modified vaccinia viruses with attenuated toxicity such as modified vaccinia Ankara (MVA), LC16m8 (derived from the Lister strain), and New York vaccinia virus (NYVAC, derived from the Copenhagen vaccinia strain) were developed. Vaccines that are applicable to various diseases have been developed based on these vaccinia viruses. In particular, vaccinia virus strains such as Western Reserve (WR), NYVAC, Wyeth, and Lister are also being developed as oncolytic viruses.

Disclosure of Invention

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

Thus, it may be possible to provide a recombinant vaccinia virus comprising a cancer therapeutic gene and optionally having suppressed expression of some genes therein, and a pharmaceutical composition for preventing or treating cancer, comprising same as an active ingredient.

It may be particularly possible to provide a recombinant vaccinia virus, comprising any one selected from the group consisting of a gene encoding soluble programmed cell death protein-1 (sPD-1), a gene encoding hyaluronidase, and a combination thereof.

In addition, it may be possible to provide a pharmaceutical composition for preventing or treating cancer, comprising the recombinant vaccinia virus as an active ingredient.

In addition, it may be possible to provide a method for preventing or treating cancer, comprising a step of administering, to an individual, a composition that contains the recombinant vaccinia virus as an active ingredient.

Advantageous Effects of Invention

The recombinant vaccinia virus comprising a cancer therapeutic gene and optionally having suppressed expression of some genes therein has an excellent anti-tumor effect. Therefore, the recombinant vaccinia virus can be usefully used to treat cancer.

Brief Description of Drawings

Fig. 1 illustrates a schematic diagram of the gene structure of IHD-W vaccinia virus (IHD-W-VV01) in which VGF, TK, and K3L are deleted. The abbreviated terms related to viral gene deletion and therapeutic gene introduction have the meanings as shown in Table 1 below.

[Table 1]

Abbreviation	Meaning
IHD-W-VV01	Recombinant IHD-W vaccinia virus in which VGF, TK, and K3L are deleted
IHD-W-VV02	Recombinant IHD-W vaccinia virus in which VGF, TK, and K3L are deleted and expression of sPD1-Fc gene is induced
IHD-W-VV03	Recombinant IHD-W vaccinia virus in which VGF, TK, and K3L are deleted and expression of PH20 gene is induced
IHD-W-VV04	Recombinant IHD-W vaccinia virus in which VGF, TK, and K3L are deleted and expression of IL-12 gene is induced
IHD-W-VV05	Recombinant IHD-W vaccinia virus in which VGF, TK, and K3L are deleted and expression of sPD1-Fc and PH20 genes is induced
IHD-W-VV06	Recombinant IHD-W vaccinia virus in which VGF, TK, and K3L are deleted and expression of sPD1-Fc, PH20, and IL-12 genes is induced

Fig. 2 illustrates a schematic diagram of the gene structure of IHD-W-VV02 in which VGF, TK, and K3L are deleted and into which sPD1-Fc gene is inserted.

Fig. 3 illustrates results obtained by performing electrophoresis of PCR product of IHD-W-VV02 gDNA, which identifies that the sPD1-Fc gene has been inserted.

Fig. 4 illustrates results obtained by performing Western blotting of proteins secreted from HeLa cells infected with IHD-W-VV02, which shows that the sPD1-Fc gene has been expressed.

Fig. 5 illustrates a schematic diagram of the gene structure of IHD-W-VV03 in which VGF, TK, and K3L are deleted and into which PH20 gene is inserted.

Fig. 6 illustrates results obtained by performing electrophoresis of PCR product

of IHD-W-VV03 gDNA, which shows that the PH20 gene has been inserted.

colorectal tumor model, IHD-W-VV05 and IHD-W-VV06, and then measuring tumor growth.

Fig. 31 illustrates results obtained by intratumorally administering, to a mouse melanoma model, IHD-W-VV05 and IHD-W-VV06, and then measuring tumor growth.

Fig. 32 illustrates results obtained by intratumorally administering, to a mouse lung tumor model, IHD-W-VV05 and IHD-W-VV06, and then measuring tumor growth.

Fig. 33 illustrates results obtained by intratumorally administering, to a mouse melanoma model, IHD-W-VV01 and IHD-W-VV06, and then measuring tumor growth.

