【DESCRIPTION】

【Invention Title】

ANTITUMOR COMPOSITION

【Technical Field】

The present invention relates to a composition for antitumor or enhancing antitumor immunity comprising a recombinant adenovirus that co-expresses interleukin-12 (IL-12) and a nucleic acid molecule inhibiting an expression of a tyrosine kinase Met (C-met).

【Background Art】

Tumor immunotherapy is a method of inducing an immune response against a tumor by enhancing the overall immune function of the body and treating tumors through the same. Research on tumor immunotherapy is actively being conducted. However, an immunosuppressive environment is almost always formed at the stage at which cancer is generated, so that, even if the body's immune system is actively working, it is difficult to easily remove the tumor. Tumor cells themselves express many abnormal antigens. These antigens elicit an eradication reaction through immune surveillance, allowing tumor cells to evade immune surveillance even if the body's immune system is active. In addition, it has been found that the phenomenon is mediated by various factors produced by tumor cells. It has been reported that tumor tissues can produce immunosuppressive molecules such as vascular endothelial growth factor (VEGF), tumor growth factor (TGF)-β and interleukin (IL)-

10, and regulatory T cells penetrate the immune-suppressed tumor. Further, stimulation of the expression of an inhibitory receptor called PD-1 continuously increases by activated T cells, and in the case of cancer cells, PD-L1, which is a ligand specifically binding to PD-1, is expressed in a large amount to inactivate activated T cells to avoid immune responses, resulting in an immune suppression microenvironment and immune tolerance in tumors.

A recent study has proved that an inhibitor capable of inhibiting the immune checkpoint of PD-1, which is involved in suppressing T cell activity, induces a strong antitumor immune response. Consequently, overcoming immune surveillance avoidance is a major strategy for immunotherapy. Therefore, as a way to overcome these limitations, research into directly introducing a cytokine gene having an immune-promoting effect into cancer cells to generate and secrete cytokines from cancer cells, and thus, specifically eliminating cancer cells through induction of antitumor immune responses is actively underway. Among the immune-enhancing cytokine genes for which antitumor effects have been reported to date, interleukin-12 (IL-12) is one of the most effective and promising cytokines.

Interleukin-12 (IL-12), which is a heterodimeric protein containing 40 kDa and 35 kDa subunits linked by disulfide bonds, is secreted from antigen presenting cells (APCs) such as activated macrophages, monocytes, dendritic cells and activated B lymphocytes, and acts directly on T cells and NK cells that can effectively eliminate cancer cells to activate the T cells and NK cells and induce the secretion of IFN-γ, as well as enhance the killing ability of the T cells and NK cells against cancer cells. Local expression of IL-12 makes tumor cells sensitive to T-cell mediated cytotoxicity, resulting in inhibition of tumor growth and establishment of systemic immunity.

However, there is a difficulty in the clinical application of IL-12 because the administration of IL-12 can cause systemic cytokine-associated toxicity that limits the patient's acceptable dose. In addition, overall downregulation of the immune effect, increased IL-10 expression in the patient's serum, and IL-12 polarization from Th1 to Th2 immunity due to decreased IFN-γ and TNF-β expression occur. For this reason, IL-12 is sometimes repeatedly administered. These clinical results show the limitations of IL-12 as a single treatment for the treatment of cancer.

Recently, studies on growth factors and receptors thereof as biological indicators reflecting the degree of malignant cancer are underway. Thereamong, studies on C-met, which is a member of the tyrosine kinase receptor family, are being actively conducted. C-met acts as a receptor for hepatocyte growth factor/scatter factor (HGF/SF), and is overexpressed in various cancers. Most patients with C-met overexpression have a poor cancer treatment prognosis. The overexpression of C-met enhances mitogenesis and cellular motility due to the HGF/SF-Met signaling system, inhibits apoptosis, forms blood vessels, and induces invasion and migration into the extracellular matrix (ECM), i.e., causes an increase in cancer malignancy. Since rapid proliferation and migration of cancer cells induced by overexpression of C-met exceed the rate of removal of cancer cells in the immune system, it is difficult to eliminate the cancer cells through an immune response. Furthermore, since the center of the immune response in a tumor microenvironment is inclined severely toward inhibition as the size of the tumor increases, it is more difficult to control tumor cells using a single therapy, and the improved antitumor effect by antitumor immunotherapy cannot be achieved.

Therefore, the development of anticancer therapies without side effects depending on dosage, etc., while having better anticancer effects by increasing antitumor immunity remains a task to be solved in the art.

【Disclosure】

【Technical Problem】

Therefore, the present invention has been made in view of the above problems, and the present inventors have tried to develop a treatment target capable of lowering the dosage while having excellent antitumor effect, and propose a method that can more effectively treat cancer by using a combination of treatment targeting IL-12 and C-met. The present inventors confirmed that it is possible to suppress tumors and overcome immune surveillance avoidance to obtain a synergistic effect in treating cancer, compared to the case of using each therapeutic target alone, by using adenovirus capable of inhibiting the overexpression of C-met while expressing interleukin-12 in the treatment of cancer, thus completing the present invention.

Throughout the present specification, a number of papers and patent documents are referenced and citations are indicated. The disclosed contents of the cited papers and patent documents are incorporated by reference in the present specification as a whole, so that the level of the technical field, to which the present invention belongs, and the contents of the present invention are more clearly described.

【Technical Solution】

In accordance with an aspect of the present invention, the above and other objects can be accomplished by the provision of a gene delivery system, comprising a gene encoding interleukin-12 (IL-12); and a gene expressing an oligonucleotide that is complementary to mRNA of C-met to inhibit expression of C-met.

In accordance with another aspect of the present invention, there is provided a pharmaceutical composition comprising a gene delivery system that comprises a nucleic acid sequence encoding interleukin-12 (IL-12); and a nucleic acid sequence expressing an oligonucleotide binding complementarily to C-met gene and inhibit expression of C-met.

The gene delivery system may be an adenovirus system, and the adenovirus system may be a recombinant adenovirus, or a recombinant adenoviral DNA.

The pharmaceutical composition may be an anticancer composition or a composition for enhancing antitumor immunity.

【Advantageous Effects】

An adenovirus system co-expressing interleukin-12 and a C-met expression-inhibiting nucleic acid molecule according to the present invention can restore immune functions in a tumor environment to remarkably enhance anticancer effects such as tumor remodeling and migration inhibition and tumor growth inhibition. Particularly, the adenovirus system can provide remarkable synergistic effects in the treatment of cancer due to co-expression of both the therapeutic genes.

【Description of Drawings】

FIG. 1 illustrates the structure of an adenovirus plasmid DNA constructed according to an embodiment of the present invention.

FIG. 2 illustrates that an adenovirus plasmid DNA constructed according to an embodiment of the present invention can express mouse interleukin-12 (mIL-12) or human interleukin-12 (hIL-12) in cells.

FIG. 3 illustrates that an adenovirus constructed according to an embodiment of the present invention increases the expression of interleukin-12 (IL-12) and inhibits the expression of C-met.

FIG. 4 illustrates anticancer adenovirus structures that co-express IL-12 and shc-met constructed according to an embodiment of the present invention.

FIGS. 5a and 5b illustrate in vivo antitumor effects of an anticancer adenovirus co-expressing IL-12 and shc-met according to an embodiment of the present invention in mouse-derived B16-F10 and CT26 cells, respectively.

FIG. 6 illustrates in vivo antitumor effects of an anticancer adenovirus pDNA/PPA complex co-expressing IL-12 and shc-met according to an embodiment of the present invention in mouse-derived B16-F10 cells.

FIGS. 7a and 7b illustrate in vivo IL-12 expression (FIG. 7a) and in vivo C-met expression inhibition ability (FIG. 7b) of an anticancer adenovirus co-expressing IL-12 and shc-met according to an embodiment of the present invention in human lung cancer cell line H1975.

FIGS. 8a and 8b illustrate cancer cell killing ability of anticancer adenoviruses expressing IL-12 and/or shc-met according to embodiments of the present invention in human lung cancer cell line H1975 under a normal oxygen condition (8a) and low oxygen condition (8b).

FIGS. 9a, 9b, 9c and 9d illustrate HUVEC cell migration (9a and 9b) and invasion (9c and 9d) inhibition effects of anticancer adenoviruses expressing IL-12 and/or shc-met according to embodiments of the present invention.

FIG. 10 illustrates tumor cell migration inhibition through Endothelial to Mesenchymal Transition (Endo-MT) inhibition in HUVEC cells by anticancer adenoviruses expressing IL-12 and/or shc-met according to embodiments of the present invention.

FIG. 11 illustrates antitumor effects in a human xenograft tumor model (H1975) by anticancer adenoviruses expressing IL-12 and/or shc-met according to embodiments of the present invention.

FIG. 12 schematically illustrates the structure of a gene delivery system that co-expresses IL-12; and a CRISPR/Cas system comprising a C-met-targeting guide RNA according to an embodiment of the present invention.

FIGS. 13a, 13b and 13c illustrate intercellular IL-12 expression effect (13a) and C-met inhibition effect (13b and 13c) by an anticancer virus co-expressing IL-12 and Lbcpf1-crMET according to an embodiment of the present invention.

FIG. 14 illustrates the antitumor effect in a human xenograft tumor model (H1975) of an anticancer virus co-expressing IL-12 and Lbcpf1-crMET according to an embodiment of the present invention.

【Modes of the Invention】

In accordance with an aspect of the present invention, the present invention provides a gene delivery system comprising a gene encoding interleukin-12 (IL-12); and a gene expressing an oligonucleotide that complementarily binds to the C-met gene to inhibit the expression of C-met.

In accordance with another aspect of the present invention, the present invention provides an anticancer composition that comprises a gene delivery system comprising a gene encoding interleukin-12 (IL-12); and a gene expressing an oligonucleotide that complementarily binds to the C-met gene to inhibit the expression of C-met; an anti-metastatic composition for cancer cells which comprises the gene delivery system; or a pharmaceutical composition for enhancing antitumor immunity which comprises the gene delivery system.

In accordance with still another aspect of the present invention, the present invention provides the use of a gene delivery system, which comprises a gene encoding interleukin-12 (IL-12); and a gene expressing an oligonucleotide that complementarily binds to the C-met gene to inhibit the expression of C-met, as an anticancer agent.

In accordance with yet another aspect of the present invention, the present invention provides a method of treating cancer, the method comprising administering a gene delivery system, which comprises a gene encoding interleukin-12 (IL-12); and a gene expressing an oligonucleotide that complementarily binds to the C-met gene to inhibit the expression of C-met, or a pharmaceutical composition comprising the gene delivery system to a subject.

