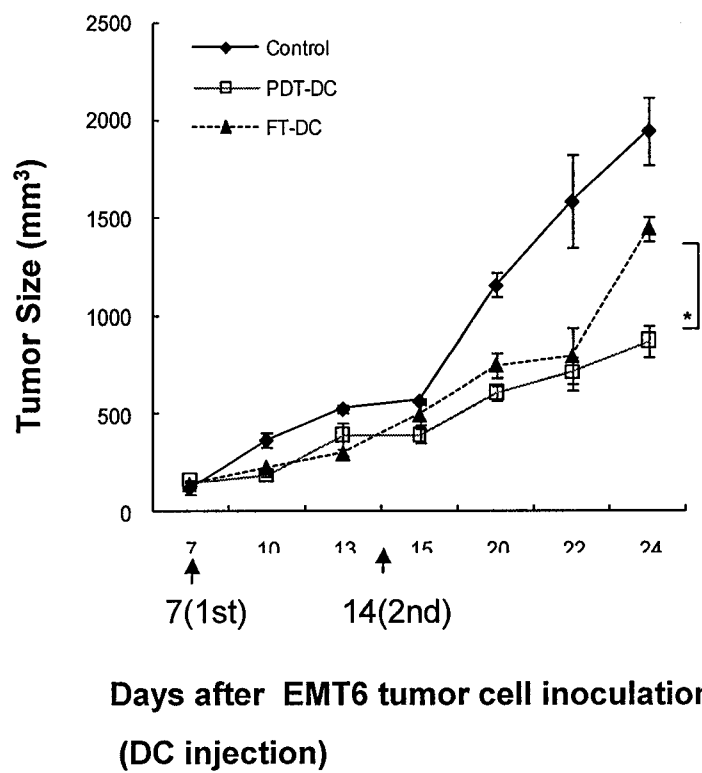
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Fig. 4c

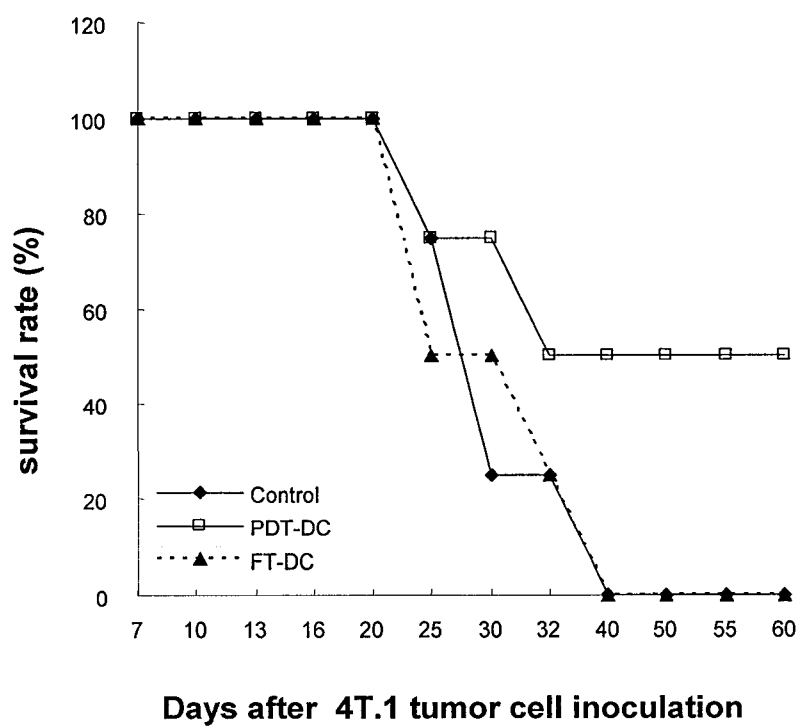
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Fig. 5a

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Fig. 5b

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Fig. 5c

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Fig. 5d