Fig. 34 illustrates results obtained by intratumorally administering, to a mouse lung tumor model, IHD-W-VV01 and IHD-W-VV06, and then measuring tumor growth.

Fig. 35 illustrates results obtained by intratumorally administering, to a mouse lung tumor model, WR-VV01 and WR-VV06, and then measuring tumor growth.

Fig. 36 illustrates results obtained by intratumorally administering, to a mouse lung tumor model, Lister-VV01 and Lister-VV06, and then measuring tumor growth.

Fig. 37 illustrates results obtained by determining *in vitro* cell-killing ability of IHD-W-VV06, WR-VV06, and Lister-VV06.

Best Mode for Carrying out the Invention

In an aspect, there is provided a recombinant vaccinia virus comprising any one selected from the group consisting of a gene encoding soluble programmed cell death protein-1 (sPD-1), a gene encoding hyaluronidase, and a combination thereof. The recombinant vaccinia virus may further comprise a gene encoding IL-12.

The term "sPD-1" as used herein refers to an extracellular domain or a fragment

The term "K3L" as used herein refers to K3L protein. The K3L protein encoded by the K3L gene is a protein having a homology to translation initiation factor-2α (eIF-2α), and can suppress an action of protein kinase R (PKR) which is an interferon activator. The K3L may be a sequence of GenBank: AAO89313.1, ABD52483.1, or AGB75754.1, but is not limited thereto. Specifically, the K3L may have the amino acid sequence of SEQ ID NO: 145. The K3L gene may be the nucleotide sequence of SEQ ID NO: 146. The K3L may have a homology of about 70% or 75% or more, and preferably about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, or 89% or more, to the amino acid sequence of SEQ ID NO: 145. In addition, the K3L gene may have a homology of about 90%, 91%, 92%, 93%, or 94% or more, preferably about 95%, 96%, 97%, 98%, or 99% or more, and most preferably about 99% or more, to the nucleotide sequence of SEQ ID NO: 146.

Suppressed expression of a gene means that the gene is not expressed or only a part of the gene is expressed by partial or entire deletion of the gene or insertion of a foreign gene into the gene, so that an activity of a protein encoded by the gene is not exhibited. A method for deleting the gene or inserting a foreign gene may be performed by methods well known in the art. For example, this may be performed by methods for inserting a foreign gene which are disclosed in Molecular Cloning, A Laboratory Manual, Second Edition, by J. Sambrook, E. F. Fritsch and T. Maniatis (2003), Cold Spring Harbor Laboratory Press, Virology Methods Manual, edited by Brian W J Mahy and Hillar O Kangro (1996), Academic Press and Expression of genes by Vaccinia virus vectors, and Current Protocols in Molecular Biology, published by John Wiley and Son (1998), Chapter 16. Specifically, in an embodiment, a foreign gene was inserted using pGEM-T Easy (Promega, Cat No. A1360) or pGEM-T (Promega, Cat No. A3600) plasmid system.

The vaccinia virus may be selected from, but is not limited to, the group consisting of Western Reserve (WR), New York Vaccinia Virus (NYVAC), Wyeth (The New York City Board of Health; NYCBOH), LC16m8, Lister, Copenhagen, Tian Tan, USSR,

Tashkent, Evans, International Health Division-J (IHD-J), International Health Division-White (IHD-W), variants thereof, and combinations thereof. Specifically, the vaccinia virus may be WR, Lister, or IHD-W vaccinia virus, and may have a sequence of GenBank: AY243312.1, DQ121394.1, or AIX98951.1. In an embodiment, the vaccinia virus may be IHD-W.

The term "V" or "virus V" as used herein refers to a recombinant vaccinia virus in which the vaccinia growth factor gene, VGF, is deleted, and the virus does not express the VGF gene due to deletion of the VGF gene.

In addition, the term "T" or "virus T" as used herein refers to a recombinant vaccinia virus in which thymidine kinase (TK) gene is deleted, and the virus does not express the TK gene due to deletion of the TK gene.

In addition, the term "K" or "virus K" as used herein refers to a recombinant vaccinia virus in which K3L gene is deleted, and the virus does not express the K3L gene due to deletion of the K3L gene.