In the present invention, the term “gene delivery system” refers to a vector system for delivering a gene into cells. In the present invention, unless otherwise specified, a gene delivery system, an adenovirus vector, an adenovirus system, or an adenovirus vector system has the same meaning. The adenovirus system of the present invention comprises both adenovirus and DNA of adenovirus (viral DNA). Preferably, the adenovirus system may be a recombinant adenovirus or a recombinant adenoviral DNA for anticancer or antitumor immunity enhancement.

The adenovirus system may include the genomic sequence of an adenovirus, so that an adenovirus is generated from an adenovirus or recombinant DNA delivered into a cell, thereby having an anticancer or antitumor immune effect. In the present invention, since the genes respectively expressing IL-12 and shc-met may be inserted into the adenovirus genome and may be delivered to cells in the form of adenovirus itself or recombinant DNA thereof, an adenovirus comprises both a recombinant adenovirus and a recombinant adenoviral DNA throughout the specification of the present invention unless otherwise stated.

In accordance with a preferred embodiment of the present invention, the adenovirus system of the present invention may be an oncolytic adenovirus (oAd) capable of specifically proliferating only in a tumor and selectively killing tumor cells. The “oncolytic adenovirus” may be used interchangeably with terms such as anticancer virus, oAd, or tumor killing virus in the present specification. All of the oncolytic adenoviruses genetically modified through conventional techniques used in the art to which the present invention pertains are included in the adenovirus system of the present invention. For example, E1A and/or E1B may be removed from the adenovirus genome to proliferate only in cancer cells, thereby not

proliferating in normal cells, or the target sequence of the viral protein may be changed to use a protease secreted from cancer cells, or a sequence expressing a ligand is inserted into a viral genome so as to express a receptor ligand to be bound to a receptor (e.g., EGFR) present in cancer cells.

In addition, to improve the killing ability of cancer cells, the TRAIL gene may be inserted into the tumor-selective oncolytic adenovirus of the present invention so that cell death occurs well, or the IFN gene may be applied to the tumor-selective oncolytic adenovirus to be inserted into cancer cells and effectively expressed therein so that an immune response occurs.

A recombinant adenovirus used in the present invention comprises a promoter operable in animal cells, preferably mammalian cells. Promoters suitable for the present invention include promoters derived from mammalian viruses and promoters derived from the genome of mammalian cells. A transgene inserted into the recombinant adenovirus is preferably inserted into an expression cassette of a promoter-trans gene poly A sequence. In this case, as the promoter, the gene expression control sequences of the present invention (HRE-TERT, HRE-E2F, HRE-TERT-E2F, HRE-E2F-TERT, HRE-E2F-5myc-TERT, and HRE may be included twice) or general promoters may be used.

General promoters bound to a transgene operate preferably in animal cells, more preferably mammalian cells, to control transcription of the transgenes. General promoters include a promoter derived from a mammalian virus and a promoter derived from the genome of a mammalian cell. Examples of general promoters include U6 promoter, H1 promoter, cytomegalovirus (CMV) promoter, the adenovirus late promoter, the vaccinia virus 7.5K promoter, SV40 promoter, HSV tk promoter, RSV promoter, EF1 alpha promoter,

metallothionein promoter, beta-actin promoter, human IL-2 gene promoter, human IFN gene promoter, human IL-4 gene promoter, human lymphotoxin gene promoter, human GM-CSF gene promoter, inducible promoter, tumor cell specific promoter (e.g., TERT promoter, PSA promoter, PSMA promoter, CEA promoter, E2F promoter and AFP promoter) and tissue specific promoter (e.g., albumin promoter), but the present disclosure is not limited thereto. It is preferred that the expression constructs for expressing a transgene comprises polyadenylated sequences bound to a downstream of the transgene. The polyadenylated sequences include bovine growth hormone terminator (Gimmi, E. R., et al., Nucleic Acids Res. 17: 6983-6998(1989)), polyadenylated sequence derived from SV40 (Schek, N, et al., Mol. Cell Biol. 12:5386-5393(1992)), HIV-1 poly A (Klasens, B. I. F., et al., Nucleic Acids Res. 26:1870-1876(1998)), β-globin polyA(Gil, A., et al, Cell 49: 399-406(1987)), HSV TK polyA (Cole, C. N. and T. P. Stacy, Mol. Cell. Biol. 5:2104-2113(1985)) or polyomavirus polyA (Batt, D. B and G. G. Carmichael, Mol. Cell. Biol. 15:4783-4790(1995)), but the present disclosure is not limited thereto.

In the recombinant adenovirus used in the present invention, the IL-12 gene sequence and the sequence expressing RNA that inhibits C-met are operably linked to a promoter. In the present specification, the term “operably linked” refers to a functional linkage between a nucleic acid expression control sequence (e.g., a promoter, a signal sequence, or an array of transcriptional regulatory factor binding sites) and another nucleic acid sequence. Accordingly, the regulatory sequence controls the transcription and/or translation of the different nucleic acid sequences.

The recombinant adenovirus of the present invention may additionally include an

antibiotic resistance gene and a reporter gene (e.g., green fluorescence protein (GFP), luciferase, and β-glucuronidase) as selectable markers. The antibiotic resistance gene comprises antibiotic resistance genes generally used in the art, and for example, may be a resistance gene against ampicillin, gentamicin, carbenicillin, chloramphenicol, streptomycin, kanamycin, geneticin, neomycin, or tetracycline, preferably a neomycin resistance gene. The selectable marker may be expressed by a separate promoter, an internal ribosome entry site (IRES), or an expression system linked by a 2A system, and IRES used in the present invention is a regulatory sequence found in RNAs of several kinds of viruses and cells (McBratney et. al. Current Opinion in Cell Biology 5:961 (1993)). In addition, "2A peptide" means a sequence encoding a cuttable small peptide (18-22 amino acids) that allows for efficient, concordant expression of discrete protein products within a single coding sequence. For example, the 2A peptide from a virus such as foot-and-mouth disease virus (F2A), equine Rhinitis A virus, porcine teschovirus-1 (P2A) or Thosea asigna virus (T2A), or any of the 2A peptides described in Szymczak-Workman, A. et al. "Design and Construction of 2A Peptide-Linked Multicistronic Vectors" may be used.

The present invention comprises both a gene encoding interleukin-12 (IL-12); and a gene expressing an oligonucleotide that is complementary to mRNA of C-met (tyrosine kinase Met) and inhibits the expression of C-met and provides a synergistic effect in enhancing anticancer and antitumor immunity by co-expressing both the genes. In addition, the present technology is significant in that it is possible to provide an excellent therapeutic effect despite the administration of IL-12 in a small amount, and to solve the problem of side effects caused by conventional cytokine administration.

In the present specification, the term “interleukin-12 (IL-12)” refers to a heterodimeric cytokine formed by a 40 kDa subunit (p40 subunit) and a 35 kDa subunit (p35 subunit) bound by a disulfide bond. IL-12 is produced by antigen-presenting cells such as macrophages and binds to receptors on cell surfaces of activated T cells, B cells and NK cells. IL-12 promotes proliferation of T cells and NK cells; enhances the cytotoxic effect of T cells, NK cells and macrophages; induces the production of IFN-γ, TNF-α and GM-CSF; and induces the activation of Th1 cells. In addition, IL-12 is known as an important co-stimulator of Th1 clone proliferation, and is known to increase the production of IgG2a antibodies in serum. In the present invention, the term “p35 subunit” and “p40 subunit” include not only the subunits exemplified in the examples, but also all analogs of subunits capable of performing unique functions of each subunit.

The adenovirus system of the present invention contains a gene encoding IL-12 in an expressible form, and secretes IL-12 after infection with tumor cells to induce a strong antitumor immune response.

The adenovirus system of the present invention may include IL-12A (p35) gene and IL-12B (p40) gene expressing IL-12A (p35) and IL-12B (p40), as subunits, so as to efficiently express IL-12. In addition, a linker sequence may be additionally included between the IL-12A (p35) gene sequence and the IL-12B (p40) gene sequence to serve to link the two subunits, or an internal ribosome entry site (IRES) sequence may be further included used to increase the efficiency of protein expression.

The linker means any sequence that can be included between two genes, and in the present invention, the linker means a sequence that can be included between the IL-12A (p35)

gene and the IL-12B (p40) gene. The linker comprises all conventional linker sequences or randomly designed sequences known to be inserted between genes, and any linkers may be included in the present invention as long as they do not adversely affect the expression of IL-12 or impair the functionality of IL-12. For example, the liker may be the sequence of SEQ ID NO: 19, but the present invention is not limited thereto.

The amino acid sequence of "IL-12A subunit (p35 subunit)" that can be used in the present invention may be those described in GenBank accession number AAD56385 (when it is desired to express the amino acid sequence of mouse p35, GenBank Accession No. AAA39292). In addition, IL-12A (p35) gene, as a sequence encoding the IL-12A subunit, may be a nucleotide sequence corresponding to a coding sequence (CDS) among sequences described in GenBank Accession No. AF180562 (when it is desired to use a mouse sequence, refer to the CDS sequence among the sequences described in M86672). In addition, IL-12A (p35) gene may have the sequence of SEQ ID NO: 1 or SEQ ID NO: 4 used in an embodiment of the present invention (in the case of a mouse, the sequence of SEQ ID NO: 7).

The amino acid sequence of "IL-12B subunit (p40 subunit)" that can be used in the present invention may be those described in GenBank accession number AAD56386 (when it is desired to express the amino acid sequence of mouse p40, GenBank Accession No. AAA39296). In addition, IL-12A (p35) gene, as a sequence encoding the IL-12A subunit, may be a nucleotide sequence corresponding to a coding sequence (CDS) among sequences described in GenBank Accession No. AF18056 (when it is desired to use a mouse sequence, refer to the CDS sequence among the sequences described in M86671). In addition, it may be the sequence of SEQ ID NO: 2 or SEQ ID NO: 5 used in an embodiment of the present

invention (in the case of a mouse, the sequence of SEQ ID NO: 8).

In an embodiment of the present invention, it was confirmed that IL-12 can be expressed in tumor cells by using the IL-12 gene of SEQ ID NO: 3 or SEQ ID NO: 6 (SEQ ID NO: 9 in the case of a mouse), particularly when expressed simultaneously with RNA for C-met, the expression level of 12 was significantly increased.

In the present specification, the term “"C-met” is one of the tyrosine kinase receptor family. C-met acts as a receptor for hepatocyte growth factor/scatter factor (HGF/SF) and is overexpressed in various cancers. Most patients with c-met overexpression have poor cancer treatment prognosis. The C-met gene of the present invention may be a nucleotide sequence corresponding to a coding sequence (CDS) among the mRNA sequences disclosed in GenBank Accession No. gi: 4557746 (when it is desired to use a mouse sequence, refer to a CDS sequence among the sequences described in Accession No. gi: 146198695).

