DESCRIPTION

Title of Invention

NATURAL KILLER CELL CONTAINING EXOGENOUS MITOCHONDRIUM AND PHARMACEUTICAL COMPOSITION COMPRISING SAME

Technical Field

The present invention relates to cell therapy products, and more particularly, to a natural killer (hereinafter referred to as NK) cell or peripheral blood mononuclear cells (hereinafter referred to as PBMCs) which comprise exogenous mitochondria, and pharmaceutical compositions comprising the same as an active ingredient.

Background Art

Recently, biopharmaceuticals have been developed to treat various incurable diseases. The biopharmaceuticals include protein drugs, antibody drugs and cell therapy products. Here, the cell therapy products refer to the pharmaceuticals used for the purpose of treatment, diagnosis, or prevention of diseases and the cell therapy products may be obtained through a series of actions such as by performing *ex vivo* isolation and proliferation or by altering biological characteristics of cells by other methods. The cell therapy products may come from autologous, allogeneic, or xenogeneic cells. Depending on the cell type, the cell therapy products can be categorized into somatic cell therapy products and stem cell therapy products.

On the other hand, immunotherapy using the patient's immune function has been paid attention in cancer therapy. In immunotherapy, properties of immune cells with diverse functions are utilized and cancer cells are eliminated through complex interactions of the immune cells. A PBMC in human blood is a blood cell with a round nucleus such as a lymphocyte or a monocyte, and includes B cells, T cells, macrophages, dendritic cells (DCs), and NK cells. Among them, NK and cytotoxic T lymphocytes (CTLs) cells directly eliminate cancer cells. Antigen-presenting cells that present antigens to these effector cells include dendritic cells or B cells. In addition, helper T cells and regulatory T cells which secrete various cytokines, and the like are involved in immune responses. Among these, NK cells are emerging and promising immune cells in immune cell therapies.

In particular, as NK cells have been shown to have an ability to kill cancer cells in a non-specific manner, many studies have been conducted on NK cells. Based on these researches, NK cell therapies in cancer, have been emerging tools for treatment of cancer cells. In particular, it has been reported that NK cells play an important role in innate and acquired immune responses achieved through cytokine secretion, against pathogens that have infected a host or cancer.

Accordingly, the present inventors have made efforts to find a new method for improving the power of cytotoxicity of NK cells and PBMCs. As a result, the present inventors have found a method of increasing the cytotoxicity of NK and PBMC, and thus provide a method of treating cancer using such cytotoxicity.

Disclosure of Invention

Aspects of the invention are defined in the independent claims. Some optional features are defined in the dependent claims.

With use of the present techniques, an NK cell with increased cytotoxicity and a pharmaceutical composition comprising the same may be provided.

With use of the present techniques, a PBMC with increased cytotoxicity and a pharmaceutical composition comprising the same may be provided.

Disclosed herein are NK cells comprising exogenous mitochondria, and a pharmaceutical composition for preventing or treating cancer or an infectious disease, comprising the same.

Also disclosed herein are PBMCs comprising exogenous mitochondria, and a pharmaceutical composition for preventing or treating cancer or an infectious disease, comprising the same.

Advantageous Effects

An NK cell and a PBMC, into which exogenous mitochondria have been introduced, not only have an increased cytotoxicity which results in increased cancer-specific killing effects, but also exhibit no side effects as immune cells existing *in vivo*. In addition, the NK cell and the PBMC are improved in terms of an ability of the cell itself, and thus can be

widely applied to various diseases in which the NK cell and the PBMC are involved. As a result, it is expected that pharmaceutical compositions comprising the NK cell and the PBMC are highly commercially applicable.

Brief Description of Drawings

FIG. 1 illustrates a result obtained by a PCR analysis method in order to investigate whether human normal hepatocyte (WRL68)-derived mitochondria have been delivered into NK cells.

FIG. 2 illustrates a result obtained by FACS analysis in order to investigate whether human normal hepatocyte-derived mitochondria have been delivered into NK cells.

FIG. 3 illustrates results obtained by fluorescence microscopy, in order to locate the human normal hepatocyte-derived mitochondria delivered into NK cells.

FIGS. 4A and 4B illustrate results showing anti-cancer activity of NK cells into which exogenous mitochondria have been delivered by a CD107a degranulation assay.

FIG. 5 illustrates a result showing the cytotoxic effects of NK cells into which exogenous mitochondria have been delivered by K562 cytotoxicity assay.

FIG. 6 illustrates a result obtained by FACS analysis in order to investigate whether mitochondria derived from umbilical cord mesenchymal stem cells (UC-MSC) have been delivered into NK cells.