In addition, the term "VT" or "virus VT" as used herein refers to a recombinant vaccinia virus in which the VGF and TK genes are deleted. Methods for inactivating expression of the vaccinia growth factor and the thymidine kinase are as described above.

In addition, the term "IHD-W-VV01" as used herein refers to a recombinant IHD-W vaccinia virus in which the VGF, TK, and K3L genes are deleted. Methods for inactivating expression of the vaccinia growth factor, thymidine kinase, and K3L proteins are as described above.

In addition, the term "IHD-W-VV02" as used in an embodiment refers to a recombinant IHD-W vaccinia virus in which the VGF, TK, and K3L genes are deleted and expression of the sPD1-Fc gene is induced.

In addition, the term "IHD-W-VV03" as used in an embodiment refers to a recombinant IHD-W vaccinia virus in which the VGF, TK, and K3L genes are deleted and expression of the PH20 gene is induced.

In addition, the term "IHD-W-VV04" as used in an embodiment refers to a recombinant IHD-W vaccinia virus in which the VGF, TK, and K3L genes are deleted and expression of the IL-12 gene is induced.

In addition, the term "IHD-W-VV05" as used in an embodiment refers to a recombinant IHD-W vaccinia virus in which the VGF, TK, and K3L genes are deleted and expression of the sPD1-Fc and PH20 genes is induced.

In addition, the term "IHD-W-VV06" as used in an embodiment refers to a recombinant IHD-W vaccinia virus in which the VGF, TK, and K3L genes are deleted and expression of the sPD1-Fc, PH20, and IL-12 genes are induced.

Specifically, as an example of the recombinant vaccinia virus, the following may be mentioned. Variants of WR vaccinia virus may include WR vaccinia viruses in which expression of at least one of the VGF, TK, and K3L genes is suppressed, and expression of at least one of the sPD1-Fc, PH20, and IL-12 genes is induced.

In addition, variants of NYVAC vaccinia virus may include NYVAC vaccinia viruses in which expression of at least one of the VGF, TK, and K3L genes is suppressed, and expression of at least one of the sPD1-Fc, PH20, and IL-12 genes is induced.

In addition, variants of Wyeth vaccinia virus may include Wyeth vaccinia viruses in which expression of at least one of the VGF, TK, and K3L genes is suppressed, and expression of at least one of the sPD1-Fc, PH20, and IL-12 genes is induced.

In addition, variants of Lister vaccinia virus may include Lister vaccinia viruses in which expression of at least one of the VGF, TK, and K3L genes is suppressed, and expression of at least one of the sPD1-Fc, PH20, and IL-12 genes is induced.

In addition, variants of Tian tan vaccinia virus may include Tian tan vaccinia viruses in which expression of at least one of the VGF, TK, and K3L genes is suppressed, and expression of at least one of the sPD1-Fc, PH20, and IL-12 genes is induced.

In addition, variants of USSR vaccinia virus may include USSR vaccinia viruses

in which expression of at least one of the VGF, TK, and K3L genes is suppressed, and expression of at least one of the sPD1-Fc, PH20, and IL-12 genes is induced.

In addition, variants of Tashkent vaccinia virus may include Tashkent vaccinia viruses in which expression of at least one of the VGF, TK, and K3L genes is suppressed, and expression of at least one of the sPD1-Fc, PH20, and IL-12 genes is induced.

In addition, variants of IHD-J vaccinia virus may include IHD-J vaccinia viruses in which expression of at least one of the VGF, TK, and K3L genes is suppressed, and expression of at least one of the sPD1-Fc, PH20, and IL-12 genes is induced.

Furthermore, variants of IHD-W vaccinia virus may include IHD-W vaccinia viruses in which expression of at least one of the VGF, TK, and K3L genes is suppressed, and expression of at least one of the sPD1-Fc, PH20, and IL-12 genes is induced.

In another aspect, there is provided a pharmaceutical composition for preventing or treating cancer, comprising, as an active ingredient, the recombinant vaccinia virus. Here, as described above, the recombinant vaccinia virus may be a vaccinia virus in which expression of the sPD1-Fc, PH20, or IL-12 gene, or combinations thereof is induced, and expression of the VGF, TK, or K3L gene, or combinations thereof is suppressed. The sPD1-Fc, PH20, IL-12, VGF, TK, and K3L genes are as described above.