The overexpression of C-met increases mitogenesis and cell motility due to the HGF/SF-Met signaling system, and induces invasion and migration into the extracellular matrix, thereby increasing the malignancy of cancer. Therefore, in the treatment of cancer, the inhibition of C-met expression is a very important issue. The present invention inhibits the expression of C-met using an oligonucleotide that is complementary to the mRNA of C-met and inhibits the expression of C-met, particularly confirmed that the inhibition of C-met expression is remarkably enhanced , compared a group in which the expression of C-met is only inhibited, when the oligonucleotide is co-expressed with IL-12.

In an embodiment, mRNA of C-met, which is an inhibitory target, in the present invention may be the sequence shown in SEQ ID NO: 10 (in the case of a mouse, SEQ ID NO:

11).

An oligonucleotide that binds to the C-met gene to inhibit the expression of C-met may be one or more selected from the group consisting of shRNA, miRNA, siRNA, antisense oligonucleotides, ribozymes, DNAzyme, triplex forming oligonucleotides (TFOs), peptide nucleic acids (PNA), and guide RNA for CRISPR.

The oligonucleotide sequence inhibiting the expression of C-met may include a sequence identical or complementary to a part of the C-met mRNA sequence of SEQ ID NO: 10, preferably comprises a sequence identical or complementary to the sequence of 4 or more consecutive nucleotides in the sequence of SEQ ID NO: 10.

In an embodiment of the present invention, it was confirmed that the expression of C-met in tumor cells can be efficiently inhibited by using shRNA, which comprises a sequence complementary to the mRNA of C-met and is capable of inhibiting the expression of C-met, as the oligonucleotide.

In the present invention, “shRNA” (small hairpin RNA or short hairpin RNA), which is an artificial RNA molecule having a hairpin structure, is used to suppress the expression of a target gene through RNA interference. shRNA is primarily transported into cells via plasmid, bacterial or viral vectors. The shRNA has the advantage of relatively low rate of degradation and turnover.

In an embodiment of the present invention, it was confirmed that the expression of C-met is efficiently inhibited by the viral system of the present invention that uses shRNA, "Hshc-met (targeting the sequence of SEQ ID NO: 13)" or "shc-met (targeting the sequence of SEQ ID NO: 15)," encoded by the gene of SEQ ID NO: 12 (human) or SEQ ID NO: 14 (mouse) as

shRNA for inhibiting the expression of C-met.

An oligonucleotide that binds to the C-met gene to inhibit the expression of C-met may be guide RNA for CRISPR. When the gene delivery system of the present invention expresses a CRISPR-CAS system, a sequence expressing Cas protein may be further included.

In the present invention, the "CRISPR-CAS system" refers to a technology of recognizing a specific nucleotide sequence present on a target DNA and cuts the DNA with a restriction enzyme to correct a gene. In the present invention, the CRISPR-CAS system is characterized in that when a sequence expressing a guide RNA for the target gene C-met is inserted into an adenovirus system, and the expressed guide RNA hybridizes to the target C-met, the target site is cleaved using Cas protein to inhibit the expression of C-met.

Unless otherwise stated, a method of constructing the CRISPR-CAS system, the type of restriction enzyme protein to be used in the CRISPR-CAS system, etc. may be used using techniques commonly used in the technical field of the present invention.

The Cas protein used in the present invention may be a Cas9 or Cas12 protein. Cas9 or Cas12 (also referred to as Cas12a or Cpf1) protein used in the present invention may be used without limitation so long as it is a Cas protein commonly used to implement the CRISPR/Cas system in the technical field of the present invention. In addition, examples of specific types of the Cas9 or Cas12 protein are as shown in Table 1 below, but are not limited thereto. The references listed in Table 1 are included in the specification of the present invention as a whole.

【Table 1】

Type	PAM	Size	Cleaved shape	Origin
SpCas9	5'-NGG	4.2kb	Blunt	*Streptococcus pyogenes*

SaCas9	5'-NNGRRT	3.15Kb	Blunt	*Staphylococcus aureus*
CjCas9	5'-NNNNRYAC	2.95Kb	Blunt	-
Cas12a (type-V Cpf1)	5'-TTTV	3.9kb	Staggered	*Lachnospiraceae bacterium* (LbCpf1);*Acidaminococcus* sp. (AsCpf1);*Francisella novicida* (FnCpf1);*Moraxella bovoculi 237* (MbCpf1)
R = purine, Y = pyrimidine. V = Not U/T (A or C or G) see: *Christine* et al., *Viral Delivery Systems for CRISPR*, Viruses 2019, 11, 28				

Cpf1 is a type of CRISPR/Cas system and is classified as Class 2 Type 5 CRISPR/Cas system. Cpf1 works as a single subunit effector module like Cas9, but is slightly different from Cas9 in terms of functionality, so that, while Cas9 requires tracrRNA to cut a gene, Cpf1 does not require tracrRNA. In the case of Cas9, a gene in a region with a protospacer-adjacent motif (PAM) containing a lot of guanine is cut off, whereas Cpf1 can efficiently cut a region having a lot of thymine. In addition, Cpf1 has a characteristic of generating a 4 to 5 nucleotide overhang when cutting a gene. Due to these various features and structural differences, Cpf1 exhibits higher accuracy even though the operating efficiency thereof is slightly lower than in Cas9.

A promoter for expression of the CRISPR-CAS system of the present invention may be any promoter, without limitation, as long as it is used in the CRISPR/Cas system in the technical field to which the present invention pertains. For example, an RNA polymerase III promoter which pertains to a type III class, or a U6 promoter may be used. In this case, since the commonly used U6 promoter requires guanosine nucleotides to initiate transcription, the use of the U6 promoter may further limit a genomic targeting site to $GN_{19}NGG$ (Mali et al. (2013) Science 339:823-826; Ding et al. (2013) Cell Stem Cell 12:393-394). T7, T3 or SP6

promoter may also be used in the vector system of the present invention (Adhya et al. (1981) Proc. Natl. Acad. Sci. U.S.A. 78:147-151; Melton et al. (1984) Nucleic Acids Res. 12:7035-7056; Pleiss et al. (1998) RNA 4:1313-1317).

In an embodiment of the present invention, it was confirmed that the expression of C-met can be efficiently inhibited through the CRISPR-CAS system of crRNA-LbCpf1 by using the 55th to 77th nucleotides in the human C-met mRNA of SEQ ID NO: 10 as the target sequence (SEQ ID NO: 17) and by using an adenovirus system comprising the gene of SEQ ID NO: 16 expressing the guide RNA for CRISPR which can be complementarily hybridized to the target sequence; and the gene of SEQ ID NO: 18 expressing Lbcpf1.

In an embodiment of the present invention, the CRISPR-CAS system was expressed using a recombinant adenovirus vector containing a gene sequence expressing LbCpf1., as shown in FIG. 13.

In the present invention, the term "complementary" is meant to encompass not only 100% complementary, but also incomplete complementarity sufficient to suppress the expression of the C-met gene through an RNA interference mechanism, and preferably 90 % complementarity, more preferably 98% complementarity, most preferably 100% complementarity. In the present specification, when expressing 100% complementarity, it is described as "completely complementary".

In accordance with an embodiment of the present invention, the shRNA sequence included in the adenovirus of the present invention comprises a sequence that is complementary to some of the sequences of SEQ ID NO: 10, preferably a sequence that is complementary to the sequence of the 1987th nucleotide to the 2007th nucleotide of the C-met mRNA sequence

of SEQ ID NO: 10.

In accordance with a specific embodiment of the present invention, shRNA, as an oligonucleotide capable of inhibiting the expression of C-met, may be encoded by the gene of SEQ ID NO: 12 or SEQ ID NO: 16 (in the case of a mouse, the gene of SEQ ID NO: 14).

"Gene or gene sequence" used in the present invention is interpreted as comprising a gene sequence showing substantial identity or substantial similarity to each sequence of the sequence numbers presented in the present invention. Substantial identity, when the sequence of the present invention and any other sequence are aligned to correspond to each other as much as possible, and when the aligned sequence is analyzed using an algorithm commonly used in the art, refers to a sequence that exhibits homology of more than 70%, preferably 80% homology, more preferably 90% homology, and most preferably 95% homology. Substantial similarity generally refers to a case in which changes in the sequence of IL-12 genes, such as deletion or insertion of one or more bases, do not affect the object of the present invention to minimize homologous recombination with the recombinant vector system. Accordingly, the IL-12 gene sequence of the present invention is not limited to the exemplified sequence lists, and it is construed as being included in the scope of the present invention so long as it does not substantially affect the activity of a desired final product of the present invention.

The adenovirus system of the present invention can achieve the treatment of cancer by using the genomic backbone of the adenovirus. Adenovirus is widely used as a gene delivery system due to a medium genome size thereof, ease of manipulation, high titer, a wide range of target cells, and excellent infectivity. Both ends of the genome contain 100 to 200 bp of an inverted terminal receptor (ITP), which is an essential component for DNA replication and

packaging. The E1 region of the genome (E1A and E1B) encodes proteins that regulate transcription and transcription of host cell genes. The E2 region (E2A and E2B) encodes a protein involved in virus DNA replication. Since only a small portion of the adenovirus genome is known to be required in cis (Tooza, J. Molecular biology of DNA Tumor viruses, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1981)), adenoviruses have the ability to carry foreign DNA molecules.

In the genome of the adenovirus system of the present invention, the E1 and E3 regions may be deleted. Particularly, the adenovirus system of the present invention may include an E1A gene and an inactivated E1B 19 gene, an inactivated E1B 55 gene, or an inactivated E1B 19 gene and E1B 55 gene.

The term "inactivation" used in relation to a gene in the present specification means that the transcription and/or translation of the gene is not performed normally, and the function of the normal protein encoded by the gene does not appear. For example, in the case of the inactivated E1B 19 gene, an active E1B 19 kDa protein cannot be produced due to mutation (substitution, addition, partial deletion or total deletion) of E1B 19 gene. When E1B 19 is deleted, cell apoptosis can be increased, and when E1B 55 gene is deleted, the specificity of tumor cells is exhibited (see Patent Application No. 2002-23760). In the present specification, the term "deletion" used in relation to a gene or a sequence has a meaning comprising not only complete deletion of a corresponding sequence, but also partial deletion thereof.