FIG. 7 illustrates a result showing the cytotoxic effects of NK cells into which UC-MSC-derived mitochondria have been delivered using K562 cytotoxicity assay.

FIGS. 8A to 8C illustrate results showing the therapeutic effects by NK cells, into which UC-MSC-derived mitochondria have been delivered in an animal model of acute myelogenous leukemia, in terms of body weight and survival rate of mice.

FIG. 9 illustrates results of expression distribution of blood tumor markers in an animal model of acute myelogenous leukemia which has been administered with NK cells into which UC-MSC-derived mitochondria have been delivered.

FIG. 10 illustrates a result showing the cytotoxic effects of PBMCs into which exogenous mitochondria have been delivered by K562 cytotoxicity assay

Detailed Description

The present disclosure provides an NK cell enriched by exogenous mitochondria.

As used herein, the term "exogenous mitochondria" refers to mitochondria introduced exogenously rather than mitochondria present in an NK cell. Here, the exogenous mitochondria may be obtained from the same subject as that from which the NK cell is obtained, but may be obtained from another subject. Here, the exogenous mitochondria may be obtained from a mammal, and preferably may be obtained from a human. For example, the exogenous mitochondria may be obtained from muscle cells, hepatocytes, fibroblasts, epithelial cells, neurons, adipocytes, osteocytes, leukocytes, lymphocytes, or mucosal cells, and preferably may be obtained from muscle cells with excellent mitochondrial activity. In addition, the mitochondria may be obtained from cells cultured *ex vivo*.

On the other hand, the exogenous mitochondria can be obtained by disrupting the cells and performing centrifugation, or by culturing the cells, disrupting the cells, and performing the centrifugation. For the method for obtaining mitochondria, a conventional method used for collecting an organelle can be used.

Here, an NK cell comprising exogenous mitochondria may be obtained by introducing mitochondria in an amount of 0.01 to 500 μg, 0.1 to 450 μg, 0.5 to 300 μg, 1 to 100 μg, or 2 to 10 μg, per 10^5 NK cells. Here, an NK cell may contain 1 to 10^3 or 10 to 10^2 exogenous mitochondria. Specifically, the number of exogenous mitochondria may be such that approximately 1, 10, 100, or 500 exogenous mitochondria are contained in one NK cell. Here, the number of exogenous mitochondria contained in NK cells can be regulated by controlling an amount of exogenous mitochondria to be introduced into NK cells. Each subject NK cell may contain a different number of exogenous mitochondria.

In addition, the NK cells may be derived from a mammal or a human. Preferably, the NK cells may be obtained from a subject intended to receive NK cell therapy. Here, the NK cells may be directly isolated from the blood of the subject and used, or may be obtained by differentiating immature NK cells or stem cells obtained from the subject and used.

Meanwhile, in order to introduce the exogenous mitochondria into NK cells, the exogenous mitochondria and the NK cells may be mixed and then the mixture may be subjected to centrifugation so that the mitochondria are delivered efficiently into the NK cells.

temperature of 0°C to 40°C, 20°C to 38°C, or 30°C to 37°C. In addition, the incubation may be performed for 0.1 to 4 hours, 0.5 to 3.8 hours, or 0.8 to 3.5 hours. In addition, the incubation may be performed for a predetermined time after centrifugation is performed so as to deliver the exogenous mitochondria into the NK cells. In addition, the incubation time can be appropriately selected depending on a cell type and an amount of mitochondria.

Also disclosed herein is a pharmaceutical composition for treating cancer or an infectious disease, comprising, as an active ingredient, an NK cell that contains the exogenous mitochondria.

Here, the cancer may be any one selected from the group consisting of gastric cancer, liver cancer, lung cancer, colorectal cancer, breast cancer, prostate cancer, ovarian cancer, pancreatic cancer, cervical cancer, thyroid cancer, laryngeal cancer, acute myelogenous leukemia, brain tumor, neuroblastoma, retinoblastoma, head and neck cancer, salivary gland cancer, and lymphoma. In addition, the infectious disease may be any one selected from the group consisting of hepatitis B, hepatitis C, human papilloma virus (HPV) infection, cytomegalovirus infection, viral respiratory disease, and influenza.

In addition, the pharmaceutical composition may be made into a preparation in liquid or frozen form. Even in a case of being thawed again after freezing, the pharmaceutical composition does not exhibit impaired cellular function and can maintain high cell viability and cell-killing ability. Therefore, the pharmaceutical composition can be easily stored and supplied in a liquid- or frozen-stored form without additional processing.

Also disclosed herein is a pharmaceutical composition which contains, as an active ingredient, NK cells exogenous mitochondria for use in the prevention or treatment of a disease.