The term "cancer" as used herein may be solid cancer or blood cancer. Here, the solid tumor may be selected from the group consisting of lung cancer, colorectal cancer, prostate cancer, thyroid cancer, breast cancer, brain cancer, head and neck cancer, fibrosarcoma, esophageal cancer, skin cancer, thymic cancer, gastric cancer, colon cancer, liver cancer, ovarian cancer, uterine cancer, bladder cancer, rectal cancer, gallbladder cancer, biliary tract cancer, pancreatic cancer, and combinations thereof. According to an embodiment, the cancer may be lung cancer, liver cancer, prostate cancer, head and neck cancer, fibrosarcoma, brain cancer, breast cancer, ovarian cancer,

pancreatic cancer, skin cancer, or colorectal cancer. In addition, the blood cancer may be selected from the group consisting of lymphoma, acute leukemia, multiple myeloma, and combinations thereof.

The pharmaceutical composition may further contain one or more pharmaceutically acceptable additives selected from the group consisting of excipients, lubricants, wetting agents, sweeteners, fragrances, and preservatives.

The composition may be formulated according to a conventional method. The composition may be formulated employing a method known in the art so as to provide rapid, sustained, or delayed release of an active ingredient, in particular after being administered to a mammal. Depending on the formulation, the composition may be appropriately administered to an individual. Such administration may be parenteral administration, and examples thereof may include intratumoral, intradermal, intramuscular, intraperitoneal, intravenous, intraarterial, subcutaneous, intranasal, epidural, and oral route. A form of a preparation for parenteral administration may be an injectable preparation.

In yet another aspect, there is provided a method for preventing or treating cancer, comprising a step administering, to an individual, a composition that contains, as an active ingredient, the recombinant vaccinia virus. Here, the cancer is as described above.

The individual may be a mammal, and the mammal may include, but is not limited to, primates (for example, humans), cattle, sheep, goats, horses, pigs, dogs, cats, rabbits, rats, mice, fish, birds, and the like. Specifically, the mammal may be a human. The composition may be appropriately administered by a person skilled in the art depending on patient's age, sex, weight, severity of disease symptom, and route of administration. The administration may be once a day or several times a day, and may be repeatedly administered on an appropriate cycle.

A preferred dosage of a composition that contains the recombinant vaccinia virus varies depending on condition and weight of an individual, severity of disease, drug

form, route and duration of administration, and may be appropriately selected by a person skilled in the art. Specifically, the dosage may be such that a patient receives virus particles, virus units having infectivity ($TCID_{50}$), or plaque forming units (pfu) of 1×10^5 to 1×10^{18}, and preferably 1×10^5, 2×10^5, 5×10^5, 1×10^6, 2×10^6, 5×10^6, 1×10^7, 2×10^7, 5×10^7, 1×10^8, 2×10^8, 5×10^8, 1×10^9, 2×10^9, 5×10^9, 1×10^{10}, 5×10^{10}, 1×10^{11}, 5×10^{11}, 1×10^{12}, 1×10^{13}, 1×10^{14}, 1×10^{15}, 1×10^{16}, 1×10^{17} or more, in which various values and ranges therebetween may be included. In addition, a dosage of virus may be 0.1 ml, 1 ml, 2 ml, 3 ml, 4 ml, 5 ml, 6 ml, 7 ml, 8 ml, 9 ml, 10 ml or more, and all values and ranges therebetween may be included.

Mode for the Invention

Hereinafter, the present invention will be described in more detail with reference to the following examples. However, the following examples are intended to merely illustrate the present invention, and the present invention is not limited thereto.

I. Production of recombinant vaccinia virus

The present inventors constructed recombinant vaccinia viral plasmids in which TK, VGF, and K3L genes are deleted or expression thereof is inactivated. Using these plasmids, recombinant vaccinia viruses in which expression of the above genes is suppressed were produced and comparison was made for properties thereof as anti-cancer substances. In addition, the present inventors constructed recombinant vaccinia viral plasmids obtained by insertion of sPD1-Fc, PH20, IL-12 genes into the above recombinant vaccinia viral plasmids. Using these plasmids, recombinant vaccinia viruses in which expression of the above genes is induced were produced and

Claims

[Claim 1]

A recombinant vaccinia virus, comprising:

any one selected from the group consisting of a gene encoding soluble programmed cell death protein-1 (sPD-1), a gene encoding hyaluronidase, and a combination thereof.