The recombinant adenovirus of the present invention may have a mutation wherein the 45th Glu residue of a nucleotide sequence encoding an Rb binding site located in the ElA gene sequence is substituted with Gly, and a mutation wherein the 121-127th amino acid sequence

thereof is entirely substituted with Gly.

In accordance with an embodiment of the present invention, the recombinant adenovirus may include the E1A site and E1B 19 kDa and E3 site (ΔE3) therein may be deleted. A recombinant adenovirus comprising E1A gene has a replicable property. The IL-12 gene and the C-met expression inhibition oligonucleotide may be inserted into the deleted E1 and E3 sites of adenovirus, respectively.

The adenovirus system of the present invention may further include a biocompatible polymer to increase the intracellular delivery ability of the adenovirus or viral DNA thereof, or to increase the remaining time, etc. by being delivered into cells. Particularly, the biocompatible polymer may be included, in a form of being combined with adenovirus or adenovirus plasmid DNA, in the system of the present invention.

The biocompatible polymer may be any one so long as it can be used in a vector system for gene delivery in the technical field of the present invention.

For example, the biocompatible polymer of the present invention may be a pH-sensitive and bioreducing polymer comprising (a) an escapable portion from immune reaction, (b) a chargeable portion, and (c) a bioreducible portion comprising a disulfide bond.

The escapable portion (a) from immune reaction functions to allow a virus, to which a polymer binds, to avoid immune responses in vivo (comprising cellular and systemic immunity reactions). A material that may be used in the escapable portion from immune reaction is particularly a polymer capable of escaping from an immune response in vivo (comprising cellular and systemic immunity reactions), more particularly PEG (polyethylene glycol), polyalkylene oxide (e.g., polyoxyethylene, polyoxypropylene or a copolymer thereof (a

polyethylene oxide-polypropylene oxide-polyethylene oxide copolymer)), polyphenylene oxide, a copolymer of PEG and polyalkylene oxide, poly(methoxyethyl methacrylate), poly(methacryloyl phosphatidylcholine), perfluorinated polyether, dextran or polyvinylpyrrolidone, more particularly, PEG, polyalkylene oxide or a copolymer of PEG and polyalkylene oxide, even more particularly PEG.

The chargeable portion (b) may impart a positive charge to the polymer at in vivo pH, specifically near a neutral pH to be bound by interaction with a surface or DNA of a negatively charged virus. Alternatively, the chargeable portion (b) may impart negative charge to the polymer, as opposed to the above, depending on the chargeability of a viral surface or DNA. Accordingly, the chargeable portion (b) comprises a material that imparts a positive or negative charge to the polymer. For example, when a negative charge is imparted, a carboxylate group may be used in the chargeable portion (b), whereas, when a positive charge is imparted, a monomer having a tertiary amine group or an amino group may be used in the chargeable portion (b).

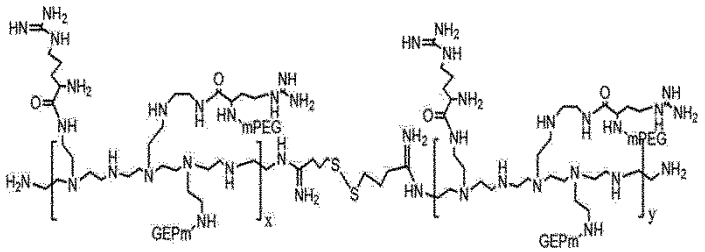
The bioreducible portion (c) comprising a disulfide bond may be included in the present invention without being limited to the type of monomer so long as it comprises a disulfide bond. The bioreducible portion (c) is reduced in an acidic environment in vivo so that a disulfide bond is converted to a sulfhydryl group, and thus the polymer structure is broken, and eventually a naked virus or DNA bound to a polymer-virus complex or a polymer-DNA complex is released.

In another embodiment, the biocompatible polymer of the present invention may include all of a polymer, a nanomaterial, a dendrimer, and a hydrogel. The biocompatible

polymer is preferably selected from the group consisting of PEI-Arg-mPEG-S-S-mPEG-Arg-PEI (PAPS), mPEG-PEI-g-Arg-S-S-Arg-g-PEI-mPEG (PPSA), PICION (pegylated and iron oxide nanoparticles-crosslinked catechol-grafted poly L lysine (PLL)), an arginine-grafted biodegradable polymer (ABP), a pegylated and PTX-conjugated polymeric micelle (APP), mPEG-b-Pip-CBA (PPCBA), PPCBA-PEI-Arginine (PPA), polyethyleneglycol (PEG), poly-lactide (PLA), polyglycolide (PGA), poly-lactide-co-glycolide (PLGA), poly-ε-caprolactone (PCL), polyethyleneamine (PEI), hyaluronic acid (HA), gelatin, chitosan, and serum albumin.

The PAPS polymer is PEI-Arg-mPEG-S-S-mPEG-Arg-PEI and may have the structure of the following Structural Formula 1:

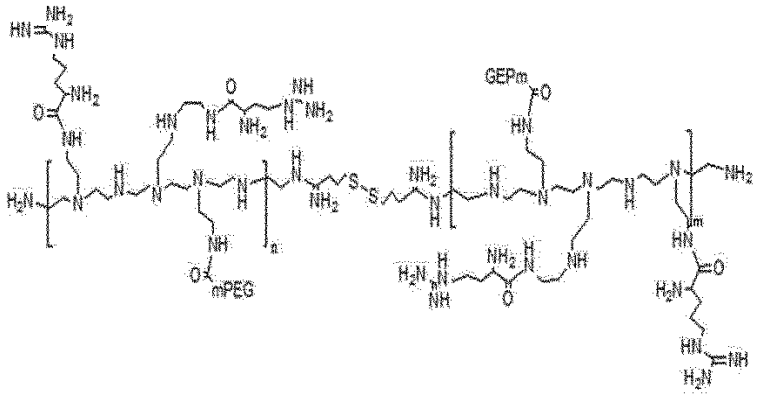
[Structural Formula 1]



In Structural Formula 1, x and y may each independently be an integer of 1 to 500.

The PPSA polymer is mPEG-PEI-g-Arg-S-S-Arg-g-PEI-mPEG and may have the structure of the following Structural Formula 2:

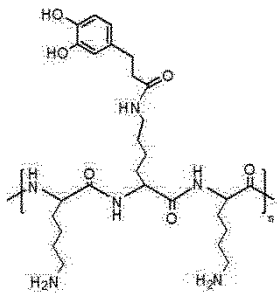
[Structural Formula 2]



In Structural Formula 2, n and m may each independently be an integer of 1 to 500.

The PICION is pegylated and iron oxide nanoparticles-crosslinked catechol-grafted poly L lysine (PLL), and may have a structure wherein the catechol-grafted poly L lysine of the following Structural Formula 3 is crosslinked with iron oxide particles, and mPEG is modified on a surface thereof:

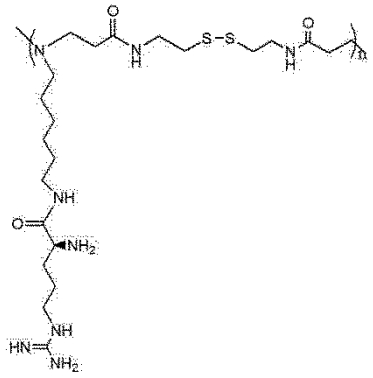
[Structural Formula 3]



In Structural Formula 3, n may be an integer of 1 to 500.

The ABP polymer is an arginine-grafted biodegradable polymer (arginine grafted bio-reducible polymer), and may have the structure of the following Structural Formula 4:

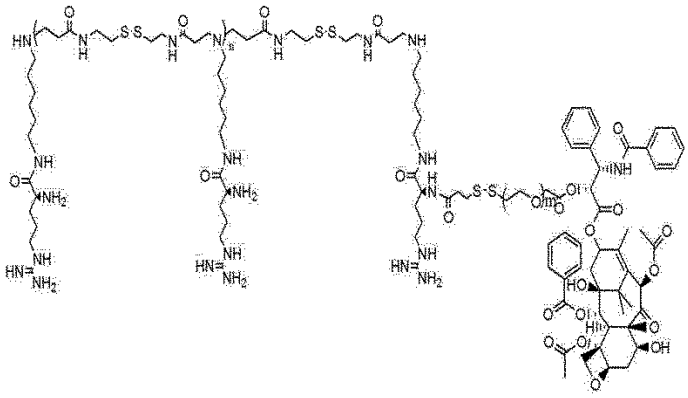
[Structural Formula 4]



In Structural Formula 4, n may be an integer of 1 to 500.

The APP is a pegylated and PTX-conjugated polymeric micelles (PEGylated and PTX-conjugated polymeric micelle, and may have the structure of the following Structural Formula 5:

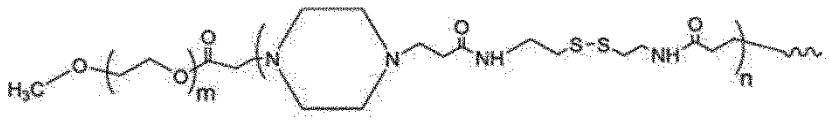
[Structural Formula 5]



In Structural Formula 5, n and m may each independently be an integer of 1 to 500.

The PPCBA polymer is mPEG-b-Pip-CBA, belongs to a pH-sensitive and biodegradable polymer, and may have the structure of the following Structural Formula 6:

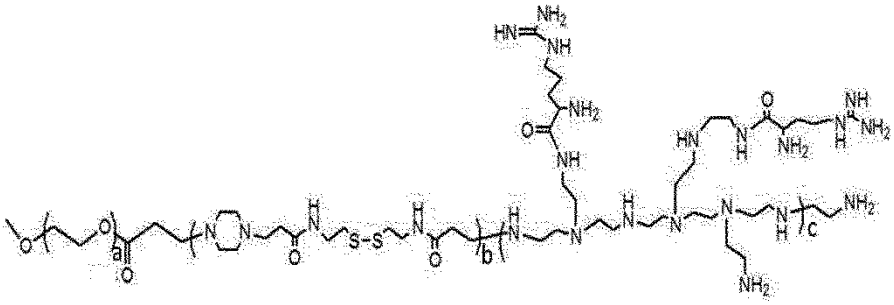
[Structural Formula 6]



In Structural Formula 6, n and m may each independently be an integer of 1 to 500.

The PPA is PPCBA-PEI-Arginine, belongs to a pH-sensitive and biodegradable polymer, and may be a polymer in which PEI and arginine are further bound to PPCBA. The PPA may have the structure of the following Structural Formula 7:

[Structural Formula 7]



In Structural Formula 7, a, b and c may each independently be an integer of 1 to 500. For example, a may be 100 to 200, b may be 1 to 10, and c may be 1 to 5, or a may be 113, b may be 6, and c may be 1, but the present invention is not limited thereto.