An effective amount of the NK cell of the present disclosure is provided for administration to a subject having a disease or a subject suspected of having a disease. For example, a cell into which exogenous mitochondria has been introduced can be provided for administration to a subject, preferably a mammal, as a therapeutic preparation. The cell may be provided for administration by an intravenous or subcutaneous route. In a case where the composition of the present disclosure is provided for parenteral administration such as by intravenous, subcutaneous, ophthalmic, intraperitoneal, or intramuscular route, the composition is preferably in an aqueous form, or it

is preferable that the composition includes a physiologically applicable body fluid, suspension, or solution. Accordingly, a carrier or vehicle is physiologically acceptable, and thus can be added to the composition and delivered to a patient. Such a carrier or vehicle does not adversely affect electrolyte of the patient. Therefore, physiological saline can be generally used as a carrier for preparations.

The pharmaceutical composition comprising the cell of the present disclosure may also comprise another drug or physiologically active substance having an effect of preventing or treating the disease, in combination with the cell of the present disclosure. A route, a time, and a dose for the combined administration can be determined depending on the type of disease, the patient's disease state, the purpose of treatment or prevention, and the other drug or physiologically active substance used in combination.

In addition, the disease may be cancer or an infectious disease. Here, the cancer may be selected from the group consisting of gastric cancer, liver cancer, lung cancer, colorectal cancer, breast cancer, prostate cancer, ovarian cancer, pancreatic cancer, cervical cancer, thyroid cancer, laryngeal cancer, acute myelogenous leukemia, brain tumor, neuroblastoma, retinoblastoma, head and neck cancer, salivary gland cancer, and lymphoma. The infectious disease may be any one selected from the group consisting of hepatitis B, hepatitis C, human papilloma virus (HPV) infection, cytomegalovirus infection, viral respiratory disease, and influenza.

Also disclosed herein is PBMC comprising an exogenous mitochondria.

As used herein, the term "peripheral blood mononuclear cell" refers to a cell with a spherical nucleus present in the peripheral blood, which is referred to as peripheral blood monocyte or PBMC. Such PBMCs may include immune cells such as B cells, T cells, macrophages, dendritic cells, and NK cells. The PBMC can be obtained through the blood of a subject. Here, the exogenous mitochondria can be obtained from tissues or cells of the subject as described above.

Here, the PBMC comprising exogenous mitochondria may be obtained by introducing mitochondria in an amount of 0.01 to 500 μg, 0.1 to 450 μg, 0.5 to 300 μg, 1 to 100 μg, or 2 to 10 μg, per 10^5 PBMCs. Here, the exogenous mitochondria may be contained in an amount of 1 to 10^3 or 10 to 100, per one PBMC. In addition, a method of introducing

the exogenous mitochondria into the PBMC can be carried out through centrifugation as described above. Also disclosed herein is a pharmaceutical composition for treating cancer or an infectious disease, comprising, as an active ingredient, the PBMC that contains the exogenous mitochondria.

Here, as described above, the cancer may be selected from the group consisting of gastric cancer, liver cancer, lung cancer, colorectal cancer, breast cancer, prostate cancer, ovarian cancer, pancreatic cancer, cervical cancer, thyroid cancer, laryngeal cancer, acute myelogenous leukemia, brain tumor, neuroblastoma, retinoblastoma, head and neck cancer, salivary gland cancer, and lymphoma. In addition, the infectious disease may be any one selected from the group consisting of hepatitis B, hepatitis C, human papilloma virus (HPV) infection, cytomegalovirus infection, viral respiratory disease, and influenza.

Hereinafter, the present disclosure will be described in more detail with reference to the following examples. However, the following examples are provided only to illustrate the present disclosure, and the scope of the present disclosure is not limited only thereto.

I. Production of NK cells into which exogenous mitochondria have been introduced, and identification of functions thereof

Example 1. Production of NK cells into which exogenous mitochondria have been introduced

Human normal hepatocytes (WRL-68) (CRL 1458, ATCC) were seeded in Dulbecco Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Gibco), 100 μg/ml of streptomycin, and 100 U/ml of ampicillin and cultured for 72 hours. After completion of the culture, the cells were washed twice with Dulbecco Phosphate Buffered Saline (DPBS; Gibco). The washed cells were treated with 0.25% trypsin-EDTA (TE; Gibco) to obtain cells. For the obtained cells, in order to extract mitochondria, a hemocytometer was used to measure the number of cells, and cells in an amount of about 3×10^6 cells/ml were collected.