[Claim 2]

The recombinant vaccinia virus of claim 1, wherein the sPD-1 comprises the amino acid sequence of SEQ ID NO: 131 or SEQ ID NO: 133.

[Claim 3]

The recombinant vaccinia virus of claim 1, wherein the gene encoding sPD-1 comprises the nucleotide sequence of SEQ ID NO: 132 or SEQ ID NO: 134.

[Claim 4]

The recombinant vaccinia virus of claim 1, wherein the hyaluronidase comprises the amino acid sequence of SEQ ID NO: 135.

[Claim 5]

The recombinant vaccinia virus of claim 1, wherein the gene encoding hyaluronidase comprises the nucleotide sequence of SEQ ID NO: 136.

[Claim 6]

The recombinant vaccinia virus of claim 1, further comprising:

a gene encoding interleukin (IL)-12.

[Claim 7]

The recombinant vaccinia virus of claim 6, wherein the IL-12 comprises the amino acid sequence of SEQ ID NO: 137.

[Claim 8]

The recombinant vaccinia virus of claim 6, wherein the gene encoding IL-12 comprises any one nucleotide sequence of SEQ ID NO: 138 to SEQ ID NO: 140.

[Claim 9]

The recombinant vaccinia virus of claim 1, wherein the vaccinia virus has suppressed expression of any one selected from the group consisting of a gene encoding K3L, a gene encoding thymidine kinase (TK), a gene encoding vaccinia growth factor (VGF), and combinations thereof.

[Claim 10]

The recombinant vaccinia virus of claim 1, wherein the vaccinia virus is any one selected from the group consisting of Western Reserve (WR), New York Vaccinia Virus (NYVAC), Wyeth (The New York City Board of Health), LC16m8, Lister, Copenhagen, Tian Tan, USSR, Tashkent, Evans, International Health Division-J (IHD-J), International Health Division-White (IHD-W), variants thereof, and combinations thereof.

[Claim 11]

The recombinant vaccinia virus of claim 1, wherein the vaccinia virus is IHD-W.

[Claim 12]

A pharmaceutical composition for use in preventing or treating cancer, the pharmaceutical composition comprising as an active ingredient the recombinant

vaccinia virus of any one of claims 1 to 11.

[Claim 13]

The pharmaceutical composition of claim 12, wherein the cancer is solid cancer or blood cancer.

[Claim 14]

The pharmaceutical composition of claim 13, wherein the solid cancer is any one selected from the group consisting of lung cancer, colorectal cancer, prostate cancer, thyroid cancer, breast cancer, brain cancer, head and neck cancer, esophageal cancer, skin cancer, thymic cancer, gastric cancer, colon cancer, liver cancer, ovarian cancer, uterine cancer, bladder cancer, rectal cancer, gallbladder cancer, biliary tract cancer, pancreatic cancer, and combinations thereof.

[Claim 15]

The pharmaceutical composition of claim 13, wherein the blood cancer is any one selected from the group consisting of lymphoma, acute leukemia, multiple myeloma, and combinations thereof.

[Claim 16]

Use of the recombinant vaccinia virus of any one of claims 1 to 11, as an active ingredient, in the manufacture of a pharmaceutical composition for preventing or treating cancer

.

[Claim 17]

The use of claim 16, wherein the cancer is solid cancer or blood cancer.

[Claim 18]

The use of claim 17, wherein the solid cancer is any one selected from the group consisting of lung cancer, colorectal cancer, prostate cancer, thyroid cancer, breast cancer, brain cancer, head and neck cancer, esophageal cancer, skin cancer, thymic cancer, gastric cancer, colon cancer, liver cancer, ovarian cancer, uterine cancer, bladder cancer, rectal cancer, gallbladder cancer, biliary tract cancer, pancreatic cancer, and combinations thereof.

[Claim 19]

The use of claim 17, wherein the blood cancer is any one selected from the group consisting of lymphoma, acute leukemia, multiple myeloma, and combinations thereof.