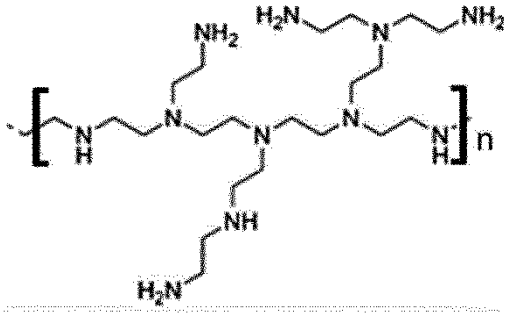
Polyethyleneamine (PEI) may include all linear or branched polyethyleneamines to which the monomer of Structural Formula 8 is bound.

[Structural Formula 8]

In Structural Formula 8, n may be an integer of 1 to 500.

The branched polyethyleneamine may also include a dendrimer wherein chains of molecules are regularly spread, in a three-dimensional manner, outward from the center according to a certain rule, in addition to general branched polyethyleneamines.

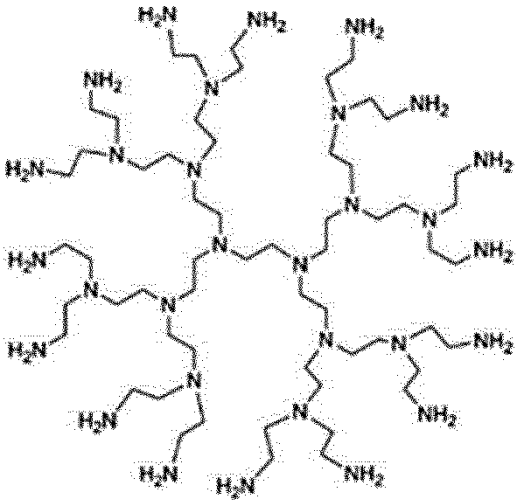
[Structural Formula 9]



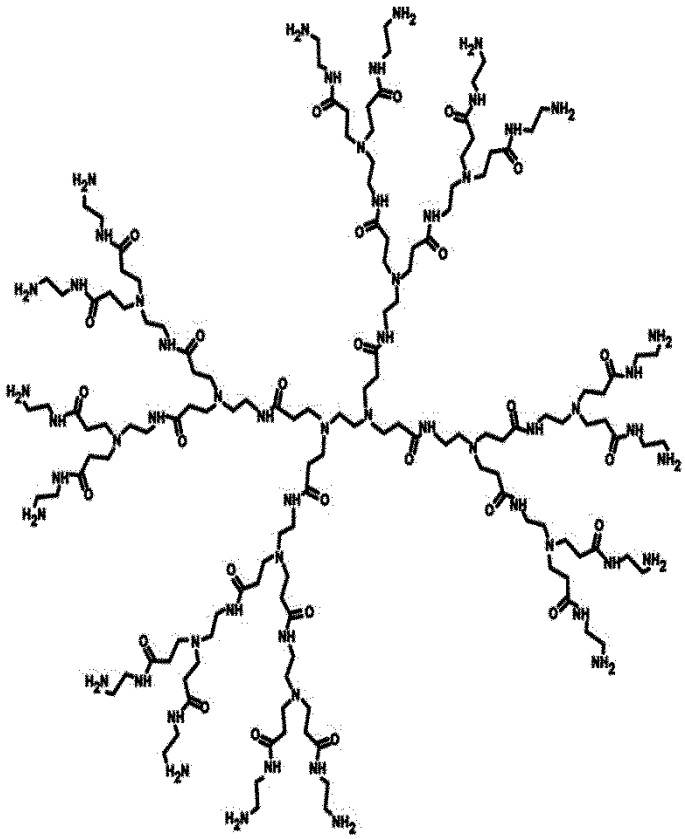
Structural Formula 9 represents an example of branched polyethyleneamine. Here, n may be an integer of 1 to 500.

The biodegradable polymer of the present invention comprises a dendrimer polymer that is an example of the polymer of Structural Formula 8 comprising ethyleneamine as a monomer. For example, a dendrimer-type branched polyethyleneamine may be the polymer of Structural Formula 10 or 11, but the present disclosure is not limited thereto. The polymer of Structural Formula 11 is a poly(amidoamine) polymer and may also be referred to as PAMAM.

[Structural Formula 10]



[Structural Formula 11]



The biocompatible polymer binds with the recombinant adenovirus or viral DNA of the present invention to increase the efficiency of inflow into cells, increases stability in an

individual, and improves delivery efficiency of a virus or viral DNA to a target site by lowering immunogenicity, thereby significantly increasing the cancer treatment effect due to the adenovirus or viral DNA of the present invention.

The biocompatible polymer and the adenovirus system may be connected by electrostatic interaction, ionic interaction or chemical bonding.

In accordance with another aspect of the present invention, the present invention provides a pharmaceutical composition that comprises a gene delivery system comprising a nucleic acid sequence encoding interleukin-12 (IL-12); and a nucleic acid sequence expressing an oligonucleotide that binds complementarily to the C-met gene and inhibits the expression of C-met. The composition may be an anticancer composition, an anti-metastatic composition for cancer cells, or a composition for enhancing antitumor immunity.

The composition may include the adenovirus system of the present invention in a therapeutically effective amount. In addition, the composition may further include a pharmaceutically acceptable carrier.

The anticancer composition serves to inhibit the survival, proliferation, and/or migration of tumor cells to inhibit the proliferation of cancer cells or to induce or promote the death of cancer cells. The recombinant adenovirus system of the present invention or a composition comprising the same can efficiently induce the death of cancer cells and inhibit migration thereof.

The anti-metastatic composition for cancer cells has an effect of inhibiting migration of cancer cells through migration. For example, it can be confirmed that the anti-metastatic

composition inhibits EMT in cancer cells, thereby inhibiting migration thereof. The anti-metastatic composition of the present invention may have both an anticancer effect through killing of cancer cells and an effect of inhibiting migration of cancer cells.

The composition for enhancing antitumor immunity is capable of treating cancer by overcoming immune surveillance avoidance of a tumor using an immunosuppressive molecule produced in tumor tissue, and may be included in an anticancer composition. Particularly, the antitumor immunity composition serves to normalize the imbalance of immune cells in tumor tissues and induce differentiation of T helper cells by IL-12, thereby activating the cytotoxicity of cytotoxic T lymphocytes and natural killer cells, resulting in enhanced anticancer immunity.

The pharmaceutical composition of the present invention expresses IL-12 to enhance anticancer immunity in a cancer subject. In addition, the expression of C-met is inhibited through shRNA against C-met, thereby further amplifying the function of IL-12. In an embodiment of the present invention, it was confirmed that, even in a mouse model injected with human-derived cancer cells, remarkable anticancer effects and tumor migration effects appeared when the IL-12 and shc-met co-expressing adenovirus of the present invention was injected.

In addition, the composition may be a composition for adjuvant treatment to enhance the therapeutic effect of standard therapy, or a composition for adjuvant treatment to improve or enhance the effect of standard therapeutic agents comprising other anticancer agents, immune checkpoint inhibitors, and immunotherapy agents, etc.

The anticancer composition of the present invention uses the recombinant adenovirus

included in the aforementioned composition for antitumor immunity enhancement. Accordingly, descriptions of the same contents are omitted to avoid excessive complexity of the present specification.

In accordance with an embodiment of the present invention, the cancer may be selected from the group consisting of gastric cancer, lung cancer, non-small cell lung cancer, breast cancer, ovarian cancer, liver cancer, bronchial cancer, nasopharyngeal cancer, laryngeal cancer, pancreatic cancer, bladder cancer, colorectal cancer, colon cancer, cervical cancer, bone cancer, non-small cell bone cancer, blood cancer, skin cancer (melanoma etc.), head or neck cancer, uterine cancer, rectal cancer, anal cancer, colon cancer, fallopian tube cancer, endometrial cancer, vaginal cancer, vulvar cancer, Hodgkin's disease, esophageal cancer, small intestine cancer, endocrine cancer, thyroid cancer, parathyroid cancer , adrenal cancer, soft tissue sarcoma, urethral cancer, penile cancer, prostate cancer, chronic or acute leukemia, lymphocytic lymphoma, kidney or ureter cancer, renal cell carcinoma, renal pelvic carcinoma, polyploid carcinoma, salivary gland cancer, sarcoma cancer, pseudomyxoma, hepatoblastoma, testicular cancer, glioblastoma, cleft lip cancer, ovarian germ cell tumor, basal cell carcinoma, multiple myeloma, gallbladder cancer, choroidal melanoma, ampulla of vater cancer, peritoneal cancer, adrenal cancer, tongue cancer, small cell cancer, pediatric lymphoma, nerve blastoma, duodenal cancer, ureteral cancer, astrocytoma, meningioma, renal carcinoma, vulvar cancer, thymus cancer, central nervous system (CNS) tumor, primary central nervous system lymphoma, spinal cord tumor, brain stem glioma, and pituitary adenoma, but the present invention is not limited thereto.

All of the aforementioned compositions of the present invention include a

pharmaceutically acceptable carrier. A pharmaceutically acceptable carrier used in the present invention is one commonly used in formulation and comprises lactose, dextrose, sucrose, sorbitol, mannitol, starch, gum acacia, calcium phosphate, alginate, gelatin, calcium silicate, fine crystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrup, methyl cellulose, methylhydroxybenzoate, propylhydroxybenzoate, talc, magnesium stearate, mineral oil, etc., but the present disclosure is not limited thereto. The pharmaceutical composition of the present invention may further include a lubricant, a wetting agent, a sweetening agent, a flavoring agent, an emulsifying agent, a suspending agent, a preservative, etc. in addition to the above components. Suitable pharmaceutically acceptable carriers and formulations are described in detail in Remington's Pharmaceutical Sciences (19th ed., 1995).

The composition of the present invention may be administered orally or parenterally. Parenteral administration may be intratumoral injection, intravenous injection, intradermal injection, subcutaneous injection, intramuscular injection, intraperitoneal injection, intrathecal injection, intracardiac injection, intrathoracic injection, intraarterial injection, intraosseous injection, intraarticular injection, transdermal administration, etc., preferably parenteral administration.

In addition, the composition may be for topical or systemic administration.

In accordance with an embodiment of the present invention, the antitumor immunity enhancement composition of the present invention is preferably administered directly intratumorally, thereby having an antitumor immunity enhancement effect.