Thereafter, the cell line was subjected to primary centrifugation at a temperature of about 4°C for 10 minutes with a speed of 350 × g. The resulting pellet was collected, and resuspended and homogenized in a buffer solution for 10 to 15 minutes. A composition containing the pellet was subjected to secondary centrifugation at a temperature of about 4°C for 3 minutes with a speed of 1,100 × g, to obtain supernatant. Then, the supernatant was

CLAIMS

1. A natural killer cell, comprising exogenous mitochondria.

2. The natural killer cell of claim 1, wherein the exogenous mitochondria are obtained from muscle cells, hepatocytes, fibroblasts, epithelial cells, neurons, adipocytes, osteocytes, leukocytes, lymphocytes, or mucosal cells.

3. The natural killer cell of claim 1 or claim 2, wherein the exogenous mitochondria are contained in an amount of 1 to 10^3 per natural killer cell.

4. The natural killer cell of any preceding claim, wherein the exogenous mitochondria are delivered into the natural killer cell by centrifuging a composition where the exogenous mitochondria and the natural killer cell are mixed.

5. A pharmaceutical composition for treating cancer or an infectious disease, comprising a natural killer cell as defined in any of claims 1 to 4 as an active ingredient.

6. The pharmaceutical composition of claim 5, wherein the cancer is selected from the group consisting of gastric cancer, liver cancer, lung cancer, colorectal cancer, breast cancer, prostate cancer, ovarian cancer, pancreatic cancer, cervical cancer, thyroid cancer, laryngeal cancer, acute myelogenous leukemia, brain tumor, neuroblastoma, retinoblastoma, head and neck cancer, salivary gland cancer, and lymphoma.

7. The pharmaceutical composition of claim 5, wherein the infectious disease is selected from the group consisting of hepatitis B, hepatitis C, human papilloma virus (HPV) infection, cytomegalovirus infection, viral respiratory disease, and influenza.

8. The pharmaceutical composition of any of claims 5 to 7, wherein the composition is in a liquid or frozen form.

9. A pharmaceutical composition comprising a natural killer cell as defined in any of claims 1 to 4 as an active ingredient for use in the prevention or treatment of a disease.

10. The pharmaceutical composition of claim 9, wherein the use in the treatment or prevention of a disease is via a route selected from the group consisting of intravenous, subcutaneous, ophthalmic, intraperitoneal, and intramuscular routes.

11. The pharmaceutical composition of claim 9 or claim 10, wherein the composition comprises another drug or physiologically active substance having an effect of preventing or treating the disease in combination with the cell.

12. The pharmaceutical composition of any of claims 9 to 11, wherein the disease is cancer or an infectious disease.

13. The pharmaceutical composition of claim 12, wherein the disease is cancer selected from the group consisting of gastric cancer, liver cancer, lung cancer, colorectal cancer, breast cancer, prostate cancer, ovarian cancer, pancreatic cancer, cervical cancer, thyroid cancer, laryngeal cancer, acute myelogenous leukemia, brain tumor, neuroblastoma, retinoblastoma, head and neck cancer, salivary gland cancer, and lymphoma.

14. The pharmaceutical composition of claim 12, wherein the disease is an infectious disease selected from the group consisting of hepatitis B, hepatitis C, human papilloma virus (HPV) infection, cytomegalovirus infection, viral respiratory disease, and influenza.

15. A peripheral blood mononuclear cell, comprising exogenous mitochondria.

16. The peripheral blood mononuclear cell of claim 15, wherein the exogenous mitochondria are obtained from muscle cells, hepatocytes, fibroblasts, epithelial cells, neurons, adipocytes, osteocytes, leukocytes, lymphocytes, or mucosal cells.

17. The peripheral blood mononuclear cell of claim 15 or claim 16, wherein the

exogenous mitochondria are contained in an amount of 1 to 10^3 per peripheral blood mononuclear cell.

18. A pharmaceutical composition for treating cancer or an infectious disease, comprising a peripheral blood mononuclear cell as defined in any of claims 15 to 17.

19. A pharmaceutical composition, comprising a peripheral blood mononuclear cell as defined in any of claims 15 to 17 as an active ingredient for use in the treatment of cancer or an infectious disease.

20. The pharmaceutical composition of claim 18 or claim 19, wherein the cancer is selected from the group consisting of gastric cancer, liver cancer, lung cancer, colorectal cancer, breast cancer, prostate cancer, ovarian cancer, pancreatic cancer, cervical cancer, thyroid cancer, laryngeal cancer, acute myelogenous leukemia, brain tumor, neuroblastoma, retinoblastoma, head and neck cancer, salivary gland cancer, and lymphoma.

21. The pharmaceutical composition of claim 18 or claim 19, wherein the infectious disease is selected from the group consisting of hepatitis B, hepatitis C, human papilloma virus (HPV) infection, cytomegalovirus infection, viral respiratory disease, and influenza.