A suitable dosage of the composition of the present invention may be varied depending upon factors such as a formulation method, an administration manner, a patient’s age, weight,

sex, pathological condition, and diet, an administration time, an administration route, an excretion rate, and response sensitivity. A daily dosage of the pharmaceutical composition of the present invention is, for example, 0.2 to 1,000 mg/kg. However, the actual dosage of an active ingredient can be determined in consideration of various related factors such as the amount of target tissue cells to be differentiated and proliferated, the route of administration, and a patient's weight, age, and sex. Accordingly, the dosage may be provided in any form without limiting the scope of the present invention.

In accordance with still another aspect of the present invention, the present invention provides the use of a gene delivery system, which comprises a gene encoding interleukin-12 (IL-12); and a gene expressing an oligonucleotide that complementarily binds to C-met gene to inhibit the expression of C-met, as an anticancer agent. The gene delivery system of the present invention co-expresses IL-12 and C-met-inhibiting RNA, thereby inhibiting tumor migration while further improving the antitumor immune effect of IL-12. The use of the gene delivery system of the present invention as an anticancer agent applies *mutatis mutandis* to the above description of the gene delivery system or pharmaceutical composition so as to avoid overlapping descriptions.

In accordance with yet another aspect of the present invention, the present invention provides a method of treating cancer, the method comprising administering a gene delivery system, which comprises a gene encoding interleukin-12 (IL-12); and a gene expressing an oligonucleotide that complementarily binds to C-met gene to inhibit the expression of C-met, or a pharmaceutical composition comprising the gene delivery system to a subject.

The gene delivery system of the present invention co-expresses IL-12 and C-met-inhibiting RNA, thereby inhibiting tumor migration while further improving the antitumor immune effect due to IL-12, resulting in a synergistic effect in cancer treatment. A method of treating with the gene delivery system of the present invention as an anticancer agent applies *mutatis mutandis* to the above description of the gene delivery system or pharmaceutical composition so as to avoid overlapping descriptions.

Hereinafter, the present invention will be described in more detail with reference to the following Examples. It will be apparent to those skilled in the art that the Examples are merely for concretely explaining the invention and therefore, there is no intent to limit the invention to the Examples.

[Examples]

Preparation of experiment and construction of recombinant vector

Preparation Example 1. Cell obtainment and culture

As a cell culture medium, Dulbecco's modified Eagle's Medium (DMEM; Gibco BRL, Grand Island, NY), Roswell Park Memorial Institute medium (RPMI; Gibco BRL), or Minimal Essential Medium (MEM; Gibco BRL) which contained 10% fetal bovine serum (FBS; Gibco BRL), L-glutamine (2 mmol/L), penicillin (100 IU/mL), and streptomycin (50 mg/mL) was used. HEK293 (expressing the adenovirus E1 site, human embryonic kidney cell line), H1975 (human non-small lung cancer cell line), and HaK (hamster kidney cancer cell line)

were purchased from American Type Culture Collection, Manassas (ATCC, VA). HaP-T1 (hamster pancreatic carcinoma cell line) was provided by Dr. Masato Abei (University of Tsukuba, Ibaraki, Japan). All cell lines were cultured under a humid environment of 37°C and 5% CO_2, and were subjected to a mycoplasma negative test using Hoeschst dye, cell culture and PCR. E. coli (Escherichia coli) was cultured at 37°C in Luria Bertani medium.

Manufacturing Example 1. Construction of oncolytic adenovirus system expressing IL-12 and/or shc-met

Manufacturing Example 1-1. Construction of pDNA expressing human-derived IL-12 and shc-met

To investigate antitumor effects and antitumor immune response using an Ad vector, two oncolytic adenovirus plasmid DNAs (referred to as Ad pDNA or oAd pDNA) recombined based on mT-Rd19-RGD so as to express an antitumor immune gene, human interleukin-12 (hIL-12), and/or a short hairpin RNA (hereinafter referred to as "shc-met") complementarily binding to C-met to inhibit the expression of C-met playing an important role in cancer cell growth and differentiation were constructed (FIG. 1): (mT-Rd19-RGD and mT-Rd19-RGD/hIL-12/shc-met).

【Table 2】

	oAd pDNA structure	Remark
Example 1	mT-Rd19-RGD/hIL-12/shc-met (Pac I site 2)	hIL-12 and shc-met co-expressed, two Pac I sites
Example 2	mT-Rd19-RGD/hIL-12/shc-met (Pac I site 1)	hIL-12 and shc-met co-expressed, one Pac I site

Manufacturing Examples 1 to 2. Construction of polymer-viral DNA complex

expressing mouse-derived IL-12 and shc-met

In addition, a gene expressing mouse-derived IL-12 p35 (SEQ ID NO: 7) and IL-12 p40 (SEQ ID NO: 8) was inserted based on HE5cT-Rd19-RGD. In addition, a shRNA was designed using 4398th to 4422th nucleotides of the mouse C-met gene of SEQ ID NO: 11 as target sequences (SEQ ID NO: 15). HE5cT-Rd19-RGD/scIL-12/shc-met plasmid DNA, into which the nucleotide sequence of SEQ ID NO: 14 was inserted, complementarily binding to the C-MET gene and expressing shc-met that was capable of inhibiting the expression of the C-MET gene was constructed. The constructed HE5cT-Rd19-RGD/scIL-12/shc-met plasmid DNA was mixed with a PPCBA-PEI-Arginine (PPA) polymer, thereby constructing a pDNA/PPA polymer complex.

Manufacturing Example 2. Construction of recombinant oncolytic adenovirus expressing IL-12 and/or shc-met

To investigate antitumor effects and antitumor immune response due to an anticancer adenovirus co-expressing IL-12 and shc-met, an anticancer adenovirus was constructed (FIG. 4, distinguishingly marked as HscIL-12 and Hshc-met when the recombinant adenovirus included a human-derived sequence):

HE5cT-Rd19-RGD: Ad vector not containing an expression gene

HE5cT-Rd19-RGD/scIL-12: Ad vector expressing only IL-12

HE5cT-Rd19-RGD/shc-met: Ad vector expressing shRNA for C-Met gene

HE5cT-Rd19-RGD/scIL-12/shc-met: Ad vector expressing shRNA for IL-12 and C-Met gene

More particularly, genes respectively expressing human-derived IL-12 p35 (SEQ ID NO: 1) and IL-12 p40 (SEQ ID NO: 2) were respectively inserted into HE5cT-Rd19-RGD. In addition, shRNA of the sequence of SEQ ID NO: 12 which targets 1987th to 2007th nucleotides of mRNA of the human C-met of SEQ ID NO: 10 as a target sequence (SEQ ID NO: 13) was inserted into a recombinant adenovirus vector.

In addition, in the same manner as in the above, each gene respectively expressing mouse-derived IL-12 p35 (SEQ ID NO: 8) and IL-12 p40 (SEQ ID NO: 9) and a shc-met-expressing sequence (SEQ ID NO: 14) targeting 4398th to 4422th nucleotides of the mouse-derived C-MET gene of SEQ ID NO: 11 as a target sequence (SEQ ID NO: 15) and binding complementarily to the nucleotides to inhibit expression thereof were inserted to manufacture an adenovirus vector co-expressing IL-12 and shcmet.

Manufacturing Example 3. Animal model establishment

3-1. Human xenograft lung cancer tumor model

When a nude mouse purchased from Orient was 6-8 weeks old, the human lung cancer cell line (H1975) was injected subcutaneously at 3×10^6 cells/50 μL into the nude mouse. Next, when the size of tumor reached an average of 100 mm^3, the anticancer adenovirus of Preparation Example 1 or 2 was administered thereto, and the effect thereof was confirmed.

3-2. Mouse tumor model

For experiments, C57BL6 (B16-F10 tumor model) mice were purchased from Daehan Biolink, and BALB/C (CT26 tumor model) mice were purchased from Orient. When mice

were 6 -8 weeks old, a mouse skin cancer cell line (B16-F10) or a mouse gastric cancer cell line (CT26) was injected subcutaneously into a mouse at $5x10^5$ cells/50 μL, and then, when the size of tumor reached an average of 100 mm^3, the anticancer adenovirus of Manufacturing Example 1 or 2 was administered and the effects thereof were confirmed.

Experimental Example 1. Confirmation of expression of mIL-12 and hIL-12 in cells by Ad pDNA

To investigate the expression of the human IL-12 (hIL-12) gene by Ad pDNA constructed according to Manufacturing Example 1, mT-Rd19-RGD and mT-Rd19-RGD/hIL-12/shc-met Ad pDNA were reacted with lipofectamine at room temperature, and then 50% confluent 293A, which is a human embryonic kidney cell line with good intracellular delivery efficiency, was reacted with the reaction product. At 72 hours after treatment, the cell culture medium was collected to perform hIL-12 ELISA.

As shown in FIG. 2, it was confirmed that, in the case of the example (mT-Rd19-RGD/hIL-12/shc-met Ad pDNA; two Pac I), hIL-12 was expressed at 97,500 ± 6,384 pg/mL. Accordingly, it was confirmed that Ad pDNA constructed according to the example efficiently induced the expression of the therapeutic gene, interleukin-12, in cells.

Experimental Example 2. Confirmation of co-expression of intercellular IL-12 and C-met by adenovirus (Ad) pDNA

In consideration of the intracellular delivery efficiency of the adenovirus pDNA constructed according to Manufacturing Example 1, the expression of hIL-12 by mT-Rd19-

RGD/hIL-12/shc-met Ad pDNA was investigated in the 293A cell line. However, C-met expression inhibition by the expression of C-met-specific shRNA was not confirmed in the 293A cell line because C-met was not over-expressed in normal 293A cells.

Accordingly, to verify the C-met expression inhibition ability by mT-Rd19-RGD/hIL-12/shc-met (two Pac I) of the example, mT-Rd19-RGD/hIL-12/shc-met adenovirus (Ad) was manufactured, a 50%-confluent human lung cancer cell line A549 overexpressing C-met in a 6-well plate was treated with 20 μl of the manufactured mT-Rd19-RGD/hIL-12/shc-met adenovirus (Ad). At 48 hours after the treatment, the cell culture medium was collected and ELISA for hIL-12 and C-met was performed. The expression amounts of hIL-12 and C-met in a non-treated A549 human lung cancer cell line were used as controls.

As shown in FIG. 3, it was confirmed that the expression (235,000 ± 20,518 pg/mL) of hIL-12 by mT-Rd19-RGD/hIL-12/shc-met Ad increased compared to the control, and the expression of C-met in the group treated with mT-Rd19-RGD/hIL-12/shc-met Ad was reduced by 1.2 times, compared to the control. Accordingly, it can be confirmed that the expression of the therapeutic genes, IL-12 and shc-met, is efficiently induced by the Ad system manufactured according to the present invention.

Experimental Example 3. Confirmation of in-vivo antitumor effects of anticancer adenovirus co-expressing IL-12 and shc-met (mouse syngeneic tumor model)

To verify the anticancer treatment effect of the anticancer adenovirus, which co-expressed mouse IL-12 and mouse C-met-targeting shc-met, constructed according to Manufacturing Example 2, respective tumor models were established using the mouse skin

cancer cell line B16-F10 or the mouse gastric cancer cell line CT26, and antitumor effects were confirmed.

After subcutaneously injecting the mouse skin cancer cell line B16-F10 or the mouse gastric cancer cell line CT26 at $5x10^5$ cells/50 μL into mice, PBS or HE5cT-Rd19-RGD/scIL-12/shc-met was respectively injected into the mice when the size of tumor reached an average of 100 mm^3. After administration into the tumor three times at intervals of two days (1×10^{10} VP), the size of the tumor was measured to observe the antitumor effect.

As shown in FIGS. 5a and 5b, it was confirmed that, using two types of mouse tumor models, tumors grew rapidly in the PBS-treated group, but tumors completely disappeared in all subjects administered HE5cT-Rd19-RGD/scIL-12/shc-met.

Experimental Example 4. Confirmation of in-vivo antitumor effects of anticancer adenovirus pDNA/PPA complex co-expressing IL-12 and shc-met (mouse syngeneic tumor model)

A complex (pDNA/PPA) of HE5cT-Rd19-RGD/scIL-12/shc-met viral DNA) and PPA polymer constructed according to Manufacturing Examples 1 to 2 was constructed to confirm the anticancer treatment effect. Particularly, tumor models were established using the mouse skin cancer cell line B16-F10, and the antitumor effects therein were confirmed. The mouse skin cancer cell line (B16-F10) was subcutaneously injected at $5x10^5$ cells/50 μL into mice, and then, when the size of tumor reached an average of 100 mm^3, PBS and the HE5cT-Rd19-RGD/scIL-12/shc-met Ad pDNA/PPA complex were respectively injected into the mouse models. After administration into the tumor 7 times daily, the size of the tumor was measured

to observe the antitumor effect.

As shown in FIG. 6, it was confirmed that the size of tumor increased up to 2514.7±202.1 mm^3 in the PBS-administered group, whereas significant antitumor effects were exhibited in all subjects of the group administered the anticancer virus, pDNA/PPA complex. The average tumor size in the group administered HE5cT-Rd19-RGD/scIL-12/shc-met Ad pDNA/PPA complex was 1387.6±171.8 mm^3, which is 44.8% lower compared to the PBS-administered group. This result indicates that the viral DNA co-expressing IL-12 and shc-met also induces improved antitumor effects.

Experimental Example 5. Confirmation of intercellular expression of IL-12 and shc-met by anticancer adenovirus co-expressing IL-12 and shc-met (human)

To investigate the expression of human IL-12 or human c-Met-targeting shc-Met by the anticancer adenovirus co-expressing IL-12 and shc-met, the human lung cancer cell line H1975 was administered each of the recombinant adenoviruses HE5cT-Rd19-RGD, HE5cT-Rd19-RGD/shc-met, HE5cT-Rd19-RGD/hscIL-12 and HE5cT-Rd19-RGD/hscIL-12/shc-met constructed according to Manufacturing Example 2 at 2 MOI (FIG. 7b) or 5 MOI (FIG. 7a). At 48 hours after the treatment, the cells and the culture medium were collected to perform ELISA for human IL-12 and western blotting for c-Met and to confirm the expression of each gene.

As shown in FIG. 7a, it was confirmed that human IL-12 was expressed in both HE5cT-Rd19-RGD/hscIL-12 (3066.3±165.7 pg/mg) and HE5cT-Rd19-RGD/hscIL-12/shc-met (94922.6 ± 185.8 pg/mg) recombinant vectors.

In addition, as shown in FIG. 7b, it was confirmed that, in the cells treated with the HE5cT-Rd19-RGD/shc-met or HE5cT-Rd19-RGD/hscIL-12/shc-met recombinant vector, the expression of C-met was inhibited, compared to the HE5cT-Rd19-RGD control. Accordingly, it was confirmed that the expression of the therapeutic genes IL-12 and shc-met inserted into the recombinant anticancer adenovirus vector manufactured in the present invention was efficiently induced.

Experimental Example 6. Verification of cancer cell killing ability of anticancer adenovirus co-expressing human-derived IL-12 and shc-met

To investigate the cancer cell killing ability of the anticancer adenovirus co-expressing human-derived IL-12 and shc-met, the human lung cancer cell line H1975 was infected with the anticancer adenovirus HE5cT-Rd19-RGD; HE5cT-Rd19-RGD/hscIL-12; HE5cT-Rd19-RGD/shc-met; or HE5cT-Rd19-RGD/hscIL-12/shc-met manufactured according to Manufacturing Example 2 at 2, 5, 10, 20, or 50 MOI, and the cancer cell killing ability of the adenovirus was observed under a normal oxygen condition (normoxia) and hypoxia condition (hypoxia).

As shown in FIGS. 8a and 8b, it was confirmed that the cancer cell killing ability of HE5cT-Rd19-RGD, HE5cT-Rd19-RGD/hscIL-12, HE5cT-Rd19-RGD/shc-met, or HE5cT-Rd19-RGD/hscIL-12/shc-met increased in proportion to virus titer under both a normal oxygen condition and a hypoxia condition. In addition, under the 50 MOI-infected conditions, the cancer cell killing ability of the group treated with a single therapeutic gene, i.e., HE5cT-Rd19-RGD/hscIL-12 or HE5cT-Rd19-RGD/shc-met, increased compared to HE5cT-Rd19-RGD, but

the co-expression group, i.e., HE5cT-Rd19-RGD/hscIL-12/shc-met, induced remarkably increased cancer cell killing ability, compared to the single-treated group. This result indicates that co-expression of IL-12 and shc-met exhibits a synergistic effect in chemotherapy.

Experimental Example 7. Verification of ability of anticancer adenovirus co-expressing human-derived IL-12 and shc-met to inhibit a migration and invasion of HUVEC

HGF expressed in cancer cells has been reported to increase the migration and invasion of cancer cells by activating the C-met signaling system in vascular endothelial cells, and to induce abnormal blood vessel formation as a result. Therefore, the following experiment was conducted to confirm whether the anticancer adenovirus co-expressing IL-12 and shc-met can reduce the metastasis and invasive ability of HUVEC.

The human lung cancer cell line H1975 was infected with HE5cT-Rd19-RGD, HE5cT-Rd19-RGD/hscIL-12, HE5cT-Rd19-RGD/shc-met, or HE5cT-Rd19-RGD/hscIL-12/shc-met, and then a supernatant was collected to preform migration and invasion assay. As controls, a non-treated medium (fresh media, 5% FBS RPMI) and a cancer cell medium (H1975-culture media, H1975-CM) not injected with an anticancer virus were used.

As shown in FIGS. 9a, 9b, 9c and 9d, it was confirmed that the migration and invasion ability of HUVEC in H1975-CM (cancer cell medium not infected with anticancer virus) increased, compared to the group treated with a non-treated medium (fresh media). On the other hand, it was confirmed that, in the group of cancer cells that were treated with a medium infected with HE5cT-Rd19-RGD/HscIL-12 or HE5cT-Rd19-RGD/Hshc-met, the migration

and invasion ability of HUVEC was reduced compared to the group infected with HE5cT-Rd19-RGD, particularly, the migration and invasion ability of HUVEC was remarkably inhibited in the cancer cell medium infected with HE5cT-Rd19-RGD/HscIL-12/Hshc-met. These results indicate that the amount of HGF expressed in cancer cells is efficiently inhibited by the anticancer adenovirus co-expressing IL-12 and shc-met, resulting in a synergetic effect in reducing the migration and invasion ability of HUVEC.

Experimental Example 8. Confirmation of epithelial-mesenchymal mutation (Endo-MT) inhibition effect of anticancer adenovirus co-expressing human-derived IL-12 and shc-met

To verify the blood vessel normalization effect induced by the anticancer adenovirus co-expressing IL-12 and shc-met, H1975 the human lung cancer cell line was infected with HE5cT-Rd19-RGD, HE5cT-Rd19-RGD/HscIL-12, HE5cT-Rd19-RGD/Hshc-met, or HE5cT-Rd19-RGD/HscIL-12/Hshc-met, and then a supernatant was collected therefrom. HUVEC cells were cultured in the supernatant, and then changes in the expression of endothelial or mesenchymal markers were investigated through western blot. As controls, a non-treated medium (fresh media, 5% FBS RPMI), and a cancer cell medium not infected with an anticancer virus were used.

As shown in FIG. 10, it was confirmed that, in the case of the groups treated with the culture medium that was collected from the cancer cells infected with HE5cT-Rd19-RGD/Hshc-met or HE5cT-Rd19-RGD/HscIL-12/Hshc-met, the expression of mesenchymal markers, N-cadherin and a-SMA, was reduced, compared to the control (fresh media-treated

group). Thereamong, the group treated with the adenovirus co-expressing IL-12 and shc-met exhibited the most excellent expression reduction effect. These results indicate that co-expression of IL-12 and shc-met not only inhibits the migration and invasion ability of HUVEC cells, but also inhibits endo-MT transition, thereby exhibiting excellent blood vessel renormalization effect.

Experimental Example 9. Confirmation of in vivo antitumor effects of anticancer adenovirus co-expressing IL-12 and shc-met in human xenograft tumor model

To verify the anticancer treatment effect of the anticancer adenovirus of the present invention co-expressing IL-12 and shc-met in a human xenograft tumor model, tumor models were established using the human lung cancer cell line H1975, and antitumor effects therein were investigated.

To establish the human xenograft tumor models, particularly, the human lung cancer cell line (H1975) at $3x10^6$ cells/50 μL was subcutaneously injected into nude mice, and then, when the tumor size reached an average of 100 mm^3, PBS, HE5cT-Rd19-RGD, HE5cT-Rd19-RGD/HscIL-12, HE5cT-Rd19-RGD/Hshc-met, and HE5cT-Rd19-RGD/HscIL-12/Hshc-met were respectively administered. After administration ($5x10^6$ pfu) into the tumor twice every two days, the tumor size was measured.

As shown in FIG. 11, it was confirmed that tumors rapidly proliferated in the PBS-administered group, but significant antitumor effects were observed in all of the groups administered with the anticancer adenovirus. Particularly, it can be confirmed that, compared to the PBS-administered group, the tumor size is reduced by 81.7% in HE5cT-Rd19-RGD,

85.5% in HE5cT-Rd19-RGD/HscIL-12, 92.2% in HE5cT-Rd19-RGD/Hshc-met, and 94.6% in HE5cT-Rd19-RGD/HscIL-12/Hshc-met. From these results, it can be confirmed that, when the anticancer adenovirus co-expressing IL-12 and shc-met is used, synergistic effects can be provided, compared to the single-treated group.

Experimental Example 10. Confirmation of IL-12 expression and C-met expression reduction in cells by anticancer adenovirus co-expressing IL-12 and Lbcpf1-crMET

Experimental Example 10-1. Construction of anticancer adenovirus co-expressing IL-12 and C-met-targeting CRISPR RNA

CRISPR RNA system (LbCpf1-crRNA system) was constructed using 3.5 generation gene scissors (CRISPR/Cpf1) that allows gene correction and desired mutation to occur in the process of cutting a target DNA, and then reconnecting the same through an intracellular repair system, so as to investigate whether the same effect is exhibited when the CRISPR system is used in the adenovirus system of the present invention. IL-12 sequence was located in a recombinant vector in the same manner as in Manufacturing Example 2, but the sequence (SEQ ID NO: 6) (indicated as HIL-12 in FIG. 12) comprising an IRES coding sequence (SEQ ID NO: 20) was disposed between the sequence encoding p35 of IL-12 (SEQ ID NO: 4) and the sequence encoding p40 of IL-12 (SEQ ID NO: 5).

C-met target guide RNA sequences were selected using a program, and then oligomer synthesis was commissioned by Bionics. Cloning was performed through annealing. The gene encoding Cpf1 protein (SEQ ID NO: 18) was inserted into the E1 site of RdB-RGD

adenovirus, and the U6 promoter was positioned upstream of a site expressing guide RNA (crC-met). In addition, the sequence encoding IL-12; and the sequence expressing C-met-targeting guide RNA (SEQ ID NO: 16) were inserted into the E3 region of RdB-RGD adenovirus, thereby constructing a recombinant adenovirus that co-expresses IL-12 and the CRISPR-CAS system. The guide RNA was designed to target the 55th to 77th nucleotides of the human C-met sequence of SEQ ID NO: 10 (FIG. 12).

It was confirmed that, when the guide RNA (crcmet) expressed by the constructed recombinant adenovirus recognizes a target site of a target gene and designates a position to be corrected, the gene scissors Cpf1 can cut a target site and thus can efficiently inhibit C-met.

Experimental Example 10-2. Investigation of C-met inhibition effect due to anticancer adenovirus co-expressing IL-12 and C-met-targeting CRISPR RNA

To investigate the expression of human-derived IL-12 or C-met expression reduction through Lbcpf1-crMET system, due to the anticancer adenovirus co-expressing IL-12 and Lbcpf1-crMET, the human lung cancer cell line H1975 was infected with the anticancer adenovirus at 5 MOI or 100 MOI, and after 48 hours, cells and culture media were collected and subjected to ELISA for human IL-12 or western blot for c-Met.

As shown in FIGS. 13a, 13b, and 13c, IL-12 expressions of 9000.0±600.0 pg/mL and 17400.0±1200.0 pg/mL were respectively confirmed in the groups respectively treated with RdB-RGD/Lbcpf1/HscIL-12 and RdB-RGD/Lbcpf1/HscIL-12-crMET. In addition, it was confirmed that the expression of C-met was inhibited in all of the groups treated with the anticancer virus. Particularly, the expression of C-met was most efficiently reduced by RdB-

RGD/Lbcpf1/HscIL-12-crMET.

Experimental Example 11. Confirmation of in vivo antitumor effects of anticancer adenovirus co-expressing IL-12 and Lbcpf1-crMET in human xenograft tumor models

Experiments were conducted to investigate whether C-met expression inhibition by CRISPR, not shRNA, can be realized together the expression of IL-12 in human xenograft tumor models.

C-met expression inhibition by CRISPR was investigated using the Lbcpf1-crMET system constructed according to Experimental Example 10-1.

To verify the anticancer treatment effect of the anticancer adenovirus co-expressing IL-12 and Lbcpf1-crMET, tumor models were established using the human lung cancer cell line H1975 and antitumor effects therein were investigated. The human lung cancer cell line H1975 was subcutaneously injected at $3x10^6$ cells/50 μL into nude mice, and then, when the tumor size reached an average of 90 mm^3, PBS, RdB-RGD/Lbcpf1, RdB-RGD/Lbcpf1/HscIL-12, and RdB-RGD/Lbcpf1/HscIL-12-crMET were respectively administered. After administration ($5x10^6$ VP) into the tumor three times every two days, the tumor size was measured to observe antitumor effects.

As shown in FIG. 14, the tumor size rapidly increased to 1486.5±221.1 mm^3 on the 25th days in the PBS-administered group, but a significant antitumor effect was observed in all of the groups treated with the anticancer virus. In the groups treated with the anticancer virus, an average of tumor size was 876.9±146.2 mm^3 (RdB-RGD/Lbcpf1), 792.0±230.3 mm^3 (RdB-

RGD/Lbcpf1/scIL-12), and 535.6±172.6mm^3 (RdB-RGD/Lbcpf1/scIL-12-crMET), respectively. That is, in the groups treated with the anticancer virus, the size of tumor was 41.0, 46.7 or 64% reduced, compared to the PBS-administered group. Particularly, in the case in which the CRISPR method was used to be co-expressed with IL-12, the tumor size was remarkably reduced. Therefore, it was confirmed that the synergistic effect due to the two therapeutic genes was exhibited also when the CRISPR system was used.

【CLAIMS】

【Claim 1】

An adenovirus system, comprising:

a gene encoding interleukin-12 (IL-12); and

a gene expressing an oligonucleotide that inhibits expression of C-met.

【Claim 2】

The adenovirus system according to claim 1, wherein the gene encoding interleukin-12 (IL-12) comprises an IL-12A (p35) gene sequence and an IL-12B (p40) gene sequence.

【Claim 3】

The adenovirus system according to claim 2, wherein the gene encoding interleukin-12 (IL-12) further comprises a linker sequence or internal ribosome entry site (IRES) sequence between the IL-12A (p35) gene sequence and the IL-12B (p40) gene sequence.

【Claim 4】

The adenovirus system according to claim 1, wherein the gene encoding interleukin-12 (IL-12) is inserted into an E1B or E3 region of adenovirus gene in the adenovirus system.

【Claim 5】

The adenovirus system according to claim 1, wherein the oligonucleotide

complementarily binds to the C-met gene to inhibit expression of C-met and is one or more selected from the group consisting of small hairpin RNA (shRNA), miRNA, siRNA, antisense oligonucleotides, ribozymes, DNAzyme, peptide nucleic acids (PNA), and guide RNA for CRISPR.

【Claim 6】

The adenovirus system according to claim 1, wherein the gene encoding the oligonucleotide inhibiting expression of C-met is inserted into an E1B or E3 region of adenovirus gene in the adenovirus system.

【Claim 7】

The adenovirus system according to claim 1, wherein the adenovirus system is a recombinant adenovirus, or a recombinant adenoviral DNA.

【Claim 8】

The adenovirus system according to claim 1, wherein, when the oligonucleotide complementarily binds to the C-met gene to inhibit expression of C-met and is a guide RNA for CRISPR, the adenovirus system further comprises a nucleotide sequence expressing Cas protein.

【Claim 9】

The adenovirus system according to claim 1, further comprising a biocompatible polymer in a form of being combined with the adenovirus system.

【Claim 10】

The adenovirus system according to claim 9, wherein the biocompatible polymer is selected from the group consisting of PEI-Arg-mPEG-S-S-mPEG-Arg-PEI (PAPS), mPEG-PEI-g-Arg-S-S-Arg-g-PEI-mPEG (PPSA), pegylated and iron oxide nanoparticles-crosslinked catechol-grafted poly L lysine (PICION), an arginine-grafted biodegradable polymer (ABP), a pegylated and PTX-conjugated polymeric micelle (APP), mPEG-b-Pip-CBA (PPCBA), PPCBA-PEI-Arginine (PPA), poly(amidoamine) dendrimer (PAMAM), polyethyleneglycol (PEG), poly-lactide (PLA), polyglycolide (PGA), poly-lactide-co-glycolide (PLGA), poly-ε-caprolactone (PCL), polyethyleneamine (PEI), hyaluronic acid (HA), gelatin, chitosan, and serum albumin.

【Claim 11】

An anticancer composition, comprising the adenovirus system according to any one of claims 1 to 10.

【Claim 12】

The anticancer composition according to claim 11 for the use in treatment of cancer, wherein the cancer is gastric cancer, lung cancer, non-small cell lung cancer, breast cancer,

ovarian cancer, liver cancer, bronchial cancer, nasopharyngeal cancer, laryngeal cancer, pancreatic cancer, bladder cancer, colorectal cancer, colon cancer, cervical cancer, bone cancer, non-small cell bone cancer, blood cancer, skin cancer (melanoma etc.), head or neck cancer, uterine cancer, rectal cancer, anal cancer, colon cancer, fallopian tube cancer, endometrial cancer, vaginal cancer, vulvar cancer, Hodgkin's disease, esophageal cancer, small intestine cancer, endocrine cancer, thyroid cancer, parathyroid cancer , adrenal cancer, soft tissue sarcoma, urethral cancer, penile cancer, prostate cancer, chronic or acute leukemia, lymphocytic lymphoma, kidney or ureter cancer, renal cell carcinoma, renal pelvic carcinoma, polyploid carcinoma, salivary gland cancer, sarcoma cancer, pseudomyxoma, hepatoblastoma, testicular cancer, glioblastoma, cleft lip cancer, ovarian germ cell tumor, basal cell carcinoma, multiple myeloma, gallbladder cancer, choroidal melanoma, ampulla of vater cancer, peritoneal cancer, adrenal cancer, tongue cancer, small cell cancer, pediatric lymphoma, nerve blastoma, duodenal cancer, ureteral cancer, astrocytoma, meningioma, renal carcinoma, vulvar cancer, thymus cancer, central nervous system (CNS) tumor, primary central nervous system lymphoma, spinal cord tumor, brain stem glioma, or pituitary adenoma.

【Claim 13】

The anticancer composition according to claim 11, wherein the composition is formulated to be intratumorally administered.

【Claim 14】

A composition for use in enhancing antitumor immunity, comprising the adenovirus system according to any one of claims 1 to 10.

【Claim 15】

The composition for use according to claim 14, wherein the composition is to be intratumorally administered.